From molecules
to life and back

FEBS 3+ Meeting

organised by the Croatian Society of
Biochemistry and Molecular Biology,
Hungarian Biochemical Society &
Slovenian Biochemical Society
OPATIJA, Croatia / 13 - 16 June 2012

Book of Abstracts

Book of Abstracts of the FEBS 3+ Meeting *From molecules to life and back*
organised by the Croatian Society of Biochemistry and Molecular Biology,
Hungarian Biochemical Society & Slovenian Biochemical Society

Editors: Jerka Dumić, Zrinka Kovarik, Jadranka Varljen
Technical Editors: Marin Tota, Dijana Detel
Edition: 320 pages, 350 copies
Publisher: Croatian Society of Biochemistry and Molecular Biology

Printed by: Fintrade&tours d.o.o., Rijeka
Cover and logo design: Sagita d.o.o., Opatija

A CIP catalogue record for this book is available from the National and University Library in Zagreb under 808047

ISBN 978-953-95551-4-4

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Hum – the smallest town in the world

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SUPORTED BY

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Dear Colleagues,

Welcome to the FEBS 3+ Meeting *From molecules to life and back*! We are pleased that the Congress is being held in Opatija, the pearl of the north Croatian coast and we hope that you will have the opportunity to experience its beauty in addition to participating in the exciting scientific programme!

FEBS 3+ Meeting is organized by the Croatian Society of Biochemistry and Molecular Biology (CSBMB), the Hungarian Biochemical Society (HBS) and the Slovenian Biochemical Society (SBS) under the auspices of the Federation of European Biochemical Societies (FEBS). Let us take this opportunity to express our gratitude to the FEBS for the generous support and sustainment, our sponsors for their donations and to all of you for your scientific involvement which certainly will contribute to the success of the Congress.

FEBS 3+ Meeting Programme was established by the FEBS with the idea of supporting and encouraging the development and improvement of the scientific collaboration among the FEBS constituent societies on the local level. Our three Societies recognised that opportunity to enhance and to advance collaboration among the scientists from our countries, but also from other parts of Europe. Being aware of the necessity of integration and interaction of the scientists from different research areas for the understanding and elucidation of the complex biological systems we tried to create the Programme which comprises all aspects of molecular life sciences. We also recognised the importance of the impact of the molecular life sciences on the society in general, policy making, legislation and ethics, so with a great support of the FEBS Science and Society Committee, we organised two sessions in which some of the most intriguing issues will be discussed.

We are pleased and honoured that the Scientific Programme is led by Professor Ada Yonath, a Nobel Prize Laureate 2009 and an outstanding group of eminent scientists, but also highly contributed by more than 190 submitted presentations. Many of them will be presented as short talks, mostly by young scientists. The participation of 49 PhD students and young post-doctoral fellows is supported thanks to the FEBS 3+ Meeting Programme and the National Societies funds.

In addition to your enthusiastic scientific contribution, we kindly invite you to participate in the social programme that will provide an excellent opportunity to exchange ideas, knowledge and experiences with colleagues, to establish new acquaintances, and to renew the old ones.

FEBS 3+ Meeting offers an occasion for a rewarding scientific and personal experience, so we hope you will enjoy early summer in Opatija as well as experience Istria, likewise the exciting journey *From molecules to life and back*.

Thank you for joining us!

Very sincerely yours,

Jerka Dumić
Chair of the
Scientific Committee

Zrinka Kovarik
Chair of the
Organising Committee

Jadranka Varljen
Chair of the Local
Organising Committee

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Information

Registration

Registration will take place at the registration desk in the Grand Hotel Adriatic at 09:30 on Wednesday, 13 June.
Registration fee for participants and students includes: admission to lectures and exhibition, Book of Abstracts, congress materials, admission to all social events and refreshments during the congress.
Registration for accompanying persons includes welcome party, the congress dinner and excursion.
The certificate of attendance will be provided at the registration desk.

Language

The official language of the congress is English. There will be no simultaneous translation.

Lectures and oral presentations

Lectures will be held in the Main Hall and in the Camelia Hall.
Oral presentations should be prepared as MS Power Point slides. We will provide laptop for presentations with Windows 7 OS, Office 2010 and Acrobat Reader software. We recommend Apple Macintosh users (without Mini Display port) to convert their presentations into PDF files to avoid compatibility issues.
Presentations need to be tested in advance. Preferably, on Wednesday, 13 June during the registration, or later during breaks, but not later than one day before the presentation. Presentations should be given on USB memory key or CD/DVD disk.

Poster presentations

Posters should be mounted according to the schedule and to the List of posters in the Book of Abstracts.

Social events

Wednesday, 13 June	20:15	Welcome Party (Grand Hotel Adriatic)
Friday, 15 June	14:00	Excursion
Friday, 15 June	20:00	Congress Dinner


Programme

Programme at a glance

Wednesday, June 13, 2012		
09:30 – 15:30	Registration	
15:30 – 16:15	**Opening Ceremony**	
16:15 – 17:00	**Opening Plenary Lecture (PL1): Ada Yonath (IL)**	
17:00 – 17:30	Coffee Break	
17:30 – 19:30	**Structure and Function of Proteins** L1-L3 and SP1-SP3	
19:30 – 20:15	**Science and Society I: Gottfried Shatz (CH)**	
20:15 – 22:30	**Welcome Party**	
Thursday, June 14, 2012		
08:30 – 10:30	**Membrane Structure and Function** L4, L5 and SP4-SP6	**Cancer Biochemistry** L6, L7 and SP7-SP9
10:15 – 11:15	**Poster Session I** + Coffee Break	
11:15 – 12:00	**Plenary Lecture (PL2): Kai Simons (D)**	
	Lunch (on your own)	
14:00 – 16:00	**Molecular Interactions and Communication** L8-L10 and SP10-SP12	**Lipidomics** L11-L13 and SP13-SP15
16:00 – 16:30	Coffee Break	
16:30 – 17:15	**Plenary Lecture (PL3): Josef Jiricny (CH)**	
17:15 – 19:15	**Immunity and Inflammation** L14-L16 and SP16-SP18	**Transporters** L17-L19 and SP19-SP21
19:15 – 20:00	**Science and Society II - Jacques-Henry Weil (F)**	
Friday, June 15, 2012		
08:30 – 10:30	**Molecular Signaling** L20-L22 and SP22-SP24	
10:30 – 11:30	**Poster Session II** + Coffee Break	
11:30 – 13:30	**Molecular Basis of Disease and Therapy** L23-L25 and SP25-SP27	**Plant Biochemistry** L26, L27 and SP28-SP30
14:00 – 23:00	**EXCURSION & CONGRESS DINNER**	
Saturday, June 16, 2012		
09:00 – 11:00	**Systems Biology and Bioinformatics** L28-L30 and SP31-SP33	**Metabolism** L31 and SP34-SP38
11:00 – 11:20	**Presentation of the Bio-Science Award 2012**	
11:20 – 12:20	**Poster Session III** + Coffee Break	
12:20 – 13:30	Lunch (on your own)	
13:30 – 14:50	**Genomics** L32, L33 and SP39, SP40	
14:50 – 15:10	Coffee Break	
15:10 – 16:45	**Regulation of Gene Expression** L34, L35 and SP41-SP43	
16:45 – 17:30	**Closing Plenary Lecture (PL4): Sandra Oršulić (USA)**	
17:30 – 17:45	**Closing Ceremony**	

Programme

Wednesday, June 13, 2012

15:30	**Opening Ceremony**	
Chairs: Israel Pecht and Vito Turk		*Main Hall*
16:15	PL1	**Ada Yonath** (Rehovot, Israel) LIFE EXPECTANCY- WISHES, PREDICTIONS AND REALITY
17:00	Coffee Break	
Structure and Function of Proteins		*Main Hall*
Chairs: László Nyitray and Boris Turk		
17:30	L1	**Boris Turk** (Ljubljana, Slovenia) PROTEASE SIGNALING: A POINT FOR THERAPEUTIC INTERVENTION
17:55	L2	**Beáta Vértessy** (Budapest, Hungary) TOWARDS A MOLECULAR SWITCH
18:20	L3	**Zrinka Kovarik** (Zagreb, Croatia) MUTAGENESIS AND NEW OXIMES ENABLE REACTIVATION OF TABUN-INHIBITED ACETYLCHOLINESTERASE
18:45	SP1	**Marko Močibob** (Zagreb, Croatia) MACROMOLECULAR COMPLEXES OF AMINO ACID:[CARRIER PROTEIN] LIGASES AND CARRIER PROTEINS
19:00	SP2	**Gábor Pál** (Budapest, Hungary) A CORRECTED MODEL OF LECTIN PATHWAY COMPLEMENT SYSTEM ACTIVATION BASED ON NOVEL in vitro EVOLVED PROTEASE INHIBITORS
19:15	SP3	**Jerica Sabotič** (Ljubljana, Slovenia) MUSHROOMS ARE A SOURCE OF UNIQUE AND VERSATILE BETA-TREFOIL PROTEINS
Chairs: Mirna Flögel and Jacques-Henry Weil		*Main Hall*
19:30	SL1	FEBS Science and Society Lecture I: **Gottfried Schatz** (Basel, Switzerland) WHAT IT TAKES TO SUCCEED IN SCIENCE - AND HOW EUROPE'S INSTITUTIONS COULD HELP
20:15	Welcome Party	

Thursday, June 14, 2012

Membrane Structure and Function		*Main Hall*
Chairs: Gregor Anderluh and Balázs Sarkadi		
08:30	L4	**Balázs Sarkadi** (Budapest, Hungary) ABC MEMBRANE TRANSPORTERS IN HUMAN PLURIPOTENT STEM CELLS

08:55 L5 **Gregor Anderluh** (Ljubljana, Slovenia)
EFFECTS OF PERFORIN ON LIPID MEMBRANES

09:20 SP4 **Endre Károly Kristóf** (Debrecen, Hungary)
ROLE OF ICAM3 AND LFA-1 IN THE INTERACTION BETWEEN HUMAN MACROPHAGES AND APOPTOTIC NEUTROPHIL GRANULOCYTES

09:35 SP5 **Sergej Nadalin** (Rijeka, Croatia)
IMPACT OF PLA2G4A AND PTGS2 GENE POLYMORPHISMS AND RED BLOOD CELL PUFAS DEFICIT ON NIACIN SKIN-FLUSH RESPONSE IN PATIENTS WITH SCHIZOPHRENIA

09:50 SP6 **Jernej Oberčkal** (Ljubljana, Slovenia)
SYNAPTOTAGMIN 1 IS A NOVEL RECEPTOR FOR AMMODYTOXIN

Cancer Biochemistry *Camelia Hall*

Chairs: Lajos Haracska and Janko Kos

08:30 L6 **Janko Kos** (Ljubljana, Slovenia)
CYSTEINE CATHEPSINS: THE TARGETS FOR DIAGNOSTIC AND THERAPEUTIC INTERVENTIONS IN CANCER PATIENTS

08:55 L7 **Lajos Haracska** (Szeged, Hungary)
REPLICATION OF DAMAGED DNA: INSIGHT INTO THE ENGINES OF MUTAGENESIS AND CARCINOGENESIS

09:20 SP7 **Mihály Kovács** (Budapest, Hungary)
MECHANISM OF REGULATION OF HOMOLOGOUS RECOMBINATION BY THE HUMAN BLOOM'S SYNDROME HELICASE

09:35 SP8 **Neda Slade** (Zagreb, Croatia)
TP53 MUTATIONAL SIGNATURE OF ARISTOLOCHIC ACID IN CARCINOMAS OF THE UPPER URINARY TRACT

09:50 SP9 **Darjan Andrejc** (Ljubljana, Slovenia)
PROTEASE INVOLVED IN POSTTRANSLATIONAL MODIFICATION OF 6-PHOSPHOFRUCTO-1-KINASE IN CANCER CELLS

10:15 **Poster Session I**, exhibition and refreshements

Chairs: Marinka Drobnič-Košorok and Gregor Anderluh *Main Hall*

11:15 PL2 **Kai Simons** (Dresden, Germany)
LIPIDS ORGANIZING CELL MEMBRANES

Lunch (on your own)

Molecular Interactions and Communication *Main Hall*

Chairs: Gordana Maravić Vlahoviček and Radovan Komel

14:00 L8 **Radovan Komel** (Ljubljana, Slovenia)
CYTOCHROMES P450 INVOLVED IN HOST–FUNGAL PATHOGEN INTERACTION ARE PROMISING TARGETS FOR NATURAL ANTIFUNGAL COMPOUNDS

14:25 L9 **Gordana Maravić Vlahoviček** (Zagreb, Croatia)
MOLECULAR INSIGHT INTO AMINOGLYCOSIDE RESISTANCE BY RIBOSOMAL RNA METHYLTRANSFERASES

14:50 L10 **László Nyitray** (Budapest, Hungary)
REGULATION OF CELL MIGRATION BY THE S100A4/METASTASIN - NON-MUSCLE MYOSIN IIA INTERACTION

15:15 SP10 **Vlatka Godinić Mikulčić** (Zagreb, Croatia)
MOLECULAR INTERACTIONS OF AMINOACYL-tRNA SYNTHETASES AND THE RIBOSOME

15:30 SP11 **Zoltán Lipinszki** (Szeged, Hungary)
THE INTERPLAY BETWEEN p54/Rpn10 PROTEASOMAL AND THE UBA-UBL EXTRAPROTEASOMAL POLYUBIQUITIN RECEPTORS IS REGULATED BY UBIQUITYLATION IN *DROSOPHILA MELANOGASTER*

15:45 SP12 **Attila Reményi** (Budapest, Hungary)
MAP KINASE INTERACTOMICS OF A NEW KIND: LINEAR BINDING MOTIF DISCOVERY BASED ON PROTEIN-PEPTIDE CRYSTAL STRUCTURES

Lipidomics *Camelia Hall*

Chairs: László Vígh and Gábor Balogh

14:00 L11 **Harald C. Köfeler** (Graz, Austria)
DETERMINATION OF MURINE HEPATIC LIPID DROPLET COMPOSITION BY AN INTEGRATED HIGH RESOLUTION LIPIDOMICS PLATFORM

14:25 L12 **Evelyn Orsó** (Regensburg, Germany)
APPROACHING CLINICAL LIPIDOMICS: LIPID BIOMARKERS FOR ENDOLYSOSOMAL AND LIPID DROPLET STORAGE IN VASCULAR AND METABOLIC DISEASES

14:50 L13 **Gábor Balogh** (Szeged, Hungary)
LIPIDOMICS OF STRESS RESPONSE

15:15 SP13 **Angela Mastelić** (Split, Croatia)
RENAL GLOBOTETRAOSYLCERAMIDE EXPRESSION IN RAT MODEL OF DIABETES TYPE 1

15:30 SP14 **Domagoj Đikić** (Zagreb, Croatia)
LIPID CHANGES IN FROG (*PELOHYLAX RIDIBUNDUS*) DURING AROUSAL FROM PROLONGED HYPOTHERMIA

15:45 SP15 **Svjetlana Kalanj Bognar** (Zagreb, Croatia)
MEMBRANE LIPIDS AND NEURODEGENERATION

16:00 Coffee Break

Chairs: László Fésüs and Beáta Vértessy *Main Hall*

16:30 PL3 FEBS National Lecture - HBS: **Josef Jiricny** (Zurich, Switzerland)
NON-CANONICAL MISMATCH REPAIR

Immunity and Inflammation *Main Hall*

Chairs: László Buday and Roman Jerala

17:15 L14 **László Fésüs** (Debrecen, Hungary)
INFLAMMASOME ACTIVATION BY DYING AUTOPHAGIC CELLS

17:40	L15	**Roman Jerala** (Ljubljana, Slovenia) MOLECULAR MECHANISM TLR4 AND MYD88-MEDIATED SIGNALING AND INHIBITION
18:05	L16	**Janoš Terzić** (Split, Croatia) A NOVEL, UBIQUITIN BINDING PROTEIN - DVC1 IS IMPLICATED IN DNA REPAIR
18:30	SP16	**Sunčica Buljević** (Rijeka, Croatia) DIPEPTIDYL PEPTIDASE IV AFFECTS NEUROPEPTIDE Y LEVELS IN INFLAMMATORY EVENTS
18:45	SP17	**Ruđer Novak** (Zagreb, Croatia) GALECTIN-3 IN MACROPHAGE DIFFERENTIAITON AND ACTIVATION
19:00	SP18	**Zsuzsa Szondy** (Debrecen, Hungary) ENGULFMENT OF APOPTOTIC CELLS TRIGGERS RETINOID SYNTHESIS IN MACROPHAGES TO ENSURE THE EFFICIENT DEATH AND CLEARANCE OF NEGLECTED THYMOCYTES

Transporters - co-organized with the Croatian Physiological Society *Camelia Hall*

Chairs: Ivan Sabolić and Hermann Koepsell

17:15	L17	**Hermann Koepsell** (Würzburg, Germany) SHORT-TERM GLUCOSE DEPENDENT REGULATION OF THE SODIUM-D-GLUCOSE COTRANSPORTER SGLT1 IN SMALL INTESTINE
17:40	SP20	**András Váradi** (Budapest, Hungary) ANIMAL MODELS FOR *in vivo* RESCUE OF DISEASE-CAUSING MUTATIONS OF ABCC6
17:55	L18	**Mladen V. Tzvetkov** (Göttingen, Germany) THERAPEUTIC CONSEQUENCES OF HIGH GENETIC VARIABILITY IN THE HUMAN ORGANIC CATION TRANSPORTER OCT1
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18:35	SP21	**Roko Žaja** (Zagreb, Croatia) THE ROLE OF ORGANIC CATION TRANSPORTERS (OCTS, *SLC22A*) IN ZEBRAFISH *(DANIO RERIO)*
18:50	L19	**Ivan Sabolić** (Zagreb, Croatia) SEX AND SPECIES DIFFERENCES IN RENAL TRANSPORTERS OF ORGANIC COMPOUNDS

Chairs: Karmela Barišić and Tihomir Balog *Main Hall*

19:15	SL2	FEBS Science and Society Lecture II: Jacques-Henry Weil (F) GENETICALLY MODIFIED PLANTS: ARE THEY USEFUL AND SAFE?

Friday, June 15, 2012

Molecular Signaling *Main Hall*

Chairs: Igor Križaj and Sonja Levanat

08:30	L20	**Sonja Levanat** (Zagreb, Croatia) THE HEDGEHOG-GLI SIGNALING IN TUMORS AND IMPLICATIONS FOR THERAPY
08:55	L21	**Igor Križaj** (Ljubljana, Slovenia) INSIGHTS INTO PATHOPHYSIOLOGY OF HUMAN SECRETED PLA_2s THROUGH THEIR TOXIC COUNTERPARTS FROM SNAKE VENOMS
09:20	L22	**László Buday** (Debrecen, Hungary) THE SCAFFOLD PROTEIN TKS4 REGULATES EGF-DEPENDENT CELL MIGRATION
09:45	SP22	**Matjaž Deželak** (Ljubljana, Slovenia) THE THIRD INTRACELLULAR LOOP OF GLUCAGON LIKE-PEPTIDE-1 RECEPTOR IS COVALENTLY MODIFIED WITH ENDOGENOUS MONO-ADP-RIBOSYLTRANSFERASE — NOVEL TYPE OF RECEPTOR REGULATION?
10:00	SP23	**Nataša Kopitar-Jerala** (Ljubljana, Slovenia) CYSTEINE PROTEINASES AND ENDOGENOUS INHIBITORS IN INNATE IMMUNE RESPONSE
10:15	SP24	**Mária Szűcs** (Szeged, Hungary) DISTINCT MOLECULAR CHANGES ACCOMPANYING OPIOID TOLERANCE IN RAT BRAIN SUBCELLULAR FRACTIONS

10:30 **Poster Session II**, exhibition and refreshements

Molecular Basis of Disease and Therapy *Main Hall*

Chairs: Damjana Rozman and Oliver Vugrek

11:30	L23	**Oliver Vugrek** (Zagreb, Croatia) NEW INSIGHTS IN INTRACELLULAR DYNAMICS OF S-ADENOSYLHOMOCYSTEINE HYDROLASE, A KEY ENZYME FOR METHIONINE METABOLISM
11:55	L24	**Damjana Rozman** (Ljubljana, Slovenia) THE ROLE OF CYP51 AND CHOLESTEROL SYNTHESIS IN PRETERM DELIVERY AND LIVER MALFORMATIONS
12:20	L25	**Balázs Sümegi** (Pecs, Hungary) REGULATION OF PI-3-KINASE-AKT PATHWAY AND MAP KINASES BY PARP-1 IS MEDIATED BY ATM KINASE AND MAP KINASE PHOSPHATASE-1 (MKP-1) IN OXIDATIVE STRESS
12:45	SP25	**Rok Keber** (Ljubljana, Slovenia) DEVELOPMENT OF A TRANSGENIC MOUSE MODELS TO DETERMINE THE ROLE OF CYP51 IN SPERMATOGENESIS AND EMBRYO DEVELOPMENT
13:00	SP26	**Zsófia Simon-Vecsei** (Debrecen, Hungary) MUTATION OF THE COMPOSITE MAIN CELIAC EPITOPE OF TRANSGLUTAMINASE 2 DIRECTLY AFFECTS ANTIBODY BINDING

13:15	SP27	**Sandra Šupraha Goreta** (Zagreb, Croatia) HEPATITIS B VIRUS: MOLECULAR GENOTYPES AND S GENE MUTANTS AMONG END STAGE CHRONIC HEPATITIS B PATIENTS

Plant Biochemistry *Camelia Hall*

Chairs: Éva Kondorosi and Maja Ravnikar

11:30	L26	**Éva Kondorosi** (Szeged, Hungary) EVOLUTION OF PLANT INNATE IMMUNITY EFFECTORS FOR SYMBIOSIS
11:55	L27	**Maja Ravnikar** (Ljubljana, Slovenia) PLANT VIRUSES: INTERACTION WITH PLANTS, DIVERSITY AND DIAGNOSTICS
12:20	SP28	**Hrvoje Lepeduš** (Osijek, Croatia) CHLOROPHYLLS CONTENT AND PHOTOCHEMISTRY OF THYLAKOID MEMBRANES IN TWO PEA CULTIVARS WITH DIFFERENT LEAF COLOURATION
12:35	SP29	**Dániel Silhavy** (Gödöllő, Hungary) HOW PLANTS KEEP THE BALANCE AMONG THREE TARNSLATION TERMINATION COUPLED EVENTS, TRANSLATION TERMINATION, READTHROUGH AND NONSENSE-MEDIATED mRNA DECAY?
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14:00	EXCURSION AND CONGRESS DINNER	

Saturday, June 16, 2012

Systems Biology and Bioinformatics *Main Hall*

Chairs: György Pósfai and Kristian Vlahoviček

09:00	L28	**Kristian Vlahoviček** (Zagreb, Croatia) ENVIRONMENTAL SHAPING OF CODON USAGE, TRANSLATIONAL OPTIMISATION, FUNCTIONAL ADAPTATION AND HORIZONTAL GENE TRANSFER ACROSS MICROBIAL COMMUNITIES
09:25	L29	**Peter Csermely** (Budapest, Hungary) KEY ROLE OF COMMUNITY-BRIDGING NODES IN NETWORKS
09:50	L30	**György Pósfai** (Szeged, Hungary) STREAMLINED-GENOME, LOW-MUTATION-RATE *ESCHERICHIA COLI*: IMPROVED CHASSIS FOR MOLECULAR/SYNTHETIC BIOLOGY
10:15	SP31	**Zoltán Gáspári** (Budapest, Hungary) STRUCTURAL PREFERENCES OF RANDOM *DE NOVO* PROTEINS
10:30	SP32	**Tamás Korcsmáros** (Budapest, Hungary) OVERLAPS IN SIGNALING NETWORKS – SYSTEMS-LEVEL IDENTIFICATION OF CROSS-TALKS AND CRITICAL NODES IN SIGNALING PATHWAYS
10:45	SP33	**Előd Méhes** (Budapest, Hungary) COLLECTIVE MOTION OF CELLS MEDIATES SEGREGATION AND PATTERN FORMATION IN CO-CULTURES

Metabolism *Camelia Hall*

Chairs: László Virág and Balázs Sümegi

09:00	L31	**László Virág** (Debrecen, Hungary) POLY(ADP-RIBOSE): A SIGNALING MOLECULE REGULATING "LIFE AND DEATH" IN DIFFERENTIATION
09:25	SP34	**Anitta K. Sárvári** (Debrecen, Hungary) ADIPOCYTE CELL DEATH AND CLEARANCE
09:40	SP35	**Judit Tóth** (Budapest, Hungary) THE Mg ION REGULATES NUCLEOTIDE HYDROLYSIS IN A NOVEL WAY IN dUTPase
09:55	SP36	**Magdolna Szántó** (Debrecen, Hungary) INTERACTIONS BETWEEN SIRT1 AND POLY(ADP-RIBOSE) POLYMERASES – NOVEL MODES OF METABOLIC REGULATION?
10:10	SP37	**Andras Horvath** (Budapest, Hungary) dUTPase IS ESSENTIAL FOR GENOME STABILITY AND IMAGINAL TISSUE DEVELOPMENT IN DROSOPHILA
10:25	SP38	**Katalin Tóth** (Budapest, Hungary) THE ROLE OF CYP3A ENZYMES IN CLONAZEPAM METABOLISM

11:00 **Presentation of the Bio-Science Award 2012** *Main Hall*

Chairs: László Fésüs and Beáta Vértessy

	AP1	**Roland Csépányi-Kömi** (Budapest, Hungary) ARHGAP25, A NOVEL RAC GTPASE-ACTIVATING PROTEIN, REGULATES PHAGOCYTOSIS IN HUMAN NEUTROPHILIC GRANULOCYTES

11:20 **Poster Session III**, exhibition and refreshement

12:20 Lunch (on your own)

Genomics *Main Hall*

Chairs: László Nagy and Simon Horvat

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13:55	L33	**Simon Horvat** (Ljubljana, Slovenia) IDENTIFICATION OF POSITIONAL CANDIDATES FOR OBESITY LOCI ON MOUSE CHR15 USING GENOMIC AND BIOINFORMATIC APPROACHES
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PIII-144 PROHEPCIDIN BINDS TO THE HAMP PROMOTER AND AUTOREGULATES ITS OWN EXPRESSION
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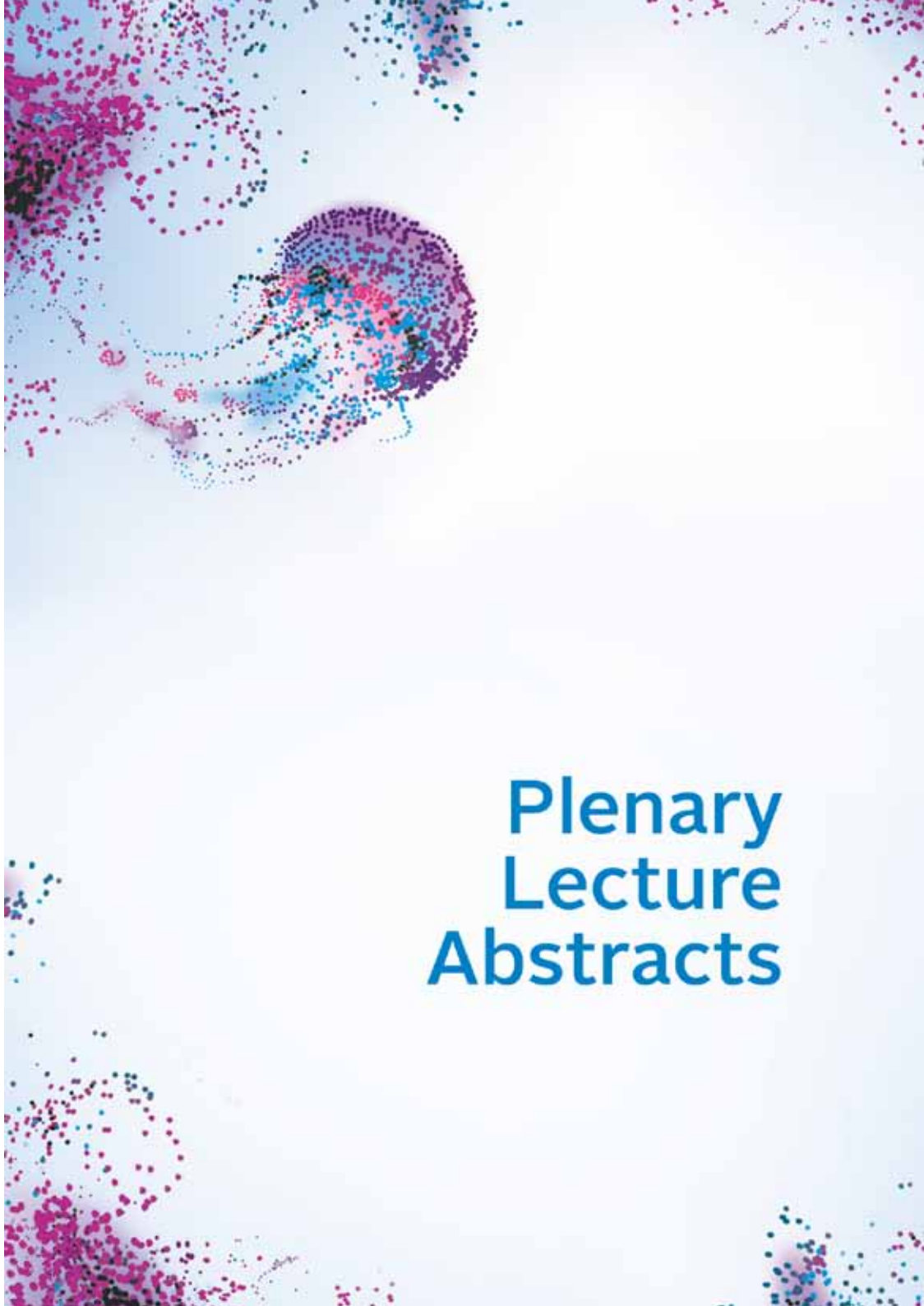

Plenary Lecture Abstracts

PL1

LIFE EXPECTANCY- WISHES, PREDICTIONS AND REALITY

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Ribosomes are the universal cellular machines that translate the genetic code into proteins. They posses spectacular architecture accompanied by inherent mobility that facilitate their smooth performance in decoding, peptide bond formation and nascent protein elongation. Owing to their fundamental role, ribosomes are targeted by many antibiotics, which paralyze the ribosomes by binding to their functional sites. The structural bases for the antibiotics binding modes, inhibitory action and synergism pathways were revealed by analyzing crystal structures of complexes of antibiotics with ribosomal particles. Issues concerning strategies for differentiation between ribosomes of patients and pathogens; mechanisms leading to bacterial resistance to antibiotics and the linkage between life expectancy and resistance to antibiotics will be discussed.

