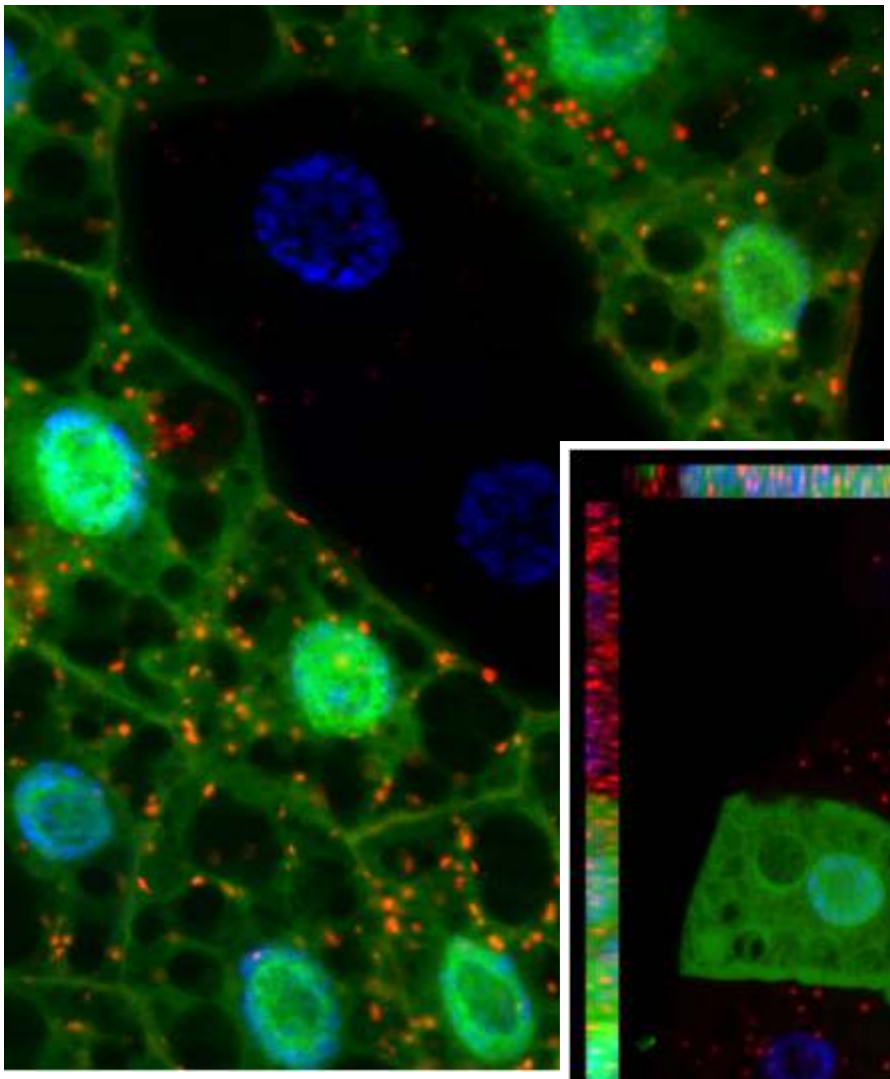
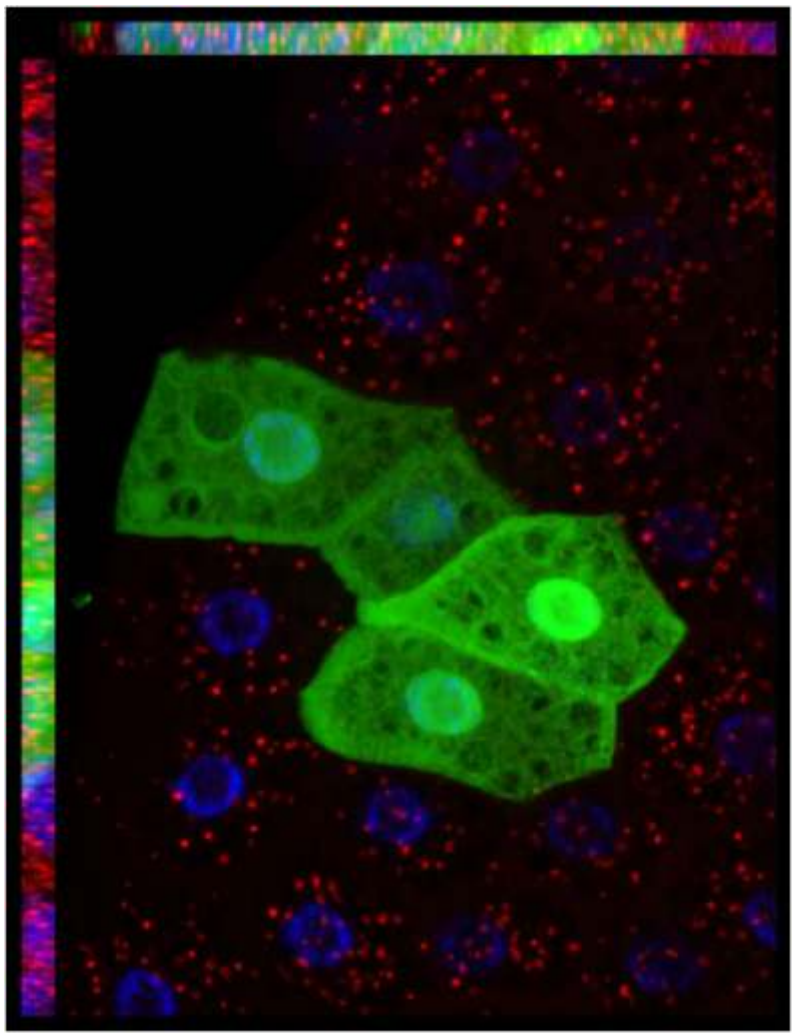
Hungarian Molecular Life Sciences 2013

5-7 April 2013





Hungarian Biochemical Society

MAGE
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Programme & Book of abstracts

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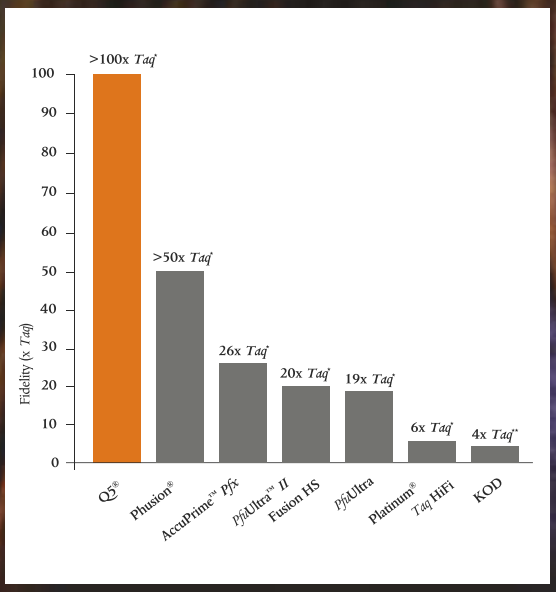

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HUNGARIAN MOLECULAR
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2013

PROGRAMME
&
BOOK OF ABSTRACTS

HOTEL AZÚR
SIÓFOK, HUNGARY
5-7 APRIL 2013

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Dear Colleagues,

Recognizing the increasing overlap and convergence of their respective research fields, the **Hungarian Biochemical Society (MBKE)**, the **Hungarian Genetical Society (MAGE)** and the **Hungarian Association for Cell and Developmental Biology (SFBT)** decided to organize a pioneering first joint conference of landmark importance, entitled

"**Hungarian Molecular Life Sciences 2013**"

in Siófok, Hungary 5-7 April 2013.

This event is expected to be the most significant national conference in life sciences in Hungary, uniting researchers within our scientific societies of long tradition. More than 400 scientists have registered, from institutions of higher education as well as from research institutes under the egis of the Hungarian Academy of Sciences or different other governmental bodies. This large-scale meeting, adhering to the traditions of each association, is concurrently will also constitute the X. Hungarian Genetics Conference, the XVII. Symposium of Cell and Developmental Biologists, and the forthcoming Convention 2013 of the Hungarian Biochemical Society. The joint conference takes advantage of integrating the fields, from their earlier relative separation, steering them toward a novel value in their history. The goal of the conference is to establish a common forum for colleagues working on the fields of classic and molecular biochemistry, cell and structural biology, developmental biology, classic and molecular genetics, the molecular biology of human diseases, systems biology, synthetic biology, genomics and bioinformatics. The conference is held in the conference center of Hotel Azúr, Siófok.

We count on your contribution to a scientific symposium of great atmosphere, elated and memorable, to a common mission for joint benefit. The official language of the Conference is English.

The congress is organized in collaboration with Diamond Congress Ltd. Their staff is happy to respond to questions you might have on organizational details.

On behalf of the organizers:

Prof. László Fésüs	**Dr. Miklós Erdélyi**	**Prof. Gábor Szabó**
Prof. Vértessy G. Beáta	**Prof. Putnoky Péter**	**Prof. Sass Miklós**
Hungarian Biochemical Society	**Hungarian Genetical Society**	**Hungarian Association for Cell and Developmental Biology**

GENERAL INFORMATION

GENERAL INFORMATION

CONGRESS ORGANISERS

- *Magyar Biokémiai Egyesület (Hungarian Biochemical Society)*
- *Magyar Genetikusok Egyesülete (Hungarian Genetical Society)*
- *Sejt- és Fejlődésbiológiai Szakosztály (Hungarian Biological Society, Section of Cell and Developmental Biology)*

ORGANISING COMMITTEEE

Miklós Erdélyi[1], László Fésüs [2], Péter Putnoky [3], Miklós Sass [4], Gábor Szabó [5], Beáta Vértessy G. [6]

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http://www.deoec.hu/

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[6] *Institute of Enzymology Research Centre for Natural Sciences of the Hungarian Academy of Sciences*
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TECHNICAL ORGANISERS

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Tel: 1-225-0209
http://www.diamond-congress.hu/

WEBSITE OF THE CONGRESS

http://www.hunlifesci.hu

WEBSITE OF THE HUNGARIAN BIOCHEMICAL SOCIETY

http://www.mbkegy.hu

WEBSITE OF THE HUNGARIAN GENETICAL SOCIETY

http://www.bioinfo.pte.hu/mage/mage.htm

VENUE

Hotel Azúr
8600 Siófok
Erkel Ferenc utca 2/C.
Tel.: 06 84 501 400

OPENING HOURS OF THE REGISTRATION

Friday, 5 April, 2013.	-	**10.00 – 20.00**
Saturday, 6 April, 2013.	-	**08.00 – 20.00**
Sunday, 7 April, 2013.	-	**08.00 – 13.00**

ONSITE CONTACT NUMBERS

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OFFICIAL LANGUAGE

Official language of the Congress is English (no translation is available).

Registration fee (incl. VAT)	Payment till 15 February	Payment after 15 February
Registration fee for industrial participants	50.000 Ft	55.000 Ft
Registration fee for senior researchers*	45.000 Ft	50.000 Ft
Registration fee for junior researchers**	30.000 Ft	35.000 Ft
Registration fee for exhibitors	22.000 Ft	22.000 Ft
Registration fee for Accompanying persons	22.000 Ft	22.000 Ft

**Only for participants with academic background*
***Junior researcher: Ph.D. and university student, or researcher under 30*

Registration fees include admission to the scientific sessions, admission to the exhibition, Welcome reception, Banquet dinner, Coffee breaks, Lunches.

Accompanying persons' registration fee and exhibitor's registration fee is not valid for admission to the scientific part of the Congress, and these fees do not include congress materials, only meals and social events.

ORAL PRESENTATIONS (PL-01 – PL-06, O-001 – O-081)

The schedule of the oral presentations can be seen in the detailed programme of this booklet. Speakers and session chairs are kindly requested to keep the time of the presentations. Make sure to bring your presentation file written on a properly closed CD ROM or USB flash drive. Presenters are kindly requested to give their presentation file to the technicians in the lecture rooms preferably half day before beginning of the corresponding session.

Oral presentations: Rooms TOSCANA I., MARBELLA II., IBIZA I.

POSTER PRESENTATIONS (P-001 – P-157)

Poster presenters can mount their posters from 10.00 on Friday, 5 April, 2013. Posters will be identified by poster numbers, which are indicated in the author index and on the floor plan (inner, back cover page of this programme booklet).

Poster sessions: P-001-P-081: TOSCANA II. - P-82-P-118: MARBELLA I. - P-119-P-157: IBIZA II.
Pins are to be provided to fix the posters by the technical organisers.

Presenting authors having **even numbers** should be at their posters at **19.00 on Friday, 5 April.** Presenting authors having **odd numbers** should be at their posters between **16.50 – 19.30 on Saturday, 6 April.**

AWARDS OF BEST JUNIOR ORAL AND POSTER PRESENTATIONS

The organisers intend to appoint a professional jury who will evaluate the best 3 presented talks and the best 3 presented posters in junior category.

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EXHIBITON

In accordance with the conventions of the congress, parallel to the scientific sessions a professional exhibition is to be organised. Please have a look at the exhibition floor plan of the booklet.

ACCOMMODATION

Hotel rooms are booked under the name of the participants. Congress participants may occupy the rooms from 14:00 on the day of arrival and should arrange the check out until 10:00. The hotel ensures a luggage room. The guarded parking lot of the hotel is available for our participants free of charge. Guests are kindly requested to settle their extra room bills (such as phone calls, drinks and minibar) prior to departure.
The room prices include buffet breakfast, the usage of wellness facilities (pools, jacuzzi, sauna park and steam bath), VAT and city tax.

SOCIAL PROGRAMMES *(incl. in the registration fees)*

Friday, 5 April, 2013	Poster reception with discussions amongst the posters
Saturday, 6 April, 2013	Banquet
Saturday & Sunday, 6-7 April, 2013	Lunches
Friday – Sunday, 5-7 April, 2013	Coffee breaks (indicated in the programme)

All participants and accompanying persons will receive a personal badge upon registration. You are kindly requested to wear your name badge when attending the meetings or social events.

Extra consumption, which is not included in the menus are kindly requested to settle prior to departure.

CANCELLATION POLICY

Cancellations on registration and hotel reservation can be made only in writing. The refund for cancellations made on and **prior to 5 March, 2013** is 100%. After this date the conference secretariat has to pay the advanced payments to the hotel, and there is no way to refund in case of latter cancellation.

PAYMENT, INVOICES

The price of the ordered services will be indicated on the final invoice according to the Hungarian official financial rules. Official final invoices and receipts for fees paid by the participants will be handed over on site at the registration desk. Please forward them to the financial department of the Institute.

LIABILITY AND INSURANCE

The organisers cannot accept liability for any personal accidents, loss of belongings or damage to private property of participants and accompanying persons that may occur during the Congress.

SCIENTIFIC PROGRAMME

SCIENTIFIC PROGRAM

FRIDAY, 5 April 2013

14:00 – 14:10 **Opening**

Plenary lectures I – Room Toscana I

Chairperson: Éva Kondorosi

14:10 – 14:40 **PL-01** **Chromosomal instability and human disease: role of common fragile sites**

Ian D. Hickson (FEBS National lecturer)
Nordea Center for Healthy Aging, Department of Cellular and Molecular Medicine, Panum Institute, University of Copenhagen, Denmark

14:40 – 15:10 **PL-02** **Arm-race between plant and viruses**

József Burgyán
Agricultural Biotechnology Center, Gödöllő

15:10 – 15:40 **PL-03** **Phosphorylation of phytochrome B inhibits light-induced signaling via accelerated dark reversion in *Arabidopsis***

Mátyás Medzihradszky[1], János Bindics[2], Éva Ádám[2], András Viczián[2], Éva Klement[3], Tim Kunkel[1], Eberhard Schäfer[1], Ferenc Nagy[2,4]
[1] Faculty of Biology, University of Freiburg, Freiburg, Germany; [2] Plant Biology Institute, Biological Research Centre, Szeged; [3] Proteomics Laboratory, Biological Research Centre, Szeged, [4] Institute of Molecular Plant Science, School of Biology, University of Edinburgh, Edinburgh, UK

15:40 – 16:30 **Coffee break**

Plenary lectures II – Room Toscana I

Chairperson: Éva Kondorosi

16:30 – 17:00 **PL-04** **An insight into regulatory roles of TFIIH-associated RNA polymerase II CTD-kinases in *Arabidopsis***

Mohsen Hajheidari[1], Sara Farrona[1], Bruno Huettel[1], Zsuzsa Koncz[1], Csaba Koncz[1,2]
[1] Max-Planck Institute for Plant Breeding Research, Cologne, Germany; [2] Institute of Plant Biology, Biological Research Center of Hungarian Academy of Sciences, Szeged

17:00 – 17:30 **PL-05** **The role of extracellular vesicles in intercellular communication**

Edit Buzás
Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

17:30 – 18:00 **PL-06** **Molecular tattooing in live zebrafish**

Miklós Képíró[1], Boglárka Várkuti[1], Katalin Kis Petik[3], Miklós Z. Kellermayer[3], Máté Varga[4], András Málnási-Csizmadia[1,2]
[1] Department of Biochemistry, Eötvös Loránd University, Budapest; [2] ELTE-HAS Molecular Biophysics Research Group, Budapest; [3] Department of Biophysics and Radiation Biology, Semmelweis University, Budapest; [4] Department of Genetics, Eötvös Loránd University, Budapest

18:00 – 18:30 **Presentation of the winner of the Bio-Science award**

19:00 **Poster reception with discussion amongst the posters**

SATURDAY, 6 April 2013

Parallel session #1 – Stem cell – Room Toscana I.

Chairpersons: B. Sarkadi – E. Gócza

9:00 – 9:20 **O-001** **Endogenous retinoic acid synthesis contributes to neural stem cell differentiation**

Barbara Orsolits[1], Adrienn Borsy[2], Emília Madarász[1], Zsófia Mészáros[1], Tímea Kőhidi[1], Károly Markó[1], Márta Jelitai[1], Ervin Welker[2,3], Zsuzsanna Környei[1]

[1] Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest; [2] Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [3] Biological Research Centre, Hungarian Academy of Sciences, Szeged

9:20 – 9:40 **O-002** **The effect of early differentiation pathway inhibitors on cell fate decision in preimplantation rabbit embryos**

Babett Bontovics[1], Jaroslav Slamecka[2,3], Pouneh Maraghechi[1], László Hiripi[1], Alexander V. Makarevich[2], Peter Chrenek[2,3], Kinga Németh[1], Zsuzsanna Bősze[1], Elen Gócza[1]

[1] Agricultural Biotechnology Center, Gödöllő; [2] Animal Production Research Centre, Nitra, Slovak Republic; [3] Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Slovak Republic

9:40 – 10:00 **O-003** **Analysis of human ABC multidrug transporter expression in human embryonic stem cells and their derivatives**

Zsuzsa Erdei[1], Tamás I. Orbán[1], Réka Lőrincz[1], Ágota Apáti[1,2], Balázs Sarkadi[1,2]

[1] Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Membrane Research Group, Hungarian Academy of Sciences, Semmelweis University and National Blood Center, Budapest

10:00 – 10:20 **O-004** **Mesenchymal stem cells induce the alternative pathway of macrophage activation**

Gyöngyi Kudlik, Beáta Hegyi, Ferenc Uher

Stem Cell Biology Unit, National Blood Service, Budapest

10:20 – 10:40 **O-005** **The role of epithelial-mesenchymal interactions during development of the avian bursa of Fabricius**

Nándor Nagy, Imre Oláh

Department of Human Morphology and Developmental Biology Semmelweis University, Faculty of Medicine, Budapest

10:40 – 11:00 **O-006** **Transcription factor mediated programming of ES cells into dendritic cells**

Emília Simó, Erika Takács, István Szatmári

Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

11:00 – 11:20 **Coffee break**

SATURDAY, 6 April 2013

Parallel session #2 – Systems biology – Room Marbella

Chairpersons: T. Korcsmáros – B. Papp

9:00 – 9:20 **O-007** **Network plasticity and rigidity determines learning and memory formation and defines optimal attack strategies**

Péter Csermely

Department of Medical Chemistry, Semmelweis University, Budapest

9:20 – 9:40 **O-008** **Autophagy Regulatory Network – an integrated resource to identify novel regulators and interactions that control autophagy**

Dénes Türei[1,2], László Földvári-Nagy[1], Dávid Fazekas[1], Dezső Módos[1,2,3], Katalin Lenti[1,3], Péter Csermely[2], Tibor Vellai[1], Tamás Korcsmáros[1,2]

[1] Department of Genetics, Eötvös Loránd University, Budapest; [2] Department of Medical Chemistry, Faculty of Medicine, Semmelweis University, Budapest; [3] Department of Morphology and Physiology, Faculty of Health Sciences, Semmelweis University, Budapest

9:40 – 10:00 **O-009** **Regulating the crosstalk between autophagy and apoptosis by stress-directed bistable switch**

Orsolya Kapuy[1], Vinod P.K.[2], Gábor Bánhegyi[1]

[1] Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2] Oxford Centre for Integrative Systems Biology, Department of Biochemistry, University of Oxford, Oxford, UK

10:00 – 10:20 **O-010** **Evolution of antibiotic hypersensitivity**

Viktória Lázár[1], Gajinder Pal Singh[1], Réka Spohn[1], István Nagy[2], Balázs Horváth[2], Mónika Hrtyan[1], Róbert Busa-Fekete[3], Balázs Bogos[1], Orsolya Méhi[1], Bálint Csörgő[1], György Pósfai[1], Gergely Fekete[1], Balázs Szappanos[1], Balázs Kégl[3], Balázs Papp[1], Csaba Pál[1]

[1] Synthetic and Systems Biology Unit, Biological Research Center, Szeged; [2] Institute for Plant Genomics, Human Biotechnology and Bioenergy, Bay Zoltán Foundation for Applied Research, Szeged; [3] Linear Accelerator Laboratory, University of Paris-Sud, France

10:20 – 10:40 **O-011** **Evolutionary potential of the underground metabolic network of *Escherichia coli***

Ferenc Pál[1], Richard Notebaart[2], Balázs Szappanos[1], Ádám Györkei[1], Balázs Bogos[1], Viktória Lázár[1], Barbara Tomor[1], Bálint Csörgő[1], Réka Spohn[1], Csaba Pál[1], Balázs Papp[1]

[1] Biological Research Centre of the Hungarian Academy of Sciences, Szeged; [2] Centre for Molecular and Biomolecular Informatics and Centre for Systems Biology and Bioenergetics, Radbound University Nijmegen, The Netherlands

10:40 – 11:00 **O-012** **Computational modeling of T and B cell development using gene co-regulatory and protein interaction networks**

Gabriel Teku[1,2], Payam Emami Khoonsari[1], Francesco Mazzarotto[1], Mauno Vihinen[2], Csaba Ortutay[1]

[1] Institute of Biomedical Technology, University of Tampere, Finland; [2] Department of Experimental Medical Technology, Lund University, Sweden

11:00 – 11:20 **Coffee break**

SATURDAY, 6 April 2013

Parallel session #3 – Developmental genetics I. – Room Ibiza

Chairpersons: R. Sinka – G. Endre

9:00 – 9:20 **O-013** **The role of ubiquitin ligases during early steps of symbiotic nodule development**

Ernő Kiss, Boglárka Oláh, Katalin Kontár, Zoltán Bozsóki, Andrea Borbola, Erzsébet Fehér-Juhász, Sándor Jenei, Gabriella Endre
Biological Research Center, Hungarian Academy of Sciences, Institute of Genetics, Szeged

9:20 – 9:40 **O-014** **Dissection of the function of a single gene belonging to a large nodule specific gene family governing bacteroid differentiation in *Medicago truncatula*-rhizobial symbiotic association**

Beatrix Horváth[1], Ágota Domonkos[1], Attila Kereszt[2], Jeremy Murray[3], Ferhan Ayaydin[4], Michael Udvardi[3], Éva Kondorosi[2], Péter Kaló[1]
[1] Agricultural Biotechnology Center, Gödöllő; [2] Institute of Biochemistry, Biological Research Center, Szeged; [3] The Samuel Roberts Noble Foundation, Ardmore, OK, USA; [4] Cellular Imaging Laboratory, Biological Research Center, Szeged

9:40 – 10:00 **O-015** **Identification and *in vivo* analysis of genes required for microtubule function in epithelial closure processes**

Ferenc Jankovics, Zsanett Lakatos, Dániel Danszky, Miklós Erdélyi
Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

10:00 – 10:20 **O-016** **Glial cells accumulate lipid droplets to promote brain development in *Drosophila melanogaster***

Viktor Kis, Mónika Lippai, Benjámin Barti, Miklós Sass
Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest

10:20 – 10:40 **O-017** **Autophagy is required for zebrafish caudal fin regeneration**

Máté Varga[1], Miklós Sass[2], Diána Papp[1], Krisztina Takács-Vellai[1], Daniel J. Klionsky[3],Tibor Vellai[1]
[1] Department of Genetics, Eötvös Loránd University, Budapest; [2] Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest; [3] Life Sciences Institute, University of Michigan, Ann Arbor, Michigan, USA

10:40 – 11:00 **O-018** **Introducing Preimplantation Genetic Diagnosis (PGD) byaCGHin Hungary: improving outcome in IVF**

Attila Vereczkey[1], Zsolt Kósa[1], Sándor Sávay[1], Marianna Csenki[1], László Nánássy[1], Beáta Dudás[1], Zsuzsanna Dömötör[2], Diána Debreceni[2]
[1] Versys Clinics, Human Reproduction Institute, Budapest; [2] Reprogenex Gene Diagnostic Laboratory, Budapest

11:00 – 11:20 **Coffee break**

SATURDAY, 6 April 2013

Parallel session #1 – Protein structure and function – Room Toscana I.

Chairpersons: B. Sümegi – L. Nyitrai

11:20 – 11:40 **O-019** **Exploring functional sites located within intrinsically disordered proteins**

Bálint Mészáros[1], Mátyás Pajkos[1], András Zeke[2], Attila Reményi[2], István Simon[1], Zsuzsanna Dosztányi[1]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Science, Budapest; [2] Eötvös Loránd University, Budapest

11:40 – 12:00 **O-020** **Blood coagulation factor XIII stabilizes Neutrophil Extracellular Trap structure by the formation of intermolecular covalent protein cross-links**

Krisztián Csomós[1], Judit Hodrea[1], Omri Rotem[1], Éva Katona[2], Zsuzsanna Bagoly[2], István Csomós[3], György Vámosi[3], László Muszbek[2], László Fésüs[1]

[1] Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen; [2] Clinical Research Center, University of Debrecen, Debrecen; [3] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen

12:00 – 12:20 **O-021** **Importance of Valine 224 in regulating the transamidation activity and calcium sensitivity of human transglutaminase 2**

Kajal Kanchan[1], Elvan Ergulen[3], Róbert Király[1], Zsófia Simon Vecsei[1], Mónika Fuxreiter[1], László Fésüs[1,2]

[1] Department of Biochemistry and Molecular Biology Medical and Health Science Centre, University of Debrecen, Debrecen; [2] Stem Cell, Apoptosis and Genomics Research Group, Hungarian Academy of Science, Debrecen; [3] UD-GenoMed Medical Genomic Technologies Ltd, Debrecen

12:20 – 12:40 **O-022** **Structural analysis of Phi11 Staphylococcal phage dUTPase**

Ibolya Leveles, Veronika Kádár, Imre Zagyva, Ábris Bendes, Gergely Róna, Kinga Nyíri, Veronika Németh, Judit Tóth, Beáta G. Vértessy

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

12:40 – 13:00 **O-023** **Investigation of catalytic properties of a key lipid biosynthetic enzyme from the malaria parasite**

Gergely N. Nagy[1], Lívia Marton[1], Beáta G. Vértessy[1,2]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Budapest; [2] Department of Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest

13:20 – 14:30 **Lunch**

SATURDAY, 6 April 2013

Parallel session #2 – DNA repair – Room Marbella

Chairpersons: B. Vértessy – L. Haracska

11:20 – 11:40 **O-024** **Allelic imbalance in DNA repair defective chicken DT40 cell lines**

Ágnes Varga[1], Nicolai J Birkbak[2], Zoltán Szállási[2], Dávid Szüts[1]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Department of Systems Biology, The Technical University of Denmark, Copenhagen, Denmark

11:40 – 12:00 **O-025** **Domain movements and sequence-specific pausing of RecQ Helicase**

Zs. S. Kocsis[1], G. M. Harami[1], Y.Sun[2], J.In[2], Y. Seol[2], S. Sarkar[2], M-P. Strub[2], K. Sarlós[1], K. C.Neuman[2], M. Kovács[1]

[1] Department of Biochemistry, Eötvös Loránd University, Budapest; [2] National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

12:00 – 12:20 **O-026** **dUTP level controls transfer of virulence genes in order to preserve integrity of the transferred mobile genetic elements**

Judit Eszter Szabó[1], Veronika Németh-Pongrácz[1], Ibolya Leveles[1], Veronika Kádár[1], Balázs Besztercei[1], Károly Liliom[1], Imre Zagyva[1], Ábris Bendes[1], Gergely Róna[1], Hajnalka Pálinkás[1], Kinga Nyíri[1], Judit Tóth[1], Beáta G. Vértessy[1,2]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Department of Applied Biotechnology and Food Sciences, University of Technology and Economics, Budapest

12:20 – 12:40 **O-027** **Cell cycle regulation of crossover timing**

Barnabás Szakál, Lajos Haracska

Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged

12:40 – 13:00 **O-028** **The COMPASS subunit Spp1 links histone methylation to initiation of meiotic recombination**

Lóránt Székvölgyi[1], Laurent Acquaviva[2], Bernhard Dichtl[4], Beatriz Dichtl[4], Christophe de La Roche Saint André[2], Alain Nicolas[3], Vincent Géli[2]

[1] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2] Marseille Cancer Research Center (CRCM), Marseille, France; [3] Institute Curie Centre de Recherche, Paris, France; [4] Centre for Cellular and Molecular Biology, School of Life & Environmental Sciences, Deakin University, Victoria, Australia

13:00 – 13:20 **O-029** **DNA damage induced polymerase exchange at stalled replication forks**

Andrea Daraba, Vamsi K. Gali, Miklós Halmai, Lajos Haracska, Ildikó Unk

Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged

13:20 – 14:30 **Lunch**

SATURDAY, 6 April 2013

Parallel session #3 – Signaling, cell-cell communication – Room Ibiza

Chairpersons: F. Nagy – L. Buday

11:20 – 11:40	**O-030**	**The circadian clock-associated small GTPase LIGHT INSENSITIVE PERIOD 1 suppresses light-controlled endoreplication and affects tolerance to salt stress in *Arabidopsis thaliana*** Kata Terecskei[1], Réka Tóth[2], Péter Gyula[1], Éva Kevei[1], János Bindics[1], George Coupland[2], Ferenc Nagy[1], László Kozma-Bognár[1] *[1] Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged; [2] Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany*
11:40 – 12:00	**O-031**	**Light quality-dependent regulation of frost tolerance in cereals** Aliz Novák[1,2], Ákos Boldizsár[1], Éva Ádám[3], László Kozma[3], Gábor Galiba[1,2] *[1] Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár; [2] University of Pannonia, Doctoral School of Molecular- and Nanotechnologies, Veszprém; [3] Biological Research Centre, Hungarian Academy of Sciences, Szeged*
12:00 – 12:20	**O-032**	**Molecular differences of NLRP3 inflammasome mediated IL-1β production in LPS- activated human monocyte-derived macrophage subtypes** Marietta Margit Budai[1], Judit Danis[1], Aliz Varga[1], László Csernoch[1], József Tőzsér[2], Szilvia Benkő[1] *[1] Department of Physiology, Medical and Health Science Center, University of Debrecen, Debrecen; [2] Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen*
12:20 – 12:40	**O-033**	**Macrophages engulfing apoptotic cells produce a novel retinoid to enhance phagocytosis** Zsolt Sarang, Gergely Joós, Zsuzsa Szondy *Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen*
12:40 – 13:00	**O-034**	**Stress-responsive regulatory mechanisms tailor innate immunity and adipogenesis** Csaba Sőti *Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest*
13:00 – 13:20	**O-035**	**Cardiac expression of a cellular calcium indicator protein in transgenic rats** Kornélia Szebényi[1], András Füredi[2], Orsolya Kolacsek[1], Ágota Apáti[1], László Héja[1], Tamás I. Orbán[1], Balázs Bender[4], Zsuzsanna Bősze[4], Ágnes Enyedi[1], Julianna Kardos[1], Balázs Sarkadi[1,3] *[1] Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Institute of Enzimology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [3] Membrane Research Group, Hungarian Academy of Sciences, Semmelweis University and National Blood Center, Budapest; [4] Agricultural Biotechnology Center, Gödöllő*
13:20 – 14:30	**Lunch**	

SATURDAY, 6 April 2013

Parallel session #1 – Molecular mechanism of diseases I. – Room Toscana I.

Chairpersons: B. Melegh – B. L. Bálint

14:30 – 14:50	**O-036**	**Human genetics in the postgenomic area: waiting for the revolution, or beginning of it?** Béla Melegh *Department of Medical Genetics, University of Pécs, Pécs*
14:50 – 15:10	**O-037**	**Second generation antipsychotic (SGA) drugs modify the differentiation program of human adipocytes inducing "browning" markers** Endre Károly Kristóf[1], Doan Xuan Minh[2], Anitta Kinga Sárvári[1], Zoltán Balajthy[1], Zsolt Bacsó[2], László Fésüs[1] *[1] Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen; [2] Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, Debrecen*
15:10 – 15:30	**O-038**	**Clonal evolution in HHD pALL: contrasting hypotheses** Róbert Mátics[1], Gábor Pajor[2], Donát Alpár[2], László Pajor[2] *[1] Department of Pathophysiology and Gerontology, Medical School, University of Pécs, Pécs; [2] Department of Pathology, Medical School, University of Pécs, Pécs*
15:30 – 15:50	**O-039**	**The rs3185480 SNP of the APCDD1 gene is associated with androgenic alopecia** Nikoletta Nagy[1,2,3], Katalin Farkas[3], Ágnes Kinyó[2], Angéla Meszes, Lajos Kemény[2,3], Márta Széll[1,3] *[1] Department of Medical Genetics, University of Szeged, Szeged; [2] Department of Dermatology and Allergology, University of Szeged, Szeged; [3] Dermatological Research Group, Hungarian Academy of Sciences, Szeged*
15:50 – 16:10	**O-040**	**Genetic and functional studies on Hungarian families with Brooke-Spiegler Syndrome** Nikoletta Nagy[1,2,3], Neil Rajan[4], Katalin Farkas[3], Lajos Kemény[2,3], Márta Széll[1,3] *[1] Department of Medical Genetics, University of Szeged, Szeged; [2] Department of Dermatology and Allergology, University of Szeged, Szeged; [3] Dermatological Research Group, Hungarian Academy of Sciences, University of Szeged, Szeged; [4] Institute of Human Genetics, University of Newcastle uponTyne, Newcastle uponTyne, UK*
16:10 – 16:30	**Coffee break**	
16:30 – 16:50	**CL-01**	**COMPANY LECTURE** **PCR goes digital pushing the boundaries of sensitivity and accuracy in nucleic acid based detection** Dénes Szilassy *Life Technologies Magyarország Kft., Budapest*
16:50 – 19:30	**Poster discussion**	
19:30	**Banquet**	

SATURDAY, 6 April 2013

Parallel session #2 – Membrane, transport, trafficking I. – Room Marbella

Chairpersons: L. Vígh – A. L. Kiss

14:30 – 14:50 **O-041** **Experimental strategies for *in vivo* rescue of disease-causing mutations of ABCC6**

András Váradi[1], Viola Pomozi[1], Krisztina Fülöp[1], Tamás Arányi[1], Jouni Uitto[2], Olivier Le Saux[3]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Department of Dermatology, Thomas Jefferson University, Philadelphia, USA; [3] Department of Cell and Molecular Biology, University of Hawaii, Honolulu, Hawaii, USA

14:50 – 15:10 **O-042** **Mutations in potential substrate- and steroid-binding sites modulate cholesterol sensing of human ABCG2**

Csilla Laczka[1], Ágnes Telbisz[2], Csilla Hegedüs[1], András Váradi[3], Balázs Sarkadi[1,2]

[1] Membrane Research Group of the Hungarian Academy of Sciences and Semmelweis University, Department of Biophysics, and National Blood Center, Budapest; [2] Biomembrane Laboratory, Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [3] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

15:10 – 15:30 **O-043** **CDK1 phosphorylation governs nuclear proteome redistribution in daughter cells after division: legacy of mother cells**

Gergely Róna[1], Máté Borsos[1], Mary Marfori[2], Jonathan J. Ellis[2], Ahmed M. Mehdi[3], Mikael Bodén[2,3,5], Zsuzsanna Környei[6], Máté Neubrandt[6], Judit Tóth[1], Bostjan Kobe[2,3,4], Beáta G. Vértessy[1]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, Australia; [3] Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia; [4] Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, Australia; [5] School of Information Technology and Electrical Engineering, The University of Queensland, Brisbane, Australia; [6] Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest

15:30 – 15:50 **O-044** **Elucidation of the catalytic mechanism of P-glycoprotein using a conformation sensitive antibody**

Orsolya Bársony[1], Gábor Szalóki[1], Dóra Türk[2], Gábor Szabó[1], Gergely Szakács[2], Katalin Goda[1]

[1] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

15:50 – 16:10 **O-045** **Substrate binding and transport by Walker-A mutant Pgps**

Gábor Szalóki[1], Dóra Türk[2], Gábor Szabó[1], Gergely Szakács[2], Katalin Goda[1]

[1] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

16:10 – 16:30 **Coffee break**

16:50 – 19:30 **Poster discussion**

19:30 **Banquet**

SATURDAY, 6 April 2013

Parallel session #3 – Apoptosis, autophagy – Room Ibiza

Chairpersons: Zs. Szondy – M. Sass

14:30 – 14:50	**O-046**	**Autophagosomal Syntaxin17-dependent lysosomal degradation maintains neuronal health in *Drosophila*** Szabolcs Takáts, Péter Nagy, Ágnes Varga, Karolina Pircs, Manuéla Kárpáti, Kata Varga, Attila L. Kovács, Krisztina Hegedűs, Gábor Juhász *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest*
14:50 – 15:10	**O-047**	**The role of Rab11 in the maturation of autophagosomes and endosomes as a negative regulator of Hook** Zsuzsanna Szatmári, Viktor Kis, Krisztina Hegedűs, Mónika Lippai, Gábor Juhász, Miklós Sass *Department of Anatomy, Cell and Developmental Biology, Eötvös Lorand University, Budapest*
15:10 – 15:30	**O-048**	**Molecular cell pathology of prion disease: the role of endosome-lysosome system and selective au tophagy** Lajos László[1], Kinga Molnár[1], Gina Puska[1], Alekszandra Gacs[1], Zsuzsanna Víg-Milkovics[1], Gábor G. Kovács[2] *[1] Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest; [2] Department of Neurology, University of Vienna Medical School, Vienna*
15:30 – 15:50	**O-049**	**Regulation of life and death decisions by poly(ADP-ribosyl)ation** László Virág[1,3], Katalin Kovács[1], Petra Lakatos[1], Csaba Hegedűs[1], Éva Szabó[2] *[1] Department of Medical Chemistry, Medical and Health Science Center, University of Debrecen, Debrecen; [2] Department of Dermatology, Medical and Health Science Center, University of Debrecen, Debrecen; [3] Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Debrecen*
15:50 – 16:10	**O-050**	**Characterization of inflammatory reactions during human adipocyte & macrophage co-incubation** Anitta Kinga Sárvári[1], Minh Doan[2], Zsolt Bacsó[2], Zoltán Balajthy[1], László Fésüs[1] *[1] Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen; [2] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen*
16:10 – 16:30	**Coffee break**	
16:50 – 19:30	**Poster discussion**	
19:30	**Banquet**	

SUNDAY, 7 April 2013

Parallel session #1 – Developmental genetics II. – Room Toscana I.

Chairpersons: P. Deák – J. Mihály

9:00 – 9:20 **O-051** **Combined genetic, molecular and cell biology approaches to reveal functions of the maternal α-tubulin isoform in *Drosophila***

János Szabad, Zsolt Venkei, Imre Gáspár

Department of Biology, University of Szeged, Szeged

9:20 – 9:40 **O-052** **Membranes along the longest sperm**

Barbara Laurinyecz[1], Viktor Vedelek[1], Péter Maróy[1], Mária Péter[3], Gábor Balogh[3], Gábor Juhász[2], Rita Sinka[1]

[1] Department of Genetics, University of Szeged, Szeged; [2] Department of Anatomy, Eötvös Loránd University, Budapest; [3] Institute of Biochemistry, Biological Research Centre, Szeged

9:40 – 10:00 **O-053** **Heat shock factor-1 intertwines insulin/IGF-1, TGF-β and cGMP signaling to control development and aging**

János Barna, Andrea Princz, Mónika Kosztelnik, Krisztina Takács-Vellai, Tibor Vellai

Department of Genetics, Eötvös Loránd University

10:00 – 10:20 **O-054** **Novel functions for a cytoskeletal actin binding protein**

Péter Vilmos[1], Ildikó Kristó[1], Szilárd Szikora[1,2], Ferenc Jankovics[1], László Henn[1], Miklós Erdélyi[1]

[1] Department of Genetics, Biological Research Center, Szeged; [2] Department of Biology, University of Szeged, Szeged

10:20 – 10:40 **O-055** **The formin DAAM functions as the molecular effector of the planar cell polarity pathway during axonal development in the *Drosophila* brain**

Rita Gombos, József Mihály

Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

11:00 – 11:20 **Coffee break**

SUNDAY, 7 April 2013

Parallel session #2 – Regulation of gene expression, epigenetics – Room Marbella

Chairpersons: L. Nagy – I. Boros

9:00 – 9:20 **O-056** **Single-strand discontinuities in the genome of lower and higher eukaryotes**

Gábor Szabó, Lóránt Székvölgyi, Éva Hegedüs, András Szántó, Péter Nánási, László Imre

Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, Debrecen

9:20 – 9:40 **O-057** **The Heat Shock Factor A4A regulates oxidative stress tolerance in Arabidopsis**

Imma Pérez Salamó[1], Csaba Papdi[1], Gábor Rigó[1], Laura Zsigmond[1], Belmiro Vilela[2], István Nagy[1], Balázs Horváth[1], Mónika Domoki[1], Csaba Koncz[3], László Szabados[1]

[1] Institute of Plant Biology, Biological Research Centre, Szeged; [2] Centre for Research in Agricultural Genomics, Barcelona, Spain; [3] Max-Planck-Institut für Züchtungsforschung, Cologne, Germany

9:40 – 10:00 **O-058** **Transglutaminase 2 (TGM2) modulates the energy metabolism of differentiating myeloid cells through repression of key regulators of mitochondrial biogenesis**

István Német[1], Péter Bai[2], Zoltán Doró[1], László Fésüs[1], Zoltán Balajthy[1]

[1] Department of Biochemistry and Molecular Biology, Medical and Health Science Center, Faculty of Medicine, University of Debrecen, Debrecen; [2] Department of Medical Chemistry, Medical and Health Science Center, Faculty of Medicine, University of Debrecen, Debrecen

10:00 – 10:20 **O-059** **Genomic analysis of HNF4a binding and the influence of ERK1/2 signaling pathway: Insights on human *ABCC6* gene transcriptional regulation**

Caroline Bacquet, Borbála Vető, Hugues de Boussac, András Váradi, Tamás Arányi

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Bdapest

10:20 – 10:40 **O-060** **Cell line development using genome engineering technologies: Reporters, effectors, producers**

Zsolt Keresztessy[1,2], Éva Nagy[1], Erzsébet Mátyás[1], József Horváth[1], Zoltán Doró[3], Bálint László Bálint[1,2], Gábor Zahuczky[3], László Nagy[2]

[1] Protein Expression and Cell Engineering Laboratory,Centre for Clinical Genomics and Personalized Medicine, Debrecen; [2] Department of Biochemistry and Molecular Biology, Medical and Health Sciences Center, University of Debrecen, Debrecen; [3] UD-GenoMed Medical Genomic Technologies Ltd, Debrecen

10:40 – 11:00 **O-061** **Application of tissue specific gene expression to improve fungus resistance in wheat**

Milán Ivanics[1], András Kis[1], Gábor Tóth[1,2], Krisztina Takács[3], Andrea Balogh[1], József Fodor[4], Klára Manninger[4], László Tamás[2], Barnabás Jenes[1]

[1] Agricultural Biotechology Center, Gödöllő, [2] Department of Plant Physiology, Eötvös Loránd University, Budapest; [3] Central Environmental and Food Science Research Institute, Budapest; [4] Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest

11:00 – 11:20 **Coffee break**

SUNDAY, 7 April 2013

Parallel session #3 – Membrane, transport, trafficking II. – Room Ibiza

Chairpersons: A. Váradi – Cs. Laczka

9:00 – 9:20 **O-062** **Inactivation of protein kinase D activity alters dendritic spine morphology, motility and hippocampal functions**

Katalin Schlett[1], Norbert Bencsik[1], Márton Gulyás[1], Sándor Borbély[1], Krisztián Tárnok[1], Diána Hazai[2], Angelika Hausser[3], Bence Rácz[2]

[1] Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest; [2] Department of Anatomy and Histology, Faculty of Veterinary Science, Szent István University, Budapest; [3] Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany

9:20 – 9:40 **O-063** **The internalization route of Transforming Growth Factor β receptor and a possible non-genomic role of Estrogen-Receptor α in the signaling during type II epithelial-mesenchymal transition**

Petra Balogh[1], Attila Patócs[2], Sándor Katz[1], Anna L. Kiss[1]

[1] Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest; [2] Department of Internal Medicine, Semmelweis University, Budapest

9:40 – 10:00 **O-064** **Thermal stress management in fission yeast: need for fat**

Attila Glatz, Mária Péter, Gábor Balogh, Katalin Kontár, Imre Gombos, Ibolya Horváth, Annamária Pilbat, Zsolt Török, László Vígh

Laboratory of Molecular Stress Biology, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

10:00 – 10:20 **O-065** **Tracking ABCB6 in the cell: from the mitochondria through the plasma membrane to the endolysosomal system**

Katalin Kiss, Anna Brozik, Nóra Kucsma, Melinda Gera, Zsófia Rakvács, Gergely Szakács

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

10:20 – 10:40 **O-066** **Dual localization of the prion protein family members: the highly conserved, N-terminal $(RXXX)_8$ motif of mouse Shadoo mediates nuclear accumulation**

Eszter Tóth[1,2], Péter István Kulcsár[1], Elfrieda Fodor[1], Ferhan Ayaydin[3], Lajos Kalmár[4], Adrienn Éva Borsy[5], Lajos László[2], Ervin Welker[1,5]

[1] Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged; [2] Department of Anatomy, Cell and Developmental Biology, Institute of Biology, Faculty of Science, Eötvös Loránd University, Budapest; [3] Cellular Imaging Laboratory, Biological Research Center, Hungarian Academy of Sciences, Szeged; [4] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [5] Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

11:00 – 11:20 **Coffee break**

SUNDAY, 7 April 2013

Parallel session #1 – Molecular mechanism of diseases II. – Room Toscana I.

Chairpersons: J. Balla – M. Széll

11:20 – 11:40	**O-067**	**The physiological role of transient receptor potential (TRP) channels in platelet calcium homeostasis and arterial thrombosis** Attila Braun[1], Dávid Varga-Szabó[1], Shuchi Gupta[1], Wenchun Chen[1], Vladimir Chubanov[3], Ina Thielmann[1], Peter Kraft[2], Guido Stoll[2], Alexander Dietrich[3], Thomas Gudermann[3], Bernhard Nieswandt[1] *[1] University of Würzburg, Rudolf Virchow Center, DFG Research Center for Experimental Biomedicine, Würzburg, Germany; [2] Department of Neurology, University of Würzburg, Würzburg, Germany; [3] Walther-Straub-Institute for Pharmacology and Toxicology, Ludwig-Maximilians University Munich, Germany*
11:40 – 12:00	**O-068**	**Blood, sweat and tears – body fluid proteomics with emphasis on antimicrobial and immunomodulatory peptide type biomarkers** Éva Csősz[1], Adrienne Csutak[2], Gergő Kalló[1], József Tőzsér[1] *[1] Department of Biochemistry and Molecular Biology, Proteomics Core Facility, Medical and Health Science Center, University of Debrecen, Debrecen; [2] Department of Ophthalmology, Faculty of Medicine, Medical and Health Science Center, University of Debrecen, Debrecen*
12:00 – 12:20	**O-069**	**Cerebrovascular alteration after X-ray exposure at low doses: in vivo and in vitro mice studies** Hargita Hegyesi[1], Boglárka Tóth Schilling[1], Fruzsina Walter[2], Nikolett Sándor[1], Mária A. Deli[2], Géza Sáfrány[1] *[1] "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest; [2] Insitute of Biophysics, Biological Research Centre, Szeged*
12:20 – 12:40	**O-070**	**Myc-driven overgrowth requires unfolded protein response-mediated induction of autophagy and antioxidant responses in *Drosophila*** Péter Nagy, Ágnes Varga, Karolina Pircs, Krisztina Hegedűs, Gábor Juhász *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest*
12:40 – 13:00	**O-071**	**Proprotein convertase furin in T cell mediated immune responses** Zsuzsanna Ortutay[1,2], Anna Oksanen[1,2], Marko Pesu[1,2] *[1] Institute of Biomedical Technology, University of Tampere, Tampere, Finland; [2] BioMediTech, Tampere, Finland*
13:00 – 13:20	**Closing**	
13:20	**Lunch**	

SUNDAY, 7 April 2013

Parallel session #2 – Microbial genetics – Room Marbella

Chairpersons: P. Putnoky – Cs. Pál

11:20 – 11:40	**O-072**	**Temperature sensitive DNA repair mutants enable highly precise genome manipulation** Ákos Nyerges, Bálint Csörgő, György Pósfai, Csaba Pál *Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged*
11:40 – 12:00	**O-073**	**Development of basic genetic manipulation system for the thermoacidophil *Thermoplasma acidophilum*** Erzsébet Baka[1,2], Csaba Fekete[1,3], Sándor Varga[4], Roland W. Knispel[5], István Nagy[5], József Kukolya[2] *[1] Szentágothai János Research Center, Pécs; [2] Central Environmental and Food Science Research Institute, Budapest; [3] Department of General and Environmental Microbiology, University of Pécs, Pécs; [4] Department of Environmental Protection & Environmental Safety, Szent István University, Gödöllő; [5] Max Planck Institute of Biochemistry, Department of Structural Biology, Martinsried, Germany*
12:00 – 12:20	**O-074**	**Genome-guided approach for identifying cryptic biosynthetic pathways and novel natural products in *Saccharomonospora azurea*** Andrea Valasek[1], Kitti Csepregi[1], Zsuzsanna Tóth[1], Éva Írisz Kiss[1], Péter Urbán[1], Ildikó Kerepesi[1], József Kukolya[2], Balázs Horváth[3], István Nagy[3], Csaba Fekete[1] *[1] Institute of Biology, Faculty of Science, University of Pécs, Pécs; [2] Central Environmental and Food Science Research Institute, Budapest; [3] Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged*
12:20 – 12:40	**O-075**	***In vivo* enzymology: Physiologic effects of dUTPase mutations in the mycobacterial cell** Rita Hirmondó[1], Anna Lopata[1], Ildikó Pécsi[1], Beáta G. Vértessy[1,2], Judit Tóth[1] *[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economy, Budapest*
12:40 – 13:00	**O-076**	**Characterization of strong antibacterial activity exhibited by nodule specific plant peptides** Gergely Maróti, Attila Farkas, Attila Kereszt, Hilda Tiricz, Kata Mikuláss, Lilla Ördögh, Éva Kondorosi *Biological Research Center, Hungarian Academy of Sciences, Szeged*
13:00 – 13:20	**Closing**	
13:20	**Lunch**	

SUNDAY, 7 April 2013

Parallel session #3 – Regulatory RNAs – Room Ibiza

Chairpersons: J. Burgyán – Z. Havelda

11:20 – 11:40	**O-077**	**Small RNA regulation of plant NB-LRR defense gene family** György Szittya[1], Simon Moxon[3], Irina Mohorianu[3], Vincent Moulton[3], Yuval Eshed[4], Tamás Dalmay[2] *[1] Agricultural Biotechnology Center, Department of Plant Biotechnology, Gödöllő; [2] University of East Anglia, School of Biological Sciences, Norwich, UK; [3] University of East Anglia, School of Computing Sciences, Norwich, UK; [4] Weizman Institute of Science, Department of PlantSciences,Rehovot, Israel*
11:40 – 12:00	**O-078**	**Maintenance of pluripotency in embryonic stem cells with microRNAs** Elen Gócza[1], Pouneh Maraghechi[1], Zsuzsanna Bősze[1], Zsuzsanna Lichner[2] *[1] Agricultural Biotechnology Center, Gödöllő; [2] Li Ka Shing Knowledge Institute of St. Michael's Hospital, Toronto, Ontario, Canada*
12:00 – 12:20	**O-079**	**Polymorphic miRNA binding sites are risk factors of diabetes mellitus** Zsolt Rónai[1], Nóra Németh[1], Anikó Somogyi[2], Mária Sasvári-Székely[1], András Guttman[3] *[1] Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2] 2nd Department of Internal Medicine, Semmelweis University, Budapest; [3] University of Pannonia, Veszprém*
12:20 – 12:40	**O-080**	**Non-canonical human microRNAs: characterization of the mirtron pathway in higher eukaryotes** Anita Schamberger[1], Balázs Sarkadi[1,2], Tamás Orbán[1,3] *[1] Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2] National Blood Center, Budapest; [3] Chemical Technology Transfer Ltd., Budapest*
12:40 – 13:00	**O-081**	**Identification of genes important in disease symptom development in virus infected plants** Éva Várallyay, Zoltán Havelda *Plant Developmental Biology Group, Agricultural Biotechnology Center, Gödöllő*
13:00 – 13:20	**Closing**	
13:20	**Lunch**	

ABSTRACTS - PLENARY LECTURES

PL-01
CHROMOSOMAL INSTABILITY AND HUMAN DISEASE: ROLE OF COMMON FRAGILE SITES

Ian D. Hickson (FEBS National lecturer)

Nordea Center for Healthy Aging, Department of Cellular and Molecular Medicine, Panum Institute, University of Copenhagen, Denmark

All proliferating cells must accurately duplicate their genomic DNA and segregate their sister chromatids during mitosis. Any perturbation of these processes, arising from either endogenous or exogenous sources, can lead to cell death or chromosomal instability. We have shown previously that common fragile site loci are difficult to segregate in anaphase and, as a result, create so-called ultra-fine anaphase bridges (UFBs). These bridges are present in virtually all cells in early anaphase and are decorated along their length by a SNF2 family protein, PICH, and a complex of the BLM, TOPO IIIa, RMI1 and RMI2 proteins (the BTRR complex). BLM is a member of the RecQ family of DNA helicases, and is the protein defective in the cancer predisposition disorder, Bloom's syndrome. We hypothesize that common fragile sites fail to complete replication in S-phase, which necessitates that action of the PICH and BTRR protein to disentangle the incompletely replicated DNA in anaphase itself. We are analyzing these processes in yeast, chicken and human cells. For example, we have generated a PICH-deficient chicken DT-40 cell mutant, and have devised a system in yeast to generate a site-specific, artificial fragile site by blocking converging replication forks. Biochemically, we are analyzing the ability of purified PICH and BTRR proteins to disentangle a DNA substrate in vitro that mimics the structure that would form as two replication forks converge. We have also recently discovered a link between the action of DNA structure-specific nuclease, MUS81-EME1, and the frequency of fragile site expression and UFB formation. Results of these studies will be discussed.

PL-02
ARM-RACE BETWEEN PLANT AND VIRUSES

József Burgyán

Agricultural Biotechnology Center, Gödöllő

RNA silencing in plants exists as a defence mechanism against viruses. Plant viruses are inducers as well as target of RNA silencing based antiviral defence. The silencing of RNA relies on host- or virus-derived 21-24 nucleotide-long small RNA (sRNA) molecules, which are the key mediators of RNA silencing-related pathways in plants and other eukaryotic organisms. Viral RNAs activate the antiviral RNA silencing generating viral siRNAs, which guide RNA-induced silencing effector complex (RISC) to cleave viral genome. We have found that AGO1 the catalytic component of antiviral RISC strictly selects for AGO1 competent viral siRNAs, which represent only approximately 1% of total viral siRNAs. Plant viruses are very efficient pathogens, which are able to infect and invade distinct plant species. They often cause severe symptoms and damage, which

suggests an efficient counter defensive strategy against the antiviral plant response. In fact most viruses evolved viral silencing suppressors (VSRs), which underlines the antiviral nature of RNA silencing and reveals a pathogen counter-defensive strategy with the active suppression of host surveillance. These VSRs often compromise not only the antiviral response but they have significant impact on the endogenous silencing pathways. Finally we have also shown, that viruses are able to harness antiviral silencing for virus benefit controlling host gene expression, demonstrating the complexity of host-pathogen interaction.

PL-03
PHOSPHORYLATION OF PHYTOCHROME B INHIBITS LIGHT-INDUCED SIGNALING VIA ACCELERATED DARK REVERSION IN *ARABIDOPSIS*

Mátyás Medzihradszky[1], János Bindics[2], Éva Ádám[2], András Viczián[2], Éva Klement[3], Tim Kunkel[1], Eberhard Schäfer[1], Ferenc Nagy[2,4]

[1] *Faculty of Biology, University of Freiburg, Freiburg, Germany*
[2] *Plant Biology Institute, Biological Research Centre, Szeged*
[3] *Proteomics Laboratory, Biological Research Centre, Szeged*
[4] *Institute of Molecular Plant Science, School of Biology, University of Edinburgh, Edinburgh, UK*

The photoreceptor phytochrome B interconverts between the biologically active Pfr (λ_{max}=730 nm) and inactive Pr (λ_{max}=660 nm) forms in a red/far-red dependent fashion and regulates, as a molecular switch, many aspects of light-dependent development in *Arabidopsis thaliana*. Phytochrome B signaling is launched by the biologically active Pfr conformer and mediated by specific protein-protein interactions between phyB Pfr and its downstream regulatory partners, whereas conversion of Pfr to Pr terminates signaling. Here we provide evidence that phytochrome B is phosphorylated *in planta* at S86 located in the N-terminal domain of the photoreceptor. Analysis of *phyB-9* transgenic plants expressing phospho-mimic and non-phosphorylatable phyB-YFP fusion proteins demonstrated that phosphorylation of S86 negatively regulates all physiological responses tested. The S86D and S86A substitutions do not affect stability, photoconversion and spectral properties of the photoreceptor, but light-independent relaxation of the phyB S86D Pfr into Pr, also termed as dark reversion, is strongly enhanced both *in vivo* and *in vitro*. Faster dark reversion attenuates red light induced nuclear import and interaction of phyB S86D-YFP Pfr with the negative regulator PHYTOCHROME INTERACTING FACTOR 3 as compared to phyB-GFP. These data suggest that accelerated inactivation of the photoreceptor phyB via phosphorylation of S86 represents a new paradigm for modulating phytochrome-controlled signaling.

PL-04
AN INSIGHT INTO REGULATORY ROLES OF TFIIH-ASSOCIATED RNA POLYMERASE II CTD-KINASES IN *ARABIDOPSIS*

Mohsen Hajheidari[1], Sara Farrona[1], Bruno Huettel[1], Zsuzsa Koncz[1], Csaba Koncz[1,2]

[1] *Max-Planck Institute for Plant Breeding Research, Cologne, Germany*
[2] *Institute of Plant Biology, Biological Research Center of Hungarian Academy of Sciences, Szeged*

Phosphorylation of the carboxy-terminal domain of RNA polymerase II largest subunit (RNAPII CTD) plays a pivotal role in the regulation of transcription in eukaryotes. The RNAPII CTD consists of conserved $Y_1S_2P_3T_4S_5P_6S_7$ repeats, which undergo phosphorylation by cyclin H-dependent protein kinases (CDKs) of the general transcription factor TFIIH. Phosphorylation of the S_5-residue stimulates transcription initiation and RNAPII-recruitment of initiation factors, Mediator and cap-binding complex (CBP), whereas phosphoserine-2 mark of the CTD mediates interaction with elongation and splicing factors and chromatin remodeling complexes. Phosphorylation of Ser-7 is so far implicated only in 3′-processing of snRNAs. In comparison to mammals, so far little is known about the regulation of RNAPII CTD phosphorylation in plants. Here we report on that serine phosphorylation of Arabidopsis RNAPII CTD is governed by CDKF;1, a unique plant specific CTD S_7-kinase. CDKF;1 is required for *in vivo* activation of functionally redundant CDKDs, which are major CTD S_5-kinases that also phosphorylate *in vitro* the S_2 and S_7 CTD residues.Inactivation of CDKF;1 causes extreme dwarfism and sterility because CDKF;1 and CDKDs also act as upstream activators of CDKA, a central regulator of cell cycle progression. In addition, CDKF;1 plays a distinguished role in the control of biogenesis of microRNAs and several silencing siRNAs. Inhibition of CTD S_7-phosphorylation in *cdkf;1*mutant seedlings is accompanied by 3′-polyadenylation defects of precursor pre-microRNAs and transcripts encoding key regulators of small RNA biogenesis pathways. The *cdkf;1* mutation decreases the levels of both precursor and mature small RNAs without causing global down-regulation of protein coding transcriptome and enhances the removal of introns that carry pre-miRNA stem-loops.Triple knockout inactivating the TFIIH-associated CDKD kinases is lethal, but a combination of a null and a weak *cdkd;3* allele in a triple *cdkd123** mutant permits semi-dwarf growth. In early phase of development, *cdkd123** seedlings show reduced CTD Ser-5 phosphorylation, accumulation of uncapped precursor pri-miRNAs and parallel decrease of mature miRNA levels.During later development of *cdkd123** seedlings, however, Ser-7phosphorylation and unprocessed small RNA levels decline similarly as in the *cdkf;1* mutant. In conclusion, co-transcriptional processing and stability of a set of small RNAs and transcripts involved in their biogenesis is sensitively affected by changes in the activities of CDKF;1 and CDKDs, which are major regulators of RNAPII CTD phosphorylation in *Arabidopsis.*

PL-05
THE ROLE OF EXTRACELLULAR VESICLES IN INTERCELLULAR COMMUNICATION

Edit Buzás

Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest

For several decades, the existence of extracellular vesicles was suggested by sporadic observations only. However, by now it became well established that the secretion of these phospholipid bilayer-bound subcellular structures represents an evolutionary conserved, active cellular process. Mediated either by their surface or cargo molecules (such as miRNAs), they play substantial roles both in physiological and pathological processes. Extracellular vesicles not only shed new light on paracrine regulation of cell functions, but also raise fundamental questions about cell autonomy. These days the field of their isolation, detection and exploitation as biomarkers, therapeutic targets or therapeutic vehicles undergoes explosion like development, and represents a very intensively investigated field of cell biology and biomedicine.

PL-06
MOLECULAR TATTOOING IN LIVE ZEBRAFISH

Miklós Képíró[1], Boglárka Várkuti[1], Katalin Kis Petik[3], Miklós Z. Kellermayer[3], Máté Varga[4], András Málnási-Csizmadia[1,2]

[1] *Department of Biochemistry, Eötvös Loránd University, Budapest*
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[3] *Department of Biophysics and Radiation Biology, Semmelweis University, Budapest*
[4] *Department of Genetics, Eötvös Loránd University, Budapest*

Until now the greatest limitation in the application of bioactive compounds has been the inability of confining them specifically to single cells or subcellular components within the organism. Recently we synthesized photoactivatable forms of bioactive compounds which can be covalently attached to their target enzymes, thus resulting in prolonged effects. Furthermore, we showed that photoactivation can be initiated by two-photon excitation, thereby confining the effect to femtoliter volumes and well-controlled spatial locations. As a test, we halted the migration of specific cells of zebrafish embryos by activating, by use of a two-photon microscope, a photoactive inhibitor of myosin. The role of cell migration and cell-cell interactions in the development of zebrafish lateral line could thus be revealed with unprecedented spatial and temporal control and detail.

ABSTRACTS - COMPANY LECTURE

CL-01
PCR GOES DIGITAL PUSHING THE BOUNDARIES OF SENSITIVITY AND ACCURACY IN NUCLEIC ACID BASED DETECTION

Dénes Szilassy

Life Technologies Magyarország Kft., Budapest

Digital PCR is a new approach to nucleic acid detection and quantification. It offers a different method for absolute quantification and rare allele detection relative to conventional real-time quantitative PCR. Digital PCR works by partitioning a sample into many individual real-time PCR reactions; some portion of these reactions contain the target molecule (positive) while others do not (negative). Following PCR analysis, the fraction of negative reactions is used to generate an absolute count of the number of target molecules in the sample, without reference to standards or endogenous controls

Researchers use digital PCR method for the following applications:

- Rare Allele Detection
- Absolute quantification of gene expression
- Absolute Quantification of Viral Load
- Absolute Quantification of Nucleic Acid Standards
- Absolute Quantification of Next-Generation Sequencing Libraries

The major differentiators of digital PCR versus the traditional real-time PCR approach are the following:

- No need to rely on references or standards
- Desired precision can be achieved by increasing the number of PCR replicates
- Highly tolerant to inhibitors
- Capable of analyzing complex mixtures
- Provides a linear response to the number of copies present to help enable analysis of small fold-change differences

Beyond providing a sensitive tool for biological discoveries, the above characteristics render digital PCR the most accurate and sensitive method in nucleic acid quantification enabling entering of the DNA or RNA based analytics into the fields of routine testing in food safety as well as in the clinical areas such as cancer profiling or infectious disease detection.

The presentation will discuss examples of various application areas for digital PCR. In addition, we will introduce a disruptive new digital PCR platform, the QuantStudio™ 3D Digital PCR System, that provides researchers with an affordable, simple and scalable -architecture solution. Due to the high-density chip technology, the system eliminates the challenges of emulsion PCR hence improving data quality, dramatically simplifying the workflow as well as enabling the utilization of the entire template sample. This is especially important when analyzing prestigious

biopsy samples for cancer profiling or in single cell analyses. An additional advantage of the zero-dead-volume of the sample is the elimination of the necessity of applying replicate runs.

The design and scalability of the QuantStudio™ 3D Digital PCR System provides the flexibility for the researchers to adapt to their experimental needs without the requirement to run a large number of samples to be cost effective.

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ABSTRACTS - ORAL LECTURES

O-001
ENDOGENOUS RETINOIC ACID SYNTHESIS CONTRIBUTES TO NEURAL STEM CELL DIFFERENTIATION

Barbara Orsolits[1], Adrienn Borsy[2], Emília Madarász[1], Zsófia Mészáros[1], Tímea Kőhidi[1], Károly Markó[1], Márta Jelitai[1], Ervin Welker[2,3], Zsuzsanna Környei[1]

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[2] *Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest*
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Retinoic acid (RA) has been widely used as a potent inducer of neuronal differentiation in various multipotent cell populations (embryonic carcinoma [EC], embryonic and neural stem [ES; NS] cells, iPS cells), in vitro. In vivo, in the central nervous system, RA action has been reported at sites of both embryonic and postnatal neurogenesis, indicating that RA signaling is involved in the regulation of neural stem cell commitment and differentiation throughout life. While NS cells respond to RA delivered to or synthetised within the brain, little is known about i; the potential of NS cells to utilize RA precursor molecules and ii; the rearrangement of the components of retinoid metabolism/signaling during NS cell development. In the presented *in vitro* study we monitored the changes in the retinoid machinery of differentiating NS cell populations derived either from embryonic or adult brain tissues. We show, that NS cells are capable to convert both vitamin A and retinyl ester to RA which serves as an auto-inductive signal to initiate the neuronal differentiation protocol.

O-002
THE EFFECT OF EARLY DIFFERENTIATION PATHWAY INHIBITORS ON CELL FATE DECISION IN PREIMPLANTATION RABBIT EMBRYOS

Babett Bontovics[1], Jaroslav Slamecka[2,3], Pouneh Maraghechi[1], László Hiripi[1], Alexander V. Makarevich[2], Peter Chrenek[2,3], Kinga Németh[1], Zsuzsanna Bősze[1], Elen Gócza[1]

[1] *Agricultural Biotechnology Center, Gödöllő*
[2] *Animal Production Research Centre, Nitra, Slovak Republic*
[3] *Faculty of Biotechnology and Food Science, Slovak University of Agriculture, Slovak Republic*

Genes that direct pluripotency in the mouse are well described, however, their expression pattern during the rabbit embryonic development is not yet known. Culture medium supplemented with different inhibitors (PD032590 (PD), CHIR99021 (CH), Y27632 (Y)and A8301 (A)) enabled embryonic stem cell (ESC) lines to be derived efficiently from mouse and rat embryos.

The aim of our work was to analyse the expression pattern of embryonic stem cell-specific markers in rabbit embryos and attached blastocysts following cultivation in different combinations of the inhibitors of GSK3 (CH), MEK (PD) and TGFβ-R (A), involved in differentiation; and a ROCK inhibitor (Y). We cultured rabbit embryos from two or from eight-cell-stage till 4.5day or 6 days,

than compared the development of rabbit embryos in different conditions. We supplemented the normal embryo cell culture medium (RDH) with 1i (RDH+PD), 2i (RDH+PD+CH), 2i+ (RDH+PD+Y), 3i (RDH+PD+CH+Y) and 4i (RDH+CH+Y+A). The expression level of epiblast-specific Oct4 and hypoblast-specific Gata4 and Gata6 were examined in blastocysts and attached embryoblasts. At 4.5 day, the expression level of Oct4 was significantly higher in blastocysts, cultured in 2i culture medium, but there was no significant difference in GATA4, GATA6 expression on mRNA and protein level, compared to embryos cultured in the other conditions. On day 6, we observed reduced expression levels of epiblast- and hypoblast-specific factors. We noticed that rabbit embryos cultured in 3i and 4i were retarded in their development.

We confirmed that the culture medium supplemented with 2i is the most appropriate condition for rabbit embryo development in terms of preserving the viability of embryos and increasing the proportion of epiblast cells in the blastocysts. We hope that we can maintain the pluripotency of the epiblast-derived rabbit embryonic stem cells in 2i medium supplemented with LIF or other factors during long-term cultivation.

This research was funded by grants Plurabbit: OMFB-00130-00131/2010 and ANR-NKTH/ 09-GENM-010-01 to ZsB, OTKA K77913, MÖB-47-1/2009 to EG and APVV-LPP-0119-09, LAGEZ-no. 26220120051 to PC.

O-003

ANALYSIS OF HUMAN ABC MULTIDRUG TRANSPORTER EXPRESSION IN HUMAN EMBRYONIC STEM CELLS AND THEIR DERIVATIVES

Zsuzsa Erdei[1], Tamás I. Orbán[1], Réka Lőrincz[1], Ágota Apáti[1,2], Balázs Sarkadi[1,2]

[1] *Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest*

[2] *Membrane Research Group, Hungarian Academy of Sciences, Semmelweis University and National Blood Center, Budapest*

Human pluripotent stem cells (hES) provide new hopes for the clinical treatment of a number of diseases and, at the same time, they are excellent models for tissue development and physiological cell differentiation. ABC multidrug transporter (ABC-MDR) proteins are primary active transporters as they utilize the energy of cellular ATP for performing a vectorial, transmembrane movement of drugs or xenobiotics. The presence of these transporters in human embryonic stem cells may significantly contribute to stem cell defense mechanisms. Our group showed previously that ABCG2 is indeed expressed by hES cells [1] and our findings support the hypothesis that functional ABCG2 expression is required to tolerate malicious effects resulting from stress conditions caused by harmful chemicals or UV light exposure [2]. In this study we examined the expression of 48 human ABC transporters in human embryonic stem cells (HUES9) and their derivatives. We differentiated the hES cells spontaneously in EB formation system and collected the cardiac-like, the nerv-like and the mesenchymal-like cells mechanically. We used TaqMan Low Density Array (TLDA) analysis to detect the mRNA level of ABC proteins in our samples. In parallel experiments we analysed mRNA levels of 98 pluripotency and differentiation marker genes. To validate the TLDA, biological replicates were used. As expected, these samples clustered together. Based on the statistical analysis, hES cells showed upregulation of pluripotency markers, whereas the derivative cells showed upregulation of the proper lineage-specific markers. Cluster

analysis showed cell-type specific upregulation of certain ABC transporters. We also confirmed the protein expression of these transporters by immunostaining. The characterisation of ABC-MDR protein expression profiles representative for hESCs and the differentiated cell types should provide a more accurate model of embryonic development, thus may improve toxicity screening and drug discovery systems.

This work has been supported by Grants from OTKA (NK83533, CK80283 and K101064) ETT (213–09, 211–09), STEMKILL (OM00108/2008), KMOP-1.1.2-07/1-2008-0003 and TÁMOP-4.2.2-08/1-2008-0015.

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O-004
MESENCHYMAL STEM CELLS INDUCE THE ALTERNATIVE PATHWAY OF MACROPHAGE ACTIVATION

Gyöngyi Kudlik, Beáta Hegyi, Ferenc Uher

Stem Cell Biology Unit, National Blood Service, Budapest

In recent years it has become clear that the therapeutic potential of mesenchymal stem or stromal cells (MSC) is related not only to their multilineage differentiation capacity but also to their ability to suppress inflammatory and immune responses. Therefore, here we studied the influence of mouse bone marrow- (BM) and adipose tissue-derived (Ad) MSCs on peritoneal macrophage (MΦ) polarization in the presence or absence of bacterial endotoxin lipopolysaccharide (LPS). We isolated and co-cultured peritoneal macrophages and mesenchymal stem cells from C57Bl/6 mice. We found that MΦs co-cultured with MSCs consistently showed a higher level of phagocytic activity, increased expression of mannose receptor (CD206), and markedly elevated IL-10, but reduced or unchanged IL-1 and TNF-α levels in the culture supernatants compared to the controls as measured by commercial ELISA assays. Even in the presence of high amounts of LPS, stromal cells were able to attenuate classical (M1) polarization of MΦs. On the other hand, the MSC induced (M2b-like) MΦ polarization appears to correlate with their enhanced ability to induce proliferation of in vivo antigen-primed T cells. Transwell co-culture system revealed that the cross-talk between MSCs and MΦs was primarily but not exclusively mediated by soluble factors that include prostaglandin E2. These results demonstrate that MSCs switch MΦs into regulatory cells characterized by low pro-inflammatory and high anti-inflammatory cytokine production, high ability to ingest pathogens and apoptotic cells, and a marked increase in their antigen-presenting potential probably aiming to control hyper-inflammation and tissue regeneration.

O-005
THE ROLE OF EPITHELIAL-MESENCHYMAL INTERACTIONS DURING DEVELOPMENT OF THE AVIAN BURSA OF FABRICIUS

Nándor Nagy, Imre Oláh

Department of Human Morphology and Developmental Biology Semmelweis University, Faculty of Medicine, Budapest

The bursa of Fabricius (BF) is a primary lymphoid organ in the birds responsible for B-cell maturation within its follicular microenvironment. During bursa of Fabricius development, extensive interactions between two embryologically different compartments (ectodermal cloacal epithelium and mesenchyme of tail bud mesoderm origin) lead to the formation of the bursal follicles into which –as a third player – dendritic cell and B cell precursors immigrate. Testosterone treatment of early chicken embryo cause chemical bursectomy, by preventing the differentiation of mesenchymal compartment and subsequently the normal development of the follicles. The aim of this study was to determine the role of epithelial-mesenchymal interactions during formation of the avian bursal follicle, the mechanism of which is poorly understood.

Using in situ hybridization and immunocytochemistry of developing bursa of Fabricius we have shown that the hindgut and cloacal epithelium expressed the Sonic hedgehog (Shh) growth factor, but not in the bursal epithelium. This distribution patter suggests that the exclusion of Shh expression from the prospective bursal epithelium is critical for normal development. The extracellular matrix (ECM), which hematopoietic cells are in contact is also important component of the bursal specific mesenchyme compartment. Fibronectin is the only ECM molecule studied that occurs uniformly in the mesenchyme, whereas we have observed a specialized distribution of tenascin expression, which is an ECM protein deeply involved in tissue interactions during fetal development and oncogenesis. Tenascin was absent from the mesenchyme of the bursal primordium but strongly expressed by the cloacal and hindgut mesenchyme. On the base of the embryonic expression of the ECM molecules we hypothesized that this specific distribution pattern of tenascin was epithelial-cell dependent.

To study the effects of epithelium and its products on the development and patterning of the extracellular matrix, we have used RCAS-Shh retroviral vectors The Shh protein overexpression in developing bursa, was combined with chick-quail chimeras and tissue recombination. Shh expressing epithelium of the hindgut was separated by 0.03% collagenase treatment from its mesenchyme and the isolated epithelial sheath was recombined with bursal mesenchyme. The recombined tissue was implanted in the chick embryo coelomic cavity for hematopoietic cell colonization. After 2 weeks of incubation, tenascin antibody staining clearly indicates the presence ectopic tenascin close to the epithelium. When Shh was ectopically expressed in the bursal mesenchyme by RCAS vector the tenascin was strongly induced and no bursal follicle developed.

Results from our embryomanipulation experiments provide evidence that the normal patterning of bursal mesenchyme is controlled by epithelial-derived factors which are critically important in the bursal morphogenesis.

O-006
TRANSCRIPTION FACTOR MEDIATED PROGRAMMING OF ES CELLS INTO DENDRITIC CELLS

Emília Simó, Erika Takács, István Szatmári

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Dendritic cells (DCs) are professional antigen presenting cells that are specialized to capture, process and present antigens to T cells in order to modulate immune response. The use of DCs to prime responses to tumor antigens provides a promising approach to cancer immunotherapy but clinically relevant responses have frequently been disappointing, partly due to the properties of the DCs obtained from adult progenitors. In addition, only limited number of cells can be obtained from adult DC progenitors (monocytes). Embryonic stem (ES) cells have the potential to give rise to any cell types in the human body, raising exciting prospects to generate DCs from this pluripotent cell source. It was already reported that DCs could be generated from ES cells (ES-DC). However ES-derived DCs had an impaired T cell activation capacity suggesting that some signaling pathways or some factors are missing from these cells. To obtain ES-DCs with enhanced antigen presenting activity we try to manipulate their development by transcription factor mediated cellular programming. We have selected 15 DC-specific transcription factors to probe the effect on ES cell-derived DC progenitors using inducible murine ES cell lines. We are currently assessing the effects of these DC affiliated transcription factors on the early phase of DC development.

O-007
NETWORK PLASTICITY AND RIGIDITY DETERMINES LEARNING AND MEMORY FORMATION AND DEFINES OPTIMAL ATTACK STRATEGIES

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Networks not only give us a visual image of complex systems providing an instant recognition of groups and important nodes, but also have a number of structural features, which are general properties of biological, social and engineered networks. Our multidisciplinary group (www.linkgroup.hu) uses networks as 'highways' making the transfer of concepts between various disciplines. This concept-transfer embeds the original idea to the entirely different context of another scientific field, and helps to solve creativity deadlocks.

Many complex systems have two, rather separate states, where one of them is more plastic (flexible), while the other is more rigid. When either Hsp60 or Hsp70 type molecular chaperones assist in the refolding of other proteins, they pull (or push) the protein enforcing its more rigid state, then release the protein invoking its more flexible, plastic state. The community structure of the protein-protein interaction network of yeast cells became more condensed (rigid) upon stress (PLoS Comput. Biol. 7, e1002187). Janos Kornai recently described capitalism as a "surplus society"

and compared it to socialism formerly characterized as a shortage society. The observed states are similar to large and small phenotypes of human metabolism. Several other examples, such flexibility changes during the learning process of human brain show flexibility-increase increases the learning potential of the system. However, an 'over-flexible' system will not have a memory, and will unable to keep changes. An increase in system rigidity increases the memory storing ability of the system. Alternating changes of flexibility and rigidity emerge as a highly efficient optimization strategy of evolutionary changes.

Flexible and rigid networks can be influenced in entirely different ways. While flexible, plastic systems dissipate the perturbations well, rigid systems do not dissipate, but transmit perturbations preserving them at their original level. Plastic systems should be optimally attacked at their central nodes. On the contrary, if rigid systems are attacked at their central nodes they may either retaliate, or break. In drug action this behavior often corresponds to side effects and toxicity. Rigid, optimized systems should be attacked at the neighborhood of their central nodes/rigid clusters. In protein structures binding sites are often at the side of rigid clusters, and binding to these sites often invokes an increase of rigidity. The inter-modular nodes of yeast cells after heat shock, chaperones, the highly dynamic, 'creative nodes' are typical examples of such indirect actions. Regulatory proteins, keystone species often act in an indirect fashion influencing highly central and rigid network segments. In social networks opinion-leaders are often persuaded best by their neighboring nodes. In drug design two strategies exist for influencing rapidly growing cells (such as bacteria or cancer), and differentiated cells (in case of practically all other diseases). The first strategy attacks central nodes, while the second their neighbors.

O-008
AUTOPHAGY REGULATORY NETWORK – AN INTEGRATED RESOURCE TO IDENTIFY NOVEL REGULATORS AND INTERACTIONS THAT CONTROL AUTOPHAGY

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Autophagy (cellular self-eating) is a cellular catabolic process that involves the sequestration of cytosolic material into double membrane vesicles termed autophagosomes for delivery to the lysosome, where the cargo is degraded by acidic enzymes.Autophagy is a key response mechanism to numerous extracellular and intracellular stresses such as nutrient and growth factor deprivation, hypoxia, protein misfolding and infection by intracellular pathogens. Autophagy is regulated by upstream signaling pathways (e.g., mTOR, Hedgehog, JAK/STAT, MAPK, TGF-β) and transcription factors, such as FOXO, NRF2 and p53. Despite the wide-spread role of autophagy and the diverse signaling processes that could control it, there is no systems-level resource, which contains autophagy components and their regulators.

Prompted by the lack of systems-level autophagy-related information, we manually collected the literature and integrated external resources to gain a high coverage autophagy database. We developed an online resource, Autophagy Regulatory Network (ARN; http://arn.elte.hu), to provide an integrated and systems-level database for autophagy research. ARN contains manually curated, imported and predicted interactions of autophagy components (3,907 proteins with 6,932 interactions). We identified and predicted 2,745 novel interactors of 56 autophagy components and integrated their transcriptional and post-transcriptional regulation from regulatory resources. We listed 157 transcription factors and 556 miRNAs that could regulate autophagy components or their protein regulators. We also connected the above mentioned autophagy components and regulators with signaling pathways from the SignaLink 2 resource containing major signaling pathways. Altogether the ARN contains 43,048 protein-protein and regulatory interactions. The user-friendly website of ARN allows researchers without computational background to search, browse and download the database. The database can be downloaded in SQL, CSV, BioPAX, SBML, PSI-MI and in a Cytoscape CYS file formats.

The ARN resource could facilitate the experimental validation of novel autophagy components and regulators. In addition, ARN helps the investigation of transcription factors, miRNAs and signaling pathway that could control the autophagic pathway. The list of such known and predicted regulators is important in pharmacological attempts against cancer and neurodegenerative diseases.

O-009
REGULATING THE CROSSTALK BETWEEN AUTOPHAGY AND APOPTOSIS BY STRESS-DIRECTED BISTABLE SWITCH

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One of the most important tasks of a cellular system is deciding between life and death to maintain its intrinsic homeostasis against external stimuli. The proper balance of secreted and membrane proteins is controlled in the endoplasmatic reticulum (ER). Accumulation of misfolded proteins due to ER stresses leads to the activation of unfolded protein response (UPR). The primer role of UPR is to reduce the bulk of damages and try to drive back the system to the former or a new homeostatic state by autophagy, while excessive stress results in apoptotic cell death. While the surviving mechanism is driven by Beclin1, the suicide processes is controlled by caspases. Interestingly, both processes are regulated by Bcl2, but the proper dynamical behaviour of the control network is yet to be clear. Therefore we approach the question from a systems biological perspective by developing a simple mathematical model of the autophagy-apoptosis crosstalk. Our analysis reveals that both Bcl2-dependent inhibition and a positive feedback driven by caspases-dependent inactivation of Beclin1 ensure that only one cellular response gets activated depending upon the stress levels. We claim this positive feedback makes the apoptotic response bistable with respect to stress levels. Moreover a presence of an additional positive feedback loop between Bcl2

and caspases renders the switch irreversible. According to this model we also demonstrate experimentally that the various combinations of different type of ER stresses (such as rapamycin, thapsigargin) have a drastic effect on the threshold of caspases-dependent cell death. Our goal is to provide a framework for further experiments and modelling.

O-010
EVOLUTION OF ANTIBIOTIC HYPERSENSITIVITY

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Multiresistant pathogenic strains are one of the relentlessly increasing global public health concerns in recent times. In the background of multidrug resistance is the well-known phenomenon of cross-resistance (that is when resistance to a certain antibiotic results in decreased sensitivity against several other antibiotics). However, very little is known about the cases in which genetic adaptation to a certain antibiotic yields enhanced sensitivity to other antibiotics (referred to as collateral sensitivity). The main objective of this study was to systematically explore the network of collateral sensitivity interactions in *Echerichia coli* for 24 clinically relevant antibiotics, using standard tools of microbial evolution experiments. Our results highlighted that collateral sensitivity indeed occurs frequently during antibiotic adaptation. From the global pattern of sensitivity interactions, populations adapted to aminoglycosides (a special group of 30S protein synthesis inhibitor antibiotics) stood out having an especially low fitness in the presence of multiple other antibiotics. Whole-genome sequencing of these laboratory evolved strains revealed multiple mechanisms underlying aminoglycoside resistance. One of the key mechanisms is to avoid aminoglycoside uptake by reducing the proton-motive force (PMF) across the inner membrane, since aminoglycosides uniquely enter the cell in a membrane potential-dependent fashion. On the contrary, efflux of many other antibiotics builds upon PMF-dependent pumps. Based on these observations, we propose a model where mutations through which aminoglycoside resistance is achieved simultaneously diminish the activity of PMF-dependent major efflux pumps leading to increased sensitivity towards many other antibiotics. More generally, our work offers a novel mean to combat resistant bacteria as well as to rationalize the design of sequential multi-drug therapies.

O-011
EVOLUTIONARY POTENTIAL OF THE UNDERGROUND METABOLIC NETWORK OF *ESCHERICHIA COLI*

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Most enzymes are capable of catalysing reactions for which they have not evolved. The importance of these weak secondary or underground reactions lies in their ability to provide evolutionary raw material for new enzymatic functions. However, it remains unclear to what extent this raw material could generate novelties in the context of the entire network. Can underground reactions contribute to the formation of novel pathways that allow utilization of novel substrates? If yes, why have not these reactions evolved in nature? To address these questions, we used a reconstruction of known underground enzyme activities in *E. coli.* By *in silico* analysis we have shown that a sizeable fraction of secondary reactions can contribute to novel pathways with important end products. We find that while these novel pathways do not improve fitness under standard nutrient conditions, they can increase growth in other environments. Based on our analysis, we propose three mechanisms limiting the evolutionary realizability of these underground pathways. First, novel compounds introduced by secondary reactions tend to be more toxic than those of native ones. Second, they confer an advantage only under few specific nutrient conditions. Third, both our *in silico* analysis and experimental studies indicate that establishment of these evolutionary novelties require multiple mutational steps. In sum, adaptation to new nutrients via recruitment of underground enzyme activities might be limited due to the presence of selectively neutral or even deleterious intermediate steps.

O-012
COMPUTATIONAL MODELING OF T AND B CELL DEVELOPMENT USING GENE CO-REGULATORY AND PROTEIN INTERACTION NETWORKS

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Computational modeling of living systems is a powerful approach to integrate the accumulated knowledge about individual components. Computational biology is capable of testing high numbers of conditions, which would be too time consuming or excessively expensive to implement with experimental tools. Using the rapidly increasing computational power modeling is capable of providing predictions about complex systems, such as the human immune system.

Several attempts have been made to develope computational models of individual immune processes, immune cells, or the immune system as whole. The reason for this is that computational models provide excellent tools for understanding immunity both in normal and disease state, finding possible treatments and drug targets to cure the abnormal functions caused by perturbations in the system.

The bioinformatics group at the Institute of Biomedical Technology, University of Tampere, has a research focus on systems biology of human immunity. In this framework, we have established an integrated Immunome KnowledgeBase during the past nine years to collect all the relevant, gene based information regarding human immunity. This information source is utilized in various systems biological studies on the human immunome.

The development of B and T cells is one of the primary focus of the group. Computational simulations were designed to identify stable gene expression patters, called attractors, which can characterize individual sub-types of these immune cells. Since the available gene co-regulation and protein interaction networks in the immunome dataset are too large to be subjects of such simulation, a unique network decomposition method was developed to determine cell type specific gene networks. The developed methodology utilizes microarray datasets and protein interaction data.

In the next step, a computational framework was developed to implement the simulations themselves. This environment uses theoretical results from agent based modeling and random Boolean networks, and it capable of simulating the changes in the gene co-regulation networks identified from the network decomposition studies.

At the moment, the simulation studies are finished for the T cells and under way for the B cell data. We were able to produce meaningful clustering of the predicted stable gene expression patterns from the simulation, and identifying some of the clusters as cell-types from the T cell development. The future aim of the project is to simulate the effect of knocked-down genes to verify our results using our collected information about primary immunodeficiencies, where mutations in known genes causes well described immunity related disease phenotypes, often elimination of selected T and B cell sub-types. In addition to this, we plan to verify predicted gene expression patterns in selected T cell sub-types in wet lab experiments.

O-013
THE ROLE OF UBIQUITIN LIGASES DURING EARLY STEPS OF SYMBIOTIC NODULE DEVELOPMENT

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Ubiquitin-mediated proteolysis is an integral part of diverse cellular functions. The E3 ubiquitin ligases -one of the three enzymes involved in linking ubiquitin to protein targets- are of particular interest as they confer substrate specificity during this process. In plants, the U-box domain containing (PUB) E3 family has undergone a large gene expansion that may be attributable to biological processes unique to the plant life cycle. Research on PUB genes from several different

plants has started to elucidate a range of functions for this family, from self-incompatibility and hormone responses to defense and abiotic stress responses.

We have recently identified the symbiotic *LIN* gene in *Medicago truncatula* and *Lotus japonicus*, codes for a predicted E3 ubiquitin ligase containing a highly conserved U-box domain [1]. Based on its mutant phenotype *LIN* functions at an early stage of the rhizobial symbiotic process, required for both infection thread growth in root hair cells and the further development of nodule primordia. Functional analysis of the cloned gene suggests that *LIN* predominantly functions during rhizobial colonization and the abortion of this process in *lin* mutants leads to a suppression of nodule development. The LIN protein with its exceptional domain organization might be an example for playing a role specialized for the regulated protein degradation during the signal transduction processes in the symbiosis.

We have also identified another U-box domain containing E3 ubiquitin ligase protein by its ability to interact with the intracellular part of the receptor kinase DMI2 involved in the early nodulation signalling. In vitro ubiquitin ligase activity of this E3 ligase and LIN was confirmed. In vivo experiments exploring their possible regulatory functions of these E3 ligases on the elements of symbiotic signalling pathway will provide insight how these plants modulate actively and precisely the complex interactions behind the symbiotic responses by means of protein turnover.

Reference
[1] Kiss E et al. (2009), Plant Physiol. 151: 1239-1249.

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O-014

DISSECTION OF THE FUNCTION OF A SINGLE GENE BELONGING TO A LARGE NODULE SPECIFIC GENE FAMILY GOVERNING BACTEROID DIFFERENTIATION IN *MEDICAGO TRUNCATULA*-RHIZOBIAL SYMBIOTIC ASSOCIATION

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The symbiotic relationship between soil bacteria termed rhizobia and legumes such as *Medicago truncatula* results in a specialized root organ called nodule, wherein the symbiotic nitrogen fixation takes place. The rhizobia are encompassed by a plant derived membrane in the nodule cells and undergo differentiation to be able to fix atmospheric nitrogen. The rhizobial-legume endosymbiotic association is tightly regulated by several bacterial and plant genes. Our aim was to identify plant genes involved in bacterial differentiation and the effective function of the root nodule. We have isolated mutant plants developing ineffective (Fix^-) nodules in a fast neutron

mutagenised M. truncatula population and the mutant plants have been used to identify plant genes required for the later stages of the symbiotic interaction. One of mutant lines designated as 6V developed nodules containing non-elongated bacteria indicating the failure of bacteroids differentiation.

We have identified the impaired gene from the 6V mutant by the combination of classical positional cloning and Affimetrix Microarray GeneChip based gene identification approach. The identified gene is a member of the nodule specific cystein-rich (*NCR*) gene family which includes more than 400 members and exists only in the IRLC-type legumes.

In order to reveal the exact function of this single *NCR* gene in the symbiotic process, we have initiated further microscopical analysis and molecular biological experiments. The subcellular localization of the fluorescent tagged fusion protein was analysed to determine the co-localisation of symbiotic bacteria and the 6V-NCR protein. We also investigated the ploidy level of the nodule cells and the bacteria isolated from the nodules by flow cytometry to investigate the differentiation of nodule cells and bacteroid development.

Our data indicate that a single gene from the large gene family of *NCR* genes can have a predominant and distinct role in bacteroid differentiation and have a unique function during *M. truncatula*-rhizobial symbiosis.

O-015
IDENTIFICATION AND *IN VIVO* ANALYSIS OF GENES REQUIRED FOR MICROTUBULE FUNCTION IN EPITHELIAL CLOSURE PROCESSES

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Whenever an organism sustains a wound it must be repaired rapidly to prevent further tissue loss and infection. The embryo of the fruit fly Drosophila melanogaster provides an excellent model system for *in vivo* analysis of the molecular and cellular events occurring during the closure of a small epithelial opening. Several developmental processes, such as dorsal closure of the embryo, involve a coordinated series of cellular activities that are very similar to those required for wound healing. Dorsal closure represents the last major morphogenetic movement during embryogenesis, when two opposed epithelial sheets converge toward the midline where they meet, sealing a hole at the dorsal surface of the embryo. Similar to wound healing, this process involves cell elongation and movements that bring two epithelial fronts toward one another such that they can eventually fuse. The fusion of the epithelial sheets takes place by a zipper-like mechanism, as the opposing surfaces are zipped together at the most anterior and posterior ends of the dorsal hole or at the wound edges. Several studies have highlighted the requirement of actin-based structures, such as filopodia and lamellipodia, for the closure eventsbut the function of the microtubulenetwork is very poorly understood.

We have shown that preceding dorsal closure, the epithelial MTs reorganize to attain an unusual spatial distribution. Instead of a radial pattern, MTs form acentrosomal arrays that are aligned along the dorsal-ventral cell axis and become restricted to the apical cell cortex. Microtubule bundles are stable, however, individual microtubules remain highly dynamic and are distributed

in the bundles overlapping in an antiparallel manner, with their minus ends. Although dorsal closure involves the coordinated action of several tissues, microtubule function is exclusively required in epithelial cells and only for the last step, the zippering, which seals the gap. We have demonstrated that epithelial microtubule control the final zippering, by promoting actin-based cell-protrusion formation.

To uncover novel components required for microtubule reorganization and function, we have applied an RNAi-based screening method combined with automated *in vivo* video microscopy. This way, the*short stop (shot)* genewas identified to be necessary for the zippering of the dorsal hole. Detailed cell biological analysis revealed that *shot*controls themorphology of the MT network in the epithelial cells by regulating dynamic properties of microtubule growth. Shot belongs to the conserved family of the spectraplakins, gigantic structural proteins with functional domains binding to actin filaments, microtubules and cell adhesion complexes. We propose thatin epithelial cells *shot* coordinates the interactions of distinct cytoskeletal components, whichenables the cells to rapidly restructure their cytoskeleton and adopt an organization appropriate to the physiological requirementsof the closure. Our work provides insights how mechanisms integrating individual cytoskeletal elements into complex, highly shaped functional patterns contribute to a developmental process at the organism level.

O-016
GLIAL CELLS ACCUMULATE LIPID DROPLETS TO PROMOTE BRAIN DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

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Lipid droplets are common organelles of eukaryotic cells. However lipid cell biology have been extensively studied in vertebrate liver and adipose tissue and in their invertebrate analogue the fat body of *Drosophila,* the cellular function of lipids in the nervous system remains unclear. In this study we used the larval brain of *Drosophila melanogaster* as a model system to study the role of lipid droplets in brain development. We showed that in the fruitfly's brain only glial cells accumulate lipid droplets during the larval development. We found that glial cells located at the brain surface accumulate the highest amount of lipid droplets which is a unique feature among glial cells. To study the molecular mechanism underlying this lipid distribution we studied the cellular and subcellular localisation of the Drosophila fatty acid-binding protein (Dfabp), previously reported to be expressed in the central nervous system. Using a variety of immunohistochemical and immunocytochemical methods we showed that Dfabp is expressed exclusively by glial cells but not by neurons. Using glial specific RNA interference we showed that depletion of *dfabp* causes a dramatic diminution in the number of lipid droplets suggesting that *dfabp* is essential for the cellular transport of lipids into brain tissue. We further showed that this lipid deficiency does not affect glial viability but causes massive neurodegeneration, a complete block in motor activity and early death. Taken together these data suggest that lipid droplets have an important role in brain development and neuronal function.

O-017
AUTOPHAGY IS REQUIRED FOR ZEBRAFISH CAUDAL FIN REGENERATION

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Regeneration is the ability of multicellular organisms to replace damaged tissues and regrow lost body parts. This process relies on cell fate transformation that involves changes in gene expression as well as in the composition of the cytoplasmic compartment, and exhibits a characteristic age-related decline. Here, we present evidence that genetic and pharmacological inhibition of autophagy — previously implicated in extensive cellular remodeling and aging — impairs the regeneration of amputated caudal fins in the zebrafish (*Danio rerio*). To our knowledge, this is the first demonstration in vertebrates that the lysosome-mediated self-degradation process of eukaryotic cells is required for injury-induced tissue renewal. We further show that upregulation of autophagy in the regeneration zone occurs downstream of MAPK/ERK signaling, to protect cells from undergoing apoptosis and trigger cytosolic restructuring underlying terminal cell fate determination. This novel cellular function of the autophagic process in regeneration implies that the role of cellular self-digestion in differentiation and tissue patterning is more fundamental than previously thought.

O-018
INTRODUCING PREIMPLANTATION GENETIC DIAGNOSIS (PGD) BYACGHIN HUNGARY: IMPROVING OUTCOME IN IVF

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Study question: What kinds of aneuploidy patterns do the day three embryos of Hungarian patients undergoing IVFdisplay?

Summary answer: Our results show similar tendencies with previous observations at numerous points, most importantly, the higher the maternal age the euploid/aneuploid ratio is lower. The occurrences of the monosomy and trisomy events in the examined embryos decreased, while complex aneuploidies increased with elevated maternal age.

What is known already: To improve the implantation rates of *in vitro* fertilization (IVF), preimplantation genetic screening (PGS) is increasingly being used to detect numerical chromosomal abnormalities in embryos. The comprehensive screening of 24 chromosomes by microarray comparative genome hybridization (aCGH) is a rapidly spreading technology in the preimplantation analysis of blastomeres. Since chromosomal aneuploidy is the major cause of

pregnancy loss, the preimplantation screening of embryos has high relevance, especially with increasing maternal age.

Study design, size, duration: Our retrospective study was performed on 421 human embryos from 107 Hungarian patients undergoing IVF from 05. 2011. to 01. 2013.

Materials and Methods: ArrayCGH analysis on single blastomeres is an accurate aneuploidy detection tool and may assist in identifying abnormal embryos at cleavage stage. We have analyzed 421 human embryos by aCGH. Day three embryos had one cell removed as a biopsy specimen, then DNA amplification and analysis were performed.

Main results and the role of chance: The first results from 107 Hungarian patients (ave.age: 36.69) are derived from the analysis of the above-mentioned samples, out of which 28.98% were normal, 34.44% had complex aneuploidy (embryos with >2 chromosome abnormalities), 11.88% were double aneuploidies, 13.78% monosomies and 10.93% trisomies. The number of complex abnormal embryos significantly increased with advancing maternal age while theoccurance of normal embryos decreased, from 37.23% and 39.89% in patients ≤35 to 69,51% and 10.97% in patients 41 and older, respectively. The number of monosomy events was higher than trisomy events in the total population (n=46, ave. age=35.97 for trisomies, n=58ave. age=36.9 for monosomies). Out of the 107 patients, 73 had normal embryos without any aneuploidy. 16 of these patients have ongoing pregnancies so far and four of them gave birth to their children.

Limitations: We should point out that this kind of investigations is not widely used yet in Hungary, further studies will punctuate confirm the present results, as this technique is becoming more widespread also in Hungary as well.

Wider implications of the findings: Despite the finding of previous studies, which described chromosome X as a chromosome with the highestfrequency of aneuploidy, in our study the aneuploidy of sex chromosomes were extremlyrare. The most common aneuploidies were for chromosomes 15, 5, 16, 10, 20 and 1. The rarest aneuploidies were instead for chromosome Y, 4, 12, 3, 11 and 2.

O-019
EXPLORING FUNCTIONAL SITES LOCATED WITHIN INTRINSICALLY DISORDERED PROTEINS

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Recent observations have shown that a large portion of the human genome encodes intrinsically unstructured/disordered proteins (IDPs) that do not adopt a stable structure in isolation. IDPs are abundant in short linear binding motifs that mediate protein-protein interactions while undergoing a disorder to order transition upon binding. These functional sites are key components of many cell signalling networks and have been associated with various diseases, especially with

cancer. However, the linear motif mediated protein-protein interactome of the human proteome and its role in diseases, is still largely unexplored.

The large-scale characterization of functional regions located within IDPs is only accessible by bioinformatic methods at the moment. There are two main frameworks that can be used to identify functional sites within IDPs. One type of approach, ANCHOR works by capturing the basic biophysical properties of regions involved in coupled folding-and-binding process: their disordered status in isolation, and the ability to form favorable interactions with globular proteins that drives the interactions. An alternative approach is based on short linear motifs (SLIMs) that correspond to short consensus sequence patterns distilled from proteins with a common interaction partner, such as PDZ, SH2 or SH3 domains. Known motifs can be used to investigate further candidate functional sites, however, the predicted functional instances of consensus motifs are overwhelmingly dominated by false positive matches that occur purely by chance. We have suggested that the combination of ANCHOR with linear motifs can yield more specific predictions with higher confidence than the two methods alone. The basis of this is the strong correlation between disordered binding regions and functional instances of linear motifs.

Prediction of functional regions located within disordered proteins can also be used to gain insights into their role in different types of cancer. Towards this aim, we analyzed mutational data from the Catalog of Somatic Mutations in Cancer (COSMIC) database and data collected from large-scale cancer genome projects. We focused on missense mutations because these provide positional information that can be specifically mapped to ordered and disordered regions of proteins. Our main finding was that while neutral polymorphisms were more likely to occur within disordered segments, cancer-associated mutations had a preference for ordered regions. However, in some cases mutations occurring in disordered regions can still have a detrimental effect on protein function. We collected several examples where protein regions displaying enrichment for cancer associated mutations aligned with disordered protein binding regions. For known motifs, we found a good agreement between the missense mutational frequency of a given amino acid and its structural role in the linear motif.

Ultimately, similar systematic analyses can discover unknown disordered binding regions with functional importance, and this strategy can help to close the gap in the functional annotation of the structured versus the disordered proteome.

O-020
BLOOD COAGULATION FACTOR XIII STABILIZES NEUTROPHIL EXTRACELLULAR TRAP STRUCTURE BY THE FORMATION OF INTERMOLECULAR COVALENT PROTEIN CROSS-LINKS

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Neutrophil Extracellular Trap (NET) composes a recently discovered remarkable mechanism of innate immune response to infection. After pathogen-induced activation neutrophils eject their DNA content into the invaded tissues forming a web-like structure. The DNA backbone is embedded with a number of antimicrobial peptides (defensines, myeloperoxidase, bacterial permeability increasing protein), proteases (neutrophil elastase, cathepsin G), and histones. This extracellular structure with its antimicrobial activity is able to collect and eradicate pathogens, thus to preclude their dissemination.

According to confocal laser scanning and electron microscopic observations NET constitutes a highly organized three-dimensional structure of DNA fascicles and protein complexes. Structural characteristics of NET raises the possibility that its texture may be strengthened by intermolecular covalent protein cross-linking carried out by one of the transglutaminases. These enzymes act on proteins by catalyzing reactions resulting in formation of protease resistent covalent bonds between a carboxylamide group of glutamine residues and the amino group of lysine residues of the polypeptide chains.

In accordance with this hypothesis we observed transglutaminase activity induction and accumulation of cross-linked high molecular weight protein complexes during NET formation of human peripheral blood derived neutrophils. Moreover, blocking of transglutaminase activity by a competitive inhibitor resulted in reduced extent and muddled structure of NET. Detailed analysis revealed that the intracellular form of blood coagulation factor XIII (plasma transglutaminase) is the only transglutaminase which is expressed by neutrophils and may be responsible for the observed protein cross-linking activity during NET formation. Taking together our results suggest that FXIII activity is induced during neutrophil activation and NET formation, that plays a decisive role in the stabilization of the NET structure.

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O-021
IMPORTANCE OF VALINE 224 IN REGULATING THE TRANSAMIDATION ACTIVITY AND CALCIUM SENSITIVITY OF HUMAN TRANSGLUTAMINASE 2

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Transglutaminase 2 (TGM2) is a calcium dependent protein crosslinking enzyme which has several additional enzymatic (GTPase, protein kinase and disulphide isomerase) and non-enzymatic (protein scaffolding, signal transduction, transcriptional regulation) activities involved in a variety of physiologic and pathologic cellular processes.

The sequence of human TGM2 was cloned in 1991 and this clone has been used in the last two decades for various biochemical, structural and cellular characterizations. This sequence contains Glycine at position 224 instead of Valine which, as recently turned out, is the amino acid present in all available sequenced TGM2 exons of humans and other mammalian species. To assess the physiological consequences of the one amino acid change, we cloned the TG2 224Val enzyme and performed its biochemical characterization. Using cellular conditions, TG2 224Val exhibited seven-fold increase in protein crosslinking transamidation activity as compared to TG2 224Gly. At the same time, a six-fold increase in calcium binding affinity of the purified protein has been observed, which could account for the high Ca^{2+} concentration milieu needed for TG2 224Gly. The similarity of substrate binding affinities and guanine nucleotide binding activities of the two variants suggests that the change in catalytic activity of TG2 upon Valine to Glycine replacement is related to differences in metal binding. While TG2 224Val showed enhanced antigenicity for celiac antibodies using conformational epitopes, the antigenicity of TG2 224Gly was more sensitive to Ca^{2+} concentration. Biochemical data in accord with bioinformatics analysis supported higher stability for TG2 224Val. In particular, Valine224 decreased the flexibility of the Ca^{2+} binding loop (first loop) and thereby significantly improved metal binding affinity, as computed by FoldX. Our data suggests that Val224 significantly increases TG2 transamidation via modulating its Ca^{2+} binding affinity and thus enables transamidation activity inside cells as opposed to the dogma that TG2 is silent as a protein crosslinking transamidase in cells.

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O-022
STRUCTURAL ANALYSIS OF PHI11 STAPHYLOCOCCAL PHAGE DUTPASE

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Preservation of DNA integrity has high importance in organisms, that is among others secured by the dUTPase enzyme family, which is a well known regulator of the dUTP/dTTP level in the cells.

dUTPases (Duts) are considered promising canonic regulatory molecules controlling relevant cellular processes. dUTP hydrolysis cycle consists of at least four distinct enzymatic steps: dUTP substrate binding, isomerization of the enzyme-substrate complex into the catalytically competent conformation, a hydrolysis (chemical) step, and rapid, nonordered release of the products.

Above these funcion several non-canonical roles are suggested in different organisms.

The role of the specie-specific dUTPase insert is deeply examined in the case of Phi 11 Staphylococcal phage dUTPase.

Staphylococcal pathogenicity island (SaPI) repression as a model, we report here that phage dUTPases induce the transfer of SaPI-encoded virulence factors.

High resolution X-rax crystallographic and kinetic data is presented in order of elucidation the external function of Phi11phage dUTPase.

The Stl protein binding partner is also identified an examined in this process, by crystallographic, EMSA, MS and fluorimetriy assays.

O-023
INVESTIGATION OF CATALYTIC PROPERTIES OF A KEY LIPID BIOSYNTHETIC ENZYME FROM THE MALARIA PARASITE

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The enzyme CTP:choline-phosphate cytidylyltransferase (CCT) from *Plasmodium falciparum* catalyze the coupling of CTP and choline-phosphate (ChoP), thereby generating the high energy intermediate within *de novo* phosphatidylcholine biosynthesis. This pathway is essential to *Plasmodium* and represents a novel validated antimalarial target. Previous studies of CCT revealed crystal structure of the conserved catalytic domain of this enzyme with bound CDP-choline product, however, there is no available crystal structural data with either of substrate. Here we characterized key elements of CCT catalysis by investigating a truncated construct containing the catalytic domain of *Plasmodium falciparum* CCT (*Pf*CCT), using biophysical, thermodynamic and

enzyme kinetic methods. Our data show that in the presence of the Mg^{2+} cofactor, the binding of the CTP substrate is attenuated by a factor of 5. The weaker binding of CTP:Mg^{2+}, when paralleled to earlier observations regarding aminoacyl tRNA synthetases, suggests that Mg^{2+} is necessary for catalysis but not for ligand binding of *Pf*CCT. We hypothesize that in lack of Mg^{2+}, positively charged side chain(s) of CCT may contribute to CTP accommodation. The formerly published CCT crystal structure indicated that cation-pi interaction mediated by a conserved tryptophan contributes to product coordination. Mutation of tryptophan to alanine revealed that this interaction is essential for enzyme efficiency. Ligand binding and kinetic investigations indicate that replacement of this residue with other aromatic residues can only partially compensate for its effect. The observed weak affinity together with fluorescence signal of ChoP substrate binding suggests that this tryptophan residue assists catalytic conversion rather than promoting substrate accommodation.

These studies provide significant new insights into understanding the mechanism of action of CCT enzymes.

O-024
ALLELIC IMBALANCE IN DNA REPAIR DEFECTIVE CHICKEN DT40 CELL LINES

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Large-scale genome rearrangements and copy number changes are a common feature of cancer cells. In particular, BRCA1 deficient tumors typically display genome instability, presumably due to the impaired function of homologous recombination repair (HR). The identification of this 'BRCAness' phenotype, which may also be caused be defects in other genes, is of prognostic value for disease progression, and may also help with treatment selection. We set out to model cancer cell genome instability using a set of mutated DT40 chicken lymphoblastoma cell lines. We compared a BRCA1 knockout line to cell lines defective in other individual genes of the HR pathway and non-homologous end joining (NHEJ) to assess their differential contribution to genome instability. To measure genome instability, we looked for copy number alteration and allelic imbalance events by single nucleotide polymorphism (SNP) array analysis. The resolution of the SNP array allows the detection of changes covering minimum 20-30 kilobases on a chromosome. After culturing wild-type cells as well as various HR and NHEJ mutants for 100 cell generations, we surprisingly did not observe a significant level copy number alterations or allelic imbalance. To accelerate the accumulation of DNA lesions, selected mutant cell lines were then subjected to a series of DNA damaging treatments with an alkylating agent. Here we observed a significant increase of allelic imbalance events in the BLM helicase mutant, but little allelic imbalance in the wild type or the BRCA1 mutants. We conclude that alternative DNA damage tolerance pathways protect the BRCA1 mutant cells from the conversion of DNA lesions into large-scale genome rearrangements, and that the relative activities of different DNA damage tolerance pathways are also likely to be important in determining the extent of genome instability in HR-deficient tumor cells.

O-025
Domain movements and sequence-specific pausing of Recq Helicase

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RecQ-family helicases play an essential role in the maintenance of genome stability via their participation in the repair and homologous recombination of DNA in all kingdoms of life. The importance of RecQ helicase functions is reflected by the fact that their mutations lead to high cancer predisposition and accelerated ageing. We apply a multi-faceted approach to elucidate the DNA-restructuring mechanism of *E. coli* RecQ and human Bloom's syndrome (BLM) helicases. We study these activities via a combination of solution kinetic, spectroscopic and magnetic tweezers single-molecule techniques. The so-called winged-helix domain adopts different conformations in the crystal structures of bacterial and human RecQ helicase family members. However, no information is available on the structural dynamics of RecQ helicases and it is not known how the possible domain rotations may contribute to the DNA-restructuring activities of these enzymes. We have engineered *E. coli* RecQ helicase for site-specific fluorescent labeling in order to detect and kinetically monitor the putative structural changes. Pyrene excimer fluorescence probes attached to neighboring domains of the protein showed an excimer (excited state dimer) signal that was sensitive to DNA binding by the enzyme, suggesting its applicability as a sensitive conformational probe. In addition, we have been focusing on understanding the sequence-specific pausing behavior observed during both dsDNA unwinding and ssDNA translocation. We compare the unwinding and pausing behavior of RecQ on different DNA sequences and in different pulling and unwinding geometries, which allow us to examine how the key unwinding properties (i.e., unwinding rate, processivity, pause location, pause duration, and their distributions) depend on DNA sequence, DNA geometry, and tension applied on the DNA. We found that DNA unwinding by wild-type RecQ helicase is interrupted by strong sequence-dependent pauses. Pausing is significantly reduced for truncated RecQ constructs lacking the auxiliary DNA-binding HRDC domain, and for point mutants disrupting DNA binding by the HRDC domain. We propose a model for sequence-dependent pausing by RecQ and highlight the potential *in vivo* ramifications of this behavior.

O-026
DUTP LEVEL CONTROLS TRANSFER OF VIRULENCE GENES IN ORDER TO PRESERVE INTEGRITY OF THE TRANSFERRED MOBILE GENETIC ELEMENTS

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dUTPases ubiquitously regulate cellular dUTP levels to preserve genome stability [*1*] Recently, several other cellular processes were reported to be controlled by dUTPases including the horizontal transfer of *Staphylococcus aureus* pathogenicity islands (SaPI) [*2*]. SaPIs are mobil genetic elements that encode virulence enhancing factors e.g. the toxins. Here, phage dUTPases were proposed to counteract the repressor protein (Stl) and promote SaPI excision and transfer. A G protein-like mechanism was proposed [*3*] which is unexpected in light of the kinetic mechanism of dUTPases [*4*, *5*].

Here we investigate the molecular mechanism of SaPI transfer regulation, using numerous dUTPase variants (phage, human, mycobacterial) and a wide range of *in vitro* methods (steady-state and transient kinetics, VIS and fluorescence spectroscopy, EMSA, quartz crystal microbalance).

Our results unambiguously show that Stl inhibits the enzymatic activity of dUTPase in the nM concentration range and dUTP strongly inhibits the dUTPase : Stl complexation. These results identify Stl as a highly potent dUTPase inhibitor protein and disprove the G protein-like mechanism which proposes dUTP as a mediator of dUTPase : Stl complexation. We propose that dUTPase can efficiently interact with Stl and induce SaPI excision only if the cellular dUTP level is low (i.e. when dUTPase resides mainly in the apo enzyme form) while high dUTP levels would inhibit SaPI transfer. This mechanism may serve the preservation of the genetic stability of the transferred SaPI genes and links the well-known metabolic role of dUTPases to their newly revealed regulatory function in spread of virulence factors. We are in the process of testing this mechanism in the *in vivo* context as well.

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O-027
CELL CYCLE REGULATION OF CROSSOVER TIMING

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Error-free DNA damage tolerance (DDT) mediating replication restart and gap-filling is crucial for genome integrity. Mechanistically, this process is driven by a switch of templates accompanied by sister chromatid junction (SCJ) formation. The highly conserved Sgs1/BLM helicase is critical for template switch intermediate processing, but the Mus81-Mms4 endonuclease, activated by Cdk1- and Cdc5-dependent phosphorylation of Mms4 in G2/M, also participates in recombination intermediate resolution. We asked how temporal deregulation of the Cdk1/Cdc5/Mus81 pathway affects genome integrity and the precedent conditions underlying its activation in mitosis. We used a series of expressional conditional systems in combination with genetic and physical approaches to unmask the temporal contribution of various regulatory pathways to DD intermediate processing. We find that persistent DDT recombination intermediates are largely resolved before anaphase via a G2/M damage checkpoint-independent, but a Cdk1/Cdc5-dependent pathway that proceeds via a previously described Mms4 activating phosphorylation. The Sgs1-Top3 and Mus81-Mms4-dependent resolution pathways occupy different temporal windows in relation to replication, with the Mus81-Mms4 pathway being restricted to late G2/M. Premature activation of the Cdk1/Cdc5/Mus81 pathway, achieved here with phosphomimicking, constitutively active Mms4 variants, as well as in S-phase checkpoint deficient genetic backgrounds, induces crossoverassociated chromosome translocations and error-prone replication accompanied by precocious processing of damage-bypass SCJ intermediates. Our results indicate that premature activation of the Cdk1/Cdc5/Mus81 recombination pathway drives genome instability by altering the uncoupling between error-free versus erroneous DDT pathways during replication. The precocious activation of the Cdk1/Cdc5/Mus81 pathway also underlies the replication defect of ATR/Mec1 replication checkpoint defective cells exposed to genotoxic stress, underscoring the importance of integrating DDT repair activities with cell cycle transitions to prevent chromosome rearrangements induced by untimely crossover recombination.

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O-028
THE COMPASS SUBUNIT SPP1 LINKS HISTONE METHYLATION TO INITIATION OF MEIOTIC RECOMBINATION

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Histone H3 lysine 4 trimethylationmarks meiotic recombination hotspots, but how this ubiquitous chromatin modification relates to initiation of Spo11-dependent DNA double-strand breaks (DSBs) has remained elusive. Here we provide evidence for a cause-effect relationship between recombination initiation and histone H3K4me3 during yeast meiosis. We show that *in vivo* targeting of*(i)* Set1, the catalytic subunit of the histone H3K4 methyl transferase complex (COMPASS) and *(ii)*Spp1,a PHD-domaincomponent of COMPASS to recombinationally cold regions is able to induce DSB formation. Interestingly, tethering of Spp1 initiates recombination independent from H3K4me3 (as opposed to Set1), therefore the presence of Spp1 at recombination sites is a prerequisite of hotspot activation. Further, Spp1 physically interacts with Mer2, a key protein of the chromosomal axis and the "core" Spo11-complexrequired for DSB formation. ThusSpp1, by interacting with H3K4me3 and Mer2, promotes recruitment of potential meiotic DSB sites to the chromosomal axis, allowing DNA cleavage at nearby nucleosome-depleted regions.

O-029
DNA DAMAGE INDUCED POLYMERASE EXCHANGE AT STALLED REPLICATION FORKS

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Genomic DNA is continuously damaged from extrinsic and intrinsic sources**.** DNA damages hinder the advance of replication forks because of the inability of the replicative polymerases to synthesize across most DNA lesions. Since stalled replication forks are prone to undergo DNA breakage and recombination that can lead to chromosomal rearrangements and cell death, cells possess different mechanisms to ensure the continuity of replication on damaged templates. Replication of damaged DNA, or DNA damage bypass, can be carried out by specialized translesion synthesis (TLS) DNA polymerases that can synthesize across DNA lesions either in an error-free or error-prone way, depending on the lesion. In the error-free way the newly synthesized strand preserves the sequence of the parental strand, in the error-prone way a mutation is introduced opposite the damage. By generating mutations in the genome, these polymerases drive carcinogenesis in humans. For example, in the absence of TLS DNA polymerase

eta that promotes error-free replication of UV damaged DNA, other error-prone TLS polymerases function that results in the variant form of *xeroderma pigmentosum,* a cancer-prone syndrome.

Since TLS polymerases are responsible for damage-induced mutagenesis, their activity must be strictly regulated. Our previous results showed that TLS DNA polymerases can interact with proliferating cell nuclear antigen (PCNA), a processivity factor of replicative DNA polymerases. We also proved that this interaction is essential for their function in DNA damage bypass. Through interaction with PCNA, TLS DNA polymerases can get access to the replication fork. However, the mechanism that allows of their replacement of the replicative polymerase at the stalled replication fork is unknown.

Using *Saccharomyces cerevisiae* as a model system, we have identified a new factor essential for DNA damage induced mutagenesis. We have characterized the role of this new factor in mutagenesis, and based on our results we set up a model for polymerase exchange at stalled replication forks.

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O-030
THE CIRCADIAN CLOCK-ASSOCIATED SMALL GTPASE LIGHT INSENSITIVE PERIOD 1 SUPPRESSES LIGHT-CONTROLLED ENDOREPLICATION AND AFFECTS TOLERANCE TO SALT STRESS IN *ARABIDOPSIS THALIANA*

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Circadian clocks are biochemical timers regulating many physiological and molecular processes according to the day/night cycles. The small GTPase LIGHT INSENSITIVE PERIOD 1 (LIP1) is a circadian clock- associated protein that regulates light input to the clock in the model plant *Arabidopsis thaliana*. In the absence of LIP1, the effect of light on free-running period length is much reduced. We showed that in addition to suppressing red and blue light-mediated photomorphogenesis, LIP1 is also required for light-controlled inhibition of endoreplication and tolerance to salt stress. We demonstrated that in the processes of endoreplication and photomorphogenesis LIP1 acts downstream of the red and blue light photoreceptors phytochrome B and cryptochromes. Manipulation of the subcellular distribution of LIP1 revealed that the circadian function of LIP1 requires nuclear localization of the protein. Our data collectively suggest that LIP1 influences several signaling cascades and that its role in the entrainment of the circadian clock is independent from the other pleiotropic effects. Since these functions of LIP1 are important for the early stages of development or under conditions normally experienced by germinating seedlings, we suggest that LIP1 is an important regulator of seedling establishment.

O-031
LIGHT QUALITY-DEPENDENT REGULATION OF FROST TOLERANCE IN CEREALS

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Cold acclimation is an integrative process where temperature, day length and developmental phase are the most important factors. In *Arabidopsis* it was proven that light-quality influences *CBF* expression, leading to increased level of frost tolerance. *CBF* genes are important regulators of frost tolerance in cereals as well. To better understand the relationship between frost tolerance and spectral differences of irradiation currently we study the effects of red, far red and blue light on the expression of CBF regulon in wheat.

The effect of monochromatic blue, red and far-red light was examined first on the winter habit einkorn (*Triticum monococcum)* 'G3116' line. The expression level of several *CBF*s and a cold-inducible effector gene (like *Cor14b)* was determined by quantitative real-time PCR (qRT-PCR) method. Our results proved that the light-quality affects the expression rate of *CBF-regulon* in the case of cereals, as well.

In the second experiment the possible effect of modulated light spectra on frost tolerance was tested studying the winter barley 'Nure', the winter wheat 'Cheyenne' and the winter einkorn 'G3116' cultivars at + 15°C. In this case the white light was supplemented by additional red or far-red or blue light for 20 days. The level of frost tolerance was quantified by electrolyte leakage measurement at different freezing temperatures measuring the degree of membrane injury of leaves. Expression levels of several CBF and other *COR* genes were determined by qRT-PCR. The survival rate of plants was tested at different freezing temperatures. It was obviously proven that the far-red light treated plants showed higher frost tolerance and higher expression level of several frost-related genes comparing to the control plants illuminated by white light.

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O-032
MOLECULAR DIFFERENCES OF NLRP3 INFLAMMASOME MEDIATED IL-1B PRODUCTION IN LPS- ACTIVATED HUMAN MONOCYTE-DERIVED MACROPHAGE SUBTYPES

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IL-1beta is a "master" cytokine that has an indispensable role in orchestrating effective innate and adaptive immune responses. It is produced in an inactive precursor form that is cleaved to active cytokine by protein complexes called Nlrp3 inflammasomes. Nlrp3 inflammasome contains Nlrp3 sensor, ASC adaptor and caspase-1 enzyme. The production of IL-1beta is strictly regulated and requires distinct signals. Some of these signals induce the expression of inactive proIL-1beta and that of the components of the inflammasome through the activation of signaling pathways. Other signals, such as the activation of ATP-sensing P2X7 receptor trigger the processing of proIL-1beta to mature IL-1beta by the multiprotein complex.

Macrophages are among the main sources of IL-1beta production. However, depending on their localization they can develop into a wide range of phenotypes. Macrophages differentiated in the presence of GM-CSF (GM-MFs) develop inflammatory phenotype, while cells differentiated in the presence of M-CSF (M-MFs) possess anti-inflammatory characteristics and have role in wound healing and tissue repair.

Our results show that GM-MFs produce high IL-1beta, while M-MFs produce low IL-1beta with a different time- and concentration kinetics. We found significant differences in basal and LPS-induced expression of Nlrp3, procaspase-1 and ASC between the two MF types both at mRNA and protein level. We found that LPS-treated GM-MFs are able to release ATP and produce IL-1beta, while M-MFs require ATP supplementation for IL-1beta secretion. Using specific inhibitors we could inhibit ATP release. We have also found expression differences in the ATP sensor P2X7 receptor as well as in the activation of key signal transduction pathways. Furthermore, inhibiting P2X7 receptor we could abolish IL-1beta production in GM-MFs. Our results altogether show that while our knowledge on the general mechanisms involved in Nlrp3 inflammasome function and on its regulatory mechanisms is rapidly increasing, it is also getting clear that the actual outcome of the activation (like IL-1beta production) is strongly depends on the cell type and on the presence or absence of various intracellular or extracellular modulators.

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O-033
MACROPHAGES ENGULFING APOPTOTIC CELLS PRODUCE A NOVEL RETINOID TO ENHANCE PHAGOCYTOSIS

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Retinoids, acting via RAR and RXR receptors, have been reported to modulate several functions of mononuclear phagocytes such as proliferation and phagocytosis of Fc-opsonized particles or yeast cells but their effect on apoptotic cell uptake have been so far not investigated. Previously it was reported that apoptotic cells promote their own clearance by triggering lipid sensing LXR receptor in phagocytes and upregulating the phagocytosis receptor Mertk, retinoic acid (RA) receptor alpha (RARα) and the multifunctional protein cross-linking enzyme and integrin co-receptor tissue transglutaminase (TG2) but the exact mechanism remains to be elucidated.

Following treatment of macrophages (MΦ) with various natural and synthetic RAR or RXR ligands we detected enhanced apopto-phagocytotic capacity and increased expression of the phagocytosis receptor Mertk, Stabilin 2, TIM4 and CD14, opsonin C1qb, lipid transporter ABCA1 and TG2 genes. Since RARα have been described as LXR target gene and previously we detected increased expression of RA producing retinal dehydrogenase 1 and 2 enzymes (RALDH1, 2) in *in vitro* LXR agonist treated MΦ or apoptotic cell engulfing thymic MΦ we hypothesized that LXR receptor activation might initiate RA production and signaling which could contribute to the observed phagocytosis enhancement following LXR activation. To test this we treated MΦ with LXR agonist for 24 hrs and found that the upregulated genes are partially overlapping with the retinoid induced ones. In line with this blocking of RA production during LXR activation attenuated the phagocytosis enhancement and abolished the induction of retinoid dependent genes suggesting the endogenous retinoid production. Using transgenic mouse strain carrying RA response element in the promoter of lacZ gene we indirectly detected the presence of RA in the peritoneal cells of LXR agonist injected mice. Since MΦ in apoptosing thymus stained positive for RALDH enzymes we searched for RA derivates in thymus and identified a novel retinoid.

Our results indicate that RAs can increase the apopto-phagocytotic capacity of MΦ by upregulating several phagocytosis related genes. We also found that MΦ not only respond to RA but following LXR activation or apoptotic cell uptake they are also able to synthetize it. The newly produced RA enhances the already described pro-phagocytotic effect of LXR activation.

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O-034
STRESS-RESPONSIVE REGULATORY MECHANISMS TAILOR INNATE IMMUNITY AND ADIPOGENESIS

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Environmental changes, called stresses impinge on diverse biological processes, thereby modulate longevity and shape evolution through stress-responsive adaptive mechanisms. Research in recent years largely increased our understanding on the pathophysiology of various age-associated human disesases, including immune and metabolic disorders. In my talk I would like to give an insight into stress-inducible regulatory mechanisms that support innate immunity and adipocyte differentiation, respectively.

Recent evidence, obtained on the versatile roundworm *Caenorhabditis elegans* indicates that innate immunity, beyond antimicrobial effectors, also relies on stress-inducible host-defensive mechanisms. It is known that both mild metabolic and oxidative stresses induce longevity in invertebrates. We observed that both reduced insulin signaling and hydrogen peroxide enhance immunity against the dangerous human opportunistic pathogen *Pseudomonas aeruginosa*. We found that increased pathogen resistance required the Nrf2 ortholog SKN-1 transcription factor, a well-known master regulator of the xenobiotic detoxification response. We observed that an optimal activation of SKN-1 increased pathogen resistance, and demonstrated a role for SKN-1 in immunosenescence. Our findings auggest that SKN-1 integrates various chemical, metabolic and microbial signals to elicit a self-protective detoxification response

Adipose tissue is an active metabolic-endocrine organ that imparts on whole-body homeostasis and it dysregulation plays a critical role in the metabolic syndrome. We found that various stresses inducing protein homeostasis disturbances reversibly inhibit differentiation in 3T3-L1 preadipocytes. These proteotoxic stresses destabilized the adipogenic master regulator PPARγ protein and compromised its transcriptional output. Investigating the potential molecular mechanism, we demonstrated that the Hsp90 chaperone is required for PPARγ stability, function, adipocyte differentiation and survival. Our results provide evidence how protein homeostasis controls cellular signaling, function and phenotype *via* chaperone-dependent support of intrinsically unstable regulators.