PL2

LIPIDS ORGANIZING CELL MEMBRANES

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The lipid raft concept introduces into membrane organization the capability of dynamic subcompartmentalization based on phase separation. Rafts form dynamic platforms with a key role in regulating membrane functions. They are dynamic assemblies of sphingolipids, cholesterol and proteins that dissociate and associate. These assemblies can be induced to coalesce to form raft clusters and these are the platforms that function in membrane trafficking, cell polarization and signalling. The most dramatic demonstration of phase separation in a cell membrane comes from our work on plasma membrane spheres produced by hypotonic swelling. We can induce large domains enriched in the gangliosides GM1 by pentavalent cholera toxin-crosslinking at 37°C. This domain formation is cholesterol-dependent and the GM1 phase is enriched in raft proteins and excludes non-raft proteins. Plasma membranes thus can phase separate like model membranes but in one key property they differ. In contrast to the phase–segregating plasma membrane spheres, the transmembrane raft proteins are excluded from the Lo phase in model membranes. The selective inclusion of transmembrane proteins in the raft phase suggests that this phase possesses a quality in addition to the lipid basis for Lo-Ld phase separation seen in model membranes. To study the protein–lipid interactions that govern partitioning of transmembrane proteins in and out of raft microdomains in membranes we have expressed and purified the EGF receptor. We have now evidence that the ganglioside GM3 in the raft phase in reconstituted proteoliposomes inhibits the tyrosine kinase activity of the receptor after EGF addition. Our working model is that transmembrane proteins become raftophilic by being lubricated by binding to raft lipids and that this capability is regulated by palmitoylation. Finally, I will describe our work demonstrating how rafts function in membrane trafficking to the cell surface, particularly how they contribute to apical membrane biogenesis as originally postulated.

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PL3 - FEBS National Lecture - Hungarian Biochemical Society

NON-CANONICAL MISMATCH REPAIR

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In this presentation, I shall discuss recent advances in our understanding of the molecular mechanisms of canonical mismatch repair (MMR), which improves replication fidelity by removing misincorporated nucleotides from the nascent DNA strand. I shall also discuss the role of MMR in other pathways of DNA metabolism, in particular somatic hypermutation (SHM). In this process, MMR and base excision repair contradict their roles as guardians of genomic integrity. Instead, they contribute towards antibody diversity through locus-specific mutagenesis, which has been postulated to require mismatch repair (MMR) proteins, monoubiquitylated PCNA and the error-prone DNA polymerase-η (pol-η). In this presentation, I shall describe non-canonical MMR (ncMMR), a hitherto uncharacterized process of DNA metabolism that is activated by a variety of lesions. ncMMR is largely independent of DNA replication, lacks strand directionality, triggers PCNA monoubiquitylation and promotes recruitment of pol-η to chromatin. Importantly, our findings show that ncMMR is limited neither to SHM, nor to B cells. In addition, we demonstrate a role for ncMMR in mutagenesis induced by alkylating damage. Thus, whereas MMR increases the fidelity of DNA replication by several orders of magnitude, activation of ncMMR by DNA damage may give rise to mutations and thus contribute to genetic diseases and cancer.

PL4

PARALLEL UNIVERSES IN CANCER

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Are we any closer to finding a cure for cancer? A cure for cancer appeared to be imminent with the early successes in treating childhood leukemia with chemotherapy. Since then, this elusive target has been moving farther away with each new technological advance that has enabled us to peer deeper into the essence of cancer. Our hope for a universal cure quickly diminished with the realization that drugs that work for leukemia are ineffective in lung cancer, colon cancer, or sarcoma. Despite many common themes in cancer, it became clear that substantial differences in cancers arising in different organs will mandate different treatment approaches. Even cancers in the same organ are vastly different and respond differently to therapy. This diversity among cancers became obvious with the recent pursuit to subdivide tumors based on their genetic alterations, which could ultimately serve as therapeutic targets. For example, breast cancers could be subdivided into four groups that have distinct biological characteristics, clinical prognoses, and responses to therapy. Recently, with the application of higher resolution techniques, this number has risen to ten distinct groups of breast cancers. And the number of subgroups will probably continue to rise until we realize that every tumor is different just as every patient is different. To effectively treat cancer, we may have to learn about each tumor individually. With the declining costs of molecular techniques, individualized therapy may be within reach. It is possible that in the near future, doctors will take a needle biopsy of a tumor, define the tumor's molecular composition, and use this information to devise a therapy that is most effective for the patient. A serious flaw in this scenario is the presumption that the tumor biopsy accurately represents the entire tumor. Recent analyses of multiple tumor biopsies from a single patient revealed that different regions of the same tumor contained distinct genetic mutations. Genetic signatures associated with both a good prognosis and a bad prognosis could be identified depending upon the area that was biopsied. The perplexing diversity of a tumor could also explain why many treatments eventually stop working. Chemotherapy often targets cells with specific mutations, which may only exist in specific parts of a tumor, allowing the remainder to expand. Analyzing more biopsies and probing deeper into the biology of cancer at the single cell level may enhance our ability to determine the best treatment options but it may also reveal additional genetic heterogeneity, to the point where an effective cancer treatment could consist of hundreds of drugs that would be more harmful to the patient than to the tumor itself. In addition to widespread genetic diversity, tumors are also a hotbed of non-genetic diversity, including differentiation status of tumor cells and metabolic derangements that allow tumors to thrive in the presence of hypoxia and nutrient deprivation. Altered tumor microenvironment, which is increasingly recognized as an active participant in tumorigenesis, adds another layer of complexity. This inherent diversity is compounded by the ability of the tumor to change over time both stochastically and in response to therapy. As a result, the last fifty years of intense scientific and clinical research has taught us that each tumor can be many diseases that are constantly evolving. Multidisciplinary approaches will be needed to translate this knowledge into cures for cancer. The FEBS3+ meeting may be one essential step towards this goal.

SL1 - FEBS Science and Society Lecture

WHAT IT TAKES TO SUCCEED IN SCIENCE - AND HOW EUROPE'S INSTITUTIONS COULD HELP

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We teach our students how to do science, but tell them little about what science is and how it shapes our view of us and the world A science degree does not automatically make one a scientist. Becoming a true scientist takes years of motivation and reflection as well as an understanding of what science demands from us.
I shall first discuss what science has given me, what I consider important about it, what I would do differently if I could start all over again, and what it takes to succeed in this unique profession. I shall then focus on several practical issues: the plight of Europe's young researchers; how to improve their career prospects; and how to ameliorate misguided governmental science policies that cripple Europe's innovative potential.

Gottfried Schatz (2005). *Jeff's View on Science and Scientists*. Elsevier, Amsterdam.
Gottfried Schatz (2011). *A Matter of Wonder*. Karger, Basel.
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SL2 - FEBS Science and Society Lecture
GENETICALLY MODIFIED PLANTS: ARE THEY USEFUL AND SAFE?

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Genetically modified organisms (GMOs) usually result either from the insertion of one (or several) gene(s) into their genome, or from the inactivation of one (or several) gene(s).
Applications of genetic engineering methods to medicine has allowed the production of proteins which have therapeutic importance, in large amounts, and devoid of contaminations by viruses or prions (for instance insulin, anti-haemophilic factors, growth hormone). Whereas these applications are well accepted, public acceptance of genetically modified plants (GM plants or transgenic plants), has sometimes been difficult, at least in some countries, especially when these plants are used as a source of food.
So far, most of the GM plants are either herbicide-resistant, so that they are not affected by the herbicide used by the farmers to kill the weeds, or resistant to plant pathogens (viruses, bacteria, fungi, nematodes, insects) which attack the plants and can cause important losses in crop yields. The introduction into a plant of a bacterial gene (from *Bacillus thuringiensis*) coding for a protein (Bt protein) which is toxic for some insects, but not for man or animals, is a very good alternative to the use of chemical substances (insecticides) which are often toxic for man and animals, and which can persist in the environment. GM plants resistant to abiotic stresses (cold, heat, drought, salt) can also be obtained by genetic engineering.
It has also been possible to obtain GM plants having improved nutritional qualities, such as a higher content in essential aminoacids, or a higher percentage of unsaturated fatty acids in their oil, or containing beta-carotene (a precursor of vitamin A) in the case of rice.
Molecular farming (or pharming) consists in the cultivation of transgenic plants producing substances of therapeutic importance (antigens, antibodies), or of industrial interest (biodegradable plastics, biofuels).
Although GM plants are cultivated on increasing areas (about 150 millions hectares, so far) and although millions of people have been eating GM plants, or products derived from GM plants, no adverse effect for human health has been documented. On the contrary, it has been possible, by gene silencing, to block the synthesis by the plant of an allergenic protein, thus resulting in the production of a non- (or less) allergenic plant (peanut, for instance).
Possible risks for the environment are sometimes mentioned, which would result from gene flow. For instance the pollen of a GM plant could pollinate the flowers of a non-GM plant of the same species grown in an adjacent field. Pollen dissemination depends on a number of factors and it can be prevented, or controlled, so that this risk has to be evaluated on a case by case basis, hence the importance of field trials, which paradoxically are sometimes destroyed by those asking for more experiments before GM plants are released.
In conclusion, gene transfer technologies have lead to important advances, not only in medicine, but also in agronomy (for the farmers and for the consumers). Possible risks should not be ignored, but it should be pointed out that all methods used for plant breeding can generate unanticipated and detrimental effects in plants. Research is therefore necessary and should be continued so that a science-based evaluation of benefits *vs* risks can be made : GM plants and plants obtained by conventional breeding should be rigorously tested and the results should be made available to the public, in order to allow rational discussions andpublic acceptance of the best solutions to the problems faced by sustainable agriculture.

Lecture Abstracts

L1

PROTEASE SIGNALING: A POINT FOR THERAPEUTIC INTERVENTION

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For a long time proteases were considered primarily as protein-degrading enzymes. However, this view has dramatically changed and proteases are now seen as extremely important signaling molecules, involved in numerous vital processes. Dysregulated protease activities can lead to pathologies, such as cancer, cardiovascular and inflammatory diseases, osteoporosis and neurological disorders. Therefore proteases represent an important class of drug targets. Some of the recent strategies in protease targeting will be discussed including identification and validation of proteases as drug targets, development of activity-based probes for imaging, and targeted drug delivery systems, which can be also applied to proteases. The major focus will be on cysteine proteases, in particular cysteine cathepsins, caspases and autophagins.

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L2
TOWARDS A MOLECULAR SWITCH

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Infectious diseases still present remarkable biomedicinal challenge due to the appearance of resistant strains of the respective causative agents. Hence, investigations into mechanisms of pathogenicity are of considerable current interest. In our previous related studies, we addressed the causative agent of tuberculosis, Mycobacterium tuberculosis, and performed multiple investigations focusing on Mycobacterium tuberculosis dUTPase, that has been suggested to be potentially essential for viability [1]. The uniquitous enzyme dUTPase is a key player in maintaining genomic integrity [2,3]. We found that in the model organism, M. smegmatis, a non-canonical segment of mycobacterial dUTPases constitutes a key element for essentiality[4].
Recently, a novel moonlighting function for yet another non-canonical dUTPase segment was suggested in Staphylococcus aureus [5]. In this study, the mechanism for derepression of gene expression from pathogenicity islands of S. aureus was investigated and a non-canonical dUTPase segment of phage phi11 dUTPase was proposed as the key element of a molecular interaction with the Staphyloccal StI repressor. Based on this information, we set out to explore around the suggested hypothesis and met unexpected results that may bear further significance, in systems separate from Staphylococcus aureus, as well. As such, we found that human and mycobacterial dUTPases may also induce similar effects.
Our results based on the crystal structures of phage phi11, human and mycobacterial dUTPases and transient and steady-state kinetics together with differential spectroscopic, limited proteolysis and quartz crystal microbalance investigations using different mutant constructs of dUTPases point towards a molecular switch model that may be exploited for molecular biology and may also be used to develop a cellular proteinaceous inhibitor of dUTPases.

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L3
MUTAGENESIS AND NEW OXIMES ENABLE REACTIVATION OF TABUN-INHIBITED ACETYLCHOLINESTERASE

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Acetylcholinesterase (AChE, EC 3.1.1.7), an important enzyme in cholinergic neurotransmission, is the primary target of organophosphorus compounds (OP) like pesticides and nerve agents such as tabun. A library of new oximes was screened for the reactivation activity of tabun-inhibited human recombinant AChE. Fifty-three out of 100 oximes reactivated wild type AChE, but only 14 of them restored full activity. Within this series, it appears that an approximate distance equivalent to 8 methylenes between two quaternary nitrogens achieved an optimal level of AChE reactivation. The mutant, Y337A, at the choline binding site was reactivated by more than 80% with only 13 of the oximes. The most efficient reactivators of Y337A appeared to be 2PAM analogs, with maximal reactivation rate constants k_{max} up to 10 times faster than those determined for the most efficient reactivator of AChE w.t. Although introducing an additional mutation into the Y337A choline binding site in double mutant Y337A/F338A reduced the enhancement observed in the Y337A mutant, the most efficient Y337A/F338A reactivators also contained the 8 methylene equivalence between two quaternary nitrogens as found for the wild type. It seems that, on average, the modification of the active site in the double mutant compromised molecular recognition reflected in the K_{ox} constant, but slightly improved the maximal reactivation rate constant k_{max}. Since all oximes were designed as reactivators of phosphorylated AChE, a limited reactivation capacity for related butyrylcholinesterase (BChE, EC 3.1.1.8) was expected. However, 37 oximes reactivated tabun-inhibited BChE more efficiently than 2PAM, and five reached maximal reactivation of 70 %. In addition, toxicity and antidotal studies with lead reactivators in mice showed significantly improved protective indexes in therapy upon tabun exposure compared to the standard antidote, 2PAM. Therefore, our findings offer a platform for further development of more potent congenic antidotes in tabun and related phosphoramidate exposure.

L4

ABC MEMBRANE TRANSPORTERS IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent (embryonic or induced pluripotent, iP) stem cells provide new possibilities to explore the development and differentiation of various cell types of the human body. We have analyzed the expression of several ABC transporter proteins, playing a key role in detoxification and protection against stress, in the pluripotent state as well as during human tissue differentiation. We found that the ABCG2 (MXR/BCRP) multidrug transporter protein was highly expressed in the pluripotent stem cells, although this expression showed a heterogeneous pattern. There was no difference between the expression of pluripotency markers in ABCG2 positive and negative hESCs, however, ABCG2 expressing cells had a higher growth rate following cell separation. Certain harmful conditions, including physical stress, chemical toxins and UV light exposure were tolerated much better in the presence of functional ABCG2 protein. This property can be explained by the transporter function which eliminates potential toxic metabolites accumulated during stress conditions. In contrast, mild oxidative stress in pluripotent stem cells caused a rapid internalization of ABCG2, indicating that certain environmental factors may induce the removal of this transporter from the plasma membrane. In the light of these results we suggest that a dynamic balance of ABCG2 expression at the population level has an advantage to promptly respond to changes in the cellular environment. Such an actively maintained heterogeneity might be evolutionarily favorable to protect special cell types, including pluripotent stem cells. During the early steps of *in vitro* stem cell differentiation (embryoid body, EB formation) the expression of the ABCG2 protein increased, while during further differentiation a highly variable, tissue-dependent expression pattern was observed. These studies may help to understand the role of xenobiotic transporters in tissue differentiation and promote the development of pharmacological agents specifically applied to affect human cell types.

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

L5

EFFECTS OF PERFORIN ON LIPID MEMBRANES

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MACPF protein family comprise a diverse set of proteins with important role in immunity, development and defence. One of the most important MACPF proteins is perforin, a pore forming protein from the immune system. It is stored in cytolytic granules and is released from cytotoxic cells of the immune system upon recognition of virus-infected or tumorigenic cells. Mutations in perforin gene were associated with a familial hemophagocytic lymphohistiocytosis, a disorder characterised by disregulated activation of T lymphocytes and macrophages. It also has a major role in graft-vs-host disease in allogenic stem cell and bone marrow transplantations. Perforin forms large holes in the membranes of virus-infected or cancer cells. Somehow it allows granzymes, proteases from cytolytic granules, to enter the cells and initiate the programmed cell death. In this way body gets rid of dangerous cells, without any damage to the nearby tissues and organs. It is not yet satisfactorily explained how perforin enables the entry of other molecules in cells and currently many models that describe this process exist. We studied perforin pore formation in membranes, which factors affect pore formation and we also determined the dimensions of its pores. Pores formed in planar lipid bilayer are extremely heterogeneous. Overall, we have identified three types of events: the formation of small and large pores and frequent membrane breakages. When studying pores in model lipid systems, such as large or giant unilamellar vesicles, we surprisingly also discovered that perforin is capable of remodelling the lipid membrane to induce invaginations and formation of secondary vesicles, which have engulfed the surrounding medium. These may be physiologically relevant for granzyme delivery in vivo. Perforin activity at lipid membranes may have implications for membrane interactions of other MACPF domain proteins.

L6

CYSTEINE CATHEPSINS: THE TARGETS FOR DIAGNOSTIC AND THERAPEUTIC INTERVENTIONS IN CANCER PATIENTS

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Lysosomal proteases, the cathepsins, are involved in various physiological processes, such as protein turnover, protein processing, modulation of immune response, etc. Their role in progression of cancer has been associated with the degradation of extracellular matrix, a proteolytic event affecting tumour cell migration, invasion, angiogenesis as well as dissemination of malignant cells from primary tumours and formation of metastasis. Degradation of extracellular matrix can be extracellular, intracellular or combination of both, and besides tumour cells, adjacent cells, such as macrophages, fibroblasts and lymphocytes could be a major source of harmful tumour associated proteolytic activity. Recent studies show that cysteine cathepsins specifically cleave several targets other than ECM and may trigger the processes in tumour apoptosis, anti-tumour immune response, and modulate tumour stem cells. The common belief is that cysteine protease inhibitors offer new therapeutic opportunities in cancer treatment and indeed, in several studies synthetic or natural inhibitors have been shown to impair tumour growth, angiogenesis and invasiveness or to reduce the metastasis formation. Cathepsins and their endogenous inhibitors can be used also as tumour markers. Their levels in tumour tissues or bodily fluids predict the risk for relapse or death in many cancer types. Additionally, they may predict the response to therapy or be used for primary diagnosis. The most promising results of prognostic and diagnostic application of cysteine cathepsins will be presented, as well as recent development of synthetic inhibitors, capable to impair tumour progression in vitro and in vivo.

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L7

REPLICATION OF DAMAGED DNA: INSIGHT INTO THE ENGINES OF MUTAGENESIS AND CARCINOGENESIS

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The genome is constantly under assault from chemical agents and radiations. This is especially deleterious in S-phase, when replication forks might stall upon encountering unrepaired DNA lesions leading potentially to mutagenesis and DNA rearrangements. The rescue of stalled replication forks is regulated by the Rad6-Rad18 ubiquitin conjugating complex dependent monoubiquitylation of PCNA, the processivity factor of replicative polymerase, which facilitates error-free or error-prone translesion synthesis providing direct nucleotide incorporation opposite DNA lesions. In addition, monoubiquitylated PCNA can be polyubiquitylated by Mms2-Ubc13 ubiquitin conjugating enzyme leading to template switching, in which copying from the undamaged newly synthesized sister strand can lead to error-free replication. After replication through the lesion, PCNA deubiquitylation can provide the restoration of the high fidelity replication machinery. Moreover, PCNA deubiquitylation can inhibit the untimely access of low-fidelity TLS polymerases and other players to the replication fork.
In the absence of PCNA ubiquitylation, recombination using the sister chromatid can provide an alternative means for fork rescue, which, however, could be disadvantageous for cells, since it creates a possibility for gross chromosomal rearrangements. The SUMO modification of yeast PCNA has been suggested to function as a guardian during replication of damaged DNA by preventing recombination and channelling to the use of Rad6-Rad18 dependent damage tolerance pathway.
We have been studying the regulatory role of ubiquitin- and SUMO-modification of PCNA on replication of damaged DNA in human cells. An overview of the research field and our recent findings will be presented.

L8

CYTOCHROMES P450 INVOLVED IN HOST–FUNGAL PATHOGEN INTERACTION ARE PROMISING TARGETS FOR NATURAL ANTIFUNGAL COMPOUNDS

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Highly conserved enzymes of fungal CYP53 family are involved in detoxification of benzoic acid, a key intermediate in metabolism of aromatic compounds in fungi. Their specificity for a narrow array of phenolic substrates and the absence of homologues in higher eukaryotes are advantageous in designing successful antifungal agents. A novel cytochrome P450, CYP53A15, was identified in the plant pathogen and opportunistic human pathogen, filamentous ascomycete *Cochliobolus lunatus*. *In vivo* and *in vitro* studies suggested that targeting CYP53A15 could help treat fungal infections. *In vitro* four phenolic compounds (isoeugenol, eugenol, vanillin, and thymol) that play a role in plant resistance to fungal infection inhibited CYP53A15. Inhibition of CYP53A15 led to increased intracellular levels of benzoic acid, which impedes fungal growth. We explored chemical properties of isoeugenol for ligand-based similarity searching, and the homology model of CYP53A15 of *Cochliobolus lunatus*, for structure-based virtual screening of a composite chemical library. Highest scoring compounds were analyzed in the spectral binding titration with CYP53A15, and assayed for antifungal activity against *C. lunatus*, *Aspergillus niger*, and *Pleurotus ostreatus*. Finally, eight compounds with antifungal potential were evaluated as inhibitors of CYP53A15 activity. Based on potent antifungal activity and good enzyme inhibition, one compound was selected for future optimization as a new lead structure, possibly suitable for pre-clinical antifungal drug development trials. In researching the mechanisms of pathogenicity, we treated *C. lunatus* with a synthetic blend of plant defense compounds and identified the fungal transcriptome. Through bioinformatic analyses, we identified pathogenicity-related genes, such as cytochromes P450, which are currently under functional analysis. Furthermore, the possibility of targeting fungal cytochromes P450 with natural plant defense compounds is studied to be applied against a bark beetle-associated fungus, a conifer pathogen.

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L9

MOLECULAR INSIGHT INTO AMINOGLYCOSIDE RESISTANCE BY RIBOSOMAL RNA METHYLTRANSFERASES

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Overwhelming problem of bacterial resistance to antibiotics is constantly increasing by emergence and spreading of both existent and novel resistance determinants. Mechanisms of resistance that until recently were observed exclusively among bacteria that produce aminoglycoside antibiotics are now being detected in a growing number of clinical strains. There are two families of enzymes that are responsible for the high level aminoglycoside resistance in pathogenic bacteria, Arm and Kam. Both types of enzymes introduce an additional methyl group onto a specific nucleotide within the aminoglycoside binding site in the 16S rRNA within the small ribosomal subunit, thus preventing the antibiotic binding. Arm enzymes methylate G1405 and the substrate of Kam enzymes is A1408, both of which are positioned in the decoding centre of the ribosome. The structure-function relationships of the methylation mechanism will be discussed in light of the recent results on the interference with the endogenous enzyme RsmF involved in ribosomal maturation and on the biological cost of this interplay. Obtained knowledge offers new directions in design of specific molecules that would serve as a defence against aminoglycoside resistant bacteria.