Our findings reveal critical stress-responsive regulators of immunity and adipocyte biology, respectively, and offer potential novel targets relevant to various human diseases.

O-035
CARDIAC EXPRESSION OF A CELLULAR CALCIUM INDICATOR PROTEIN IN TRANSGENIC RATS

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GCaMP2 is a genetically engineered calcium sensor in which the fluorescence of the green fluorescent protein (GFP) is modulated by changes in calcium ion (Ca^{2+}) concentration. The expression of this protein provides a powerful tool for visualizing intracellular calcium transients in single cells, thus enabling the detection of ligand-induced signaling pathways. Moreover, in contrast to small molecule Ca^{2+} sensor dyes (e.g. Fluo-3, Fluo-4, Rhod-2 or X-Rhod-1), the tissue-specific stable expression of GCaMP2 allows to target specific cell types or tissues for signaling studies. Previously, we have documented that the use of the CAG promoter provides high level GFP expression in human embryonic stem cell derived cardiomyocytes, while other cell types show only lower expression levels of this protein [1]. We utilized this feature of the CAG promoter to generate transgenic rats for expressing the GCaMP2 Ca^{2+} indicator specifically in the heart. The transgenic rats were generated by injecting rat embryos with a transposon construct containing the GCaMP2 coding sequence under the transcriptional control of the CAG promoter. A transposase-dependent gene delivery system was used to obtain stable genetic modification of the embryos. Here we demonstrate that, based on this technology, GCaMP2 is applicable to follow cellular calcium signaling events in tissue slices prepared from the heart of transgenic rats, and also in primary cardiomyocytes isolated from the rat ventricle. In confocal microscopy experiments cardiac-expressed GCaMP2 proved to be sensitive enough to visualize Ca^{2+} transients of spontaneous contractions, or those elicited by adrenalin or ATP in the isolated primary cardiomyocytes. As a summary, we have generated a rat model overexpressing a genetically engineered, tissue-specific Ca^{2+} sensor with the ability to measure in vivo Ca^{2+} signals in rat cardiomyocytes. We suggest that GCaMP2 expressing primary rat cardiomyocytes are suitable for cardiotoxicity testing and drug screening of pharmacological agents which may affect intracellular Ca^{2+} concentrations, causing unwanted side effects or decreased treatment efficiency. By crossing of these calcium sensor expressing model animals with those providing specific (knock-out or knock-in) disease models, we plan to investigate the role of alterations in Ca^{2+} signaling under specific disease conditions. In order to extend the use of this technology, we also plan to apply other tissue-specific promoters for calcium sensor protein expression in transgenic rats.

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O-036
HUMAN GENETICS IN THE POSTGENOMIC AREA: WAITING FOR THE REVOLUTION, OR BEGINNING OF IT?

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President Bill Clinton called the human genome as the "most important, most wondrous map ever produced by humankind" and he supposed that "It will revolutionize the diagnosis, prevention and treatment of most, if not all, human diseases". Soon after the publication of the draft sequence in 2001, a rapid progress in the monogenic disease research could be observed: there was an exponential increase in the new monogenic-disease entities identified. In association with the new gene discovery a rapid increase was also seen in the number of deleterious mutations published in the scientific literature. These events made the monogenic diseases the first winners of the postgenomic area. Albeit in 2007 the Science magazine declared the array based population screening as the "Breakthrough of the year", which launched the spread of the genomewide association studies, the results of this promising area, even with discovery of thousands of susceptibility genes, could not achieve real clinical significance; meanwhile, the biobanks and biobanking networks emerged still point toward huge perspectives. By the anniversary of the Human Genome Project, almost all of the leading scientific journals published their reviews about the decade elapsed, we have to confess that most doctors have not embraced the genomic revolution, according to leaders of medical professional groups, because they have trouble seeing how it will benefit their patients. Including the achievements of the pharmacogenomics, only a small percent of doctors thought they had enough knowledge to use tests when prescribing drugs, the personalized medicine, of P4 medicine, made only just few steps ahead. We also had to face that almost no progress was seen on the therapy side, therefore a new initiative, the International Rare Disease Research Consortium (IRDiRC) was launched in 2012 with the ambitious goal of introducing therapy for 200 rare diseases till 2020. Indeed, the research of the rare disease is really a promising new field by the disseminations of next generation robust techniques, and there is indeed a chance for realization of chapters of the scenario envisaged a decade ago.

O-037
SECOND GENERATION ANTIPSYCHOTIC (SGA) DRUGS MODIFY THE DIFFERENTIATION PROGRAM OF HUMAN ADIPOCYTES INDUCING "BROWNING" MARKERS

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Although brown adipose tissue (BAT) can be found only in small amounts in the human body after infancy, recent studies revealed that it has a major importance in regulating the energy balance of the entire body. Its oxidative metabolism contributes to energy expenditure during cold exposure and diet or physical exercise induced thermogenesis triggering the recruitment of "brown adipocyte-like" or "beige" cells interspersed in subcutaneous white adipose tissue depots in a process called "browning". A very high negative correlation has been found between obesity and BAT amount in humans. Targeting the currently known and unknown regulatory systems of "browning" might open up better strategies to specifically stimulate BAT in obese individuals to aid weight reduction.

We observed that in vitro antipsychotic drug treatment at clinically administered concentrations reprogrammed the gene expression pattern of differentiating human adipocytes, surprisingly leading to overexpression of the major BAT marker gene, UCP1 (Uncoupling Protein 1). Our aim was to clarify whether a commonly used second generation antipsychotic drug (SGA) was able to induce a browning program on differentiating human adipocytes. Furthermore, we intend to establish an in vitro model of human brown adipocyte differentiation and to set up a panel of measurements that can discriminate between white and brown adipocytes. It includes determination of expression of white, brown and general adipocyte markers by Real Time Quantitative-PCR, immunoblotting or immunocytochemistry and changes in morphology (size and number of lipid droplets) or mitochondrial properties (Heme content, distribution and number of mitochondria) by Laser Scanning Cytometry.

Human preadipocyte cell line or primary preadipocytes (adipose-derived stem cells) obtained from herniotomy were differentiated into white or brown adipocytes with or without long or short-term SGA treatment. SGA administration resulted in significant overexpression of several brown adipocyte marker genes (UCP1, ELOVL3, CIDEA, CYC1, PGC1A) and Ucp1 protein while SGA treated cells had more and smaller lipid droplets than the control ones. We have concluded that SGA treatment can induce a browning program in differentiating human white adipocytes in vitro, while the background of weight gain, its common in vivo side effect has not been elucidated yet.

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O-038
CLONAL EVOLUTION IN HHD PALL: CONTRASTING HYPOTHESES

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Pediatric acute lymphoblastic leukemia is a disease with an early outbreak and quite good chances of survival. The high hyperdiploid (HHD) subtype shows one of the best survival probability. Hypotheses about the etiology involve many types of chromosomal rearrangements as well as numerical aberrations, the latter being in focus of our studies. With exclusion of the most common structurals [i.e. t(9;22) (q34;q11.2) BCR-ABL, t(v;11q23) MLL, t(12;21) (p13;q22) TEL-AML1 (ETV6-RUNX) and t(1;19) (q23;p13.3) E2A-PBX1 (TCF3-PBX1)] the numericals were used to test the 4 current hypotheses about the evolution of the disease (tetrasomy followed by losses; near-haploidy followed by duplication; simultanous gains and sequential gains). We investigated the most frequently affected 8 chromosomes with iFISH and analysed the pattern with networking methods. The results point in a different direction from the current view. The aim of the presentation is to contrast the hypotheses and discuss them.

O-039
THE RS3185480 SNP OF THE APCDD1 GENE IS ASSOCIATED WITH ANDROGENIC ALOPECIA

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Adenomatosis polyposis coli down-regulated 1 (APCDD1) gene is an inhibitor of the Wnt signaling pathway therefore it may have a role in the development of the skin appendages, moreover a mutation of this gene has been shown to be associated with a rare hair condition, hereditary hypotrichosis simplex. In this study we aimed to investigate whether the single nucleotide polymorphisms (SNP) of APCDD1 gene contribute to the development of a common hair disease, androgenic alopecia. 210 patients with androgenic alopecia and 98 controls have been enrolled to the study. The severity of the androgenic alopecia was classified according to Hamilton-Norwood in the case of men and according to Ludwig in the case of women. The genotypes of 9 SNPs in the coding region of the gene have been determined with direct sequencing. We found a significant difference in the distribution of the genotypes of the c.1781C/T, p.L476L SNP (rs3185480) of the APCDD1 gene in exon 5, causing a 3.5 and a 2.8 times increased risk for the development of androgenic alopecia for the homozygote (CI 0.933 – 13.125; Nominal Regression p=0.063) and the heterozygote carriers (CI 1.086 – 7.217; Nominal Regression p=0.033) of the alleles respectively. Based on our results we conclude that the c.1781C/T, p.L476L SNP

(rs3185480) of the APCDD1 gene contributes to the development of androgenic alopecia. Regarding the possible function of the rs3185480 SNP, an *in silico* investigation suggested that this polymorphism is located in an exonic splicing regulatory element thus may alter mRNA splicing. Moreover, this SNP alters the codon usage of leucine from a preferred codon (CTC) to a rare codon (CTT), which might influence the efficacy of the translation and thus the APCDD1 protein level. Currently we are working on the functional characterization of this rs3185480 APCDD1 SNP.

O-040
GENETIC AND FUNCTIONAL STUDIES ON HUNGARIAN FAMILIES WITH BROOKE-SPIEGLER SYNDROME

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by the development of skin appendage tumors, such as cylindromas, trichoepitheliomas and spiradenomas. BSS develops due to mutations in the tumor suppressorgene, *CYLD*. In this study we aimed to perform genetic and functional studies on Hungarian BSS families. Genetic screening of the *CYLD* gene identified a novel heterozygous missense mutation (c.2613C>G; p.His871Gln) in a Hungarian BSS pedigree located in Szeged. Functional studies on the novel missense mutation demonstrated, that the CYLD-interactor NEMO protein immunoprecipitated from fibroblasts carrying the mutation is more ubiquitinated than the one immunoprecipitated from control cells. Since NEMO is a well-known negative regulator of the NF-κB signaling pathway, we hypothesize that this novel missense mutation of the *CYLD* gene might influence the NF-κB signaling pathway. In another family screening we identified a recurrentheterozygousnonsense mutation (c.2806C>T, p.Arg936X)in a BSS family located in the region of Szekszárd. The same recurrent nonsense mutation was identified in an Anglo-Saxon pedigree in the UK (region of Newcastle). Haplotype analysis of the Hungarian and Anglo-Saxon BSS pedigrees carrying the same nonsense mutation revealed independent mutation events in the background of the disease. Our results suggest that the position of the identified recurrent nonsense mutation is a mutational hotspot on the gene.

The genetic screening of BSS pedigrees may identify the causative mutation on the *CYLD* gene thus we can offer presymptomatic screening and prenatal diagnosis, which may have a huge impact on family planning.

O-041
EXPERIMENTAL STRATEGIES FOR *IN VIVO* RESCUE OF DISEASE-CAUSING MUTATIONS OF ABCC6

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Mutations in *ABCC6* can cause chronic or acute forms of dystrophic mineralization described in disease models such as pseudoxanthoma elasticum (PXE, OMIM 26480) and generalized arterial calcification of infancy (GACI, OMIM 614473) in human. The ABCC6 protein is a large membrane-embedded organic anion transporter and we recently firmly established that the protein is localized in the basolateral compartment of the plasma membrane of hepatocytes. The major aim of our study was to identify mutants with preserved transport activity but failure in intracellular targeting, as these mutants are candidates for functional rescue. Ten missense mutations were investigated; we found that seven variants retained significant transport activity. All mutants were transiently *in vivo* expressed in mouse liver via hydrodynamic tail vein injections. R1138Q and R1314W displayed endoplasmic reticulum localization, therefore we tested whether 4-phenylbutyrate (4-PBA), a drug approved for clinical use, could restore their intracellular trafficking to the plasma membrane in mouse liver. The cellular localization of R1314W was significantly improved by 4-PBA treatments, thus potentially rescuing its physiological function.

Silencing of the Abcc6a gene in zebrafish with sequence-specific morpholinos triggered a developmental phenotype which can be rescued by injecting wt human ABCC6 mRNA. Consequently, zebrafish provides an in vivo model to study disease-associated ABCC6 variants functionally. Indeed, we have established that all ten of disease-causing mutants fail to rescue the phenotype, irrespective whether they are transport- or localization mutants. Interestingly, one mutant, V1298F, which was found to be correctly situated in the plasma membrane, but showing minimal residual activity in the transport assay gave a partial (30%) rescue. These results indicate that – although the (patho)physiological consequence of lack of ABCC6 function in human and zebrafish is different, zebrafish provides a second animal model to study ABCC6 mutations.

Our work demonstrates the feasibility of the *in vivo* rescue of cellular maturation of ABCC6 mutants in physiological conditions very similar to the biology of the fully differentiated human liver and could have future human allele-specific therapeutic application. Furthermore, our studies can provide a model for systematic investigation of disease-causing mutations of membrane proteins and for pharmacologically assisted maturation of this class of proteins.

O-042
MUTATIONS IN POTENTIAL SUBSTRATE- AND STEROID-BINDING SITES MODULATE CHOLESTEROL SENSING OF HUMAN ABCG2

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Human ABCG2 is a plasma membrane glycoprotein expressed in many tissues especially in those with barrier function. ABCG2 is an active transporter that extrudes various compounds from the cells, therefore it can protect the body and also cancer cells against a large number of molecules. We and others have demonstrated earlier that the activity of human ABCG2 is modulated by cholesterol, however, the sensing site(s) for cholesterol have not yet been identified. Arg 482 and a potential steroid-binding element (SBE, aa 555-558) of ABCG2 have been reported to modulate the transporter's function. In our present work we have characterized the role of aa 482 and the SBE in cholesterol sensitivity of human ABCG2. Nine ABCG2-R482 and three SBE mutants were expressed in Sf9 insect cells, containing relatively low amounts of plasma membrane cholesterol, thus allowing the investigation of the effect of different membrane cholesterol levels on ABCG2 function. Our data delineate two clusters of aa 482 variants. Mutants having smaller amino acids at position 482 (G, S, T, N and D) are not significantly activated by cholesterol. However in the case of R482 variants with larger aa side chains (I, K, M and Y) membrane cholesterol level greatly enhanced ATPase activity and transport capacity, similarly to the wild-type protein. Interestingly, Leu to Ala replacements in the SBE, besides altering substrate specificity, also modulated cholesterol sensing in ABCG2. The activity of these variants was apparently cholesterol-independent. By using purified ABCG2 in a reconstituted system with well-controlled cholesterol levels, we document here that cholesterol is an essential activator of the ABCG2 activity. Surprisingly, in contrast to the data obtained in native membrane environment, in the purified, reconstituted system, the apparently "cholesterol-insensitive" mutant variants of ABCG2 (R482G and L558A) all required cholesterol for full activity, although showed greatly variable cholesterol sensitivities. Our data reveal important sterol regulation of ABCG2 and suggest that the sterol sensing regions examined here represent potential targets for pharmacological modulation.

O-043
CDK1 PHOSPHORYLATION GOVERNS NUCLEAR PROTEOME REDISTRIBUTION IN DAUGHTER CELLS AFTER DIVISION: LEGACY OF MOTHER CELLS

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Eukaryotic cells control many biological processes by regulating the movement of macromolecules into and out of the nucleus. Several nuclear proteins share CDK1 phosphorylation site near their NLS signal which affects their nuclear transport. However, in most cases neither the role, nor the structural background of this regulation is understood. We would like to provide deeper insights into these mechanisms through dUTPase and other proteins which are subjected to CDK1 phosphorylation.

Phosphorylation may abolish nuclear import of the protein if it is properly situated in the proximity of its NLS. We have gathered known CDK1 substrates, where this phosphorylation was known to disrupt binding to importin-alpha. We here propose a scheme that clearly identifies the exact sequence position where a negative charge will necessarily disrupt NLS function. Based on this proposed scheme, further candidates for such regulation were identified by screening the human genome for CDK1 targets, where the phosphorylation event is located in the vicinity of NLS signals. We explored the annotation of Gene Ontology terms assigned to this group and found proteins involved in DNA damage recognition and repair, as well as several transcription factors, and RNA-editing proteins. For any of these functions, strict and regulated scheduling of nuclear availability has clear and imminent significance, arguing for the need for further study. In order to experimentally test the effect of phosphorylation on nuclear import of our candidate proteins, we designed an efficient and sensitive model system. We choose DsRed labeled beta-galactosidase, a well-described bacterial protein, as an inert fluorescent cargo core upon which different NLSs can be loaded in a cloning strategy to test the effect of phosphorylation on their localization pattern. Use of this model in a test system generated convincing results indication that the bioinformatic screening gave valid results.

The exact molecular mechanism underlying the disruption of the interaction with importin-alpha and its phosphorylated cargo, has also been investigated with numerous biophysical methods, and X-ray crystallography.

Our data show that the nuclear proteome composition of daughter cells after cell division is notably determined by phosphorylation events occurring in the mother cell. This could be applied to several known, and newly proposed CDK1 substrates.

O-044
ELUCIDATION OF THE CATALYTIC MECHANISM OF P-GLYCOPROTEIN USING A CONFORMATION SENSITIVE ANTIBODY

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P-glycoprotein (Pgp) belongs to the family of ATP-binding cassette proteins, which bind and hydrolyze ATP to catalyze the translocation of their substrates through membranes. The basic architecture of these proteins is highly conserved, consisting of two homologous halves built from a transmembrane domain (TMD) and a cytosolic nucleotide binding domain (NBD). According to three-dimensional models based on crystal structures of ABC proteins the TMDs are always in outward facing conformation in the nucleotide-bound form of the protein, as opposed to the inward facing nucleotide-free form, where the bundle of transmembrane helices is closed on the extracellular side. The discontinuous extracellular epitope of the UIC2 mAb is sensitive to the switch between the above conformational states, as reflected by antibody binding. It was previously shown by Druley et al. (1) that nucleotide binding to the ABC domains decreases the UIC2 reactivity of Pgp in a concentration dependent manner in cells permeabilized by *Staphylococcus aureus* alpha toxin. Following the same strategy, we are studying how substrates, hydrolyzable and non-hydrolyzable nucleotides and phosphate analogues affect the formation of the above catalytic intermediates discriminated on the basis of UIC2 reactivity, in wild-type and in single or double Walker A mutants (K433M, K1076M, K433M/K1076M).

We have found that nucleotide binding itself is sufficient to switch Pgp from the inward facing (UIC2 binding) to the outward facing (UIC2-nonbinding) TMD conformation in both wild-type and single Walker A mutant Pgp variants. Similarly to wild-type Pgp, single Walker A mutants can also be trapped in the post-hydrolysis state in the presence of ATP and vanadate (V_i) or BeF_x, suggesting that these variants are capable of ATP hydrolysis. Formation of the BeF_x-trapped complex is facilitated by substrates both in the wild-type and the single Walker A mutant Pgp variants. In drug transport measurements we have found that single mutants also show cyclosporine A-sensitive drug efflux, but this activity is very weak compared to that of wild-type Pgp. Interestingly, cells expressing single or double Walker A mutant Pgp variants accumulate the Pgp substrate vinblastine-bodipy in their plasma membranes. Based on confocal microscopic images vinblastine-bodipy staining strongly co-localized with Pgp molecules and could be prevented by CsA or V_i treatment. The above data support a model in which Pgp molecules seesaw between the nucleotide-free inward facing conformation characterized by high drug affinity and the nucleotide-bound outward facing conformation(s) with low drug affinity. Binding of ATP is sufficient to switch Pgps from the inward facing to the outward facing conformation.

Pgps trapped in the post-hydrolysis state by V_i are still in the low drug affinity conformation, while release of nucleotides resets them to the high drug affinity conformation.

Reference
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O-045
SUBSTRATE BINDING AND TRANSPORT BY WALKER-A MUTANT PGPS

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P-glycoprotein (Pgp) is an ABC transporter that is able to extrude a large variety of chemotherapeutic drugs from cells, causing multidrug resistance of cancer cells. The protein consists of twelve transmembrane alpha-helices forming the substrate binding site, and two nucleotide binding domains (NBD) involved in ATP binding and hydrolysis. Based on crystal structures of several ABC transporters, Pgp is believed to alternate between an inward and an outward facing conformation, characterized by high and low substrate binding affinities, respectively.

Despite accumulating structural and functional data, it is still unknown how ATP binding and hydrolysis are connected to the conformational changes that allow transmembrane transport. To elucidate partial catalytic reactions, we studied Pgp variants carrying mutations in the conserved Walker A region (K433M and K1076M) of either the N-terminal or C-terminal ABC domains or both. Although mutation of these key residues have been shown to abolish ATPase and transport activity, we found that single mutants possessed a residual drug efflux activity (the double mutant variant was indeed inactive). Confocal microscopic image analysis showed that both the single and double mutant Pgp variants sequester vinblastine-bodipy in the plasma membrane, whereas wild-type Pgp can efficiently catalyze transmembrane transport. Fluorescence cross-correlation analysis proved that the sequestered vinblastine-bodipy strongly co-localize with the mutant Pgp molecules. Since the vinblastine-bodipy staining of the plasma membrane could be competed with Pgp substrates, these results suggest that mutations of the key Walker A lysines stabilize Pgp in the inward open, substrate binding conformation. Using transition state analogs, single mutants could be trapped in the outward open (low substrate affinity) conformation, suggesting that single mutations allow the transition between the two conformations. Detailed analysis of transport rates indicates that the two NBDs may not be functionally equivalent.

Taken together, we show that mutation of a single Walker A lysine is compatible with a residual transport activity. Analysis of the partial catalytic reactions suggests that ATP binding brings about the conformational change needed to switch Pgp from the inward facing to the outward facing conformation.

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O-046
AUTOPHAGOSOMAL SYNTAXIN17-DEPENDENT LYSOSOMAL DEGRADATION MAINTAINS NEURONAL HEALTH IN *DROSOPHILA*

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O-047
THE ROLE OF RAB11 IN THE MATURATION OF AUTOPHAGOSOMES AND ENDOSOMES AS A NEGATIVE REGULATOR OF HOOK

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Autophagy is a well-known bulk degradation process characterized by the formation of double membrane vesicles called autophagosomes. After the closure of the isolation membrane autophagosomes undergo fusion events with late endosomes and lysosomes, thus its content is degraded. The exact molecular mechanism of autophagosome maturation and fusion events remain unclear.

Rab11 has role in maturation of autophagosomes in cultured mammalian cells as it has been reported earlier. Our present study aimed to reveal the functions of Rab11 in autophagy in an in vivo system, in developing *Drosophila melanogaster*.

We found that Rab11 is required for maturation of autophagosomes since depletion of active Rab11 protein was resulted in accumulation of autophagosomes because of the autophagic flux was impaired. Surprisingly, we could detect the accumulation of enlarged late endosomes as well at the same experimental circumstances. Our further results indicated that Rab11 is required for amphisome formation and both endogenous and transgenic Rab11 localized to autophagosomes and amphisomes. An autophagy-induction dependent translocation of Rab11 form Rab4 positive recycling compartment to Atg8a positive autophagic structures was also observed.

Our co-immunprecipitation experiments revealed a molecular interaction between Rab11 and Hook both in cultured *Drosophila* S2 cells and in vivo. However, hk (Hook) mutant flies did not show the same phenotype as rab11 mutants. Autophagy was impaired in these mutants but we could not detect the accumulation of late endosomes.

Since Hook is a negative regulator of endosome maturation we asked how the interaction of Rab11 with Hook can be involved in the regulation of autophagy. We found that Rab11 colocalizes with Hook and the ratio of this colocalization increased upon autophagy induction. Like Rab11, Hook showed an autophagy-induction dependent colocalization with Atg8a, although Hook could not be found on autophagosomes. Our further results showed that induction of autophagy by amino acid starvation decreases the colocalization of Hook with the late endosomal marker Rab7, while Rab11-Rab7 colocalization was hardly affected. In addition to these, depletion of Rab11 caused accumulation of Hook on enlarged late endosomes. Our results suggest that upon autophagy induction Rab11 causes the relocalization of Hook from late endosomes to autophagic structures thereby allowing maturation of endosomes and fusion of autophagosomes with these matured late endosomes.

Taken together our data provide a mechanistic insight into the maturation processes of autophagosomes and endosomes.

O-048
Molecular cell pathology of prion disease: the role of endosome-lysosome system and selective autophagy

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To define the roles of endosome-lysosome system (ELS) and autophagy in prion disorders we analyzed the subcellular location of normal and pathological isoforms of prion protein (PrP) and their interactions with neurons and glial cells. We performed immunogold electron microscopy and double immunolabeling for endosomal (rab5, rab7, M6PR) and autophagy (LC3, p62) markers as well as for ubiquitin and mitochondrial proteins to monitor the extra- and intracellular fate of PrP, and to evaluate its cytopathogenic effects in different experimental cell and animal models of prion diseases and in human CJD cases.

The co-localization of the endosomal markers and the disease-associated PrP (PrP^d), and the accumulation of PrP^d in multivesicular bodies support our former findings that the ELS plays a pivotal role in the biogenesis, and the intracellular and cell-to-cell transportation of PrP^d, and in the cytopathogenesis caused prions. Ultrastructural and immunogold-EM examinations revealed intense PrP accumulation in partially ubiquitin and p62-positive aggresome-like structures (ALIS) and in autophagic vacuoles (AV) sequestering smaller ALIS in genetic CJD and its animal model.

Our observations of intracellular PrP accumulation support the notion that the permanent production of mutant PrP (mutPrP) undermines the collaborative protein-processing defence mechanisms. This shifts the balance towards to aggresome formation and selective proteinophagy,

which might be cytoprotective efforts to sequester mutPrPs. Our immune-EM observations on the significant accumulation of AVs sequestering damaged mitochondria in dystrophic neurites surrounding PrP-plaques in human CJD and its animal models confirm the cytoprotective role of mitophagy in prion diseases.

In conclusion, our findings support the notion that the same highly sophisticated and aligned network of intracellular stress response systems involving chaperones, ubiquitin-proteasome system and selective macroautophagy may also exist in prion diseases as well as in the other comformational neurodegenerative disorders like Alzheimer's and Parkinson's.

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O-049
REGULATION OF LIFE AND DEATH DECISIONS BY POLY(ADP-RIBOSYL)ATION

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Poly(ADP-ribosyl)ation (PARylation) is a reversible protein modification carried out by poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) enzymes. PARPs cleave NAD to nicotinamide and ADP-ribose and attach the latter to proteins followed by the addition of further ADP-ribose units resulting in a branched polymer. PARG and ARH3 (ADP-ribosyl hydrolase 3) are responsible for the rapid degradation of PAR. Reversible PARylation regulates various cellular processes including chromatin organization, replication, transcription, proliferation and DNA repair. It also plays a dual role in cell death regulation: it contributes to cell survival by assisting repair of mild DNA damage but in severe genotoxicity it mediates a necroptotic cell death. Here we wish to overview recent developments in the field of PARylation-related cell death and survival. The focus will be on the different PARylation-mediated cell death pathways, the role of PARG, SIRTs and mitochondria.

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O-050
CHARACTERIZATION OF INFLAMMATORY REACTIONS DURING HUMAN ADIPOCYTE & MACROPHAGE CO-INCUBATION

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Nowadays obesity is an epidemic health problem worldwide, enhancing the risk for metabolic disorders such as type 2 diabetes, nonalcoholic fatty liver disease, metabolic X-syndrome and cardiovascular diseases. Hypertrophic adipose tissue is associated with a rise of free fatty acids (FFA), adipokines and proinflammatory molecules (IL6, TNFalpha, MCP-1). Weight gain correlates with adipocyte size expansion and an increased number of dying adipocytes. The elevated levels of FFA and proinflammatory cytokines attract monocytes into the hypertrophic adipose tissue. These recruited monocytes differentiate to activated macrophages, which are situated around dead adipocyts, in a "crown like" structure, release more pro-inflammatory cytokines, which cause an inflammatory vicious cycle in white adipose tissue.

The types of adipocyte cell death and their connection with macrophages is not completely characterized yet. We have created a human *in vitro* model for adipocyte cell death and a phagocytosis assay involving human adipocytes and macrophages. As an adipocyte source, we use SGBS human preadipocyte cell line and precursor cells isolated from human subcutaneous adipose tissue differentiated *in vitro* into adipocytes. The macrophages are derived from primary human monocytes. We have characterized the morphological changes and cell death types of adipocytes during differentiation. The lipid content of differentiating adipocytes, and their cell death profile has been measured on a time curve by laser scanning cytometry. We studied the cytokine profile during phagocytosis of adipocyte corpses by macrophages. The ideal time point for studying interaction of adipocytes with macrophages and the resulting pro-inflammatory effect has been determined.

We detected lipid accumulation, shrinkage of nuclei and an increasing level of spontaneous adipocyte cell death during the progress of adipogenic differentiation. Differentiating adipocytes became sensitive to apoptotic stimuli, such as TNFα + cycloheximide (CHX), and hypoxia treatment. Dying adipocytes show apoptotic feature due to Annexin V positivity and their anti-inflammatory effect. Significant phagocytosis of adipocytes, dying spontaneously or due TNFα+CHX treatment could be detected. Co-incubation of differentiated adipocytes and macrophages leads to IL6 production which is a phagocytosis dependent phenomenon. These data may lead to better understanding of the complex regulatory processes which take place between differentiating/dying adipocytes and macrophages.

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O-051
COMBINED GENETIC, MOLECULAR AND CELL BIOLOGY APPROACHES TO REVEAL FUNCTIONS OF THE MATERNAL A-TUBULIN ISOFORM IN *DROSOPHILA*

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To identify factors essential for the commencement of embryogenesis in *Drosophila,* we induced, isolated and characterized a set of dominant female-sterile mutations (*Fs*). Some of them allow the formation of seemingly normal eggs that are fertilized, however embryogenesis does not commence inside. We assumed that the *Fs*-identified normal gene is engaged in the initiation of embryogenesis. In principle, by making use of the *Fs* mutations as "tools", we may have an access to the normal genes and elucidate their molecular functions. We first mapped the studied *Fs* mutation and then determined its nature. Once it turned out to be of dominant negative type (i.e. the *Fs*-encoded gene product hinders function of the normal one), we subjected the *Fs* allele to second mutagenesis and generated loss-of-function, recessive alleles of the gene. The recessive alleles allow the determination of the loss-of-function phenotype and thus a deduction of the presumptive normal gene function. Should the recessive alleles be the results of transposon mutagenesis, the gene can be cloned (through e.g. the inverse PCR technique), its site and molecular function can be elucidated.

It has turned out that the $Kavar^{18c}$ and the $Kavar^{21g}$ *Fs* mutations of *Drosophila* identify the *αTub67C* gene. Product of this gene, α4-tubulin, is the so-called maternal α-tubulin isoform. The α4-tubulin molecules form and become deposited into the egg cytoplasm during oogenesis. They help life of the future zygote during the initial steps of its life when the zygotic genes are not yet expressed.

$Kavar^{18c}$ originated through the G^{224}→A transition that lead to a Glu^{82}→Lys (E82K) replacement. Glu^{82} plays key role in binding GTP, a structural component of the tubulins. Only very short microtubules form in presence of the $Kavar^{18c}$-encoded E82K-α4-tubulin and thus embryogenesis cannot commence.

$Kavar^{21g}$ originated through the G^{1276}→A transition that lead to a Glu^{426}→Lys (E426K) replacement. Analysis of the $Kavar^{21g}$-related defects revealed that the Glu^{426} sites (Glu^{415} in the canonic α-tubulins) are those points where the ADP-bound kinesins clasp (with their Arg^{346} and Arg^{350}) the microtubules. A the kinesins drop off the microtubules at the E426K-α4-tubulin sites, the transport speed decreases and leads to death of the embryos. The ATP-bound kinesins bound to the same Glu^{426} (through their Ser^{343} residue). Starting from E426K-α4-tubulin we revealed key features of the kinesin-based transport processes along the microtubules.

There are no α4-tubulin molecules in eggs of the females homozygous for $kavar^{null}$, a complete loss-of-function *αTub67C* mutant allele. Comparison of such and normal eggs/embryos showed that α4-tubulin is essential for (i) fast growth of the microtubules and (ii) attachment of the so-called interpolar microtubules to the nuclear envelope. These microtubules push to the opposite poles - along the nuclear envelope - the daughter centrosomes where they organize the spindle apparatus. In the absence of α4-tubulin the formation of the microtubules is so slow that it cannot keep up

with the Cyclin/Cdc-controlled cyclic events and thus the centrosomes fail to separate, spindles do not form, the embryos die and the females are sterile.

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O-052
MEMBRANES ALONG THE LONGEST SPERM

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The existence of similar male sterile phenotype in flies, mice and human strongly suggests that many of the genes required during spermatogenesis have been evolutionary conserved. In *Drosophila*, the adult testis is a single tube in which the developmental stages are laid out in chronological order from the stem cells at the tip to mature spermatozoa at the base, with particular stages occurring at specific regions along its length. It is a steady state system with primary gonial cells being constantly generated from stem cells at the tip and 64 mature sperms per primary gonial cell being transferred into the seminal vesicles at the base.

Our aim is to identify new membrane transport related genes involved in the early and late stages of spermatogenesis and describe the precise molecular function of these genes.

To achieve this goal we tested publicly available transposon insertion collections for male sterility. Many membrane reorganizing processes happen in the fly testis during spermatogenesis. We started the genetic characterization of mutant lines in which membrane transport related genes are affected. One of them is the phosphatidate cytidyltransferase, CdsA enzyme. It catalyzes the synthesis of CDP-DAG, which is involved in phosphoinositol (PI) and cardiolipin biosynthesis. Phosphorylated forms of PI play important roles in lipid signaling and membrane trafficking, while cardiolipin is an important component of the inner mitochondrial membrane. The elongated cyst contains 64 synchronized spermatids. Spermatid individualization is especially interesting cellular process because it requires an unusual amount of membrane remodelling using a well-defined actin structure. Drosophila spermatids increase 150-fold in length and the total surface area following individualization is ~5 fold greater than in early round spermatids. We found that the sperm's mitochondria and axonemes are elongated and investment cones do form in the mutant testis, but individualisation does not occur in the cysts. Mutant cysts have unsynchronized actin cones and abnormal mitochondria, which can be responsible for the phenotype. Both mitochondria and the axial membrane show abnormalities in $CdsA^{ms}$ mutants. Mitochondria are not synchronised in the cyst and mitochondria become extremely large. Later stage cysts show signs of apoptosis in $CdsA^{ms}$ mutants. The sperm cell has a characteristic polarized morphology and its surface is also highly differentiated into different membrane domains. To understand better the lipid composition of the developing *Drosophila* sperm and the function of CdsA during

individualization, we extracted lipids and analyse the lipid composition of wild type and lipid metabolic mutant *CdsA*^{ms} using mass spectrometry.

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O-053
HEAT SHOCK FACTOR-1 INTERTWINES INSULIN/IGF-1, TGF-B AND CGMP SIGNALING TO CONTROL DEVELOPMENT AND AGING

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Temperature affects virtually all cellular processes. A quick increase in temperature challenges the cells to undergo a heat shock response to maintain cellular homeostasis. Heat shock factor-1 (HSF-1) functions as a major player in this response as it activates the transcription of genes coding for molecular chaperones (also called heat shock proteins) that maintain structural integrity of proteins. However, the mechanisms by which HSF-1 adjusts fundamental cellular processes such as growth, proliferation, differentiation and aging to the ambient temperature remain largely unknown.

Most target genes of HSF-1 identified so far encode heat shock proteins. However, it has been shown that besides activating expression of molecular chaperone genes, HSF-1 also regulates the transcription of genes involved in cell differentiation and development. Our aim was to determine new direct target genes of HSF-1 in *Caenorhabditis elegans in silico* and we planned to prove that HSF-1 directly regulates the transcription of a given target gene using standard methods of molecular biology and genetics.

In *C. elegans*, the DAF-7/TGF-β (transforming growth factor-beta), insulin/IGF-1 (insulin-like growth factor-1) and DAF-11/GC (cyclic guanosine monophosphate) signaling regulate aging, stress response and dauer larva formation. Dauer larva is a highly stress-resistant, long-lived larval form induced by unfavorable environmental conditions. These three distinct signaling systems regulate the synthesis of the steroid hormone dafachronic acid (DA). DA promotes reproductive growth and inhibits dauer larva development. A key step of DA synthesis is catalyzed by DAF-9/CYP450 (cytochrome P450).

We identified in the *C. elegans* genome putative HSF-1 binding sites *in silico*. We found that one of these sites is located in the 5′ regulatory region of *daf-7* encoding a TGF-β ligand, and also in the *cis*-regulatory sequence of *daf-9* which encodes a cytochrome P450 protein. Thus, *daf-7* and *daf-9* are potential direct transcriptional targets of HSF-1.

We showed that in *C. elegans* HSF-1 represses the expression of *daf-7* to induce young larvae to enter the dauer stage. Under favorable conditions HSF-1 is inhibited by crowding pheromone-sensitive guanylate cyclase/cGMP and systemic nutrient-sensing insulin/IGF-1 signaling. Loss of HSF-1 activity allows DAF-7 to promote reproductive growth. Thus, HSF-1 interconnects the insulin/IGF-1, TGF-β and cGMP signaling pathways to control development and longevity in

response to diverse environmental stimuli. Furthermore, HSF-1 upregulates *daf-9*/cytochrome P450 thereby promoting reproductive growth.

HSF-1 plays a dual role in the regulation of *C. elegans* larval development. First it promotes dauer larva formation by repressing *daf-7*, and second, inhibits dauer development via activation of *daf-9*. The overall impact of HSF-1 is to enhance the ability of *C. elegans* populations to survive (dauer larvae) and reproduce (reproductive adults) simultaneously under harsh environmental conditions.

Together, these results provide mechanistic insight into how temperature, nutrient availability and population density coordinately influence development, behavior, stress response and lifespan through HSF-1.

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O-054
NOVEL FUNCTIONS FOR A CYTOSKELETAL ACTIN BINDING PROTEIN

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The most dynamic component of the cytoskeleton in every eukaryotic cell is the microfilament network of linear polymers of actin subunits. Extensive research in the past decade has significantly broadened our view about the role actin plays in the life of the cell and added at least two novel aspects to actin research. One of these is the discovery of the existence of nuclear actin which became evident only recently. Another new and interesting perspective in actin research is the investigation of the role actin plays in mitotic chromosome segregation: despite long-held skepticism recent works seem to have revealed the existence of the actin-based, so called spindle matrix.

Members of the actin binding Ezrin-Radixin-Moesin (ERM) protein family of vertebrates are major regulators of actin dynamics in the cytoplasm. In *Drosophila melanogaster*, Moesin is the sole representative of the ERM protein family. To the present view Moesin, similarly to its vertebrate counterparts, is responsible for the crosslinking of membrane proteins to the cortical actin network. Surprisingly, we found both in cultured cells and Drosophila embryos that beyond its obvious cytoplasmic localization Moesin is present in the interphase nucleus and co-localizes with the mitotic spindle. In the nucleus Moesin accumulates at the nuclear envelope and it can be detected both in the nucleoplasm and on the chromosomes where it binds actin in a very dynamic manner. On the chromosomes Moesin localizes to the euchromatic regions and the analysis of polytene chromosomes strongly suggest that Moesin plays role in transcription. Our results also revealed that Moesin transportation to the nucleus is an active process, however the predicted single nuclear localization signal is not necessary for the nuclear localization of the protein.

During prophase Moesin level rapidly increases in the nucleus and the protein co-localizes with the actin networksurrounding the mitotic spindles throughout mitosis. Based on the findings that

the mitotic localization of Moesin meets all the distinguishing requirements for spindle matrix components and that it exhibits genetic interaction with them, we conclude that Moesin is a new member of the spindle matrix. This idea is supported further by our observations that during mitosis F-actin together with the microtubules is responsible for the compaction and alignment of the mitotic chromosomes and that it is required for the formation of the microtubule spindles as well as chromosome segregation.

This work was supported by TÁMOP-4.2.2/B-10/1-2010-0012

O-055
THE FORMIN DAAM FUNCTIONS AS THE MOLECULAR EFFECTOR OF THE PLANAR CELL POLARITY PATHWAY DURING AXONAL DEVELOPMENT IN THE *DROSOPHILA* BRAIN

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Planar cell polarity (PCP) signaling is renowned for its ability to coordinate cellular polarization within the plane of a tissue, often resulting in the formation of polarized cellular architectures. Recent studies established that the PCP pathway is also important for various aspects of nervous system development and function. Although many pieces of evidence suggest that the main effect of PCP signaling is to regulate actin dynamics, it remained elusive how PCP proteins are linked to directed cytoskeletal rearrangements, particularly within the developing brain. Recently, we investigated the connection between the *Drosophila* formin *DAAM*, that has previously been shown to be required for embryonic axonal morphogenesis, and the core PCP genes during adult brain development. We found that *dDAAM* plays a pivotal role during axonal growth and guidance of the mushroom body (MB) neurons. We used a combination of genetic interaction and biochemical assays to demonstrate that the core PCP proteins act in concert with dDAAM during correct targeting of the MB axons. We found that dDAAM acts downstream of Dishevelled, and the actin assembly activity of dDAAM is essential for axonal growth regulation, suggesting that dDAAM acts as a molecular effector by linking the PCP system to direct changes in the growth cone actin cytoskeleton. In addition, it appears that the axonal guidance function of the core PCP/DAAM regulatory module is likely to be conserved during evolution, whereas the tissue polarity function is restricted to vertebrates.

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O-056
SINGLE-STRAND DISCONTINUITIES IN THE GENOME OF LOWER AND HIGHER EUKARYOTES

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Live, nonapoptotic eukaryotic cells, from yeast to human, disassemble into ~ loop-size ds fragments upon protein denaturing treatments. Applying comet assay run in field inversion mode we could analyze DNA fragmentation also at the individual cell level, having concluded that ds fragmentation is related to endogeneous ss breaks and that these nicks may be juxtaposed with RNA/DNA hybrids (Székvölgyi et al., PNAS 104(38):14964, 2007). These structures have been identified as R-loops, sensitive to RNA polymerase inhibitors. We have developed a method to label with biotinylated nucleotides the immediate vicinity of nicks, and a reverse South-Western (rSW) blot procedure to detect incorporated labeled nucleotides, and R-loops, separately in the complementary strands (Hegedüs et al., NAR, 37(17):e112, 2009), using anti-biotin and anti-RNA/DNA hybrid antibodies, respectively. Making use of these procedures, we mapped endogeneous nicks and R-loops in HCHO-fixed S. cerevisiae, by ChIP-chip on tiling arrays, and by rSW within the repetitive rDNA locus. These studies confirmed the co-localization of these two structures, in line with our complementary results on mammalian cells. Having developed a laser scanning cytometry (LSC) based method to measure superhelicity and size of the DNA loops present in the „halos" of salt-extracted mammalian cell nuclei, we could show that the nuclear domain harbouring nicks and the other one exhibiting superhelicity are topologically separated, in G1, S as well as in G2 phases of the cell cycle.

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O-057
THE HEAT SHOCK FACTOR A4A REGULATES OXIDATIVE STRESS TOLERANCE IN ARABIDOPSIS

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Reactive oxygen species (ROS) accumulating in response to biotic and abiotic stress represent important signalling molecules in the coordination of stress-acclimation pathways with metabolism and development. Heat-shock factors (HSFs) are principal regulators of plant responses to heat and oxidative stress. Here we show that estradiol-inducible overexpression of

HSFA4A in Arabidopsis confers high level of salt tolerance, as well as reduced sensitivity to heavy metals, anoxia and oxidative agents. Consistently, the *hsfa4a* T-DNA insertion mutant is hypersensitive to salt stress. HSFA4A overexpression decreases, whereas the *hsfa4a* mutation elevates hydrogen peroxide accumulation and lipid peroxidation. Genome-wide transcript profiling indicates that HSFA4A co-ordinately regulates the expression of a large set of genes responding to oxidative stress. HSFA4A shows dimerization in yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays, which is reduced when conserved Cys are replaced by Ala residues. HSFA4A is phosphorylated by the mitogen-activated protein kinases MPK3 and MPK6 that interact with HSFA4A in yeast and plant cells. Our results suggests that HSFA4A acts as a downstream regulator in MPK3/6-dependent stress response pathways.

O-058
TRANSGLUTAMINASE 2 (TGM2) MODULATES THE ENERGY METABOLISM OF DIFFERENTIATING MYELOID CELLS THROUGH REPRESSION OF KEY REGULATORS OF MITOCHONDRIAL BIOGENESIS

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TGM2 is a member of the transglutaminase enzyme family that covalently cross-link proteins in a Ca^{2+}- dependent manner. Besides this activity, it could act as a protein disulphide isomerase or protein kinase. At physiological Ca^{2+} level, by the binding of GTP to the enzyme, it loses transamidating activity and becomes a G protein. TGM2 could be localized to various compartments of cells, it could be cytosolic, nuclear, mitochondrial, or could be found in cell membrane, even in the extracellular matrix. It is involved in the terminal differentiation of immune cells and increased TGM2 activity is a common feature of several inflammatory diseases.

The NB4 cell line is an acute promyelocytic leukemia cell line which could be differentiated into *neutrophil granulocytes with all-trans retinoic acid (ATRA) treatment. We have previously published that the expression of TGM2 was highly increased during differentiation process of NB4 cells, and using shRNA mediated knock down of TGM2 expression in NB4 cells we could confirm that TGM2 can enhance the differentiation process, migration, adherence, phagocytotic ability and CCL chemokine production in NB4 cells (*Z Balajthy, et al., Blood, 2006 108, 6., K Csomos, et al., Blood. *2010 116).*

The aim of this work is to explore the effect of TGM2 expression on the energy metabolism of differentiating myeloid cells. Our previous results indicated that expression of six proteins, namely glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, lactate dehydrogenase A and B, enolase A and phosphoglycerate kinase *were modulated by TGM2 as they were expressed at higher levels in NB4 TGM2-KD cells. To validate these data, real time measurement of both glycol*ytic (as an extracellular acidification rate) and mitochondrial (as oxygen consumption) activity was carried out which revealed a significantly higher metabolic activities in NB4 TGM2-KD and TGM2 KO mouse bone marrow derived neutrophil granulocytes. Treating the cells with a mitochondrial oxidative chain

uncoupling agent called carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone (FCCP), NB4 TGM2-KD and TGM2 KO neutrophils showed higher respiratory capacity. Since RT-qPCR measurements of glycolytic enzymes did not reveal significant differences in mRNA levels, changes in protein levels are probably due to posttranslational modifications. The expression of Sirtuin 1 (SIRT1) and its downstream targets, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and β, which are key regulators of mitochondrial biogenesis, were significantly upregulated both at the mRNA and protein levels in NB4 TGM-KD cells. Similarly, mRNA levels of PGC1α, β and SIRT1 in mouse bone marrow derived neutrophil granulocytes were also significantly up-regulated. In addition, several components of mitochondrial oxidative phosphorylation chain were also induced both in knock-down and KO cells.

Based on previous publications, our working hypothesis is that during NB4 differentiation the increasing amount of TGM2 modifies histones in the promoter region of SIRT1 or PGC1α, controlling the expression of components in oxidative phosphorylation and energy metabolism. To validate this hypothesis chromatin immunoprecipitation experiments are in progress to confirm the presence of TGM2 in the promoter region of SIRT1 and PGC1α.

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O-059
GENOMIC ANALYSIS OF HNF4A BINDING AND THE INFLUENCE OF ERK1/2 SIGNALING PATHWAY: INSIGHTS ON HUMAN *ABCC6* GENE TRANSCRIPTIONAL REGULATION

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Pseudoxanthoma elasticum (PXE), a rare recessive genetic disease characterized by the calcification of elastic fibers, is due to loss-of-function mutations of the human *ABCC6* gene. The expression pattern of *ABCC6* is tissue specific: it is mainly present in the liver and at a lower extent in the kidneys and the intestines, while in other tissues *ABCC6* expression is barely detectable. Until now little is known about the physiological regulation of the gene, although our previous data demonstrated that the activation of the ERK1/2 cascade in hepatocytes downmodulates the expression of *ABCC6*. The aim of the present study was to study the epigenetic modifications and transcription factors associated with the *ABCC6* promoter region, in order to identify major regulators of its expression as well as factors involved in its tissue specificity. We used the chromatin immunoprecipitation technique (ChIP), which consists of *in vivo* crosslinking of the chromatin, followed by precipitation with antibodies specifically directed against the proteins of interest, DNA extraction and detection of genomic regions using quantitative real time PCR or genome sequencing (ChIP-seq). Using HepG2 cells as a model system expressing *ABCC6*, we were able to detect the enrichment of Hepatocyte nuclear factor 4a (HNF4a) and CCAAT/Enhancer binding protein alpha and beta (C/EBPa and C/EBPb), in two different regions of the *ABCC6* promoter, which have been previously described as activators of the expression of the gene. We chose HNF4a as a key target of the ERK1/2 pathway, and subsequently performed ChIP-seq and

whole genomic analysis of the HNF4a binding sites. Treatment of the cells with epidermal growth factor (an ERK1/2 activator) caused the loss of enrichment of HNF4α in these regions. This effect is seen even after very short treatments (30 min), suggesting that the regulation may be established by direct phosphorylation of HNF4α. In vitro phosphorylation assay showed that ERK1 indeed phosphorylates HNF4α. This effect is also reflected in the chromatin state of the *ABCC6* promoter, where histone acetylation is quickly lost upon EGF treatment. As both of HNF4a and C/EBPb are playing a major role in the regulation of hepatic metabolic equilibrium we suggest that ABCC6 might have a unique feature among the other ABC transporters as an indicator of the metabolic state of hepatocytes.

O-060
CELL LINE DEVELOPMENT USING GENOME ENGINEERING TECHNOLOGIES: REPORTERS, EFFECTORS, PRODUCERS

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Recent specific genome editing technologies allow us to precisely position deletions, modifications, and transgenes in the genomes of living cells. As a result, stable cell lines can be generated with controlled characteristics and predictable transgene functioning for the purpose of e.g. fundamental research, bioassays, and recombinant protein production.

Using various sequence-specific meganucleases and TAL effector nucleases engineered to recognise integrated target sequences or natural genomic loci of mammalian cells (Jurkat, HEK, YT, THP-1, CHO etc.), we have successfully generated various stable monoclonal cell lines expressing membrane-bound antigens, cell surface receptors, or monoclonal antibodies, to operate as target cells or effector cells in bioassays, or potential biopharmaceutical protein producers.

The concept of sequence-specific genome editing is based on the fact that genomes are ordered and controlled mostly by sequence-specific DNA binding proteins, transcription factors. Recently, thanks to the accumulating information on DNA sequence recognition by such proteins, the principles of those interactions have been increasingly exploited to engineer tools for targeted genome modifications. Firstly, a nuclease is engineered to cleave the desired target sequence in the genome, creating a double-strand break (DSB). Secondly, the cellular mechanisms of DNA DSB repair, which are (i) non-homologous end joining (NHEJ) or (ii) homologous recombination (HR), operating in the absence and presense of a template DNA, respectively, are exploited to introduce the desired modifications at the chosen genomic location. To date, engineered nucleases, which were successfully used in specific genome manipulations are restricted to Meganucleases at some extent, but mostly to Zinc-Finger Nucleases (ZFNs), and recently, to TAL-like effector nucleases (TALENs).

This cutting-edge technology, which is fully operational in both cell lines and primary cells, including oocytes, has made us able to rationally disrupt target genes at single nucleotide resolution (sure knock-outs) *via* the introduction of small insertions or deletions at the endonuclease TALEN) cleavage site in a cell line. Also, we were able to insert the gene of a recombinant cell surface antigen into precisely-defined location for efficient expression without harmful efects on normal cell functioning in a cell line of choice. In addition, we specifically integrated therapeutic antibody genes into a highly expressive and well defined genomic locus of CHO cells to achieve efficient protein production.Specific genome editing technologies are still in the initial evolutionary phase (it is true especially for TAL effector nucleases and some upcoming new approaches), that means, besides the substantial optimisation work one need to carry out, one also has to innovate a lot in the adaptation of the commercially available systems (e.g. Cellectis Bioresearch or currently the System Biology Division of Life Technologies) to the specific applications.

Therefore, and as a result, while going along our genome editing routes, we were forced to implement and developed new technologies (i) to assess genome modifications at early stages of TALEN transfections, based on specific amlification and capture of modified sequences for more accurate quantification and sequencing, (ii) generally applicable strategies and tools for the detection of, and direct enrichment for assisted KO cells using e.g. flow cytometry, and (iii) new approaches to map TALEN specificity *in vivo* in automated and HTS assays. Most promisingly, we are keen (iv) to device and test some new/proprietary genome editing approaches, which are completely divergent from the current state of the art.

O-061
APPLICATION OF TISSUE SPECIFIC GENE EXPRESSION TO IMPROVE FUNGUS RESISTANCE IN WHEAT

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Rusts (*Puccinia spp.*) are considered one of the most important fungal diseases affecting wheat (*Triticum aestivum* L.). Leaf rust (*Puccinia recondita* f. sp. *tritici*) and stem rust (*Puccinia graminis* f. sp. *tritici*) cause very severe loss of quality and quantity in cultivated wheat in Hungary. Wheat is one of the major agricultural crops in Hungary as well as in many countries all over the world with high economical and scientific interest due to conventional and molecular wheat breeding. The conventional breeding methods were not able to achieve a breakthrough in providing considerably tolerant genotypes. Thus it is necessary to take new approaches to develop wheat resistant against rust diseases and to combine genetic transformation with classical plant breeding. The basic objective is stabilization of yield quality and yield safety by the increased resistance against rust fungus diseases by the use of the available tools and processes of biotechnology.

Plants attacked by fungi produce PR (pathogenesis related) proteins as chitinases, glucanases. Nevertheless in most of the cases the induced self-defence mechanisms do not provide enough protection since they are either not effective or they are activated too late through a multitude of different metabolic pathways. Several pathotypes of rust fungus play a role in the rust infection, therefore it is almost impossible to achieve a so-called 'general resistance' in the majority of the cases.

Our aim was to transfer the *cmg1* gene, coding for a 83.2 kDa beta-1,3-exoglucanase enzyme of endoparasite fungus *Coniothirium minitans* into wheat through direct genetransfer.

Due to the constitutive gene regulation, the developed resistant plants produce the protein with hydrolase activity in all parts of the plants. To overcome this, we applied a tissue-specific way for the regulation of the transgene, focusing only on the green tissue-specific regulation, so that the recombinant protein is present only in the green tissues of the transgenic plants. In this way the beta-1,3-exoglucanase cannot be detected in the starchy endosperm of the wheat kernel.

To achieve this goal the *cmg1* gene had to be inserted into a gene construction under the regulation of the wheat's own ribulose-1,5-bisphosphate carboxilate-oxigenase (rubisco) gene's promoter.

In addition we developed a modified biotest system, based on the standard method, in order to test the biological efficiency of the modified wheat's 'general resistance'. After the PCR, RT-PCR and Western Blot analysis of the transgenic plants, they were subjected to the modified biotest, in which all the pathotypes of leaf rust available in Hungary were used for provoked infection. The implementation of the biotest revealed that 12 of the GM wheat lines showed considerably high-level resistance against *P. recondite* under greenhouse conditions.

O-062
INACTIVATION OF PROTEIN KINASE D ACTIVITY ALTERS DENDRITIC SPINE MORPHOLOGY, MOTILITY AND HIPPOCAMPAL FUNCTIONS

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The protein kinase D (PKD) family has been recently described as a separate family among serine/threonine protein kinases. PKD is highly expressed in CNS neurons and is known to regulate dendritic arborisation and the intracellular transport of transmembrane receptors.

Dendritic spines are actin-rich protrusions that comprise the postsynaptic sites of synapses and receive the majority of excitatory synaptic inputs in the CNS. Dendritic spines change significantly in shape, volume and number within short time courses, depending on synaptic activity. Memory formation and consolidation, e.g., is normally linked to increased synaptic efficacy, which is accompanied by enlarged dendritic spine heads and stabilised synaptic connections. The morphology and motility of dendritic spines depend greatly on actin elongation and branching.

PKD is known to influence actin polymerization and cell motility in non-neuronal cells via regulating cofilin or cortactin activity. So far its functions in dendritic spines and synaptic efficancy, however,have not been elucidated.

In the present work, we have investigated the consequences of impaired PKD functions on the dendritic spines of embryonal hippocampal neurons and in pyramidal neurons of adult transgenic mice. PKD inactivation was achieved by introducing a point mutation in the kinase domain leading to a dominant negative kinase inactive mutant form of PKD (kdPKD). Fluorescently tagged kdPKD (kdPKD-EGFP) was transfected into cultivated hippocampal neurons while for in vivo studies,kdPKD-EGFP expression was induced by 8 weeks of doxycycline treatment of CaMKrtTAxkdPKD-EGFP mice, leading to the expression of EGFP-tagged mutant PKD in an inducible, forebrain-specific manner.

Quantitative fluorescent microscopy was used to analyse the distribution and morphology of the dendritic spines of the transfected pyramidal neurons. Motility of the dendritic spines was followed by fluorescent live cell imaging and wasanalysed by a custom-written semi-automated software. Electron microscopy (EM) analyses were carried out in the CA1 and CA3 region of the hippocampus, after visualizing kdPKD-EGFP expressing neurons by anti-GFP immunohistochemistry.The effects of mossy fiber stimulation on LTP formation were measured in the CA3 and CA1 region of hippocampal slices. Behavioural analyses of transgenic animals were also fulfilled.

Our confocal and EM data prove that the inactivation of endogenous PKD functions leads to a reduced number of mushroom-shaped, more mature spines and a decrease in average spine profile area while increases the ratio of filamentous spines which have weaker synaptic connections. Accordingly, filamentous spines expressing kdPKDwere more motilein live cell imaging analyses. LTP formation was reduced in hippocampal slices and spatial memory formation was impaired inkdPKD expressing transgenic mice. Thus, inactivation of protein kinase D functions affects the formation and/or maintenance of dendritic spines, influencing the strength of synaptic connections and synaptic efficacy.

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O-063

THE INTERNALIZATION ROUTE OF TRANSFORMING GROWTH FACTOR B RECEPTOR AND A POSSIBLE NON-GENOMIC ROLE OF ESTROGEN-RECEPTOR A IN THE SIGNALING DURING TYPE II EPITHELIAL-MESENCHYMAL TRANSITION

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Transforming growth factor β (TGF-β) superfamily consists of numerous cytokins that regulate various cellular processes. TGF-β, the prototype of the family, signals through its cell surface serine/threonin kinase receptors (tpye I and type II) and it is well known to be able to induce

epithelial-mesenchymal (EMT) transition via both Smad- pathway and MAPK- pathway. Type II EMT is described to be associated with wound healing, tissue regeneration, organ fibrosis and is induced upon inflammatory stimuli. It can be triggered by secretion of growth factors such as TGF-β, EGF.

Different endocytic routes are used for the internalization of TGF-β ligand and its receptors and these pathways can control the activity of downstream events. Internalization via clathrin-coated vesicles promotes the signaling while the caveola-mediated endocytosis supposingly plays important role in the termination of the events. There are data suggesting that the early endosome does not only maintain a special environment for the effective signaling but can direct the internalized cargos towards degradative pathways (multivesicular bodies, lysosomes). Thus, this compartment serves as a signaling center where the further fate of proteins is decided and they are sorted.

There are data that indicate the role of estrogen-receptor (ER) α as a negative regulator of TGF-β pathway by increasing the degradation of nuclear Smad proteins. Although the role of ER α localized in the plasma membrane is still less clear but might modulate the activity of the signaling via MAPK pathway.

Aims: We examine type II EMT in rat mesenteric mesothelial cells upon inflammatory stimuli induced by Freund's adjuvant treatment. Our aim is to reflect the role of different cytoplasmic compartments in TGF-β signaling. We follow the internalization route of type II TGF-β receptor and examine whether it is localized in early endosome marker (EEA1) positive or caveolin-1 marker positive compartments and how the localization of TβRII changes at the different times of the inflammatory events *in vivo*.

Our aim also is to prove the possible role and non-genomic action of ERα in mesothelial cells upon inflammation from a morphological point of view.

Results: The Western-blot analysis shows that TGF-β is secreted into the peritoneal fluid upon inflammation indicating its role in EMT. The results of immunocytochemistry and also our electron microscopical records show that after internalization TβRII is localized first in EEA1 positive compartments and after the culmination of inflammation it appears to occur in both caveolin-1 and EEA1 positive compartments showing that indeed early endosome plays role in the degradative events as well. EEA1 and caveolin-1 markers also co-localize at the peak time of inflammation and afterwards.

The ER-α is proved to be presented mainly in the apical plasma membrane of mesothelial cells and it is found to co-localize with caveolin-1 marker indicating that ER α is localized in the lipid rafts when it is in the plasma membrane and it might activate MAPK pathways and downstream events by interacting with Src-418.

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O-064
THERMAL STRESS MANAGEMENT IN FISSION YEAST: NEED FOR FAT

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Using different prokaryotic models, we have earlier demonstrated that cellular membranes are not only the passive endurers of the heat shock, but might function as thermosensors, as well. Our "membrane as thermosensor" theory relies mainly on results showing that the expression of molecular chaperones can be induced under isothermal conditions by disturbing the physical order of membranes. Furthermore, the chaperones (especially the small heat shock proteins, sHSPs) are able to bind and stabilize membranes either *in vitro* or *in vivo*. To extend our hypothesis,we have initiated similar research using higher eukaryotic organisms. The fission yeast (*Schizosaccharomyces pombe*) is relatively simple, well-characterized creature, the nickname "micromammal" well describes the suitability as a model.

S. pombe contains two sHSPs, both are iduced upon heat shock. The purified proteins are able to associate to yeast lipids with different affinity. Both HSP null mutant cells show thermosensitive phenotype similar to the trehalose-deficient strain. The temperature threshold of both sHSP heat induction is also influenced by cellular trehalose level .The three mutant strains show altered lipid composition under all temperatures tested, indicating the tight co-operation of the lipids, trehalose and sHSPs in this organism. Surprisingly, the level of fatty acid desaturation of triacylglycerol (TAG) shows opposite tendency compared to that of the membrane-forming lipids. TAG is the main component of the lipid droplets, which where previously regarded as simple fat storage. Emerging number of experiments indicate that this should not be the case, therfore nowadays cellular functions of lipid droplets are under revision. Our data strongly support this notion, suggesting that besides the known thermoprotectants, lipid droplets might also play a crucial role in thermal stress management.

O-065
TRACKING ABCB6 IN THE CELL: FROM THE MITOCHONDRIA THROUGH THE PLASMA MEMBRANE TO THE ENDOLYSOSOMAL SYSTEM

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Determination of the correct subcellular localization represents an important basis toward characterization of ATP-binding cassette (ABC) transporters and resolution of their roles in cellular physiology. ABCB6, a member of the adenosine triphosphate–binding cassette (ABC) transporter family, has been proposed to be responsible for the mitochondrial uptake of porphyrins. In contrast, we have shown that ABCB6 is a glycoprotein present in the membrane of mature

erythrocytes and in exosomes released from reticulocytes during the final steps of erythroid maturation. We confirm the mitochondrial localization of ABCB7, ABCB8 and ABCB10, suggesting that only three ABC transporters should be classified as mitochondrial proteins. Using confocal microscopy, we followed ABCB6 from the ER to the Golgi and the plasma membrane. Modulation of different endocytic steps with a panel of inhibitors or the overexpression of wild type and mutant Rab GTPases revealed that surface ABCB6 enters via a Rab5-dependent step to early endosomes. Treatment with chloroquine, a lysosomotropic agent, resulted in the lysosomal trapping of ABCB6. Taken together, our results suggest that ABCB6 resides in the endo/lysosomal compartment of cells, performing a yet unknown function.

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O-066
DUAL LOCALIZATION OF THE PRION PROTEIN FAMILY MEMBERS: THE HIGHLY CONSERVED, N-TERMINAL $(RXXX)_8$ MOTIF OF MOUSE SHADOO MEDIATES NUCLEAR ACCUMULATION

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The prion protein (PrP) – known for its central role in transmissible spongiform encephalopathies – has been reported to possess two nuclear localization signals and localize in the nuclei of certain cells in various forms. Although these data are superficially contradictory, it is apparent that nuclear forms of the prion protein can be found in cells in either the healthy or the diseased state. There are growing numbers of evidences about the appearances of various intracellular forms of the prion protein, which might play a role in prion diseases. In this respect, it is intriguing that another prion protein superfamily member – the Shadoo (Sho) – is also found in the nucleocytoplasm of some neurons.[1]

Here we report the accumulation of Shadoo in the nucleus of several neural and non-neural cell lines as visualized by using an YFP-Sho construct. This nuclear localization is mediated by the (25-61) fragment of mouse Sho encompassing an $(RXXX)_8$ motif. Bioinformatic analysis shows that the $(RXXX)_n$ motif (n=7-8) is a highly conserved and characteristic part of mammalian Shadoo proteins. Experiments to assess if Shadoo enters the nucleus by facilitated transport gave no decisive results; however, these experiments revealed that Shadoo's interactions in the nucleolus and in the rest of

the nucleus are markedly different.[2]The most pivotal questions are tracing the journey of various forms of Shadoo from translation to the nucleus and discerning the potential nuclear function and binding partner(s) of PrP and Shadoo.

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O-067
THE PHYSIOLOGICAL ROLE OF TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS IN PLATELET CALCIUM HOMEOSTASIS AND ARTERIAL THROMBOSIS

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Elevation of the intracellular Ca^{2+} concentration is an essential step during platelet activation. Inhibition of Ca^{2+} channels in platelets alters integrin activation, degranulation and thrombus formation in vivo. Transient receptor potential (TRP) channels are a group of divalent cation sensitive channels located on the plasma membrane with TRPC isoforms mediating Ca^{2+} influx and TRPM isoforms mediating Ca^{2+}/Mg^{2+} influx. Previously, it was proposed that store-operated Ca^{2+} entry (SOCE) through TRPC1 was the major SOC channel in human platelets; whereas TRPC6 regulated diacylglycerol (DAG) mediated receptor operated Ca^{2+} entry (ROCE). Using genetically modified mouse models, we have demonstrated that TRPC1 is not the SOC channel in murine platelets and has no functional significance in platelet activation and arterial thrombosis. On the contrary, we could identify the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) as a key regulator of SOCE and Orai1 as the only relevant SOC channel in murine platelets. Furthermore, we found that DAG activated ROCE was abolished in *Trpc6*$^{-/-}$ platelets establishing TRPC6 as the only DAG induced ROC channel in murine platelets. Surprisingly, this abolished ROCE did not affect platelet activation and thrombus formation in vivo. In order to understand the functional redundancy between SOCE and DAG mediated ROCE, platelets lacking both Ca^{2+} channels were analyzed. We found that Orai1-mediated SOCE dominated the process of platelet activation, aggregation and thrombus formation, whereas TRPC6 played a minor role in these processes. Besides the redundant functions we found that TRPC6 together with Orai1 regulated Ca^{2+} store content in murine platelets. Another member of the TRP family, TRPM7 was also detected in platelets. However, the role of this channel in platelet physiology and thrombosis has not been investigated. TRPM7 is a chanzyme comprising a cation channel at the N-terminus and an α-type Ser/Thr kinase domain at the C-terminus. It has been shown to play an important role in the pathogenesis of ischaemic stroke; hence knock-down of TRPM7 resulted in enhanced viability of

neurons under hypoxia. Using a kinase dead TRPM7 knockin mouse model, we have established the physiological role of TRPM7 kinase as a key modulator of Ca^{2+} store release and SOCE in murine platelets, and highlighted the role of this new signaling mechanism in the pathogenesis of thrombosis and cerebrovascular events.

O-068
BLOOD, SWEAT AND TEARS – BODY FLUID PROTEOMICS WITH EMPHASIS ON ANTIMICROBIAL AND IMMUNOMODULATORY PEPTIDE TYPE BIOMARKERS

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Antimicrobial and immunomodulatory peptides and proteins are active constituents of the chemical barriers providing a first line defense system in our body. They can be present on skin surface, in sweat, tears, saliva, airways secretions etc., practically at all sites where our body meets the outer environment containing potentially harmful invaders. The antimicrobial and immunomodulatory peptides can be induced by proinflammatory cytokines produced during inflammation.

Our main focus was the analysis of the antimicrobial and immunomodulatory peptides/proteins in blood, sweat and tears of patients with type 2 diabetes and Alzheimer disease. Both pathological conditions develop on inflammatory background and the proinflammatory cytokine release was demonstrated in case of diabetic retinopathy and Alzheimer disease as well. The level of antimicrobial and immunomodulatory peptides/proteins was not studied in these conditions so far, thus we developed targeted proteomics method for identification and quantification of antimicrobial and immunomodulatory peptides in body fluids of these patients. Some components of this family were identified as tear biomarker candidates for the proliferative stage of diabetic retinopathy and Alzheimer disease.

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O-069
CEREBROVASCULAR ALTERATION AFTER X-RAY EXPOSURE AT LOW DOSES: IN VIVO AND IN VITRO MICE STUDIES

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A radiation-induced blood-brain barrier (BBB) breakdown has been evoked, but clearly demonstrated only at high doses, to explain the acute radiation toxicity syndrome and delayed brain radiation injury. We have investigated impairment in BBB integrity induced by low (100mGy) or moderate (1-2Gy) dose of X-ray exposure. The observations have been confirmed on an in vitro astocyte/pericyte/endothelial cell co-culture model of the BBB. Because endothelial progenitor cells move towards injuries within blood vessels, detecting their presence can lead to a diagnostic system for various vascular injuries. Therefore the aim of the present study was also to detect EPCs in peripheral blood in locally head irradiated mice.

The effect of local cranial irradiation on the permeability of BBB was investigated in Bl/6 mice at 10 days old age or10 week's old age or in the offspring of in utero exposed animals. One day, one week, one and six month after X-ray exposure brain extravasations was assessed with Evans blue which binds serum albumin, brain edema was evaluated using the wet/dry method. Circulating endothelial progenitor (EPC) was measured using colony assay (CFU-EPC). EPC colonies were identified as the CD34+, CD31+ and Griffonia simplicifolia lectin + (GS-l).

In the 10 weeks of age irradiated group one week after X-ray exposure at the dose of 100 mGy increase of the permeability was observed. At the dose of 2 Gy no significant modification was measured. In 10 days old age irradiated mice, one month after exposure, we obtained significant increases in the BBB permeability to both doses. Increased permeability was measured at 6 months after exposure at 1 Gy in the offspring of in utero treated mice. We observed a significant age-dependent decline in the radiosensitivity of mice.

A significant increase in circulating EPCs was observed in mice 24 and 168 hours after 100mGy irradiation compared to unirradiated control. Circulating EPC outgrowth colonies were significantly reduced in 2Gy irradiated mice at 24 hours but later the number of EPCs returned to the control level. It is concluded that mice exposed to low dose irradiation have increased numbers of circulating EPCs and accordingly, increased capacity for tissue repair.

On the in vitro model, the effects of X-ray were observed on the cellular density of endothelial and glial cells by nuclear staining and counting. Tight junction protein ZO-1 and adherens junction β-catenin were detected by immunocytochemistry and the permeability of the endothelial monolayers to Evans-blue-albumin and fluorescein was determined until 5 days after radiation exposure. DNA double-strand breaks were also estimated by phospho-γ-H2AX FACS analysis. Our results showed that X-ray irradiation induced an increase in paracellular permeability and a progressive decrease in the number of endothelial and glial cells.

Our in vivo experimental data demonstrated that cellular injury, as judged by impairment of permeability, was delayed and less pronounced in the immature brains. A better understanding

and awareness of this phenomenon are essential for designing appropriate treatment modality in brain radiotherapy or in diagnostic radiology.

O-070
MYC-DRIVEN OVERGROWTH REQUIRES UNFOLDED PROTEIN RESPONSE-MEDIATED INDUCTION OF AUTOPHAGY AND ANTIOXIDANT RESPONSES IN *DROSOPHILA*

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O-071
PROPROTEIN CONVERTASE FURIN IN T CELL MEDIATED IMMUNE RESPONSES

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Furin is a member of the proprotein convertase enzyme family, and it is present in all human tissues. It has a crucial role in processing physiological proproteins, like growth factors, hormones,

plasma proteins and receptors, because of this furin has an indispensable physiological role; furin deficiency is embryonically lethal. Additionally, it activates pathogens (e.g. HIV, *B. anthracii* toxins) and upregulates metastatic activity of cancers; it takes part in the pathogenesis of cystic fibrosis. This is the cause why furin inhibitors have been suggested in treatment of a wide variety of diseases.

Furin expression in T cells is critical for host-defense against intracellular pathogens; it cleaves several proteins known to be indispensable for T cell functions, including TNFα converting enzyme (TACE), Notch1 (Notch homolog 1, translocation-associated (Drosophila)), and TGFβ (tumor growth factor beta) family cytokines. Although furin is essential in maintaining peripheral tolerance via the regulation of TGFβ production, furin deficient effector T cells were found to be insensitive to regulatory T cell mediated suppression, which refers to additional roles of furin in T cell activation. In this work, the focus of the research was on the influence of furin on interleukin-2 (IL-2) expression, a critical regulator of the magnitude and duration of T cell immune response.

Impact of furin was investigated by using a T cell specific furin knock out mouse model and stable human Jurkat T cell lines that express either normal or elevated amount of furin or an enzymatically inactive form of this protein. Furin knock out naive T cells show initially elevated IL-2 production, but activation via T cell receptor (TCR) cause a decreased enhancement of the expression of this cytokine compared to the furin producing wild type cells. Furin overexpressing human T cells produce more IL-2 than cells with ordinary furin expression level. TCR activation triggered IL-2 production can be blocked using furin inhibitor or shRNA. We also investigated the activation of T cell receptor associated transcription factors to find the mechanism by which furin influence the cytokine expression. The promoter region of IL-2 gene contains several binding sites for AP-1 (activator protein 1), NFAT (nuclear factor of activated T cells) and NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells). We found elevated activity of these transcription factors in furin overexpressing cells.

The results of this work show that furin expression highly influences/regulates the interleukin-2 production of T lymphocytes. An explanation of this process could be the higher activation level of the examined transcription factors. These results affirm that furin has a prominent role in T cell activation.

O-072
TEMPERATURE SENSITIVE DNA REPAIR MUTANTS ENABLE HIGHLY PRECISE GENOME MANIPULATION

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Oligonucleotide mediated allelic replacement is an efficient tool for bacterial genome manipulation (Wang et al. Nature 460:894; F. J. Isaacs et al. Science 333:348). Mutants with endogenous mismatch repair (MMR) system deficiency have been shown to be a beneficial background for oligonucleotide mediated allelic replacement, due to the increase of mismatch incorporation efficiency and unbiased mutation spectra. However, inactivation of the host's mismatch repair

system results in a dramatically elevated general mutation rate and therefore accumulation of unwanted background mutations across the genome.

We present a novel strategy for mismatch repair evasion using temperature sensitive DNA repair mutants and a method for temporal inactivation of the mismatch repair protein complex in *Escherichia coli*. This method enables the transient suppression of DNA repair during mismatch carrying oligonucleotide integration, but allows normal mismatch correction during cell growth and electrocompetent cell preparation stages. This advanced technique further increases the precision of oligonucleotide mediated allelic replacement and enables more predictable cell programming.

O-073
DEVELOPMENT OF BASIC GENETIC MANIPULATION SYSTEM FOR THE THERMOACIDOPHIL *THERMOPLASMA ACIDOPHILUM*

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Thermoplasma acidophilum is a thermoacidophil archeon which on one hand is able to cope with the harsh environment (58 °C and pH 2) and on the other hand became an important and exceptional model organism of proteomic studies due to its physical characteristics (small cell size, lack of cell wall), its low complexity proteome and its resemblance of several metabolic pathways to eukaryotic cells. Despite the last decade extensive research on *Thermoplasma* genomics and proteomics, the basic genetic tools (including routine clonal work, transformation system, and available selection markers) were missing that hindered the further work greatly.

The scope of our work was to develop the yet missing genetic tools for *T. acidophilum*. First of all, as growth on solid medium is necessary for clonal selection, we developed and optimised a Gelrite-based solid medium amended by native yeast extracts as growth promoting substance. In parallel, numerous pro- and eukaryotic antibiotics were tested and finally novobiocin and rifampicin were found as the most appropriate selection agents for *T. acidophilum*. Based on the available selection markers (novR *gyr*B and *arr*2-rifampicin) vector constructs were developed either for chromosomal integration or for individual replications. Finally several different transformation methods were tested. Unfortunately neither the widely used methods like electroporation nor the more specialised, not commonly applied techniques like lipofection, magnetofection or gene gun particle delivery brought the desired results. We have developed a unique transformation system with our vector constructs, based on simple natural transformation methods. By the use this transformation system novobiocin and rifampicin resistant *T. acidophilum* cell lines were obtained.

We have developed functioning genetic tools for *T. acidophilum* however; further improvements and fine tuning are still needed for direct applications like KO mutagenesis. Application of the new genetic tools in *T. acidophilum* will facilitate the research on proteome and genetic machinery of this model archaeon.

Keywords: Thermoplasma acidophilum, genetic tools, cultivation

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O-074
GENOME-GUIDED APPROACH FOR IDENTIFYING CRYPTIC BIOSYNTHETIC PATHWAYS AND NOVEL NATURAL PRODUCTS IN *SACCHAROMONOSPORA AZUREA*

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Microbes are prolific sources of many bioactive secondary metabolites including antibiotics, antitumor compounds, immuno-suppressants, antiviral and antiparasitic agents. However cell's metabolic network that evolved in nature is not optimized for practical application; many biosynthetic gene clusters appear to be expressed poorly, or not at all, under laboratory growth conditions. The recent developments of the genomics-guided approaches for bioactive metabolites clearly indicate that the second golden age of the microbial natural product discovery was born.

In order to provide new insights into the molecular basis of secondary metabolite biosynthesis, the recently published draft genome of *S. azurea* strain SZMC 14600 was further analyzed. The incomplete draft genome consists of a 4,973,727 bp long chromosome with a G+C content of 70.3%. In total 4,604 genes were predicted, from which 4,554 responsible for protein-coding, and 69 RNAs were also identified. The majority of the protein-coding genes (69.1 %) were assigned with a putative function, while 406 genes were annotated as hypothetical proteins. Genome sequence analysis of *S. azurea* revealed 253 genes (5.56 %) associated with COGs functional categories of secondary metabolites biosynthesis, transport and catabolism. It is well known that a wide range of biologically active products are synthesized by thiotemplate modular systems (TMS) including polyketide synthase (PKS), non-ribosomal peptide synthetase (NRPS) and hybrid synthase/synthetase (PKS/NRPS) enzymes. Comparative analysis of the genome sequence of the actinomycetes bacterium *S. azurea* SZMC 14600 has revealed numerous secondary metabolite biosynthetic gene clusters including a giant type I modular polyketide synthase (PKS) gene cluster, which is composed of 37 ORFs, nine of which encode PKSs (*Sapk*21A, *Sapk*22B, *Sapk*24C, *Sapk*25D, *Sapk*26E, *Sapk*27F, *Sapk*28G, *Sapk*29H, *Sapk*30I) and spans more than 180 kbp, making it one of the largest polyketide biosynthetic gene clusters described to date. In order to identify additional

natural products and biosynthetic pathways numerous *in silico* methods have been applied (e.g. ClustScan, SBSPKS toolbox, antiSMASH etc.). As a result of these efforts several hypothetical and/or previously undescribed NRPSs and PKS/NRPS hetero-gene clusters were recognized.

We hope that our structural genomics efforts will form a foundation for the subsequent research steps, such as prediction of putative physico-chemical properties of a metabolic product that makes intelligent drug design possible.

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O-075
IN VIVO ENZYMOLOGY: PHYSIOLOGIC EFFECTS OF DUTPASE MUTATIONS IN THE MYCOBACTERIAL CELL

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Thymidine biosynthesis is essential in all cells as dTTP is one of the nucleotide building blocks of DNA. Normally, three major pathways exist for dTTP synthesis, but only one of these is present in mycobacteria. This one involves the dUTPase reaction which also serves to eliminate excess dUTP to prevent DNA uracilation.Due to the exclusive biosynthetic role of dUTPase in mycobacteria, these organisms are ideal toinvestigate the physiology of the dUTPase reaction in the living cell.

Therefore, we aimed to relate the previously deciphered *in vitro*reaction mechanism of dUTPase to its physiological role inlive cells.

Mycobacterium smegmatis was used as a model for the investigation of mycobacterial thymidine biosynthesis. We found that mycobacterial thymidilate biosynthesis is highly conserved. Theknock-out of the conventional monofunctional dUTPase (*dut)* resulted in lethality, which could be reverted by complementation with the wild-type or several other dUTPase mutants with *in vitro* characterized enzyme activity.The mutant strains carrying dUTPases of variably decreased enzyme activity were viable and did not show any growth defect under stress-free conditions. Nevertheless, their genomic uracil content was increased as well as their mutation rates. Importantly, the increase in both phenomenadirectly correlated with the decrease in the*in vitro* enzyme activity.We also found that a wild-type enzyme activity of *dut* may be necessary to maintain growth under various stress-conditions.

We conclude that dUTPase activity may be dispensable for a few generations under stress-free conditions. However, the accumulating uracil content and probably in consequence of this the increased mutation rate result in slow deterioration of the dUTPase deficient population. To unravel further details of the molecular mechanism behind the observed phenotypes we conduct nucleotide pool studies in the mutant strains.

O-076
CHARACTERIZATION OF STRONG ANTIBACTERIAL ACTIVITY EXHIBITED BY NODULE SPECIFIC PLANT PEPTIDES

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Antimicrobial peptides (AMPs) are considered as natural antibiotics produced by all kinds of living organisms including bacteria, plants and animals. Certain AMPs show narrow while others exhibit broad spectrum of activity to fight microorganisms, including Gram-positive and Gram-negative bacteria, viruses, fungi, parasites and even multidrug resistant pathogens. Plants represent one of the most abundant and largely undiscovered source of AMPs. Symbiotic host cells in *Medicago truncatula* produce nodule-specific cysteine-rich (NCR) peptides that resemble antimicrobial peptides of the innate immune system.

Our specific goal was the detailed antimicrobial characterization of selected members of the M. truncatula NCR peptide family. We have aimed the identification of various peptide targets and the disclosure of the structural requirements of the examined peptides for their in vitro antimicrobial activity.

Solid-phase synthesized peptides were used to investigate the peptides' antimicrobial characteristics. By treating log phase bacteria with the peptides, membrane permeabilization was detected by the uptake of the PI (propidium-iodide) dye in a peptide- and concentration-dependent manner. Certain NCR peptides induced bacterial cell death, as indicated by decreased colony forming units in plating assays and by the loss of respiratory activity.

Beside the peptides' membrane penetrating activity, possible intracellular targets of the NCR peptides were identified in various bacteria indicating the peptides' multi-level mechanism of action. Affinity protein purification experiments using tagged peptide derivatives were performed to isolate intracellular interacting protein partners. Characteristic interacting protein patterns were observed for certain peptides.

O-077
SMALL RNA REGULATION OF PLANT NB-LRR DEFENSE GENE FAMILY

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Plant genomes contain large numbers of resistance (R) genes with nucleotide binding (NB) and leucine-rich repeat (LRR) domains which function as an innate immune system that recognize specific pathogen effectors and trigger resistance responses. Although they provide protection

from diverse pathogens, their large numbers and cell death trigering activity are potential threats to plant fitness. Despite the enermous agricultural importance of R-genes, the mechanisms regulating their expression are not well understood. Recently, small RNAs (sRNAs) emerged as an important gene expression regulators of various cellular processes. Inplants based on their biogenesis we distinguish two main classes of sRNAs: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Despite of the differences in their biogenesis, one group of siRNAs also regulate protein coding genes very similarly to miRNAs and are called trans-acting siRNAs (ta-siRNAs). The formation of tasiRNAs from a noncoding RNA (TAS) precursor is dependenton a miRNA trigger. We have recently identified new tasiRNA producing TAS loci in tomato that are homologous to NB-LRR resistance genes and require the miR-482 family to trigger their production. Tasi-RNAs are produced from the NB-LRR homologous conserved P-loop region of TAS genes and are down-regulated during pathogen infection. This suggest that miR-482 family and ta-siRNAs derived from TAS loci homologous to R genes play an important role in R gene regulation during pathogenic interactions.

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O-078
MAINTENANCE OF PLURIPOTENCY IN EMBRYONIC STEM CELLS WITH MICRORNAS

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miRNAs are a class of endogenous, small RNA molecules that do not code for proteins, and regulate expression of their target molecules. miRNA target recognition is highly redundant and the outcome of miRNA expression or its alteration is always dependent on the target pool available in situ. Therefore miRNAs act in networks and integrate with the classical regulatory associations of proteins. This allows miRNAs to fine tune the spatial and temporal gene expression, making them uniquely suitable for the regulation of developmental processes. miRNAs were proved to be essential for the regulation of pluripotency, differentiation, axis formation, cell cycle regulation, development and maturation of the nervous system, organogenesis as well as for pathological conditions such as skeletal and growth defects, cardiomyopathies and different cancers. Key experiments showed that the miRNA pathway is indispensable for stem cell maintenance and differentiation in mammalian systems and recent work pointed out its importance in iPS cell generation. There is increasing evidence that cancer pathogenesis and progression shares some aspects of embryonic development (eg: epithelial-mesenchymal transition), dedifferentiation and stem cell maintenance. Since miRNAs are crucial participants of these processes, stem cell biology and cancer molecular pathology will both benefit from the deeper understanding of miRNA regulation in embryonic stem cells. I will highlight the most common theories about the way they establish simple regulatory networks with their targets. I will also discuss, in more details, the role of ES cell-specific miRNAs in the maintenance of pluripotency of the mouse embryonic stem cells (ES cells), and their connection to regulation of cell cycle.

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O-079
POLYMORPHIC MIRNA BINDING SITES ARE RISK FACTORS OF DIABETES MELLITUS

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***Aims*:** Wolframin is a major protein of the endoplasmic reticulum, it is expressed in most tissues and clinical data demonstrate its significant connection to diabetes mellitus. Loss of function mutations of the *WFS1* gene result in the monogenic Wolfram-syndrome, characterized by optic atrophy, diabetes insipidus, early onset diabetes mellitus and deafness. Accordingly, polymorphic variants of the gene, which cause only minor alteration in protein function are putative risk factors of diabetes. Our aim was the association and molecular analysis of two SNPs (rs1046322 and rs9457) in the 3′ UTR region of the *WFS1* gene, which are supposed to alter the binding of miRNA-668 and miRNA-185, respectively, according to *in silico* data.

Materials and methods: Association analysis of the polymorphic loci and diabetes mellitus was carried out by case–control setup. 617 patients and 1147 health controls participated in the study. Genotype analysis was carried out using PCR and allele-specific probes, functional analysis of miRNA binding was investigated by luciferase reporter system.

Results: Our results suggested that rs9457 SNP "C" allele was significantly more frequent among patients with type 2 diabetes mellitus (p = 0.0008), whereas the rs1046322 variant showed a significant association with the type 1 form of the disease. Haplotype analysis confirmed the role of the polymorphic variants in the genetic background of diabetes. Results of the luciferase reporter experiments confirmed the data of the sequence analysis: the rs1046322 and the rs9457 SNPs altered the binding of miRNA-668 and miRNA-185, respectively.

***Conclusion*:** Our results suggest that the rs9457 and rs1046322 polymorphisms are the genetic components of diabetes mellitus. Earlier studies showed an association between a third variant of the 3′ UTR, rs1046320 and diabetes, however no biological function of the SNP could be observed. We suggest that this result is due to the strong linkage disequilibrium between rs9457 and rs1046322, thus the latter polymorphism can be a genetic marker of rs9457 miRNA-SNP.

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O-080
NON-CANONICAL HUMAN MICRORNAS: CHARACTERIZATION OF THE MIRTRON PATHWAY IN HIGHER EUKARYOTES

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microRNAs (miRNA) are small non-coding RNAs influencing most cellular processes due to their important role in gene expression regulation. Most of these 20-24 nucleotide long molecules are formed via a canonical pathway, but there are also emerging data on various alternative miRNA maturation routes. The most prominent of them is the mirtron pathway, which was first descibed in *Drosophila melanogaster* and *Caenorhabditis elegans*. miRNAs of mirtron origin are localized in short introns where the whole intron is essentially equivalent to the so called pre-miRNA form of the canonical maturation process. This allows that the first step of the mirtron processing is different from the canonical one: the pre-miRNA is cleaved out from the primary transcript by the splicing machinery instead of the Drosha/DGCR8 complex. Since bioinformatic predictions and small RNA data analyses have suggested the existence of mirtrons in higher eukaryotes, we decided to examine several predicted human mirtron sequences. We investigated their processing and gene silencing capacity from their endogenous or a heterologous (GFP) coding context. We demonstrated that functional human mirtrons can be produced and this is a coding context-independent process. Furthermore, we showed that mir-877 can liberate functional mature miRNAs from both of its 5'- and 3'-arms simultaneously in various cell types examined. In light of this result, it seems to be important to also include the "passenger" strand when exploring the relevant physiological functions of different miRNAs. We have proven the existence of the human mirtron biogenesis pathway in two different ways: we investigated the splicing dependency using splicing mutant forms of mirtrons while the independence of Drosha/DGCR8 complex was proven in a DGCR8 deficient cell line. For functional testing, we used luciferase assays while for the detection of mature miRNAs we applied *Northern blot* analysis and quantitative real-time PCR (miRNA TaqMan). For reproducible measurements, we had to optimize the latter technique. Based on these results (e.g. the influence of plasmid DNA contamination on the measurements) for mature miRNA detection we used cell lines stably expressing the examined mirtrons. These were established by *Sleeping Beauty* transposon based technology. Taken together, our results emphasize the need for functional testing of both arms of miRNAs, as well as the experimental validation of human mirtrons since in spite of being localized in a short intron, predicted species could be expressed via other miRNA maturation pathways. Furthermore, we demonstrate that accurate detection of mature miRNA species by stem-loop real-time PCR needs to be thoroughly optimized.

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O-081
IDENTIFICATION OF GENES IMPORTANT IN DISEASE SYMPTOM DEVELOPMENT IN VIRUS INFECTED PLANTS

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In virus infected plants the invading virus spreads thorough the plant and interferes with the host metabolism forming typical symptoms characteristic for the particular plant-virus interaction, causing massive loss in crop yields. Severity of symptoms marks the magnitude of gene expression alterations and the efficiency of viral movement which is controlled by RNA silencing, small RNA based protection network of the plant. Persistent down regulation of prominent housekeeping genes (shut-off phenomenon which occurs in the nucleus at transcription level) in systemically infected leaves has been described for several plant-virus interactions and its presence was linked to the appearance of severe disease symptoms.

We made genome wide analysis of gene expression changes in different virus-host combinations to get further insight into the gene expression changes in compatible interactions. Gene expression patterns of *N. benthamiana* plants infected with different viruses, inducing „shut-off" or not, were compared in microarray hibridization experiments. Heat map analysis of the results demonstrates that changes in gene expression pattern of crucifer infecting tobamovirus (crTMV) and cymbidium rinspot virus (CymRSV) infected plants („shut-off" present) are significantly similar. In striking contrast turnip crinkle virus (TCV) infection („shut-off" not present) caused only limited changes. In TCV infected plants not only the lack of down regulation of housekeeping genes (no shut-off) but also the inhibition of the up regulation of common stress genes were observed. It means that in compatible plant-virus interactions viruses act in different ways: either by profoundly altering or inducing only limited changes in the genome wide mRNA expression patterns. We have identified differently expressed genes which action can possibly explain the development of severe symptoms.

Since RNA silencing is the key protection mechanism of the plant against virus infection we investigated its role in altering disease symptoms. Argonaute1 (AGO1) is the central molecule of the miRNA pathway, regulating very important developmental processes, and the antiviral system of the host. In virus infected plants we showed that miR168, which controls *AGO1* mRNA, level is increased. Using mutant plants, transient expression and target mimicry constructs we demonstrated that miR168 can translationally block the expression *AGO1* mRNA activity. We further showed that one of the miR168 precursor genes is induced in virus infected plants probably by the action of viral RNA silencing suppressor proteins. Moreover, down regulation of AGO1 can lead to enhanced accumulation of miRNA target mRNAs altering the virus specific symptoms.

Our research was funded by OTKA (PD78049; K78351).

ABSTRACTS - POSTER PRESENTATIONS

P-001
THE ROLE OF AUTOPHAGY IN THE DEVELOPMENTALLY PROGRAMMED CELL DEATH IN THE *DROSOPHILA* LARVAL SALIVARY GLAND

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During postembryonic development, the larval organs of the common fruit fly *(Drosophila melanogaster)* are degraded, as well as their salivary glands. In the degradation of these organs, apart from apoptosis, autophagy is suggested to play an important role. The way autophagy contributes to the destruction of the gland cells is still unclear. Both mechanisms act as catabolic, cell physiological processes regulated in inverse proportion to growth, and they take a joint part in the death of the salivary gland cells.

We conducted a detailed research on the role of autophagy in programmed cell death in salivary glands of *Drosophila*. The GAL4/UAS system has been used for salivary gland-specific ectopic gene expression. Silencing of *TSC1* (gene of an autophagy regulating protein), *Atg1* and *Atg6* (genes of key proteins of the autophagic process) expression reduced autophagy (based on mCherry-Atg8a fluorescence). Salivary glands normally disintegrate within 17 to 19 hours after puparium formation, while in TSC1, Atg1 and Atg6 deficient larvae they survived up to 21 to 23 hours.

We used a genetically encoded caspase sensor (apoliner) that can be detected in fixed tissue by standard fluorescent microscopy. The sensor comprises two fluorophores, monomeric red fluorescent protein (mRFP) and enhanced green fluorescent protein (eGFP) that are linked by an efficient and specific caspase-sensitive site. Upon caspase activation, the sensor is cleaved and eGFP translocates to the nucleus, leaving mRFP at membranes. We managed to trace early caspase activity in salivary glands originated from 13 hours old pupae. This phenomenon implies the cooperation of autophagy and apoptosis. The prolonged lifespan of salivary glands with silenced autophagy suggests that this process has a role in eliminating this organ.

Keywords: Apoptosis, Autophagy, Drosophila, Salivary gland

P-002
THE EFFECT OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) ON STRESS-AND APOPTOSIS-RELATED PROTEINS IN NORMAL MAMMARY AND BREAST CANCER CELL LINES

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide with special regulatory effect on different organs. Moreover, it is a well known antiapoptotic factor. In the present study, the effect of PACAP on the apoptotic and stress-related proteins was investigated on human mammary epithelial (HMEC) and breast cancer cell lines (MDA-MB-468 and MCF-7) exposed to H_2O_2 induced oxidative stress. The PAC-1 receptor expression was detected by confocal laser scanning microscopy and the mitochondrial depolarization was visualized by JC-1 dye on MCF-7 cells. The pro- and antiapoptotic factors were detected by human cell stress and apoptosis array kits. Our results showed that PAC-1 receptor was detected in all the investigated cell lines. PACAP-treatment could attenuate the oxidative stress-induced damage in the mitochondrial depolarization on MCF-7 cells. The sublethal dose of H_2O_2 increased the expression of almost all the stress-related proteins on HMEC, and PACAP co-incubation decreased the expression of COX-2, pp53, pp38, PON1 and Bcl-2. PACAP had no effect on the cell survival after high dose of H_2O_2 treatment measured by MTT. The proapoptotic proteins randomly elevated after oxidative stress in all of the three cell lines, while a fairly regular pattern was observed in the changes of antiapoptotic factors. The members of the IAP family, such as livin, XIAP and survivin showed decrease on H_2O_2 treated cells, which was partly compensated by PACAP co-incubation. These data suggest that proteins of the IAP family might be potential targets of PACAP in modulating the apoptotic processes in the mammary gland.

P-003
THE ROLE OF *DROSOPHILA* ATG6 IN RECEPTOR MEDIATED ENDOCYTOSIS

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Receptor mediated endocytosis is an evolutionary conserved function of the eukaryotic cells internalizing selected molecules in a clathrin-dependent manner. Since the cargo molecule is specifically bound by receptors on the cell surface, this process allows the sorting of the internalized molecules and route them to the recycling pathway or towards lysosomal degradation. The physiological function of this process is diverse. For example this type of endocytosis can be used to modulate several signaling pathways by degrading signaling receptors

which result in silencing the signal transduction or recycle them to the cell surface which in this case, will result in elongated signaling activity.

As this process could terminate in the degradation of ligands – and their receptors as well – many protein complexes involved in the regulation of receptor mediated endocytosis (such as ESCRTs/Endosomal Sorting Complex Required for Transports) are also involved in the mechanism of autophagy, which process is also used for degradation. Vacuolar protein sorting 34 (Vps34) is the catalytic subunit of a protein complex, producing phosphatydilinositol-3-phosphate (PI(3)P) and is required for the early steps of endocytosis and autophagy as well. Atg6 (Autophagy related gene 6) is a binding partner of Vps34 and is required for autophagy and for the transport of certain proteins into the vacuole in yeast. In higher eukaryotes this protein is mostly known by its essential and crucial function in autophagy induction. Recent studies in cell cultures suggest that the role of Atg6 cannot be restricted to autophagy; it may participate in cytokinesis and endocytosis as well.

Here we show that *Drosophila* Atg6 is an essential regulator of endocytosis, as the knock-down of Atg6 by RNAi results in several serious defects in the development of the wing tissue. Electron microscopy revealed that abnormal endosomal structures, such as dense multivesicular bodies and multilamellar bodies appear in the developing pupal wing cells. These structures show acid phosphatase activity, suggesting that these structures could be enlarged, defective endolysosomes. We showed that Atg6 knocked-down wing imaginal disc cells accumulate Notch which results in enhanced Notch signaling. Taken together, we assume that the RNAi of Atg6 results in the disruption of receptor mediated endocytosis, which may cause abnormal differentiation and development. This is further approved by the observation that in these cells the junctional and adhesion complexes are malformed and there are serious problems with planar cell polarity as well. To examine whether Atg6 has a role in other types of endocytosis we performed an *ex vivo* essay where wing imaginal disc cells were allowed to take up Texas Red (TR) conjugated avidin. We observed that Atg6 RNAi imaginal disc cells are unable to internalize TR avidin. Since the uptake of the fluorochrome-labeled avidin is carried out by pinocytosis, our result implies that Atg6 is required for endocytosis in general.

Our current studies are aimed to examine the molecular markers of the abnormal structures of the Atg6 knocked-down cells and to localize the Atg6 protein in *Drosophila* cells to reveal the exact functions of Atg6 in endocytosis.

P-004
IMPAIRED PROTEASOMAL DEGRADATION ENHANCES AUTOPHAGY VIA HYPOXIA SIGNALING IN *DROSOPHILA*

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Two pathways are responsible for the majority of regulated protein catabolism in eukaryotic cells: the ubiquitin-proteasome system (UPS) and lysosomal self-degradation through autophagy. Both processes are necessary for cellular homeostasis by ensuring continuous turnover and quality control of most intracellular proteins. Recent studies established that both UPS and autophagy are capable of selectively eliminating ubiquitinylated proteins and that autophagy may partially compensate for the lack of proteasomal degradation, but the molecular links between these pathways are poorly characterized.

We show that autophagy is enhanced by the silencing of genes encoding various proteasome subunits (α, β or regulatory) in larval fat body cells. Proteasome inactivation induces canonical autophagy, as it depends on core autophagy genes *Atg1, Vps34, Atg9, Atg4* and *Atg12*. Large-scale accumulation of aggregates containing p62 and ubiquitylated proteins is observed in proteasome RNAi cells, but loss of *p62* does not block autophagy upregulation, suggesting that compensatory autophagy is not simply due to the build-up of excess cargo.

One of the best characterized substrates of UPS is the HIF-1α subunit of hypoxia-inducible transcription factor, which is continuously degraded by the proteasome during normoxic conditions. Hypoxia is a known trigger of autophagy in mammalian cells, and we show that genetic activation of hypoxia signaling also induces autophagy in Drosophila. Moreover, we find that proteasome inactivation-induced autophagy requires *sima*, the Drosophila ortholog of *HIF-1α*. Thus, we identify a novel genetic link mediating upregulation of autophagy by impaired proteasomal activity.

Keywords: Autophagy, Drosophila, HIF-1α /sima, Hypoxia, p62/Ref2P, Proteasome

** P. Lőw and Á. Varga contributed equally to this work*

P-005
INHIBITION OF AUTOPHAGIC CELL DEATH AND RADIOSENSITISATION WITH SILENCING OF TP53INP1 IN HUMAN FIBROBLAST CELLS

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Tumor protein 53-induced nuclear protein-1 (TP53INP1) encodes two nuclear protein isoforms (TP53INP1α and TP53INP1β) and transcription is activated by p53. In response to stress TP53INP1 takes part in p53-mediated cell death and cell cycle arrest. Overexpression of this gene promotes apoptosis and cell cycle arrest in many cases. Moreover, TP53INP1 interacts with p53 thus modifies the transcriptional activity of p53 on several other genes. The objective of this study was to assess what radiation-induced cell responses the TP53INP1 take plays a role in.

The importance of TP53INP1 expression for radiation responses was investigated using F11hTERT (hTERT immortalized human fibroblasts) knockout for TP53INP1 with TP53INP1 shRNA.

We examined the following areas in the modified cells related to normal fibroblast: cell cycle distribution analyzed by flow cytometry, the autophagy detected by Acridine Orange staining, the senescent cells stained with SA-β-Gal, the expression of TP53INP1, GDF-15, GADD45A and CDKN1A measured by real-time PCR, and the kinetic of repair of double strand breaks by γH2AX staining. Irradiated fibroblast and bystander cells were compared for survival, by the colony-forming assay and amount of mitochondrial DNA deletion after irradiation, determined by real time PCR.

We demonstrated that TP53INP1 deficient cells showed resistance of the G2-delay, and the proliferation rate was higher compared with the F11hTERTcells. Furthermore the lack of TP53INP1 gene function results in autophagy decline following irradiation, while it has not effect on the senescence. TP53INP1 was required for ionizing radiation induced maximal elevation of CDKN1A and GDF-15. At DNA repair we found that in gene silenced cells the reparation was delayed. Bystander effect decrease was also observed in cells with arrested TP53INP1. Finally, we show that TP53INP1 proficiency is important for clonogenic survival after radiation.

These data reveal novel functional roles for TP53INP1 in cell cycle, survival and responses to ionizing radiation.

P-006
TGF-ß SIGNALING PATHWAY AND AUTOPHAGY

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Autophagy is a highly conserved process of eucaryotic cells that is responsible for degradation of dysfunctional organelles and macromolecules. It is one of the basic mechanisms of defense against stress. Beyond this crucial function of autophagy it has also a proven role during morphogenesis as it elevates to an extraordinary high level and results in cell death in Drosophila melanogaster. Considering the current knowledge of the process and molecular regulation of autophagy, it has strong biomedical, social and economic significance.

During insect metamorphosis, larval organs are eliminated by intensive autophagy. Many studies show that in several cases cells can avoid apoptosis by autophagy in certain cases of development. Besides the increased levels of autophagic processes in these surviving cells an intensification of transforming growth factor (TGF)-ß signaling pathway has been observed. TGF-ß pathway is a highly conserved pathway that has an important role in aging. Since autophagy related genes (Atg) also have a deep influence on lifespan, certain relationship can be hypothesized between autophagy and TGF-ß signaling. Our goal was to examine the possible relationship between TGF-ß signaling and autophagy in Drosophila melanogaster.

SARA is a member of TGF-ß signaling pathway. We used a dominant negative form of sara, which carried an amino acid change on the protein phosphatase binding domain. Due to the amino acid change, protein phosphatases were unable to inactivate the TGF-ß receptor. In cells, where the dominant negative form of sara was overexpressed, autophagy couldn't be induced by starvation, and these cells were unable to perform developmental autophagy as well.

Taking the above into account we suggest that in Drosophila melanogaster TGF-ß may have role in the repression of autophagy.

P-007
THE ROLE OF THE GATA-TYPE TRANSCRIPTION FACTOR *SERPENT* IN THE REGULATION OF AUTOPHAGY

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Autophagy is an evolutionarily conserved process of eukaryotic cells for degrading their components – damaged, nonfunctioning or defective cellular organelles and proteins – by the lysosomal system. Autophagy can be induced by physical and chemical influences and it is also activated under stress conditions such as starvation. This cell biological event can be well studied in the degrading larval organs of the holometabolous insects since these organisms eliminate their obsolate larval tissues by intensive autophagy (programmed cell death type II) at the time of metamorphosis.

The GATA-like transcription factor gene *serpent* (*srp*) is necessary for the regulation of several biological processes in the fly. Srp is a key regulatory transcription factor in hematopoiesis, embrionic fat-cell differentiation and acts as a selector gene to specify the developmental cell fate of the endoderm. Although Gln3, the ortholog of Srp in yeast, regulates the transcription of certain genes which are necessary for the induction of autophagy (e.g the *Drosophila atg14* ortholog *apg14*), Srp has not been implicated in the regulation of autophagy.

We carried out RNAi-mediated silencing of *srp* in *Drosophila* larval fat body cells using the FRT/FLP recombinase system. In *srp* RNA-silenced clones, mCherryAtg8 positive autophagotic structures and Lysotracker Red-labeled autolysosomes were observed under fed conditions. Similar results were obtained after overexpression of *srp*. We found that there is also a significant induction of autophagy in the *srp* null mutant cells.

The function of Srp is likely to depend on its localization. To reveal the role of Srp in the regulation of autophagy, we examined its localization in the larval fat body cells under fed and starved conditions. Under nutrient-rich conditions nuclear localization was observed while during starvation Srp remained in the cytoplasm. The localization of Srp in *srp*-RNAi silenced and *srp*-overexpressed clone cells was also investigated and we found that Srp can not enter into the nucleus in either case.

In yeast, there are several phosphatases and kinases which are involved in the regulation of Gln3. Experiments were carried out to investigate the effect of silencing, overexpression and/or dominant negative mutation of *Drosophila* orthologes of these genes (mts, PP4 19C and TOR) on autophagy to find presumable interaction partners.

Our results suggest that Srp plays a role in the regulation of autophagy probably by inhibiting the transcription of certain genes which are necessary for the induction of autophagy.

P-008
ULTRASTRUCTURAL CHANGES DURING *DROSOPHILA MELANOGASTER* LARVAL SALIVARY GLAND DEGRADATION

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Cell death is one of the basic biological processes and plays a critical role in both health and disease. Programmed cell death (PCD) represents an integral part of development and homeostasis in animals, plants, and even some single-cell organisms. While multiple forms of PCD are known, apoptosis and autophagy are the best characterized ones.

Apoptosis is genetically programmed death and its execution via the caspase cascade is well documented. Yet, apoptosis does not always function alone. In most cases, autophagy, a lysosomal degradation mechanism, which enables the cell to eliminate damaged or unnecessary organelles and macromolecules to recycle them, has been shown to be engaged in a complex interplay with apoptosis. With the increasing understanding of its biological functions, the involvement of autophagy in the cell death and cell survival process becomes a critical point of concern.

During the metamorphosis of insects, larval tissues are eliminated via PCD to allow the formation of new adult structures. Therefore, insects represent particularly good experimental systems for defining the regulatory mechanisms that mediate PCD, and thus can be extended to higher organisms.

Investigation of the histolysis of Drosophila larval salivary gland gives us an ideal opportunity to understand the regulation of PCD. This process is regulated by the molting hormone (20-hydroxyecdysone).

Many contradictory data can be found in the literature about the exact process of salivary gland degradation. Although, autophagy and apoptosis of this organ have been extensively studied at light microscopic level, just a few ultrastructural data are available on these processes. Therefore, the main aim of our work is to perform detailed, comprehensive ultrastructural investigations to disclose the basic morphologic phenomenons of this process.

P-009
INTERACTION OF HOPS COMPLEX WITH SYNTAXIN17 IS REQUIRED FOR AUTOPHAGOSOME CLEARENCE IN *DROSOPHILA*

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P-010
GENERATION AND CHARACTERIZATION OF *VPS13A* MUTANTS IN DROSOPHILA

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Autophagy and endocytosis are intensively studied essential membrane-transport mechanisms of the eukaryotic cell. Perturbation of either of these processes by mutations in their regulator genes leads to abnormal cell function and human diseases.

Amongst many other genes several members of the VPS (Vacuolar Protein Sorting) gene family play key role in one or both of these processes (e.g. components of the ESCRT machinery – Endosomal Sorting Complex Required for Transport). Although these proteins have well-characterized functions in autophagy and/or endocytosis in yeast, some VPS genes have not been investigated in multicellular organisms.

Yeast *vps13* has four homologues in human. One of them, VPS13A is mutated in patients showing the symptoms of chorea-acanthocytosis, a neurological disorder associated with uncontrollable movements (chorea) caused by striatal neurodegeneration and with spiny red blood cells (acanthocytes). The exact biological functions of the VPS13A protein (chorein) are unknown.

One of the well-established model systems to study autophagy and endocytosis is the fruitfly *Drosophila melanogaster*. Yeast *vps13* has three orthologues in Drosophila, the counterpart of the human VPS13A is CG2093 (*vps13a*). Some *in vitro* data suggests a possible role for *vps13a* in endocytosis, however no mutants have been generated and reported yet.

Our aim was to generate *vps13a* mutants in Drosophila, and the investigation of the mutant phenotypes mainly in the aspects of autophagy and endocytosis.

By imprecise immobilization of a P-element in the 5′ UTR (Untranslated Region) of the gene, we generated a 2.144 base long deletion that covers the first three exons of *vps13a* (including the atg-sequence as well). Eighty percent of the flies homozygous for the mutation (hereafter referred to as *vps13Δ*) dies at late pupal stages, males have no dissectable testes. Surviving adult homozygous *vps13Δ* males have apparently normal testes but show complete male sterility. Homozygous females have swollen abdomen, crowded with eggs, likely due to the impairment of normal egg laying. Additionally, these females often show abnormal wing morphology.

At the cellular level *vps13Δ* clone cells generated in the fat body (a tissue widwly used to investigate autophagy in flies) show no changes in the number, size or distribution of autophagic structures compared to the control cells, suggesting that *vps13a* has no function in the autophagic process of this tissue.

However, abnormal wing morphology may suggest a role of *vps13a* in endocytosis. Thus, our further goal is the investigation of *vps13Δ* clone cells in the wing disc using endocytosis-specific markers.

P-011
FIP200 PROMOTES PHAGOPHORE ASSEMBLY AT PERILYSOSOMAL P62 AGGREGATES BY ACTIVATION OF ATG1 IN *DROSOPHILA*

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P-012
CHARACTERIZATION OF PRIMARY CELLS DERIVED FROM HUMAN UMBILICAL CORD

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Even today it is a big challenge to develop a method to generate stable cell lines from primary cell cultures. Most primary cell cultures, regardless of the numerous methods used to sustain them, suffer from limited lifespan and genomic abnormality. This can hinder establishing standardized experimental conditions to gain relevant and reproducible results.

We have a large collection of primary cells derived from Human Umbilical Cord. In the last three years 95 cell lines have been established, most of them derived from newborns with a genetic risk for celiac disease (parents or siblings are celiac). We have three morphologically different cell types: endothelial (HUVEC), human arteric cell (HAC) like cells and myoblast (MB) like cells.

Our study goal is to characterise these three different cell types before we use them in our experiments to identify genetic factors that contribute to celiac disease development.

To identify the three morphologically different cell types we investigated the expression of 30 cell marker proteins by FACS. Cluster analysis of the FACS results clearly separates the three cell types. HUVEC cells keep their endothelial characteristics, HAC and MB cells are more similar to an early progenitor cell type. By analysing the growth curve of the three cell types we found significant differences in their growth rates, HUVEC cell cycle length is approximately 2.5 days, HAC cells' 10 days, while MB cells show very high proliferative potential, cell cycle length is cc. 1 day. Under in vitro condition HAC and MB cells could be differentiated into different kind of muscle cells. By immuno-histochemical staining we could demonstrated that HAC cells express smooth muscle actin and cytoceratin 19, MB cells also express smooth muscle actin, but desmin and dystrophin as well. Further characterization of the cells revealed that MB cells display anchorage-independent growth, while the HUVEC and HAC like cells did not. Karyotype analyses detected genomic instability in several HUVEC cell line, aneuplody, mostly trisomy for chromosome 11. HAC cells display normal karyogram. Most interestingly, in all examined MB cell lines the chromosomes appeared abnormal, with many translocations. This may imply that the phenotypic plasticity of the cells could be based on chromosome instability, which call further research.

Our data suggest that the morphologically different primary cells derived from human umbilical cord are also differ in their growth pattern, function, differentiating potential and genomic stability. Further investigation of the MB and HAC cells can shed light on the possible lineages they could differentiate to and help identifying more precisely these cell types.

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P-013
THE ROLE OF TRANSGLUTAMINASE 2 (TGM2) IN RETINOIC ACID AND ARSENIC TRIOXIDE INDUCED DIFFERENTIATION PROGRAM OF ACUTE PROMYELOCYTIC LEUKAEMIA CELLS

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Acute promyelocytic leukemia (APL) NB4 cells can be differentiated to neutrophil granulocytes by all-trans-retinoic-acid (ATRA) treatment used as a differentiation therapy in patient with APL to result in terminal differentiation of promyelocytic cells. This differentiation method results in up and down regulation of several thousand genes to generate functional neutrophil granulocytes with abilities for quick immune responses. One of the most up-regulated genes in ATRA induced maturation of NB4 cells is transglutaminase 2 (TGM2). Silencing of TGM2 expression in NB4 cells revealed functional contribution of TGM2 to adhesion, migratory, phagocytic capacity of neutrophils and superoxide (ROS) production. (Balajthy Z, et al. Blood. 2006; Csomós K, et al. Blood. 2010)

Arsenic trioxide (ATO) has recently been identified as an effective drug for treatment of APL. ATO combined with ATRA shows a synergistic effect, which further prolongs survival of APL patients. ATO also affects transactivation of transcription factors resulting in activation of the cellular signaling pathways leading to ROS generation by the NADPH oxidase system during arsenic exposure. These events may explain the ability of ATO to induce partial differentiation and apoptosis leading the complete remission in relapsed APL patients.

Retionic acide syndrome or differentiation syndrome (DS) is a complication associated with the treatment of APL with ATRA and ATO, in which induction and secretion of CC chemokines (CCL2/MCP-1, CCL3/MIP-1a, CCL22/MDC, CCL24/MPIEF-2) and cytokines (IL1B and IL8) result in both tissues infiltration by neutrophils and then organ damage through ROS production of differentiating APL cells. Knocking down TGM2 expression in ATRA differentiated NB4 cells suppressed both chemokines/cytokines and ROS production suggesting a role of TGM2 in triggering DS (Csomós K, et al. Blood. 2010). ATRA/ATO combine treatment could further enhance the already robust induction of TGM2 by ATRA. Surprisingly, this combined treatment did not increase but suppressed ROS production. When we knocked down the TGM2 expression in ATRA/ATO treated cells ROS production was inhibited further. Future experiments can reveal the various regulatory pathways, dependent or not on TGM2, which influence ROS production in of differentiating promyelocytes upon ATRA or ATRA/APO treatment. Nevertheless, it is worth considering to modify TGM2 expression together with these therapeutic protocols for the attenuation the inflammatory phenotype of differentiation syndrome.

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P-014
THYROID HORMONE DEPENDENT DIFFERENTIATION OF M/L-CONES IN ORGANOTYPIC CULTURES OF THE RAT AND SYRIAN HAMSTER RETINA

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Purpose: Literature data indicate that thyroid hormones play an important role in M/L-cone differentiation of the mouse. Little data exist however about cone development in other species of mammals, mainly due to the lack of proper methods. In a previous work we demonstrated the expression of TRβ2 thyroid hormone receptor in the photoreceptors of the rat and the Syrian hamster. The aim of the present study was to examine if the hormone indeed controls M/L-cone development in these rodent species.

Methods: Retinas of early postnatal (P0-P4) Sprague-Dawley rats and Syrian golden hamsters were explanted onto semiporous membranes, and kept in culture for 14-28 days. Culturing media contained a 1:1 mixture of DMEM and F12, supplemented with vitamins, amino acids and hormones, with or without serum (FCS, 10%) added. Thyroid hormone (T3) was added to, or omitted from the cultures. After fixation, general retinal morphology was compared on radial sections and opsin expression patterns were analyzed using immunocytochemistry and PCR.

Results: The retina of both species exhibited a near normal differentiation pattern in control cultures, with M/L-cone densities and morphology comparable to that observed in vivo, even without serum added to the medium. The structure was also maintained under prolonged culturing conditions. Withdrawal of T3 from the media affected only the cultures lacking serum substitution, indicating that serum alone contains enough thyroid hormone to allow full differentiation. The lack of hormone under serum free conditions significantly altered the staining pattern and number of M/L-opsin expressing cells. Even after 15 days in vitro, labeling with monoclonal probe completely disappeared, and the number of elements stained by polyclonal antiserum decreased significantly. The change was more prominent after 3 or 4 weeks in culture. M/L-opsin expression could still be detected in all cultures using PCR reactions, but with a prominent down regulation of the M/L-, and up regulation of the S-opsin levels respectively, in serum and T3 free cultures.

Conclusions: Our results show that thyroid hormone plays an important role in M/L-cone differentiation also in the two rodent species reported, and indicate that it may be a universal regulator in mammals. Comparing our results with previous experiments clearly indicates that thyroid hormones although necessary, alone not sufficient, and other jet unknown factors must also pay important roles in M/L-cone maturation. The organotypic culturing technique enables us to study potential factors influencing photoreceptor development, and to point out similarities and differences in retinal maturation between species.

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P-015
PRION PROTEINS IN DIFFERENTIATING AND MATURE NEURAL CELLS

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Prion diseases include Creutzfeldt-Jacob Disease, Fatal Familial Insomnia, Chronic Wasting Disease and Bovine Spongiform Encephalopathy best known as mad cow disease. The infectious Prion protein involved in these diseases originates from a conformational change of the cellular Prion protein, which is part of the prion superfamily also including the glycoproteins Shadoo and Doppel. Shadoo, a recently discovered member of the family shares several properties with the Prion protein. However, its physiological function and possible role in prion disease are not known. Initial experimental results suggest that Shadoo and Prion protein may complement each other. We investigated the expression of Shadoo at the mRNA and protein levels and examined its subcellular localization both during in vitro neural differentiation and in the adult mouse brain. The expression patterns of Shadoo were compared to those of the Prion protein.

P-016
TRKB EXPRESSION ON THE DEVELOPING CONES OF THE RAT RETINA: MORPHOLOGICAL AND FUNCTIONAL ASPECTS

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Some results indicate that the TrkB neurotrophin receptor is expressed in M-cones of the adult rat retina. In the present study we determined the temporal and spatial pattern of cone-specific TrkB expression during development and tested if its specific blockade can affect cone differentiation.

Retinas of Sprague-Dawley rats were collected in different developmental ages and analyzed by immunocytochemistry. For the functional studies, organotypic retina cultures allowing M-cone development were prepared from P2 rats and allowed to develop in culture for 19 days. In order to neutralize the endogenous neurotrophins acting via the TrkB receptor, cultures were treated with BDNF- and NT-4-specific antibodies continuously. After histological processing, the morphology and cone densities of treated and non-treated control retinas have been compared.

The expression of TrkB receptor started at P10, at the same time when the M-opsin appeared. During development, the TrkB receptor appeared in dual cones expressing both S- and Mopsins. In adults only the mature M-cones expressed the receptor. The overall retinal architecture was well preserved both in control and anti-NT-4 treated cultures. In these groups every retinal layer was well developed and no major differences could be detected. Although the thickness of the photoreceptor layer was comparable to that of the control and anti-NT-4 treated retinas, anti-

BDNF treatment resulted in a remarkable thinning of the inner retina. The calculated M-cone density in the control cultures was ~4450 ±870 cell/mm^3.

Interestingly, neither the anti-BDNF nor the anti-NT-4 treatment had detectable effect on the M-cone number and similar density values with no significant differences were calculated in both treated groups. The TrkB receptor is expressed in developing and mature M-cones as well. Its developmental pattern suggests that neurotrophins may be involved in M-cone development and function, however, their role in the regulation of M-opsin expression is unlikely.

P-017
IMMUNCYTOCHEMICAL ANALYSIS OF MISPLACED ROD CELLS IN THE DEVELOPING RETINA OF RODENT SPECIES

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Purpose: In the developing retina of rod-dominant rodent species the rod photosensitive pigment –rhodopsin– can be detected with immunolabeling as soon as on postnatal day 1 (P1). Besides the increasing staining of the forming rods in the photoreceptor layer, an appreciable number of rhodopsin-positive neuron-like cells are also labeled in the inner nuclear and ganglion cell layers during the first weeks of postnatal development. The aim of the present study was to characterize and compare the morphology, the number and the staining characteristics of this peculiar cell population in various rodent species.

Method: Retinas of Sprague-Dawley rats, Siberian and Syrian golden hamsters and mice of early postnatal ages (P0-P24) were studied. Rhodopsin was detected by immunocytochemical labeling with various rhodopsin-specific antibodies on retinal cryosections. To investigate, if rhodopsin-positive dendritic cells can be identified with, or related to retinal cell types other than rod cells, double immunolabelings were performed with a rhodopsin antibody on one hand and with several antibodies recognizing different opsin molecules as well as distinct retinal cell types (bipolars, ganglion cells, amacrine and horizontal cells, microglia) on the other. Sections were also double stained against several proteins of the phototransduction cascade. Additionally, an antibody against caspase-3 was used to investigate the possibility of apoptosis.

Results: Dendritic, neuron-like cells expressing rhodopsin were consistently observed in the inner nuclear and ganglion cell layers of early postnatal retinas in all four species studied. They comprised a few percent of all rhodopsin-positive cells, often appeared in smaller clusters and morphologically resembled resident bipolar, amacrine or ganglion cells. They could be reliably detected using several different antibodies, raised against either the C-, or the N-terminal of rhodopsin molecule, respectively. These cells first appeared at the 4th postnatal day (P4), reached their maximum density at P14 and disappeared entirely by the 4th week. Double labeling could not confirm the presence of any other type of photopigment (cone opsins, melanopsin) in these cells. Furthermore, staining with antibodies specific to various retinal cell types showed no colocalization with bipolar, horizontal, amacrine and ganglion cells. The rod-like nature of the rhodopsin-positive dendritic cells was corroborated by the presence of rod arrestin and recoverin, other proteins of the phototransduction cascade were only detectable in the minority of the

population. These cells were shown to form synapses but although completely missing from adults, no apoptotic signs were detectable in the sections of P14-21 retinae.

Conclusions: Our results showed that during development, a few percent of all rhodopsin-expressing cells are not located in the photoreceptor layer but are displaced to the inner retinal layers. Although most displaced rods lose morphological features of photoreceptors, they contain some, but not all elements of the phototransduction cascade, indicating that they are most probably misplaced rods that fail to complete differentiation and integrate into the photoreceptor mosaic.

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P-018
ESTABLISHING EXPERIMENTAL SYSTEMS TO PROVE THE EFFECT OF MAGNETIC FIELD ON PLANT GROWTH

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The effect of magnetic fields has been clearly demonstrated for several organisms and in some cases even the molecular mechanisms were elucidated. In plants, however, it's still an open question whether the ability of magnetic field sensing evolved parallel to the sessile lifestyle and if yes, whether it has a visible influence on plant growth or development. The main purpose of our experiments was to establish a system suitable to reproducibly study the effects of magnetic field on plants both at macroscopic and molecular level.

On the basis of literature data we choose *Vicia faba* and *Arabidopsis thaliana* to investigate the effect of static magnetic field on the growth of shoot and hypocotyl, respectively. The cylinder shaped magnets were placed vertically so that field orientation was parallel to Earth's N-S axis and the homogenous field strength was ~40 mT.

Treating *Vicia* plantlets in cylinder magnets we found that the vertical force field enhanced the growth of internodes that were in their early differentiation stage when the magnetic field was applied. Except for the lengthened internodes no other alterations in growth were detected, but we observed a change in leaf shape when compared to the control.

During germination of wild type *Arabidopsis* under white light we observed hypocotyl growth inhibition in cylinder magnets. It was shown previously in the literature that the magnetic field asserts its effect through cryptochromes in several species. Therefore, to verify this hypothesis we investigated the effect of magnetic field on the double defective cryptochrome mutants *cry1-304 cry2-1* as well. In the latter we have not detected significant differences in hypocotyl length between control and magnetic field-grown plants. Based on these result we suggest that a strong static magnetic field can inhibit hypocotyl growth and this inhibition may be mediated by cryptochromes. To confirm our hypothesis, we have started to analyze gene expression on some cryptochrome-related genes (*CRY2, COP1, phyB, HY5, CO*). Depending on the results of the semi-quantitative RT-PCR we plan to expand our experiments on other cryptochrome-mediated

physiological processes and to perform histological studies to clarify the histological background of the observed morphological alternations.

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P-019
CELL-AUTONOMOUS AND NON-CELL-AUTONOMOUS HEMOCYTE DIFFERENTIATION IN *DROSOPHILA MELANOGASTER*

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The immune defense of *Drosophila* is based on the tight cooperation of the cell mediated and humoral immune responses. The origin of the specialized immune cells (the hemocytes) can be traced back to the early stages of embryonic development. In the larva, the hemocytes form hematopoietic compartments, the circulation, the lymph gland and the sessile tisse, where the proliferation and differentiation of the immune cells is taking place.

Our previous experiments revealed that the hematopoietic compartments of the *Drosophila* larva arise from separate embryonic blood cell lineages. We also found that these compartments all take part in the differentiation of the effector hemocytes, the plasmatocytes, crystal cells and lamellocytes, in response to parasitic infection.

Lineage tracing experiments showed that plasmatocytes, which were initially considered as terminally differentiated hemocytes may transform into lamellocytes following immune induction. The immune response to parasites involves significant changes in gene expression, but the molecular background of these events is sparsely characterized. It is still unclear whether the development of effector cells is cell-autonomous, or hemocytes are able to send signals to each other, and can therefore trigger a concerted action of factors in the course of blood cell differentiation.

We tackled this issue by investigating the involvement of key regulators in different signal transduction pathways in the development of lamellocytes. These experiments required a transgenic expression system, which is insensitive to gene expression changes that take place in differentiating cells. With this in mind, we used the *in vivo* lineage tracing system to silence and overexpress regulatory factors in specific hemocyte lineages. Apart from *in vivo* experiments, we also characterized the hemocytes using our molecular marker panel.

Our results revealed that the overexpression of specific factors within a cell lineage can result in the differentiation of lamellocytes independent of the target lineage (aka. non-cell autonomous differentiation), while other regulators cause the differentiation of exclusively lineage-autonomous lamellocytes. These results lend support to the idea that the differentiation of hemocytes is based on a hierarchical, multi-level regulatory network of factors.

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**These authors contributed equally to this work.*

P-020
NEW MOLECULAR GENETIC DIAGNOSTIC METHODS IN PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

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Aims: A deviation from the embryo's normalchromosomal structure (aneuploidy) standsmost of the timein the background of multiplefailed *in vitro* fertilization (IVF) cycles andmiscarriages. Errors of meiosis I and post-zygotic events are the most frequentaccountants of embryonic aneuploidy.

With advancing maternal age, a reduced chance for fertilization and decreased implantation rate can be observed, while the number of numerical chromosomal abnormalities of oocytes increases. According to the data of ESHRE (European Society of Human Reproduction and Embriology) - which are based on the examination of all the 24 chromosomes - almost 70% of women's oocytes above 40 years might carry chromosome aneuploidies. To improve the implantation rates of in vitro fertilization, preimplantation genetic screening (PGS) is increasingly being used to detect numerical chromosomal abnormalities in embryos. The comprehensive screening of 24 chromosomes by microarray comparative genomehybridization (aCGH) is a rapidly spreading technology in the preimplantation analysis of cleavage stage embryos.

Materials and Methods: ArrayCGH analysis is carried out on a single cell, which is removed from the 6-8 cells of the developing pre-embryo without affecting its viability. The aCGH analysis of the removed cells explores which embryos are affected in a certain genetic disorder (preimplantation genetic diagnosis, PGD), furthermore, the screening of the complete chromosomal structure of pre-embryos (preimplantation genetic screening, PGS) reveal possible numerical abnormalities and gross structural defects of chromosomes.

The main indications of ESHRE to perform PGS are the followings: i.) Advanced maternal age (over 36 years); ii.) Three unsuccessful IVF cycles, or the implantation of at least ten good quality embryos without a complete successful IVF cycle; iii.)multiple, recurrent spontaneous abortions without an identified reason; iv.) Severe male infertility; v.)Previous diseases in the family anamnesis, which are due to chromosomal abnormalities.