L10

REGULATION OF CELL MIGRATION BY THE S100A4/METASTASIN – NON-MUSCLE MYOSIN IIA INTERACTION

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S100A4/metastasin is a member of the S100 family of small EF-hand Ca^{2+}-binding proteins and directly involved in promoting tumor metastasis and likely in other non-malignant human diseases such as rheumatoid arthritis and psoriasis. In the extracellular space it exerts cytokine-like function, while inside the cell it regulates cellular motility by binding to non-muscle myosin IIA (NMIIA). The Ca^{2+}-dependent interaction of S100A4 with NMIIA prevents filament assembly and promotes filament disassembly. It is thought that inhibition of myosin activity at the leading edge of polarized cells decreases retrograde actin flow and maturation of focal adhesions, which increases actin polymerization-mediated protrusions and thereby cell migration. The increase in cytoskeletal dynamics upon elevated S100A4 expression could also indirectly fine-tune the specific communication pathways that regulate cell migration. Here, I will present the high-resolution structure of S100A4 in complex with a 45-residue-long myosin heavy chain fragment that comprises the C-terminal end of the coiled-coil tail overlapping with the so-called assembly competence domain (ACD) and part of the non-helical tailpiece. The crystal structure of the S100A4–NMIIA complex reveals a novel mode of interaction in the S100 family: A single, predominantly α-helical myosin chain is wrapped around the Ca^{2+}-bound S100A4 dimer occupying both hydrophobic binding pockets. Moreover, thermal denaturation and modeling experiments of coiled-coil forming NMIIA fragments indicate that the coiled-coil tail partially unwinds upon S100A4 binding. Based on these results, we propose a two-step model for NMIIA filament disassembly: An S100A4 dimer first catches the random coil tailpiece then the C-terminal residues of the coiled-coil are unzipped and rolled over the dimer partially disrupting the ACD and resulting in filament dissociation. The description of the complex will facilitate the design of specific molecules that could interfere with the S100A4–NMIIA interaction and could have therapeutic application in metastasis and/or other S100A4-related human pathologies.

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L11

DETERMINATION OF MURINE HEPATIC LIPID DROPLET COMPOSITION BY AN INTEGRATED HIGH RESOLUTION LIPIDOMICS PLATFORM

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Lipidomics is driven by rapid advances in analytical technologies such as mass spectrometry and by getting insights at the level of lipid molecular species in understanding lipid metabolism and its dysregulation. The plethora of different lipids in biological systems requires the highest possible mass spectrometric resolution and mass accuracy, information about specific fragments by MS/MS and chromatographic pre-separation. In this project we developed an analytical method using a LTQ-FT MS hyphenated with RP-UHPLC to characterize the lipidome of lipid droplets (LDs) isolated from hepatocytes of WT C57BL/6 mice subjected to normal diet, high fat diet and fasting conditions. In addition to the analytical challenge we have to cope with bioinformatic demands of handling the large amount of generated data. This required the development of an automated software tool in form of a stand-alone, platform-independent Java application called Lipid Data Analyzer (LDA). The main findings are that phospholipid and diacylglycerol lipid classes remain by and large untouched, whereas molecular triacylglycerol species are enriched in fasting and high fat diet conditions, with a distinctive shift towards long chain polyunsaturated fatty acids under fasting conditions. Finally we demonstrate that lipidomic analysis of hepatocyte LD enables phenotyping the organ's physiological state. The TG lipidome is best suited for such phenotyping, whereas structural analysis of TG, DG and PL molecular species furnishes metabolic insights.

L12

APPROACHING CLINICAL LIPIDOMICS: LIPID BIOMARKERS FOR ENDOLYSOSOMAL AND LIPID DROPLET STORAGE IN VASCULAR AND METABOLIC DISEASES

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Recent developments in lipidomics provide novel opportunities for clinical applications including identification of biomarkers, monitoring of diseases or adverse drug effects and environmental hazards. Lipid storage in diverse cells induces metabolic adaptation and transdifferentiation of the corresponding tissue/organ leading to foam cell formation and atherosclerosis, fatty liver, obesity and myopathy as hallmarks of energy overload diseases. Major cellular lipid storage occurs either in the endolysosomal compartment as phospholipidosis in response to metabolic overload (e.g. macrophage foam cell formation), drug toxicity (e.g. drug-induced phospholipidosis), genetic defects (e.g. Niemann-Pick type C /NPC/ disease), or in lipid droplets filled with accumulated acylglycerols and/or sterol esters.
During endolysosomal degradation of ingested lipids several lipid hydrolases interact with the surface of luminal multivesicular bodies (MVB), which are negatively charged due to the presence of the endolysosomal signature lipid bis(monoacylglycerol)phosphate (BMP). Inefficient MVB processing is associated with impaired docking of lipid hydrolases leading to their proteolytic degradation and endolysosomal phospholipidosis. MVB-bound BMP and the mitochondrial signature lipid cardiolipin (CL) are formed from their common precursors, the endoplasmic reticulum-associated phosphatidylglycerol and CDP-diacylglycerol. In order to characterize the pathways contributing to lipid trafficking among these compartments, control and NPC (i.e. defective endosomal cholesterol egress) cells (i.e. monocyte-derived macrophages and fibroblasts) were compared by transcriptomic and lipidomic analyses. Macrophages were challenged either by endolysosomal phospholipidosis induction (i.e. loading with oxidized low density lipoprotein /oxLDL/) or by lipid droplet induction (i.e. loading with enzymatically modified LDL /eLDL/).
The professional endolysosomal lipid degradation in macrophages is paralleled with an increased mitochondrial activity and enhanced CL levels. By contrast, fibroblasts have limited endolysosomal and mitochondrial capacities, and the NPC defect can not be compensated by alternative routes, leading to endosomal phospholipidosis with BMP accumulation and mitochondrial dysfunction.
The combination of lipid mass spectrometry with transcriptomic, epigenomic and proteomic analysis of cells and body fluids rapidly expands our current knowledge of diverse disease pathologies.

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L13

LIPIDOMICS OF STRESS RESPONSE

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Membrane lipids adapt rapidly in response to various environmental perturbations. Upon stress elicited by a wide range of stimuli, membranes as stress sensors give rise to compositional changes of membrane lipids, microdomain reorganisation and produce a unique set of lipid mediators participating in the activation of stress protein signalling pathways. Nowadays, mass spectrometry provides an ideal tool for lipidomic studies which allows simultaneous analysis of thousands of lipids and their alterations. Evaluation of the large amount of data can be assessed by unsupervised and supervised multivariate statistical approaches.
The present study aimed to establish a mechanism for the possible interconnection between specific changes in lipid composition, membrane perturbation and the concomitantly altered expression of heat shock proteins (HSPs). Untargeted lipidomics revealed that modulations in membrane fluidity achieved either by heat or a fluidizing agent resulted in highly specific alterations in membrane lipid composition. The accumulation of lipids with raft-forming properties under stress conditions may explain the condensation of ordered plasma membrane domains detected by fluorescence microscopy. The loss in polyenoic lipid molecular species with a parallel increase in saturated species was a consequence of the activation of phospholipases. Using targeted approaches, the PLA_2 and PLC – DAG lipase – MAG lipase pathways were identified in B16 cells, which contributed significantly to the production of several lipid mediators after stress.
Next, the effect of culture conditions on lipid homeostasis and its correlation with the stress response of B16 cells were examined. Importantly, variations in plating density may profoundly alter the PUFA status and microdomain organisation of cells, and may result in the disturbance of both membrane-associated sensing and signalling events. Our data emphasise the pivotal role of nutrient lipid supply in modifying responses to stress and highlight the need for the careful control of culture conditions when assessing cellular responses in vitro.
Furthermore, the relationship between stress response, HSP expression and lipid droplet (LD) biogenesis will be discussed. Fever-like heat stress promotes TG synthesis in mammalian cells and in yeast (S. pombe). On the base of the apparent interconnection between the abundance of HSPs and LDs we suppose that LDs may have some unexpected functions in stress management.

L14

INFLAMMASOME ACTIVATION BY DYING AUTOPHAGIC CELLS

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Pathogen-activated and damage-associated molecular patterns activate the inflammasome in macrophages. We have previously found that that during engulfment by human macrophages of MCF-7 or 293T cells undergoing autophagic death caspase-1 was activated and IL-1β was processed, then secreted in an MyD88-independent manner (Autophagy 7,1-10, 2011). In our current experiments it was observed that mouse macrophages release IL-1β while co-incubated with pro-B (Ba/F3) cells dying by apoptosis with autophagy as a result of IL-3 withdrawal, but not with living, apoptotic, necrotic or necrostatin treated cells. NALP3-deficient macrophages display reduced IL-1β secretion, which is also inhibited in macrophages deficient in caspase-1 or pre-treated with its inhibitor. We show that activation of NALP3 depends on phagocytosis of dying cells, ATP release through pannexin-1 channels of dying autophagic cells, P_2X7 purinergic receptor activation, and on consequent potassium efflux. Injection of dying autophagic Ba/F3 cells intra-peritoneally in mice recruits neutrophils and thereby induces acute inflammation. These findings demonstrate that NALP3 performs key upstream functions in inflammasome activation in mouse macrophages engulfing dying autophagic cells, which can lead to pro-inflammatory responses.

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L15

MOLECULAR MECHANISM TLR4 AND MYD88-MEDIATED SIGNALING AND INHIBITION

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Innate immune response plays an essential role for the defence of all multicellular organisms. TLR4 is in many ways an exception among the Toll-like receptors as it is the only one that signals through the two signalling pathways. TLR4 has a role in many chronic and infectious diseases. Cellular signalling of most TLRs is mediated by an adapter protein MyD88 through TIR domain interactions. Monomeric TIR domains inhibit activation of TLR signalling, which is exploited by pathogens to suppress the innate immune response. Whereas the structure of the Death-domain inflammasome comprising death domains of MyD88-IRAK4 and IRAK-2 has been determined, the mechanism of TIR domain mediated activation is not understood.
We discovered the important role of TLR4 in sensing the oxidative stress, which is particularly relevant in chronic inflammation. Partially oxidized phospholipids in microvesicles from patients with rheumatoid arthritis mediate activation of TLR4 signalling pathway. Activation of TLR4 by MVs mimics the molecular mechanism of activation by LPS, demonstrated by the effects of MD-2, mutations, inhibitors and receptor complex dimerization. We reconstituted the biologically active MVs from synthetic phospholipids by partial oxidation. Signal from pathogens (LPS) and endogenous danger signal (MVs) induced significantly different expression profile response in mouse BMDMs with strong inflammation resolving component induced by the endogenous signal. In order to investigate the molecular mechanism of TLR activation mediated by TIR-domain interactions we prepared a tethered TIR dimer. Dimeric TIR domain platform has a unifying role both for the immunosupression by bacterial virulence factors TCPs (TIR domain-containing proteins) and for the proinflammatory signalling in cancer. Coiled-coil dimerization segment present in many bacterial TCPs such as the TcpB from Brucella is required for the potent suppression of TLR/IL1R innate immunity signalling. The addition of an artificial coiled-coil dimerization segment conferred superior inhibition of broad spectrum of TLRs and prevents the constitutive activation by a dimeric TIR platform. Based on our results we propose a molecular model of activation of TLR signalling based on the dimeric TIR-domain platform as the rate-limiting step of activation.

L16

A NOVEL, UBIQUITIN BINDING PROTEIN - DVC1 IS IMPLICATED IN DNA REPAIR

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DVC1 (**D**NA damage-associated **V**CP/p97 **C**o-factor**1**) is a novel ubiquitin biding protein containing SHP, MPD, MIU and UBZ domains as well as PIP box. Its interacting partners include several DNA damage response proteins like p97/VCP, PCNA and p53BP1. DVC1 is responsible for VCP/p97 recruitment to the sites of double-stranded DNA brakes and for maintenance of nuclear membrane integrity. Interaction with VCP/p97 protein is established via DVC1 SHP domain. According to its structure and its role in DNA repair it is reasonable to expect involvement of DVC1 in human cancer.

L17

SHORT-TERM GLUCOSE DEPENDENT REGULATION OF THE SODIUM-D-GLUCOSE COTRANSPORTER SGLT1 IN SMALL INTESTINE

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Absorption of D-glucose in small intestine is mainly mediated by glucose uptake into enterocytes across the brush-border membrane (BBM) via Na^+-D-glucose cotransporter SGLT1 and by basolateral glucose efflux via the passive transporter GLUT2. During a glucose-rich meal the absorptive capacity of small intestine is increased. After gavage of mice with glucose, the maximal velocity (Vmax) of Sglt1 mediated glucose uptake into BBM vesicles and the amount of Sglt1 protein in the BBM were increased 2.5-4fold (*1*). The Vmax of Glut2 mediated glucose uptake into BBM vesicles and the amount of Glut 2 protein in the BBM were increased 2fold (*1*). The capacity of glucose uptake across the BBM via Sglt1 was 8.7 times higher compared to the capacity of glucose uptake via Glut2. The glucose dependent recruitment of Sglt1 to the BBM occured within 2 min.

The intracellular regulatory protein RS1 (gene *RSC1A1*) is involved in posttranscriptional regulation of SGLT1 in small intestine (*2*). RS1 is colocated with SGLT1 at the *trans*-Golgi network (TGN) (*3*). We studied the function of RS1 in oocytes of *Xenopus laevis.* After expression of SGLT1 we injected fragments of RS1 protein without and together with an inhibitor of the TGN and/or glucose. One hour later we measured SGLT1 mediated glucose uptake. The experiments showed that RS1 inhibits the release of SGLT1 containing vesicles from the TGN provided the intracellular concentration of D-glucose is below 100 µM. In the presence of an intracellular glucose concentration >200 µM the inhibitory effects of the RS1 fragments were blunted (*4*).

In mice in which Rs1 was removed (*5*) (*Rs1-/-* mice) the concentration of Sglt1 protein in the BBM of jejunum and Sglt1 mediated glucose uptake into BBM vesicles was increased 3-10fold. After gavage of *Rs1-/-* mice with glucose, the Vmax of Sglt1 mediated glucose uptake into isolated BBM vesicles and the amount of Sglt1 protein in the BBM was not increased further. The data suggest that RS1 is critically involved in the glucose dependent upregulation of small intestinal glucose absorption after a glucose rich meal.

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L18

THERAPEUTIC CONSEQUENCES OF HIGH GENETIC VARIABILITY IN THE HUMAN ORGANIC CATION TRANSPORTER OCT1

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The human organic cation transporter OCT1 is highly genetically polymorphic. Nine percent of Europeans have substantially reduced or completely lack OCT1 activity due to loss-of-function polymorphisms in the OCT1 gene. World-wide this percentage varies from more than 90% (South America) to less than 1% (Asia).

OCT1 is strongly expressed in the liver sinusoidal membrane. Therefore, variations in OCT1 activity may affect the hepatocellular uptake of cationic and weak basic drugs in the liver. This may result in limited metabolism and variations in the pharmacokinetics and activity of these drugs.

Using cell line models, we showed that OCT1 strongly mediated the uptake of weak basic drugs with low membrane permeability: tropisetron, morphine, debrisoquine and O-desmethyltramadol (the active metabolite of tramadol). The increased cellular uptake by OCT1 may accelerate hepatic drug metabolism, as demonstrated for the CYP2D6 model drug debrisoquine. Furthermore, the cellular uptake was abolished or substantially decreased if any of the common loss-of-function amino acid polymorphisms Arg61Cys, Cys88Arg, Gly401Ser, Gly465Arg, or Met420 deletion were present in the OCT1 gene.

In humans, the presence of these lost-of-function OCT1 polymorphisms was associated with increased plasma concentrations of tropisetron, ondansetron, morphine and O-desmethyltramadol. The higher plasma concentration resulted in increased efficacy of tropisetron, ondansetron and O-desmethyltramadol.

In contrast, OCT1 had no effect on the cellular uptake of drugs with high membrane permeability like tramadol and codeine. Also, the plasma concentrations of tramadol and codeine were not affected by the presence of OCT1 polymorphisms.

In conclusion, common genetic polymorphisms in OCT1 affect the hepatocellular uptake, and thus modulate the plasma concentration and efficacy, of some cationic and weak basic drugs. Natural membrane permeability of the drug is important determinant of its dependence on OCT1 for hepatocellular uptake.

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L19

SEX AND SPECIES DIFFERENCES IN RENAL TRANSPORTERS OF ORGANIC COMPOUNDS

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In the mammalian kidneys, transport of various organic compounds (organic anions and cations, glucose) and water is mediated by specific proteins localized in the luminal and/or contraluminal cell membrane domains along the nephron, largely in proximal tubules. These transporters contribute to reabsorption and/or secretion of endogenous and xenobiotic organic compounds, including various anionic and cationic drugs that are used in human and veterinary medicine. Recent studies have shown that some of these transporters ("drug transporters") contribute to development of drug resistance, drug-drug interactions, and drug-induced nephrotoxicity, whereas their malfunction due to truncated forms of proteins or point mutations in their genes can cause genetic diseases. In rodents (rats, mice, rabbits), many renal transporters exhibit sex differences in their protein and/or mRNA expression, whereas in pigs and humans, some transporters are absent, some exhibit localization in the cell membrane domains different from that in rodents, but the sex-related expression of thus far tested transporters could not been confirmed. For some transporters common to rodent, pig and human kidneys, species differences were observed in selectivity for substrates, distribution along the nephron, expression of mRNA and/or protein, sensitivity to inhibitors, and regulation of the activity. In addition, recent findings in this field in rodents revealed the species-related discrepancies in the expression of some transporters at the level of protein and their mRNA, e.g., sex differences in the transporter protein can exist with or without equivalent differences in the expression of its mRNA. Moreover, these two parameters can be in an opposite relationship. We, therefore, conclude the following: a) the data on renal transporters in one sex and species can not simply be regarded as relevant for other sex and species, b) posttranscriptional regulation may represent crucial mechanism in controlling the sex- and species-related protein expression of the renal transporters, c) various physiological, pharmacological, and toxicological findings related to the transporter-mediated handling of organic substances and water in the rodent kidneys do not reflect the situation in the pig and human kidneys, and d) the kidneys in pigs, not in rodents, may represent much better model for studying the human-related expression and functions of various renal transporters.

L20

THE HEDGEHOG-GLI SIGNALING IN TUMORS AND IMPLICATIONS FOR THERAPY

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The Hedgehog-Gli (Hh-Gli) signaling pathway is a developmental pathway, which is often found aberrantly active in various tumors. The pathway is a highly coordinated and orchestrated network involving binding of the ligand Hedgehog (Hh) to its receptor, a twelve transmembrane protein, Patched (Ptch). This causes Ptch to release its repression over the coreceptor Smoothened (Smo), a seven transmembrane protein and triggers a cascade of events in the cytoplasm leading to activation of the transcription factor Gli and transcription of target genes. The Gli proteins are regulated by the Suppressor of Fused (SuFu), Protein Kinase A (PKA), Glycogen Synthase Kinase 3β (GSK3β) and Casein Kinase 1 (CK1).

The involvement of the Hh-Gli signaling pathway in a variety of human cancers is still not completely elucidated but, preclinical *in vitro* and *in vivo* data demonstrate a role of this pathway in cancer pathogenesis, self-renewal and chemotherapy resistance. To date several Smo inhibitors have been proposed as potential candidates for cancer therapy either as a single agent or in combination regimens with conventional chemotherapy.

Our results on several different human cancers show different levels of pathway alterations. We found that the increased level of GSK3β is essential for survival of colon cancer cells and this alters Gli3 processing, keeping the Hh-Gli signaling pathway active. After GSK3β inhibition, Gli3 is processed into its repressor form, the pathway is downregulated and the proliferation of colon cancer cells is decreased. This suggests a major role for the interplay of GSK3β and Gli3 in the regulation of this pathway in colon cancer. On the other hand, in ovarian tumor pathogenesis we found a difference in *SHH* gene expression between borderline tumors and carcinoma, with significantly higher expression in borderline tumors compared to carcinoma. However, the upregulation of Hh-Gli signaling in almost all tested samples suggests that this is an early event in ovarian tumorigenesis regardless of tumor type.

Our data support the role of the Hh-Gli signaling pathway in cancer and emphasize the need for a better understanding of the modes of Hh-Gli pathway regulation in different tumors, its role in tumor response to traditional therapy, as well as the interactions with other signaling pathways in order to develop better therapies based on combinations with inhibitors of the Hh-Gli signaling pathway.

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INSIGHTS INTO PATHOPHYSIOLOGY OF HUMAN SECRETED PLA_2s THROUGH THEIR TOXIC COUNTERPARTS FROM SNAKE VENOMS

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Secreted phospholipases A_2 ($sPLA_2$s) constitute, physiologically and pathologically, a very important family of enzymes. Abundantly present in some snake venoms these enzymes can be neurotoxic, myotoxic, anticoagulant, procoagulant, cardiotoxic or cytotoxic. Orthologues of the venom $sPLA_2$s have been discovered in other animals including humans. In mammals, eleven $sPLA_2$ paralogues have been described so far. Similarly as the snake venom $sPLA_2$s, also human $sPLA_2$s are multifunctional proteins being implicated in multiple physiological and pathological settings for example innate immunity, neurotransmitter release, neuritogenesis, angiogenesis, embryogenesis, pain perception, apoptosis, ARDS, endotoxic shock, pancreatitis, rheumatoid arthritis, Alzheimer's disease, proliferation and, related to the latter, also in different forms of cancer. Recent insights into the molecular basis of action of presynaptically neurotoxic (β-neurotoxic) $sPLA_2$s will be presented and discussed. Mechanistic descriptions of the multitude of actions of $sPLA_2$s is today one of the most exciting and promising research areas. Based on discoveries with the β-neurotoxic snake venom $sPLA_2$s explanations of some activities of mammalian $sPLA_2$s have been suggested. It appears to be considered as the interplay of the receptor-binding and the enzyme function of these proteins.

L22

THE SCAFFOLD PROTEIN TKS4 REGULATES EGF-DEPENDENT CELL MIGRATION

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Tks4 belongs to a family of scaffolding proteins recently shown to be involved in podosome formation and cell invasion. Mutations in the SH3PXD2B gene coding for the Tks4 protein are responsible for the autosomal-recessive Frank-ter Haar syndrome. Recently, we have shown a novel role for Tks4 in the EGF signaling pathway. It has been found that upon EGF treatment Tks4 translocates to the cell membrane where it associates with the activated EGF receptor and becomes tyrosine phosphorylated. We have identified the Src kinase to be responsible for this phosphorylation. In addition, association between the EGFR and Tks4 is not direct and requires the presence of Src. Treatment of cells with LY294002, an inhibitor of PI 3-kinase, or mutations of the PX domain reduces tyrosine phosphorylation and membrane translocation of Tks4. Furthermore, a PX domain mutant (R43W) Tks4 carrying a reported point mutation in a Frank-ter Haar syndrome patient shows aberrant intracellular expression and reduced phosphoinositide binding. Finally, silencing of Tks4 has been shown to markedly inhibit HeLa cell migration in a Boyden chamber assay in response to EGF or serum. Our results therefore reveal a new function for Tks4 in the regulation of growth factor-dependent cell migration.

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L23

NEW INSIGHTS IN INTRACELLULAR DYNAMICS OF S-ADENOSYLHOMOCYSTEINE HYDROLASE, A KEY ENZYME FOR METHIONINE METABOLISM

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S-adenosylhomocysteine hydrolase (AHCY) deficiency is a rare metabolic disorder of the methione pathway that was discovered recently in Croatia. AHCY deficiency is considered a methylation disorder and therefore provides an opportunity for studying the underlying mechanism in disease development and pathology, and serves as a model system in methylome research.

Predominantly, AHCY is a cytoplasmic enzyme, but some portion of the protein is located to the nucleus. Indeed, it is proposed that the efficiency of transmethylation might profit from a close proximity between methyltransferases and AHCY due to its particular function of rapid removal of S-adenosyl homocysteine (SAH), the by-product of transmethylation reactions. Rapid removal of SAH is crucial to avoid product inhibition of methyltransferases because SAH is one of the most potent methyltransferase inhibitors. In an effort to focus on the functional implications of mutations in the AHCY gene found in a number of patients, and on the intracellular localization and function of AHCY, here we present some new insights in intracellular dynamics of S-Adenosylhomocysteine hydrolase. We present data using live cell imaging, fluorescence recovery after photobleaching (FRAP), mobility dynamics of AHCY in different cellular compartments, and mutational studies targeting nuclear import (NLS) and export (NES) domains of AHCY.

L24

THE ROLE OF *CYP51* AND CHOLESTEROL SYNTHESIS IN PRETERM DELIVERY AND LIVER MALFORMATIONS

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Cholesterol synthesis is essential in embryonal development as evidenced from the mouse knockout models. Earlier the gene appears in the pathway, earlier the phenotype occurs. In humans, two genes from cholesterol synthesis (*Hmgcr, Dhcr7)* associate with preterm delivery and lower birth weight. Lanosterol 14α-demethylase belongs to the late portion of cholesterol synthesis. It encodes a cytochrome P450 enzyme CYP51 that is evolutionarily the most conserved in the CYP superfamily. The gene spans 22 kb on human chromosome 7 and was so far not linked to malformations in humans. We performed initial *CYP51* genotyping on population of 188 Caucasian women who had a spontaneous preterm delivery and 188 unrelated preterm infants born at <37 weeks. Within ten amplicons covering exons, untranslated regions (UTR) and intron-exon borders we identified 22 *CYP51* polymorphisms, where 11 are rare novel variants. An T/G transversion in exon 3 causes potentially damaging Y145D substitution in the CYP51 substrate recognition site. Sequencing this amplicon in further 1000 premature infants shows low frequency, suggesting little contribution to preterm delivery. TaqMan genotyping of common variants in larger population is in progress, together with further sequencing of the 5' and 3'-UTRs. Our data indicate that *Cyp51* and normal cholesterol synthesis are crucial also for normal liver development. The liver *Cyp51* conditional knockout mice show severe liver malformations, from hepatomegaly to cholagiopathy (4% of the progeny, almost exclusively males), accompanied by cases of hepatocyte mitosis and apoptosis. These sex-dependent changes aggravate by aging while the blood lipid profile reflects disrupted lipid homeostasis. Albeit the relation between human *CYP51* and liver diseases has yet to be established, the discovered rare novel variants are promising candidates for further genotyping of patient groups.

L25

REGULATION OF PI-3-KINASE-AKT PATHWAY AND MAP KINASES BY PARP-1 IS MEDIATED BY ATM KINASE AND MAP KINASE PHOSPHATASE-1 (MKP-1) IN OXIDATIVE STRESS

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It has been suggested that the release of nuclearly formed ADP-ribose polymers, or ADP-ribosylated-proteins, could be responsible for the cytosolic and mitochondrial effects of poly(ADP-ribose) polymerase-1 (PARP-1) activation in oxidative stress. We provide a novel mechanism for the regulation of MAP kinases and PI-3-kinase-Akt pathway. Reactive oxygen species (ROS)-activated PARP-1 regulates the activation of JNK and p38 mitogen-activated protein kinases (MAPKs) because inhibition of PARP-1 by pharmacons, or suppression by siRNA resulted in the inactivation of MAPKs. This regulation was achieved by increased expression and enlarged cytoplasmic localization of MAPK phosphatase-1 (MKP-1) upon PARP-1 inhibition which reflected in the phosphorylation states of JNK and p38. Silencing of MKP-1 attenuated the protective effect of PARP inhibition. We observed that silencing ATM kinase prevented the PARP-1 inhibition induced activation of Akt which indicate a role of ATM in PARP-1 inhibition induced Akt activation. We showed that inhibition of PARP-1 increased cytoplasmic ATM level, and facilitated the interaction between ATM and NEMO which is required for the nuclear export of ATM. NEMO-ATM complex in the cytoplasm bind to Akt1 and this complex can provide the microenvironment where Akt1 can be activated.

We provided a novel mechanism of PARP-1 inhibition induced suppression of MAP kinase activation based on PARP inhibition induced expression of MKP-1. PARP-1 inhibition induced Akt activation is regulated by shifting ATM localization, where PARP inhibition facilitates the interaction between ATM and NEMO which activates the nuclear export of ATM and the formation of ATM-NEMO-Akt1 complex in cytoplasm facilitating Akt1 activation. These data show novel mechanisms by which PARP-1 activation/ inhibition can regulate expression of regulatory proteins, and can shift the Crm1 independent nuclear export of ATM to regulate Akt1.

L26
EVOLUTION OF PLANT INNATE IMMUNITY EFFECTORS FOR SYMBIOSIS

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Symbiosis between legume plants and *Rhizobium* soil bacteria results in the formation of nitrogen fixing root nodules. This relationship is mutually beneficial as bacteria support plant growth by reduction of the atmospheric nitrogen to ammonia inside the nodule cells while the plant supplies the bacteria with photosynthetic products and the energy for nitrogen fixation. Nodule development is induced by bacterial lipochitooligosaccaride signal molecules, the Nod factors. Nodule maturation and functioning require, however, further and still uncovered signaling events between the partners. Symbiotic cell functioning in *Medicago truncatula* and closely related species (belonging to the IRL Clade), necessitates a remarkable, plant controlled differentiation process of the bacteria resulting in living, non-dividing nitrogen fixing bacteria called bacteroids that are polyploid, largely elongated and branched with definitive loss of cell division capacity. Thus, in these cases, the bacteroids cannot return to the free living state and the plant has more advantage in the symbiosis than the bacteria.
We have identified a wide spectrum of nodules specific peptides in *M. truncatula* resembling antimicrobial peptides of the innate immunity that govern this irreversible terminal differentiation of bacteroids [1]. The majority of these peptides (>600) belong to the NCR family. *NCR*s are only expressed in the symbiotic cells but at different stages of bacteroid development. The NCR peptides are targeted to the bacteroids via the secretory pathway of the nodule cells and are essential for bacteroid differentiation [2]. Direct action of NCRs on bacteria, inducing features of terminal bacteroid differentiation, has been demonstrated both *in planta* and *in vitro*. Our present studies are focused on identification of plant peptides present in the bacteroids, their bacterial targets and mode of action and how their concerted actions control bacterial cell number and cell fate in symbiosis.
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L27
PLANT VIRUSES: INTERACTION WITH PLANTS, DIVERSITY AND DIAGNOSTICS

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Plant viruses are the cause of the most devastating diseases since there is no cure available for infected plants. Therefore it is crucial to understand the key steps on the disease development and the mechanisms of resistance. New functional genomics approaches help us to study such processes in the potato – Potato virus Y (PVY) interaction. In addition, precise diagnostic techniques are needed to follow the virus in the studied plants. Until now, numerous serological and molecular methods were developed for the detection of Potato virus Y isolates. For the particular case of serological methods, in order to facilitate the selection of the best anti-PVY antibodies, a Surface Plasmon Resonance (SPR) approach was developed. The method might be used in the future to find new host molecules that interact with the virus.
PVY is the most economically relevant virus infecting potato. Data on the movement and distribution of PVY within the potato plants are very scarce, but essential for exploring the plant-virus interaction. Therefore a complex approach to monitor simultaneously the localization of PVY RNA and PVY viral particles in the potato plant was developed. RT-qPCR detection system enabled us to estimate the amounts of PVY RNA in the different tissues of systemically infected plants. The viral RNA amounts were in concordance with the relative viral particle concentrations estimated by negative staining transmission electron microscopy (TEM). Additionally, ultra thin sections of resin embedded potato tissues were investigated by TEM for the sub cellular localization of PVY proteins. For better insight into the viral RNA accumulation, an in-situ hybridization method for the detection of PVY RNA in potato tissue was developed.
In all previous omics experiments, the disease development and resistance mechanisms were studied using the whole tissue like, i.e., whole leaves. In order to determine infected and virus-free regions within each tissue, like local lesions on inoculated leaves, even before the visual symptoms of infection appears, a PVY-GFP infective clone was prepared to follow the virus spread between cells. An improved knowledge on the distribution of the virus within individual tissues and cell types of the organs above ground will, in addition, contribute greatly to the understanding of aphid-mediated transmission used by PVY to infect new hosts.

L28

ENVIRONMENTAL SHAPING OF CODON USAGE, TRANSLATIONAL OPTIMISATION, FUNCTIONAL ADAPTATION AND HORIZONTAL GENE TRANSFER ACROSS MICROBIAL COMMUNITIES

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Microbial communities are ubiquitous and represent the largest portion of the Earth's biomass. Metagenomics projects use high-throughput next-generation sequencing to survey these communities and shed light on the myriad of genetic capabilities that enable microbes to inhabit virtually every corner of the biosphere. Computational analyses of genetic content in numerous completed metagenomic projects to date have been limited to counting genes, and their respective functions, found in an environment, and to phylogenetic classification of sequenced samples. By exploring well-established concepts of translational optimization through codon usage adaptation that were proven relevant at the level of single bacterial genomes, we show that the constituents of microbial communities share similar codon usage bias, regardless of their phylogenetic distribution. Community-wide bias in codon usage facilitates horizontal gene transfer and enables adaptation to environmental conditions by translational optimization similar to single bacterial genomes. In this respect, microbial communities effectively behave as meta-genomes. These findings set up a platform for the identification of genes important for functional adaptations of entire microbial communities to diverse environments.

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KEY ROLE OF COMMUNITY-BRIDGING NODES IN NETWORKS

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Our multidisciplinary group (www.linkgroup.hu) uses the general properties of networks as 'highways' making the transfer of concepts between various disciplines rather easy. This allows the utilization of the 'wisdom' of biological systems surviving crisis events for many billions of years. As an example of 'crisis' in biological systems the community structure of the protein-protein interaction network of stressed yeast cells was studied using our Moduland program, which is a novel method family to detect pervasively overlapping communities (PLoS ONE **7**, e12528, www.linkgroup.hu/modules.php). Upon heat shock the compactness of yeast protein communities increased and the number of community-bridging nodes decreased (PLoS Comput. Biol. 7, e1002187). The stress-induced decrease of inter-modular connections was beneficial, since it A.) allowed a better focusing on vital functions, and thus spared resources; B.) localized damage to the affected communities; C.) reduced the propagation of noise; D.) allowed a larger 'degree of freedom' of the individual communities to explore different adaptation strategies; and E.) allowed a more adaptive re-organization of the network from pre-formed elements during/upon relief from stress. From this and other studies community reorganization emerges as general and novel systems level mechanism of cost-efficient adaptation, evolvability, learning and memory formation. Our studies showed that community-bridging nodes play a particularly important role in adaptive processes. In yeast stress inter-community contacts were maintained and developed by key proteins of cell survival. Our signaling database, SignaLink (www.SignaLink.org) revealed that cross-talks between signaling pathways are much more characteristic to humans than to *C. elegans* or *Drosophila*. Community-bridging nodes have a key role in protein structure, metabolic and social networks. We proposed to call highly dynamic community-bridging nodes as creative nodes in 2008. These nodes can be identified by their efficiency in perturbation-propagation using our recently developed Turbine program (www.linkgroup.hu/Turbine.php) or by their game centrality, i.e. the ability of a node or edge to establish or break cooperation in a repeated social dilemma game using our program NetworGame (www.linkgroup.hu/NetworGame.php). Community-bridging nodes emerge as novel regulators of adaptation, evolvability, learning and memory formation.

L30

STREAMLINED-GENOME, LOW-MUTATION-RATE *ESCHERICHIA COLI*: IMPROVED CHASSIS FOR MOLECULAR/SYNTHETIC BIOLOGY

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Our laboratory focuses on the significant simplification of the *Escherichia coli* genome to produce a reduced-complexity model organism and an improved biotechnological tool. The genome of the K12 strain was streamlined by deleting most genes irrelevant for laboratory or industrial applications. Elimination of the genetic ballast (selfish DNA, unknown genes, laterally transferred islands) resulted in improvements (e.g., fast growth, high uniformity, increased tolerance) for practical applications. Recently, further improvements were achieved by eliminating mutation-generating mechanisms. While molecular mechanisms generating genetic variation provide the basis for evolution and long-term survival of a population in a changing environment, they might be dispensable in stable, laboratory conditions. In fact, newly emerging, evolved features might be undesirable when installing artificial genetic circuits. Spontaneously arising mutations can relieve the cell from the metabolic burden imposed by an engineered (e.g., protein-overexpressing) genetic construct and the deteriorated clone can quickly overgrow the population. Elimination of prophages, IS elements and diversity-generating, error-prone DNA polymerases involved in induced mutagenesis resulted in reduced evolutionary capacity and in significant stabilization of the genome. While retaining robust growth, the cells showed a significant decrease in overall mutation rates, most notably under various stress conditions, and allowed relatively stable maintenance of toxic protein-expressing clones. The low-mutation rate, high-fidelity, reduced-genome strain, while genetically less adaptable in a changing environment, represents an improved host in various synthetic and molecular biological applications, allowing more efficient production of growth-inhibiting biomolecules.

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L31

POLY(ADP-RIBOSE): A SIGNALING MOLECULE REGULATING "LIFE AND DEATH" IN DIFFERENTIATION

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Poly(ADP-ribosyl)ation (PARylation) is a covalent protein modification mediated by poly(ADP-ribose) polymerase (PARP) enzymes. Enzymes of the 17 member PARP family cleave NAD into nicotinamide and ADP-ribose and polymerize the latter onto glutamate or aspartate residues of acceptor proteins such as PARPs themselves (auto-PARylation), histones, transcription factors and other proteins (trans-PARylation). The polymer is degraded by poly(ADP-ribose) glycohydrolase (PARG) enzymes. Some PARPs such as PARP-1 and PARP-2 are typically activated by DNA breakage, however, alternative pathways (e.g. phosphorylation) have also been described. PARylation and PARP/PARG enzymes regulate diverse biological processes ranging from DNA repair, replication, transcription and protein stability. The molecular basis of these regulatory activities are also diverse and include covalent PARylation of target proteins, non-covalent binding of (free or protein bound) PAR to certain proteins, protein-protein interactions, modulation of cellular NAD level and competition with other NAD-dependent enzymes such as SIRTs. These effects lead to modulation of complex cellular functions such as metabolism, proliferation, differentiation and cell death. In the lecture a comprehensive overview of PAR biology will be provided with focus on cell death and differentiation.
Work in the authors' laboratory is supported by the following grants: OTKA K75864, K82009, PD83473, TAMOP-4.2.2-08/1-2008-0019 and TÁMOP 4.2.1./B-09/1/KONV-2010-0007, the National Innovation Office (Baross program Seahorse grant).

L32

GENOME-WIDE ANALYSES OF TRANSCRIPTION: THE LANDSCAPE OF RXR REGULATED TRANSCRIPTIONAL ACTIVITY IN MOUSE BONE MARROW DERIVED MACROPHAGES

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A key issue in biology is the differentiation and maturation of cells. This process is principally regulated by transcription factors binding to DNA and regulating the expression of the genome. We have been studying the role of a specialized family of transcription factors, nuclear hormone receptors, in the differentiation and lineage specification of myeloid-derived immune cells macrophages and dendritic cells. We have found that lipid receptors heterodimerizing with the Retinoid X Receptor (RXR) form an interrelated network providing the cells with means to sense and interpret their lipid environment and re-program their gene expression and thus shaping their immunphenotype.
In order to understand the interrelationship of the various nuclear receptors, transcription factors linked to cytokine signaling and also transcriptional co-factors and histone modifications we have initiated a set of genome-wide approaches to determine the genomic localizations (cistromes), binding sites and interactions of these various factors. By using chromatin immonoprecipitation followed by next generations sequencing (ChIP-Seq), steady state RNA determination (RNA-Seq) and nascent RNA determination (Global Run On Sequencing, GRO-Seq) we have created a comprehensive map of RXR binding and activity in mouse bone marrow derived macrophages. The ramification of such analyses to transcription biology and nuclear receptor signaling will be discussed.

L33

IDENTIFICATION OF POSITIONAL CANDIDATES FOR OBESITY LOCI ON MOUSE CHR15 USING GENOMIC AND BIOINFORMATIC APPROACHES

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Obesity is a risk factor for a number of chronic diseases including diabetes, cardiovascular diseases and cancer and is controlled by a complex interplay between genetic and environmental factors. Monogenic forms of obesity are rare and hence the attention has turned to searching for genes of the more common polygenic form of obesity by genomics approaches such as quantitative trait locus (QTL) mapping and bioinformatics analyses. Additional genes need to be identified to elucidate the mechanisms of susceptibility or resistance to obesity development. We previously identified a large segment on mouse Chromosome 15 (*Fob3*) affecting obesity-related traits in lines of mice selected on high (Fat line) and low (Lean line) body fat content that represent a unique model of polygenic obesity. Follow up studies revealed that *Fob3* consists of at least three separate linked QTLs *Fob3a*, *Fob3b1* and *Fob3b2*. One objective of this study was to identify candidate genes and narrow down the genetic intervals of *Fob3a* and *Fob3b2* QTL using congenic line F_2 crosses, interval-specific haplotype analysis and comparative genomics. The second objective was to identify differentially expressed candidates for *Fob3a* and *Fob3b2* using Affymetrix GeneChip 1.0 ST microarray and quantitative RT-PCR analyses. Our results identified strong positional candidate genes for *Fob3a* and *Fob3b2* on Chr15. Only a handful of positional candidates that exhibited differential expression were revealed that in combination with aforementioned genomic and bioinformatics analyses helped to significantly narrow down the list of candidates causal for *Fob3a* and *Fob3b2*. A comprehensive physiological characterization of top candidate genes is under way – some results revealing functional and physiological perturbations that might be responsible for the observed phenotypic effects will be presented. Identifying new obesity-susceptible and/or obesity-resistant loci should help to uncover important inherited risk factors and provide novel targets for diagnostics and development of therapies.

L34

RIBOSOMAL PROTEINS AND P53 REGULATION

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The exposure of cells to various DNA-damaging stressors activates p53 to preserve cellular and genetic stability, preventing tumor development in mice and humans. The critical role of p53 in tumor suppression is supported by the observation that approximately 50% of all human cancers have mutations within this gene. Although it was largely accepted that common to all p53-activating stresses is DNA damage, research over the last decade has shown that disruption of ribosome biogenesis activates p53 via binding of several ribosomal proteins (RP) to Mdm2 and inhibition of its E3 ubiquitin ligase activity against p53 independently of DNA damage. The observation that many DNA-damaging stressors inhibit ribosome biogenesis and consequently disrupt nucleoli suggests that signalling pathways involved in p53 activation by DNA damage and defects in ribosome biogenesis might share common components. However, despite huge research efforts in this field, the molecular mechanisms underlying transduction of the p53-activating signals by specific RPs remain largely obscure. Recently, we demonstrated the key role for RPL5 and RPL11 but not other previously suggested "p53-activating" RPs in p53 activation by various stressors and provided a novel mechanistic insight into this regulation.

L35

ON THE FUNCTION AND SPECIFICITY OF GCN5-CONTAINING HISTONE ACETYLTRANSFERASE COMPLEXES

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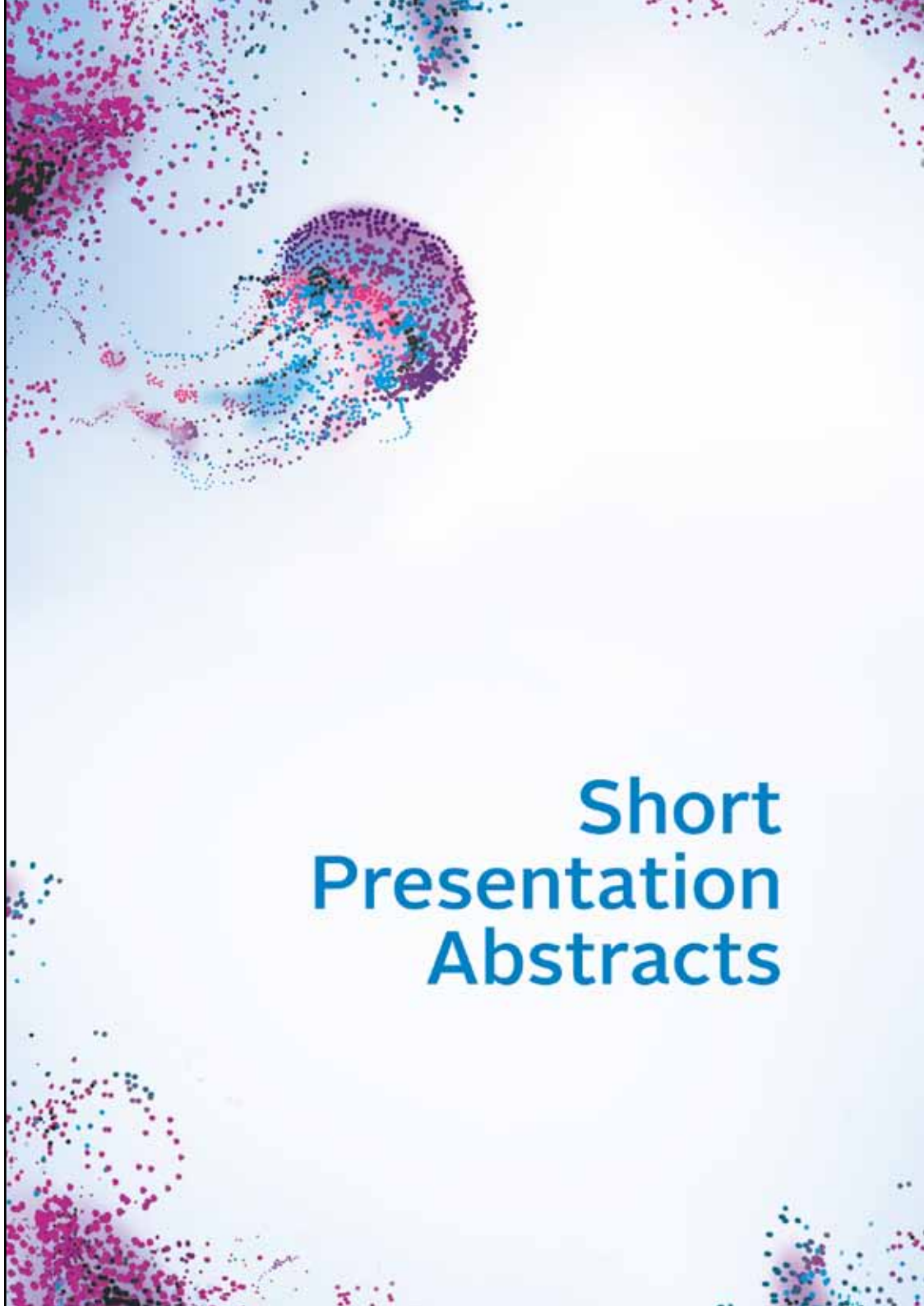
Histone acetyltransferases (HATs) are important chromatin regulators acting frequently as components of large multiprotein complexes. GCN5 (general control nonderepressed 5), the first histone acetyltransferase described, is present in two functionally distinct metazoan complexes. The ATAC (Ada2a containing) and SAGA (Spt-ADA-GCN5-containing) complexes have similar HAT modules as they share GCN5, ADA3 and SGF9 subunits, but they differ in containing one of two complex-specific ADA2 type-adaptors; ADA2a or ADA2b. Despite that, the two HAT complexes have clearly distinct functions: Our earlier genetic analysis revealed that *Drosophila* mutations removing ADA2b from SAGA results in a decrease in histone H3K9ac and H3K14ac levels, while mutations affecting dADA2a, an ATAC subunit result in decrease in H4K5ac and H4K12ac levels. Transcriptome analysis of *Ada2a* and *Ada2b* mutants also indicated that the SAGA and ATAC complexes effect the expression of different groups of genes. Since the presence of either dADA2a or dADA2b adaptor protein in ATAC and SAGA complex respectively, correlates with the histone specificity of the specific complex, we generated hybrid ADA2 proteins to reveal molecular interactions that determine HAT complex specificity. Our findings demonstrate that the ADA2 C-terminal regions play important role in the specific incorporation of ADA2 into SAGA or ATAC type complexes, what in turn determines H3 or H4 specific histone targeting. These results are in accord with previously collected genetic data and underline the different histone specificity of the two *Drosophila* GCN5-containing HAT complexes. The demonstration of the important contribution of ADA2 C-terminal regions to complex formation poses questions and opens ways to follow in the exploration of subunit interactions in order to elucidate the roles of these GCN5-containing complexes in chromatin regulation.



Short Presentation Abstracts

SP1

MACROMOLECULAR COMPLEXES OF AMINO ACID:[CARRIER PROTEIN] LIGASES AND CARRIER PROTEINS

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Aminoacyl-tRNA synthetases have well established and fundamental role in protein biosynthesis. They catalyze attachment of amino acids to cognate tRNAs, which are subsequently used as substrates for the ribosomal translation of mRNA. We have recently discovered and characterized bacterial homologs of atypical archaeal seryl-tRNA synthetases (aSerRS). These novel aSerRS homologs lack N-terminal tRNA-binding domain and, curiously, they transfer activated amino acids to phosphopantetheine prosthetic group of small carrier proteins (CPs) instead to tRNA. Therefore, they were named amino acid:[carrier protein] ligases (aa:CP ligases).

In order to gain insight how these aSerRS homologs recognize substantially different macromolecular substrate, the crystal structure of aa:CP ligase 1 from *Bradyrhizobium japonicum* in complex with cognate carrier protein was solved. One CP molecule binds to each subunit of homodimeric aa:CP ligase. The phosphopantetheine group of carrier protein enters deep into the active site of the same subunit, from the opposite side than tRNA to aSerRS active site. The structure of the complex revealed that interaction with cognate CP relies on the α-helix idiosyncratic to aa:CP ligases, while kinetic and pull-down experiments showed that recognition of cognate CP is specific. Therefore, a hybrid protein of *Bradyrhizobium japonicum* aa:CP ligase was constructed, in which the helix involved in CP interaction was replaced with equivalent one from *Agrobacterium tumefaciens* aa:CP ligase. The hybrid protein displayed altered CP specificity, preferentially recognizing heterologous *A. tumefaciens* CP. Deletion of the helix resulted in loss of aa:CP ligase interaction with CP. The properties of hybrid protein and deletion variant confirmed that interaction of aa:CP ligases and CP is solely dependent on the identified region. The crystal structure of the hybrid protein in the complex with *A. tumefaciens* CP was also solved, and revealed unanticipated, slightly different CP orientation compared to *B. japonicum* complex. The crystal structures of aa:CP ligase complexes with CPs, combined with biochemical experiments, unravel fundamentally different recognition of macromolecular partners by these close aminoacyl-tRNA synthetase relatives.

SP2

A CORRECTED MODEL OF LECTIN PATHWAY COMPLEMENT SYSTEM ACTIVATION BASED ON NOVEL *in vitro* EVOLVED PROTEASE INHIBITORS

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The complement system is part of the immune system. It protects us against invading pathogens and dangerously altered self-cells. The system can be activated through three distinct routes, the classical, the alternative and the lectin pathway. Each pathway is triggered by specific danger signals but eventually culminate in a common effector route. Unlike the classical pathway, which relies on the existence of already developed specific antibodies, the lectin pathway provides an immediate antibody-independent defense. It is triggered when large pattern recognition proteins recognize common signatures on pathogens or altered host cells. This leads to activation of associated MASP (mannan-binding lectin-associated serine protease) zymogens. *In vitro* studies showed that MASP-2 has all activities needed for igniting the pathway. It can auto-activate and cleave complement components C2 and C4 forming a centrally important cascade element, the C4b2a C3 convertase. MASP-2 has therefore been recognized as the autonomous pathway activator. MASP-1 can also auto-activate and cleave C2, but it cannot cleave C4. Consequently, MASP-1 has been recognized as an auxiliary pathway component. We evolved a pair of unique, monospecific MASP-1 and MASP-2 inhibitors. Both inhibitors completely block the lectin pathway indicating that the genuine role of MASP-1 has been overlooked. With a series of experiments we revealed a completely novel mechanism of lectin pathway activation. We show that MASP-1 is an essential component as it is the dedicated activator of MASP-2. Since unregulated activation of the lectin pathway plays a dominant role in provoking massive tissue damage upon myocardial infarct and stroke, these inhibitors are also lead molecules for subsequent drug development.

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SP3

MUSHROOMS ARE A SOURCE OF UNIQUE AND VERSATILE BETA-TREFOIL PROTEINS

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Mushrooms are a rich source of protease inhibitors and lectins that show unique characteristics and are exclusive to basidiomycetes. We have characterized several proteins, isolated from mushrooms, at genetic, biochemical and structural levels. Clitocypins (Merops family I48) from *Clitocybe nebularis* and macrocypins (Merops family I85) from *Macrolepiota* procera inhibit cysteine proteases by a distinct mechanism, whereas serine protease inhibitors cnispin (*Clitocybe nebularis*) and cospin (*Coprinopsis cinerea*) of the Merops family I66 utilize standard canonical mechanism for inhibition of trypsin but at a distinct reactive site. Furtermore, we have functionally characterized a GalNAcβ1-4GlcNAc-specific lectin CNL from *Clitocybe nebularis*, which showed antiproliferative activity against Jurkat human leukemic T cells and exhibited toxicity against the fruit fly (*Drosophila melanogaster*), the Colorado potato beetle larvae (*Leptinotarsa decemlineata*), the mosquito *Aedes aegypti*, the amoebozoa *Acanthamoeba castellanii*, and against a hypersensitive strain of nematode *Caenorhabditis elegans*. All these exceptionally stable proteins share similar biochemical properties, which are summarized in their three-dimensional structure, namely they all have a beta-trefoil fold. The β-trefoil fold is formed by the core six-stranded β-barrel which supports 11 loops that differ in shape and composition and provide a versatile surface for interaction with several types of proteins and carbohydrates.
Versatility as well as specificity, selectivity and other unique features of protease inhibitors and lectins found in mushrooms supported in a stable molecular scaffold reveals mushrooms as a valuable source of bioactive proteins suitable for applications in the fields of biotechnology, medicine and agriculture.
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SP4

ROLE OF ICAM3 AND LFA-1 IN THE INTERACTION BETWEEN HUMAN MACROPHAGES AND APOPTOTIC NEUTROPHIL GRANULOCYTES

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Apoptotic cells express eat-me signals on their surface which are recognized by several receptors mainly on professional phagocytes of the mononuclear phagocyte system. This „engulfment synapse" can define a safe and effective clearance of apoptotic cells in order to maintain tissue homeostasis in the entire body. Our previous TaqMan Low Density Array measurements predicted important role of some apopto-phagocytic genes in phagocytosis of apoptotic neutrophils by macrophages, because their expression level heavily elevated during the early stage of the phagocytosis. After they (ADORA2A, FPRL1, ICAM3, THBS1) were silenced by RNA interference, significant decrease in phagocytic capacity was observed only after silencing ICAM3. Our goal was to investigate the role of ICAM3 transmembrane protein and its interacting partners in phagocytosis and in the anti-inflammatory effect of apoptotic neutrophils on macrophages. Human monocytes were isolated from 'buffy coats' of healthy blood by CD14 specific magnetic separation. To examine the phagocytic capacity of 5 day differentiated macrophages, apoptotic neutrophils were isolated from human blood by Histopaque density-gradient centrifugation. The phagocytosis assay was performed using fluorescent labelled cells and the incorporated cell-rate was measured by flow cytometry, immediately after pre-incubation of macrophages or apoptotic cells with blocking antibodies. The localization of the investigated receptors was visualized by indirect immunostaining. The release of TNFa and IL-6 by knock-down or blocked macrophages which were treated with LPS and co-incubated with apoptotic neutrophils was determined by ELISA. Significant reduction of phagocytosis was noticed after blocking of ICAM3 from both sides. In macrophages but not in neutrophils silencing and blocking components of LFA-1, which can strongly bind ICAM3, resulted in a decreased phagocytosis of apoptotic cells. Engulfing portals formed in macrophages during phagocytosis are characterized by accumulation of ICAM3 and the subunits of LFA-1 which show co-localization on the surface of the phagocytes suggesting that ICAM3 and LFA-1 act as recognition receptors in the phagocytosis portals of macrophages for engulfment of apoptotic neutrophils. Furthermore, silencing and blocking of ICAM3 and the components of LFA-1 moderately decreased the anti-inflammatory effect of apoptotic neutrophils on LPS treated macrophages.