In a retrospective study, we have analyzed 421 human embryos by aCGH in order to assess the aneuploidy patterns. Day 3 embryos had one cell removed as a biopsy specimen and were analyzed.

Results: The first results from 107 Hungarian patients (ave.age: 36.69) are derived from the analysis of the above mentioned samples, out of which 28.98% were normal, 46.32% had complex aneuploidy (embryos with ≥2 chromosome abnormalities), 13.78% monosomies and 10.93% trisomies. Complex abnormal embryos significantly increased with advancing maternal age while normal embryos decreased, from 37.23% and 39.89% in patients ≤35 to 69.51% and 10.97% in patients 41 and older, respectively.

Our results on Hungarian patients show similar tendencies at numerous points with previous observations, however we should point out that this kind of investigations is in the early phase, further studies will punctuate the present results, as this technique is going to be wider.

Conclusion: The microarray technology is appropriate to examine numerical and major structural abnormalities of all human chromosomes at the same time, within 12 hours. The aim of PGS is therefore to increase the chances of embryo implantation, help to avoid multiple pregnancies, and reduce the number of spontaneous abortions by selecting and transferring embryos which are free from chromosomal defects.

P-021
DEVELOPMENT OF MT ASSAYS TO DISCOVER POTENTIAL $5HT_6$ ANTAGONISTS FROM FOCUSED LIBRARIES

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5-Hydroxytryptamine receptor 6 (5-HT_6) and is a promising target for drug development in the therapy of diseases of the central nervous system. $5HT_6$ is implicated in the pathogenesis of Alzheimer's disease, schizophrenia and mood disorders.

We initiated a research program aiming at identifying new potential antagonists of 5-HT_6. First, we developed MT assays for screening antagonists of 5-HT_6. We applied a method which followed the change of intracellular Ca^{2+} level released upon receptor activation. In this method the Ca^{2+} signal was detected by using a luminescent indicator, aequorin. In this system the apoenzyme is in complex with a hydrophobic prostetic group, coelenterazine and molecular oxygen. Upon Ca^{2+} binding, the coelenterazine is oxidized to coelenteramide releasing carbon dioxide and emitting light.

Due to the fact that random screening of a discovery library often results in a poor hit rate (≤ 0.1%) we have decided to select 5-HT_6 focused libraries for screening. 2D similarity comparison of chemical structures allows rapid selection of focused libraries from huge, commercial repositories. The $5HT_6$ target-focused libraries were selected by a multi-stage 2D selection and filtering method. First, the Reference Space was defined by collecting 49 known $5HT_6$ antagonist („seeds") from available literature and databases, and its property space was determined by calculating 6 physico-chemical parameters. The searchable Chemical Space was composed by major vendor databases (cca. 5M compounds). The 2D similarity search was performed by setting a similarity treshold (0.65 Tanimoto) and resulted in 3775 analogous compounds. Matching the „reference property space" (872) and applying diversity selection and visual inspection finally resulted in 91 compounds which were involved in the 1st round screening. At 10 μM concentration 12 compounds showed higher than 70 % antagonist activity (hit rate = 13.1 %) and 4 compounds had IC_{50} values below 1 μM. Based on the 1st round screening results considering 11 best hits we applied two methods for the selection of the compound set for the 2nd round screening. Repeated 2D similarity search and 3D pharmacophore matching resulted in 112 compounds that are currently under screening in vitro. The presentation will discuss the virtual and in vitro screening

methods as well as analyze the performance of this two-stage library generation and evaluation process.

In the poster we describe the library selection and its biological evaluation applying the assays developed.

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P-022
LIMITATION IN USAGE OF VIGS VECTORS IN DETERMINATION OF FUNCTION OF ENDOGENOUS GENES IN PLANTS

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In the post genome era the major challenge of plant biology is to determine functions of all genes in the genome. To solve this problem RNA interference based methods can be used because of their high specificity and efficiency.

RNA interference is an important defense mechanism against viruses, transgenes and other molecular parasites. Viruses induce a defense response in their hosts involving recognition by the Dicer enzyme, which cleaves long viral double-stranded RNA (dsRNA) molecules into short, 20-25 nucleotide long double stranded fragments, siRNAs. One strand of the siRNA is incorporated into RNA-induced silencing complex (RISC) which cleaves or translationally inhibit all RNAs having sequence complementarity to the incorporated siRNA. Virus-induced gene silencing (VIGS) based upon this phenomenon is one of the most widely used tools for gene function studies in plants. Application of this method starts with the constraction of recombinant virus carrying fragment of the targeted plant gene. Upon infection, the modified virus induces gene silencing not only against viral sequences but also against the inserted plant gene. This will lead to the degradation of the corresponding plant mRNAs and mutant, loss of function phenotype will appear.

However, in compatible plant–virus interaction, the virus can alter the gene expression pattern of the host. Viruses are able to induce efficient host gene mRNA down regulation ("shut-off") of important housekeeping genes, but the presence of "shut-off" differs from one interaction to another.

In our experiments we studied the effects of VIGS vectors on the host gene expression in plants. We infected *Nicotiana benthamiana*, *Solanum lycopersicum* and *Triticum aestivum* with tobacco mosaic virus (TMV), potato virus X (PVX), tobacco rattle virus (TRV), and barley stripe mosaic virus (BSMV), to find out whether "shut-off" is present in these interactions or not. Investigation of the expression level of different housekeeping genes showed that "shut-off" is present almost in all of these plant-virus interactions. These viruses served as a base for the most widely used VIGS vector systems and are widely used to study function of endogenous genes in different hosts. Target genes tested by us (GAPDH, Rubisco, elongation factor) are commonly used as reference value genes in quantitative PCR measurements in these VIGS studies. To find out that the particular

VIGS systems are also able to alter the host gene expression we have infected *N.benthamiana*, *S.lycopersicum* and *T.aestivum* with TMV, PVX, TRV and BSMV based VIGS vectors.

We can concluded that the expression of the widely used reference genes was almost always drastically down regulated. Our data point out the necessity of careful selections of both genes, to be investigated and used as a reference control in the planned experiments.

Our work was supported by OTKA (PD78049).

P-023
STUDYING THE FUNCTION OF THE SECRETORY PATHWAY DURING GERM CELL FORMATION

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In many cases the malfunction of the sperm is the underlying factor in human fertility problems. However it is a significant issue, our knowledge is rather limited on the mechanism of the molecular structure and the development of the sperm. In the testis of *Drosophila* the whole process of spermatogenesis can be studied as from stem cell through every step to the mature sperm. During the last few years many membrane traffic mutants were identified with male sterile phenotype, what suggests that proteins involved in membrane trafficking has an important role to play in germ cell formation.

Vps54 (Scat) protein, a subunit of the Golgi associated retrograde protein (GARP) complex, is participating in the retrograde transport (transport from the extracellular space or organelles of the secretory and endosomal-lysosomal system towards the Golgi and the endoplasmatic reticulum).

Our research confirmed that *scat* homozygous mutants show male sterile phenotype however females are fertile. Detailed investigations of the testes showed that cysts are normally formed at early stages of spermatogenesis in mutants, but at later stages their polarization fail and the individualization of elongated spermatids does not occur.

We found that actin cones which plays a very important role in the process of spermatid individualization were not composed in s*cat* mutants. Studying the elongating cysts we found that polarization (normally spectrin localized to one pole of the cyst and nuclei to the opposite) fail as both spectrin and nuclei shows a scattered phenotype within the cyst.

In addition the terminal part of testes shows a stronger Acridine-Orange and LysoTracker staining what may refer to apoptosis. Furthermore, no mature sperms were visible with EM in the cysts. Examining the additional function of GARP complex we found that in *scat* mutant testes the acrosome (a spermium specified organelle, recruited from Golgi and lysosome and localised to the nucleus) could not be detected.

In summary we could demonstrate the effect of GARP complex both on acrosome formation and spermatid cyst elongation and polarity.

We do hope that our research contributes to gain a better insight into the function of Golgi associated retrograde protein transport during spermatogenesis. Also studies of GARP complex

may open new perspectives as retrograde protein transport is scarcely investigated even so its importance is recognized.

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P-024
THE *DROSOPHILA* FORMIN DDAAM IS REQUIRED FOR AXON GROWTH IN THE DEVELOPING MUSHROOM BODY

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The connections between neurons are necessary for the formation of a functional nervous system. A major prerequisite of this process is the outgrowth and precise navigation of axons toward their target cells. The growing axons choose their specific path by sensing the environmental cues. In this process, the key structure is the growth cone, formed at the tip of the growing axon. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as formins, which promote actin assembly by associating with the fast-growing end of the actin filaments, and facilitate the formation of unbranched filaments.

We have previously examined the function of the *Drosophila* formin dDAAM in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that dDAAM plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. We noticed that *dDAAM* exhibits a strong expression in certain regions of the larval and adult brain as well. Specifically, in the developing mushroom body dDAAM is highly enriched in the newly born axons suggesting that dDAAM might be a general regulator of *Drosophila* axonal development. Consistently, by loss of function analysis we detected axonal projection defects in the mushroom body. To identify proteins that may act together with dDAAM in the regulation of axonal growth, we carried out a genetic interaction analysis. We demonstrated that dDAAM interacts with *Ena* and *chic*. Moreover, we identified Rac as the most likely activator of dDAAM in the developing nervous system. Our poster will provide a detailed analysis of the axonal growth defects exhibited by *dDAAM* mutants in the adult brain.

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P-025
THE RNA CAP MODIFIER TRIMETHYLGUANOSINE SYNTHASE (TGS1) IS REQUIREDFOR *DROSOPHILA OOGENESIS*

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Drosophila Trimethylguanosine Synthase 1 is encoded by the *dtl* gene, from which a single mRNA is transcribed that codes two ORFs. TGS1 is translated from the second ORF. The TGS1 protein has an unique role in the 2,2,7-trimethylguanosin modification of snRNA and snoRNA molecules. These RNA species play an important role in rRNA modification, splicing, and RNA maturing.

In the absence of TGS1 in the germline, female sterility is observed. The size and the number of the mature eggs are decreased, the dorsal appendage is shortened, and elevated levels of apoptosis is observed inthe middle of oogenesis, at stages 8-9. The developmental stages of oogenesis are shifted. Furthermore, the nuclei of nurse cells are unable to disperse their polythene chromosomes at the stage 5. As a result of these malfunctions, famales lay a small number of eggs and only a smallportion of those eggs develop to adulthood.

From these observations we conclude that the trimetylguanosine modification of a special subset of RNAs plays a crucial role in the process of polytene chromosome dispersion and the oogenesis in *Drosophila*. The fact, that in rare occasions fertile eggs can observed suggests that this modification is not essential in oogenesis, but significantly contribute to this process.

P-026
RNAI BASED SCREEN REVEALED THE ROLES OF *FASCETTO* AND *MEI-P26* GENES IN DROSOPHILA GERM LINE DEVELOPMENT

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The formation, maintenance, migration of germ cells and their incorporation into gonads have several phylogenetically conserved aspects among the animal species. The sophisticated molecular and genetic methods available in Drosophila research make this organism an ideal model for studying germ cell development. In Drosophila, more than 500 genes were identified so far that are expressed in the embryonic germ line or gonads. We performed an RNAi based functional genomic analysis of these genes. Maternally provided and zygotically expressed transcripts were silenced by microinjection of gene specific dsRNAs into syncytial blastoderm stage embryos. The resulted phenotypes were analyzed by making use of *in vivo* video microscopy. This way, we found that 48 genes have essential role in germ line development. To further investigate the function of the identified genes, we used gene and tissue specific artificial microRNAs. Silencing of zygotically expressed genes by these microRNAs revealed the role of 18 out of the 48 genes in larval and adult germ cell development.

We present the results of detailed cell biological analyses of two genes identified in our RNAi screen: *fascetto* and *mei-P26*. Silencing of a microtubule-associated protein coding gene, *fascetto* caused reduction of germ cell number in larval stage and formation of rudimentary adult gonads. The intracellular localization pattern of Fascetto protein in the germ cells suggests a role in the regulation of germ cell mitosis. *mei-P26* is a gene with known function in germ line stem cell renewal in adult gonads. We demonstrate that *mei-P26* functions in the germ line throughout the whole life cycle. We provide evidence that *mei-P26* ensures the maintenance of germ cell fate presumably by protecting germ cells from somatic differentiation.

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P-027
STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE SESSILE TISSUE IN *DROSOPHILA MELANOGASTER*

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P-028

SELECTIVE ELIMINATION AND RNAI SILENCING OF FMRFA-RELATED PEPTIDES AND THEIR RECEPTORS IN THE CNS DECREASE THE LOCOMOTOR ACTIVITY IN *DROSOPHILA MELANOGASTER*

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Three neuropeptide genes are classified in the FMRF-related (FaRP) group: the dFMRFamide-, dromyosuppressin (DMS) and drosulfakinin (DSK) genes coding for 8, 1 and 3 peptides, respectively. To compare their effects on the locomotor activity of Drosophila adults, we made RNAi knockdown of the peptides and their specific receptor genes. In addition, we constructed Gal4 drivers with three distinct parts of the FMRF gene's 5' regulatory sequence (RS8-Gal4, RS11-Gal4, RS17-Gal4), and used them to ablate FMRF-positive neurons inducing apoptosis by expressing the *rpr* gene. We examined the locomotor activity of flies by measuring the mean velocity of movement (MVM) after startling with repeated air-puffs. Locomotor activity was decreased by RNAi (double-stranded RNA-interference) knockdown induced in the CNS by the *elav-Gal4* driver. According to the MVM curve profiles, RNAi knockdown most effectively decreased the velocity when the *DMS-R1* and *DMS-R2* genes were silenced together (*RNAiDMS-*

R1/elav-Gal4;RNAiDMS-R2/+). Similar effect was observed in *RNAiDSK/ elav-Gal4;RNAiDSK-R2/+*, while moderate effects in three other combinations (*RNAiFMRF/ elav-Gal4;RNAiFR/+, RNAiDMS/ elav-Gal4; RNAiDMS-R2/+, RNAiDSK-R2/ elav-Gal4; RNAiDSK-R1/+*), and weak effect in *RNAiDMS-R2/ elav-Gal4; RNAiDMS-R1/+*. Male and female flies were not different in this respect.

In the cell ablation experiment, the MVM profiles of the female flies were different from the controls when the *UAS-reaper* transgene was driven by *RS8-Gal4* or *RS17-Gal4*. The *RS11-Gal4* and *FMRF-Gal4* drivers were ineffective. In the males only the *RS17-Gal4* showed a weak effect. RNAi silencing of the FaRP and FaRP-receptor genes effectively decreased the startle-induced locomotor activity of flies. Ablation of FMRFa-positive neurons by the *RS8-Gal4* and/or *RS17-Gal4* drivers also reduced the flies' activity.

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P-029
MLD/DTS-3 GENE IS ZYGOTIC AND REGULATES ECDYSONE PRODUCTION OF LARVAE

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We have shown the classic mutation *l(3)DTS-3* (Dominant Temperature Sensitive-3) (Holden and Suzuki, 1973) causes ecdysone (E) deficiency phenotype in larvae that can be rescued by ring gland (RG) implantation (Holden et al.,1986). At restrictive temperature (29°C) *l(3)DTS-3* shows extended L3 lethal phenotype. Upon E feeding, the mutant larvae can be rescued to form pupae. We had isolated two recessive, P insertion alleles of *l(3)DTS-3* (Deak et al,1997). *l(3)DTS-3* is also allelic to the amorph mutations of *mld* gene (*molting defective*, Neubueser et al., 2005). The phenotype of the recessive amorph alleles of *mld* is L1 lethal. In feeding/rescue experiments with *mld* alles, we found, the block in E biosynthesis is in between 7dehydro-cholesterol and ketodiol (2,22,25-trideoxy ecdysone), within the "Black box" of the pathway. The pleiotropic phenotypes of *l(3)DTS-3* include hypertrophic ring gland (Holden and Ashburner, 1978), an abnormality commonly observed in endocrine glands of mammals, if hormone biosynthesis is blocked.

The *mld* gene is not essential to embrygenesis. *l(3)DTS-3* homozygotes exhibit recessive embryo lethality and *l(3)DTS-3* females halt egg production at restrictive condition, suggesting maternal role of the gene. *mld* transcripts were detected during embryogenesis, and ovary, suggesting maternal origin of the transcript. Exploiting the Dominant Female Sterile technique, we had generated germ line chimeras with amorph *mld/mld* genotype. We found oocyte production comparable to positive control, with full fertility and normal embryogenesis. From this we conclude, *mld* gene is not essential to the embryogenesis, rather it is zygotic in nature.

The mld is hypostatic to E biosynthesis gene, *sad*. Mutations of genes coding for enzymes of E biosynthesis, like *sad* are embryo lethal, with developmental arrest at around st. 14/15 when E titer drops low. However the phenotype of amorph *mld* mutants is extended L1, without compromised embryo viability. The phenotype of *sad mld* double mutant is embryo lethal in st 15, the same time as the *sad* alone. Therefore *sad* is fully epistatic to *mld*. The lack of *mld* function in the double

mutant does not enhance the phenotype of *sad*. From these observations we conclude, *mld* gen is not involved in ecdysone biosynthesis in the embryo.

***The mld* function is required in L1 only for a short period, when ecdysone titer is high.** Conditional silencing of *mld* results extended L1 condition. The silencing was effective only for a short period of L1 development. After heath shock between 16-20h-age the larvae failed to molt, remained L1 for days, until perished. The behaviour and the characteristics of the mld-silenced larvae are the same as the *mld* amorph phenotype. The critical period coincides with the elevated E titer, known to be required for the subsequent L1/L2 molting.

The hypertrophy of ring gland in *mld* mutants is the result of the lack of ecdysone signaling. The ring gland, the source of E in larvae, is hypertrophic in *mld-/mld-* and *l(3)DTS-3/+* larvae. In *mld/mld*, *+/+* mosaics in RG, generated by Flp/FRT, marked with lacZ or GFP, no difference was seen in the size of cells or nuclei of homozygous mutant, wild type spots or heterozygous tissue in RG. We conclude, the lack of *mld* function is not autonomous to the RG cell size phenotype. Pharmacological inhibition (fenarimol) of E biosynthesis also results hypertrophic RG. Ecdysone feeding to homozygous amorph *mld*, or fenarimol treated larvae rescues the extended larva phenotype and the RG hypertrophy. This suggests the involvement of ecdysone signaling in determination of RG size. Indeed, RG specific silencing of ecdysone receptor (*EcR*), i.e. inhibition of E signaling, results hypertrophic RG. We conclude the E-EcR complex has inhibitory effect to the growth of RG.

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P-030
IDENTIFICATION OF THE MOLECULAR INTERACTION PARTNERS OF THE FORMIN DDAAM

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The formin proteins are an important and evolutionarily well conserved class of actin binding proteins with essential biological functions, including cell division, cell migration and organelle transport. In these processes the best understood molecular role of formins is to promote the nucleation and elongation of unbranched actin filaments, although some formins have also been implicated in the regulation of microtubules. We have previously shown that the single Drosophila DAAM ortholog, dDAAM, is involved in multiple aspects of trachea development and axonal growth regulation, however the molecular mechanisms underlying these morphogenetic functions remain to be uncovered. To gain a better understanding of the molecular functions of dDAAM, we aim to identify the protein interaction partners of dDAAM with biochemical and genetic methods. The biochemical interaction partners will be identified by affinity chromatography. To this end, we will use a dDAAM-Flag fusion protein by tagging the dDAAM gene *in situ* by site specific mutagenesis. To complement the biochemical approach, interaction partners will also be identified by a genetic interaction screen using the hypomorphic $dDAAM^{EX1}$ allele. The results of these efforts will be presented on my poster.

P-031
IMPACT OF *DROSOPHILA* DAAM ON LARVAL LOCOMOTION AND BODY WALL MUSCLE STRUCTURE

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Drosophila DAAM (dDAAM) is the sole DAAM formin ortholog in fruit fly. Formin proteins are important regulators of actin dynamics involved in filament nucleation and elongation. Although their molecular roles in actin regulation have been clearly demonstrated in vitro, their functions at the cellular or organism levels are still poorly understood. Our previous studies, focused on the analysis of the indirect flight muscle, revealed that dDAAM is a key player in myofibrillogenesis and sarcomere formation. To address if dDAAM plays a more general role in muscle development, we examined the larval heart tube and the body wall muscles. We analysed the dDAAM mutant larval muscles at the structural level by microscopic approaches, whereas functional studies involved the detailed examination of the locomotory activity of the larvae. We found that the sarcomeric structure is highly affected in the dDAAM null mutants, and the strictly regulated larval locomotion is also seriously altered.

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P-032
REGULATION OF GERM CELL DEVELOPMENT AND FUNCTION

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Interest in sperm function has greatly intensified for two reasons: first, it is becoming increasingly apparent that human infertility could be traced to male factors, including alterations in sperm proteins, and second, there is increasing number of empirical evidence that sperm provide essential factors, both nucleic acid- and protein-based, to early zygote development possibly beyond their function during fertilization. The basic principles of sperm structure and the differentiation of the individual spermatid components (acrosome, nucleus, mitochondria, flagellum) are conserved across the whole animal kingdom. There are a high degree of similarities between the testicular histopathology in infertile patients and certain male sterile mutants of both *Drosophila* and mouse. Male sterile mutations of *Drosophila* exhibit a broad range of phenotypes and affect all stages of spermatogenesis. *Drosophila* spermatogenesis is an excellent system in which the differentiation of germ cells from gonial precursor cells to motile sperm, including regulation of germ cell proliferation, meiosis, mitochondria derivative formation, flagella formation and individualization can be analyzed genetically. Our aim was to identify new genes involved in the early (gonial mitosis) and late stages of spermatogenesis (meiotic division, spermatid elongation and individualization), and describe the precise molecular function of these proteins. The advent of collections of precisely mapped transposon element insertion lines, which are available for almost each gene, allows us to identify male sterile mutations without new

mutagenesis experiments. The „semi-lethal" lines of the collections provide an enriched source of sterile mutations. We used P element, *piggyBac* and *Minos* element insertion lines. We categorized the available insertions based on their insertion site (exon, intron) and the mRNA expression profile. We started the fertility tests on homozygotes males and females of exonic insertions, which have high expression in testes or ovary. We tested approximately 1000 lines and found 26 male or female sterile lines. We present the genetic and molecular characterization of these lines.

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P-033
A NEW MECHANISM FOR THE HUMAN PCNA UBIQUITYLATION

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The DNA in our cells is continuously damaged by different agents. These agents are changing the structure of the DNA molecule. DNA repair mechanisms are able to set back the original structure of the DNA double helix but some damages get to the S phase of the cell cycle where they can cause the stalling of the replication fork, chromosomal breaks and cell death. To avoid these possibilities the DNA damage bypass pathway has evolved which can protect the stalled replication fork by different ways.

The main step of the pathway is the monoubiquitylation of the PCNA protein, which is the processivity factor of the polymerases by Rad6/Rad18 complex at the lysine 164 position. After this modification the replicative polymerase can be changed by an alternative polymerase, which is able to synthesize through the lesion. The monoubiquitylated PCNA can be polyubiquitylated by the Mms2/Ubc13/HLTF complex through lysine 63 residues, therefore HLTF can reverse the replication fork. On this way the stalled strand can be finished using the newly synthesized sister strand as a template.

Our study is focusing on the better understanding of the function and regulation of the DNA damage bypass pathway, focusing on the ubiquitin protein. We are trying to identify new ubiquitin conjugase and ligase proteins, which can regulate the activity and interactions between the members of the DNA damage bypass pathway through ubiquitylation. Our ultimate goal is to shed light on the whole molecular mechanism of the damage bypass.

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P-034
RAD51 INDEPENDENT D-LOOP FORMATION IN HUMANS

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Cellular DNA is continously damaged by numerous damaging agents. Since the damaged DNA often induces the stalling of the replication forks it can lead to generation of gapped DNA or double strand breaks (DSBs). The homologous recombination (HR) system accurately repairs DSBs in an error-free manner. The mechanism is based on the ability of Rad51 protein to form a presynaptic filament on ssDNA and a search for the homologous region of the sister chromatid. This filament invades donor duplex DNA and forms a D-loop structure with the help of many accessory proteins such as Rad52, Rad54 and Rad55/Rad57.

The repair of the stalled forks is also coordinated by Rad6/Rad18 pathway through damage bypass using translesion (TLS) polymerases or template switch (TS) mechanism. Template switch is thought to be facilitated by fork regression or by a strand invasion dependent mechanism. Fork regression has been shown to be carried out by HLTF but the strand invasion dependent sub-branch is not well characterized. Here we report that HLTF also facilitates the DNA strand invasion of the homologous region and formation of a D-loop structure. The 3' end of the invading strand is then used by a polymerase for the extension. The HLTF-dependent D-loop formation and its consequent extension are not dependent on the classical HR enzymes such as Rad51 and Rad54, and only oversaturated amount of RPA is inhibiting it. Therefore HLTF can be responsible not only for the fork regression but also for the strand invasion dependent mechanisms of the template switching.

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P-035
C1ORF124 A NEW REGULATOR OF THE PCNA UBIQUITYLATION

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Stalled replication machinery on the DNA is a critical threat to the cell, since it can collapse, leading to the accumulation of genetic changes or cell death. Stalling can occur when the replicative polymerase is unable to process beyond a particular point for any reason, such as when DNA damage is encountered through which the polymerase cannot replicate. Upon stalling of the replication fork cell will die if there is no resolution to this problem. However, there are several strategies that the cell may employ to rescue the replication fork. These are often collectively called damage tolerance pathways, since the lesion is not repaired, but "tolerated" as the cell finds a way

to overcome the defect of replication stalling. These mechanisms include DNA damage bypass, homologous recombination (HR)-dependent repair and non-homologous end-joining (NHEJ)-dependent repair to deal with fork collapse. Although replication stalls frequently a delicate balance of damage bypass, homologous recombination and non-homologous end-joining could ensure survival and at the same time effectively prevent increased mutagenesis, gross chromosomal rearrangement, and carcinogenesis.

Genomic instability has been documented as a preceding step for multiple inactivations of tumor suppressor genes and activations of proto-oncogenes that can lead to cancer. In our study we are focusing on the regulation of the ubiquitylation of PCNA to give more insight into the regulation of DNA damage tolerance pathways. We identified a new player which have role in regulation of PCNA ubiquitylation.

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P-036
DETECTION OF PRESENCE AND REMOVAL OF INTERSTRAND CROSS LINKS DURING POSTREPLICATION REPAIR IN HUMAN CELLS BY REVERSE BROMODEOXYURIDINE (BRDU) COMET ASSAY

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Covalent crosslinking of the two strand of DNA, known as interstand cross-links (ICLs) caused by cisplatin, mitomycin-C and other anticancer agents, act as bidirectional barriers to polymerases, thereby blocking DNA replication and transcription. Multiple biochemical activities must be orchestrated to excise these lesions and restore the original DNA sequence. In mammalian cells, much repair of ICLs occurs during S-phase, coupled to replication. Failure to repair ICLs can lead to cell death and genome abberrations. Inaccurate ICL repair is a characteristic of individuals affected with a genetic disorder, named Fanconi anemia (FA). Until recently, 15 genes have been identified, that when mutated, result in FA or FA-like syndrome, and the coordinated interaction of the FA proteins was called Fanconi anemia pathway. The processing of ICLs require concerted action of the Fanconi anemia pathway, excison repair, translesion synthesis and homologous recombination but the participants of this mechanisms and their mode of actions is far not understood yet.

Here, we presented a modified version of comet assay which was able to identify the presence of crosslinks in human cells in S-phase. The reverse comet assay, based on combination of BrdU labeling and DNA releasing effect of a H_2O_2 treatment, was also capable of detecting the kinetics of formation and removal of ICLs at single cell level. Our method was applied to follow the postreplication repair (PRR) of ICLs after treatment with crosslinking agents. With this method we have detected different level of defficiency in PRR of ICLs in various human cell line bearing mutation in relevant genes of cross-link repair.

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P-037
POSSIBLE ROLES OF DAMAGE BYPASS GENES IN INTERSTRAND CROSS-LINK REPAIR

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Chemotherapeutic interstrand cross-linking agents like cisplatin, 8-mop + UVA or the nitrogen mustard are not just able to alter bases, but they are able to crosslink both strands of DNA which is inhibiting the separation of DNA strands. Therefore interstrand cross-links (ICLs) are probably the most effective replication blocking damages which are able to inhibit replication from both directions. In case of yeast and bacteria, a single unrepaired ICL leads to cell death. We addressed the question, how *Saccharomyces cerevisiae* could deal with ICLs, during replication. Studies of ICL damage repair in yeast identified numerous genes involved in the processes including NER genes, *Rad1*, *Rad3*, *Rad4*, *Rad14*, checkpoint genes, *Mec1*, homologous recombination genes, *Rad51*, *Rad52* and also damage bypass genes *Rad18*, *Rev3*, *Rev7*.

During our experiments we were focusing on the characterization of damage bypass genes and their relationship with other repair genes in case of ICL damage, with the intent to find the most prominent ones. We also tested the epistatic relations of the players in *Rad6-Rad18* pathway. Our data suggesting that the damage tolerance pathway has exceptional role in the survival of ICL damage. According to our current model, the preferences of the damage bypass system in case of crosslinking agents, is very close to the preferences in case of other agents, like MMS.

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P-038
URACIL CONTAINING DNA IN HOLOMETABOLA INSECTS

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Uracil can incorporate into DNA via two mechanisms: instead of thymine, through DNA polymerases or via cytosine deamination. Base-excision repair and regulation of nucleotide pools are responsible for the prevention of uracil accumulation in DNA. The two key enzymes in this process are uracil-DNA glycosylase and dUTPase [1]. In Holometabola insects UNG, which is primarily responsible for the recognition and excision of uracil, is not encoded. In addition, there is no dUTPase expression in differentiated tissues of fruit fly. It is only expressed in actively dividing tissues [2]. As a consequence of lack of these two enzymes, we measured high uracil content in fruit fly larvae. It was demonstrated that dUTPase silencing causes cell death and DNA fragmentation in dividing tissues that induces pupae lethality [3].

In my work, I aim to extend our knowledge about uracil DNA processing not only in fruit fly but also in other insects belonging to different taxonomical categories among Holometabola. To achieve this, I would like to determine genomic uracil content and dUTPase expression levels of embryos and different stages of larvae. I would like to examine this in the following insects: another midge *Drosophila virilis*, the silkworm (*Bombyx mori*), a butterfly that has industrial significance, an agricultural pest beetle *Tribolium castaneum*, and the western honey bee (*Apis mellifera*). To measure the uracil content, I use an approach, which uses a qPCR-based assay that takes advantage of uracil-DNA discrimination of *Pyrococcus furiosus*'s DNA polymerase [4]. Furthermore I am also trying to develop a robust and fast dot-blot based technic, using uracil-specific binding of a catalytically inactive mutant UNG. With this method, I would like to quantify uracil content of DNA.

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P-039
DROSOPHILA LONG ABCC PROTEIN, DMRP, GIVES INSIGHT TO UNIQUE INTRA-MOLECULAR DOMAIN INTERACTIONS OF ABCC PROTEINS

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Eukaryotic ATP Binding Cassette (ABC) transporters are ubiquitous multi-domain export pumps. They transport a wide range of structurally unrelated compounds at various physiological barriers, such as the Blood-Brain Barrier. Moreover, they confer multidrug resistance to cancer cells, therefore are the major obstacle of chemotherapy. The functional ABC transporters consist of two Nucleotide Binding Domains (NBDs) and a minimum of two transmembrane domains (TMDs). Membrane spanning TDMs form the substrate binding sites and translocation channel, and cytoplasmic NBDs fuel the transport process via ATP hydrolysis. Among ABC proteins C-type ABC transporters are characteristic for their unprecedented and yet un-revealed transport mechanisms.

DMRP, the only "long ABCC-type protein" in *Drosophila*, has a magnitude higher activity in *in-vitro* assays than its human homologues. Taking advantage of its enormous activity we used wild type DMRP and DMRPs harbouring mutations in the critical Walker-A motives, as a model to gain insight to the enigmatic mechanism of C-type ABC transporters.

We have provided the first detailed characterization of the N-terminal conservative Walker-A motive mutant C-type ABC protein reporting altered kinetics and retained substrate and inhibitor

patterns. Moreover our study revealed a so far unreported complex modulatory effect of substrates on the catalytic cycle of DMRP. Our findings provide detailed experimental evidences for unprecedented intra-molecular crosstalk of the nucleotide binding domains and the transmembrane domains of C-type ABC transporters that results in their unique transport mechanism.

P-040
THE ROLE OF PARP10 ENZYME IN THE MITOCHONDRIAL METABOLISM

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Poly(ADP-ribose) polymerase-10 (PARP10), belongs to the super family of poly ADP ribose polymerase (PARPs). The encoding gene of PARP10 is located on chromosome 8 in humans. The translation of PARP10 gene results a 109kDa size protein. This protein is composed of a DNA binding domain and a catalytic domain. PARP10, regulate gene transcription by altering chromatin organization by mono-ADP-ribosylating to histones and other proteins. PARP10 overeypression leads to apoptosis in Hela cells. Recently the involvement of PARP10 in the regulation GSK3 activity had been proposed. As GSK3 is an important metabolic regulator, we aimed to investigate the role of PARP10 in the regulation of cellular energy homeostasis.

We used a Hela cell line harboring an inducible PARP10 overexpression construct. PARP10 expression was induced by doxycyclin (DOXY) treatment (2 mg/ml, 8 hours). The induction of PARP10 by DOXY decreased mitochondrial membrane potential as evidenced by DioC6 and TMRE measurements. Interestingly, superoxide production did not increase as mitochondrial membrane potential decreased. We continued the characterization of mitochondrial function by assessing changes in mitochondrial DNA, whereby we observed the reduction in mitochondrial DNA in DOXY-treated PARP10 cells. Currently we are trying to identify whether there are mitochondrial genes showing characteristic changes upon the induction of PARP10 overexpression that could provide a possible explanation for the blunted mitochondrial. As another possibility we assessed the activity of certain cellular energy sensors and found the dysregulation of Akt whereby GSK3 phosphorylation was reduced further accentuate inhibition of GSK upon PARP10 overexpression.

The metabolic reactions governed by PARPs is ever growing (reviewed in Bai and Cantó, Cell Metab. 16:(5) 290-295. 2012) and hereby we identified PARP10 as a novel “metabolic PARP”. It is known that PARP1 impacts on mitochondrial activity. PARP1 leads to cell death wherein the mitochondrial dysfunction is a major component. Interestingly, cell death evoked by PARP10 overexpression does not share the same features as the one induced by PARP1. Namely we did not observe mitochondrial uncoupling that suggest a markedly different mode of cell death. As future plans, we will continue explore rearrangements in cellular metabolism upon PARP10 overexpression and depletion and assess molecular determinants of these changes.

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P-041
INFLAMMATION INDUCED EPITHELIAL-TO-MESENCHYMAL TRANSITION: HOW THE MESOTHELIAL CELLS GAIN A MIGRATORY CHARACTER

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Intraperitoneal injection of Freund's adjuvant induces acute peritonitis resulted in significant morphological changes on the mesothelial cells: the flat, simple squamous cells became rounded, cuboidal shaped, many of them have lost their connection with the neighbouring cells and detached from the basement membrane, they start to express macrophage markers (ED1, OX43 and CD68). The cytokeratin (epithelial cytoskeletal marker) was gradually replaced by vimentin (mesenchymal intermediate filament protein), indicating that mesothelial cells gradually lost their epithelial character and gained mesenchymal phenotype. It is well known that during epithelial-to-mesenchymal transition (EMT or transdifferentiation), epithelial cells lose their apical and basolateral polarity, break their intercellular tight junctions and degrade basement membrane components to become migratory mesenchymal cells. The TGFß signalling seems to play important role in this inflammation induced EMT.

In our recent work we studied the mechanism leading to break down the tight junctions and the degradation of the basement membrane. We supposed that during elimination of the tight junctions, occludin (one of the main tight junction protein) is internalized. Using double labelling immunocytochemistry we followed the intracellular trafficking of occludin. We found that both clathrin coated vesicles as well as caveolae showed occludin labelling. At the third day of inflammation the internalized occludin was present in EEA1 positive early endosomes. A few occludin labelling was detected in CD63 and Rab7 positive late endosomes as well, indicating that a small amount of occluding was degraded. The urokinase–type plasminogen activator receptor (uPAR) regulates proteolysis cell-ECM interaction and controls migration. By the time of the inflammation the expression of the urokinase receptor (uPAR) in mesothelial cells is increased. As the inflammation progressed strong caveolin-1 labelling was found inside of the cytoplasm (in perinuclear localization) indicating that inflammation induced caveolar internalization that might be a determining step of the signalling events.

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P-042
DETECTION OF FLUORESCENT NANOPARTICLES IN BIOLOGICAL MATERIAL BY FLUORESCENCE SPECTRUM ANALYSIS

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Nanoparticles have unique properties what follow from their sub micron size and surface characteristics. These characteristics make them eligible candidates for various biomedical applications. While targeted delivery of nanoparticles has increasing importance in therapeutic applications, the distribution and clearance of nano sized particles in the living organisms is not fully understood.

Large-scale imaging of nanoparticles at the cellular level faces many difficulties. Here we present data on optimalisation of spectral imaging (Nikon A1R Confocal Laser Microscope System) method to visualise and determine the location of fluorescent nanoparticles in cells and tissues. The procedure allowed demonstration of 40-60nm size fluorescent nanoparticles in different type of neural cells and mouse tissues.

In order to get optimum resolution, the fluorescence spectrum of particles were determined under different conditions including dry state, suspended in PBS or protein containing solutions, in interaction with fresh tissue slices, in contact with moviol mounting material, and at different instrument settings with changing the excitation wavelength, optical filters and detection ranges. We found that because of strong auto-fluorescence, the wavelength providing maximum excitation of particles does not necessarily results in optimum resolution.

Corrected spectral analysis was able to distinguish particle specific fluorescence from cell auto-fluorescence. After identifying particles, Z-stack analysis was carried out to separate active cellular accumulation of nanoparticles from surface adhesion of aggregated forms.

P-043
DYNAMIC COMPARTMENTALIZATION OF THE KV 4.X CHANNELS, HMGB1AND SAP97 ALTERED DURING STRESS EFFECTS

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Background and aim: The transient outward potassium current I(to) (encoded by Kv4.3 (KCND3) plays an important role in the repolarization phase I of cardiac action potentials in the heart.

Extracellular high-mobility group box-1 (HMGB1) is a potent innate signal for the initiation of host defence or tissue repair. This nuclear factor released by necrotic cells but its role in the injury of the heart remains unclear. The synapse-associated protein 97 (SAP97) anchors the Kv-type ion channels regulating the subcellular localization of channels. We compared the expression and distribution of Kv4 channels and their modulators like HMGB1 and SAP97 in the presence of 3-anhydroophiobolin A (3-AOA) as an external stress by qRT-PCR and immunofluorescence in cardiomyocytes and malignant cells. 3-AOA is a member of the ophiobolin complex of secondary fungal metabolites, and it possesses antitumor, antibacterial, antifungal activities.

Objectives: Human ventricle samples and cell lines were analysed at mRNA and protein levels.

Results: mRNAs of Kv 4.2 and Kv4.3 α-subunits were slightly down-regulated but KChIP2 β-subunits decreased significantly to 50% in the dilated cardiomyopathic (DCM) patients versus controls. Immunostaining revealed that the SAP97 and both Kv4.3 and Kv4.2 channel subunits colocalize at the sarcolemma of undiseased cardiomyocytes and the distribution of these complexes has changed in the dilated cardiomyopathic (DCM) heart. HMGB1 has a strong effect as well as the 3-AOA for Kv4 ion channel expression in cardiomyocytes and malignant cells.

Conclusion: DCM remodels the expression of Kv4 channels. HMGB1 plays a major role of the injury of myocytes and malignant cells resulting in the activation of proinflammatory pathways and enhanced myocardial damage representing possible novel therapeutic strategy in cardiomyopathic injury and malignancy.

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Keywords: I_{TO} current, Kv4 ion channels, HMGB1, SAP97, ophiobolins, cardiomyopathy

P-044
FROM PROCARYOTES THROUGH HORIZONTAL GENE TRANSFER TO ASPERGILLOSIS

Designing of uniqe single and dual copy TaqMan qPCR assays for the detection and identification of *Aspergillus fumigatus* and *A. terreus* species based on the detection of the Streptomyces factor C orhologs

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Despite the rapid development in antifungal therapy during the past decade invasive aspergillosis is the main cause of infection-associated mortality in patients being treated with haematological malignancies and is an emerging disease in solid organ transplant recipients. Mayor causative agents of the highly devastating systematic mycoses are mainly the opportunistic filamentous fungi of the *Aspergillus genus*, such as *Aspergillus fumigatus*, *A. terreus.* The saprophytic *Aspergillus* species are ubiquitous in our environment. The exposure to *Aspergillus* spores or conidia is therefore almost constant. Due to the immunocompromised state of these individuals aspergillosis can become invasive and cause systemic infections. In spite of the fact that in the status of the primary disease improvement may appear, the secondarily evolved infections lead to death. The only means of the survival is the antifungal therapy initiated early enough. For an improved patient outcome early, sensitive and reliable diagnosis is mandatory. The most reliable microbiological and histopathological methods are time consuming, signs and symptoms of systemic diseases caused by *Aspergillus* species are non-specific and patients are often unable to undergo invasive diagnostic procedures. Real-time PCR technique is a highly sensitive method detecting small amount of fungal DNAs in biological samples and supporting diagnosis. We have developed species specific, highly sensitive quantitative real-time PCR diagnostic assays for detecting and identifying *Aspergillus fumigatus* and *A. terreus* species in different biological samples. The assays are based on the detection of the fungal orthologs of the Streptomyces facC gene. Due to the fact that facC orthologs are almost exclusively found in *Aspergillus fumigatus* and *A. terreus*, the high rate of false positive results that is given from the presence of other pathogen species obtained by using other multi copy target genes in DNA based assays will be reduced to zero. Our real-time assays are able to detect 1-5 GE in biological samples both in manual and in automated DNA extraction systems. Routine clinical tests are in progress.

P-045
INVESTIGATION OF MISSENSE DISEASE-CAUSING MUTATIONS OF ABCC6 *IN VITRO* AND *IN VIVO*

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The ABCC6 transporter, a member of the ABC protein family, is expressed mainly in the liver and to a smaller extent in the kidney and small intestine, in the basolateral plasma membrane compartment of the cells. It has been demonstrated that mutations in the *ABCC6* gene are responsible for the development of pseudoxanthoma elasticum (PXE OMIM 26480). Furthermore, a missing allele of *ABCC6* is a genetic risk factor in coronary arterial disease (CAD), and recently mutations in the *ABCC6* gene were found to be the genetic background of Generalized Arterial Calcification of Infancy (GACI).

In order to better understand how mutations in the *ABCC6* gene lead to the development of abnormal calcification symptoms, we have been investigating the biochemical properties and the subcellular localization of the wild type (wt) transporter and ten disease-causing mutants. We have established a complex experimental strategy to determine the structural and functional consequences of disease-causing mutations in the human ABCC6 protein. The transport activity of the mutants was determined by biochemical transport assays. Subcellular localization of wt and mutant proteins was investigated using MDCKII cell lines overexpressing the transporter *in vitro*, and also expressing the protein in mouse liver via hydrodynamic tail vein injections *in vivo*. The major aim of our study was to identify mutants with preserved transport activity but failure in intracellular targeting, as these mutants are candidates for functional rescue. Sodium 4-phenylbutyrate (4PBA), an FDA-approved drug, has been shown to act as a chemical chaperon and to restore the reduced cell surface expression of certain mutated plasma membrane proteins. For example it can partially restore the cellular trafficking (thus function) of the ABCC7 cystic fibrosis mutant (DF508).

We tested both in our *in vitro* and *in vivo* systems whether 4PBA treatment could restore the plasma membrane targeting of ABCC6 mutants that retained substantial transport activity. The mutants were also tested for functional rescue in a zebrafish model system.

The data we present here demonstrate that the combination of *in vitro* and *in vivo* approaches we developed are suitable for comprehensive studies of disease-causing missense mutations of the human ABCC6 transporter. These experimental systems are also suitable for testing pharmacological compounds with the aim of improving the localization/stability of human mutant proteins, which may open new ways for clinical-oriented research.

P-046
INFLUENCE OF THE LIPID ENVIRONMENT ON THE ACTIVITY OF THE HUMAN ABCG2 MULTIDRUG TRANSPORTER PURIFIED AND RECONSTITUTED IN LIPOSOMES

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ABC transporters are membrane proteins found in nearly all kinds of living organisms, from bacteria to mammals or plants. These proteins have two conservative intracellular ABC domains responsible for energizing the transport process by hydrolyzing ATP in cooperation. Transmembrane domains are less conservative and they ensure transported substrate recognition by the transporters. Substrate binding, transport and ATP hydrolysis are connected in a complicated transport cycle. Multidrug transporters are involved in the chemical defense mechanism of cells by pumping out several different, mostly hydrophobic, but otherwise chemically unrelated toxic compounds. These transporters have important roles in developing multidrug resistance, which is a major problem in the therapy of several diseases, including tumors or chronic infections. ABCG2 is one of the three important multidrug transporters localized in several therapeutically important tissues, e.g. the intestine, the blood-brain barrier, tumor cells, stem cells, hepatocytes and kidney cells. We have studied the substrate interactions, transport cycle and structure-activity relationships of the ABCG2 protein. Earlier we established *in vitro* experimental systems by producing the human ABCG2 protein in insect cell cultures. Isolated membrane fractions of these cells were suitable for measuring ABCG2 ATPase and drug transport activity. Recently we established a new *in vitro* system by using a purified, reconstituted ABCG2 protein. Polyhistidine tagged ABCG2 was solubilized from insect cell membranes and isolated by affinity purification. Purification of functional membrane proteins is a difficult task; therefore a careful optimization was required for maintaining ABCG2 functionality. We found that the use of mild detergents and excess lipids was the key factor in retaining the activity and the homodimer structure of ABCG2 throughout the process. The transporter had no measurable activity in a detergent, but regained activity after reconstitution in a proper lipid environment. We found that optimum transporter activity was obtained in natural lipid extracts, obtained from either E. coli or mammalian brain. Moreover cholesterol was an essential component for obtaining maximum ABCG2 activity. In insect cell membranes ABCG2 shows a significant drug-independent, so called "basal" ATPase activity that can be increased by substrate drugs. Experiments by using the reconstituted ABCG2 clarified that this basal activity is not caused by an unknown drug-substrate present in the insect membrane, but it is an intrinsic feature of the ABCG2 protein. We found that this basal ATPase activity was reduced by different sterol-derivatives, e.g. bile acids, while substrate-stimulated ATPase activity was unaltered. All these findings show that the ABCG2 multidrug transporter activity is fine-tuned by the lipid environment. Our new *in vitro* system establishes new functional assays for assessing the lipid effects. The ABCG2 protein reconstituted in liposomes can be directly used in selective vesicular transport assays. The generation of the functional, isolated and purified ABCG2 protein is an important step for allowing structural studies.

P-047
A MEMBRANE PROTEIN IN THE NUCLEUS

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Prion and Shadoo proteins, which belong to the prion protein super family, are membrane proteins. They have a secretion signal on their N-terminus therefore they use the secretory pathway for folding and trafficking. On their C-terminus they have a gycosylphosphatidylinositol (GPI) anchor which attaches them to the cell membrane. While studying the subcellular localisation of these proteins with the use of fluorescent protein tags we found the Prion protein in the cell membrane of mammalian cells but, surprisingly, the Shadoo protein showed nuclear localisation in a fraction of cells in addition to the normal membrane localisation. My aim was to explore the cause of this duality.

I examined the localisation of several fusion fluorescent protein constructs in mammalian cells to reveal which part of the Shadoo protein is responsible for the extraordinary localisation. There are particular segments in both proteins which are capable to direct them to the nucleus from the cytosol but the question remains: How can a secretory protein get out of the secretory pathway to the cytosol?

My results suggest that the GPI anchor signals of the two proteins have different effects on the nuclear localisation of secretory proteins: in the presence of the Prion protein GPI signal the nuclear localisation is almost entirely eliminated. In contrast, the presence of the Shadoo protein GPI signal on the same protein results in an increased nuclear localisation. Our results suggest that there may be a pathway for proteins to transit from the secretory pathway to the cytosol. This may be of great importance concerning proteins which are involved in signal transduction or which have dual functions. Our results call for further examinations to reveal the mechanism of this transition.

P-048
PROTEIN PHOSPHATASE Z IS INVOLVED IN THE OXIDATIVE STRESS RESPONSE IN FUNGI

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The catalytic subunit of protein phosphatase Z (PPZ) is encoded by the *ppzA* gene in the filamentous fungus *Aspergillus nidulans* and by *phzA* in the closely related opportunistic pathogen *Aspergillus fumigatus*. When PpzA and PhzA were expressed in *Saccharomyces cerevisiae* or *Schizsaccharomyces pombe* they partially complemented the deleted phosphatases in the *ppz1* or the *pzh1* mutants, and they also mimicked the effect of Ppz1 overexpression in *slt2* MAP kinase deficient *S. cerevisiae* cells. Although *ppzA* acted as the functional equivalent of the known PPZ enzymes in *S. cerevisiae* and *S. pombe*, its disruption in *A. nidulans* did not result in the expected phenotypes since it failed to affect salt tolerance or cell wall integrity. However, the inactivation of *ppzA* resulted in increased sensitivity to oxidizing agents like *tert*-butylhydroperoxide, menadione, and diamide. We assessed whether the new phenotype is specific to *A. nidulans* or is generally valid. We found that the deletion of the orthologous PPZ genes *S. cerevisiae PPZ1* and *Candida albicans CaPPZ1* also caused oxidative stress sensitivity. Thus, our work reveals a novel function of the PPZ enzyme in *A. nidulans* that is conserved in very distantly related fungi.

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P-049
LOWER MAINTENANCE ENERGY REQUIREMENT OF STREAMLINED-GENOME *E. COLI* COMES AT THE EXPENSE OF REDUCED ADAPTATION CAPABILITY

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Using approaches of synthetic biology, we are focusing on the rational large-scale remodeling of the genome of *E. coli* K-12 (Pósfai et al., 2006, Science). A cell with a streamlined, semisynthetic genome could serve as an improved model organism, and as a programmable cellular chassis for industrial applications. It was hypothesized that deletion of the mobile genetic elements and other

unneeded genomic islands would result in a cell displaying lower complexity, higher genetic stability, and lower energy consumption. Lower complexity is evident, higher genetic stability has been previously demonstrated (Umenhoffer et al., 2010, Microb Cell Fact; Csörgő et al., 2012, Microb Cell Fact). Regarding cellular energetics, we show here in a series of growth experiments in a chemostat that the streamlined-genome cell has indeed a reduced maintenance (zero growth) energy requirement, presumably due to the elimination of unnecessary, energy-consuming processes (e.g., lack of flagella synthesis). However, when the energy consumption is compared to that of the wild-type strain, this gain is diminished by the lack of ability to further adapt to the growth conditions. Streamlined-genome strains display a low initial maintenance energy requirement, which than remains basically unchanged for a hundred generation. In contrast, wild-type cells display a higher initial maintenance energy requirement, but quickly develop genetic changes that result in a phenotypically heterogeneous population and in a decrease of energy consumption. Whole-genome sequencing of these adapted cells is underway to elucidate the genetic basis for this difference in the adaptation capabilities of the wild-type and streamlined-genome strains. In conclusion, our experiments show that artificially created lower complexity and higher genetic stability come at the expense of reduced adaptability.

P-050
THE JANUS-FACE OF PROPHAGES IN THE *E. COLI* BL21 STRAIN

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P-051
A SURPRISING ROLE FOR MG^{2+} TRANSPORT IN THE REGULATION OF THERMOTOLERANCE IN *SALMONELLA*

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As part of the investigation of the determinants of thermotolerance in *Salmonella enterica* serovar Typhimurium, we isolated *chr* (*c*onstitutively *h*eat *r*esistant) mutations that substantially increased the resistance of the cells to lethal high temperature. Two of these mutations, *chr-1* and *chr-3*, are in the transcriptional control region of the *mgtA* Mg^{2+} transport gene of the organism, and the third one, *chr-2*, is the transcriptional control region of the *mgtCBR* operon, in which the *mgtB* gene specifies a paralogous Mg^{2+} transporter. The *chr-1* and *chr-3* mutations cause high level constitutive transcription of the *mgtA* gene, and *chr-2* confer high level constitutive expression of *mgtCBR*. We obtained physiological evidence suggesting that the *chr* mutations increase the cytosolic free Mg^{2+} concentration. Mg^{2+} has been implicated to be an important regulator of pathogenesis genes in *Salmonella*, and our results now reveal that this cation also has a role in the regulation of thermotolerance. Increased Mg^{2+} accumulation might enhance thermotolerance by protecting the integrity of proteins or membranes, by mitigating oxidative damage, or by acting as an inducer of thermoprotective functions.

P-052
SUPPRESSION OF OXIDATIVE MUTAGENESIS INHIBITS THE EVOLUTION OF ANTIBIOTIC RESISTANCE IN MUTATOR BACTERIA

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In recent years it has been demonstrated that antibiotics are very active participants in the emergence of resistance against them, through increasing genetic variation by means of mutations. Recent works have established that the final lethal effect of the bactericide antibiotics is due to a common mechanism, the generation of highly deleterious reactive oxygen species (ROS), which subsequently leads to cell death.Based on these findings the same works suggest potentiation of oxidative stress as a possible strategy to enhance the efficiency of current antibiotic strategies.

However, the lethal effect of ROS production is only one side of the coin since the same process is also responsible for providing the mutation-supply for the evolution of antibiotic resistance.By employing series of short term laboratory evolutionary experiments we demonstrated that suppression of antibiotic mediated oxidative mutagenesis by the ROS scavenger thioureasignificantly decreases the capacity of *Escherichia coli* to develop resistance against a wide range of bactericide antibiotics. Additionally we show that this effect applies to mismatch repair deficient mutatorpopulations as well, which are known to be facilitators in the emergence of resistance during clinical infections.

Our work indicates that the mutagenic effect of ROS generated by antibiotics is a critical promoting factor in the evolution of resistance. Hence not the potentiation but on the contrary, the suppression of oxidative damage may afford for a novel therapeutic strategy to arrest the rise of resistant bacteria.

P-053
ORIENTATION EFFECT IN THE TRANSPOSITION OF IS30 – ROLE OF SUBTERMINAL SEQUENCES

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The mobile genetic elements (transposons and IS elements) play important role in horizontal gene transfer, in generation and movement of pathogenicity islands and in re-shuffling of genes, finally resulting in the appearance of new bacterial variants. The mobile element IS30 belongs to a growing class of ISs known to transpose through an intermediate formed by abutting the inverted repeats (IR) of the element. These intermediates, minicircles or IS-dimers, carry a joined left and right IR junction with a 2-bp spacer between the ends. The formation of minicircles or dimers can be explained by a site specific deletion, where the IRs of the element or the transposon join to each other. The activity of IS30-based transposons are influenced by the relative orientation of the IS elements (head-head; tail-tail; head-tail). While the head-tail transposons harbouring two IS30 copies in same orientation may transpose as a unit, the head-head and tail-tail transposons of IS30 showed no detectable transposition activity. Moreover, the targeted end in the integration reactions determines the direction of IS30 insertion. The target and the integrating IS30 copies are always attached by their left and right ends leading to a head-to-tail orientation of the elements by joining their left and right inverted repeats. All of these observations suggest, that IS30 able to distinguish between own ends resulting in a so called “orientation effect”.

Here, we demonstrate that the enhancer elements identified previously in the subterminal part of IS30 are the main cause of the orientation effect. These enhancers are located within the 51 bp internal part next to the left IR (AAAC repetitive elements) and within the 17 bp internal part next to the right IR (decanucleotide, 5’-GAGATAATTG-3’). First, it was demonstrated that the nucleotide differences between the 26 bp left and 26 bp right IR sequences do not influence the joining activity. Additionally, both the 26 bp left and right IRs (without the enhancer sequences) are able to form junctions both in vivo and in vitro. However, we can not detect in vivo the connections between the same 68 bp left, nor the same 65 bp right IR ends if they contained the enhancer sequences. On the contrary, the joined left-left and right-right junctions were observed in

"in vitro" experiments. Based on these results, it was predicted that the connection of identical IS*30* ends is significantly inhibited, but not rejected by the presence of the enhancer elements. Moreover the "incorrect" left-left or right-right end connection results in 100-120 bp palindromic sequences in the transposition intermediates, which stimulated deletion formations of the plasmids used. Consecutive reaction of these unstable intermediates (integration into the target sequence, deletions, inversions) may eliminate these palindromic sequences, thus a kind of transposition product (usually deletions) can be isolated.

The role of repetitive sequences was also confirmed by in vivo transposition experiments using a transposon harbouring the minimal 26 bp IR of IS*30* together with an indifferent repetitive DNA sequence. It can concluded, that the joining of identical ends of longer than 65 bp occurs not only at a reduced frequency, but causes serious stability defect.

All of these data indicate that the subterminal sequences have a specific role in the orientation effect.

P-054
GENERATION OF GENETIC DIVERSITY IS THE PRIMARY FUNCTION OF THE INDUCIBLE DNA-POLYMERASES IN*E. COLI*

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In response to stress, the level of three minor DNA-polymerases (PolII, PolIV, PolV) increases 10-20-fold in *E. coli*. These polymerases have the ability to synthetize DNA on damaged templates bypassing replication blockages, however, they introduce mutations during translesion synthesis. PolIV and PolV perform low fidelity synthesis, and the generally accurate PolII is also error-prone at certain lesions. While mutagenesis is mostly harmful, it can contribute to the survival of the population by generating beneficial mutations occasionally. It has been a question for a long time, what the main function of inducible polymerases can be: rescuing stalled replication forks or generating genetic diversity under stress. Literature data are controversial: some studies indicate that their primary role is to rescue cells from replication arrest and the mutagenesis is an unavoidable consequence of this function, whereas other data show that they are not essential to replication repair (other DNA repair systems are sufficient to carry out the task) and their only function is the generation of genetic diversity. In order to settle the question, we examined two isogenic strains (MDS42, MDS42pdu) differing only in the presence of the inducible DNA-polymerases. Comparing the growth properties and surviving abilities of the two strains in response to the DNA-damaging agent mitomycin C (mC), it was found that: (1) various mC concentrations cause similar growth inhibition in the two strains, (2) applying low concentrations of mC (which is able to induce polymerases, but causes only moderate growth inhibition), the lack of the polymerases does not influence growth and short-term survival, (3) in the presence of higher concentrations of mC (enhanced stress, increased DNA-damage), the number of living cells decreases more severely in the absence of inducible polymerases, (4) under stressful conditions, stress-resistant mutants arise much more frequently when the polymerases are present. Our results show that under normal growth conditions or under moderate stress damaged DNA sites can be

fixed without the minor polymerases. The repairing function of inducible polymerases becomes significant only under enhanced stress/damage, when other repair systems (nucleotide-excision repair, recombination-mediated repair etc.) become presumably exhausted. On the other hand, the role of the polymerases in generating stress-resistant mutants was clearly demonstrated. In conclusion, we found that generation of genetic diversity is the primary function of the inducible DNA-polymerases.

P-055
EFFECT OF METAL NANOPARTICLES ON THE EXPRESSION AND ACTIVITY OF MMPS AND ON THE VIABILITY OF TUMOR CELLS

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Silver nanoparticles are widely used in household products due to their antibacterial properties. Over the last decade, substantial research capacity was dedicated to assess their distinct physical, chemical and biological properties to broaden the application of silver and other metal nanoparticles in biomedicine, such as in biomedical imaging, in photothermal and in cancer therapy. Such studies demonstrated that for example the size and the shape of noble-metal (gold, silver, platinum) nanoparticles influence their optical, electromagnetic and catalytic properties. Metal-nanoparticles represent a potential new class of probes for tumor detection, diagnosis and for targeted and non-targeted therapy as well. However, for a large number of metal-nanoparticles the precise biological properties, mechanism of action, signalling pathways and the set of the affected genes and their expression changes have not been fully explored. Tumorigenesis and disease progression of cancer is a complicated process including cell transformation, evasion of host defenses, angiogenesis, invasion and metastasis. These events are not only regulated by a complex interaction of various growth factors and cytokines, but also influenced by proteolytic enzymes such as matrix metalloproteases (MMPs).

The goal of the present study was to determine the grade of cytotoxicity of various metal-nanoparticles on tumor cells and to find out whether the treatment of these cells by nanoparticles could influence the expression of metalloproteinases (MMPs) thereby eventually moderating the angiogenic and metastatic characteristics of the cells. Different silver and gold nanoparticles have been synthetized with chemical reduction technology. The citotoxicity of the particles on U2OS osteosarcoma and Capan-1 adenocarcinoma cell cultures have been investigated, viability of the cells was assessed by Trypan blue staining test. The expression of MMP-2, -3, -9, -10, -12 was analyzed by real-time PCR, and the protein expression of MMP-3, MMP-10 and MMP-12 by Western blotting, the gelatinase activity of MMP-2 and MMP-9 was assessed by in-gel zymography.

Our results indicated that silver nanoparticles drastically reduce the viability of both types of cells compared to gold particles. The cytotoxicity of the particles was also dependent on the stabilizing material used by the synthesis, where xanthan- and starch-stabilized silver was proven to be the most cytotoxic. The expression of pro- and active forms of MMP-3 showed the most pronounced

increases following the treatment of both Capan-1 and U2OS cells with silver or gold nanoparticles. Increased gelatinase activity of MMP-2 has been proven in both tumor cell cultures after the administration of silver nanoparticles. These results indicate that noble-metal nanoparticles in fact influence the expression and activity of various MMPs, however further investigations are required to analyze the precise mechanism of action of these nanoparticles.

P-056
INFLAMMATORY PROCESSES IN AMIODARONE INDUCED CYTOTOXICITY

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Introduction: Amiodarone is a type III agent used in the treatment of severe cardiac arrhythmias. Since the administration of the drug is prolonged, numerous side effects and complications can develop. In our work, we focused on the citotoxicity of amiodarone. We examined its effect on cell viability, investigated the activation of signalling pathway following amiodarone administration, and identify those that are involved in the cell damage and in the development of the side effects.

Materials and Methods: We examined amiodarone induced cell death by MTT method. We analyzed the apoptotic or necrotic nature of the amiodarone-induced cell death by flow cytometry following double-staining with annexin V and PI fluorescent dyes. We treated cultured cells with amiodarone and monitored the activation and regulation of intracellular signalling pathways at the protein level by immunoblotting. Furthermore, we assessed the expression of key members of the signalling pathways by analysing mRNA levels using real-time PCR.

Results: Treatment with high dose amiodarone induces cell death in cell cultures. We found that the most appropriate effective concentration of amiodarone was 30 μM. We treated cultured cells with amiodarone and monitored the activation and modification of the signalling pathway at the protein level by immunoblotting. We found a transient activation on the PI3 kinase pathway, and detected a contious activation of Jnk and NFkB, COX-2 pathways. We detected alteration of COX-2 at the mRNA level using real-time PCR. We are also investigating inhibition of amiodarone-induced NFkB and Cox-2 activation and its effect on the amiodarone (and desethyl-amiodarone)-induced inflammatory pathway.

Conclusions: Our results and published data indicate the involvement of the TNF-α , NFkB, Cox-2 inflammatory pathway in the amiodarone induced inflammatory processes. Our results are suggesting that patients receiving NSAID prophylaxis (non specific COX inhibition), could have a lower risk in developing amiodarone induced inflammatory processes, and in the incidence of side effects.

P-057

KNOCKING OUT THE TRANSIENT RECEPTOR POTENTIAL VANILLOID-1 (TRPV1) GENE PROMOTES THE DEVELOPMENT OF AGE-RELATED OBESITY IN MICE: AN INSIGHT INTO MECHANISMS

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We have recently shown that mice genetically lacking the transient receptor potential vanilloid-1 (TRPV1) channel become significantly heavier than their wild-type littermates as a function of age. It remained unknown how the lack of TRPV1 influences the activity of the main effectors of energy balance regulation in aging mice and how the change in the activity of effector(s) will lead to the development of a higher body weight in older age.

Male TRPV1 knockout (KO) and wild type mice of different age groups were used in the study. The body weight of the animals was measured on the same day of every week. Mice were randomly assigned into two experimental groups: (i) in loosely restrained animals, colon temperature and oxygen consumption were measured; (ii) in freely moving mice, abdominal temperature and locomotor activity were registered with telemetry.