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SP5

IMPACT OF PLA2G4A AND PTGS2 GENE POLYMORPHISMS AND RED BLOOD CELL PUFAS DEFICIT ON NIACIN SKIN-FLUSH RESPONSE IN PATIENTS WITH SCHIZOPHRENIA

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OBJECTIVE: Attenuated niacin skin-flush response in schizophrenia marks cytosolic phospholipase A2/cyclooxygenase-2 (cPLA2/COX-2) cascade abnormalities and has been proposed as an endophenotype in this illness. We investigated whether, and to what extent, niacin flush response in patients with schizophrenia, could be related to polymorphisms in genes of the cPLA2/COX-2 cascade. We also tested possible correlation between niacin response and fatty acid (FA) profile of their red blood cells (RBC).
METHODS: Patches containing 0.1M, 0.01M, 0.001M and 0.0001M of niacin solution were kept for 5 minutes on the forearm skin in 79 patients and 80 controls. Visual evaluation of flushing rated from zero to three was done in 5-minute intervals by two independent raters. The genotyping of BanI polymorphism of the PLA2G4A gene (cPLA2) and A/G variant (rs689466) of the PTGS2 gene (COX-2) of the subjects that underwent niacin sensitivity testing was revealed by PCR-RFLP analysis. Total RBC lipids were extracted, converted into FA methyl esters and further analyzed by gas chromatography. We determined the relative amount of different saturated FAs (SFAs), monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) from n-3 and n-6 series. We introduced several indices and ratios into analysis.
RESULTS: Both polymorphisms diminished niacin flushing to a small, although significant extent (1.7% and 1.8%; $P<0.01$) in the patient group. Analysis of the RBCs' FAs content revealed that double bond index/peroxidizability index (DBI/PI) ratio accounted for 44.6% - 47.3% of flushing variability (at 0.01M niacin concentration and two time-intervals). Negative and high correlation between niacin response and DBI/PI ratio (β_1 = -0.69 and β_2 = -0.67) indicated an association between decreased relative RBC content of, especially, long-chain PUFAs (LC-PUFAs) and attenuated niacin response in patients. Significant decreases in relative content of eicosapentaenoic acid, docosahexaenoic acid, total PUFAs, linoleic acid, DBI, PI, and several ratios (PUFAs/SFAs, PUFAs/MUFAs), and significant increase in the relative SFA content contributed to this finding ($P<0.01$ or $P<0.001$).
CONCLUSIONS: Both polymorphisms as well as deficits in RBC LC-PUFAs significantly contributed to blunted niacin response in patients with schizophrenia. FA profile of their RBC membranes affected niacin flushing substantially while PLA2 and COX-2 gene variations affected skin response to a negligible extent.

SP6

SYNAPTOTAGMIN 1 IS A NOVEL RECEPTOR FOR AMMODYTOXIN

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Ammodytoxin (Atx) is a phospholipase A_2 with presynaptic neurotoxicity from the venom of the nose-horned viper (*Vipera ammodytes ammodytes*). The exact mode of its neurotoxic action is not known yet but it seems to be also intracellular. Atx was demonstrated to enter the cell cytosol *in vivo*. The mechanism of this translocation is, however, still unknown. Synaptotagmin (Syt), a protein spanning the synaptic vesicle (SV) membrane, is known to participate at translocation of botulinum neurotoxin into the motoneuron. In this study we report that Atx specifically binds to the isoform 1 of Syt (Syt1). Our aim is to further characterize this interaction to proceed then with investigation of involvement of Syt1 in translocation of Atx over the plasma membrane.
Crude mitochondrial–synaptosomal fraction of porcine cerebral cortex was subjected to Atx-affinity chromatography, SDS-PAGE analysis of the eluates and subsequent mass spectrometry of the excised gel slices. The molecular masses of tryptic peptides obtained from two analysed gel slices corresponded to that of Syt1. To confirm Syt1 as an Atx-binding protein, co-immunoprecipitation of proteins in PC12 cell lysates was performed, using Atx and anti-Atx IgG and protein A-Sepharose. Anti-Syt1 immunoblotting confirmed the presence of Syt1 in the immunoprecipitate. Affinity labelling by photoreactive sulfoSBED-AtxC revealed the presence of specific Atx-binding proteins also in porcine cerebral cortex SVs and some of these were immunoreactive to anti-Syt1 antibodies. The interaction between Atx and Syt1 seems to be Ca^{2+}-independent. Specific interaction between Atx and Syt1 was ultimately confirmed using recombinant Syt1. To localize the Atx-binding site(s) on Syt1, the recombinant cytosolic fragment of Syt1 was used. Contrary to our expectations, AtxC specifically labelled this fragment, locating one of the Atx-binding sites to this part of Syt1. To determine whether Syt1 possesses also a luminal Atx-binding site, affinity labelling of the full length Syt1 was performed in the presence of excessive amount of antibodies directed either to the cytosolic or to the luminal part of Syt1. Specific labelling of Syt1 was obtained in the presence of both kinds of antibodies leaving the problem unsolved. Immunocolocalization studies of Syt1 and Atx on PC12 cells, mapping of the interaction site between the two proteins and the surface plasmon resonance measurements will be presented.

SP7

MECHANISM OF REGULATION OF HOMOLOGOUS RECOMBINATION BY THE HUMAN BLOOM'S SYNDROME HELICASE

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The most toxic form of DNA damage is the double-stranded DNA break (DSB). To avoid the harmful consequences of DSBs, cells use homologous recombination (HR)-based error-free DNA repair mechanisms. Human Bloom's syndrome DNA helicase (BLM), a member of the RecQ family, plays crucial roles in HR progression and regulation. In the early steps of HR a three-stranded DNA structure, a displacement loop (D-loop), is generated by the Rad51 recombinase. BLM is able to perform quality control of HR by disrupting D-loops. We generated a series of truncated mutants of BLM to monitor dissolution kinetics. Surprisingly we found that all investigated constructs are able to disrupt D-loops, but the different constructs use distinct processing mechanisms. Our results demonstrate how the different domains of BLM regulate D-loop processing. Using solution biochemical and single-molecule studies we showed that the different actions of BLM during HR take place in different oligomeric forms of the enzyme. During single-stranded DNA translocation, which serves as a basis for quality control of HR via disruption of Rad51 nucleoprotein filaments, BLM functions as a monomer. However, more complex DNA structures resembling HR intermediates, including D-loops and Holliday junctions, induce partial oligomerization of BLM. The results indicate that BLM exists in a dynamic equilibrium between different assembly states, which is modulated by the structure of DNA intermediates encountered during HR.

SP8

TP53 MUTATIONAL SIGNATURE OF ARISTOLOCHIC ACID IN CARCINOMAS OF THE UPPER URINARY TRACT

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Endemic (Balkan) nephropathy (EN), a chronic renal disease affecting residents of rural villages situated near tributaries of Danube River, is strongly associated with transitional cell (urothelial) carcinoma of the upper urinary tract (UUC). Aristolochic acid (AA), a powerful nephrotoxin and human carcinogen, was shown recently to be the causative agent in EN. In EN, exposure occurs through ingestion of bread prepared from flour contaminated with AA. After metabolic activation AA forms covalent DNA adducts in renal cortex and urothelial tissues. Aristolactam-DNA adducts generate unique mutational spectra in p53 tumor suppressor gene, which together with the presence of DNA adducts in the renal cortex serve as biomarkers for aristolochic acid nephropathy and associated urothelial carcinomas. TP53 mutation spectrum was dominated by A:T→T:A transversions located almost exclusively on the non-transcribed DNA strand with unique „hot spots" at several splice sites and at codons 131 and 209. *TP53* gene mutations at this position have not previously been reported. The mechanism underlying the observed strand bias appears to be a selective failure to excise AL-DNA adducts by global genomic nucleotide excision repair. This factor also may account for the remarkable persistence of these adducts in human tissues (in some cases more than 50 years). In summary, aristolochic acid joins vinyl chloride and aflatoxin as human chemical carcinogens with a definitive mutational signature. This important information, coupled with the use of AL-DNA adducts as a biomarker, should prove useful in establishing the role of AA ingestion in countries with a high prevalence of UUC.

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SP9

PROTEASE INVOLVED IN POSTTRANSLATIONAL MODIFICATION OF 6-PHOSPHOFRUCTO-1-KINASE IN CANCER CELLS

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The switch from oxidative phosphorylation to aerobic glycolysis (Warburg effect) is one of the most consistent characteristics of malignant cell metabolism. Deregulated metabolic flow through glycolysis induces larger glucose consumption of tumor cells and the conversion of the majority of glucose into lactic acid. Recently, we described a phenomenon of posttranslational modification of the key regulatory glycolytic enzyme 6-phosphofructo-1-kinase (PFK1) in cancer cells. After proteolytic cleavage of the native enzyme an active, shorter fragment was formed that was resistant to feed back inhibition. Modification of PFK1 might be the pivotal factor of deregulated glycolytic flux in tumors. The mechanism of posttranslational modification proposed is through the overexpressed/mutated cytosolic protease, which is active only in malignant cells and could cleave off the C-terminal part of PFK1. In order to understand the process of posttranslational modification of PFK1, the protease involved will be characterized. In silico analyses of cleavage sites narrowed the protease selection to serine proteases, probably a family of kallikreins. To test the hypothesis a lysate of mouse melanoma cell line B16-F10 was screened for proteolytic activities using fluorescently quenched (FRET) peptide containing one (out of two possible) cleavage sequence of the native PFK1 enzyme. First the lysate was fractionated with ammonium sulfate and separated with hydrophobic interaction chromatography. Final step of isolation was performed with size exclusion chromatography and the level of purification determined by SDS-PAGE. In the poster the results of purification procedure and characterization will be shown. In the future the fraction with isolated protease will be checked for ability to cleave the native PFK1 enzyme in vitro. In the case of correct cleavage highly active shorter PFK1 fragments should be detected. Final identification of the protease will be preformed by MS. Data obtained by the identification of protease might be used for the design of specific inhibitors that could prevent the formation of highly active shorter PFK1 fragments in cancer cells.

SP10

MOLECULAR INTERACTIONS OF AMINOACYL-tRNA SYNTHETASES AND THE RIBOSOME

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The composition and complexity of the translation machinery differ markedly between the three domains of life. Organisms from the domain Archaea show an intermediate level of complexity, sharing with eukaryotes several additional components of the translation machinery, that are absent in Bacteria. Since protein-protein interactions play a key role in numerous events that take place in a cell, most cellular processes are regulated by multiprotein complexes. Several aminoacyl-tRNA synthetases (aaRS) are located in multi-synthetase complexes (MSC) in all three domains of life. Archael seryl-tRNA synthetases (SerRSs) diverge into two major and disparate types of enzymes (bacterial and methanogenic type). We have revealed protein partners of methanogenic type SerRS in *Methanothermobacter thermautotrophicus*, identifying arginyl-tRNA synthetase (ArgRS) and a number of ribosomal proteins as interactors. Interaction of SerRS with ArgRS improves the activity of SerRS while the presence of SerRS did not lead to significant enhancement of ArgRS activity. This aaRSs complex may constitute a part of the thermo- and osmoadaptation mechanisms of thermophilic methanogenic Archaea, by providing an optimal microenvironment that facilitates stable tRNA aminoacylation under a range of conditions. We used the yeast two-hybrid system with several biophysical approaches to investigate interactions between aaRS and ribosomal proteins. Our results indicate that aminoacylated tRNAs to be delivered to the ribosome in the form of a ternary complex with elongation factor EF1A and GTP, may be actually generated by ribosome-bound synthetases. In support, several lines of evidence suggest that during the elongation step of translation in the cytoplasm of eukaryote cells tRNAs flow in a closed circuit. The assembly of proteins within stable or transient complexes plays an essential role in this process. The structure of *M. thermautotrophicus* ribosome also provides a snapshot of the reductive evolution of the archaeal ribosome and offers new insights into the evolution of the translation system in archaea. Our observations have conceptual implications for understanding how translation machinery is organized in Archaea.

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SP11

THE INTERPLAY BETWEEN p54/Rpn10 PROTEASOMAL AND THE UBA-UBL EXTRAPROTEASOMAL POLYUBIQUITIN RECEPTORS IS REGULATED BY UBIQUITYLATION IN *DROSOPHILA MELANOGASTER*

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Polyubiquitin receptors execute the targeting of polyubiquitylated proteins to the 26S proteasome. *In vitro* studies indicate that disturbance of the physiological balance among different receptor proteins impairs the proteasomal degradation of the substrates. To study the physiological consequences of shifting the *in vivo* equilibrium between p54/Rpn10 proteasomal and Dsk2 extraproteasomal polyubiquitin receptors, transgenic *Drosophila* lines were constructed in which the overexpression or knocking-down of these receptors can be induced. Flies overexpressing $p54^{Flag}$ were viable and fertile, without any detectable morphological abnormalities, although detectable accumulation of polyubiquitylated proteins demonstrated a certain level of proteolytic disturbance. $p54^{Flag}$ was assembled into the 26S proteasome and could fully complement the lethal phenotype of the p54 null mutant (Δp54). The overexpression of $Dsk2^{Flag}$ caused severe morphological problems and lethality, accompanied by a huge accumulation of polyubiquitylated proteins, that could be rescued in a double transgenic line coexpressing $Dsk2^{Flag}$ and $p54^{Flag}$, suggesting genetic interaction between these two genes.
We found significant differences in Dsk2-26S proteasome interaction in *Drosophila* as compared with *Saccharomyces cerevisiae*. In yeast, Dsk2 can interact only with ΔRpn10 proteasomes and not with the wild-type one. In *Drosophila* Dsk2 does not interact with Δp54 proteasomes, but the interaction can be fully restored by complementing the Δp54 deletion with the functional $p54^{Flag}$. We have also demonstrated that p54 physically interacts with the UBL domain of Dsk2 via its UIM3 motif, which is also responsible for the coupled ubiquitylation of the conserved terminal lysines of p54. Gel-filtration chromatography revealed that the extraproteasomal p54 was extensively ubiquitylated, while only very modest modification was detected in the proteasome-assembled subunit. Moreover, the ubiquitylation of p54 seriously jeopardizes the interaction of UIM3 with the UBL domains of Dsk2 and Rad23 extraproteasomal polyubiquitin receptors. This modification supports the previous notion that p54 is a shuttling subunit of the proteasome with an essential extraproteasomal function. This assumption is supported by the observation that, while the transgenic $p54^{Flag}$ can fully rescue the lethal phenotype of p54 null mutation, its derivative from which the conserved lysines were deleted can not rescue the Δp54 mutation.

SP12

MAP KINASE INTERACTOMICS OF A NEW KIND: LINEAR BINDING MOTIF DISCOVERY BASED ON PROTEIN-PEPTIDE CRYSTAL STRUCTURES

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It is of utmost importance to organize physical protein-protein interactions specifically for correct physiological function in intracellular signaling networks. A systems level approach that could identify all functionally relevant protein-protein interactions between signaling network components, such as protein kinases, and their partner proteins would be a great asset for making a blueprint of regulatory networks controlling intracellular information processing. Classical top-down approaches may give interesting insights into the global organization of these networks, however they mostly fail in capturing the whole complexity of a specific system. In my talk I will present how insights obtained at the lowest level (at atomic level on the physical nature of a specific interaction type) successfully governed the design of top-down approaches that gave novel insights into the complexity, the abundance, and tentatively into the evolution of signaling links at systems level.
For mitogen-activated protein kinases (MAPK), their physical connections with their partner proteins are formed through short linear motifs binding to a dedicated protein-protein interaction surface, the so-called docking groove, that is common to all MAPKs. Because MAPK docking grooves are shallow “open” interfaces and topographically they are all similar, it has been enigmatic how flexible linear motifs (docking motifs) located in unstructured regions of MAPK partners can be the pivotal elements in determining the physical, and ultimately the physiological specificity of ubiquitous MAPK networks. In our recent work we have explored the structural basis underlying the specificity of MAPK-docking motif interactions. Insight into the physical nature of these interactions was key to devise a proteome wide linear motif search procedure that identified hundreds of proteins with putative MAPK-binding linear motifs in the human and in other proteomes. The result of this analysis suggests that linear motifs directly binding to MAPKs constantly neofunctionalise ubiquitous MAPK networks – similarly to the role of modular and structured protein-protein interaction domains in the evolution of other signaling systems.

SP13

RENAL GLOBOTETRAOSYLCERAMIDE EXPRESSION IN RAT MODEL OF DIABETES TYPE 1

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Globotetraosylceramide (Gb4Cer, GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer) has been identified among the major renal neutral glycosphingolipids. Gb4Cer molecules are found in membrane lipid rafts where they can influence the function of receptors and transporters embedded in these domains. Apical membranes of the kidney proximal tubule epithelial cells contain lipid rafts that support absorptive strategy for nutrient reabsorption. Considering diabetic nephropathy and changed glycosphingolipid metabolism in diabetes, the aim of our study was to determine renal Gb4Cer expression in rat model of diabetes type 1.
Diabetes was induced with streptozotocin (55 mg kg^{-1}) injection two weeks before Gb4Cer analysis in the kidney of Sprague-Dawley rats weighing 140-160 g (8 males and 5 females) and compared to respective control animals (3 males and 3 females). Gb4Cer fractions in the tissues of diabetic and control rats were determined by high performance thin-layer chromatography (HPTLC), followed by immunostaining with specific anti-Gb4Cer polyclonal antibody.
Diabetic male rats showed increased expression of Gb4Cer. In addition, two bands of Gb4Cer derivative (corresponding to sulphated Gb4Cer), chromatographed bellow Gb4Cer, were also increased in diabetic male rats compared to control. In contrast to control male rats, control female rats had lower Gb4Cer and only one Gb4Cer derivative band. Diabetic female rats showed lower expression of Gb4Cer and Gb4Cer derivative compared to control female rats.
Results of this study are significant in the view of the risk to develop end-stage renal disease that is doubled in men compared with women when age at diabetes type 1 onset is ≥15 years. Knowing that estrogen treatment decreases the content of Gb4Cer in rat kidney, our results point at the role of Gb4Cer in nephropathy development particularly in males.

SP14

LIPID CHANGES IN FROG (*PELOHYLAX RIDIBUNDUS*) DURING AROUSAL FROM PROLONGED HYPOTHERMIA

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Studies on endothermic animals showed that lipid metabolism plays an important role in hibernating animals as main source of metabolic fuel during bouts, contrary to normometabolic states in which glucose metabolism is a main energy source. Data on changes of liver and serum lipid levels in hibernating ectotherms is scarce. Frogs are excellent model for studies of hypometabolic changes in hibernating ectothermic animals. We present the analysis of the dynamics of lipid changes in frogs *(Pelophylax ridibundus)* exposed to 8°C for 30 days after which they were gradually acclimatized to temperature of 23°C over the period of 24 h, as an artificial induced arousal from hibernation. The entire procedure was recorded using IR camera due to exact temperature measurement during first 148 minutes to establish the exact termal changes of body parts. Species was confirmed by RFLP analysis. Frogs (N=4/time group) were sacrificed immediately after taking them out of hibernaculum, after 1, 2, 4 and 24 hours. Liver lipids were analyzed by TL chromatography. Blood serum lipid (triglycerides, cholesterol, HDL, LDL) levels were analyzed to establish the correlation changes between liver and serum lipid levels at each sampled group. Results showed that temperature rise and arousal from induced hibernation is in correlation with liver lipid mobilization, but on the contrary serum lipids changes have different dynamics within 24 hours.

SP15

MEMBRANE LIPIDS AND NEURODEGENERATION

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Precise control and regulation of brain lipids metabolism is required during complex processes of dynamic rebuilding and remodelling of neuronal membranes (neurite outgrowth, synaptogenesis, myelination). Also, it has been confirmed that lipid metabolism is altered in neurodegeneration and that changes in membrane properties and structural-functional integrity contribute to neurodegeneration process. The alterations of membrane physicochemical properties are not merely a consequence of primary pathology, but may be involved in early pathogenesis of the disease. It is of particular interest to investigate the intermolecular interactions within highly organized lipid microdomaines - lipid rafts - enriched in cholesterol, sphingolipids and specific protein molecules. Here we focus to gangliosides, membrane glycosphingolipids bearing sialic acid residues, which are attributed with diverse functions such as intercellular interactions, cell recognition, neurotransmission, and signal transduction. The highest concentration and variability of ganglioside structures are found in the human brain. Specific temporal and regional distribution of brain gangliosides has been reported. Moreover, gangliosides may serve as markers of neurodevelopmental stages, aging and neurodegeneration. Brain ganglioside content and composition as well as ganglioside metabolism are altered in Alzheimer's disease. It appears that alterations of membrane ganglioside composition are involved in the early pathogenesis of Alzheimer's disease through documented effects on amyloid precursor protein proteolytic processing and amyloid aggregation. Investigations of glycolipid metabolic alterations which accompany neurodegenerative disorders provide insight into pathogenetic mechanisms and enable recognition of diagnostic markers as well as molecular structures interfering with cascade of pathological events.

SP16

DIPEPTIDYL PEPTIDASE IV AFFECTS NEUROPEPTIDE Y LEVELS IN INFLAMMATORY EVENTS

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Proteases have been proposed as one of the key factors in the occurrence of inflammatory processes due to their ability to metabolize different biologically active molecules implicated in inflammatory events. Dipeptidyl peptidase IV (DPP IV/CD26), a multifunctional glycoprotein found in both soluble and membrane-bound form, regulates circulating bioactive peptides and participates in the modulation of the immune response through its enzymatic function and expression on various immune cells. Neuropeptide Y (NPY), a substrate of DPP IV/CD26, is produced by central and peripheral nervous system as well as by immune cells and has pleiotropic effects on both innate and adaptive immune system. Truncation alliterates the affinity of NPY to its receptors and the relevance of the DPP IV/CD26 - NPY connection in autoimmune and inflammatory diseases has been indicated.
We hypothesized that DPP IV/CD26 through its neuroimmunomodulative properties plays an important role in rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD). Since clinical studies suggested the importance of DPP IV/CD26 and NPY in autoimmunity, we investigated their systemic and local levels in adult patients affected with RA and osteoarthritis, latter representing a control group. Activity and concentration of DPP IV/CD26 were significantly decreased in both synovial fluid and serum, while, inversely, immunodetection showed elevated levels of NPY. Furthermore, our previous studies showed altered concentration of DPP IV/CD26 in serum of IBD patients, hence our research was broaden to an experimental model of IBD in order to investigate the effects of DPP IV/CD26 deficiency on the circulating and tissue levels of NPY in CD26 deficient and wild-type mice with induced colitis. During colitis development, decreased DPP IV/CD26 activity was found in serum, colon and brain in wild type mice, while CD26 expression was increased in colon. Inflammatory events in the colon lead to an increase in serum and colon NPY concentrations in both mice strains. Colitis induced an increase in brain NPY concentration in the acute phase in wild type mice and, adversely, a decrease in CD26 deficient mice.
In conclusion, mechanisms activated upon inflammation induce changes in NPY secretion systemically and at the sites of inflammation, in both IBD and RA, which further confirms the impact of DPP IV/CD26 on its bioactive substrate NPY in chronic autoimmune disorders.

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SP17

GALECTIN-3 IN MACROPHAGE DIFFERENTIAITON AND ACTIVATION

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Galectin-3 (Gal-3) is a β-galactoside lectin which modulates many processes of innate and acquired immunity. Generally considered a potent pro-inflammatory signal, Gal-3 triggers/promotes monocyte respiratory burst, acts as a monocyte/macrophage chemoattractant and promotes the survival of inflammatory cells. In response to a broad range of environmental cues, monocytes and macrophages exhibit different biological and biochemical characteristics, but two main subtypes, classically (M1) or alternatively (M2) differentiated and activated macrophages have been recognized. The aim of this study was to explore the expression of Gal-3 in the physiology of said human macrophages.
Human monocytes from healthy blood donors were differentiated into M1 or M2 cells using macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively. Obtained macrophages were activated classically by IFN-γ and LPS, or alternatively, using IL-4/IL-10 to generate M2a/c cells. Macrophage polarization was confirmed by flow cytometric profiling of secreted cytokines and distinct surface markers expression. Gene and protein expression levels of intra- and extracellular Gal-3 were investigated by qRT-PCR, Western-blot, flow cytometry, imunoprecipitation and ELISA, while surface Gal-3 receptor expression was analyzed by flow cytometry.
Obtained results imply that differentiation of monocytes into classically and alternatively activated macrophages is followed by marked changes of Gal-3 expression and proteolitic cleavage. Furthermore, its expression and secretion were tightly regulated and significantly differed among classically and alternatively activated macrophages. Interestingly, considerable differences in galectin-3 expression profiles were observed among the same macrophage subtypes obtained from different blood donors. In response to IFN-γ/LPS, classically activated macrophages polarize into two distinct populations with respect to the membrane galectin-3 expression. Human monocytes have a high amount of free galectin-3 receptors, while on both types of activated macrophages the receptors were fully saturated.
Specific expression and secretion patterns of Gal-3 in M1 *vs.* M2a/M2c macrophages contribute to better understanding of its role and regulation in these cells and provide an important new insight into the biological characteristics of these cells.

SP18

ENGULFMENT OF APOPTOTIC CELLS TRIGGERS RETINOID SYNTHESIS IN MACROPHAGES TO ENSURE THE EFFICIENT DEATH AND CLEARANCE OF NEGLECTED THYMOCYTES

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The thymus provides the microenvironment, in which thymocytes develop and reach the mature T cell stage.
Due to the random nature of T cell receptor (TCR) production, 90% of CD4+CD8+ thymocytes produced express a TCR that does not recognize peptide loaded self MHC molecules found in the thymus and will undergo a default death pathway named "death by neglect". The thymocyte apoptosis program *in vivo* is completed by the clearance of apoptotic cells by professional phagocytes. Here we show that lipid content of the engulfed apoptotic cells will trigger the synthesis of retinoids in macrophages. The retinoids produced enhance the phagocytosis capacity of macrophages by activating retinoid X receptor (RXR) dependent signalling pathways, which upregulate the expression of various phagocytosis receptors. In addition, the retinoids are released and enhance the apoptosis of neglected thymocytes. Retinoids induce apoptosis via activating retinoid receptor (RAR)γ, which initiates an apoptosis program dependent on the synthesis of Nur77. We show that Nur77 acts partly as a transcription factor in the nucleus (inducing the synthesis of various apoptosis-related molecules, such as Fas ligand, TRAIL, NDG-1, Grp65 and Bid), and also translocates into the mitochondria, where it induces the exposure of the BH3 domain of Bcl_{xL}. The program is caspase-8 dependent and involves the mitochondrial pathway of apoptosis. Glucocorticoids are thought to be produced locally by thymic epithelial cells to initiate apoptosis in the neglected thymocytes. Retinoids enhance the glucocorticoid-dependent apoptosis of thymocytes acting via RARα/RXR. We show that the ligated receptor directly interacts with the glucocorticoid receptor to stimulate its transcriptional activity. In addition, retinoids induce the expression of transglutaminase 2, a protein, which in macrophages will promote phagocytosis of apoptotic cells and in thymocytes will contribute to the apoptotic program. Our data indicate that a complex crosstalk between apoptotic cells and macrophages ensures the efficient death and clearance of neglected thymocytes, and one of the mediators of this crosstalk is a new, not yet characterised retinoid.
Supported by OTKA K77587, by the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project (implemented through the New Hungary Development Plan, co-financed by the European Social Fund), and a Sanofi Aventis Scholarship.

SP19

MOLECULAR CHARACTERIZATION OF A NOVEL ORGANIC ANION TRANSPORTING POLYPEPTIDE, ZEBRAFISH Oatp1d1 (*Slco1d1*)

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Membrane transport proteins from the OATP family (Organic anion transporting polypeptides; solute carrier SLC21/SLCO) are involved in the uptake of various endogenous and foreign compounds across the plasma membrane. However, their role has not been comprehensively investigated in non-mammalian species. The goal of our study was molecular characterization of physiologically and toxicologically relevant uptake transporter Oatp1d1 in zebrafish (*Danio rerio*). Using phylogenetic analysis, we confirmed that Oatp1d subfamily is present in teleosts and absent from amphibians and higher vertebrates. Oatp1d members are present in all five analyzed teleost fishes, confirming that Oatp1d1 is not a zebrafish specific gene. Tissue expression profiling using qPCR revealed sex differences: in liver, kidney and gonads where Oatp1d1 is expressed higher in male fish. Using transiently transfected HEK293 cells and radioactively labeled model substrate [H^3] estrone-3-sulfate, we performed a detailed functional characterization of Oatp1d1. We found that Oatp1d1 transports steroid hormones and their conjugates, mostly estrogenic derivatives, but also dehydroepiandrosterone (DHEAS), a testosterone precursor. Among thyroid hormones, Oatp1d1 transports triiodothyronine (T3), but not thyroxine (T4). Zebrafish Oatp1d1 is involved in the uptake of bile salts with preference towards taurine conjugates, as well as in the uptake of bilirubin. A series of model substrates and inhibitors of OATP1 subfamily was further tested in order to compare substrate specificity of Oatp1d1 with mammalian OATP1 members. This analysis revealed higher similarity to OATP1A2, OATP1B1 and OATP1B3 then to OATP1C1. However, despite partly overlapping substrate range, Oatp1d1 differs from mammalian OATP1 members in terms of substrate affinities and inhibitor potencies for certain compounds. Furthermore, we have found that Oatp1d1 transport activity is dependant upon inwardly directed [H^+] gradient, with possible involvement of conserved histidine residue in the third transmembrane domain. This pH dependence might be physiologically significant in kidney, where tissue acidification in the distal part is noted under normal conditions in teleosts. In conclusion, the described characterization of a novel Oatp1 gene in zebrafish reveals its role in the maintenance of steroid and thyroid hormones, bile acids and bilirubin balance in fish, as well as in disposition and excretion of foreign compounds.

SP20

ANIMAL MODELS FOR *in vivo* RESCUE OF DISEASE-CAUSING MUTATIONS OF ABCC6

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Calcification of cardiovascular tissues occurs in a variety of pathological conditions, including vascular injury, renal failure, atherosclerosis, and aging. Loss-of-function mutations in *ABCC6* can cause chronic or acute forms of dystrophic mineralization described in disease models such as pseudoxanthoma elasticum (PXE, OMIM 26480) in human and murine dystrophic cardiac calcification. The ABCC6 protein is a large membrane-embedded organic anion transporter primarily found in the plasma membrane of hepatocytes. We have established a complex experimental strategy to determine the structural and functional consequences of disease-causing mutations in the human ABCC6. 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; using biochemical transport assays, we have identified seven variants that retained significant transport activity. All mutants were transiently expressed *in vitro* in MDCKII cells and *in vivo,* in mouse liver via hydrodynamic tail vein injections. The mutants showed intracellular accumulation indicating abnormal trafficking. 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. Microinjecting zebrafish embryos with full-length human ABCC6 cDNA results in full rescue of the morpholino-induce developmental phenotype in zebrafish (while the R114X nonsense mutant triggered no rescue at all). These results indicated 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.

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SP21

THE ROLE OF ORGANIC CATION TRANSPORTERS (OCTS, *SLC22A*) IN ZEBRAFISH *(DANIO RERIO)*

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Polyspecific organic cation transporters (OCTs) of the *SLC22* family in mammals play important roles in distribution and elimination of cationic drugs and toxins. In mammals, three OCT members (OCT1-3) have been characterized so far. Although zebrafish has been adopted as an important model species in life sciences, OCTs have not been studied in zebrafish. Therefore, our study was the first attempt directed to identification, molecular characterization and understanding of physiological role of zebrafish OCTs.
Through genome and phylogenetic analysis we identified two zebrafish OCT co-orthologs: OCT1/2a and OCT1/2b. Using the real-time PCR we quantified their expression in seven zebrafish tissues (brain, liver, kidney, intestine, testes/ovaries, muscle, gills). Zebrafish OCT1/2b exhibited high expression in testes, moderate in kidney and relatively low expression in other tissues. On the contrary, OCT1/2a, which is more closely related to mammalian OCT1 and OCT2 orthologs, showed extremely high expression in kidney and high expression in liver and testes of male zebrafish. Moderate expression was found in male's brain and female's liver.
In order to determine the substrate specificity of zebrafish OCTs, we transiently expressed the transporters in HEK293 cell line. The OCT1/2a transported fluorescent cations with high affinity: 4-(4-Dimethylaminostyryl)-N-methylpyridinium (ASP+; Km=0.14 μM) and ethidium bromide (Km=0.11 μM). The uptake of fluorescent cations was 8-fold higher in the OCT1/2a transfected cells in comparison to the non-transfected cells. Using the ASP+ as fluorescent probe we tested 50 compounds known to interact with human OCT1-3, in order to determine substrate/inhibitor specificity of zebrafish OCT1/2a. Among the tested physiological substrates, high affinity was found for hormones androstenodione (Ki=1.6 μM) and progesterone (Ki=2.8 μM), while moderate affinity was found for β-estradiol (Ki=29 μM) and testosterone (Ki=16 μM). Among the tested drugs, the highest affinity was found for verapamil, diltiazem and prazosine with Ki values of 1.1 μM, 2.2 μM, and 3.6 μM, respectively. Taken together, our results imply pivotal role of zebrafish OCT1/2a in uptake of hormones in testes as well as their clearance from blood through kidney and liver. Our further research will be focused on characterization OCT1/2b ortholog to determine the relative importance of the OCTs in zebrafish and to elucidate their true physiological role.

SP22

THE THIRD INTRACELLULAR LOOP OF GLUCAGON LIKE-PEPTIDE-1 RECEPTOR IS COVALENTLY MODIFIED WITH ENDOGENOUS MONO-ADP-RIBOSYLTRANSFERASE — NOVEL TYPE OF RECEPTOR REGULATION?

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Glucagon-like peptide-1 receptor (GLP-1R) is a member of secretin/vasointestinal peptide receptor family B. Previous studies revealed the main role of the third intracellular loop of GLP-1R in G-protein activation, where the receptor phosphorylation seemed to be the only regulatory mechanism. To further study signaling mechanisms we investigated the effect of the third intracellular loop-derived peptides on endogenous mono-ADP-ribosyltransferase (ART) mediated mono-ADP-ribosylation of G-proteins' β subunit in CHO cells. Mono-ADP-ribosylation, like protein phosphorylation, has all the characteristics of a regulatory mechanism and is yet confirmed to regulate the activity of β subunit of G proteins.

Results showed an inhibitory effect of IC_3 peptide on mono-ADP-ribosylation of β subunit, obviously via the mechanism of competitive inhibition. Excluding the activity of this inhibition via pertussis toxin-sensitive G proteins and validation of IC_3 peptide activation of G-protein αβγ trimer, the direct functional coupling of IC_3 and ART was confirmed. IC_3 peptide exerts its competitive inhibition in micro molar range around 50 μM which is also the active amount of peptide in other biological processes. According to the CD spectroscopy, a change in the secondary structure of IC_3 peptide happens at the same concentration range. Considering the fact that the well-known sequence recognized by ART is found within the IC_3 peptide sequence, the most credible candidate for modified amino acid residue, Arg^{348}, was proposed. Peptide IC_3(R348A) was tested and, in comparison with the wild type peptide, its mono-ADP-ribosylation was reduced significantly but not eliminated completely. Therefore, an alternative amino acid residue modification was suggested and Cys341 was finally confirmed. Arg348 is modified predominantly and the sum of mono-ADP-ribosylation of both mutants correlates with IC_3 peptide modification. Modification of Cys341 possibly evolved recently as an alternative mechanism of receptor desensitization in the case of Arg348 mutation. Double mutant IC_3(R348A,C341A) has no effect on modification of β subunit and its own mono-ADP-ribosylation is nearly completely lost.

In conclusion, we showed that mono-ADP-ribosylation of GLP-1R is possible *in vitro* and very likely represents a novel type of receptor activity regulation *in vivo*. Additionally, our findings can be very supportive in development of new treatment procedures for diabetes mellitus type 2 and obesity.

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SP23

CYSTEINE PROTEINASES AND ENDOGENOUS INHIBITORS IN INNATE IMMUNE RESPONSE

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Macrophages and natural killer (NK cells) are key players of innate immune response and several studies have focused on reciprocal regulation between myeloid and NK cells. Innate immune respons against viruses and bacteria consist of recognition of pathogen associated molecules via membrane associated Toll-like receptors (TLRs) and cytosolic and the nucleotide-binding domain leucine-rich repeat–containing receptors (NLRs). Recently several studies have shown the involvement of endosomal cathepsins in TLR3, 7 and 9 mediated immune response and at the crosstalk of macrophages and natural killer cells (NK). In our present work we have investigated the regulation of endosomal cysteine proteinases by endogenous inhibitors - cystatins in macrophages and NK cells. Stefin B (cystatin B) is present in the cell nucleus and in the cytosol. Bone marrow macrophages (BMM) derived from wild type (WT) and stefin B-deficient mice ($StB^{-/-}$) were primed with interferon gamma and treated with lipopolysaccharide (LPS) and /or CpG DNA. We determined that rather than inducing tolerance, CpG pretreatment primed macrophages for the cytokine production. Cathepsin B was reported to play a role in a direct NLRP3 activation by crystals and amyloid beta (Halle et al. 2008; Hornung et al. 2008). We investigated the role of cytosolic cysteine proteinase inhibitor (stefin B) in inflammasome activtion. Cystatin F is found in endosomes of NK cells and myeloid cells. It could be also secreted into extracellular medium, only as a dimer. Cystatin F dimer is converted into an active monomer by proteolytic cleavage in endosomes. The truncated form of cystatin F inhibits cathepsin C, which is necessary and sufficient for granzyme A processing. Cystatin F expression was found to be highly upregulated in LPS activated macrophages. Our results suggest that the cystatin F, secreted from activated macrophages, is uptaken by NK cells, processed by endosomal cathepsins in order to efficiently inhibit cathepsin C. Research was financed by Slovenian research Agency (ARRS) grants (J3-0612) to NKJ and grant (P-0140) to BT.

SP24

DISTINCT MOLECULAR CHANGES ACCOMPANYING OPIOID TOLERANCE IN RAT BRAIN SUBCELLULAR FRACTIONS

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Opioid drugs remain the gold standard for the treatment of severe pain. However, their clinical use is hampered by unwanted side effects such as tolerance, dependence, respiratory depression and constipation. It has become apparent that analgesic tolerance and dependence are complex phenomena and growing data question prevailing explanations of tolerance. This includes the hypothesis that receptor desensitization and internalization are the underlying molecular mechanisms of physiological tolerance.. In the present work, we test the hypothesis that the ability of a ligand to cause receptor internalization is closely related to its efficacy in signaling. We have found that chronic *intracerebroventricular (icv)* treatment of rats with an appropriate dose of the prototypic mu-opioid peptide agonist, DAMGO and its chloromethyl ketone derivative resulted in analgesic tolerance accompanied by a decrease in mu-receptor density in synaptosomal membranes of rat brain. A proteolytically stable analog of the endogenous agonist of mu-receptors, cis-1[(S,2R-aminocyclohexanecarboxylic acid]^2-endomorphin-2 (ACHC-EM2) displayed longer-lasting antinociceptive effect in the tail-flick test with delayed development of tolerance compared to that of the parent compound, endomorphin-2 (EM-2). Chronic *icv* treatment (20 μg icv for 11 days, twice daily) with the new analog increased the mu-receptor density in the microsomal fraction while internalization of the surface sites was not evident. Similar results were found with morphine (Fábián et al. J. Pharmacol. Exp. Ther. (2002) 302: 774). Since DAMGO and ACHC-EM2 behaved like full agonists and morphine was a partial agonists in G protein activation assays, these results imply that the efficacy of opioid ligands does not directly correlate with their ability to induce receptor internalization. Delineation of the molecular mechanisms involved in drug tolerance/ dependence and their temporal interrelationships represent an important scientific challenge.

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SP25

DEVELOPMENT OF A TRANSGENIC MOUSE MODELS TO DETERMINE THE ROLE OF CYP51 IN SPERMATOGENESIS AND EMBRYO DEVELOPMENT

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Lanosterol 14α-demethylase encoded by the *Cyp51* gene controls one of the key steps in cholesterol biosynthesis thereby playing an important housekeeping role. Additionally, *Cyp51* produces an intermediate in germ cells, meiosis-activating sterol (MAS) that accumulates by many fold in germ cells compared to other somatic tissues. MAS has been implicated in the control of meiosis and germ cell maturation by *in vitro* experiments. However, the *in vivo* role of *Cyp51* and MAS in the germ cell development as well as in other tissues and processes is unclear. To test if *Cyp51* is essential for embryo development and how lack of function of this enzyme affects cholesterol *de novo* production, we first developed a full knockout model that exhibited several prenatal Antley-Bixler (ABS)-like features leading to lethality at E15.0. We ascribe lethality to heart failure due to hypoplasia, ventricle septum, epicardial and vasculogenesis defects suggesting that *Cyp51* deficiency is involved in heart development and coronary vessel formation. As the most likely downstream molecular mechanisms alterations in sonic hedgehog and retinoic acid signaling pathways were identified. Knockout of *Cyp51* provides evidence that this gene is essential for embryogenesis. Owing to vast similarity of *Cyp51* knockout developmental defects with the ABS syndrome abnormalities, it presents an animal model for studying this syndrome in humans. To examine the effect *Cyp51* inactivation on spermatogenesis and test the hypothesis of the essential role of *Cyp51* and its intermediate MAS in germ cell development we generated male germ-cell specific *Cyp51* knockout mice. The inactivation of gene *Cyp51* in germ cells was 89 % efficient. Phenotypic analyses revealed no significant changes in the morphology of the germ cells, testis weight, daily sperm production and reproduction capacity in the *Cyp51* knockout testis comparing to wild types. It is possible that only minute amount of MAS is sufficient to perform the biological function. MAS could therefore be transported from the interstitial compartment and contributed to the normal development of the germ cells. It is also plausible that the incomplete inactivation of *Cyp51* gene resulted in the rescue of the phenotype. Our results demonstrate the essential role of *Cyp51* in embryo development, while the absolute requirement of *Cyp51* in germ cell development has not been shown conclusively.

SP26

MUTATION OF THE COMPOSITE MAIN CELIAC EPITOPE OF TRANSGLUTAMINASE 2 DIRECTLY AFFECTS ANTIBODY BINDING

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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 (aa) R19, E153, E154 and M659 to form a composite main celiac epitope. Human recombinant TG2 mutants were expressed in *E. coli* in a His-tagged form 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 as well as of patient-derived monoclonal antibody fragments. As CD antibodies recognize highly conformational binding sites, we next investigated whether they indeed bind to the surface composed by R19, E153, E154 and M659 or the RE and REM mutations affect folding and thus indirectly change the binding to a distant yet unknown binding site. The folded structure of R19S, E153S and R19S/E153S was confirmed by CD spectra. Mutant R19S had similar transamidating activity as wildtype TG2 (WT) indicating proper conformation. Aa changes in the core domain (E153S) resulted in decreased transamidating activity, which can be explained by the overlap with one of the Ca^{2+} -binding sites of the enzyme. The E154Q mutation alone did not alter the binding of CD antibodies, which propose intact binding site in this case. For further evidence we investigated the possible similarity of this site between TG2 and factor XIII (fXIII), which is not antigenic in CD. The crystal structure of fXIII is available and we found only one aa difference on this surface part: a negatively charged aa in TG2 (E154) and the homologue aa with positive side chain in fXIII (K199). As this difference is tolerated without conformational change in natural fXIII, we created mutant E154K. E154K had similar antigenicity as E153S (remaining antibody binding 66.6%) and when it was combined with further aa changes, R19S/E154K/M659S, the remaining binding was only 22.0%. Mutant TG2s could bind fibronectin in a same extent as WT. We could prove that the identified celiac epitope directly mediates antibody binding and the decreased binding is not due to the alteration of the overall structure of the protein. Our recent work proposes to confirm these results with recombinant mutant TG2s expressed in mammalian cell culture system.

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SP27

HEPATITIS B VIRUS: MOLECULAR GENOTYPES AND S GENE MUTANTS AMONG END STAGE CHRONIC HEPATITIS B PATIENTS

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Hepatitis B virus (HBV) infection is an infectious inflammatory liver disease that affects more than 2 billion people in the world. Genotypically, HBV genomes have been classified into ten groups; designated A-J, based on an intergroup divergence of approximately 8% based on S gene sequence. The S gene encodes for the hepatitis B surface antigen (HBsAg), which indicates current hepatitis B infection. It is well known that the genotypes vary geographically and that certain genotypes results in higher rates of chronicity, severity of disease clinical course, and different anti-viral drug response. Twelve serum samples obtained from end stage chronic hepatitis B patients immediately before liver transplantation were studied. Patients were selected based on their clinical and serology data, and the stage of the disease. The aim of the study was to determine molecular genotypes of HBV in the selected patients and to identify mutations in HBV S gene, which afford HBV variants a distinct survival advantage, permitting the mutant virus to escape from the immune system. S gene mutations affect HBsAg immunogenicity, and are often induced under immunosupression (vaccines or hepatitis B immune globulin therapy, HBIg. They are also responsible for reactivation of the disease, diagnostic assay failure and occult hepatitis B infection. Amplification of target HBV-DNA region were carried out by nested polymerase chain reaction (PCR) using selected primers, corresponded to the conserved regions among the different genotypes, allowing the distinction of HBV genotypes. The products of nested PCR were sequenced, using the second-round primers. The obtained sequences were then submitted to the Blast program in order to determine their similarity to other HBV strains deposited in GenBank. Genotypes of HBV were found to be type D (8 patients) and type A (4 patients). Twenty one point mutations detected in the S gene of HBV mainly affect 'a' determinant, located on the major hydrophilic region (MHR) of HBsAg. Changes in that region disrupt the antigenicity of HBsAg in multiple ways and have impact on the severity of the disease. Five of detected mutations (G102N, F127L, C147Y, G159A and G159R) were identified to be the new ones.

SP28

CHLOROPHYLLS CONTENT AND PHOTOCHEMISTRY OF THYLAKOID MEMBRANES IN TWO PEA CULTIVARS WITH DIFFERENT LEAF COLOURATION

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Photochemical features of thylakoid membranes and chlorophyll content were investigated in two pea (*Pisum sativum* L.) cultivars different leaf colouration: Assas (green leaves) and Arvika (red leaves). Particularity of Arvika cultivar is the appearance of red leaves in juvenile developmental stages (2-3 leaves), in the part of year when low temperatures in the morning are combined with medium irradiation. *In vivo* measured fast transients of chlorophyll fluorescence (OJIP) was used to evaluate overall photosynthetic efficiency (expressed as performance index - PI_{ABS}) as well as for advanced analysis of main photochemical processes per active reaction centre (RC) of photosystem II (PSII), such as absorption of light energy (ABS), trapping of exciton (TR_0), excess energy dissipation (DI_0) and electron transport (ET_0) beyond primary acceptor (Q_A^-). Further, we challenged investigated plants with short-term high light conditions and evaluated response of photosystem II driven linear electron transport (relETR) and non-photochemical quenching (NPQ). It was revealed that in spite of prevailing red colour, Arvika leaves had identical levels of chlorophylls *a* and *b*. Field measurements of OJIP transients showed that red leaves grown at incident irradiation (500-800 µmol $m^{-2}s^{-1}$) had decreased PI_{ABS} and ET_0 per RC, while other fluxes per RC showed no significant differences in comparison with green leaves. Experiment with high light (~2000 µmol $m^{-2}s^{-1}$) in laboratory conditions also revealed impaired relETR accompanied with increased NPQ values in red leaves. In summary, here we demonstrated that red colour that emanates from anthocyanins located in epidermal cells does not affect neither chlorophylls levels neither the light capture features of PSII. In spite of equal levels of chlorophylls and PSII photochemistry upstream of Q_A^- in red and green leaves, red leaves had reduced overall photosynthetic efficiency due to impaired linear electron transport in thylakoid membranes.

SP29

HOW PLANTS KEEP THE BALANCE AMONG THREE TARNSLATION TERMINATION COUPLED EVENTS, TRANSLATION TERMINATION, READTHROUGH AND NONSENSE-MEDIATED mRNA DECAY?

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When a translating ribosome reaches a stop codon three things, translation termination, translational readthrough (RT) or Nonsense-mediated mRNA decay (NMD) can happen. The outcome depends on the stop context and the level of the eRF1 termination factor. At normal stop context, the eRF1 binds to the stop and terminates translation, while RT occurs when the stop is in an improper context. High eRF1 level intensifies termination and reduces RT. If the 3'UTR contains NMD cis elements (unusually long 3'UTR or a spliced intron in the 3'UTR), the translation is terminated, however, the mRNA is quickly degraded as the eRF1 recruits the NMD complex.

We show that in plants an elegant regulatory system has evolved that controls the eRF1 level via RT and NMD, thereby ensuring the balance among termination, RT and NMD. We found that in plants, unlike in other eukaryotes, eRF1 is a multicopy gene and that expression of one eRF1 allele (eRF1-1) is regulated by both RT and NMD. Relevantly, RT rescues the eRF1-1 mRNAs from NMD. We prove that this structure can buffer the fluctuations in eRF1 level and/or NMD intensity.

Normally, the eRF1-1 RT is weak and eRF1-1 mRNAs are moderately targeted by NMD. However, if eRF1 level is increased, the eRF1-1 RT is reduced, thus the NMD will target eRF1-1 mRNAs more efficiently. Hence, the eRF1-1 protein expression will be reduced and the normal concentration of the eRF1 protein will be restored. Moreover, as we found that the intensity of plant NMD correlates with the eRF1 protein level, this regulatory circuit can also buffer the fluctuations of NMD efficiency. For instance, enhanced NMD leads to reduced eRF1-1 mRNA expression, decreased eRF1 protein level, and consequently to attenuated NMD activity. We also show that this specific eRF1 regulation is extremely conserved within higher plants.

SP30
PLANT SERYL-tRNA SYNTHETASES: FIDELITY AND MACROMOLECULAR RECOGNITION

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Aminoacyl-tRNA synthetases (AARSs) catalyze the attachment of amino acids to their cognate tRNAs to establish the genetic code. To obtain the high degree of accuracy that is observed in translation, these enzymes must exhibit strict substrate specificity for their cognate amino acids and tRNAs. Many AARSs participate in macromolecular associations that improve their function or allow them to participate in various cellular processes, other than translation.

Among eukaryotes, the recognition elements of $tRNA^{Ser}$ were analyzed in detail only in yeast and humans. To determine identity requirements in plant serine system, cross-species complementation test and aminoacylation assay were performed. Maize cytosolic SerRS (SerZMc) efficiently recognized bacterial and eukaryotic $tRNAs^{Ser}$ indicating that SerZMc can accommodate various types of $tRNA^{Ser}$ structures. Genetic experiments using *E. coli* tyrosine-specific tRNA suppressor showed that the discriminator base G73 is crucial for recognition by SerZMc. The above data indicate that maize cytosolic SerRS has a broad tRNA specificity and a very low requirement for tRNA identity elements. Although SerZMc efficiently recognized bacterial $tRNAs^{Ser}$, which are similar to maize organellar $tRNAs^{Ser}$, GFP tagging experiments indicated its exclusive cytosolic localization. Since maize nuclear genome contains numerous organellar $tRNA^{Ser}$ genes, we hypothesize that some of them are expressed and their corresponding tRNAs are recognized by SerZMc in the cytosol.

Fidelity of translation is important for normal cell functioning as well as for recombinant protein production of biotechnological importance in plant cytosol or chloroplasts. Therefore, we compared the fidelity of maize cytosolic and dually targeted organellar SerRS (SerZMo) with respect to amino acid recognition. Both enzymes weakly misactivated near-cognate amino acids threonine and cysteine, SerZMo being slightly more accurate enzyme compared to SerZMc. However, both enzymes were capable of hydrolytic editing implying their high overall accuracy.

Thus far, macromolecular associations of plant AARSs were not reported. We used high throughput interaction technologies to determine protein partners of plant SerRS. Potential interactors identified in Y2H screen could not be confirmed *in vitro*, possibly due to transient nature of their interactions with SerRS. Preliminary data of TAP/MS experiments indicate various translation factors as putative interactors of plant SerRS.

SP31
STRUCTURAL PREFERENCES OF RANDOM *DE NOVO* PROTEINS

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Proteins can emerge *de novo* from the translation of previously noncoding DNA segments [1]. However, present-day proteins seem to have evolved to avoid high propensity for aggregation, a potential hazard to living cells [2]. This rises the question how large the aggregation potential of *de novo* proteins can be, i.e. whether they aggregate at a higher rate than extant proteins by chance. To address this question, we have generated *in silico* random DNA segments with systematically altered GC-content, translated them, and subjected the resulting 160-residue protein sequences to consensus predictions to assess their propensity to form disordered regions, transmembrane helices and aggregates. We identified clear trends in the investigated properties based on the GC-content of the underlying DNA segments: polypeptides translated from segments of high GC content tend to be disordered and not prone to aggregation or to form transmembrane helices. The three-dimensional region defined by the three properties of sequences translated from random DNA with a GC-content of 40, 50 and 60 % lies practically entirely within that spanned by the properties of the human proteome. The largest observed difference between these two data sets is the higher occurrence of transmembrane helices in the human proteome. Our results suggest that aggregation is not a serious risk for *de novo* proteins, at least not higher than for extant ones.