In accordance with our earlier results, we did not find a statistically significant difference between the body weights of young TRPV1 KO and control mice, but as they became older, TRPV1 KO mice were heavier than controls ($p<0.05$ at the age of 12 months). In experiment (i), we have observed that – as a result of their lower metabolic rate – basal colon temperatures of TRPV1 KO mice were lower than those of controls in both young (35.9±0.2 vs. 36.3±0.2°C at night) and older age groups (35.8±0.3 vs. 36.6±0.1°C at night). In experiment (ii), we have found that young as well as older TRPV1 KO mice were hyperactive and had higher abdominal temperature than their age-matched controls, although the difference in body temperature was smaller in aged mice than in young animals.

Our findings support the hypothesis that the genetic disruption of TRPV1 contributes to the development of age-related obesity. We have shown that the presence of TRPV1 is necessary for the prevention of excess weight gain with age. If TRPV1 is absent, the aging animals become overweight compared to controls, which is the result of their lower basal body temperature (metabolic rate) and the failure of their locomotor compensation. We can conclude that TRPV1 channels are needed for the maintenance of basal metabolic rate and – over the long term – for the preservation of normal body weight.

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P-058
AN ANIMAL MODEL FOR NOVEL HUMAN DISEASES ASSOCIATED WITH MUTATIONS OF TYPE IV COLLAGEN COL4A1 AND COL4A2

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We are focusing on the recently discovered human diseases in patients carrying mutations in type IV collagen genes COL4A1 or COL4A2. The corresponding proteins are basic constituents of the ubiquitous basement membrane. The clinical manifestations include perinatal cerebral hemorrhage and porencephaly, hereditary angiopathy, nephropathy, aneurysms, and muscle cramps (HANAC), and Walker-Warburg Syndrome. Additionally, ocular dysgenesis and myopathy were noted in mice. The latest reports show systemic tissue degeneration and pleiotropy of the mutations and confirm the experimental observations that COL4A2 mutations phenocopy COL4A1 lesions. We have recently identified conditional, dominant temperature-sensitive mutations in an allelic series of the type IV collagen gene, *col4a1* in *Drosophila*, causing severe myopathy in striated muscles of the oviduct, and visceral myopathy in smooth muscles of the gut (Kelemen-Valkony et al., 2012A, 2012B). These results support our hypothesis that *col4a1* gene mutations result in muscular dystrophy in *Drosophila* (Kiss et al., 2012). In our preliminary experiments we determined the mutation sites that all were glycine substitutions within the collagenous domain of the COL4A1 protein. We observed allelic heterogeneity of muscle fiber degeneration: The N-terminal mutations trigger at fast, whereas the C-terminal lesions cause myopathy at low progression with marked differences in the morphology of the muscle fibers. We have demonstrated aberrant laminin C expression and localization in $col4a1^{G552D1}$ mutant (Kelemen-Valkony et al., 2012A) and observed a similarly aberrant immunodetection pattern for beta PS integrin. We have generated monoclonal anti-COL4A1 antibodies that were confirmed in Western analysis to provide a signal at the expected size. We have detected cellular degradation of Malpighian tubules (corresponding to a single glomerolus of mammals) and of the indirect flight muscle. These results collectively suggested a systemic disorder affecting multiple tissues and organs. We anticipated that the exposed cytoplasm of the degenerating cells triggers a chronic inflammation in the mutant flies. Indeed, in our microarray-aided transcriptional profiling experiments remarkable upregulation of genes encoding antimicrobial peptides (Diptericin, Metchnikovin, Attacin) suggested a robust immune reaction. We have explored the proteolytic mechanism leading to the noted clearance of actin from the muscle fibers and detected actin cleavage at SKR/GILTLKY, corresponding to a furin recognition site in the $col4a1^{G233E}$ mutant (Mink et al., 2011).

Further preliminary experiments were aimed at alleviating the consequence of mutations in heterotrimers ($[COL4A1]_2COL4A2$) by chemical chaperons or by genetic interactions. Glycerol treatment or over-expression of lysyl oxidase DmLOXL-1 (a collagen cross-linking matrix enzyme) both resulted in significantly elevated survival rates of the $col4a1^{G552D1}$ mutant animals.

Although several clinical genetic studies reported patients with COL4A1 mutations with basement membrane defects and pathological manifestation in multiple tissues, in depth histology, cell-

based and biochemical studies of the mutant muscle tissue are lacking and significant details of the underlying pathomechanism remain unknown. Therefore, our *Drosophila col4a1* mutant series serves as effective model system to uncover the mechanisms by which COL4A1 mutations result in compromised myofiber-basement membrane interactions and manifest in aberrant muscle function in this emerging COL4A1-associated disorder.

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P-059
THE ROLE OF OSTEOPONTIN EXPRESSION IN MELANOMA PROGRESSION

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It has been shown that osteopontin is overexpressed in the majority of cancers and associated with its pathogenesis. Besides multiple intra- and extracellular functions it has a crucial role in cell adhesion, chemotaxis, prevention of apoptosis, invasion, migration and growth of tumor cells. In our previous studies we performed global gene-expression profiling on a series of primary melanoma samples resulted in that OPN is the strongest upregulated gene associated with poor clinical outcome.

The aim of the present study was to validate our previous microarray data and to determine the OPN expression at both mRNA and protein levels in primary melanoma samples and melanoma cell lines. Our goal has been inhibition of OPN expression by RNA interference (RNAi) in melanoma cell lines with high osteopontin expression. Furthermore, our aim was to clarify the role of osteopontin in signaling pathways which play important role in melanoma progression.

The mRNA expression of OPN was analyzed by Q-PCR on primary melanoma tissues (n=28) and melanoma cell lines (n=12). Immunohistochemistry was carried out on Tissue Microarray (TMA; 53 samples). TMA sections were digitized and evaluated by MIRAX Viewer Software. Indirect immunofluorescence labeling of Nfκβ p65 and osteopontin protein was made on fixed cells and results were assessed by confocal laser scanning microscope. Using OPN-specific small interfering RNA (siRNA) was inhibited OPN expression in melanoma cell lines. OPN silencing was evaluated with Q-PCR for mRNA levels.

The mRNA expression of OPN was significantly higher in melanomas with higher thickness (> 4 mm) and ulcerated surface. The high expression level was mainly detected in late stage tumors (Clark level IV and V). More than 50% of tissues showed high protein expression and was significantly associated with ulceration. High levels of OPN mRNA expression were detected in the majority of cell lines and these results we verified by immunfluorescence labeling of OPN

protein. Nuclear translocation of the Nfκβ p65 protein was observed in one of these cell lines. However, cell lines with low OPN expression exclusively featured cytoplasmic staining. siRNA mediated knockdown of OPN in melanoma cell lines led to decreased expression of OPN at mRNA levels.

In summary, the high OPN expression was confirmed by Q-PCR in 28 primary melanomas. Based on our data high level mRNA and protein expression of OPN were found which are associated with less favorable clinical outcome of melanoma patients. Nuclear translocation of the Nfκβ p65 protein in cells with high OPN mRNA expression was detected which refers to activation of Nfκβ pathway. Silent effect showed that OPN-specific siRNA suppressed OPN mRNA expression *in vitro*. Our future plan is to perform functional biological studies in order to define the role of OPN in signal transduction because might be a specific candidate molecule that can promote tumor growth and invasion, therefore it might be potential target for therapeutic intervention in control of melanoma progression.

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P-060
INVESTIGATION OF MOLECULAR ALTERATIONS ASSOCIATED WITH THE INVASION OF MELANOMA CELL LINES

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Melanoma, the most aggressive type on skin cancer, is characterized by high metastatic potential, but the complete molecular mechanism leading to invasion and metastasis formation is not well characterized yet. It is assumed that aggressiveness of the disease is associated with the high degree of genetic heterogeneity of melanoma cells.

In order to investigate melanoma cell invasion potential and define the mechanisms by which tumor cells acquire invasive phenotype in a heterogeneous cell population, we applied in vitro Matrigel Invasion Assay of selected cell lines derived from primary (N=9), metastatichuman melanomas (N=5) and mouse xenografts (N=3). By real-time quantitative RT-PCR we determined the relative mRNA expression levels of the*MMP-2*,*PTEN*, *CCND1* and *CAV1* genes. To inhibit the invasion of melanoma cells we used an anti-carcinogenic glycoalkaloid called α-solanine.

Based on the Matrigel Invasion Assay,ten melanoma cell lines were invasive. Three of these were developed from primary melanomas, three from melanoma metastasis, and all cell lines derived from mouse xenografts were invasive.The most invasive cell line was the M24 derivedM24 met xenograft. According to the gene expression analysis, M24 and M24 met had specific expression pattern, because all ofthe examined genes were highly overexpressed. In the rest of the cell lines decreased relative PTEN mRNA level was detected. The lack of PTEN expression can promote cell migration by actin filament remodeling, but in the case of M24 and M24 met it was not observed.

CCND1 overexpression was detected in four non-invasive cell lines, and down-regulated in five invasive cell lines. The low mRNA level of CCND1 is probably associated with high migratory capacity which is characteristic in the resting G_0 phase of the cell cycle. We detected low CAV1 mRNA expressionin the majority of cell lines, except four non-invasive, primary derived cell lines, which had normal or high caveolin-1 mRNA level. MMP-2 overexpression was noticed in all invasive, and seven non-invasive cell lines in different levels. To inhibit the invasion potential, non-toxic doses of α-solanine treatment was performed in four cell lines derived from primary tumor, metastatic melanoma and mouse xenografts. The maximal dose of α-solanine can inhibit the number of invasive cells with the average of 68%in three cell lines, compared to the control samples. In contrast, α-solanine had no effect on M24 met cell line, which can be the result of high MMP-2 expression.

In summary, invasion can markedly suppressed by α-solanine in three cell lines, probably through the reduction of matrix metalloproteinase activities and inhibiting invasion related signaling pathways. The down-regulation of the PTEN gene in both primary and metastatic melanoma cell lines is probably the result of the deletion/mutation of this tumorsuppressor gene that can occur at the early stage of the disease. The low expression of CCND1 gene most likely promote melanoma cells' invasion. The role of caveolin-1 during cell migration is still controversial; the decreased expression of CAV1 in metastatic melanomas was observed, but information about its role during melanoma invasion is still missing.

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P-061
THE PRESENCE AND ESTROGEN-MEDIATED UPREGULATION OF THE TRPV1 RECEPTOR IN RAT ENDOMETRIUM

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In recent years, the presence of the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor has been described in various non-neuronal cells (epithelial and smooth muscle cells, keratinocytes) besides the central and peripheral nervous system. Upon activation by capsaicin, noxious heat, protons, lipid mediators, it generates pain and neurogenic inflammation. TRPV1 expression is upregulated by estrogen in the hippocampus and dorsal root ganglia which may play a role in the increased nociception and its variation with the reproductive cycle observed in women. Our aim was to detect the expression and estrogen mediated upregulation of the TRPV1 receptor in rat endometrium.

We examined young (age: 4 weeks) and adult (age: 4 months) female rats. The animals received the synthetic estrogen analog diethylstilbestrol (DES) for 8 and 12 days (n=6-6). Another group (n=4) was ovariectomized. Uterus biopsy samples were homogenized in TRI reagent. The isolated RNA was reverse transcribed into cDNA and quantitative polymarase chain reaction (qPCR) was

perfomed with TRPV1 specific primers and probes. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), hypoxanthine phosphoribosyltransferase 1 (HPRT1) and peptidylprolyl isomerase A (Ppia) reference genes were used. Immunohistochemistry was performed with polyclonal rabbit TRPV1 first antibodies and horseradish peroxidase (HRPO) conjugated second antibodies on paraffin-embedded sections with diamino-benzidine (DAB) development.

We detected the TRPV1 receptor on the mRNA and protein levels in rat endometrium. There was no significant difference between young and adult animals in mRNA expression. In untreated adults, marked immunopositivity was observed in the stroma and glands and weak positivity in the epithelial layar. In control young rats, there was weak immunopositivity in the epithelial layer. DES treatment significantly upregulated non-neuronal TRPV1 mRNA expression an average 3-4 fold compared to all three reference genes. In young animals, the epithel layer, while in adults the stroma showed elevated TRPV1 immunopositivity after DES treatment. Interestingly, ovariectomy also upregulated mRNA expression and immunopositivity in the epithel layer although to a smaller extent.

We demonstrated for the first time the estrogen-mediated upregulation of the TRPV1 receptor in rat endometrium. Further investigations are planned to elucidate its functional relevance.

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P-062
OLIGOMER-SPECIFIC ALPHA-SYNUCLEIN ANTIBODY REVEALS NEW INSIGHTS INTO MOLECULAR CELL PATHOLOGY OF PARKINSON'S DISEASE AND OTHER SYNUCLEINOPATHIES

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Lewy body dementia (LBD), Parkinson's disease (PD) and multiple system atrophy (MSA) are characterised by the deposition of disease-associated, aggregated α-synuclein. Indeed, recent studies, demonstrated that oligomers of α-synuclein might be potentially useful to distinguish diseased from non-diseased conditions. A newly developed 5G4 monoclonal antibody reacted with higher molecular forms of α-synuclein may be suitable for re-evaluation of neuropathological archival material and reveals widespread α-synuclein pathology [1]. Besides the neuronal immunpositivity the preliminary light microscopic investigation disclosed 5G4 immunolabeled astrocytic profiles in some cases correlated significantly with microglia activation and astrogliosis.

In the present study we performed 1) a systematic comparation of light microscopy and pre- and postembedding immunogold electron microscopy after low temperature substitution embedding to map the cellular localization patterns of oligomeric α-synuclein in neurons and glial cells in human post-mortem samples, and 2) we correlated the density of different deposits with the severity of micro- and astrogliosis in cases with and without clinically detectable dementia.

In addition to Lewy bodies and Lewy neuritic deposits in substantia nigra (SN), we detected synuclein immunreactive citoplasmic granular deposits, filamentous network and filaments ordered in line with each other not only in neurons but likely in glia cells in other brain regions such as putamen/striatum and amygdala. The immunogold labeling clearly demonstrates the lack of α-synuclein in lipofuscin granules and neuromelanin pigments of dopaminerg cells. Our observations comfirm the notion reported previously [2] that the Lewy bodies themselves are not the most relevant morphological hallmarks for the clinical symptoms.

Our data confirm the preivous supposition that the fine ultrastructural localization of desease associated α-synuclein revealed by oligomer specific 5G4 monoclonal antibody may substantially contribute to understand the early steps of molecular pathology of synucleinopathies in human nervous system.

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P-063
PR-TYPE GLUCOHYDROLASES AS PART OF THE DEFENCE AGAINST LEAF RUST IN NEAR-ISOGENIC WHEAT LINES CARRYING DIFFERENT RESISTANCE GENES

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Up to now, in the interaction between wheat and its pathogen, leaf rust, more than 60 host resistance genes have been identified in cultivated species or in their close wild relatives. Still, in most cases not only the molecular mechanism of the developed resistance (attended often by different symptoms) is hardly known but – except for *Lr1, Lr10* and *Lr21* – even the product of *R* genes has not been revealed. We investigated how *Lr1* and *Lr9* affect the response of seedlings against *P. triticina* pathotype 43722. Earlier, both resistance genes conferred efficient protection against field strains, but due to a shift in the pathotype spectrum over the last decade, Lr1 and Lr9 carrying genotypes have started differing in their success of resistance under field conditions in Central Europe.

To compare their putatively different stress response, proteomic analyses of the apoplast as a first barrier to invading pathogens were carried out. 1- and 2-D PAGE followed by MS-analysis revealed that several members of at least six PR families (PR9-type peroxidases, PR2 glucanases and chitinases of PR3 and -4, thaumatin-like proteins (PR5)) and a GDSL-like lipase seem to be involved in the response of the infected leaf tissue – with different timing and intensities. Since,

amongst them, the group of inducible or constitutively expressed endo-1,3-glucanases and chitinases are known contributors in many but not all resistance types against wheat leaf rust, our aim was to specify if their activity and/or expression dynamics could be correlated with the defense potential of the tested genotypes. Therefore, specific and consensus primers derived partly from MS-identified sequences were used for RT-(q)PCR combined with restriction analyses and cloning to follow induction and the rate of contribution of the main phylogenetic subgroups of PR2 (subfamily A of GH17) and PR3 (GH19 family) during the progress of invasion or resistance response (0-7 dpi).

Melting analysis as well as restriction of consensus chitinase qPCR products showed that expression of both class II and IV chitinases is typical in the early stage of infection as well as in mock-infected samples, but members of class I are induced later. In the early stage no convincing difference was observed in the general chitinase transcript level between the resistant lines and the sensitive cv. Thatcher (Tc) control. At the same stage (3 dpi) however, class II type *Chi1* showed a much higher level of induction in both resistant lines than in Tc.

For the total set of endo-1,3-glucanases, a gradual increase of expression was found with levels ranking in the following order Tc < Lr9 < Lr1, similar to previous measurements of enzyme activity. The most pronounced induction differences between resistant and sensitive lines were found in glucanase cluster I. Inducible isoforms (*TaGlba,b,f*) of cluster III were not detected in mock-treated samples but were expressed at higher level in the two resistant lines.

The detailed analysis of chitinase and glucanase expression and its comparison to former enzyme assays indicated that, especially in the case of chitinases, there is no direct correlation between mRNA expression profiles of these enzyme groups and their enzyme activity, suggesting that further, posttranscriptional regulative events might severely influence the outcome of infection-induced gene expression.

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P-064
THE ROLE OF THE GPI-ANCHOR AND GLIAL CELLS IN THE PATHOGENESIS OF PRION DISORDERS

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The prion diseases are conformational neurodegenerative disorders such as Parkinson's, Alzheimer's and Huntington disease. The main step in the pathology of neurodegenerative diseases is the appearance of a pathological protein conformer. The disease-associated forms of these proteins cause fatal degenerative changes in the central nervous system. The conformational conversion of the cellular prion protein (PrP^{C}) to a misfolded form termed scrapie-prion protein (PrP^{Sc}) results in the clinico-pathological phenotype of the prion disease called transmissible spongiform encephalopathy (TSE), and are characterized by spongiform vacuolization, expansive axon degeneration, intense regional and selective neuronal cell death, and astrocyte and microglial proliferation (called gliosis). These changes are usually caused by the accumulation – many times amyloid-like aggregation – of the protease-resistant PrP^{Sc} and the consequently failured

intracellular protein degradation systems such as ubiquitin-proteasome system (UPS) and autophagy.

Brain tissue of normal PrP^{C}-expressing wild-type mouse infected with PrP^{Sc} shows characteristic histo-pathological features of TSE. Interestingly, spongiform lesions, remarkable neuronal death and astrogliosis are not observable in the case of PrP^{Sc}-infected transgenic mice expressing cellular prion protein lacking the glycosylphosphatidylinositol (GPI) membrane anchor (PrP^{GPI-}), in spite of extracellular plaques are frequently noted. Microglial cells – activated by stress signals rised from permanent plaque formation – try to separate and eliminate the mass of extracellular protease resistant prion protein (PrP^{res}) accumulations. Our light- and electronmicroscopic and immunogold-EM observations reveal an intimate contact of plaques and microglial cells, and demonstrate a series of progression of degenerating glia cells. This touch seems to be a „fatal embrace" for most of microglial cells. Subsequently, the stress caused by the permanent formation of PrP-plaques and microglia degeneration reached neurons. One of the most prominant neuronal stress response is the formation of huge distrophyc neurites containing morphologically recognizable damaged mitochondria. Fine immuno-electronmicroscipical analysis suggests that these mitochondria are segregated and eliminated by selective macroautophagy called mitophagy. This might be a cytoprotective process for preventing apoptotic cell death.

Our observations draw attention to that the neurotoxicity is not triggered by the extracellular protein plaques as it is perceptible and well-founded in the case of Alzheimer-disease. It is known that intracellular disease-associated form of β-amyloid protein – not the extracellular accumulation of the β-amyloid – causes pathological features in Alzheimer patients. We can suppose that the neuropathological changes of the PrP^{GPI+} scrapie-infected mice are similar to the cytopathological hallmarks of the group of other neurodegenerative disorders such as synucleinopathies (Parkinson's disease and multiple system atrophy) and tauopaties (Alzheimer's disease and frototemporal dementia). The common and similar molecular mechanisms of their pathology make rather more important the comparative morphological investigations.

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P-065
IDENTIFICATION OF „EARLY" CELIAC EPITOPES UTILIZING RECOMBINANT TRANSGLUTAMINASES FROM DIFFERENT PROTEIN EXPRESSION SYSTEMS

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Celiac disease (CD) is a pathologic immune reaction to ingested gluten and it is characterized by the production of disease-specific autoantibodies against the enzyme type-2 transglutaminase (TG2). The pathogenic role and exact binding properties of these antibodies to TG2 are still unclear. During our work we have identified by molecular modeling key amino acids R19, E153, E154 and M659 to form a composite main celiac epitope. Human recombinant TG2 mutants were expressed in *E. coli* and the binding properties of celiac autoantibodies were examined by ELISA.

The mutations RE=R19S/E153S and REM=R19S/E153S/E659S caused 93% reduction of the binding ability of celiac serum antibodies in earlier studies. As the main celiac epitope is conformational, we intended to produce the mutant and wild type (wt) antigens in mammalian protein expression system, where more accurate folding and proper posttranslational modifications are expected. We aimed to characterize the binding sites of celiac antibodies derived from prospectively followed celiac children from the PreventCD study, using their first serum sample with TG2 positivity.

We created stably transfected 293AD cell lines for protein expression and purified the proteins with affinity chromatographyin His-tagged form. We monitored the purified TG2s with Western blot analysis and characterized their fibronectin (FBN) binding capacity and transglutaminase activity as well. To test the antigenicity of the mutants we either directly coat the plate with them in enzyme linked immunosorbent assay (ELISA) or we used the FBN-bound TG2s as antigen. We compared the antigenicity of TG2s from mammalian and bacterial protein expression system.

TG2 mutants from 293AD cells showed similar FBN-binding capacity as wt TG2. Their relative transglutaminase activities were decreased (25-85%) compared to wt enzyme, while they were higher than the relative activities of proteins from bacterial system. In the direct ELISA early coeliac antibodies bound to the mutants with decreased capacity, the highest reduction was 78% for mutant RE. The reduction was 89% in the case of mutant RE of bacterial origin. In FBN-TG2 ELISA measurements we found 9% remaining binding for the mammalian RE and REM mutants and 6% remaining binding for bacterial double mutant. In contrast IgA anti-TG2 antibodies of a celiac patient with delayed diagnosis did not show so prominent diminished binding to the mutants (40-60% remaining binding for RE and REM).

Recombinant mutant TG2s expressed in mammalian expression system provide good tools in epitope mapping studies. The binding pattern of celiac antibodies to the proteins from different

expression system was similar, but the reduction was higher in the case of bacterial mutants. The characterized "early" celiac epitope is overlapping with the earlier found main binding site, however celiac patients with a delayed diagnosis may have different epitope pattern due to epitope spreading.

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P-066
INVESTIGATION OF THE EFFECTS OF COELIAC MINIBODIES ON TRANSGLUTAMINASE ACTIVITY AND EPITOPE MAPPING STUDIES

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Coeliac disease (CD) is a genetically-determined chronic inflammatory intestinal disease caused by intolerance to gluten proteins. Tissue transglutaminase (TG2) was identified as the major autoantigen of coeliac disease. According to recent data the antibody response is directed against conformational epitopes and our research group has identified the main binding site of these antibodies, which is conformational and is built up by amino acids from the I (19R), II (153E), and IV (659M) domains of TG2. The detection of anti-TG2 IgA antibodies has a significant role in the diagnosis of the disease and autoantibodies may be involved in the pathogenesis. In a previous collaborative study with our Italian partners we isolated total RNA from intestinal biopsy lymphocytes of an adult patient with coeliac disease and high serum anti-TG2 antibody level. With the amplification of IgV region total antibody library was cloned and this was selected against TG2 utilizing phage display. We subcloned the transglutaminase-positive single chain variable fragments (ScFvs) into pcDNA3.1/Hygro vector containing also the CH2 and CH3 constant regions of human IgG1.

Two of these clones (4.1, A5) were transfected to 292T cells to obtain stable transfected cell lines for minibody expression. Minibodies were purified from cell culture supernatant with ACTA Prime using protein A column. After pooling and concentration of the fractions containing the minibodies protein samples were analyzed with SDS-polyacrylamide electrophoresis. We characterized the epitopes of the minibodies with TG2 mutants and with competition ELISA measurement between minibodies and ScFvs. In addition we investigated their bindings to the open and closed conformations of TG2 utilizing commercially coated plate (Zedira GmbH). The minibodies were compared with natural celiac antibodies by clinical diagnostic methods (Anti–EndoMysial Antibody immunofluorescent assay, EMA). We also tested the effects of the minibodies on the transglutaminase activity of wild type TG2 with microtiter plate assay based on the incorporation of 5-(biotinamido) pentylamine into immobilised *N,N*-dimethylated casein.

Two coeliac minibodies with slightly different epitope specificity were successfully cloned and produced in highly concentrated purified soluble form. Both showed a typical positivity in the EMA assay and a higher binding to the open conformation TG2 similarly to natural coeliac

antibodies. Minibodies slightly elevated the transglutaminase activity of TG2 when they were administered in 5 ◎g/ml concentration. Minibody 4.1 competed with several other phage-expressed coeliac ScFv antibodies but not with the phage-bound A5 ScFv. Minibody A5 competed with the own clone A5 ScFv but not with other ScFvs under the conditions we used. This clone showed a lower reactivity with the N-terminal R mutant. This diversity may reflect epitope variations in adult coeliac patients. Further competition studies with differently coated TG2 antigens are planned to see if the binding sites have any overlap.

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P-067
ACTIVATION OF HSP72, HSP90α AND HEAT SHOCK FACTOR-1 IN OXIDATIVE STRESS BY BGP-15, A NEW TYPE OF INSULIN SENSITIZER

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BGP-15 is a new type of insulin sensitizer (R,S–O-(3-piperidino-2-hydroxy-1-propyl)–nicotinic–acid-amidoxime). Its effects has been demonstrated in several animal models [0] and also in insulin resistant human individuals [0]. The drug candidate is presently in human IIB clinical development phase (trial identifier). Inhibition of JUN-kinase activation by the stimulation of stress protein (HSP72) expression by BGP-15 has been demonstrated as a novel insulin sensitizing mechanism [0]. One of the most important known mechanisms protecting cells from various stresses - like oxidative stress - is the heat-shock response which results in the induction of the synthesis of heat-shock proteins (HSPs) through activation of HSF-1 [0]. BGP-15 - has been shown previously to act as a co-inducer of several HSPs, enhancing the amount of these proteins produced following a heat shock compared to heat shock alone [0]. Monomer form of HSF-1 exists in the cell under tight negative regulation by a multi-subunit complex that includes HSP90 [0]. HSP90-based chaperone system plays an essential role in the stabilization, assembly and function of nNOS and eNOS. In addition the active form of both enzymes may exist exclusively in complexes with HSP90 [0,0].

In the present study the effects of BGP-15 was examined on activation of HSPs in hyperglycaemic HaCaT cells, as a model for oxidative stress. BGP-15 treatment resulted in increased appearance of HSP72 in weak detergent (NP-40) soluble fraction after 1 min of BGP-15 treatment and the levels continuously increased up to 1h. Increased translocation of HSP90α was also observed to soluble fraction until 60 minutes reaching 2-fold elevation. Parallel to these changes the level of n-NOS became detectable after 2 minutes in the soluble fraction and its level continuously increased until 60 minutes. Kinetics of e-NOS appearance was somehow different: it became only visible after 30 minutes. Formation of NO-Tyr in cytosolic fraction was also observed suggesting indirectly the production of NO by either nNOS and eNOS. The trimmer form of HSF-1 significantly increased in nuclear fraction after 30 and 60 minutes of treatment. Small fraction of the trimmer form of HSF-1 is still detectable even after 24 hours.

These data suggest that BGP-15 induces client-change of HSPs and appearance in the easily extractable cytosolic fraction. In addition it automatically contributes to the activation of HSPs by releasing it from the binding. These changes results in restoration of NO production and elevation of HSP expression that lead to insulin sensitization.

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P-068
LIVE IMAGING OF ADHESION COMPLEXES HANDLING IN COELIAC AND NORMAL CELLS

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Coeliac disease, also known as celiac sprue and gluten-sensitive enteropathy, is a multifactorial disorder of the small intestine triggered by cereal gluten proteins in genetically susceptible persons. The gluten-induced autoimmune reaction against transglutaminase 2 (TG2) eventually leads to severe damage of the small intestinal villous architecture.
Cell – matrix adhesions play essential roles in important biological processes including cell motility, cell proliferation, cell differentiation. The adhesion of the extracellular matrix is mediated by specialized regions of the plasmamembrane, called focal adhesions. The purpose of the study was to investigate the dynamic changes of these complexes in living cells by labelling two main cytoskeletal proteins, talin and actin.

Methods: Endothelial and muscle cells were isolated from fresh human umbilical cord vein and Wharton jelly of normal control subjects and from risk newborns with a celiac first-degree relative. Risk children were followed clinically until the disease manifested and proven by small intestinal biopsy and anti-TG2 antibody positivity. We investigated the adhesion of the normal and coeliac cells in real time by measuring the metabolic capacity of cells in reducing resazurin to a pink, high fluorescent resorufin (Cell-Titer Blue® Assay, Promega). Red fluorescent protein-conjugated actin

and green fluorescent protein talin were expressed by BacMam technology (Cell Light®, Life Technologies) based on baculovirus delivery. Cells were plated and after counterstaining the nuclei by Hoechst®, they were photographed on Olympus IX81 workstation in time-lapse experiments.

Results & Conclusions: Both proteins were successfully expressed and became visible after 4-6 hours without disturbing the viability of the cells. In our experiments we observed a number of anomalies related to cell adhesion, motility and shape, especially when the cells were photographed in time-lapse experiments. Coeliac cells had decreased adhesion capacity compared to the normal cells. In living cells we observed differences in the cytoskeletal structures between the control and coeliac cells in different cell lines.

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P-069
COMBINED GENE COPY NUMBER AND GENE EXPRESSION PROFILING OF MATCHED PRIMARY AND METASTATIC MELANOMAS

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Metastatic process is the predominant cause of melanoma-specific death, decreasing survival dramatically, and resulting in difficulties in the effective cancer treatment as well. To gain insights into the molecular alterations behind cancer progression, and to determineto what extent the profiles of primary melanoma and the corresponding metastasis are similar or distinct, we performed high-throughput expression profiling using Affymetrix Gene 1.0 ST microarrays and CNV/SNP profiling using AffymetrixCytoScan750K microarrays of three matched melanoma tissue pairs.Genetic analysis revealed differences between the distinct pairs, but similar pattern of the matched samples.One-copy-loss of 9p21 (coding CDKN2A and B), gains at 6p25.3 (IRF4 and DUSP2) and 8q23-q24 (including MAPK15) loci, and LOH at 17q22-q23.2 (including MAP3K3, ICAM2 or MIR21) and 18q12.1-q12.2 (coding MAPRE2, necessary for spindle symmetry during mitosis) regions were common genetic events in both primary and metastatic melanomas. Furthermore, primary tumors were characterized by gains of 1p36.33, 20q13.33 and 22q11.22loci, losses at 9p22.3-p24.1, and the LOH/gain of 13q21.31, whereas the metastatic tumors exhibit frequent gains at 1p13.2-p12 (including NRAS and NOTCH2), 1p31.1-p22.3 and 5p15.33 chromosomal regions, losses at10q25.3 and 22q12.1 loci, and LOH at 10q21.3 and 11q12.1 regions.Hierarchical clustering of the gene expression results revealed a distinct separation of the melanoma tissue pairs from each other.Using stringent criteria, we assigned 118 genes out of the 6013 pre-filtered genes, whose expression significantly altered (fold-change more than 2)in metastatic lesions compared to the primary melanoma samples. Forty-three genes were upregulatedand enriched in the migration (e.g. L1CAM, a melanoma specific gene), WNT and JAK-STAT signaling pathways, influencing survival and invasion of the melanoma cells. Whereas 75 genes weredownregulated and significantly enriched in the cell cycle regulation, DNA damage

response, cell adhesion and anchorage(e.g. MMP1 and MMP7) pathways.Fifteen genes out of the 118 differentially expressed genes can also be assigned to allelic events. Among these alterations,10 genes (e.g. L1CAM and FAM46C) showed both copy number gain and more than two-fold increase inmRNA level, whereas 4 genes (e.g. TYRP1) exhibited both copy number loss along with a decreased mRNA level. Allelic loss of the 17q23.1 locus containing MIR21 gene was also observed. MIR21encodes microRNA-21 whose plasma concentration could be a potential prognostic and diagnostic marker of glioblastomas and colorectal cancers, and was found to be downregulated in our metastatic samples compared to the primary lesions.Our data may provide a deeper view of changes in advanced melanoma relative to primary tumors and reveal possible new targets that can be used in assessing prognosis.

(TÁMOP-4.2.2/B-10/1-2010-0024)

P-070
EXOME AND CUSTOM ENRICHMENT TARGET SEQUENCING TO MAXIMIZE THE EFFICIENCY OF NEXT GENERATION SEQUENCING

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Next Generation Sequencing (NGS) technologies enable rapid generation of data by sequencing massive amounts of DNA, re-define genomics and their effects become increasingly widespread. The price for sequencing has dropped exponentially with the development of NGS, still the present platforms of NGS are expensive enough for routine whole genome sequencing. At the present time, targeted re-sequencing allows us to focus efficiently and cost-effectively on regions of interest, for instance custom designed candidate regions from 100 kb to up to 60 Mb or the entire protein coding portion of the genome, called the exome. Here, we confer the experimental approach of selected sequencing of breast cancer susceptibility genes using the Agilent SureSelect target capture technology. Also, we demonstrate the application of Roche-Nimblegen whole exome capture system for the ultimate genotyping of all genes in a single sample experiment to discover potential monogenic association of schizophrenia. In our target capture experiments first we generated whole genome libraries in order to hybridize to the oligo-probe library, representing the total human exome or selected target genes. After rigorous washing and elution of captured libraries we measured the relative fold enrichment of selected genes in pre-capture and post-capture libraries by qPCR. The result of qPCR showed 100-4000x enrichment of various target genes. Only the target captured and successfully enriched libraries were submitted to deep sequencing, providing valuable information of 1-2% of the total genome or representing the 600 Kb targeted genes region. First and second level bioinformatic analysis showed that both exome

capture and selected target capture experiment achieved coverage of target region with Poisson-like distribution with a small IQR value.

P-071
DNAJ AS INTERACTING PARTNER OF HUMAN TRANSGLUTAMINASE 2

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Human Transglutaminase 2 (TGM2) is a multifunctional cross linking enzyme which has a large number of interacting partners contributing to its diverse biological and pathological functions such as cell growth, differentiation, adhesion, migration, apoptosis, neurodegenerative disorders, liver diseases, metastasis and cancer. However the molecular interactions between TGM2 and its interacting partners which govern these processes are largely unknown because of the lack of adequate information regarding these interacting proteins. It is important to identify specific binding partners of TGM2 indifferent cellular compartments asit may have an impact on the signaling pathways functioning in these cellular processes. Thus the present study aims to identify novel interacting partners of TGM2 and finally explore its functional significance.To achieve this we use NB4 cell line as a model because TGM2 expression is undetectable in wildtype NB4 cell line butupon ATRA (all*trans*retinoicacid) treatment TGM2 expression increases several folds. The differential gene expression analysis of NB4 cell line and TGM2 knocked down NB4 cell line after ATRA treatment revealed that TGM2 was involved in expression of large number of ATRA-regulated genes.

To identify the proteins interacting with TGM2 weemployed GST pull down assays and subsequent mass spectrometry analysis. We obtained various novel TGM2 binding candidates namely Tubulin α, Histone H2A and heat shock protein 40 (HSP 40)/DNAJ and in addition to some known interacting partners such as human Glutathione S Transferase (hGST-P1) validating the experimental approach. Since DNAJ and human TGM2 has been reported to be involved in regulating the aggregation of proteins in Huntington and Alzheimer disease models we chose DNAJ as one of the candidate protein for further analysis. We performed co-immunopreceipitation and co-immunostaining studies. DNAJ couldpull down TGM2 while colocalization of botht hese proteins could be detected in cytoplasm via confocal microscopy.

We have also cloned and purified recombinant DNAJ for direct binding assays. TGM2 has four domain structure andweplantouse TGM2 domain mutants to determine the exact binding domain/site of DNAJ. These experiments would be executed using Biacore and ELISA methods. In future we also plan to explore the physiological and biological significance of TGM2 and DNAJ interaction using cellular models.

P-072
SPECTINOMYCIN RESISTANCE AS CHLOROPLAST MARKER IN ALFALFA

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Alfalfa *(Medicago sativa L.)* is the second most important forage crop in Hungary, and the fourth one in the world. Because of its high fibre and nutritional quality, it is used in ruminant and other livestock feeding in various forms as silage, hay or grass. Alfalfa is also an important source of biological nitrogen fixation and is known for its high biomass production.

Chloroplasts are plant cell organelles, their main function is photosynthesis. Their number varies up to a 100 in the cell and each chloroplast contains 10-100 copies of the prokaryotic type genome. Spectinomycin inhibits protein synthesis on the prokaryotic type 70S plastid ribosomes by binding to the helix H34 in the small subunit and induces bleaching in tissue culture. In the majority of the plant species chloroplast shows maternal inheritance, one of the exceptions is the *Medicago* genus, where it inherits biparentally.

Mutants resistant to spectinomycin were isolated in tissue culture on media containing 30 mg/L spectinomycin. The starting material was young, healthy leaves from greenhouse grown plants of *Medicago sativa* RegenSY line. The surface sterilized leaves were cut and placed on callus inducing media containing spectinomycin. After embryo inducing the calluses were transferred to hormone free spectinomycin containing media for further development. The whole plant regeneration process took 24-28 weeks. In order to have homoplastomic plants the whole process was repeated with leaves of the regenerated plants two more times on elevated spectinomycin level. After the third regeneration cycle 27 independent green, spectinomycin resistant mutant lines were placed into soil for greenhouse cultivation.

DNA was isolated from the spectinomycin resistant plants and the control wild type plant. The 16S rRNA region of the chloroplast DNA was PCR amplified and sequenced. Comparing the mutant sequences to the original RegenSY sequence we identified different mutations, all were located at a conservative region of the 16s rRNA. In one region (1013-1018) we identified G-C and C-A, and in the other region (1138-1144) A-T, A-C, C-G, C-T nucleotide substitutions. After molecular characterization the plants were self fertilized and crossed with wild type plants. The selfed seed progeny was 100% spectinomycin resistant, and the out-crossed progeny showed segregation for the trait. The lines were morphologically characterised and in some lines we measured dry-material and chlorophyll content. All plants were morphologically indistinguishable from the wild type RegenSY line, and neither of the other two parameters showed significant differences.

The mutations can be used as markers in chloroplast inheritance studies, marker associated selection. The mutant plants can also be used for pollen tracing (gene flow) and in plant breeding for selection or identification.

Our research was founded by OTKA K-82037 research application.

P-073
NEXT-GENERATION SEQUENCING AND HLA-TYPING: METHOD VALIDATION USING 1000 GENOMES PUBLIC SAMPLES

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Although the main focus areas of next-generation sequencing (NGS) techniques are whole-genome, whole-exome and targeted sequencing, these techniques gaining importance in other fields as well like HLA-typing. The MHC region containing the HLA genes is the most polymorphic part of the human genome and this makes the usual reference based alignment of NGS reads highly unreliable in this complex. We have developed algorithms to solve HLA typing by directly mapping NGS reads to the IMGT/HLA database to determine the best matching HLA type from whole genome and whole exome data. We are presenting methods to determine HLA types up to four field if possible using paired Illumina reads of the 1000 Genomes (KG) samples. Samples of the HapMap set with known HLA types (A,B,C) were used for validation. Criteria for typing confidence and accuracy is also presented. Typing a single sample takes minutes on a commodity PC and usually needs no laborious human expert interaction. Results show that although the KG data was not intended to use for high-resolution HLA typing, read sets with proper read-length and quality can provide the correct types above 90% concordance.

P-074
ISOLATION AND CHARACTERIZATION OF PROMOTERS OF GENES INVOLVED IN POLYAMINE METABOLISM IN STRAWBERRY

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Promoter regions of two genes, *SAM-decarboxylase* and *Sperimidne-synthase* (showing alteration in gene expression during fruit ripening, hence supposed to be ripening-specific) were isolated and characterized by bioinformatic methods. PLANTCARE database was used for this bioinformatic description, wich contains cis regulating elements of plant promoters, and suitable for identifying sequence motifs of interest. Polyamines – similarly to ethylene – can regulate numerous plant-physiologycal processes, additionally the SAM (*S-adenosyl-methionine*) is a common precursor of both ethylene- and spermidine-biosynthesis. The *Spermidine-synthase* is the gene encoding the keyenzyme of the polyamine-biosynthesis. Increased spermidine and spermine level in plant cells extends abiotic stress-resistance, and influences fruit ripening, as researches showed.

Ethylene-linked cis regulating elements were identified in the promoter regions of the sequences of *Sperimidne-synthase* and *SAM-decarboxylase* genes.

Transient expression system were developed for the investigation of the function of the regulating regions in these promoters using *Nicotiana benthamiana* as a model. Operation and inducibility of

the promoters were examined by deletion promoters and sGFP riportergene in different tissues and developmental stages to allocate the accurate function in strawberry.

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P-075
THE ROLE OF USP5 IN THE REGULATION OF APOPTOSIS IN *DROSOPHILA MELANOGASTER*

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Removal of ubiquitin from polyubiquitylated proteins is performed by deubiquitylating enzymes (DUBs) that catalyze the cleavage of isopeptide bonds between target proteins and ubiquitin. Although the study of DUBs intensified in the last few years, understanding of their functions remains considerably limited. Genetic analysis of mutant phenotypes in the well-characterized model organism, *Drosophila melanogaster* can provide important information to elucidate the function of DUBs.

RNAi knockdown of one of the DUB genes coding for the Drosophila ortholog of human Usp5 (*DmUsp5*) causes early pupal lethality. Late lethality of these animals is accompanied by an increase in the number of apoptotic cells in the larval brain and imaginal discs. The development of homozygous null *DmUsp5* animals stops in L3 and they die in this stage after a 5 day long stagnation period. Acridine orange staining of L3 larval brains and wing discs revealed a very high incidence of apoptosis in these animals. In addition to this, the expressions of *p53*, *reaper* and *hid*, but not *grim* pro-apoptotic genes have been elevated in the *DmUsp5* mutant larval brains and imaginal discs. A heterologous complementation experiment confirmed functional homology between *DmUsp5* gene and yeast *Ubp14*. Western blot analysis and polyubiquitin specific ELISA assay demonstrate the accumulation of polyubiquitins and polyubiquitylated proteins in *DmUsp5* mutants similarly to the yeast orthologue that further support functional conservancy. The expression of the ubiquitin stress responsive *Ubp6* increased highly in *DmUsp5* mutants indicating the ubiquitin stress in these animals.

Based on these observations we conclude that Drosophila *DmUsp5* DUB enzyme appears to be involved in regulating apoptosis and moderating ubiquitin stress.

P-076
KINETIC CHARACTERIZATION OF HUMAN T-CELL LYMPHOMA VIRUS TYPE 3 PROTEASE

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Human T-cell lymphoma virus type 3 is member of the deltaretroviridae subfamily of retroviruses discovered in Cameroon. HTLV-1 and HTLV-3 are closely related. HTLV-3 is associated with neurological disorders, tumors and immunodeficiency. Unlike most HTLV viruses, HTLV-3 can infect both CD4+ and CD8+ T-cells. As it is the case with other retroviruses, HTLV-3 protease has an important role in the viral life-cycle. After the assembly of the immature virions, the protease cleaves the viral precursor proteins into functional units.Thus the protease is a good target for antiretroviral therapy. Our aim was to determine the kinetic parameters for the enzyme and to compare them with those of HTLV-1 protease, furthermore, to determine the inhibition profile of the enzyme. To facilitate these studies, we have optimized HTLV-3 protease expression, preparation and purification. We expressed the protease in BL21(DE3) *E.coli* cells using a pET expression plasmid. The enzyme was purified by reversed-phase HPLC chromatography. Kinetic parameters for peptides representing HTLV-3 and HTLV-1 cleavage sites were performed using an HPLC-based assay system. Furthermore, the specificity of the enzyme was also compared to that of HTLV-1 using a single substitution-containing set of an HTLV-1 protease substrate. Inhibition experiments showed that both IB268 and IB269 inhibited the protease, but unlike with the HTLV-1 enzyme, IB268 was a more potent inhibitor for HTLV-3 protease.

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P-077
SPATIOTEMPORAL EXPRESSION OF MICROGLIA RELATED ANTIGENS IN THE DEVELOPING CHICKEN CENTRAL NERVOUS SYSTEM

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Micrgolia are fundamental elementswithin the central nervous system sustaining tissue homeostasis by phagocyting tissue debris and mediating various processes to either neurons or glial cells. According to recent notion microglia differentiate from primitive myeloid cells emerging from the yolk-sac mesoderm. Progenitors populate the intraembryonic mesenchyme and subsequently the neuroepithelium.Different tracing approaches were usedto track the homing of microglial precursors, but the stages of differentiation are still poorly mapped. In our previous works we reported that chicken microglia express CD45 (protein tyrosine phosphatase receptor type C), CD44 (hyaluronate receptor), MHC class II (major histocompatibility complex II) antigens.

As a novel finding we demonstrated the expression of ChB6 (formerly known as Bu-1; chicken B-cell specific surface glycoprotein) by avian microglia.

In the present immunolabellingwork we report the sequential maturation of microglial cells.The expression of the above mentioned antigens is timely regulated. Patterning was initiated by the homing of CD45+ cells showing subsequent CD44+ and MHC II+ phenotype. Microglial cells undergoing ramification started to express ChB6 from the 8-10th day of embryonic development.In the subventricular zone of the telencephalon the cells preserved a less ramified phenotype showing intense immunoreactions.These areas interestingly overlap the main locations of glio- and neuroblasts.Our results showthat besides the hematopoietic cell markers antibodies against ChB6, a particular B-cell surface protein, represent useful tools to monitor chicken microglial cells in the developing and mature central nervous system.

P-078
EXPLOITING THE EUROPEAN *MEDICAGO TRUNCATULA TNT1* INSERTIONAL MUTANT COLLECTION

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The use of tagged mutant collections is essential to reveal gene functions and to uncover genetic interactions such as those that underlie plant responses to the environment and development. An insertion mutant collection based on the use of Tnt1 and MERE1 retroelements was developed in the *M. truncatula* model legume (Tadege et al., 2009; Rakocevic et al., 2009) during the EU GLIP project (www.eugrainlegumes.org) in parallel to the one already existing at the Noble Foundation (http://bioinfo4.noble.org/mutant/). In the case of the GLIP collection – that was generated in the *M. truncatula* Jemalong background –, however, only the construction of the mutant lines was financed. Consequently, only a limited number of mutants were characterized in this collection, despite the value of such characterized collections for the community. Recently, a bilateral Hungarian-French collaborative project was launched to further develop this specific genomic tool in *M. truncatula* and to facilitate the molecular characterization of new loci involved in nodule and root development. One part of this project deals with the isolation and description of symbiotic mutants of this collection through large symbiotic screens. Another important aim of the project is to sequence the insertion sites of the Tnt1 copies and also those of the endogenous MERE1 active retrotransposon (Rakocevic et al., 2009) in at least 2000 mutagenized lines and contribute to the *Medicago* flanking sequence tag (FST) database to advance further reverse genetics screens. To achieve this goal, the development of a high throughput sequencing method has been initiated.

Large symbiotic screens are currently in progress to isolate Nod$^-$, Fix$^-$ and Nod^{+++} mutant lines in the collection. The identified lines will subsequently be confirmed for their symbiotic character, and the responsible mutated gene will be defined.

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P-079
CHLOROPLAST POLYMORPHISM IN CULTIVATED ALFALFA VARIETIES

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Due to its high nutritive value alfalfa (*Medicago sativa L.)* is one of the most valuable crops used for livestock feeding and also plays an important role in sustainable agriculture by increasing soil fertility and nitrogen content. Alfalfa is an autotetraploid, allogamus, insect pollinated, perennial plant; both cultivars and wild populations are highly heterogeneous.

Chloroplasts are autonomous organelles with strong nuclear communication to maintain all functions. Their genome, due to the prokaryotic origin of the chloroplast, is relatively small, haploid and circular, and 10 to 100 copies per chloroplast are compartmentalized in up to 100 plastid per cell. Polymorphisms are the result of mutations during evolution, which are increasing the heterogeneity in the population. Chloroplast polymorphisms can be used as molecular markers for studying chloroplast inheritance or phylogenetic relationships and can be useful in breeding processes.

We analyzed five regions of the alfalfa chloroplast genome for polymorphism in six Hungarian cultivars selected from three breeding stations (Kisvárda, Kompolt, Szarvas) and two commercially available seed mixes, one of Canadian and one of European origin, sold for human consumption. Our studies were focused both on the differences between cultivars and on the variability within cultivars.

For our study, we have chosen two intergenic regions, *trnI-rpl23, trnE-trnT,* two coding regions, *accD, trnH* and the replication slippage region of alfalfa. We used region specific primers based on the sequence of *Medicago truncatula*, except in the replication slippage region where *Medicago sativa* sequence is available. We isolated DNA from at least 15 individuals from each cultivar and performed PCRs using the region specific primers. The PCR products were separated by gel electrophoresis for AFLP analysis. To validate our results, we sequenced the PCR fragments of a few individual plants from each cultivar.

Our results showed that the *trnI-rpl23* intergenic region and the replication slippage region are highly polymorphic; we found considerable variations between cultivars and between individuals in each cultivar. In the *accD* gene only a few individuals displayed polymorphism and those differences were similar in the different cultivars. Both the *trnE-trnT* intergenic region and the *trnH* coding region were highly conserved; we found only one base pair difference in one cultivar.

The *trnE-trnT* intergenic region and the *trnH* coding region are not suitable for marker development because they lack polymorphism and so does the *accD* region because it has only a few AFLPs, which are the same in the different individuals.

Based on the large number of AFLPs found in the *trnI-rpl23* and the replication slippage regions, both sites are suitable for future marker development. The markers could be used directly or developed into molecular markers for studying chloroplast inheritance.

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P-080
VALIDATION OF MANGALICA SPECIFIC DIAGNOSTIC REAL-TIME PCR METHOD IN A INTERNATIONAL RING TRIAL

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The international ring trial was performed in the framework of the MANGFOOD project aiming at: Improving the competitiveness of Mangalica products using complex portfolio.

The aim of the project is to enhance the competitiveness of Hungarian Mangalica products by providing tools to detect adulteration and supply information for building a comprehensive legal framework. The genomics of this special „Hungaricum" pig breed was investigated with the help of the latest genome sequencing and molecular biological technologies, leading up to the development of a validated DNA-based analytical procedure to detect and quantify Mangalica breed in foodstuffs, catering for the needs of the food industry and the control authorities to ensure traceability and ethical market behavior.

The Mangalica breed is an endemic Hungarian breed of pig, and the conservation and maintenance of its genetic stock is a major and central question.

The MANGFOOD project aimed at developing a DNA-based analytical procedure to detect and quantify Mangalica breed in foodstuffs. The developed real-time PCR method was validated within an international ring trial. We were looking for practiced and reliable laboratories experienced in molecular diagnostic procedures for the validation process.

Fourteen molecular biological laboratories took part in the international ring trial, of which eleven are located in Hungary and three laboratories are from abroad. The selection of the participating laboratories was carefully chosen in order to cover a broad spectrum of manufacturers and instruments, so we could demonstrate the operation of the system developed to be device-independent.

According to the statistical analysis of results that were sent back by the laboratories, it is stated that our self-developed Mangalica-specific TaqMan® based real-time PCR diagnostic system corresponds to the expectations in the investigated 0-50% Mangalica content range.

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P-081
TRANSGLUTAMINASE VARIANTS IN LARGE HUMAN POPULATION

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Transglutaminases catalyze the posttranslational modification of proteins by transamidation of available glutamine residues. Transglutaminase 2 (TGM2) is an unique member of the family with multiple enzymatic activities and interacting partners but its full physiological and pathological role is far from understood.The unusual multiple roles of TGM2 encoded into one polypeptide chain suggest that genomic variations in the TGM2 gene should be limited. At present efforts are in progress to relate human phenotypes to variations at DNA level, of which most are single nucleotide variations (SNVs). Single nucleotide variations research gives insights into genetic basis of complex human diseases. The 1000 genome project (1KG) and Exome sequencing project (ESP) are the two ongoing large scale sequencing projects to detect single nucleotide variants of exons. In this study, we screened these databases for non-synonymous SNVs in the exons of close to 15 thousandsTGM2 Genes and compared them with other transglutaminase family members (TGM1, TGM3-7 and FXIIIa). Based on the avaliable data we conclude that there are different selection pressures on members of the human transglutaminase family and TGM2 is the least tolerant to sequence changes. Although a high proportion of the SNVs potentially may lead to loss of protein function, TGM2 has not shown so far any pathological phenotype associated with SNV's in humans.

We have also carried out a comparative genomics study between humans, primates and mouse sequences which may shed light on the functionaldifferences between transglutaminases of these species and their relevance tohuman physiology and pathology.

P-082
RECOMBINANT MUTAGENIZED FUSION PROTEIN SUBSTRATES OF ALPHAVIRUS PROTEASES

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Alphaviruses are enveloped mRNA viruses of the Togaviridae family. They are distributed worldwide and pose a potential threat to livestock and humans causing encephalitis- and arthritis-like syndromes. Their life-cycle requires four non-structural proteins (nsPs) for the regulation of the viral replication. NsP2 is especially essential since the formation of nsPs depends on its proteolytic activity. Previous studies from our laboratory have characterized the activity of three alphavirus nsP2 proteases using oligopeptide substrates representing the native alphaviral cleavage sites, as part of a study to demonstrate whether nsP2 proteases are suitable for processing recombinant fusion proteins. It was concluded that nsP2 proteases on their native substrates do not provide significant advantages over the already successfully utilized proteases. In order to improve the efficiency of proteolysis by nsP2 proteases, we planned to optimize the substrate sequences. For this purpose substrates were modified by random mutagenesis in their P1' position and were inserted into a recombinant fusion protein. In this construction, oligopeptides are fused to a hexahistidine (His_6) tag followed by maltose binding protein (MBP) on their N-terminal end to ease purification and improve solubility, and to different fluorescence proteins (FPs) on the C-terminal side to facilitate detection. The expression plasmid due to its size (> 8Kb) is not suitable for mutagenesis, therefore we designed a 'restriction cassette' which allows the insertion of the modified substrate sequences that were cleaved out from the mutagenized plasmid by proper endonucleases. We also intend to demonstrate the use of this system for substrate optimization and to show how it is suitable for generating modified alphaviral nsP2 substrates.

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P-083
HOMOLOGS OF *MEDICAGO TRUNCATULA* SYMBIOTIC PROTEINS IN PLANTS

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Symbiotic association with soil microbes proved to be a powerful strategy for plants to overcome nutrient limitations of their habitat. These symbioses provide valuable macronutrients (e.g. P or N) for the host and protective environment rich in sugar compounds for the microbe. An ancient type of coexistence is the symbiosis with arbuscular mycorrhiza (AM) fungi. Its origin can be dated back to the appearance of the first land plants, currently this kind of symbiosis is present in the majority of land plant families. Another, more recent symbiosis exists between nitrogen-fixing soil

bacteria and a narrow range of plants involving phylogenetically closely related species from the Fabales, Fagales, Cucurbitales and Rosales clades. It is called root nodule symbiosis (RNS), during which a new specific organ, the root nodule is formed where the microsymbionts settle down and the nitrogen fixation takes place. Plants capable of symbiotic nitrogen fixation are essential in the natural nitrogen circulation of ecosystems. Crop rotation with nitrogen fixing legumes has been a successful method for centuries, by which crop lands were refreshed for horticultural cultivation and higher yields could be obtained. Still, biological nitrogen fixation is cost-efficient and effective solution for the continuously growing nitrogen demands of agricultural cropping all around the globe.

Improving the efficiency of nitrogen fixing symbiosis may be possible, however, to achieve this goal not only subcellular and molecular processes should be well-understood, but genes necessary and satisfactory for nitrogen fixing symbiosis need to be identified and well-characterized in nodulating plants. Our present knowledge of the molecular apparatus needed for these symbiotic associations is mainly due to intensive ongoing research on symbiotic mutants of two chosen model organisms: *Medicago truncatula* (Mt) and *Lotus japonicus* (Lj). Genetic analyses showed that the AM and RNS systems share genes, supporting the idea that already existing elements of the more ancient program were recruited during the evolution of root nodule symbiosis. Therefore exploring and studying the homologs of symbiotic genes in non-legume plants are of great importance as well.

Homologous counterparts of legume symbiotic genes had been identified in non-legume plants too. Some of them are able to partially or fully accomplish the symbiotic functions of their legume homolog as it was proven by a number of studies. Our goal was to search plant genomes by symbiotic genes to identify the respected homologs, thereby to show to what extent these genes are conserved outside the nodulating clades. A thorough comparison could give a hint whether which genes are more likely able to fulfill symbiotic functions based on their sequence similarity to legume symbiotic genes.

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P-084
SRUCTURAL ANALYSIS OF THE HUMAN TRPV1 CHANNEL

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Transient receptor potential vanilloid 1 (TRPV1) is a nonselective, Na^+/Ca^{2+} permeable cation channel. It is involved in pain sensation and in a wide range of non-pain-related physiological and pathological conditions. TRPV1 can be activated by different stimuli, including heat, acidification,

exogenous compounds such capsaicin and resiniferatoxin, or the endogenous anandamide. It is considered to be a polymodal integrator of painful stimuli and inflammatory processes and is a subject of intensive pharmacological research. Revealing the relationship between the function and main structural determinants could lead to the exploration of potentially useful drugs and development of new targeted therapies.

To investigate the fine structure of TRPV1 in particular details we produced the full length protein in the yeast *Schizosaccharomyces pombe* and in the insect *Spodoptera frugiperda* (Sf9) cell line for later X-ray crystallography. The N-terminal ARD-repeats and the C-terminal domain responsible for tetramerization, calmodulin- and PIP2 binding, were expressed in *Escherichia coli* bacteria and purified for binding assays, NMR and X-ray crystallography studies. We have examined the ion selectivity of the pore region and demonstrated the pore-blocking activity of some bivalent heavy metal ions in the following order Co^{2+}> Cd^{2+}>Ni^{2+}>Cu^{2+}>Zn^{2+} , respectively. We have also analyzed some selected amino acid mutants of the channel pore. Based on these succeedings, homology remodelling and *in silico* data mining we'd like to confer the functional, structural and computational results for better understanding of this interesting receptor.

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P-085
EXPLORING THE CONFORMATION AND ACTIVITY OF TRANSGLUTAMINASES BY FLUORESCENCE RESONANCE ENERGY TRANSFER

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Four crystals of transglutaminase (TGM) 2 have been resolved so far by X-ray crystallography yielding Exploring the conformation and activity of transglutaminases by fluorescence resonance energy transfer.

structures that attest to major domain reorganizations upon allosteric effector and/or substrate binding. Homologue models and resolved structures of other members of the TGM family suggest a very similar functional organization.

We are developing Förster resonance energy transfer (FRET)-based methods to detect these predicted conformational transitions in real time, to show how they are brought about by regulatory molecules, Ca^{2+} and GTP, and above all, leveraged by the correspondance between conformational and activity states, to detect enzyme activation directly in simple biological models, such as live cultured cells. Besides TGM2 we are focusing on the least studied TGM6 and 7.

FRET has gained wide acceptance as a technique to observe dynamic structural changes in proteins. We are following the common strategy of appending fluorophores to the ends of the protein domains involved using small biarsenical dyes – an emerging alternative to fluorescent proteins – which bind to short peptide motifs engineered into TGM2.

Working with purified recombinant protein *in vitro* we hope to calculate physical distances between the labeled tags at the termini relying on the strict dependence of the FRET phenomenon on distance.

In a second approach we are using fluorescently labeled specific substrate peptides to detect FRET with the labeled enzymes in the enzyme-substrate complex.

We are also investigating the possibility of using various FRET producing constructs in live cells as biosensors of TGM-activity exploiting the membrane permeability of the biarsenical dyes or using cell penetrable peptide additions to substrate peptides. We hope to obtain a subcellular activity map with spatiotemporal resolution and expect that the probes will reveal intracellular sites and biochemical processes where TGM2 is active as a transamidase.

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P-086
TYROSINE PHOSPHORYLATION-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES OF SH3 DOMAINS

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SH3 (Src homology 3) domain is one of the best characterized and studied protein interaction modules. It belongs to the family of proline-rich motif binding domains; recognize polyproline-II helices, generally the PXXP core motifs as a central element.

Protein phosphorylation is one of the most fundamental regulatory events in eukaryotic cells. The importance of reversible tyrosine phosphorylation in function of cells is emphasized by the fact that tyrosine kinases comprise the largest group of oncoproteins.

It has recently been established that the SH3 domain can get tyrosine phosphorylated, but only little is known about the possible structural and functional implications of this covalent modification.

In the course of our work, we demonstrated the phosphorylation of a neuronal scaffold protein Caskin1 on its SH3 domain by EphB1 receptor tyrosine kinase. Two tyrosine residues were identified to be phosphorylated within the domain: Tyr296 and Tyr336. Tyr296 is localized in the flexible RT loop linking the first two beta-strands, while Tyr336 is present in the more compact fourth beta-strand. For further analysis, we expressed and purified the isolated SH3 domain and phosphorylated it in vitro by a recombinant active EphB1. The time-course of the phosphorylation was followed by native gel electrophoresis.

The first data referred to the structure was obtained by CD-spectroscopy. Comparing the far-UV spectra of phosphorylated and non-phosphorylated SH3 no major difference was detected. Far-UV spectroscopy provides information about the secondary structure. Significant change was

observed in the near UV spectra upon tyrosine phosphorylation of the SH3 domain. It seems that the chemical environment of these residues is clearly sensitive to phosphorylation suggesting that tertiary structure of the SH3 domain is likely altered around the phosphorylated tyrosines.

Comparing HSQC spectra of phosphorylated and non-phosphorylated SH3 domains, the second and third ß-strands and also the N-Src loop seemed to change.

Since interaction partners are not yet identified for Caskin-1 SH3 domain, similar investigation of an additional SH3 domain – the domain of Grb2 adaptor protein - has been started. Phosphorylation of this protein within the SH3 domain has been already published, and it is known that it causes the release of a nucleotide-exchange factor Sos.

Our goal is to identify the general mechanism by which SH3 domain phosphorylation regulates protein-protein interaction.

P-087
A ROLE FOR RASGAPS IN ASSOCIATIVE LEARNING AND MEMORY IN *C. ELEGANS*

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A conserved subfamily of the GTPase activating proteins (RasGAPs) increases the intrinsic catalytic activity of Ras and downregulates the Ras/MAPK pathway. The function of RasGAPs is well characterized in development and tumorgenesis, however, their role/involvement in learning and memory is unclear/unknown.

Orthologues of gap genes can be found in many species including *Caenorhabditis elegans*. The nematode *C. elegans* is the only organism where animals carrying single RasGAP mutation and even double mutants are viable. *C. elegans* is an excellent model system due to its short lifespan, widely explored genome and the available and proven experimental methods. Its simple nervous system of 302 neurons can produce surprisingly complex behavioral patterns similar to higher organisms including humans. Here, we investigated the function of the different RasGAP genes in learning and memory.

First, loss-of-function single mutants of the *gap-1 (ga133)*, *gap-2 (tm748)*, *gap-3 (ga139)* genes and double mutants (*gap-1;gap-2*, *gap-1;gap-3* and *gap-2;gap-3*) were tested for motility and chemotaxis towards volatile compounds to exclude possible sensory and motor defects.

In aversive olfactory associative learning the intrinsic attraction to diacetyl was associated with starvation, which resulted in the acquisiton (learning) of an aversive behaviour.

The retention of conditioned behavior over time (short-term memory) was tested after a recovery phase. Multiple conditionings and longer test intervals (16 and 24 hours) provided data about long-term memory.

Our results show that *gap-1* alone is required for olfactory associative learning, while short-term and long-term memory is regulated both by *gap-2* and *gap-3* function. Additionally, the *gap-1;gap-2* and *gap-2;gap-3* show both learning and memory defects. *gap-1;gap-3* mutant worms have chemosensory defect. These findings demonstrate a complex and redundant action of the different RasGAP proteins that act in concert to ensure proper associative learning and memory.

As a next step, we also established an *in silico* protein-protein interaction network of RasGAPs in order to find the possible signaling pathways that could be the essential mediators of the RasGAP function in learning and memory. In future, we will combine the *in silico* data with the power of *C. elegans* genetics to elucidate the molecular role of RasGAPs in learning and memory.

Our results demonstrate that the different RasGAPs act in concert to ensure proper learning and memory. Due to functional conservation amongst species, our findings will likely contribute to better understand the role of different RasGAP isoforms and the regulation of the Ras/MAPK pathway in vertebrate cognitive functions.

P-088
THE SELECTIVE AUTOPHAGY CARGO P62 FACILITATES AUTOPHAGOSOME FORMATION BY BINDING MULTIPLE ATG PROTEINS IN *DROSOPHILA*

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P-089
STUDIES ON THE FULL-LENGTH HUMAN NOD-LIKE RECEPTOR FAMILY CARD DOMAIN CONTAINING 5 (NLRC5) PROTEIN

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Members of NOD-like receptor (NLR) family are pattern recognition receptors having important roles in the immune system. They have key role in the regulation of pathways after the recognition of microbial- and danger-associated molecular patterns in vertebrates.The NOD-like receptor family CARD domain containing 5 (NLRC5) is a unique member of the NLR protein family. It is the largest member of this family containing unusually high number of leucine-rich repeats (LRRs) and an untypical CARD (caspase activation and recruitment domain) effector domain. NLRC5 is an intensively studied receptor with various functions, including the capabilityfor transcriptional regulation of MHC class I, and its regulatory role in various signal transduction pathways has also been reported. It is still controversial whether the NLRC5 is involved in the inflammasome activation. However, NLRC5 has proposed function in viral recognition but its direct ligand is still unidentified. Furthermore, there are only few structural data about NLRC5 due to the lack of crystal structures of full-length NLRs and NLRC5, the low number of template structures for homologous modeling and the unique domain architecture of NLRC5.

This work was initiated with the aim to analyze the sequences of NLRC5 and some NLRC5 related proteins and to build a homologous model for the full-length human NLRC5. Sequence alignment of NLRC5 and NLRC5 related proteins resulted in the recognition of consensus patterns within the studied LRR sequences and two typical types of consensus LRR sequences were identified in the RI and NLRC5 proteins. Investigation of LRR sequences helped to identify LRRs in NLRC5 and to predict their number and position within the protein. In spite of the presence of an untypical CARD and unusually long LRR domains of NLRC5 we were able to construct a homologous model for both the monomeric and a homo-heptameric full-length human NLRC5 protein having open conformations for the monomers. To determine the molecular weight of NLRC5, we expressedflag-tagged NLRC5 in 293T cells. To determine its molecular weight gel filtration analyses was performed.

Our model can help the better interpretation of NLRC5 structure studies in the future and could identify critical residues of the molecule. Further optimization of NLRC5 overexpression and purification system can support the functional studies of NLRC5.

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P-090
INTRACELLULAR LOCALIZATION OF NCB5OR OXIDOREDUCTASE ENZYME

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Introduction: NAD(P)H cytochrome b5 oxidoreductase (Ncb5or) is a recently described natural fusion protein which shows close structural similarity to two enzymes of fatty acid desaturation. The function of Ncb5or has not been clarified yet, moreover, the literature about its intracellular localization is also contradictory. In Ncb5or knock-out mice, as a consequence of the increased stress sensibility of β-cells, diabetes is developed. To elucidate the protecting effect of Ncb5or on β-cells, our first aim was to clarify the exact intracellular localization of this soluble enzyme.

Methods: The intracellular localization of endogenously expressing Ncb5or was analyzed using human HEK293T cell line. Cells were harvested and homogenized, the subcellular fractions were separated by subsequent (ultra)centrifugations. The purity of subcellular fractions was confirmed, characteristic proteins of cell organelles were detected by immunoblot. The intracellular localization of Ncb5or was further analyzed by fluorescence microscopy. Green fluorescence fusion protein was generated by subcloning Ncb5or into pEGFP-N1 vectorial background. The construct was transiently transfected into HEK293T cells and analyzed by microcopy. As a control, nucleus and endoplasmic reticulum were also stained.

Results: Expression of endogenous Ncb5or could be demonstrated in our HEK293T cell line both at mRNA and protein levels. Purity of the generated nuclear, microsomal, mitochondrial and cytosolic cell fractions were confirmed by the detection of proteins characreristic for the given cell compartment. Ncb5or could only be detected in the cytosolic fraction using an antibody either against the protein, or against the tag which was used for labeling. This observation could be confirmed using fluorescent microscopy. Ncb5or fusion protein was observed in the cytoplasm of the cells in a diffuse manner, but no co-localization could be detected with fluorescent markers labeling either the nucleus or the endoplasmic reticulum.

Conclusions: Our results clearly prove, that Ncb5or is localized in the cytoplasm, consequently the earlier presumption, suggesting that the enzyme uses reducing equivalents directly from the lumen of the endoplasmic reticulum could be ruled out. On the other hand our observations agree with the putative role of the enzyme in the fatty acid metabolism, however further research is necessitated to clarify this mechanism in detail.

P-091
CHARACTERISATION OF ISOPEPTIDASE ACTIVITY OF TRANSGLUTAMINASE 2

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Transglutaminase 2 (TGM2) is a multifunctional protein composed of four domains and well conserved in primates and humans. It takes part in intra- and extra-cellular processes and is involved in neurodegenerative, oncogenic and immunological disorders, but its exact roles are not fully understood in these diseases. TGM2 has various enzymatic activities and the best studied one is the Ca^{2+}-dependent transglutaminase activity which covers several kinds of protein modifications: formation of γ-glutamyl-ε-lysine bond between polypeptide chains, mono- or polyaminylation by transamidation and deamidation of protein-bound glutamine by hydrolysis. TGM2 has a poorly studied isopeptidase activity when the previously formed γ-glutamyl-ε-lysine or –amine bonds are cleaved.

Our aim was the detailed biochemical characterisation of the isopeptidase activity of human TGM2 and to compare the Val224 wild type form, as revealed by large scale exon sequencing, to the so far widely used Gly224 variant derived from a cloning error.

Fluorescent kinetic assay was used in which transglutaminase 2 cleaves the isopeptide bond in the oligopeptide substrates accompanied by the release of the cadaverine linked dark quencher increasing the fluorescence. After the optimisation we determined the kinetic parameters, Ca^{2+}-dependence, GTP inhibitory effect of both purified recombinant proteins. Our results show that the Gly224 mutant has lower activity and calcium sensitivity but similar substrate affinity compared to the wild type. Our data suggest that Val224 has significant role in the Ca^{2+}-binding and the regulation of the catalytic processes, including isopeptidase activity of TGM2.

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P-092
PDBTM: PROTEIN DATA BANK OF TRANSMEMBRANE PROTEINS AFTER EIGHT YEARS

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The PDBTM database, the first comprehensive and up-to-date transmembrane protein selection of the Protein Data Bank (PDB), was launched in 2004. The database was created and has been continuously updated by the TMDET algorithm that utilizes a geometric algorithm and is able to distinguish between transmembrane and non-transmembrane proteins using their 3D atomic

coordinates only. The TMDET algorithm can locate the spatial positions of transmembrane proteins in lipid bilayer as well. During the last eight years not only the size of the PDBTM database has been steadily growing from approx. 400 to 1700 entries but new structural elements have been identified as well, in addition to the well-known α-helical bundle and β-barrel structures. Numerous "exotic" transmembrane protein structures have been solved since the first release, which has made it necessary to define these new structural elements, such as membrane loops or interfacial helices in the database. The PDBTM also defines the biologically active oligomer form of transmembrane proteins. The present paper reports the new features of the PDBTM database that have been added since its first release, and our current efforts to keep the database up-to-date and easy to use so that it may continue to serve as a fundamental resource for the scientific community involved in the research of transmembrane protein structures. The PDBTM database is available at http://pdbtm.enzim.hu.

P-093
STUDIES ON THE HIV-2 PROTEASE AS A CHEMOTHERAPEUTIC TARGET

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Since its discovery in 1986, the human immunodeficiency virus type-2 (HIV-2) along with HIV-1 have infected more than 34 million worldwide. Once thought to be limited to west Africa where it originated, the incidence of HIV-2 infection is on the rise in Europe and America. The HIV protease is a homodimericaspartyl protease that is crucial for the viral life-cyclecleaving proviralpolyproteins, hence creating mature protein components that are required for the assembly of an infectious virus. With diagnostic measures and clinically used protease inhibitors focusing on HIV-1, studies of the efficacy of those inhibitors on HIV-2 protease remain widely lacking. With the help of a wild-typeHIV-2 vector backbone and cloning techniques we have developed a cassette system where the efficacy of clinically used protease inhibitors can be studiedfor various serotypes of HIV-2 protease both *in vivo* and *invitro*. *In vitro*, the enzyme was expressed and its activity was characterized by using high-performance liquid chromatography,the stability of the enzyme was then determined by studying its autodegradation. Furthermore, we have measured the $IC_{50}$and K_i values of some of the most widely used clinical inhibitors (nelfinavir, indinavir, ritonavir andamprenavir) using an oligopeptide substrate-based assay. In our experiments, optimization of the expression protocol led to a very stable enzyme with high folding efficiency. Indinavir and ritonavir provided the best *in vitro* inhibition, while amprenavir showed a much lower efficacy.We believe that the combination of *in vitro* and *in vivo* studies performed with our cassette system will provide an accurate measure of the efficacy of currently used protease inhibitors, bearing in mind the rising incidence of HIV-2 and the scarce studies of its protease, the characterization of the inhibition profiling is indeed crucial.

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P-094
PUTATIVE ACTIVATION MECHANISM OF A PLANT SPECIFIC MAPK

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The three tier mitogen-activated protein kinases (MAPKs) cascades are fundamental modules of signal transduction in all eukaryotes. The hallmark of the MAPKs is the TXY phosphoacceptor site which needs to be phosphorylated on both amino acids by MAPKKs to fully activate the kinase. Sequence analysis of plant MAPK pathway components has revealed that in addition to conserved motifs, plants have also evolved unique features. The majority of plant MAPKs possesses the classical ERK type TEYmotif in their T-loop and the C terminally located MAPKK binding site, known as common docking domain (CD). This subtype is divided into three subfamilies, A-C, and a panel of upstream, activating MAPKKs of these MAPKs has been described. The other subtype comprises one subfamily (D) which holds a plant specific TDY amino acid pattern at the corresponding position and is devoid of CD domain. Furthermore, no interacting MAPKKs of D type MAPKs have been identified yet, thus mechanism of their activation is presently unknown.

Our protein of interest, AtMPK9, is an *Arabidopsis* representative of MPK subfamily D, it possesses both the TDY activation loop and the long carboxy-terminal extension with unknown function, another distinctive mark of the members of this MPK subfamily. We aimed to investigate the activation mechanism of AtMPK9 by analyzing kinase activities and phosphorylation patterns of different *in vitro* mutagenized variants of the kinase. We inserted AtMPK9 and the created mutants into specific translational vectors and produced proteins by wheat germ based, cell-free *in vitro* translation. Our results indicate that phosphorylations of threonine and tyrosine of activation loop are equally inevitable for the kinase activity. Interestingly, experiments implemented with the kinase-dead mutant suggest that the phosphorylation of these amino acids relies on autophosphorylation. According to mass spectrometry analysis, in contrast to *in vitro* trasnlated wild-type AtMPK9, neither threonine nor tyrosine of TDY was phosphorylated of kinase-dead mutant. In accordance with the autophosphorylation hypothesis, the in vitro translated AtMPK9 showed similar level of *in vitro* kinase activity than AtMPK6 – a member of subfamily A – activated with its gain-of-function MAPKKs. Presently, we study the mechanism and regulation of AtMPK9 homodimer formation.

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P-095
CYTOSINE-TO-URACIL DEAMINATION BY SSSI DNA METHYLTRANSFERASE

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A few prokaryotic DNA(cytosine-5) methyltransferases (C5-MTase) catalyze cytosine to uracil deamination of the target cytosine in the absence of the methyl donor S-adenosyl-methionine (SAM) (e. g. M.HpaII, Cell 1992, 71: 1073-1080). The reaction appears to be dependent on transient covalent bond formation between the active site cysteine thiol and carbon 6 of the cytosine leading to a 5,6-dihydrocytosine intermediate with an enhanced tendency for deamination. It remains to be determined how general this feature is for C5-MTases.

One of these enzymes (M.SssI) shares the specificity (CG) of eukaryotic DNA MTases, and thus has special importance as an experimental tool in the study of eukaryotic DNA methylation. The possibility to use M.SssI as a CG-specific cytosine deaminase would greatly increase the value of this enzyme in epigenetic research. There are conflicting results in the literature with regard to the ability of M.SssI to deaminate cytosine therefore we started to re-investigate this phenomenon. We used a sensitive genetic reversion assay in which a C to U transition in a mutant kanamycine resistance gene restores the kanamycine resistant phenotype.

We found that incubation with M.SssI *in vitro* in the absence of SAM led to a 3-fold increase of cytosine deamination over the spontaneous rate. The SAM analog 5'-amino-5'-deoxyadenosine increased M.SssI-catalyzed deamination 10-fold. A mutant enzyme M.SssI(G19D), presumably impaired in SAM binding, exhibited high cytosine-deamination activity *in vivo* in the presence of SAM, acting as a mutator enzyme.

Experiments aimed to determine whether M.SssI can deaminate 5-methyl cytosine to thymine, are in progress.

P-096
LIPOMA-PREFERRED PARTNER PROTEIN (LPP), A GENETIC MARKER FOR COELIAC DISEASE PREDISPOSITION IS INTERACTING WITH TRANSGLUTAMINASE 2

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Objectives and Study: Coeliac disease is an intestinal disorder triggered by dietary gluten from wheat, rye and barley and is characterized by small-intestinal lesion with villous atrophy, crypt hyperplasia and generation of autoantibodies against type-2 transglutaminase (TG2). It is a permanent gluten intolerance segregating in families and is inheritable. The genetic risk for celiac disease is determined by the presence of HLA-DQ2 or DQ8 and othernon-HLAgeneswithlower contribution. The LPP geneencoding a structural protein important for cell motility and smooth muscle cells shows high association with celiac disease in four consecutive intronic single nucleotide polymorphisms. It is currently unknown how LPP in involved in the disease pathogenesis. The aim of this study was to investigate whether LPPcan associate with transglutaminase 2 (TG2) or targeted by serum antibodies from celiac disease patients.

Methods: Biopsy samples from 6 normal and 5 coeliac disease patients as well as human umbilical cord endothelial vein(HUVEC) and arterial muscle cells derived from normal and celiac subjects were analysed for LPP expression by real time PCR, Western blot and immunohistochemistry. Human recombinant LPP, fulllength TG2 and its domain deletion fragments were expressed in E. coli and used for interaction studies by enzyme-linked immunoassay (ELISA) and surface Plasmon resonance real-time binding analysis.

Results: LPP was similarly expressed in normal and coeliact issues. Both TG2 and LPP localised to focal adhesion complexes and associated with thecytoskeleton of muscle cells maintained in culture. The expression in HUVECs was very low. Recombinant LPP bound dose-dependently to TG2 and the interaction involved multiple domains of TG2. Serum IgA antibodies from celiac disease patients showed a higher reaction with recombinant LPP in ELISA than those from non-coeliac controls.

Conclusion: LPP is directly binding to TG2, the main celiac autoantigen.

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P-097
EXAMINATION OF NUCLEIC ACID BINDING OF THE NEWEST PRION PROTEIN, SHADOO, USING AGAROSE GEL SHIFT ASSAY

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Transmissible spongiform encephalopathies including Creutzfeldt-Jakob disease, fatal familial insomnia, bovine spongiform encephalopathy and scrapie are fatal, incurable neurodegenerative disorders. Transmissible spongiform encephalopathies are associated with the formation of the infectious isoform of the prion protein which is generated by a conformational conversion from the cellular prion protein. The prion protein family includes two additional members besides the cellular prion protein, the Doppel and the newest prion protein, the Shadoo. In 2008 an evolutionary conserved RGG-box motif, which is known to be able to bind RNA, was identified *in silico* in the amino acid sequence of Shadoo. Recently, nucleic acid binding ability of Sho was demonstrated in *in vitro* experiments.

Here we study the nucleic acid binding features of Shadoo and aim to map its nucleic acid binding region utilizing the wild type and deletion and fusion mutants of Shadoo fused to a mCherry fluorescent protein in pRSET-B bacterial expression vectors. Three regions of Shadoo were studied: a short predicted nuclear localization signal, the RGG-box, as well as the glycine and arginine rich $(RXXX)_8$ motif that is contained in region K25-R61 of the Shadoo protein. Proteins were expressed in *E. coli* BL21 strain and partially purified using Ni-NTA agarose beads. The nucleic acid binding was examined with a newly developed Agarose Gel Shift Assay method, which exploit the advantages of a fluorescent protein fusion tag.

P-098
INVESTIGATION IN THE PUTATIVE ROLE OF NCB5OR REDOX ENZYME IN LIPID DESATURATION USING IN VITRO TRANSLATION AND RECONSTITUTION IN PROTEOLIPOSOMES

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NADH cytochrome b5 oxidoreductase (Ncb5or) is a novel natural fusion protein of unrevealed function. Pancreatic β-cells of Ncb5or (-/-) mice show highly increased sensitivity to lipotoxicity; and the animals develop insulin dependent diabetes mellitus at age 4-6 weeks. The two redox domains of this soluble enzyme are homologous to Cytochrome b5 (Cyb5) and Cyb5 reductase (Cyb5R) integral membrane proteins of the endoplasmic reticulum (ER). Due to the well characterized roles of Cyb5/Cyb5R couple in microsomal fatty acid desaturation, it has been

hypothesized that Ncb5or can also provide electrons to stearoyl-CoA desaturase isoenzymes (Scd1 and 5), which catalyzes conversion of stearoyl- to oleoyl-CoA.

We aim to reconstitute a functional human desaturase system in vitro and elucidate the putative physical and functional interaction between Ncb5or and Scds.

The soluble form of His6-Cyb5R is expressed in E. coli BL21(DE3) in auto-induction medium and purified in His-Select Cobalt Affinity Gel. The coding sequences of glutation-S-transferase-tagged Cyb5 and Ncb5or as well as the histidine tagged Scd1 were inserted in pEU3 vector for cell free in vitro tanslation. The two membrane proteins (Cyb5 and Scd1) were synthesized in the presence of liposomes (Avanti Polar Lipids, Alabaster).

The original catalytic triad can be reconstituted by mixing soluble Cyb5R and proteoliposomes containing both Cyb5 and Scd1. The hypothesized role of Ncb5or will be addressed by testing its potential to replace Cyb5R and Cyb5 in the complex. The enzyme activity will be assessed by HPLC detection of saturated and unsaturated fatty acyl-CoAs. Physical protein-protein interactions of differently tagged purified enzymes will be examined by high resolution Alpha Screen method. The functional reconstituted enzyme complex is also suitable to investigate the effect of various human mutations in the individual components.

The expected results of our investigations can clarify the contribution of Ncb5or to fatty acid desaturation, which in turn will help to understand the role of this novel enzyme in the protection of pancreatic β-cells against lipid-induced oxidative and ER-stress. Since both Scd1 and Ncb5or are implicated in the pathogenesis of diabetes, our studies may reveal potential new drug targets for this epidemic.

P-099
PHENOTYPIC HETEROGENEITY PROVIDES ADAPTIVE ADVANTAGE UNDER HIGH SELECTIVE PRESSURE

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Isogenic individuals within a population generally show a certain level of phenotypic variability. However, the role of this phenomenon in evolutionary processes is highly debated and needs to be explained.

To investigate this phenomenon, two synthetic constructs were used in the same yeast background, resulting in isogenic strains which show different heterogeneity of gene expression of *PDR5* efflux pump. In our strains the mean expression level of *PDR5* pump is similar but the coefficient of variation is different.

Evolutionary experiments were carried out in the presence of the antifungal agent, fluconazole, which is a known substrate of Pdr5p. Based on our experiments the heterogeneity is neutral when the selective pressure is low. In contrast, heterogeneity provides advantage when population faces a higher selective pressure: the survival subpopulation is greater which provides increased chance of accumulation of beneficial mutations.

Our results provide experimental evidence which supports the hypothesis that the phenotypic heterogeneity of an isogenic population can contribute to adaptive responses in changing environments.

P-100
HISTONE ACETYLATION BY ATAC COMPLEX REGULATES STEROID HORMONE BIOSYNTHESIS

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Steroidogenic factor 1 (*Sf-1*) is a nuclear receptor which plays an important role in adrenogonadal development and steroidogenic gene expression. The activity of *Sf-1* is controlled by posttranslational modifications. Phosphorylation at Ser203 and acetylation by GCN5 and p300 have been reported to enhance *Sf-1* function.

The *Sf-1* shows tissue specific expression being highly expressed in the adrenal cortex, testis, ovary, hypophysis, ventromedial hipothalamus, skin and spleen. Its mutation or disregulated expression can lead to tumor formation.

Recently we have reported that the lack-of-function mutations of the GCN5 histone acetyltransferase (HAT)-containing ATAC complex influence steroid biosynthesis. In contrast, the lack of the other GCN5-containing HAT complex, SAGA had only mild effect on steroid biosynthesis. The mechanism by which ATAC affects steroid synthesis, although remains to be discerned. The two most probable scenarios could be that ATAC influences the transcription of genes involved in steroid hormones biosynthesis directly by histone acetylation at their promoters, or that it acetylates SF1 and by this regulates the transcription of steroid converting gene indirectly. In order to gain information on which of these possibilities might occur we studied the presence of ATAC complex at the regulatory regions of *Cyp11a1* gene that catalyzes the first regulatory step of steroid hormone biosynthesis in Y1 (mouse adrenocortical tumoral) cell line.

We studied whether ATAC- (H4K5ac) or SAGA- (H3K9ac) specific histone acetylation is present at the promoter, initiator and 3'UTR (untranslated) regions of the *Cyp11a1* gene. We found that H4K5 acetylation can be detected at all three regions, while we could not detect SAGA specific H3K9 acetylation. We also investigated whether the acetylation level is altered upon TSA (trichostatin A) histone deacetylase inhibitor treatment. We found that H4K5 acetylation level was significantly increased at all three gene regions after TSA treatment.

Based on our findings we conclude that the ATAC HAT complex plays a role in steroid hormone biosynthesis through histone acetylation. To provide further proofs to this conclusion we have started experiments to detect the presence of the ATAC complex at the regulatory regions of the *Cyp11a1* gene.

P-101
THE FUNCTION AND USE OF THE H4 REPLACEMENT GENE (H4R) OF *DROSOPHILA MELANOGASTER*

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The basic building unit of chromatin is the nucleosome, which contains a protein core composed of histone proteins wrapped around 146 bp of DNA. Histone proteins are evolutionally conserved and their covalent modifications (acetylation, methylation, phosphorylation, etc.) regulate gene expression and several other chromatin related processes.

Histones in higher eukaryotes are encoded by two types of genes. Genes for replication-dependent histones are active predominantly in the S phase of the cell cycle. These genes are usually present in multiple copies, contain no introns and their transcripts are not polyadenylated, therefore have a short half lifetime. In contrast with these, replacement histone genes are usually present in a single copy, contain introns and produce polyadenylated mRNAs.

In *Drosophila melanogaster* the only replacement gene for histone H4 is *H4r*, which encodes via an intron-containing polyA mRNA a protein that is identical in amino acid sequence with its replication-dependent H4 counterpart. This poses the question: what is the point to have replacement histone if its structure is identical with that of its canonical form?

To find an aswer to this question we aimed to obtain new information on the function of *Drosophila H4r* by studying the expression of the *H4r* gene.

We studied *H4r* promoter function by transfecting *H4r* promoter-luciferase riporter genes into *Drosophila* S2 cells. We found that the *H4r* promoter is functional in S2 cells, thus is useable for another *in vitro* and *in vivo* experiments. One possibility could be that the *H4r* gene is activated through stress signals. To test this assumption, we used Actinomycin D and UVB-irradiation to determine the transcriptional response of the *H4r* promoter for DNA damaging agents induced stress. However, we found no significant change in *H4r* gene expression in S2 cells following replication or transcription block.

We investigated the change in *H4r* gene expression after replication and transcription block using RT-PCR assay. We are not found a significant change in *H4r* gene expression in S2 cells, therefore we aimed to research further the function of *H4r* promoter *in vivo*.

We injected wild type *Drosophila* embrios with a plasmid, which contains in fusion of the endogen *H4r* promoter (1000bp) and a GFP (Green Flourescent Protein). We examined the intensity and pattern of GFP signal for interphasis cells in dissected salivary glands of L3 larvae and to compare the cells in different stages of cell cycle in neuroblasts using by confocal microscope. Our further aims to research for *H4r* gene expression through abovementioned stressors *in vivo* and therefore to identify the possible function of *H4r* gene in the cell.

P-102
HOW DEEP IS YOUR DEEP SEQUENCING? – A MATHEMATICAL APPROACH FOR THE CHARACTERIZATION OF SAMPLE BEHAVIOUR IN CHIP SEQUENCING

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Deep sequencing technologies are generating unprecedented ammount of data and we are getting more and more insight into the details of gene expression regulation. One of the most frequently used technology in functional genomics is chromatin immunoprecipitation (ChIP). Combining ChIP with deep sequencing (ChIP Seq) we can identify the binding sites of specific transcription factors in a whole genome approach.

We performed mathematical modelling of sample behaviour of the ChIP datasets and tested the model on experiments with more than 50 million reads per sample. Our results show that high quality ChIP Seq datasets always show a saturating behaviour. We investigated several methods to predict the saturation of the identified peaks. Our datasets show that the identified peaks can be clearly divided in at least two large populations that have adistinct behaviour. The highest peaks show a distinct motif enrichment compared to the majority of peaks that have a relatively low tag number.

The investigated datasets can be well described by Zipf's law with characteristcs following Heap's law. Weibull aproximation and the three parametric asymptotic aproximation can be usefull to predict the maximum saturation point but a Zipf saturation modell based on lexicon size and variability investigations performed equally good. Similar to the languange investigations, predictions are reliable only if the predictor datasets are at least 50% of the predicted maximum. The distribution of the peaks follows a double power law distribution, with unexpectedly high differences in the power value. Striking similarities can be observed with the analysis on n-grams in public datasets. These similarities suggest that the identified binding sites can be subdivided in a core group that is constant in majority of the cells investigated, and a variable group that reflects the variability of individual cells in a population.

P-103
TRANSPOSONAL HYPOMETHYLATION AND LOCAL DEMETHYLATION OF PRIMARY MELANOMAS ARE ASSOCIATED WITH COPY NUMBER LOSS OF DNMT1 AND UNFAVOURABLE CLINICAL OUTCOME

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The best described factor involved in melanoma epigenetics is DNA methylation, however, is important to distinguish between genome wide hypomethylation and localized hypermethylation.In melanoma, the most aggressive form of skin neoplasm, the presence of genome wide hypomethylation has been reported by several groups. Although the degree of global demethylation has not been proved to be sufficient to distinguish benign naevus from melanoma and no demethylation-based distinction has been drown among diverse melanomas with different clinical behaviours. In contrast to the transposable widespread hypomethylation, the so-called hypermethylation is usually strictly localized into the transcriptionally active gene parts and promoters inhibiting directly the gene expression.There are currently substantial amounts of data available that refer to gene silencing associated with the localised CpGhypermethylation of a specific gene promoter, however, most of the provided data are derived from cell lines or used single-gene approach.

Our main goal was to persuade a study to obtain better insights into how distinct types of DNA methylation changes associate with melanoma progression. As Knudson's two-hit hypothesis is often achieved through a combination of differing typesof genomic alterations, we also aimed to investigate whether methylation patterns are associated with other types of somatic alterations such as the most frequent mutation and DNA copy number alteration.

Using quantitative pyrosequencing, we found that transposonalhypomethylation was associated with shortened relapse free survival of melanoma patients, however, Cox regression pointed direct relationship of the overall loss of 5-methyl-cytosins and metastatic potential. To study methylation patterns of 1,505 promoters related CpGs, we applied Illumina Golden Gate assay, hypermethylation of 57 CpGswas uniquely accompanied by $BRAF^{V600E}$ mutation. Studying the effects of hypermethylation on patients' survival, we summarized 6 genes (DSP, EPHB6, HCK, IL18, IRAK3 and KIT) whose hypermethylation were associated with lower overall survival rate and 4 out of 6 genes (DSP, HCK, IL18 and KIT) exhibited significantly differences. However, when we included patients' age, gender and $BRAF^{V600E}$ mutation status in the survival risk prediction model, only KIT remained still significant.Remarkably, the methylome which was detected at early stage samples (Breslow thickness ≤ 2mm) gradually decreased during the medium and late stages (Breslow thickness: 2-4mm and Breslow thickness>4mm). Such inverse relation among other predefined groups (histological subtype, ulceration and metastasis formation) and promoter methylation was also revealed, however, Breslow thickness, which is a continuous variable

allowed the most precise insights into how promoter methylation had decreased from stage to stage. We also integrated our methylation results with high throughput copy number alteration dataset. Apart from the lack of local correlation between copy number alterations and DNA methylation, loss of 19p13.2 (harbouring DNMT1 gene) only seen in the advanced stages of melanomas.

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P-104
MAPPING THE ARRANGEMENT OF NICKS AND R-LOOPS AT THE RDNA LOCUS OF *SACCHAROMYCES CEREVISIAE*

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Agarose embedded, deproteinized *S. cerevisiae* chromatin exposed to S1 nuclease digestion, or upon denaturation, yield loop-sized DNA fragments (~50-150 kbp), due to the presence of preformed single-strand (ss) discontinuities bordering these loop-size chromatin domains detected using either FIGE or CHEF gel electrophoretic systems.

In order to compare the distribution of ss discontinuities in the different chromosomes, they were separated by CHEF prior to S1 digestion or denaturation, and analysed in a 2nd dimension. These experiments have lead to the conclusion that the incidence of nicks is similar in each of the chromosomes.

To determine if loop relaxation by a random, initial nicking event may prevent further lesions within the same loop, leading to ~loop-size ss DNA fragments, the effect of the intercalating dye ethidium bromide was studied. These experiments have excluded superhelicty as a major player in determining the incidence of nicks.

For mapping of nicks within the naturally amplified rDNA units, we have developed a reverse South-Western protocol. The nicks present in the deproteinized chromatin of agarose-embedded S. cerevisiae could be nick-translated by DNA polymerase I using biotinylated nucleotides. Labeling was limited to the immediate surroundings of the nicks using terminator nucleotides. After restriction digestion and field-inversion gel electrophoresis in urea-agarose (Hegedüs et al., NAR, 37(17):e112, 2009), the DNA fragments of rDNA were blotted onto nylon membrane and the restriction fragments containing nicks were detected by anti-biotin antibody. This method was also used for the detection of R-loops in rDNA units, but in this case we used the RNA/DNA hybrid specific S9.6 antibody. We have compared the distribution of nicks and R-loops within the rDNA cluster in the case of various yeast cell strains.

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P-105
IN VIVO VALIDATION OF CANDIDATE CATTLE REGULATORY SNPS IN INDICATOR TRANSGENIC MOUSE MODELS

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Cattle (*Bos taurus*) are considered to have been one of the first animals domesticated by man for agricultural purposes. The world has now over 1.3 billion cattle – about one for every five people on the planet.

Advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect economic traits. Several of these genetic markers are now available and used in industry marker-assisted selection programmes. Genomic selection is a form of marker assisted selection in which genetic markers covering the whole genome are used to estimate breeding values (genomic breeding values). It is crucial to develop strategies to predict functionally relevant polymorphisms to pinpoint more rapidly causative genetic variants underlying phenotypes of interest. Most studies of regulatory polymorphisms were conducted on a gene-by-gene basis; but recent attempts analysed them on a large scale, albeit not in cattle.

Because of its economical importance cattle was one of the first mammals to have its genome sequenced in August 2006. During the sequencing of the cattle genome more than 2.2 million putative single nucleotide polymorphisms (SNPs) have been identified and deposited in public databases.

The aim of the Regulomix project is to develop a large-scale approach to identify and characterise bovine SNPs that alter the regulation of gene expression (rSNPs). We have identified hundreds of real rSNPs in the cattle genome. Several putative rSNPs were selected for the test phase, based on the function and/or location of the corresponding genes. For each potential rSNP, a pair of 2,000 bp-long DNA fragment which harbours one of the possible allelic variant have been constructed. For each rSNP, the two allele-specific promoter fragments were cloned into a single promoter-less vector with two fluorescent protein genes (GFP and DsRed). The pre-selected vectors with the reporter genes and the allele-specific promoter sequences have been microinjected into FVB/N mouse zygotes obtained from superovulated donor females. Following micromanipulation the embryos have been transferred into surrogate mothers according to standard protocols. Mice born from embryotransfer underwent transgene specific analysis to identify the founder animals, which were bred to deliver the F1 generations. The individuals of transgenic F1 population were examined with RT-PCR, QRT-PCR. In order to compare the expression levels of the two promoter allele, the expression level of GFP cloned after appropriate promoters were determined. We used the other reporter gene (DsRed) to normalise the data.

The rSNP identification and validation strategy that will be developed during this project could be easily adapted to other organisms of interest. The panel of validated rSNPs, resulting from this project, could be used in future association studies, linkage analyses or gene expression quantitative trait projects to pinpoint more rapidly causative genetic variants.

P-106
THE ROLE OF ACETYLATION IN THE REGULATION OF SF-1 AND STEROID CONVERSION

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Steroid hormone biosynthesis is regulated by cytochrome P450 enzymes that show tissue specific expression. The nuclear receptor SF-1 (Steroidogenic Factor 1) is expressed in the steroidogenic tissues and as a transcription factor it plays an important role in the regulation of the steroid hormone biosynthesis. Among others, SF-1 also regulates the *Cyp11a1* gene expression that is responsible for the first enzymatic step of the steroid biosynthesis. The SF-1 function is controlled by posttranslational modifications (acetylation, phosphorylation and sumoylation) and interacting protein partners. The histone acetyl transferase (HAT) GNC5 enzyme which is the main catalytic subunit of both ATAC and SAGA HAT complexes has an important role in the acetylation of the SF-1 protein.

Our goal was to study the effect of acetylation on the regulation of steroid conversion. Previously, it was shown that GCN5-mediated acetylation activates SF-1. Supposedly, the activated SF-1 is located in the nuclei and regulates the expression of the steroidogenic genes.

We show here that indeed, both the intracellular localization and the transactivation capablity of SF-1 depends on acetylation. We also demonstrate the effect of histone deacetylase inhibitors on the expression of *Sf-1* and its target gene *Cyp11a1*. By the use of luciferase reporter assay we show that acetylation modifies the transactivation capability of SF-1. We found that transfected SF-1 protein localized in the cytoplasm when cells were treated with histone acetyltransferase inhibitor (HATi IV) but was found in the nuclei when the cells were treated with the histone deacetylase inhibitor TSA. Furthermore, we also show that inhibition of histone deacetylase activity modulates the transcribed mRNA level of *Sf-1* and *Cyp11a1* genes in the steroidogenic Y1 cell line.

P-107
NUCLEAR FUNCTION FOR THE ACTIN BINDING CYTOSKELETAL PROTEIN, MOESIN

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Moesin, the well-known cytoplasmic actin binding protein is the only member of the evolutionary conserved mammalian ERM (Ezrin, Radixin, Moesin) protein family in *Drosophila melanogaster*. ERM proteins are responsible for the organization of the cortical actin network and an chormembrane proteins to it. Our laboratory has demonstrated previously that Moesin is present in the interphase nucleus but the biological significance of this localisation remained unknown.

We are studying the exact localisation and function of Moesin in the interphase nucleus in *Drosophila* larval salivary gland cells. The localisation experiments showed that Moesin accumulates as a ring at the nuclear envelope; it is present in the nucleoplasm, in some chromosome regions and occasionally in the nucleolus. We found that the quantity of Moesin in the nucleus increases upon heat stress, which suggests that Moesin transportation to the nucleus is an activeprocess.

To further analyse the chromosomal localisation, we performed immunostaining experiments on larval polytene chromosomes. Moesin was found to localise complementary to the dense heterochromatic regions, which means tha tMoesin binds to the euchromatic bands. Moesin also showed colocalisation with the active form of RNA Polimerase II and the intensity of the accumulation of the two proteins on the chromosomes was identical. Moesin staining was especially strong in the chromosome puffs which are special euchromatic regions of extremely active transcription sites in the polytene chromosomes.

The analysis of heat shock genes revealed that Moesin level increased in the heat shock puffs in response to heat stress. Inducing transcription on a transgene regulated by a heat shock promoter resulted in an extra Moesin band in the corresponding chromosome region suggesting tha tMoesin is required for transcription rather than the formation of the puff structure.

In summary, our results strongly argue that Moesin participates in the process of transcription where it is either required for the remodelling of the chromatin structure orit is directly involved in the assembly ormaintenance of the transcription complex.

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P-108
NUCLEOSOME-DNA COHESION IS HIGHLY SENSITIVE TO CERTAIN H3 MODIFICATIONS AND TO SUPERHELICAL TWIST

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We have developed a sensitive and high throughput method, applying a laser scanning cytometer (LSC), for the analysis of histone mobility features. Our method offers sensitive means to determine, quantitatively and in a cell-cycle phase specific manner, a major component of global histone mobility: nucleosome-DNA cohesion. After salt elution of agarose-embedded isolated nuclei the remaining histone levels are determined by immunofluorescence labeling, using modification-specific antibodies. H3K4me3 modified histones were eluted from isolated nuclei at much lower salt concentration than H3K27me3 modified histones, in various cell types, including mouse embryonic stem (ES) cells and their differentiated counterparts (NPCs). Nucleosome-DNA cohesion appears to be highly sensitive also to superhelical twist. Nucleosomes containing these two kinds of modifications could also be distinguished based on a differential sensitivity to intercalators.

P-109
FUNCTIONAL CHARACTERIZATION AND GENE EXPRESSION PROFILING OF *DROSOPHILA MELANOGASTER* SHORT DADA2B ISOFORM-CONTAINING DSAGA COMPLEXES

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In eukaryotes the genetic material is present in a compact chromatin structure consisting of DNA and histone proteins. Histone acetyltransferase (HAT) complexes play a role in chromatin structure modifications which might lead to changes in gene expression. The GCN5 protein is the catalytic component of several multiprotein HAT complexes which modify chromatin structure by acetylating specific lysine residues at the N-terminal tails of histone H3 and H4. ADA2 proteins, together with ADA3, SGF9 and GCN5 form the acetyltransferase module of GCN5-containing HAT complexes, which play roles in modulating HAT activity and specificity of the complexes. In *Drosophila melanogaster* our group has described two ADA2 proteins (dADA2a and dADA2b) in two GCN5-containing HAT complexes, ATAC and dSAGA which have different histone

specificities. The dADA2b-containing dSAGA complex is involved in the post-translational modification of nucleosomal histone H3 at lysine (K)9 and K14. Furthermore, analysis of the *dAda2b* gene revealed that by alternative splicing it gives rise to two mRNAs (dAda2bS and dAda2bL).

We examined whether the two dSAGA specific dADA2b subunits could contribute to the functional complexity of dSAGA in transcription regulation. Here we present findings showing that during *Drosophila melanogaster* development the two dADA2b isoforms (dADA2bS and dADA2bL), which differ in their C-terminal domains, are expressed at various levels. Genetic complementation experiments indicate that dADA2bS alone can support development but cannot fully complement *dAda2b* mutations. In the presence of dADA2bS, the SAGA-specific histone H3 acetylation level is partially restored in *dAda2b* mutants. Comparison of whole transcriptome profiles of *dAda2b* null and *dAda2bS* transgene-carrier *dAda2b* null larvae indicates partial overlap between affected genes. mRNA levels corresponding to selected genes which are either up- or down-regulated in *dAda2b* mutants are altered by dADA2bS expression to different extents, ranging from complete restoration to wild type levels to no restoration at all. The short (dADA2bS) isoform of dADA2b seems to be more capable of restoring lost dSAGA functions that cause mRNA level up-regulation than those that lead to decreased mRNA levels.

The data presented here are in accord with results of genetic complementation experiments, and support the hypothesis that different isoforms of dADA2b contribute to the functional variations of dSAGA multiprotein HAT complexes.

P-110
CHIP EXPERIMENTS WITH SPIKE CONTROLS

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Chromatin immunoprecipitation is a widely used, valuable method for the detection of protein-DNA interactions in vivo, primarily for transcription factors and histone modifications. Coupled with real- time quantitative PCR and deep sequencing methods, ChIP would be a promising tool to reveal disease-associated changes in transcription factor recruitment thus providing us a deeper insight into the chromatin-level variances in the case of many multifactorial diseases and cancer. Our aim is to develop a method that allows the introduction of chromatin immunoprecipitation into clinical research. Several protein-DNA complexes were tested, and shown to be able to serve as spike-in controls. Such controls will allow us to monitor the immunoprecipitation efficiency, to titrate the epitope-binding capacity of an antibody and also to monitor batch-to-batch variation of policlonal antibodies. By now, we have controls for HDAC1, MECP2, CTCF, RXR and ER. We tested a polyclonal RXR-specific spike control on chromatin samples from 293T HEK cell line, and also carried out epitope-selection to further improve specificity.

P-111
TAF10 PROTEINS INDICATE STRUCTURAL AND FUNCTIONAL LINKS BETWEEN HISTONE ACETYLTRANSFERASE AND BASAL TRANSCRIPTION FACTOR COMPLEXES

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TATA-binding protein associated factors (TAFs) have been identified as subunits of the TFIID basal transcription factor complex required for RNA polymerase II initiation. More recent studies indicate that TAFs are also present in histone acetyl transferase complexes which regulate transcription initiation and the organization of the chromatin structure. This observation raises the possibility of complex „transmutation" by which due to changes in subunit composition one type of multiprotein complex is converted to an other type as transcription initiation is progressing.

Of the two Drosophila GCN5 histone acetyltransferase (HAT)-containing complexes SAGA and ATAC, TAF10 subunits are present in the former while they are missing from the latter. Despite that we found that the gene expression alterations in *taf10* mutants are very similar to those observed in ATAC subunit (Ada2a, Ada3) mutants. First, we aimed to find out whether only *taf10* mutants have similar gene expression alterations to ATAC mutants or other TAFs mutants also show the ATAC specific gene expression patterns. For this we studied the gene expression pattern of *Drosophila* stocks in which *taf5*, *taf10*, or *taf8* was downregulated by RNAi.

We have recently shown that *Halloween* genes, which are expressed in the prothoracic gland and regulate ecdysone synthesis are regulated by the ATAC HAT complex. As a result of ecdysone syntesis failure ATAC mutants arrest development at the larval-prepupal transition though they do not present any evident defect during larval development. We silenced *taf5*, *taf8*, *taf10* genes specifically in the ring gland where ATAC-regulated ecdysone synthesis occurs at late larva stage and observed developmental arrest before the prepupal transition, while there was no effect detectable on the other larval developmental stages. This phenotype is similar that seen in ATAC mutants. 20-hydroxyecdysone feeding rescue TAF mutant L3 stage larvae and they reach pupa stage. These data showed that TAFs influence the ecdysone synthesis similarly as it was observed in ATAC-specific *Ada2a* mutants. Furthermore, decreased expression of TAF proteins in the wing discs results in notched wing phenotype which suggests that similarly to ATAC, TAF proteins also play role in apoptosis induction.

Our data suggest a functional interconnection between the ATAC HAT complex and the basal transcription factor TFIID. By further studies we aim to elucidate the details of the structural and functional interrelationship of the two complexes.

P-112
EXPRESSION OF ABCB1 TRANSPORTERS IS REGULATED BY SEVERAL DIFFERENT MECHANISMS IN DRUG RESISTANT RAT HEPATOMA CELLS

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Multidrug resistance (MDR) is the ability of cells to extrude cytotoxic agents with the use of energy-dependent ABC transporters. In the presence of drugs MDR expression is up-regulated by different mechanisms, though the molecular background of increased MDR expression is mostly unknown. Recent studies suggested that epigenetic modifications might play an important role in this process.

The aim of our study was to reveal epigenetic modifications responsible for the increased MDR level in multidrug resistant cell lines.

The cell lines we used in our experiments were a drug-sensitive parental rat hepatoma cell line (D12), a medium (col500) and a highly (col1000) drug-resistant variant of it.

Rodents, similarly to other mammalian cell types, have several MDR genes. In order to determine which of these play a major role in drug efflux in the drug resistant hepatoma cell lines, first we compared the drug removal capacity of drug-sensitive D12 and drug-resistant col500 and col1000 cells. We detected an increased Abcb1 activity in the drug-resistant cell lines compared to the drug-sensitive D12 cells. In contrast with that, the activity of Abcc1 was decreased significantly in the colchicine-selected cells. Next, we distinquished between Abcb1a and Abcb1b activities and found that the elevation of Abcb1b activity is responsible for the MDR phenotype in the resistant col500 and col1000 cells. By comparing the expression of these genes we found that the mRNA levels of both *Abcb1a* and *Abcb1b* were increased in the drug resistant cell lines compared to the parental D12. On the other hand, neither the copy number of these genes, nor the half life of their mRNA product showed a significant change in resistant versus sensitive cells. To treatment of histone deacetylase inhibitors (HDACi) surprisingly, *Abcb1a* and *Abcb1b* genes responded in an opposing way: the expression of *Abcb1a* was decreased, while the expression of *Abcb1b* was increased in cells treated with HDACi. Nonetheless, HDACi-treatment had no effect on the drug-efflux capacity either in the sensitive or in the resistant cells. Since HDACi treatment on the other hand resulted in an increased level of acetylated H3K9 and H3K14 in the regulatory regions of both genes, we concluded that elevated *Abcb1* gene expression is not necessarily coupled to histone acetylation changes, and conversely, the levels of H3K9 and H3K14 acetylation do not necessarily predict the expression level of the *Abcb1* genes.

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P-113
EFFECTS OF HISTONE POST-TRANSLATIONAL MODIFICATIONS AT THE LEVEL OF CHROMATIN LOOPS AND CHROMOSOMES

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Disassembly of chromatin to loop-sized fragments has been observed in a variety of organisms including non-apoptotic mammalian cells and in yeast spheroplasts. Single-strand specific nuclease digestion of intact, chromosome-sized DNA can recapitulate this surprisingly uniform fragmentation pattern, raising the possibility that single-strand discontinuities (nicks) delimit chromatin loops and sites of active transcription.

In order to elucidate the role of transcription in the establishment of these higher-order chromatin structures, we studied the relationship between chromatin fragmentation and histone modifications contributing to the transcriptional landscape. A number of histone gene substitution mutants – defective in post-translational histone modifications – as well as RNA polymerase I, II, III conditional mutants were screened for karyotype changes and loop-size fragmentation patterns.

We also performed Nick-ChIP and R-loop ChIP, two novel genome-wide methods developed in our lab, on chromatin samples prepared from yeast cells cultured in different carbohydrate conditions. We found that alteration of the transcriptional profile - elicited by the use of alternative carbon sources - correlated with the presence and distribution of R-loops and single-strand discontinuities. Further, the amount and distribution of R-loops and of nicks positively correlated with the rate of transcription. Our results support the notion that preformed nicks and chromosomal R-loops flank the sites of active (coding and/or non-coding) transcription.

P-114
HSF-2 IS A NOVEL HEAT SHOCK FACTOR ORTHOLOG IN *C. ELEGANS*

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The heat-shock response is a conserved protein homeostasis defense mechanism, which is essential for survival and protects against neurodegenerative diseases. It mainly functions *via* the activation of the major heat shock transcription factor-1 (HSF-1) with a potential and poorly characterized contribution of three additional mammalian heat shock factors. Among them, HSF-2 appears to play a role in embryonic and neuronal development and it cooperates with the HSF-1 in the regulation of the heat shock response. Until now, only one HSF has been described in invertebrates. Here, we report a novel invertebrate HSF-2 and its involvement in the heat-shock response in the roundworm *Caenorhabditiselegans.*

We employed the *hsf-2(tm4607)* and *hsf-1(sy441)* loss-of function strains. Mutants were backcrossed to the genetic background 6 times to eliminate potential mutations. We found a normal

development and reproduction of *hsf-2(tm4607)*, contrasting the retarded growth and decreased egg-laying ability of *hsf-1(sy441)* compared to N2 (wild type), respectively. Moreover, we observed a markedly induced thermotolerance and *hsp* mRNA expression by a mild heat shock in the wildtype strain, which was entirely absent in *hsf-1(sy441)* and was reduced in the *hsf-2(tm4607)* mutant strain. Further experiments investigating the mechanism and kinetics of this effect are in progress.

Our findings demonstrate that HSF-2 facilitates the optimal heat-shock response in *C. elegans* and imply that a complex regulation of heat-stress adaptation appeared early during evolution. Our work may contribute to a deeper understanding of the human heat-shock response.

P-115
UVB AND INHIBITION OF HISTONE DE-ACETYLATION RESULT IN SUPERINDUCTION OF MMP GENES IN HUMAN KERATINOCYTES

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UVB radiation is an important environmental agent that affects cutaneous carcinogenesis, photoaging, inflammation and immune suppression. Like other cellular stressors, UVB influences the transcription of many genes through the activation of distinct signaling pathways which in turn determine the cellular response for the damage. UV radiation also causes global changes in histone posttranscriptional modifications and as well in gene-specific histone acetylation. Here we report that genes encoding *ATF3* and *COX2* are upregulated in UVB response of HKerE6SFM human keratinocyte cell line. Histone deacetylase inhibitor trichostatin A (TSA) influenced the UVB dependent activation of *ATF3* and *COX2* and caused superinduction of these genes. An analysis of the alterations in the global transcriptome of HKerE6SFM cell upon UVB and TSA revealed the induction of members of the matrix metalloprotease (MMP) gene cluster – *MMP13*, *MMP12*, *MMP3*, *MMP1* and *MMP10* – at 11q22.3 region. Surprisingly, the expression of *MMP9* was not altered significantly. Results of chromatin immunoprecipitation indicated that polymerase II enrichment at the initiation regions of genes of the MMP cluster paralleled the expression pattern of the genes. Curiously, the Pol II density was also high at the *MMP9* promoter in UVB treated keratinocytes. The level of acetylated histone H3K9 was also elevated upon UVB irradiation except at the *MMP10* and *MMP9* promoters in correlation with the mild and no-significant alteration in the expression level of these genes, respectively. These findings suggest that acetylation of histoneH3 at lysine 9 plays important role in the UVB response of MMP genes in the 11q22.3 region.

P-116
ROLE OF MIRNAS IN *ARABIDOPSIS THALIANA* SEED AND FRUIT DEVELOPMENT

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MicroRNAs (miRNAs) constitute a class of small (21-24 nucleotide in length) non-coding endogenous RNAs that regulate gene expression by targeting mRNAs for degradation or translational repression. MiRNAs are intensively investigated and they have been found to be the pivotal component of developmental regulation processes. Recent studies showed the non-cell autonomous function of several miRNAs.

We investigated the role of miRNAs in seed and fruit development. Using our *in situ* hybridisation results together with the latest online databases and scientific literature we selected miRNAs present in *Arabidopsis thaliana* seed or fruit. We studied the role of these miRNAs (miR166, miR827, miR169, miR828, miR390, miR824, miR858, miR775, miR857, miR394, miR472) in fruit development by transgenic over-expression of miRNA precursor genes in transgenic plants. To our surprise only miR824 and miR857 over-expressing transgenic plants showed significant phenotipical changes during silique formation. Transgenic plants over-expressing miR824 produced abnormally small siliques, while miR857 over-expressing plants, produced siliques with fewer seeds than wild type plants. We analysed the expression of miR824 and miR857 using small RNA Northern blot and we found correlation between the severity of the phenotype and enhanced accumulation of the investigated miRNAs. Currently we are investigating the level of potential target mRNAs in the transgenic plants.

During our experiments we analyzed the spatial accumulation pattern of selected conservative miRNAs in *Arabidopsis thaliana* embryonic tissues with *in situ* hybridisation. The majority of the investigated miRNAs showed uniform accumulation across the embryo demonstrating their role at this developmental stage. In the case of miR167, however, we detected a gradient like expression profile which is usually considered to be the hallmark of the non-cell autonomous activity of miRNAs. Using a previously described GUS reporter assay we analyzed the spatially highly coordinated expression of the four precursor genes producing mature miR167. We found that one of precursors, *MIR167a,* also showed a gradient like expression pattern in the embryo. These data point to the necessity of careful investigation of gradient like expression patterns of miRNAs since this phenomenon can be generated not only by the non-cell autonomous action of miRNAs but also by the specific expression characteristic of miRNA precursor genes.

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P-117
A VERSATILE METHOD TO DESIGN STEM-LOOP PRIMER-BASED QUANTITATIVE PCR ASSAYS FOR DETECTING SMALL REGULATORY RNA MOLECULES

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Short regulatory RNA-s have been identified as key regulators of gene expression in eukaryotes. They have been involved in the regulation of both physiological and pathological processes such as embryonal development, immunoregulation and cancer. One of their relevant characteristics is their high stability, which makes them excellent candidates for use as biomarkers. Their number is constantly increasing as next generation sequencing methods reveal more and more details of their synthesis. These novel findings aim for new detection methods for the individual short regulatory RNA-s in order to be able to confirm the primary data and characterize newly identified subtypes in different biological conditions. We have developed a flexible method to design RT-qPCR assays that are very sensitive and robust. The newly designed assays were tested extensively in samples from plant, mouse and even human formalin fixed paraffin embedded tissues. Moreover, we have shown that these assays are able to quantify endogenously generated shRNA molecules such as shRNAs from the urine samples of healthy pregnant patiens. The assay design method is freely available for anyone who wishes to use a robust and flexible system for the quantitative analysis of matured regulatory RNA-s.

P-118
ARTIFICIAL MIRNA-MEDIATED VIRUS RESISTANCE IN BARLEY

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In Hungary among the cereal viral diseases the Wheat Dwarf Virus (WDV) infection is the most significant resulting in severe disease symptoms such as yellowing of leaves and dwarf growth. This virus is a member *Mastrevirus* genus of *Geminiviridae* family and is transmitted by the leafhopper *Psammotettix alienus*. The WDV genome consists of single stranded DNA (ssDNA). The host plants are infected usually in autumn developing weak root system consequently the majority of the infected plants dies during the wintertime. Classical breeding efforts to produce WDV resistant barley cultivars were not successful so far because of the lack natural resistance genes. To evade this problem we plan to develop WDV resistant barley lines by a biotechnology approach known as "artificial miRNA" (amiRNA).

The miRNA pathway is an important post transcriptional gene regulatory mechanism involved in developmental processes. MiRNAs are ~21 nucleotide long non-coding RNA molecules which are generated by sequential processing of longer precursor molecules. The plant miRNA loci encode capped and polyadenylated transcripts (pri-miRNAs), which are processed to pre-miRNAs in the nucleus by the Dicer-like enzyme DCL1. The genetically defined pre-miRNAs possess a self-complementary fold- back structure that is processed to a double stranded intermediate comprising the miRNA and the complementary miRNA* strands, respectively. The miRNA-miRNA* duplex is unwound and the miRNA is subsequently loaded in the RISC (RNA-induced Silencing Complex) and guide the RISC to degrade any RNA possessing sequence complementary to the miRNA. It can be specify RNA targets by remodeling the miRNA precursors rendering them to produce artificial miRNA.

We intend to establish the WDV resistant barley by utilizing the amiRNA technology, without the production of protein. To avoid the adaptation of the target virus, due to the high viral recombination frequency, as well as to increase the efficiency of the resistance we prepare a polycistronic construction of amiRNAs. Five WDV-specific amiRNA entities will be expressed under the control of barley regulatory elements in the transgenic plants. We isolated a barley miRNA precursor (hvu-mir171) suitable for producing amiRNA and it was successfully tailored specifically for WDV genes encoding the replicase (Rep and RepA) and the movement protein (MP). We tested the processing of the generated amiRNAs in transient expression system in Nicotiana benthamiana applying small-RNA Northern hybridization. The biological activity of amiRNAs has been assessed by using GFP-target-sensor construction. The selected amiRNAs will be introduced as polycistronic-amiRNA construct into barley by Agrobacterium tumefaciens based transformation. The barley lines regenerated from the transformed cell cultures will undergo molecular analysis and infection test by WDV carrier Psammotettix alienus insect vector. Promoter sequences of three barley ubiquitine genes have been isolated and are tested for suitability as cis-regulatory element.

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P-119
FRANK-TER HAAR SYNDROME PX DOMAIN MUTANT PROTEIN SHOWS ABERRANT INTRACELLULAR EXPRESSION AND FORMS AGGRESOME

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Aggresomes form from unfolded proteins when the proteasomal system failed or overwhelmed. It has significant importance because protein unfolding and aggregation have been involved in a wide variety of disease known as amyloidosis, including Parkinson's, Alzheimer's and prion disease.

Ina previous study we found that the PX (Phox homology) and SH3 (Src homology) domain-containing Tks4 protein has a role in the EGFreceptor signalling.In response to growth factor treatment the cytoplasmic protein is translocated to the plasma membrane through its Src-binding and lipid-binding PX domain. At the plasma membrane, Tks4 is tyrosine phosphorylated by Src kinase and regulates actin polymerization leading to membrane ruffle formation or cell migration.Tks4 was also implicated in the production of reactive oxygen speciesby tumor cells, and in the differentiation of white adipose tissue.

Mutations in the SH3PXD2B gene coding for the Tks4 protein are responsible for the autosomal-recessive Frank-ter Haar syndrome. Tks4 substitution mutant results in the change of the conserved arginine 43 to tryptophan in the PX domain (R43W).We found previouslythat the R43W mutant protein showed reduced phosphoinositide binding and forms juxtanuclearaggregates in transiently transfected COS7 cells.In the present study we characterized these aggregates which were very similar to the so-called aggresomes described by other authors. It has beenwell known from the literature that the aggregated proteins transported by microtubule-dependent manner to the microtubule organizing centre (MTOC) where they are eliminated by autophagocytosis. Therefore first we examined the role of microtubule network in the aggregated protein trafficking by immunocytochemistry. We found that aggregates colocalized with microtubules and the formation of perinuclear aggresome prevented by nocodazole, a microtubule depolymerising agent. To confirm the transport of aggregates to the microtubule organizing centre we detected colocalization between aggresome and a MTOC marker centrin 2 protein.One of the most characteristic components of aggresomes is the vimentin intermediate filament that forms a cage surrounding the aggresomes. We found that the cells transfected with TKS4 R43W mutant showed reorganization of the vimentin filaments which formed a cage-like structure around the aggresome. The role of proteasome system in the clearance of aggresomes was examined by proteasome inhibitor MG-132. The inhibition is significantly increased the level of aggresomes which proved the contribution of proteasomes in the elimination of these structures.The aggregated proteins in aggresomes are often insoluble in non-denaturing detergents. We observed that Tks4 mutant was primarily found in the detergentinsoluble part of the lysate contrary to Tks4 wild type that predominantly accumulated in the soluble fraction.

Thus, our work reveals that the Frank-ter Haar syndrome PX domain mutant protein Tks4 forms aggregates, which shows several defining characteristics of aggresome.

P-120
INTERACTION OF LYSOPHOSPHATIDIC ACID WITH PH DOMAINS

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Signaling phenomenons proceeding through cell surface receptors lead to direct physiological reactions or gene transcritpions in eukaryotic cells. In these processes multicomponent complexes are formed under the cell membrane involving proteins and lipids as well. The many modules in the structure of signaling proteins enables the formation of protein-protein and protein-lipid interactions in signaling complexes. Protein- and lipid-binding domains are wide-spread and conserved among eukaryotes.

The aim of this study is to understand and characterize the mechanism of particular protein-lipid interactions which can play important roles in the physiology of cells.

Pleckstrin homology (PH) domain is one of the most canonical lipid-binding domains of signaling proteins which can bind phosphoinositide derivatives, such as phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$). In our work we have studied the interaction of the PH domain of Akt1, Grp1 and gelsolin with lysophosphatidic acid (LPA). Akt1 promotes cell survival, Grp1 has GEF activity on Arf1, while gelsolin regulates the dynamics of actin cytoskeleton. LPA is a structurally simple bioactive lysophospholipid which has effects on basic cellular processes, such as proliferation, survival, and motility. These effects are elicited through LPA-specific G-protein-coupled receptors (LPA_{1-6}) and/or PPARγ, a nuclear transcription factor.

It has been reported previously that LPA binds to the $PI(4,5)P_2$ binding site of gelsolin, possibly interfering the inositol polyphosphate binding at this region of the protein. We hypothetised that gelsolin contains a PH domain, thus we cloned a part of the protein, showing the highest similarity to known PH domains, expressed it and studied its interactions with bioactive lysophospholipids. We also expressed and studied the known PH domains of Akt1 and Grp1 for lysophospholipid binding. In binding studies utilizing intrinsic tryptophane fluorescence we have found that LPA could bind to the PH domains only when the lipid formed micelles in the solution. CD spectroscopy measurements served some information about changes in the secondary structure elements of these domains in case of LPA binding. Quartz Crystal Microbalance technique and Isothermal Titration Calorymetry were applied to determine the strength of the interactions between the domains and the lipid. K_d values are in the nanomolar range suggesting strong binding of LPA to these PH domains after micelles appearing in the system.

Our preliminary results show that LPA can act a second messenger-like fashion on membrane surfaces via recruiting protein domains. LPA, in a clustered form mimicked by micelles in vitro, can bind to at least some PH domains, revealing new aspects of lysophospholipid- protein interactions.

P-121
CALCIUM HOMEOSTASIS AND PURINERG MODULATION IN THE SUPPORTING CELLS OF THE ORGAN OF CORTI

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Sensorineural hearing loss (SNHL) is the most common sensory deficit among people. The injury of the inner ear is the source of the sensorineural hearing loss for example presbyacusis, high level noise induced hearing loss, or caused by ototoxic drugs like the aminoglycosyde antibiotics family. Currently we can't cure these because we don't have enough knowledge about it's physiologycal and patophysiologycal mechanisms, neither have the appropriate drugs or medical treatments. However the molecular background is well known, based on experimental data on purinergic transmission which has an important role in cochlear physiology and patophysiology. ATP has a protective role as it can reduce the endocochlear potential and induce Ca^{2+} waves among outer, inner hair cells and supporting cells.

Despite of this, until now we didn't have enough knowledge about sensorineural hearing losses, as the hearing mechanisms were examined only in rodent embryos and organotypic cultures. It is known that during development the purinergic receptor pattern changes, explaining why these methods are not the most appropriate for modelling sensorineural hearing loss in adult individuals. Therefore we introduced a novel acute hemicochlea preparation which was made from rodents with fully developed hearing system. These preparations allowed us to measure $[Ca^{2+}]_i$ changes in individual cells in the organ of Corti, while the imaged cells kept their original tissue environment. We could also measure the basic Ca^{2+} concentracions (baseline) in the supporting cells and demonstrated that ATP responses are dose dependent. Repeated ATP application resulted in receptor desensitisation. Our results prove that our newly developed method may serve as a useful tool in investigting the mechanisms behind sensorineural hearing loss.

P-122
DYNAMIC O–GLCNAC MODIFICATION ON VARIOUS NEURONAL STRESS-MODELS

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O-glycosylation (O-linked β-N-acetylglucosamine; O-GlcNAc) is a unique, dynamic posttranlational modification on the Ser/Thr residues of proteins, similar to phosphorylation. Numerous forms of cellular injury can lead to elevated levels of O-GlcNAc in both in vivo and in vitro models. The elevation of O-GlcNAc levels after cellular injury was shown to be protective in models of heat stress, oxidative stress, ER stress, ischemia reperfusion injury and trauma hemorrhage. Together, these data suggest that *O*-GlcNAc is a regulator of the cellular stress response. However, the molecular mechanism(s) by which *O*-GlcNAc regulates protein function and improves cell survival have not been identified. Recently, several studies also suggested that O-GlcNAc represents a key regulatory modification in the brain, contributing to transcriptional regulation, neuronal communication and neurodegenerative disease.

Our present aim was to study the dynamic progress of O-GlcNAc levels over short (0- 4 hours) and medium terms (24-72 hours) following a prompt, modest stress. We used two fairly distinct models: oxidative stress (H_2O_2) in a neuronal cell line (SH-SY5Y), and sub-lethal brain electroshock on anaesthetized Wistar rats. Following stress we collected cells and brain-tissue samples at similar times (0, 30, 60 min, 2, 4 hours, 1, 2, 3 days). We measured OGT (O-GlcNAc transferase) mRNA levels, protein O-GlcNAc levels by Western blot and by immunfluorescence staining in both SH-SY5Y and rat brain-tissue. We also determined the rate of apoptosis in SH-SY5Y by flow-cytometry after propidium iodide/Annexin-V-FITC staining.