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SP32

OVERLAPS IN SIGNALING NETWORKS – SYSTEMS-LEVEL IDENTIFICATION OF CROSS-TALKS AND CRITICAL NODES IN SIGNALING PATHWAYS

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Signaling pathways control a large variety of cellular processes and they are now viewed as a set of intertwined pathways forming a single signaling network. Reliable analyses of this system need uniform pathway definitions and curation rules. Accordingly, we created SignaLink, a signaling resource containing 8 major signaling pathways from the nematode *C. elegans*, the fly *D. melanogaster*, and humans. With SignaLink we found that in humans any two of the 8 pathways can cross-talk. We provide several visualizations of the cross-talk network. SignaLink is available at http://SignaLink.org.
Recently, we extended the coverage and depth of SignaLink by creating Reguling http://Reguling.org), an integrated database of the regulation of signalling. Here, we linked scaffold proteins and proteins involved in endocytosis to pathway proteins. This led us to identify cross-talk organizing endosomes. Next, we extended the network with directed protein-protein interactions. The direction and the confidence for each interaction were calculated based on domain-domain and domain-motif interactions. In the next step, we included the underlying regulatory network: (1) downstream transcription factors and their subnetworks, based on manual curation of primary literature; (2) interactions between transcription factors and transcription factor binding sites of genes, using OregAnno, JASPAR, and MPromDB; (3) mRNA transcripts (from ENSEMBL), miRNA transcripts (from miRBase, miRGen and PutmiR), and their interactions (from miRecords and Tarbase). The database can be freely downloaded in various network file formats (BioPAX, SBML, CSV, etc.) via a BioMART-like download page, where users can filter the datasets. Reguling will allow us and the research community to analyze signalling cross-talks in a more complex layer, via transcription and post transcriptional regulation.
These resources allowed us to investigate cross-talk regulation to specify signalling flow. We analyzed critical nodes, a group of proteins previously defined as isoform proteins with partly different function and regulation, where at least one isoform has cross-talk to another pathway. Within a critical node the ratio of each isoforms determines the direction of the signalling flow. We identified critical node proteins on a systems-level and examined their dispensability in the global signalling flow. Moreover, we analyzed their role in evolution and during tumorgenesis.

SP33

COLLECTIVE MOTION OF CELLS MEDIATES SEGREGATION AND PATTERN FORMATION IN CO-CULTURES

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Pattern formation by segregation of cell types is an important process in various biological events including embryonic development. We show that an experimentally yet unexplored mechanism based on collective motion of segregating cells enhances the effects of known pattern formation mechanisms such as differential adhesion. We used fluorescent time-lapse videomicroscopy in two-dimensional mixed cell cultures composed of various pairs of cell types with different inherent motility characteristics. We also altered the directionality of cell migration by the inhibition of Rac1 GTP-ase know as a switch between random versus directional cell migration. We demonstrate that more correlated cell migration, characterized by longer directional persistence length of migrating cells, results in faster and more extensive segregation into homogeneous cell clusters, monitored by various statistical physical methods. The growth of the characteristic scale of cell clusters generally follows an algebraic scaling law and this process is faster, characterized by higher exponent values, in the presence of collective motion, compared to random motion. This work is based on and presented with time-lapse live cell imaging videos.

SP34
ADIPOCYTE CELL DEATH AND CLEARANCE

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Nowadays obesity is an epidemic health problem worldwide, enhancing the risk for metabolic disorders such as type 2 diabetes (T2DM), nonalcoholic fatty liver disease (NAFLD), metabolic X syndrome and cardiovascular diseases. Hypertrophic adipose tissue is associated with a rise of free fatty acids (FFA), adipokines and proinflammatory molecules (such as IL6, TNFα, 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 adipocyets, in a “crown like” structure, release more proinflammatory 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 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 which is differentiated *in vitro* into adipocytes. The macrophages are derived from primary human monocytes. We intended to characterize the cell death types of adipocytes, and to determine the cytokine profile of adipocytes during differentiation and the cytokines released during phagocytosis of adipocyte corpses. The lipid content of differentiating adipocytes has been measured on a time curve by laser scanning cytometry (LSC), to identify the stages of differentiation, and their cell death profile has been analyzed. The ideal time point for studying interaction of adipocytes with macrophages and the resulting proinflammatory effect has been determined.

We detected an increasing level of spontaneous adipocyte cell death during the progress of adipogenic differentiation. Macrophages can efficiently phagocytose differentiated, dying adipocytes. Coincubation of differentiated adipocytes and macrophages leads to IL6 production, and they are not able to block LPS induced IL6 and TNFα production. Dying adipocytes can inhibit LPS induced IL6 and IL1β production. 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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SP35
THE Mg ION REGULATES NUCLEOTIDE HYDROLYSIS IN A NOVEL WAY IN dUTPase

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Mg^{2+} regulates enzymes in various ways but usually acts as a significant activator. Mg^{2+} is the most prevalent physiological cofactor for nucleotide hydrolysis as well. Here we report that dUTPase, a nucleotide diphosphate hydrolase, uses the Mg^{2+} cofactor in a unique manner, only as a conformational rectifier. Although Mg^{2+} is the physiological cofactor for dUTPase, its presence at the active site only increases the steady-state hydrolysis activity twofold. The dUTPase enzymatic activity is indispensable to balance cellular dUTP/dTTP levels. Lack of the enzyme leads to erroneous DNA synthesis and finally cell death. To investigate the influence of Mg^{2+} on the catalytic mechanism and the structure of human dUTPase, a broad array of techniques were employed ranging from transient kinetics to crystallography and molecular dynamics simulations. As reported by fluorescence spectroscopy, of two predominant substrate conformations Mg^{2+} facilitates the formation of the catalitically competent “gauche” conformation at the alpha-phosphate group allowing the nucleophilic attack of the catalytic water on the alpha-phosphorus atom. Our molecular dynamics simulations reinforced that the Mg^{2+} -complexed nucleotide prefers the “gauche” conformation within the active site in contrast to the uncomplexed nucleotide, which adopts two predominant conformations. The proton inventory assay indicated that in the absence of Mg^{2+} only one proton is transferred in the transition state at pH 7.5 instead of the two protons liberated in saturating Mg^{2+}. Otherwise, the basic mechanism of the hydrolysis reaction seems to be unaffected by Mg^{2+}. The effect of Mg^{2+} on the structural integrity of the homotrimeric enzyme is more important. We revealed that the human dUTPase has two structural metal-binding sites within the central channel of the enzyme with different binding affinities toward the Mg^{2+} ions. The presence of Mg^{2+} in the central channel results in increased melting temperature and resistance towards tryptic digestion. In conclusion, our findings indicate a yet undescribed enzyme regulation mechanism by the Mg^{2+} ion.

SP36

INTERACTIONS BETWEEN SIRT1 AND POLY(ADP-RIBOSE) POLYMERASES – NOVEL MODES OF METABOLIC REGULATION?

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SIRT1 is a NAD^+-dependent protein deacetylase and its NAD^+-dependence enables SIRT1 to act as a sensor of cellular metabolism. SIRT1 is activated by increases in NAD^+ levels, or indirectly by different small molecule activators. SIRT1 activation leads to the deacetylation and activation of numerous metabolic transcription factors such as PGC-1 and FOXOs that culminates in the enhancement of mitochondrial biogenesis. Activation of SIRT1 in mice results in higher whole-body energy expenditure (EE) and protection against high-fat diet (HFD)-induced obesity abd diabetes. NAD^+ dependence of SIRT1 activity suggested that artificially silencing other NAD^+ consumers, such as PARPs, could effectively induce SIRT1 activity. Our results demonstrate that the deletion of either PARP-1, or PARP-2 in mice, equally results in a leaner phenotype, higher EE and SIRT1 activation that was protective against HFD-induced obesity and diabetes. PARP-1 and SIRT1 are interrelated due to the competition for the same limiting NAD^+ pool. However, the link between PARP-2 and SIRT1 is based on a direct interaction between PARP-2 and the proximal SIRT1 promoter, where PARP-2 acts as a supressor. We further tested our observations in a model of Doxorubicin (DOX)-induced cardiovascular damage. DOX enhances free radical production and hence induce DNA damage, PARP activation, and cellular and mitochondrial dysfunction. PARP-1 knockout mice are protected against DOX-induced damage, what prompted us to investigate whether the deletion of PARP-2 could be equally protective. Aortae from PARP-2 knockout mice were partially protected against DOX-induced smooth muscle damage. The depletion of PARP-2 does not drastically reduce PARP activity suggesting a different protective mechanism than in PARP-$1^{-/-}$ mice. Therefore we hypothesized that SIRT1-induced mitochondrial stabilization could be the protective factor. We have observed the induction of SIRT1 upon PARP-2 depletion both *in vivo* and *in vitro*. Induction of SIRT1 induced mitochondrial biogenesis and protected mitochondria against DOX-induced damage. The results presented hereby depict a new modality for the activation of mitochondrial biogenesis and presents different means of applicability.

Supported by: Bolyai fellowship to PB, National Innovation Office (FR-26/2009, Baross program Seahorse grant), OTKA CNK80709, K82009, K75864, PD83473, TÁMOP-4.2.2/B-10/1-2010-0024 and TAMOP-4.2.2-08/1-2008-0019 and UD MHSCMecenatura (Mec-8/2011).

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SP37

dUTPase IS ESSENTIAL FOR GENOME STABILITY AND IMAGINAL TISSUE DEVELOPMENT IN *DROSOPHILA*

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Proper S phase progression during cell cycle requires a balanced and abundant nucleotide pool. Impairment in the nucleotide biosynthesis may lead to DNA replication errors and DNA damage checkpoint activation and also can violate genome integrity. dUTPase has the role to maintain uracil-free genome by eliminating dUTP from the nucleotide pool. However, physiological fate of the unusual uracil-substituted DNA is not described in details. Depletion of dUTPase in some organisms (*E. coli*, *S. cerevisiae*, *A. thaliana*, *C. elegans*) revealed its conservative role to preserve genome integrity and avoid DNA repair responses. Among uracil-DNA glycosylases, UNG is the most important DNA repair enzyme that is responsible for the majority of uracil-DNA processing, and whose depletion can complement dUTPase deficiency at least partially. Since *Drosophila* lacks the UNG enzyme, it provides a hopeful model to study the fate of uracil-substituted DNA.

We showed that dUTPase expression is restricted mainly to undifferentiated proliferating tissues. Furthermore, genomic uracil substitution depends on the dUTPase expression pattern in *Drosophila* larval tissues, since absence of UNG allows the persistence of uracil-DNA in differentiated tissues. Perturbing this pattern by dUTPase silencing increased the rate of genomic uracil substitution in undifferentiated tissues as well. However, lack of UNG does not guarantee complete tolerance to uracil-DNA. We detected an increased rate of DNA strand breaks in imaginal wing precursor tissue and developmental defects in pupal stage. Our suggested candidate for uracil-DNA processing is the mismatch-specific TDG homologue uracil-DNA glycosylase, Thd1, whose silencing complemented pupal lethality of dUTPase silenced animals.

SP38

THE ROLE OF CYP3A ENZYMES IN CLONAZEPAM METABOLISM

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Clonazepam is a benzodiazepine-type drug, acting as post-synaptic $GABA_A$ receptor modulator. It has potent anticonvulsant, muscle relaxant, anxiolytic, and hypnotic properties, and as such is often prescribed for the treatment of panic attacks, generalized anxiety and social phobias. It is also used in the treatment of epilepsy for its anticonvulsant effect, as it raises the threshold and can prevent generalized epileptic seizures from occurring. Clonazepam is metabolized by the CYP3A4 enzyme catalyzing nitro-reduction into 7-amino-clonazepam, which is a pharmacologically inactive metabolite.
In our study the effect of CYP3A polymorphisms on clonazepam metabolism was examined in human liver microsomes using cytochrome P450 selective inhibitors. The levels of the 7-amino-clonazepam metabolite were determined by LC-MS/MS. During the *in vitro* inhibition studies, CYP3A4/5 selective ketoconazole was found to significantly inhibit clonazepam metabolism.
The differences in clonazepam metabolism observed in psychiatric patients can be explained by the genetic polymorphism of *CYP3A5* and the changes in CYP3A4/5 expression due to various environmental and endogenous factors. Patients' drug-metabolizing capacity can be qualified as poor (individuals with low or no activity for a given isoenzyme) and extensive metabolizers (individuals with faster than average substrate metabolism rates). The *CYP3A5*3/*3* homozygous mutant genotype, resulting in the lack of CYP3A5 expression, is present in 90% of the white population. The *CYP3A5*1/*3* heterozygous genotype results in CYP3A5 enzyme expression, which leads to an increase in the metabolism of CYP3A substrates, as the enzyme activity of CYP3A5 is additive to the CYP3A4 activity.
The CYP3A5 genotype and CYP3A4 enzyme expression levels for psychiatric patients treated with clonazepam were determined by quantitative real-time PCR, and serum levels of clonazepam by LC-MS/MS. Of the 19 patients in the study two were found to have *CYP3A5*1/*3* genotype, while the others were of the *CYP3A5*3/*3* typical for the white population. Based on CYP3A4 mRNA levels, seven patients were characterized as poor metabolizers, with the rest being intermediate metabolizers.
Our results show that *CYP3A5* genotype does not influence serum levels, whereas CYP3A4 phenotype (CYP3A4 mRNA expression) has a significant effect on clonazepam serum concentrations.
This study was funded by the European Union, and co-funded by the European Regional Development Fund (GOP-1.1.1-09/1-2009-0001).

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SP39

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 core promoters (H3K4trimethyl) the enhancers (H3K27 acetylation) or the binding sites of specific transcription factors in a whole genome approach. In order to generate high quality date we designed to standardize the sample preparation procedure and to develop a methematical modell that explains sample behaviour in ChIP Seq.
A basic question in the planning of these experiments is the quality controll of the sample. Although our lab had signifficant experience in ChIP QPCR, several steps of the sample processing had to be changed to generate high quality data in the ChIP Seq system. Intrestingly the optimal fragment length for sequencing on Illumina system (300bp) seems to be difficult to be achieved by sonication without the disruption of large protein-DNA complexes of the sample. As a negative controll we decided to use IgG controll, a non targed genomic region and if possible genetically engeneered samples that lack the specific protein. A critical step of the ChIP Seq experiments is the lack of process controlls. We designed and generated a set of spikes that can be added to the chromatin sample and their enrichment shows the performance of the protocoll. While early ChIP Seq experiment were using up to 3 million reads for the characterization of samples, the development of the sequencing technology allows us to have sequencing depth of 50 million reads or even higher. For an optimal design of the ChIP Seq experiments we designed an algorithm that allows us to characterize the sample behaviour from a smaller set of reads. We will present our results and how this algorithm could be used for the characterization ofother complex samples like mapping of rearrangements in cancer samples by deep sequencing.

SP40

HISTONE VARIANTS INDEXING THE PLASMODIUM FALCIPARUM EPIGENOME

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Plasmodium falciparum, the causative agent of malaria, is responsible for almost 1 million deaths annually. No efficient vaccine is available and resistance against the current and only available medication is spreading. Drugs influencing the epigenetic regulatory processes are promising candidates to battle this devastating disease, but our knowledge about the mechanisms that constitute it's epigenome is limited.

After optimising next-generation sequencing technology to the extremely AT-rich genome, we performed high-resolution genome-wide profiling (ChIP-seq) of the 4 histone variants encoded in the *P. falciparum* genome. Interestingly, all variants primarily localize to the euchromatic part of the *P. falciparum* epigenome, whereas silent heterochromatic domains (encoding amongst others antigenic variation gene families) are largely devoid of variants and are mainly occupied by canonical histones. Within this euchromatic domain the histone variants show distinct localisation: i) H3.3 is primarily present in the coding body of genes and its occupancy correlates with the transcriptional activity; ii) H2A.Z and H2Bv containing nucleosomes are constant features of the intergenic regulatory regions; iii) while CenH3 is strictly confined to an approximately 4.5kb centromeric region present once on each chromosome.

Our data provides a complete map of the histone variant occupancy in the *P. falciparum* epigenome and strong indication for the use of histone variants in indexing functionally different parts of the malaria genome.

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SP41

ABCC6, THE PSEUDOXANTHOMA ELASTICUM DISEASE GENE, IS A METABOLIC SENSOR

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Pseudoxanthoma elasticum (PXE), a rare recessive genetic disease causing xanthomas and cutis laxa is characterized by the calcification of elastic fibers. The disorder is due to loss-of-function mutations of the *ABCC6* gene but the pathophysiology of the disease is still not understood. Here we investigated the transcriptional regulation of the gene, using DNase I hypersensitivity assay followed by luciferase reporter gene assay. We identified three DNase I hypersensitive sites (HS) specific to cell lines expressing *ABCC6*. These HS are located in the proximal promoter and in the first intron of the gene. We further characterized the role of the HS by luciferase assay and demonstrated the transcriptional activity of the intronic HS. We identified the CCAAT/Enhancer binding protein ß (C/EBPß) as a factor binding the second intronic HS by chromatin immunoprecipitation and corroborated this finding by luciferase assays. We also showed that C/EBPß interacts with the proximal promoter of the gene. We propose that C/EBPß forms a complex with other regulatory proteins including the previously identified regulatory factor HNF4α. This complex would account for the tissue-specific expression of the gene and might serve as a metabolic sensor. Our results point toward a better understanding of the physiological role of *ABCC6*.

SP42

IMPORTANT, DISTINCTIVE ROLE OF THE RSC COMPLEX IN CHROMATIN STRUCTURE REMODELLING AT THE YEAST *PHO* PROMOTERS

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The massive transition of chromatin structure at the yeast *PHO5* promoter from a repressed to an active, open state was clearly demonstrated to be a prerequisite for promoter activation. We have previously shown that out of 15 non-essential chromatin-remodelling ATPases examined, chromatin structure remodelling at the *PHO5* promoter involved the SWI/SNF and Ino80 remodelling complexes. In the absence of either SWI/SNF or Ino80 complex, chromatin opening kinetics were strongly delayed and by simultaneous inactivation of both complexes synthetic effect was observed, but eventually complete opening was accomplished. Therefore no essential remodelling complex at the *PHO5* promoter has been identified yet. The RSC complex is essential, the most abundant chromatin-remodelling complex in yeast and has been shown to disassemble nucleosomes *in vitro*. As inactivation of the RSC ATPase subunit Sth1 is lethal, we have used a temperature sensitive *sth1^{td}* mutant to assess the possible effect of RSC inactivation on the *PHO5* chromatin remodelling. The rate of chromatin opening and the consequent activation of the *PHO5* promoter upon physiological induction in phosphate-free medium were significantly delayed by Sth1 depletion and this effect was even more pronounced under weaker induction conditions. Chromatin remodelling and consequent activation of a Gal4-activated *PHO5* promoter variant, induced through GAL-signalling pathway, was similarly affected by RSC inactivation showing that the observed effect was independent of the induction conditions and transactivator involved. However, simultaneous inactivation of SWI/SNF and RSC complexes completely prevented remodelling of the native *PHO5* promoter, as well as the promoter variant, showing a functional interplay of the two complexes in the remodelling process. Also, inactivation of the RSC complex in the *isw1 chd1* double mutant, which by itself showed a significant delay in the kinetics of *PHO5* chromatin remodelling, completely abolished remodelling. Altogether these results pointed out a crucial, distinctive role of the RSC complex for the remodelling process in the absence of dedicated remodellers like SWI/SNF, Isw1 and Chd1. Interestingly, inactivation of the RSC complex had no significant effect on chromatin remodelling at the other two *PHO* promoters, *PHO8* and *PHO84*, which are coactivated with *PHO5*.

SP43

CREM IN ADRENAL CIRCADIAN REGULATION: EPIGENETIC ACTIVATION OF *CYP17A1* AND IMPACT ON *PER1,2* EXPRESSION IN *CREM* KNOCKOUT MICE IN COMPLETE DARKNESS

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cAMP responsive element modulator CREM encodes multiple activators and repressors. The best known are activator CREMτ with essential role in spermatogenesis (*Crem-/-* males are infertile) and repressor ICER involved in circadian regulation of melatonin synthesis in the pineal gland. Due to ubiquitous expression of ICER and its involvment in cAMP autoregulatory loop, we hypothesized that *Crem* isoforms might have a more general role in circadian regulation of cAMP-responsive core clock genes and metabolic output genes. By applying *Crem* knockout (ko) mice we show that in complete darkness (DD) CREM isoforms contribute to circadian expression of steroidogenic cytochromes P450 (CYP) in the adrenal. Most striking is the CREM dependent hypo-methylation of *Cyp17a1* promoter at ZT 12 that results in higher *Cyp17a1* mRNA and protein expression in ko adrenals. (*Kosir & Prosenc-Zmrzljak et al., FEBS J. 2011)*. The epigenetic repression of mouse *Cyp17* in wild type adrenals results in different corticosteroids in mouse (corticosterone) *versus* the human (cortisol). In contrast to our expectations, the liver and adrenal circadian expression did not differ significantly between wt and *Crem* ko mice for majority of evaluated genes, suggesting a minor role of *Crem* products in circadian regulation in DD *in vivo*. For *Per1* and *Per2* the phase-delay is indicated in *Crem* ko adrenals and ICER binds to CRE1 promoter element of *Per1* alone or together with CREB. The current topic is to evaluate whether a broader physiological role of CREM signalling on the periphery is linked to the light phase.

AP1

Bio-Science AWARD 2012

REGULATION OF PHAGOCYTOSIS BY THE NOVEL RACGAP ARHGAP25

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Background: Members of the Rho family small GTPases play essential role in the signal transduction of innate immunity. GTPase activating proteins (GAPs) decrease the amount of the GTP-bound, active form of small GTPases and contribute to the termination of biological signals. According to *in silico* data ARHGAP25 is specifically expressed in haematopoietic cells which suggest its role in immune responses. The aim of our study was to reveal the role of ARHGAP25 in human neutrophils.
Materials and methods: GAP activity of ARHGAP25 was measured in classical GAP assay and in ARHGAP25-overexpressing COS7 cells. ARHGAP25-silenced PLB-985, ARHGAP25 knock down primary human macrophages and ARHGAP25-overexpressed COS*phox*FcγR cell lines were used for functional studies. We prepared the loss-of-function mutant ARHGAP25R192A to investigate the role of GAP activity in phagocytosis.
Results: Our Northern blot experiment confirmed that ARHGAP25 was highly expressed in haematopoietic cells and we could detect the protein in the major white blood cell types. We found that ARGAP25 acts as a RacGAP *in vitro* and *in vivo* as well. Silencing of ARHGAP25 increased the phagocytosis in PLB cells and in human macrophages. In knock down PLB cells we found increased amount of filamentary actin and an increase in phagocytosis-induced superoxide production. In COS*phox*FcγR cells, overexpression of the protein blocked phagocytosis. Mutation of the critical arginine in the GAP domain abolished this inhibitory effect.
Conclusion: We suggest that ARHGAP25 RacGAP regulates phagocytosis of neutrophils by controlling Rac-dependent F-actin reorganisation.

Poster Abstracts

PI-1

INTERACTION OF LYSOPHOSPHATIDIC ACID WITH PH DOMAINS

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In signaling the activation of cell-surface receptors leads to selective gene transcriptions or immediate physiological reactions. In these processes many multicomponent complexes are formed at the membranes containing several proteins as well as membrane-lipids. The structure of signaling proteins is modular by which domain-domain or domain-lipid interactions are evolved. Some of these domains are responsible for lipid binding and are evolutionarily conserved among eukaryotes.
The aim of this study is to understand the mechanistic details of some protein - lipid interactions in signaling. Several cell membrane-associating proteins contain domains which directly interact with lipids, in our case plecktsrin homology (PH) domains. Our attention turned to physiologically important lysophospholipid mediators, especially lysophosphatidic acid (LPA), as it previously has been reported that LPA binds to the PH domain of gelsolin, possibly interfering the inositol polyphosphate binding of this domain, but the molecular details of the interaction is not known.
In the course of our work we aimed at characterizing the interaction between several PH domains and LPA using biochemical and biophysical methods in vitro. After expression of the PH domain and the entire protein of gelsolin, fluorescence and CD spectroscopy as well as isothermal titration calorimetry measurements indicated that LPA can bind to the PH domain of gelsolin over its critical micelle concentration. Thus the protein interacts with the lipid in its associated form instead of the individual molecule. This interaction is specific to LPA with a nanomolar Kd value. Based on our findings we have looked for other PH domain containing proteins, namely Vav2, Akt1 and Grp1, seeking for similar interactions. We expressed the PH domains of both proteins and characterized their interaction with LPA. We also carried out live-cell confocal microscopy with GFP-tagged PH domains of Akt1 and Grp1. We studied the changes in spatiotemporal localization of these GFP-PH domains by modifying the intracellular level of LPA, either by stimulating or inhibiting its metabolizing enzymes.
Our 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 with high affinity, revealing new aspects of lysophospholipid-protein interactions.

PI-2

INFLUENCE OF GROWTH CONDITIONS ON FATTY ACID AND NEUTRAL LIPID COMPOSITION OF THE MITOCHONDRIA OF THE BOTTOM-FERMENTING BREWER'S YEAST

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Strain of Saccharomyces cerevisiae yeast used for the production of lager beer is known as the bottom-fermenting brewer's yeast. It undergoes propagation and fermentation during which it is exposed to various types of stresses among which the strongest effect on the cells have anaerobic conditions and ethanol toxicity. Ethanol tolerance is closely related to the lipid composition of the mitochondria, which play an important role in brewing yeast physiology and fermentation performance.

The aim of this study was to determine fatty acid and neutral lipid composition of the mitochondria of the bottom-fermenting brewer's yeast in aerobically grown pure culture (0^{th} generation) and in the 1^{st} and 4^{th} recycled generations grown anaerobically. Mitochondria were isolated after enzymatic disruption of the cell wall. Lipids were extracted by Folch method. Fatty acids were determined by GLC of corresponding methyl esters. Neutral lipids were separated by two-step TLC of total lipid extract and quantified by densitometry. The content of saturated and unsaturated fatty acids was almost the same in the pure culture, while in the recycled generations unsaturated acids prevailed (56 and 57%, respectively). C16-acids prevailed significantly in all generations accounting from 64 to 72%. Palmitic acid was the main in the 0^{th}, while palmitoleic acid in the 1^{st} and 4^{th} generation. Among neutral lipids, only ergosterol and squalene were present in significant amounts. The concentration of ergosterol was the highest in the 0^{th} generation (9.7 μg/mg proteins). The mitochondria of all analysed generations contained substantial amounts of squalene (4.0 – 7.1 μg/mg proteins).

In conclusion, we may say that growth conditions influenced fatty acid and neutral lipid composition of the mitochondria of the bottom-fermenting brewer's yeast strongly and in a very peculiar way. We assume that the accumulation of squalene has an assigned function in the regulation of membrane fluidity and permeability as a part of adaptive mechanism.

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PI-3

DESIGNED SELF-ASSEMBLING POLYPEPTIDE TETRAHEDRON

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Self-assembly is one of the most powerful concepts in nature contributing to its rich diversity. DNA origami provides examples of spectacular artificial structures defined at the nanometric scale[1,2]. However design of polypeptide fold is significantly more complex as it involves a large number of cooperative interactions. Inspired by the DNA origami, we aimed to design self-assembling polypeptide nanostructures. We circumvented the polypeptide folding problem by using a principle of nanostructure design based on the assembly from the connected rigid rod-like segments with intervening flexible hinges. The defined order of segments enables formation of structures based on the predictable pair-wise interactions among segments. In this way we can build different polyhedral structures where coiled-coil dimeric segments represent the edges of the assembled structure[3]. We designed a single polypeptide chain, comprising concatenated natural and designed coiled-coil-forming peptide segments. Gene was codon optimized for *E. coli*, where the polypeptide was expressed and purified. Refolding of the denatured polypeptide was performed by dialysis under conditions that promote self-assembling. Formation of the predicted coiled-coil content was confirmed by circular dichroism measurements. Dynamic light scattering analysis showed homogeneous size distribution of assemblies in solution, with sizes around 7 nm. The direct evidence for the polypeptide assembling into tetrahedron is provided by microscopic images using TEM and AFM. Described polypeptide design represents a new platform for the creation of bionanostructures with properties unseen in nature.