We found remarkably similar O-GlcNAc pattern in both models: OGT as well as O-GlcNAc significantly increased and peaked between 2-4 hours following stress. In the cell culture model, the initial O-GlcNAc elevation coincided with the highest rate of apoptotic events. In brain-tissue slides we observed the highest nuclear accumulation of O-GlcNAc proteins 2 hours after stress. After 24 hours, O-GlcNAc levels almost returned to the basal level and medium-term O-GlcNAc levels remained relatively stable in both models.

Our data suggest that the elevation of O-GlcNAc is a general, conserved response to a stress situation. Following short-term stress, O-GlcNAc levels immediately increase but after a short-period, return to normal levels. The correct timing of this dynamic cellular response is probably crucial to avoid unnecessary cellular events (e.g. Ca^{2+}-overload) to happen.

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P-123
CUSTOM CYTOKINE PRODUCTION SERVICES DEVELOPMENT OF A RESEARCH CYTOKINE PRODUCTION PIPELINE

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Cytokines are widely used in the field of immunology and stem cell research but the cost of such biological reagents can mount to extremities in case of complex systems and can consume a large proportion of grant budgets. Purified recombinant cytokines tend to be hugely overpriced partly due to marketing and shipping costs. Locally provided cytokine products produced by university core facilities may be a way of reducing research expenses or re-allocating financial resources.

In our laboratory, we aim to provide the research community with high quality cytokines at affordably low prices. We established an infrastructure and a development/production workflow suitable for the generation of cytokine products with thorough biochemical, toxicological, and standard biological activity testing and quality control. In collaboration with a number of departments and labs within the university, we established a network of independent/external research laboratories testing our cytokine products in their own experiments.

Recombinant human cytokines are produced from optimised synthetic genes in HEK293T cells (DMEM, 10% FBS) *via* transient transfection or cytokine expressing stable HEK293 AD cell lines secreting the proteins into the medium. The proteins are glycosylated, contain affinity and epitope tags, specific cleavage sites for tag removal. Cytokines are available as conditioned medium or as purified by Ni-affinity chromatographic technique.

We set up a series of standard biological assays in the labs to test and ensure quality of our recombinant cytokines. These assays are widely adapted by worldwide manufacturers, and therefore, we are able to make direct comparison with commertially available cytokine products e.g. with respect to specific activity

In summary, we are offering high quality, highly active, mammalian cell derived cytokines (IL-4, GM-CSF, IL-2, LIF) for research use, at an affordably low price on a non-profit bases. We provide thorough biochemical and biological characterisation data for the products: cytokine conditioned media or purified proteins. Also, we are open up our cytokine development pipeline for your specific needs – you name a cytokine – we produce it for you withun a couple of months.

P-124
HSP90 CHAPERONES PPARΓ AND REGULATES DIFFERENTIATION AND SURVIVAL OF 3T3-L1 ADIPOCYTES

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The peroxisome proliferator-activated receptor-γ (PPARγ) is a key regulator of adipocyte differentiation and function, as well as a target of insulin-sensitizing drugs. The Hsp90 chaperone stabilizes a diverse set of signaling 'client" proteins thereby regulates eukaryotic cell proliferation and survival.

Here, we report that the Hsp90 inhibitor, geldanamycin inhibits the differentiation and survival of murine 3T3-L1 adipocytes. Moreover, Hsp90 inhibition disrupts an Hsp90-PPARγ complex, leads to the destabilization and proteasomal degradation of PPARγ and inhibits the expression of PPARγ target genes, identifying PPARγ as a *bona fide* Hsp90 client. A similar destabilization of PPARγ and a halt of adipogenesis also occur in response to protein denaturing stresses caused by a single transient heat shock or proteasome inhibition. Recovery from stress restores both PPARγ stability and adipocyte differentiation.

Thus, our findings reveal Hsp90 as a critical regulator of adipocyte physiology, linking proteostasis with adipogenesis through PPARγ stability, and offer a potential novel therapeutic target in obesity and diabetes.

Keywords: adipogenesis / diabetes / obesity / proteostasis / stress

P-125
CASKIN1-MEDIATED POSTSYNAPTIC EFFECTS ON THE DENDRITIC SPINES OF HIPPOCAMPAL NEURONS

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In the central nervous system, the majority of glutamatergic excitatory inputs terminates on dendritic spines. Based on morphology, three types of dendritic spines are characterised: there are stubby, filamentous or mushroom spines. Mushroom spines have enlarged heads and extended postsynaptic density (PSD), providing a multiprotein complex responsible for anchoring neurotransmitter receptors near sites of neurotransmitter release and providing a connection to the actin cytoskeleton. Synaptic stimuli can modify the morphology of dendritic spines, leading to changes in synaptic strength – this process is known as synaptic plasticity and forms the basis of learning and memory. Synapses formed on mushroom spines are functionally stronger therefore these spines are regarded as more stable, "memory" spines.

Caskin1 (Cask interacting protein 1) is a scaffold protein which is expressed in the brain in high amounts and is located at synapses. Caskin1 is a multidomain protein possessing six ankyrin repeats, a single SH3 domain and two SAM domains in the N-terminus while it has a long, intrinsically disordered proline-rich C-terminal region. Caskin1 can be one of the molecular linkers in the pre- or postsynaptic structures, however its role in the formation and function of the postsynaptic density (PSD) is yet unknown.

In this work we have investigated the effects of Caskin1 on dendritic spine morphology in mouse hippocampal neuronal cultures. Caskin1 and/or the green fluorescent protein (EGFP) were overexpressed in transfected hippocampal neurons and dendritic spines were analysed by quantitative confocal microscopy. Our data revealed that Caskin1 was present predominantly in the somatodendritic region of neurons and was enriched especially in dendritic spine heads. Importantly, overexpresson of Caskin1 increased the amount of more mature, mushroom-shaped dendritic spines in the expense of filamentous spines of transfected neurons. Moreover, Caskin1 protein always colocalised with PSD95, a postsynaptic scaffold protein. Electronmicroscopy is in progress to further confirm the postsynaptic localisation of Caskin1 protein.

Taken together, it is likely that Caskin1 affects dendritic spine morphology and function via interacting with the PSD multiprotein complex.

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P-126
HSP90 IS A REGULATOR OF THE FOXO3 FORKHEAD TRANSCRIPTION FACTOR

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The forkhead transcription factor family has been shown to play a role in diverse biological processes, such as cell growth and apoptosis. The forkhead member FOXO3 and its ortologs are master regulators of the oxidative stress response, innate immunity and contribute to longevity in various model organisms, including the roundworm *Caenorhabditis elegans*. The conserved Hsp90 chaperone stabilizes a plethora of thermodynamically unstable signaling proteins, so called 'clients' and provides a scaffold for cellular signaling networks. In this study, we investigated the interaction between Hsp90 and mammalian FOXO and its *C. elegans* ortolog DAF-16.

In mammalian cells, we observed that the specific Hsp90 inhibitor geldanamycin (GA) induces the destabilization of both endogenous and overexpressed FOXO3 in a concentration-dependent manner. Moreover, we demonstrated that Hsp90 and FOXO3 form a GA-insensitive physical complex. Using the proteasome inhibitor MG132, we showed that GA induces a proteasomal degradation of FOXO3, suggesting an Hsp90-dependent targeting of unfolded FOXO3 to the proteasome. Indeed, reporter gene assays showed that GA inhibits both basal as well as MG132-induced FOXO3-dependent promoter activation. Currently, using genetic approaches and RNA interference, we investigate how the Hsp90 ortolog DAF-21 modulates DAF-16/FOXO expression, localization and function in *C. elegans.*

Our findings identify mammalian FOXO3 as a novel Hsp90 client and may provide an insight into the evolutionary history and potential organismal impact of this interaction. The Hsp90-dependent regulation may have broad implications in aging and various human diseases.

P-127
THE ROLE OF PROTEIN KINASE D ACTIVATION IN RESPONSE TO OXIDATIVE STRESS-INDUCED NEURONAL CELL DEATH

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Protein kinase D (PKD), a serine/threonine kinase is highly expressed in the mammalian brain and fulfills many roles in cellular physiology, including the modulation of signaling pathways activated in response to cellular stress. According to studies on non-neuronal cells, reactive oxygen species originating from the mitochondria during oxidative stress activatePKD. Our knowledge on the relation between oxidative stress and PKD-mediated changes in neurons, however, is still limited.

In the present work we show that PKD undergoes a rapid and transient activation upon H_2O_2 treatment in mouse primary cortical neuronal cultures. Activation of PKD was verified by the increased autophosphorylation level of PKD as well as by PKD FRET reporters. We demonstrated that treatment with PKD-specific inhibitors significantly attenuated cell death induced by 24 hoursof oxidative stress. Neuroprotective effect was the most profound when neurons were preincubatedwith PKD inhibitors for 2 hours before the onset of the H_2O_2 treatment. This can be explained by the finding that PKD inhibitors activate NFkB, which might help neurons withstand oxidative stress.

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P-128
GUT EPITHELIUM DERIVED SONIC HEDGEHOG REGULATES THE EXTRACELLULAR MATRIX DURING FORMATION OF THE INTESTINAL NERVOUS SYSTEM

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The enteric nervous system (ENS) is principally derived from vagal-level neural crest cells that migrate rostrocaudally along the entire length of the gastrointestinal tract, giving rise to neurons and glial cells in two ganglionated plexuses. Incomplete migration of vagal-derived enteric neural crest cells (ENCCs) leads to Hirschsprung's disease, a congenital disorder characterized by the absence of enteric ganglia along variable lengths of the distal intestine. Inductive interactions between gut epithelium and mesenchyme have been suggested to regulate the migration and differentiation of ENCCs. However, little is known about the function of epithelial derived factors, such as Sonic hedgehog (Shh), how they influence the hindgut mesenchyme derived factors and how they regulate extracellular matrix formation during ENS development.

To study the signaling role of epithelium derived Shh protein, dissected hindgut from 6 day old chicken embryo was cultured in collagen gel for 72 hours in the presence of 4 μg/ml Shh protein or 1μM cyclopamine (Shh signaling inhibitor). When cultured in the presence of Shh protein, no ganglia formation is seen and the hindgut is aganglionic, while in the presence of cyclopamine large, ectopic ganglia developed in the hindgut. To test whether the delayed migration was a result of alteration of the extracellular environment, we compared hindgut sections through the neural crest migratory wavefront in Shh, cyclopamine and control guts using immunohistochemistry for matrix proteins either permissive (laminin, fibronectin, collagen I), or non-permissive (versican, collagen IX, tenascin) for the NCC migration. Shh treatment strongly induced the expression of versican, whereas cyclopamine reduced the expression pattern of this inhibitory matrix molecule. These results indicate that versican is a candidate for mediating the effects of Shh on ENCC migration. Abnormalities of NCC migration and extracellular pattern formation are characteristic of two human intestinal disorders, Hirschsprung's disease and intestinal neuronal dysplasia. Our results support an essential role for epithelial-mesenchymal interactions in these aspects of ENS development and provide a basis for further investigation of these proteins in the etiology of neuro-intestinal disorders.

P-129
MONITORING THE MCHERRY AND GFP EXPRESSION IN INDUCIBLE EMBRYONIC STEM CELL LINES AND INEMBRYONIC STEM CELL DERIVED BLOOD CELL PROGENITORS

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Nowadays, much attention has focused on reprogramming factors, transcriptional network of embryonic stem (ES)cells. The transcriptional networks regulating the diversification of myeloid lineages are currently being elucidated.The long term goal of our laboratory is to modify the transcription programs of ES cell-derived progenitors via perturbing the expression of lineage determining transcription factors.For these studies we intend to use a tet-inducible cell system in which genesare forced to express in the presence of the antibiotic tetracycline or one of its derivatives (e.g. doxycycline). To accelerate our understanding about the kinetic and magnitude of this inducible system we have engineered a panel of inducible mouse ES cell clonescontainingEGFPormCherry reporter genes.The advantage of this system is that the expression level of the reporter can be monitoredin individual cells by microscopic or flow cytometric analysis.

Our data clearly indicated that the reporter genes were expressed upon doxycycline treatment although the magnitude of the induction of the individual cell clones was different.We separated the mCherry/GFP negative and positive cells by cell sorting to assess the secondary inducibility of the reporter transgenes. Interestingly, we observed almost 100 % mCherry/GFP expression after the restimulation of the positive cells,however the negative cells showed a poor inducibility after the second doxycycline treatment. These results demonstrated that the sorted positive cell population retained the induction capacity suggesting that our system is genetically and epigenetically stabile. Finally we tested the differentiation capacity of the reporter cells upon blood cell development using the OP9 co-cultured procedure. We also assessed the inducibility of the report genes after 11 days of differentiation. It is important to emphasize that similar induction was detected inthe differentiated cells as in the original ES cells.

P-130
DEVELOPMENT OF TYPE II PNEUMOCYTES IN THE CHICKEN'S LUNG

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The anatomical and histological structure of the mammals and birds are highly different. The bronchi of the bird's lung do not divide dichotomically. Thus, from the secondary bronchus three or more parabronchi can open. The histology of the lung shows more significant differences. In mammals the lung produces bag-like alveoli, in which the Type I and Type II pneumocytes are "mixed". The bird's lung has air caplillaries, which is lined by Type I cells, while the Type II or granular pneumocyte locate in the wall of parabronchi and atria, resulting in clear topograhical separation of the two types of pneumocytes. Type II cells emege in the sixteenth day of incubation, when the air capillaires are still closed. Shortly, after this embryonic day the Type II cells appear in the entrance of the atria. The molecular mechanism of the Type II cell differentiation is unknown. The separate location of the Type I and Type II pneuomocytes may be indicated that the two types of cells develop independently from an undifferentiated endodermal cell. We hypothetise, that the mesenchym of the parabronchi and the atrial wall induces the Type II pneumocytes, while the mesenchyme of the air capillaries does not have capability for inducing Type II pneumocyte. The separate location of the two types of cells provides very convenient model to study the molecular background of the granular cell differentiation in birds.

P-131
INDUCTION OF THREE DC SPECIFIC TRANSCRIPTION FACTORS ON EMBRYONIC STEM CELL-DERIVED BLOOD PROGENITORS

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Since dendritic cells (DCs) were first described by Steinman et al. in 1973, many of their functions have been revealed. As major antigen presenting cells DCs have essential role in activation of cellular and humoral immune response. Dendritic cell based therapeutic approaches were applied in several clinical studies as tumor vaccines. However a serious obstacle of these procedures is to produce appropriate quantity of DCs. Thus understanding and manipulating their development is essential to take advantage of their wider application in human medicine.

Ex vivo DC generation from embryonic stem (ES) cells is a promising way to produce scalable amount of DCs for clinical applications.The long term goal of our research group is to stimulate the ES derived-DC development by transcription factor mediated cellular programming. Recently several transcription factors were identified that required for the *in vivo* DC differentiation. For

example, DC development is greatly affected by Irf8, Batf3 and Ikzf1 (Ikaros). In this study we probed the effects of these three transcription factors onstem cell derived DC progenitors. We have generated inducible mouse ES cell lines in which one of these three genes (Irf8, Batf3 and Ikzf1) can be upregulated by doxycycline treatment (Tet-on system).Our preliminary data indicated that the induction of these transcription factors has a detrimental effect on ES cell-derivedmesodermal/blood cell progenitors. Interestingly, Ikzf1 induction positively modulated the early blood cell development, in contrast the force expression of Batf3 suppressed the generation of myeloid blood cells. Finally we failed to modulate the GM-CSF driven myeloid development by ectopic expression of Irf8.

P-132
INVESTIGATION OF THE ROLE OF TISSUE TRANSGLUTAMINASE IN HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are very attractive in the development of regenerative medicine as they can be extensively expanded in culture while maintaining a stable phenotype and multilineage potential. MSCs can be induced to differentiate into myocytes, adipocytes, osteoblasts, chondrocytes, tenocytes and hematopoietic-supporting stroma under proper stimulation. Nevertheless, therapeutic application of MSCs has inherent limitations due to the insufficient efficiency for producing mature cell types. Thus, investigation of the molecular mechanism of cell differentiation will certainly lead to improved clinical methodology. Tissue transglutaminase (TGM2) can be a notable target for augmenting differentiation potential as the expression pattern for TGM2 suggests that it promotes differentiation to clinically important cell types. Although, similarly to other several cell types, TGM2 is essential for integrin-mediated survival of MSCs, the exact role of TGM2 through regulation of differentiation processes of MSCs is not understood yet. We have detected the presence of TGM2 in human bone marrow, adipose tissue and umbilical cord derived primary MSCs and in immortalized MSC lines obtained from adipose tissue. According to our results there is considerable amount of TGM2 in the non-differentiated MSCs, but TGM2 level decreases during adipogenic differentiation. In order to clarify the effect of TGM2 on differentiation processes we overexpress TGM2 in MSCs using lentiviral transduction. Our investigation will lead to our better understanding on the role of TGM2 in the molecular mechanism of stem cell differentiation and may have a significant contribution to development of new techniques on human MSC differentiation.

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P-133
DEVELOPMENTAL MAPPING OF CD45+ CELLS IN EARLY AVIAN EMBRYO

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Almost every organ contains stellate-shaped cells expressing CD45 hematopoietic marker and MHC class II antigen. The embryonic origin of these cells, how they colonize a given organ primordium, and their tissue distribution and phenotype are virtually unknown. The avian embryo is well suited for studying the origin, differentiation and tissue specific colonization of hematopoietic cells. The accessibility of the embryo allows manipulations not feasible in mammalian systems.

The aim of our study was to determine the embryonic origin of the chicken CD45+ cells in the different embryonic tissue and their differentiation in loco. In the chicken embryo the first CD45+ cells emerge in the blood island of the yolk sac at 48 hours of incubation, which is followed by their accumulation in the intra-aortic cells clusters about 12-16 hours later. The circulating CD45+ cells are round or ovoid shaped, but in the mesenchyme scattered, stellate-shaped, CD4+ cells also occur. By 120 hours of incubation stellate-shaped CD45 positive cells colonized all organ rudiments, even they appear in the mesenchyme of the limb buds. The round-shaped CD45+ cells are concentrated around the aorta and in the splanchnic mesenchyme they are getting to form a "sheath" around the splanchnic arteries. In the mesenchyme, the CD45 hemopoietic cells co-express MHC class II antigen, which makes them capable for antigen presentation.

To study whether the CD45+ stellate-shaped cells of the avian embryo can be considered a different subpopulation of the circulating CD45+ cells or instead they have a common stem cells originating from the extraembryonic blood islands, yolk-sac without embryo were cultured in the egg for additional 48 hours and the dissected embryo was cultured in vitro in three-dimensional collagen gel matrix. In the case of cultured yolk sac large number of CD45+ stellate cells differentiated, while in the explanted embryo only CD45+ cells with round-morphology developed.

It is concluded that the hemopoietic stem cells for the stellate CD45+ cell series originate from some source other than the intraembryonic round CD45+ cells, and that this source must be extraembryonic, namely the yolk-sac blood island. On the base of the embryonic expression of the CD45 antigen we hypothesize, that yolk-sac derived CD45+ stellate precursor cells actively migrates through the embryonic mesenchyme and colonizes each organ primordia to differentiate into CD45 and MHC class II double positive stellate cells.

P-134
MICRORNAS IN RABBIT PREIMPLANTATION EMBRYOS AND EMBRYONIC STEM-LIKE CELLS

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In the context of biomedical research, genetic engineering in rabbits (Oryctolaguscuniculus) would be precious model to generate genetically defined rabbit models of human diseases, as well as transgenesis for production of pharmaceutical proteins.

Since ECSs are derived from early blastocysts, they can reflect the potential characteristic of their founder embryonic population. It is important to compare the expression pattern of both miRNAs and proteins known to play regulatory roles during early lineage specification.

We aimed to explore ESC-specific miRNA expression pattern using SOLiD™ System Small RNA Analysis from early embryonic stages to early rabbit embryonic stem-like (ES-like) cell passages to get more insight into their potential regulatory mechanism in embryonic development.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology. In the current study, we described and analyzed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells for the first time. The rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498) were identified in rabbit preimplantation embryos and ES-like cells. The ocu-miR-302 cluster was highly similar to its human homolog, while ocu-miR-290 revealed a low level of evolutionary conservation with its mouse homologous cluster. The expression of ocu-miR-302 cluster members began at 3.5 dpc early blastocyst stage and they stayed highly expressed in rabbit ES-like cells. In contrast, high expression level of ocu-miR-290 cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells. Differential expression of the ocu-miR-302 cluster and ocu-miR-512 miRNA was detected in rabbit trophoblast and embryoblast. The predominant expression of ocu-miR-512 in rabbit trophoblast may propose its trophoblast specificity.

We suggest that the expression of ocu-miR-302 cluster is more characteristic for the rabbit ES-like cell specific, while the ocu-miR-290 cluster may play a crucial role during early embryonic development. This study represents the first identification of pluripotency-associated miRNAs in rabbit early embryonic development and ES-like cells, which can open up new way to investigate the regulatory function of ocu-miRNAs in embryonic development and stem cell biology.

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P-135
COMPARISON THE GENE EXPRESSION PATTERN OF MACROPHAGE AND DENDRITIC CELL SPECIFIC TRANSCRIPTION FACTORS IN MOUSE EMBRYONIC STEM CELLS- AND BONE MARROW- DERIVED DENDRITIC CELLS

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Monocytes and macrophages are critical effectors and regulators of inflammation and innate immune response, the immediate arm of the immune system. Dendritic cells (DCs) initiate and regulate the adaptive immune responses and are central for the development of immunologic memory and tolerance. Recent in vivo experimental approaches in the mouse have unveiled new aspects of the developmental and lineage relationships among these cell populations. The discovery of DCs as a distinct lineage of mononuclear phagocytes, specialized in antigen presentation to T cells and the initiation and control of immunity, revealed additional roles of these cells in shaping the immune response to pathogens, vaccines, and tumors.

DCs can be generated from various precursors including embryonic stem (ES) cells (ES-DC), but ESderived DCs had an impaired T cell activation capacity. Our hypothesis is that some key transcription factors may be missing from the ES-DCs, and this contributes for the impaired T cell activation capacity of these cells. The main goal of my study is to compare the gene expression pattern of the embryonic stem (ES) cell- versus bone marrow (BM)-derived DCs.

We have monitored the mRNA level of fifteen myeloid/DC specific transcription factors (Irf8, Relb, Egr1, Egr2, Sfpi1 (Pu1), Irf2, Mafb, Tcf4, Maf, Ikzf1, Batf3, Spib, Runx3, Irf4, Id2) with real-time quantitative PCR from ES- or BM- derived DCs and their progenitors. These 15 factors were selected based on previous data that demonstrated these genes are essential for DC or macrophage development.Our data revealed that eleven genes out of the 15 showed a very similar expression pattern in both type of cells (ES vs BM derived DCs). Interestingly 3 genes showed a lower expression in ESderived DCs suggesting that upregulation of these factors might modify the phenotype of the ES derived antigen presenting cells.

P-136
7H3 IS A NOVEL BURSAL STEM CELL ANTIGEN

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The avian embryo provides an excellent model system for studying the development of lympho-myeloid organs because it is amenable to in vivo experimental manipulation throughout embryogenesis. A further advantage of avian model is the B-cell development takes place in a separate organ, the bursa of Fabricius. As an effort to learn how lympho-myeloid cell diversification is regulated in the birds we have produced a large panel of monoclonal antibodies (mAbs) by immunizing mice with cell suspension of spleen and bursa of Fabricius of guinea fowl (*Numida melegaris*).

One of these mAbs (clone: 7H3) was found to recognize a cell surface antigen (molecular weight: ~ 70 kDa) expressed by CD45+ hematopoietic cells in the early embryo. In bursa of Fabricius, spleen, and thymus from embryonic day 16, nearly all lymphoid progenitor cells carried the 7H3 antigen. By the end of the embryonic period, double immunolabeling proved that all B cells of embryonic bursa expressed the 7H3 antigen. However, after hatching the 7H3 expression in both the cortex and medulla of the follicles gradually diminished, and it was lost, except a subpopulation of cortical B cells and CD3+ T cells. Other cells of lympho-myeloid origin, macrophages, dendritic cells, granulocytes did not react with 7H3 mAb.

According to previous cell transplantation experiments, the postnatal bursa of Fabricius may contain an undifferentiated B cell population (called bursal stem cells) which regenerates the bursal follicles after B cell depletion. On the base of the embryonic expression of the 7H3 antigen we hypothesized that our mAb could be a candidate marker for these bursal stem cells. To examine the ontogeny of 7H3+ cells during bursal regeneration we induced B cell depletion in 10 week old birds with cyclophosphamide (Cy) treatment. Three days CY treatment caused virtual absence of bursal lymphoid cells and later, destruction of the normal bursal architecture. Two weeks after treatment, bursal recovery starts by infiltration of 7H3+ cells in the cortex and 15 days later followed the medulla.

Taken together, these data suggest that 7H3 mAb is a novel hematopoietic cell marker, which recognizes bursal stem cells of the adult bursa of Fabricius.

P-137
ANTI-INFLAMMATORY EFFECTS OF MOUSE MESENCHYMAL STEM CELLS ON MICROGLIA

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The immunosuppressive and anti-inflammatory properties of mesenchymal stem or stromal cells (MSC) have been demonstrated on a wide range of innate and adaptive immune cells.

It is well-known that neuroinflammation plays a crucial role in both pathogenic and regenerative processes of the brain and is largely mediated by microglia and astrocytes, therefore we investigated the effects of mouse bone marrow-derived MSCs on microglia.

We prepared primary mixed glia cell cultures and then selectively isolated primary microglia cells, co-cultured with MSCs and investigated the phagocytic and antigen presenting capability of microglia and the attendant production of pro- and anti-inflammatory factors in the presence or absence of bacterial endotoxin lipopolysaccharide (LPS).

We found that MSCs in co-cultures and also in transwell cultures inhibited the activation of microglial cells and changed the ratio of the secreted pro-inflammatory tumor necrosis factor-α (TNF-α) and anti-inflammatory IL-10 in the supernatants. In addition the phagocytic activity of microglial cells were significantly increased by MSCs. The MSCs also enhanced the antigen presenting capability of microglia and induced morphological changes in these cells. Based on transwell experiments we hypothesize that the cross-talk between these cells is mainly but not exclusively mediated by soluble factors, including prostaglandin E2 (PGE2).

These findings underline the theory that in the presence of MSCs microglia cells undergo alternative activation and gain M2-like (anti-inflammatory) phenotype similarly to peritoneal macrophages. In summary, the observed beneficial effects of MSCs may have relevance to treatment strategies for inflammatory diseases of the central nervous system, apparently by alternative activation of microglia.

P-138
METABOLIC CHANGES DURING DIFFERENTIATION OF NEURAL STEM CELLS

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According to previous results[1], neural stem cells survive at much lower oxygen supply than neurons, both in vivo and in vitro. In order to understand the diverse O_2-demand, metabolic analyses were carried out on one-cell derived populations of neural stem cells representing progenitors of the neural plate/early neural tube (NE-4C[2]) and the adult neurogenic zones (HC_A and SVZ_M)[3]. The embryonic (E9) neuroectoderm derived NE-4C neural stem cells displayed very low O_2 consumption, and it was further decreased by starvation. Differentiating NE-4C progenies on the other hand, increased O_2 consumption in response to starvation indicating that neural precursors gain energy from catabolizing own cellular material. Depending on origin and developmental stages, different stem cells displayed different responses in response to supplementing the "starvation" medium with single metabolites (glucose, lactate, β-OH-butyrate, amino acids). Non-differentiated NE-4C cells increased O_2-consumption in response to any of the metabolites. In contrast, neuronal derivatives of NE-4C cells decreased O_2-consumption and increased H^+ production in response to glucose, indicating that glucose is not used for mitochondrial energy production by these cells. Adult-derived non-differentiated neuronal stem cells also decreased O_2-consumption in response to glucose. In these cells, addition of amino acids resulted in a sudden but transient increase, while ketone bodies caused a slow but permanent increase of oxygen consumption. The data indicate that the basic metabolism shifts with the advancement of neural differentiation, and the metabolic profile reflects the origin and stage of differentiation of distinct neural stem/progenitor populations.

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Keywords: neural stem cell, metabolism, neuronal differentiation, oxygen consumption

P-139
CLINICAL AND SCIENTIFIC ASPECTS OF THE FUTURE THERAPEUTIC USE OF MESENCHYMAL STEM CELLS IN THE FIELD OF PHYSIOTHERAPY

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Introduction: Mesenchymal stromal cells (MSCs) are multipotent adult stem cells with unique characteristics that make them promising candidates in future therapies. MSCs are easy to isolate and culture, show great plasticity, and their regenerative and immunomodulatory potential has already been successfully used in several preclinical and clinical trials. For MSC-based therapies to become routine clinical practice, both the optimal source of stem cells, and the exact molecular mechanisms underlying their therapeutic effects need to be determined.

Aim: We aimed to survey what is the anatomical origin of the cells that are used recently by MSC-based clinical trials and what proportion of these trials was aimed to treat musculoskeletal disorders. Using professional software we also re-analysed data from an already published study that compares the gene expression profile of MSCs isolated from young and adult mice. We were hoping to uncover new consequences on the optimal donor age that could also prove to be useful in future human therapies.

Materials and methods: We analysed online available data by computer: *in silico*, with the so called „dry lab" bioinformatic method, seeking still unexplored connections. During our first analysis we used on-line available databases such as Clinical Trials and PubMed, for the re-evaluation of expression data from a previously reported study we used Ingenuity Pathway Analysis software.

Results: We established that close to one third (31%) of all clinical trials using MSCs aim to treat musculoskeletal disorders, in most cases (22%) osteoarthritis. The majority of the clinical trials (42%) used bone marrow derived MSCs, but cord blood (16%), adipose tissue (10%) and other tissues (32%) can also serve as a stem cell source for therapeutic applications. *In silico* analysis of the gene expression data on young and adult bone marrow MSCs revealed that transcription of several genes involved in the alkaline phosphatase activation pathway or associated with tumour formation increased with the donor's age.

From the 81 genes that were upregulated, 61 could be associated with cancer and 8 were alkaline phosphatase activation related, some of them are important transcription factors.

Discussion: Our survey of the current clinical trials suggests that new MSC based therapies are about to be introduced in the field of physiotherapy. Bone-marrow was the most commonly reported MSC source in clinical trials. Gene expression changes in the bone-marrow derived MSCs of older donors indicate a higher possibility of tumour formation. At the same time increased alkaline phosphatase activity was observed, which is the characteristic of MSCs committed towards osteogenic differentiation. We can conclude that the younger the bone marrow-derived cells, the more suitable for medical purposes they are, because of the higher plasticity and the

lower risk of tumour formation. *In silico* methods can provide a tool for physiotherapists to expand their research to the molecular level.

P-140
ESTABLISHMENT OF AN *IN VITRO* MODEL OF SYNOVIAL SARCOMA ONCOGENESIS IN MESENCHYMAL STEM CELLS

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Synovial sarcoma is a rare mesenchymal tumor with aggressive clinical behavior and dismal therapeutic outcome. Synovial sarcoma is invariably associated with the chromosomal translocation t(X;18) which results in an in-frame fusion between the truncated forms of the SYT gene and one of the SSX family members SSX1, 2, or 4. Although SYT-SSX fusion oncoproteins have been shown to be involved in epigenetic reprogramming, and mesenchymal stem cells are strongly suspected as the targets of SYT-SSX-related malignant transformation, regulatory alterations that convert benign stem cells to cancer are largely unidentified. To help elucidate details of this process, the synovial sarcoma-associated fusion oncogene SYT-SSX1 was amplified from tumor cDNA, tagged C-terminally with a self-designed dual epitope tag (influenza virus hemagglutinin - vesicular stomatitis virus glycoprotein), and cloned into a fluorescent marker-expressing lentiviral vector. Similar vector constructs containing the separate fusion partners SYT and SSX1 were created as controls. All vectors were verified by sequencing. After testing the constructs in human embryonic kidney (HEK293) cells, C2C12 murine myoblasts and human mesenchymal stem cells were transduced, and transgene-expressing cells were enriched by flow cytometry sorting. Co-expression of the transgenes with the fluorescent marker and correct nuclear localization of the epitope tags were confirmed by RT-qPCR and immunofluorescence labeling. The established cell populations allow analysis of both short- and long-term effects (i.e., from 72 hours to several months) of SYT-SSX1 expression on multiple levels of gene regulation, and will contribute to a better understanding of SYT-SSX1-related oncogenesis.

P-141
EXPRESSION AND FUNCTION OF TENASCIN-C DURING COLORECTAL ENTERIC NERVOUS SYSTEM DEVELOPMENT

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The intestinal microenvironment contains several extracellular macromolecules that play important roles during enteric nervous system (ENS) development. Tenascin-C is an extracellular matrix glycoprotein that regulates cell migration, possibly by modulating the adhesiveness of neural crest cells to their substratum. The role of tenascin-C in enteric neuronal development is unknown. In this study we show that tenascin-C is dynamically expressed during ENS development in the chick gut. It is absent from the cecal region prior to the arrival of crest-derived cells, and then is found to colocalize with the migrating wavefront. Experimentally generated aganglionic hindgut leads to loss of tenascin-C expression in the submucosal area. Analysis of FACS-sorted neural crest cells by qRT-PCR and generation of chick-rat intestinal chimeras both demonstrate that vagal-derived enteric neural crest cells produce tenascin-C, whereas sacral-derived crest cells do not. Based on vitro migration assays, we find that tenascin-C promotes the migration of enteric neural crest cells. We conclude that vagal-derived enteric neural crest cells produce tenascin-C to promote their migration during ENS development.

P-142
VALIDATION OF RABBIT MICRORNA SEQUENCES IDENTIFIED BY SOLID SEQUENCING

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Over the past few decades microRNAs have emerged as important regulators of gene expression networks. Since then, numerous studies tried to identify microRNAs and analyse their function. It is proven, that they have important regulatory roles in proliferation, apoptosis, cell differentiation, and link to numerous types of cancer specific microRNA expression patterns are known.

MicroRNAs are 21-26 nucleotide long, non-coding, single stranded RNAs, which have an important role in the sensitive adjustment of basic functions by posttranscriptional gene regulation. They target sites are in the three prime untranslated regions (3′ UTR) of messengerRNA (mRNA), where they repress the translation or cleavage the mRNA.

According to data of miRbase, more than 1000 human (Homo sapiens) and more than 800 mouse (Mus musculus) microRNAs are known, but not any yet in rabbit (Oryctolagus cuniculus). Numerous studies try to identify specific gene expression patterns of specific cell types, or cancers.

In this study we would like to test the hypothesis according to which the number of reads, what we get by Solid sequencing, could be used to estimate the gene expression level. Moreover, we would like to devise a method to compare the data of Solid sequencing with the results of real-time PCR analysis. With this object we searched known human and mouse microRNA sequences in sequenced mouse fibroblast, rabbit fibroblast and 13.5-day-old rabbit embryo. After that, we isolated RNA from mouse and rabbit fibroblasts, and rabbit 13.5-day-old embryo, than analysed it by real-time PCR to identify some of the searched microRNAs.

As a result of comparison, it could be established that the read numbers of Solid sequencing cannot be directly compared to the relative expression levels of real-time PCR analysis. Although in some cases we could estimate the order of magnitude, these are not really reliable, and we could not identify exact scales or expression patterns.

We suppose it could be possible to perform more exact comparison, if we used RNAzol Reagent for RNA isolation. It let us to isolate separately the microRNA fraction, and we could use cDNA for the real-time PCR from microRNA fraction. In this case it could be possible to compare each microRNA's expression to the all microRNA expression level. It could be done the same analysis with the read numbers of Solid sequencing, because we know the number of the total microRNA reads. So, we could make more exact comparison between the two methods.

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P-143
EMBRYOID BODY AND OP9 CO-CULTURE: TWO WAYS OF BLOOD CELL DEVELOPMENT FROM EMBRYONIC STEM CELL

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Embryonic stem cells represent (ESCs) a pluripotent population of cells capable of self-renewal, large-scale expansion, and differentiation into various cell lineages including cells of hematopoietic lineage. Therapeutic application of ES cells is becoming clinical realities for treating various diseases, including certain cancers (e.g. leukemia, solid tumors). Recently, considerable efforts have been made to differentiate ES cells *in vitro* into hematopoietic stem cells (HSCs), and mature blood cells including dendritic cells (DCs). Dendritic cells are considered the most influential antigen presenting cells (APCs) in the body because of their unique capacity in initiating immunity against various antigens. In addition, DCs are involved in the maintenance of immunologic self-tolerance in the periphery, inducing regulatory T cells or anergy of autoreactive T cells. ES cells have the potential to become a scalable source of cells for DC-mediated immunotherapy.

In this study, we compared two methods to differentiate ESCs into myeloidDC precursors.The first method uses OP9 bone marrow stromal supportingcellsin a co-culture system, while the second

method isbased on the formation of embryoid body (EB) at the first stage of differentiation. To monitor the mesoderm and blood cell differentiation capacity of these developing cells, we have assessed the cell surface expression of several markers at day 5 and day 11,moreover we have conducted real-time quantitative PCR experimentsto measure the expression level ofMafb transcription factor.

Our data revealed that ES cells were differentiated into cells of the hematopoietic lineage by both methods. However, the EB derived cells showed higher expression of MafB and mesodermal/hematopoietic surface markers(CD41, Flk1) at the early stage of development (day 5). In addition, high percent of CD45 and CD11b positive cells were detected after 11 days of culture by both methods, however we observed higherCD11c positivity from the EB derived cells. Together these results demonstrated that the EB based protocolhas a superior capacity to convert ESCs into early blood cell progenitors.

P-144
EXPRESSION OF PLURIPOTENCY MARKERS MAY IDENTIFY A THERAPEUTICALLY SUPERIOR SUBPOPULATION OF MESENCHYMAL STEM CELLS

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Background and aims. Mesenchymal stem cells (MSCs) are adult stem cells that hold great promise in regenerative medicine and immunomodulation. Under the current standards, MSCs isolated from various tissues are identified by plastic adherence, expression of a defined set of surface markers, and the ability to differentiate toward the osteogenic, chondrogenic, and adipogenic lineages *in vitro*. While these criteria are useful to describe cell isolates on the whole population level, MSC cultures are known to be largely heterogeneous on the level of individual cells with regard to important properties such as expansion and differentiation potential or pro-regenerative effect. Specifically, less committed cells are thought to be therapeutically more relevant than those closer to terminal differentiation. Our aim was to identify subpopulations expressing or co-expressing several pluripotency markers in MSC isolates derived from various tissue types.

Materials and methods. MSCs were prepared by conventional techniques from human adipose tissue (Ad), Wharton's jelly (WJ), periodontal ligament (PDL), and bone marrow (BM). Isolates were characterized by flow cytometry, immunofluorescence, quantitative RT-PCR, as well as ABC transport and differentiation assays. Human embryonic stem cells (HUES) were used as controls for pluripotency marker expression.

Results. Isolates from all sources were confirmed to uniformly (over 95%) express standard MSC markers CD13, 29, 44, 73, 90, 105, and 166. Also, adipogenic and osteogenic differentiation ability

was demonstrated in all cases. The pluripotency marker SSEA4 was expressed by ~40% of WJ MSCs, but SSEA4-positive cells were present up to 8-15% in MSCs from other sources as well. Alkaline phosphatase (ALP) was expressed by 45-50% of BM MSCs and 20-25% of other MSCs; however, ALP may also be an indicator of osteogenic commitment. The early pluripotency factor SOX2 was clearly seen in a subset of WJ MSCs while showed only weak expression in others. The stemness-related plasma membrane transporter ABCG2 was detected in a remarkably high proportion (~40%) of PDL cells, and the same cells exhibited ABCG2-specific transport activity.

Conclusions and perspective. MSC populations isolated from extraembryonic and postnatal tissues contain pluripotency marker-expressing cells in varying proportions. Co-expression of these markers is currently being investigated in order to narrow our focus on a close-to-pluripotent subset of adult tissue stem cells. Verification of the observed expression patterns at the RNA level and further characterization of the selected subpopulations are in progress. It remains to be clarified whether expression of these markers is a reliable indicator of uncommitted state.

P-145
A POSSIBLE NEW RECIPE FOR CELL AND GENE THERAPY: INDUCED PLURIPOTENT STEM CELLS COMBINED WITH ARTIFICIAL CHROMOSOMES

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In 2006, the direct reprogramming of mouse fibroblasts to induced pluripotent stem (iPS) cells was achieved by the overexpression of four transcription factors (Oct4, Sox2, Klf4, cMyc). Differentiated cell types derived from iPS cells would be useful in cell and gene therapy applications to treat patients of various diseases and injuries. Human iPS cells can help to understand the development and function of human tissues and to discover and test new medicines. The usage of these cells in human therapy is presently problematic: i) the applied oncogenes should be removed from cells; ii) safer gene-carriers should be used to avoid insertional mutagenesis; iii) reliable technologies should be developed to acquire considerable amount of differentiated cell types for therapies.

Recently, several types of vectors and protocols have been developed to exclude transgene integration during reprogramming. These technologies should be examined with regard to quality of individual iPS cells, the efficiency of reprogramming and genome integrity. Moreover, iPS cells should be removed before therapy to avoid unexpected and undesired side-effects (e.g. tumor formations).

Therefore, we aimed to achieve reprogramming of somatic cells into pluripotent state by utilizing mammalian artificial chromosomes (MACs). MACs have a virtually unlimited transgene carrying capacity, they are non-integrating vectors and autonomously maintained throughout cell divisions as a unique chromosome.

We attempted to generate vector- and transgene-free iPS cells using the MAC to express the four reprogramming proteins. From the factor-producing chinese hamster cell lines, nuclear extracts were prepared and added to the cell culture medium every day. Moreover, we also applied the mRNAs of these factors for iPS cell generation in separate experiments. The iPS cells derived from these simple, non-mutagenic and highly controllable technologies would be applicable to a range of MAC-based tissue-engineering tasks.

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P-146
GENERATION AND CHARACTERIZATION OF A CELL TYPE-SPECIFIC SELECTIVE MARKER FOR ISOLATION OF HEPATOCYTE-LIKE CELLS FROM STEM CELL-DERIVED CULTURES

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P-147
EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON SPERMATOGENESIS

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Pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide with diverse effects, was originally isolated as a hypothalamo-hypophyseal peptide. Subsequent studies showed highest levels of PACAP in the testis after the brain, suggesting that it influences the development and functioning of spermatozoa. Indeed, it has been proven that PACAP has an effect on spermatogenesis, both locally and via influencing the hypothalamo-hypophyseal-gonadal axis. The aim of the present study was to investigate sperm motility, morphology and expression of key determinants of spermatogenesis in the testis of mice lacking endogenous PACAP. Motility of sperm cells was investigated using a computer aided sperm analysis system. Sperms isolated from the epididymis of PACAP KO mice showed a decrease in sperm motility. The morphological analysis of spermatozoa isolated from wild type and PACAP KO mice showed that the sperm head diameter was significantly smaller in PACAP KO mice. The shape of the heads investigated with transmission and scanning electronmicroscopy, did not show marked differences between the two groups. However, we found more abnormal tail forms among PACAP KO cells. The family of Sox transcription factors play key roles in spermatogenesis. We investigated Sox 9 and Sox 10 in the testis of PACAP KO mice by immunohistochemistry and Western blotting. We found that while Sox 9 expression was markedly reduced, Sox 10 was significantly increased in PACAP KO mice. The phosphatase PP2A was also increased in mice lacking PACAP. Our results show that there are marked differences in sperm morphology, biochemistry and function between wild type and PACAP KO mice, suggesting that endogenous PACAP plays an important role in spermatogenesis.

P-148
COMPPI: COMPARTMENTALIZED PROTEIN-PROTEIN INTERACTION DATABASE

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Protein-protein interaction networks (interactomes) help system level understanding of the organization and functions of intact and diseased cells. Intracellular protein localization data have been rarely incorporated to interactomes. Therefore, currently used interactomes have a large number of false positive interactions, because the putatively interacting proteins are not localized at the same subcellular compartment.

To filter false-positive interactions we assigned subcellular localization data to participating proteins. We extended the interactome of four species (*S. cerevisiae, C. elegans, D. melanogaster* and *H. sapiens*) with subcellular localization data and assembled a compartmentalized protein-protein interaction database, ComPPI (www.linkgroup.hu/comppi). Subcellular localizations were standardized by Gene Ontology terms to a non-redundant localization tree.

Based on the subcellular localization of the proteins, the quality of localization data, and the topology of the interactome neighborhood of affected proteins a confidence score was assigned to each protein-protein interaction, which enables the creation of high-confidence interactomes. On the webpage the user may search for interactions of individual proteins, or download full, or partial interactomes of whole cells or their organelles.

Analysis of the compartmentalized protein interactome showed that protein localization data induce profound changes in the properties of currently used interactomes. Thus, ComPPI database offers a great help to find those protein interactions, which have a high confidence regarding their subcellular localization and/or change their subcellular localization in disease or during the aging process. These data also offer additional opportunities for the treatment of illnesses with complex pathomechanisms, like diabetes or cancer.

P-149
RAPID COMPENSATORY ADAPTATION FOLLOWING GENE LOSS IN *SACCHAROMYCES CEREVISIAE*

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It has been revealed previously that existance of genes frequently vary across related species. The general knowledge is limited about how a seemingly indispensable gene can be lost without the eradication of the resulting population by purifying selection. Hypothetically,non-essential gene loss can be compensated by accumulation of trans-acting mutations as a result of an evolutionary process.

It is also equivocal that how rapid is the compensatory process and to what extent does it depend on biochemical and functional properties of the lost genes, and whether is there any cost of the compensation.

To characterize the genomic mechanisms triggered by gene loss, an evolutionary experiment was carried out with over 194 haploid single gene knock-out yeast strains.All of the strains initially show slow growth in standard rich laboratory medium (more than 10% fitness defect compared with wild type). The rate of compensation was measured. After only 400 generations of laboratory evolution, 58% of knock-outscompensatedatleasthalf of theinitialdefect.Tendency for evolutionary compensation is not restricted to certain gene classes, and is not severely affected by position in various biological networks. Possible pleiotropic effects of compensation were examined by carrying out another large scale phenotypic screens in 14 different laboratory media.The results provided examples of prevalent pleiotropy of the compensatory mutations.

Our results demonstrate that compensatory evolution following gene loss is rapid and pervasive in the laboratory. If compensational genomic responses provide a robust advantage in different environments, they could get fixed in the population, thus these mechanisms could be powerful tools of evolution generating essentially new variations and might lead to peaks on the fitness landscape unreachable by solely adaptive steps.

P-150
DIRECTED EVOLUTION OF ANTIMICROBIAL PEPTIDES TO INVESTIGATE RESISTANCE MECHANISMS

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The emergence of multi-resistant bacterial strains is going to result in a serious public health hazard if new therapeutic strategies are not developed in the foreseeable future. The multitude of antimicrobial peptides (AMPs) that are currently in clinical trials shows that these molecules can play an important role in addressing this situation. Unlike with traditional small molecular drugs, resistance against AMPs evolves with a lower probability, and their effectiveness can be increased with directed evolution. In this project we are developing a miniaturised high throughput screening assay that may enable the directed evolution of AMPs with a screening capacity (10^7 clones/h) that is inaccesssible with current methodologies. In this technology – *In vitro compartmentalization* (IVC) combined with microfluidic systems – the screening units are monodisperse aqueous droplets dispersed in an oil phase (water-in-oil droplets) that can miniaturise a cytotoxicity assay to the single cell level. By evolving the bacterial killing effect of AMPs, we are looking for answers to the questions (i) Can AMPs co-evolve with bacterial resistance (ii) Is there any difference in the evolvability of peptide groups with different mechanism of action (iii) What mechanisms help the peptides adapt to resistant strains.

P-151
IS TRANSCRIPTIONAL REPROGRAMMING FOLLOWING GENE DELETION ADAPTIVE IN YEAST?

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One of the most surprising discoveries of the post-genomic era thatmost genes can be removed individually without apparent growth defect. One theory suggests that this robustness may be achieved by transcriptional reprogramming and predicts that most expression changesare adaptive and contribute to compensating the absence of the gene. However, due to lack of large-scale transcriptome data on knockout strains, we know very little about the general rules governing transcriptome reprogramming upon gene deletion.Besides the issue of functional compensation via adaptive reprogramming, it remains also unclear what features of a given gene determinethe extent of transcriptional response following its deletion and how often the deleted gene and the responsive genes are functionally linked A recent systematic dataset from the Holstege lab allowed us to address these questions on an unprecedented scale. The dataset contains information on genome-wide gene expression changes for1412 viable knock-out yeast strains.

Our preliminary results indicate that the number of upregulated genes show strong positive correlation with both the fitness contribution and the degree of pleiotropy of the deleted gene. While we revealed a significant overrepresentation of functional relatedness between the deleted and the responsive genes, this effect is small and most changes do not occur within functional modules.Finally, by overlaying the transcriptome data on large-scale genetic interaction datasets, we revisited the hypothesis of functional compensation by adaptive reprogramming. A key prediction of this hypothesis is that pairs of genes that compensate each other's deletion (i.e. show synthetic genetic interactions) should achieve this via specifically upregulatingthe interacting partner.In contrast to this expectation, we only found a marginal enrichment of transcriptional reprogramming between pairs of synthetic lethal genes, suggesting that most of the expressional changes observed do not provide functional compensation.

P-152
CROSS-TALK MATRICES: A NOVEL APPROACH TO EXAMINE NETWORK LEVEL EXPRESSION CHANGES DURING COLON TUMORIGENESIS

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Signalling rewiring transforms normal cells into aggressive, malignant tumour formations. Altered function of signalling pathways (e.g., WNT, Hedgehog, TGF-β and Notch), and their malfunctioning cross-talks have been identified in different cancer cells. However, how the signalling network rewires, and how its various cross-talks change during tumourigenesis, have not yet been explained in detail.

First, we searched the Gene Expression Omnibus and the EBI Array Express resources to acquire expression data on the different stages of colon carcinogenesis. We used all available Affymetrix HGU133Plus2 microarray chip based studies. We collected altogether 44 normal colon, 102 colon adenoma and 441 colon carcinoma arrays. We combined the expression data with the network of the SignaLink 2 database. SignaLink 2 (http://SignaLink.org) is a well annotated and large-scale signalling pathway database that contains manually curated protein-protein interactions for seven major signalling pathways as well as transcriptional and post-transcriptional regulators of these pathways. Thus, SignaLink 2 allows the examination of cross-talks in three levels: post-translational, transcriptional and post- transcriptional.

Statistical analysis of mRNA and miRNA expression levels showed significant differences between the three major cancer stages (normal, adenoma and carcinoma). To facilitate our analysis we developed a pathway based representation method for signalling networks, called "cross-talk matrix". With the matrix-representation method we found important changes in the expression

patterns of cross-talks indicating specific rewiring processes. For example when normal colon tissue transitioned into adenoma, almost all the cross-talks have been up-regulated, however expression changes within the pathways were minor, and the cross-talks through miRNAs were only slightly up-regulated between these two stages. During the malignization process, except for a few cases, all the cross-talks were down-regulated. The interactions within the pathways were down-regulated as well, but the transcriptional cross-talks were up-regulated. We found only a few master transcription factors (such as the hypermethylated oestrogen receptor) to be responsible for this effect.

In conclusion we analysed more than 500 microarray datasets and developed a new way to present pathway based expression changes during colon tumorigenesis. We found substantial difference in the expression change of the protein-level, transcriptional and post-transcriptional-level cross-talks. We hope that the presented approach will facilitate future research works to better understand cancer progression.

P-153
LEARNING AS A NETWORK LEVEL PHENOMENON AND ITS IMPLICATIONS IN BIOLOGICAL NETWORKS

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Networks are a useful abstraction for modeling the behavior of highly complex systems occurring in biology. The most studied type of biological networks is by far the protein-protein interaction network. We are using an in-house developed program called Turbine (www.linkgroup.hu/Turbine.php) which enables network-level dynamic simulations on large networks. With a small modification of the basic dynamic model termed “communicating vessels”, we have introduced a learning aspect to the perturbation analysis. In this generalization of the Hebbian-type model the actively used links gain strength, while unused links get weaker. This model already shows an interesting phenomenon: a network which was affected by perturbations for a longer period of time shows a large slowdown in its ability to accommodate to and dissipate new types of perturbations. In this poster, we discuss this effect and its implications in biological networks – particularly the aforementioned protein-protein interaction network – as well as in learning and aging phenomena.

P-154
INTEGRATED ANALYSIS OF SIGNALING NETWORKS IN COLON TUMORIGENESIS POINTED OUT THE IMPORTANCE OF FIRST NEIGHBORS OF CANCER-RELATED PROTEINS

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During tumor progression of colorectal cancer the signaling network of the cells changes. To investigate the effect of such tumorigenic rewiring and to identify key components we have integrated and analyzed data from multiple different sources.

In our study we downloaded all accessible Affymetrix HGU133plus2 microarray expression datasets of three stages of colorectal cancer progression (normal, adenoma carcinoma) from Gene Expression Omnibus and Array Express resources. The widespread mutations of colorectal cancer were downloaded from COSMIC database. We searched methylation data from the literature. Then, we integrated the data of expression, mutation and methylation with the SignaLink 2 (http://signalink.org) signaling network database. SignaLink 2 contains 7 major signaling pathways, their protein-protein interaction, as well as their transcriptional and post-transcriptional regulations. We analyzed the networks with the ModuLand method and examined the general properties of the networks. For the functional annotation of the signaling processes we used the Gene ontology (GO) resource's Biological Process domain.

We found that the differentially expressed, mutated and methylated genes encode cancer-related proteins that were not functioning in the core of the signaling pathways. In addition, these cancer-related proteins did not appear more often among the proteins that form cross-talks between pathways. On the contrary, the first neighbors of such cancer-related proteins were significantly more common among proteins present in the core of the pathways and protein that connects them through cross-talks. Next we examined the system-level effect of the cancer-related proteins. With the Moduland modularization method, we found three major modules in the signaling network of normal colon that fell into five modules in colon adenoma and remarkably reassembled in colon carcinoma. First neighbors of cancer-related proteins were also significantly more common among the proteins that determined the stage-specific module structure. We also found novel functional connections between ligands and transcription factors in colon carcinoma, such as "mitosis" and "death", indicating an important effect of rewiring.

In conclusion, we found that the central part of the signaling network did not change, but the first neighbors of cancer-related proteins were indeed affected during the malignization process. These proteins could generate substantial, systems-level changes in the regulation of the signaling network.

P-155
APPLICATIONS OF TURBINE:DESIGNING INTERVENTIONS IN METABOLIC NETWORKS

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Networks quickly became an important component of understanding the functions of biological systems. Protein structure networks, protein-protein interaction networks and metabolic networks show the promise of attaining a high-level synthesis of the previously accumulated large body of knowledge in the life sciences. Our in-house developed program Turbine (www.linkgroup.hu/Turbine.php) was designed for the purpose of enabling full dynamic analysis on large, complex networks. We have developed an extension for Turbine, called Turbine:Solver capable of planning an intervention to shift the current network state into a certain more desirable goal state. A key use of this program is to generate novel drug targets for complex diseases by shifting the gene expression state of a cell to a desired 'healthy' state from the 'diseased' state. However, to our knowledge, no dynamic model is currently available for modeling system-wide changes of gene expressions. Therefore, we demonstrate the prediction power of the Turbine toolkit by presenting an example application on the human metabolic network where the law of mass action dynamics is available for system-wide analysis.

P-156
SIZE DOES MATTER – A COMPREHENSIVE METHOD FOR HIGH-THROUGHPUT COLONY SIZE MEASUREMENT

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Systems biology studies require the acquisition of reliable phenotypic data on a large scale. In unicellular model organisms, one of the most informative phenotypic data is fitness (i.e. growth rate or colony size), since it reflects the overall state of cellular processes upon genetic or chemical perturbations. However, measuring microbial growth on a large-scale is challenging. First, quantitative fitness measurement requires small measurement errors and, ideally, absence of systematic biases. Second, the method needs to be scalable, hence fast and cheap. While several methods have been published to measure fitness in microbes on a large scale they either i) fail to handle systematic bias, ii) are designed for some very specific assays (genetic interaction screens), or iii) require time series measurements, which are costly. To achieve an optimal balance between accuracy and costs, we developed a robust workflow for comparing colony sizes of microbial cultures grown on agar surface in a high-throughput manner. Our method relies on both an optimized experimental workflow to minimize random and systematic errors and on a computational pipeline that performs a series of bias corrections to minimize experimental

artifacts. A key step in the experimental workflow is the inoculation of independent colonies using the same inoculum size to minimize random noise. This is achieved by employing a robotized system equipped with an ordered array of uniform metal pins to perform the inoculation on agar plates at high densities (e.g. 384 and 768). Next, a robust computational algorithm detects colonies on photos taken at a single time-point and calculates colony sizes. Raw colony size data is then corrected for within plate effects (e.g. colonies at the edge of the plate are usually larger) and normalized across multiple plates using internal controls. The method is currently validated for *Saccharomyces cerevisiae* colonies and being optimized for *E. coli*.

P-157
NETWORK-LEVEL RECONSTRUCTION OF THE 'UNDERGROUND' METABOLISM OF *ESCHERICHIA COLI*

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Secondary or 'underground' reactions are weak catalytic activities of enzymes other than for which they evolved. It is widely held that these secondary reactions play important roles in the evolution of new enzymatic functions. While a number of case studies examined the properties and evolution of secondary reactions of individual enzymes, it remains unknown how these reactions might contribute to the evolutionary expansion of existing metabolic networks. To fill this gap in our knowledge, we reconstructed, for the first time, the underground metabolism of an organism by compiling the known secondary activities of *E. coli* enzymes. We extended an existing network representing the native metabolism of *E. coli* with 260 secondary reactions compiled from databases and literature. We report that more than 45% of secondary reactions can be seamlessly integrated into the native metabolic network and can contribute to novel pathways with important end products. Statistical analysis of the network position of underground reactions revealed that, due to chemical constraints, native and secondary activities of the same enzyme tend to be located close to each other on the metabolic map. Taken together, our results suggest that while a large fraction of the underground catalytic repertoire can contribute new links to the native metabolic network, their topological positions are biased and might generate non-random patterns in pathway organization.

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JEGYZET:

	Friday, 5 April 2013		
14:00-14:10	Opening		
14:10-15:40	Plenary lectures I - Chairperson: Éva Kondorosi /PL-01-PL-03/		
15:40-16:30	*Coffee Break*		
16:30-18:00	Plenary lectures II. - Chairperson: Éva Kondorosi /PL-04-PL-06/		
19:00	Poster discussion, reception at the posters' area (posters with even numbers)		

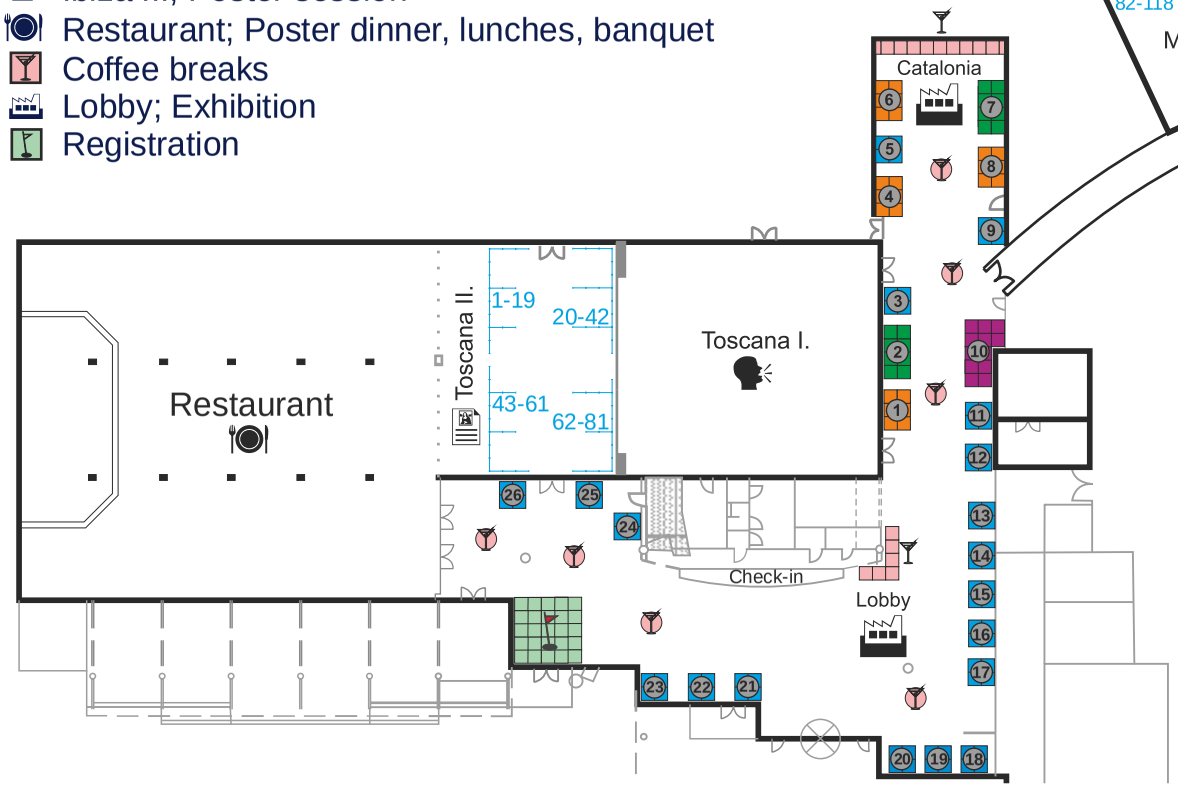
	Saturday, 6 April 2013		
	I. Parallel Session - Room Toscana I.	II. Parallel Session - Room Marbella	III. Parallel Session - Room Ibiza
9:00-11:00	Stem Cell Chairpersons: B. Sarkadi - E. Gócza O-001-O-006	Systems biology Chairpersons: T. Korcsmáros - B. Papp O-007-O-012	Developmental genetics I. Chairpersons: R. Sinka - G. Endre O-013-O-018
11:00-11:20	*Coffee Break*		
11:20-13:20	Protein structure and function Chairpersons: B. Sümegi - L. Nyitray O-019-O-023	DNA repair Chairpersons: B. Vértessy - L. Haracska O-024-O-029	Signaling, Cell-cell communication Chairpersons: F. Nagy - L. Buday O-030-O-035
13:20-14:30	*Lunch*		
14:30-16:10	Molecular mechanism of diseases I. Chairpersons: B. Melegh - B. L. Bálint O-036-O-040	Membrane, Transport, Trafficking I. Chairpersons: L. Vígh - A. L. Kiss O-041-O-045	Apoptosis, Autophagy Chairpersons: Zs. Szondy - M. Sass O-046-O-050
16:10-16:30	*Coffee Break*		
16:30-16:50	Company lecture CL-01		
16:50- 19:30	Poster discussion (posters with odd numbers)		
19:30	***Banquet***		

	Sunday, 7 April 2013		
	I. Parallel Session - Room Toscana I.	II. Parallel Session - Room Marbella	III. Parallel Session - Room Ibiza
9:00-11:00	Developmental genetics II. Chairpersons: P. Deák - J. Mihály O-051-O-055	Regulation of gene expression, Epigenetics Chairpersons: L. Nagy - I. Boros O-056-O-061	Membrane, Transport, Trafficking II. Chairpersons: A. Váradi - Cs. Laczka O-062-O-066
11:00-11:20	*Coffee Break*		
11:20-13:00	Molecular mechanism of diseases II. Chairpersons: J. Balla - M. Széll O-067-O-071	Microbial genetics Chairpersons: P. Putnoky - Cs. Pál O-072-O-076	Regulatory RNAs Chairpersons: J. Burgyán - Z. Havelda O-077-O-081
13:00-13:20	Closing, award ceremony		
13:20-	*Lunch*		

Map & exhibition floor plan

Function rooms:

- Toscana I.; Plenary lectures, session room
- Toscana II.; Poster session, poster dinner
- Catalonia; Exhibition
- Marbella I.; Poster session
- Marbella II.; Session room
- Ibiza I.; Session room
- Ibiza II.; Poster session
- Restaurant; Poster dinner, lunches, banquet
- Coffee breaks
- Lobby; Exhibition
- Registration



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1. Kvalitex Kft.
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3. Kromat Kft.
4. Izinta Kereskedelmi Kft.
5. Izotóp Intézet Kft.
6. G&G Instruments Kft.
7. Roche Magyarország Kft.
8. AP Hungary Kft.
9. Biomedica Hungaria Kft.
10. Life Technologies Magyarország Kft.
11. Merck Millipore Kft.
12. Sigma-Aldrich Kft.
13. Biocenter Kft.
14. Bio-Science Kft.
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