PI-4

THE INTERACTION OF NEP1-LIKE PROTEINS WITH LIPIDS

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The family of necrosis- and ethylene-inducing peptide 1 (NEP1)-like proteins (NLPs) elicit diverse defense reactions and cell death in dicotyledonous plants. They are cytolytic proteins, structurally similar to actinoporins, pore forming toxins from marine invertebrates. A membrane disrupting activity was demonstrated for NLPs [1]. The other proteins that have similar structure are fungal lectins. All these proteins interact with specific structures at the membranes surface; actinoporins with sphingomyelin and lectins with carbohydrates. When plasma membranes were pretreated with different proteases and glycosidases the NLP proteins still bound to the membranes, therefore, the target molecule on the host cell is most probably of lipid origin. We have used multilamellar vesicles prepared from lipid extract from tobacco leaves in a binding assay for different NLPs (NLPPp from Phytophthora parasitica, NLPPcc from Pectobacterium carotovorum subsp. carotovorum and NLPPya from Pythium aphanidermatum). The secondary structure of proteins at different pH values was evaluated by using circular dichroism. Crystal structure of NLPPya reveals a single-domain molecule with a fold consisting of a central β-sandwich, with 3 strands in the first sheet and a 5-stranded antiparallel second sheet. Three helices encompass the second sheet at the top of the sandwich. At the base of the protein an uneven surface is established mainly by 3 broad loops [1]. We investigated the protein structure by using different pH. According to the far UV circular dichroism spectra the proteins do not undergo structural changes upon pH change from 7.4 to 5.5. When comparing the near UV circular dichroism spectra we observed changes when lowering pH to 5.5 in the range where phenylalanine has a characteristic profile.

[1] A common toxin fold mediates microbial attack and plant defense. Ottmann C, Luberacki B, Küfner I, Koch W, Brunner F, Weyand M, Mattinen L, Pirhonen M, Anderluh G, Seitz HU, Nürnberger T, Oecking C. Proc Natl Acad Sci U S A. 2009 Jun 23;106(25):10359-64. Epub 2009 Jun 11.

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PI-5

INVESTIGATING TRANSEPITHELIAL PERMEABILITY OF PROTEINS USING CACO-2 CELL BASED *IN VITRO* EPITHELIUM MODEL

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Due to increasing importance of biopharmaceuticals, which are structurally mostly protein drugs, and a long lasting desire for enable their oral delivery, there is a need to establish a good and reliable intestinal epithelium model for evaluation of protein drugs transepithelial permeability. Cell culture based *in vitro* models are commonly used for predicting transepithelial permeability in drug delivery studies and Caco-2 cell intestinal epithelium model represents the most widely used one. Although most of oral drug delivery studies on Caco-2 model were performed with low molecular substances, the aim of our study was to use this model for investigating transepithelial permeability of proteins. Few protein drugs were used in permeability experiments on Caco-2 model and apparent permeability coefficients (Papp) were calculated. It has been found out that all tested proteins have almost identical dynamics of transport across Caco-2 and a very low permeability was determined. Average Papp values were at approximately 4 X 10^{-10} cms^{-1} for different proteins whereas free diffusion through permeable support without a cell layer was about 1000 x higher, indicating a very wide detection range. According to low basic permeability of proteins, an enhancer of paracellular permeability was used to temporarily lose intercellular connections and to lower transepithelial electric resistance. Consequently protein permeability rose up close to a level of flow in cell-free setup. Comparison to permeability of low molecular permeability markers in the same conditions revealed similar response as for paracellular permeability marker. Our results thus suggest involvement of paracellular route in protein transepithelial transport.

PI-6

DOMAIN ORGANISATION OF MACPF PROTEINS

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Proteins with MACPF (Membrane Attack Complex/Perforin) domain are classified in a large MACPF protein family which includes both lytic and nonlytic proteins. In lytic members, MACPF domain is necessary for insertion of protein into the membrane or for oligomerization and formation of a transmembrane pore. Several MACPF proteins, especially lytic ones, contain additional domains, necessary for binding of protein to lipid membranes.
In the present study we examined domain organisation of protein sequences that contain MACPF domain. These were acquired by searching public databases using PSI-BLAST, BLASTP and TBLASTN algorithms. As a probe we used MACPF domain sequences of human perforin, Bth-MACPF from *Bacteroides thetaiotaomicron* and Plu-MACPF from *Photorhabdus luminescens*. We were able to retrieve 809 different protein sequences containing the MACPF domain from 192 different species. 91.6 % of all sequences belong to 142 different eukaryotic species, while 8.3 % of sequences derive from 49 distinct prokaryotic species. We also found one virus protein with a MACPF domain in a virus *Anguillid herpesvirus 1*.
Presence of other domains was examined using InterProScan tool. Out of 410 examined MACPF-domain containing protein sequences, half contain additional protein domains. Domain organisation was found to be more complex in eukaryotes. We found 39 and 22 different additional domains in MACPF protein sequences from Metazoa and Bacteria, respectively. Extra domains found in Bacteria were distinct from the ones found in Metazoa group. In 97 % of bacterial MACPF protein sequences extra protein domains were found on the C terminal part of sequence, while for animal sequences the same was true in 54 % of cases. Some of these additional domains may be responsible for binding to either membranes (*e.g.* MABP or C2) or carbohydrates (*e.g.* Ricin B lectin or MIR). Interestingly, seven sequences supposedly contain several MACPF domains; *e.g.* predicted protein sequence from *Taeniopygia guttata* was found to contain two MACPF domains, while hypothetical protein from *Babesia bovis* strain T2Bo contains even three MACPF domains.

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PI-7

CELL-ADHESION MOLECULES ARE MARKERS OF PLASTICITY IN HIPPOCAMPAL TISSUE AFFECTED BY ALZHEIMER'S NEURODEGENERATION

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Alzheimer's disease (AD) is characterized by up-regulated expression of plasticity molecules, particularly in hippocampus and entorhinal cortex, reflecting activation of compensatory mechanisms and reorganization of remaining cellular structures. In this study, the expression of neuroplastin was analyzed by immunohistochemistry in human hippocampal tissue affected by Alzheimer's neurodegeneration. Neuroplastin immunoreactivity distribution was compared with expression of polysialylated neural cell adhesion molecule (PSA-NCAM), in AD and control hippocampal tissue. Both neuroplastin and PSA-NCAM are cell surface glycoproteins, highly abundant in mammalian brain tissue, and are involved in developmental processes such as neuronal migration, neurite outgrowth and synaptogenesis. A role of PSA-NCAM in central nervous system plasticity was previously described. Our results on distribution of neuroplastin immunoreactivity confirmed its extracellular localization in both control and AD hippocampal sections. The overall intensity of neuroplastin immunoreactivity was higher in AD than in control hippocampi, and was most notably expressed in neuronal population of dentate gyrus inner molecular layer. PSA-NCAM immunoreactivity in AD hippocampal tissue was similarly distributed with most notable differences in dentate gyrus, as compared with healthy tissue. We suggest that in addition to PSA-NCAM, neuroplastin may serve as plasticity marker, as its expression is increased in AD neurodegeneration due to described reorganization, neuronal remodelling and plasticity reactivation.

PI-8

EQUINATOXIN MEMBRANE BINDING AND PORE FORMATION

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Equinatoxin II is a member of the actinoporins, a unique family of 20 kDa pore forming proteins, originally isolated from sea anemones. After initial binding to a sphingomyelin containing cell membrane, an N-terminal α-helix is inserted into the membrane and cation selective pores are formed by oligomerization of several monomers. Recently, a nonameric prepore oligomer has been suggested. We used a stopped flow fluorescence spectroscopy to study a putative prepore formation. Preliminary results do not show significant difference between the helix insertion and oligomerization kinetics. In addition, a mutant equinatoxin was prepared, which cannot undergo a conformational change under oxidizing conditions. However the toxin is still able to bind to the target membrane. A helix insertion and calcein release is much slower compared to the wild type protein, when toxin first binds membrane in an oxidized state and a reductant is added afterwards. Altogether results indicate that prepore is not required for equinatoxin pore formation. We also investigated the effect of lipid phase coexistence on equinatoxin membrane binding using novel droplet-on-hydrogel bilayer technique. Fluorescently labeled equinatoxin first concentrates in the lipid phase boundary. Unexpectedly, after prolonged time it dissolves in the liquid disordered lipid phase, where the functional pores can be found using calcium influx and Fluo-8 indicator. To get a further insight into equinatoxin pore structure we were using single molecule fluorescence approach. Equinatoxin monomers labeled with Cy3B fluorophore were mixed with lipid vesicles to achieve oligomerization and fused with a glass cover slip afterwards. Bright spots were photobleached and fluorescence intensity traces analyzed to detect photobleaching step of each Cy3B dye. The number of clear steps in the fluorescence trace represents the number of monomers in equinatoxin pore.

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PI-9

ANALYSIS OF THE UBIQUITYLATION OF THE HUMAN PCNA PROTEIN

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The DNA in our cells is continuously damaged by different agents, such as UV irradiation, reactive oxygen species, metabolites and chemicals. These agents are changing the structure of the DNA molecule. To avoid these mutations many DNA repair mechanisms have evolved. These 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. In an other error free mechanism the monoubiquitylated PCNA becomes polyubiquitylated by the Mms2/Ubc13/HLTF complex through lysine 63 residues, therefore HLTF can reverse the replication fork. On this newly emergent so-called chicken foot structure the stalled strand can be finished using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism.

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.

PI-10

INDUCTION OF APOPTOSIS IN MELANOMA CELLS USING DYNLL/LC8 DYNEIN LIGHT CHAIN BINDING PEPTIDES

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The key molecular mechanism in the progression of malignant melanoma is the constitutive activation of mitogen-activated protein kinase (MAPK) and AKT signaling pathways, due to mutations in genes N-Ras and B-Raf. MEK and ERK kinases have essential roles in these cascades; their permanent activation leads to enhanced cell proliferation, invasion, angiogenesis and inhibition of apoptotic mechanisms. MEK inhibitors are potential candidates for the blockade of MAPK pathway and they are currently undergoing clinical evaluation. The release of B-cell lymphoma 2 (Bcl-2) homology 3 (BH3)-only pro-apoptotic proteins Bim and Bmf from the cytoskeleton is necessary for the intrinsic mitochondrial apoptotic pathway. During apoptosis, these proteins are translocated to mitochondria and block their anti-apoptotic ligands, thus inducing programmed cell death. It was recently shown that Bim and Bmf are sequestered to the cytoskeleton through LC8 dynein light chain (DYNLL). It is also known that melanoma cell lines exhibit a variable sensitivity to MEK-inhibitors, and a mechanism retaining Bmf in the cytoskeletal compartment can be a major contributing factor to the lack of apoptosis in resistant cells. The binding partners of DYNLL contain a short linear motif. Based on a high throughput *in vitro* directed evolution technique (phage display), we have previously identified a short peptide (VSRGTQTE) having a ten times higher affinity than the strongest known binding peptide (Bmf). The dimerization of the peptide (fused to a Leu-zipper) enhances the affinity to the subnanomolar range (Rapali et al. 2011, PLoSOne, 6(4): e18818). Both the monomer and dimer forms of the peptide are possible competitive inhibitors of DYNLL. Simultaneously treating MEK-inhibitor resistant human melanoma cell lines (e.g. MeWo) with high affinity DYNLL binding peptides (using transient transfection or the form of octa-arginine mediated penetrating peptides) and MEK-inhibitor (CI-1040), leads to the induction of apoptosis. The translocation of Bmf from the cytoskeleton to mitochondria is an evidence of its release and it increases the apoptotic rate. Experiments are in progress to elucidate the detailed mechanism of Bmf sequestration to and release from the actin cytoskeleton. Inhibition of the DYNLL-Bmf interaction could be considered as a potential therapeutic approach in fighting against melanomas.

Supported by OTKA NK81950 and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003.

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PI-11

EPIGENETIC SILENCING OF SECRETED PHOSPHOLIPASES A_2 IN HUMAN BREAST CANCER CELL LINES

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Epigenetics encompasses reversible and heritable phenotypic changes arising independently of the DNA sequence, such as alterations in DNA methylation patterns and histone modifications, playing important roles in various diseases, including breast cancer[1]. Secreted phospholipases A_2 ($sPLA_2$s) are enzymes and receptor ligands that participate in colon, prostate and gastric cancer[2]. The aim of this study was to determine the involvement of epigenetic mechanisms in the regulation of $sPLA_2$ gene expression in mammary cell lines, representing different stages of breast cancer development.

First, DNA methylation was assessed by treating cell lines with a DNA-methyltransferase inhibitor, 5-aza-2'-deoxycytidine (DAC), and analysing gene expression by quantitative PCR. Reexpression of human group IIA, III and X (hGIIA, hGIII and hGX, respectively) $sPLA_2$s was observed in several cell lines indicating that DNA hypermethylation is responsible for $sPLA_2$ gene expression silencing in breast cancer. Sequence analysis of hGIII $sPLA_2$ promoter region suggested that Sp1, ESR1 and MZF1 transcription factors were crucial for hGIII $sPLA_2$ gene reexpression upon treatment with DAC.

Second, a significant increase in the expression of all three $sPLA_2$s was observed in the most tumourigenic cell line upon treatment with a histone deacetylases inhibitor, trichostatin A (TSA), and it was even further augmented upon treatment with both DAC and TSA. This indicates that both DNA hypermethylation and histone acetylation are involved in $sPLA_2$ gene expression silencing in breast cancer, particularly in highly tumourigenic and invasive cells.

Our results confirm the importance of epigenetic factors in downregulation of hGIIA, hGIII and hGX $sPLA_2$ expression in breast cancer, suggesting a functional importance of these enzymes in development and progression of the disease.

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PI-12

DETRIMENTAL AND CELL CYCLE ARRESTING EFFECTS OF SODIUM SELENITE TREATMENT ON GLIOBLASTOMA MULTIFORME CELL LINES AND PRIMARY CELLS

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Aim: Selenium is an extraordinary trace element that can incorporate into amino acid and protein structures. Since it was discovered by Berzelius in 1817, it has been investigated in several studies, especially cancer research as a chemopreventive agent. Glioblastoma multiforme (GBM) is the most aggressive and invasive type of primary brain tumor. The aim of this study is to analyze the effects of sodium selenite on glioblastoma in terms of cell death and cell cycle arrest.
Material-Methods : In this study, we used U87 GBM cell line and two different primary GBM cell lines. Cells were treated with sodium selenite (0,5-20 µM) for 24 hours. Cell death was assessed with the MTT assay and cell cycle arrest analysis was performed by using propidium iodide with flow cytometer.
Results : As a result of the MTT assay, U87 and one of the primary cell lines did not respond to sodium selenite against the dose range we used for cell death analyses. However, the other primary cell was responsive to sodium selenite treatment; resulting in G2-M arrest, increase in Sub-G1 cells, genomic instability and significant death in a dose dependent fashion.
Conclusions: In conclusion, one primary GBM cell line was responsive to sodium selenite treatment, while others were not. These differences between GBM cells may be due to drug resistance. Drug resistance is the one of the problems in brain tumours, especially GBM. The unresponsive cells should be treated with increased doses of sodium selenite in the future. This study will yield further studies focused on the possibility of using selenium against some types of GBM.
Acknowledgement: We thank Dr. Nurten Saydam and Dr. Okay Saydam (Vienna Medical University) for kindly donating the cell lines and for their laboratory support.

PI-13

THE ROLE OF ΔNp73α IN RESPONSE TO GENOTOXIC STRESS IN NORMAL HUMAN FIBROBLASTS

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p73 exists in multiple isoforms which could be divided into two groups: one containing transactivation domain (TA) and another amino-terminally truncated (ΔN) isoforms. While TAp73 isoforms show tumor-suppressive functions similar to those of wild-type p53, ΔNp73 isoforms inhibit wild-type p53 as well as TAp63 and TAp73, and are considered as potential oncogenes. ΔNp73 isoforms are overexpressed in many tumors correlating with enhanced chemoresistance and poorer disease outcome. The aim of our work is to investigate how ΔNp73α overexpression (OE) affects cell cycle regulation and DNA damage response in normal human cells. To this end we infected wild-type human dermal fibroblasts (HDF-WT) as well as HDF expressing human papilloma virus HPV16-E6 oncoprotein (HDF-E6), which promotes p53 degradation, with retroviral vectors carrying ΔNp73α gene. Our video-microscopy experiments show that in HDF-WT ΔNp73α OE abrogates G2 cell cycle arrest in the presence of topoisomerase II inhibitor ICRF-193. In agreement with this result, we found lower p21 expression in cells with ΔNp73α compared to control ones after ICRF-193 treatment.
To determine whether ΔNp73α plays a specific role at the G2/M checkpoint, HDF-WT and HDF-E6 were synchronized at G1/S boundary and exposed to γ-irradiation (12 Gy) after the release from the block. Surprisingly, FACS analysis did not reveal significant difference between cells expressing ΔNp73α and control cells. To explain these results more detailed analysis of mitotic progression should be carried out together with the analysis of DNA damage response pathways. Further studies should contribute to understanding the mechanisms by which ΔNp73 isoform exerts its oncogenic function by perturbing the cell cycle regulation in normal cells, shedding new light on the process of tumorigenesis in general.

PI-14

A NEW REGULATING PROTEIN OF THE UBIQUITYLATION OF HUMAN PCNA

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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 has role in regulation of PCNA ubiquitylation.

PI-15

FORK REVERSAL BY Rad5: MOLECULAR BASIS

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Replicating cells are seriously threatened by stalled replication forks, because without their repair there are high risk of apoptosis, as it may result in double stranded DNA breaks. These dangerous structures can be formed by the effect of DNA damaging agents, unrepaired lesions or dissociation of the members of the replicating apparatus.
Rad5 is a yeast protein with human homologs having a proposed replication fork reversal activity, which may facilitate the restart of stalled replication forks. Rad5 is a SWI/SNF ATPase having no canonical helicase activity. It does not unwind unbranched dsDNA substrates, but it can process four-armed forks with homologous arms. Beside full-length Rad5, we also used a 160 aminoacid N-terminal truncated construct. As they resembled each other at the basic constants we used the truncated hereinafter.
The relatively low basal (DNA-free) ATPase activity of Rad5 is accelerated by both ssDNA and dsDNA. Poly-dT ssDNA and dsDNA enhance this activity 5 and 50 times, respectively. The M13 phage DNA showed activity increase as the dsDNAs, so we demonstrated the distinguishment of the secondary structure. We concluded that the Rad5 should have different mechanism binding and functioning on the double and single stranded DNA. We observed limited ssDNA and dsDNA length dependence of the ATPase activity. They are both present at the stalled replication forks and the discrimination have importence in the fork reversal. Our results indicate a large Rad5 effective binding site of 60 nt for ssDNA. We found also a small break in specificity constant per dsDNA length at 35 bp.
Now we are investigating the activity of Rad5 on a new substrate, designed for short-distance fork reversal assays. We are employing the advantage of single round conditions, developed by traping the dissociating Rad5, to shed light on the molecular mechanism.

PI-16

THE ROLE OF P21$^{WAF1/Cip1}$ GENE IN AUTOPHAGY AND SENESCENCE AS RESPONSES TO CISPLATIN TREATMENT OF COLON CARCINOMA CELLS

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Autophagy is a process of lysosomal degradation of cellular components operating in physiological conditions, but it can also be activated as a stress response. It appears to be very important in tumor development, progression and chemosensitivity. Cellular senescence, an irreversible cell cycle arrest, usually occurs upon telomere shortening, but can also be induced with certain chemotherapeutics to accomplish tumor growth inhibition. Interestingly, recently, it was discovered that autophagy might mediate mitotic senescence transition. Due to apoptotic cell death deficiencies, chemoresistance often occurrs in tumor cells. Therefore, senescence and autophagy are becoming potential alternative mechanisms for antitumor therapy.

p21$^{WAF1/Cip1}$ is a well characterized cyclin-dependent kinase inhibitor that negatively modulates the cell cycle progression by arresting G1, G2 or S phase of the cell cycle. Moreover, p21 has a role in cell differentiation, senescence and apoptosis, where it can act as either inhibitor or activator of apoptosis. However, its role in autophagy is completely underexplored and only recently addressed. Because of its complex and contradictory functions, it is important to study its roles in different cell death responses to DNA-damage treatment. The aim of this study was to investigate if autophagy and cellular senescence are activated upon p21 overexpression. In addition, the influence of cisplatin treatment-response upon p21 gene overexpression was assessed. Moreover, the role of p21 in autophagy mediated senescent transition was also investigated. SW480 and HCT116 colon cancer cells were treated with cisplatin upon adenovirus-mediated p21 overexpression. Both treatments alone or in combination induced autophagy and cellular senescence thereby preventing tumor growth. It was demonstrated that autophagy modulation influences senescence induction. However, downregulation of the basal p21 gene expression had no statistically significant influence on autophagy modulation, while it inhibited senescence activation upon cisplatin treatment. These results give additional insights into interconnected mechanisms of cell-responses to therapy and determine whether and how p21 characterizes chemosensitivity of tumor cells. We hope that the discovery of precise mechanisms of cell-response to therapy and the role of p21 gene in their modulation could lead to the design of new and more effective treatments of cancer.

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PI-17

MODULATION OF UROKINASE PLASMINOGEN ACTIVATION SYSTEM BY PARP-1 INHIBITION

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Plasminogen activation system is a complex system regulating extracellular proteolysis involved in various physiological and pathological processes. It is precisely regulated on the level of transcription and mRNA degradation and inhibition of its components, urokinase plasminogen activator, its inhibitors PAI-1 and PAI-2 and its receptor. Our aim was to elucidate the role of poly(ADP-ribosyl)ation in the induction of urokinase activity in A1235 glioblastoma cell line, after alkylation damage. A1235 glioblastoma cell line was treated with alkylating agent, alone or in combination with PARP-1 inhibitor, and the cell growth, cell cycle and appearance of senescent cells was analysed. Urokinase activity was determined in conditioned media and gene expression from isolated RNA. DNA damage was assessed by comet assay. As A1235 cells are DNA repair deficient and sensitive on alkylation agents, low doses of these agents caused growth arrest, changes in cell morphology and perturbances in cell cycle, as well as appearance of senescent cells. DNA damage caused by alkylation appeared proportional to the applied agent concentration, and PARP-1 inhibitor addition increased the level of damage. The pattern of modulation of urokinase activity after alkylation damage, caused by addition of PARP-1 inhibitor, indicate the indirect involvement of PARP-1 in urokinase induction, through influence on the DNA damage. We concluded that induction of urokinase activity resulted from the change in net balance between urokinase and PAI-1 transcription, and these processes are regulated through different signalling pathways.

PI-18

EXAMINATION OF CYTOTOXIC COMPOUNDS ON DIFFERENT TUMOR CELLS TO CELL VIABILITY AND TO THEIR MIGRATION PROPERTIES WITH A NOVEL CELL-MICROELECTRONIC SENSING TECHNIQUE

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We examined the effects of cytotoxic compounds on the migration and proliferation properties of human glioblastoma, liver carcinoma and melanoma cells with a novel cell microelectronic sensing technique.
Real-Time Cell Analyzer (RTCA) DP is a novel cell migration and invasion assay system that uses the Boyden Chamber principle but does not involve any fixation, labeling or counting of the cells. The core of the system is the CIM-Plate device, composed of an upper chamber and a lower chamber. The upper chamber has 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate or polyester membrane. The median pore size of this membrane is 8 µm. The membrane contains microelectronic sensor arrays that are integrated on its bottom surface. Migration of cells will occur through these electrodes, which changes impedance, and will increase cell index, a unitless parameter , which is calculated as a relative change in actual impedance. The more cells migrate, the higher the cell index will be. RTCA SP is also a microelectronic cell sensor method, where microlectrodes are integrated in the bottom of a microtiter plate (96-well E-plate) and measures adhesion, proliferation or cytoxicity. One of the most important advantages of the RTCA SP and DP system compared to other cell invasion and migration assays that this system does not require any labels or reporters, so that the experiment is physiologically more relevant. The real-time measurement can detect changes continuously, which means that the system can give information at any stages of the experiment.
Our results based on the migration experiments were validated by the RTCA SP system. We have tested the real-time effects of numerous molecules in single and in combination treatments. The RT-CES method, when used as described here, is advantegous for pharmaceutical screening of molecular libraries compared to other classical methods.

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PI-19

EXAMINATION OF SUBCUTANEOUS B16 MELANOMA CELL-DERIVED PRIMARY TUMOR GROWTH IN STAT6 DEFICIENT MICE

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Tumor tissue contains different immune cells which can generate an immune response against tumor cells. Paradoxically, the developing tumor can often induce a protumoral immune-microenvironment which inhibits anti-tumor immunity while enhancing tumor growth, invasion and vascularization.
$CD4^+$ Th1 type T cells and M1 macrophages are essential components of the anti-tumor immune response activating $CD8^+$ cytotoxic T cells and reducing tumor growth by tumor cell destruction. In contrast, Th2 cells and M2 macrophages inhibit anti-tumor immunity as well as promote tissue remodelling and tumor progression. However, some in vitro results suggest that M2 macrophages can also inhibit tumor cell proliferation. STAT6 transcription factor has a central role in the development of Th2 cells and M2 macrophages, since it transmits the signal of Th2 type cytokines (IL-4, IL-13) towards the nucleus controlling the expression of hundreds of target genes. Th2 type cytokines can be produced by tumor-infiltrating immune cells and, in some cases, also by tumor cells. In the absence of STAT6 an increased Th1 response, $CD8^+$ cytotoxic T cell and M1 macrophage differentiation can be observed.
The main goal of our work was to determine the role of STAT6 transcription factor in tumor development using subcutaneous B16 mouse melanoma tumor model in STAT6 knock-out and wild type mice. Primary tumors were removed 14 days after inoculation and individual tumor weights were measured. We also examined the basal structure and macrophage content of tumors using immunohistochemistry, and characterized the tumor's immunological status with measurement of different immune cell markers by quantitative PCR.
Our results show an increased primary tumor growth in STAT6 deficient mice compared to the wild type animals. Basal structure of the tumor tissue was similar in both groups, but peripherial tumor cell infiltration to the neighboring tissues was enhanced in STAT6 deficient mice. Gene expression analysis of the tumor tissue revealed significant down-regulation of M2 macrophage marker arginase 1 and anti-inflammatory cytokine IL-10 in STAT6 knock-out mice-derived tumors. These results suggest that STAT6 may have an influence on primary tumor growth and have a role in controlling the phenotypic switch of tumor-infiltrating immune cells.

PI-20

INTERACTION OF SPONGE NON-METASTATIC GROUP I NME GENE/PROTEIN WITH TELOMERES

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The Nme family, initially called nucleoside diphosphate kinases (NDPK) are evolutionarily conserved enzymes present in all three domains of life. Human Nme1 is the most studied representative of the family and the first identified metastasis suppressor. NDPK is involved in the maintenance of the cellular NTP pool, transferring the phosphate group through the histidine phosphointermedier. Interestingly, it seems that this biochemical feature of NDPK is not responsible for its antimetastatic activity. Several other biochemical functions have been assigned to this protein: histidine kinase activity, 3'-5' exonuclease and DNA cleavage activity, and transcriptional regulatory activity. The Nme family is known to participate in numerous crucial biological events such as proliferation, differentiation, development and apoptosis, as well as in adhesion, migration, and vesicular trafficking. In spite of the comprehensive scientific activity in this area it is still unclear which biochemical/biological activities are responsible for Nme's antimetastatic role. Vertebrate Nme enzymes can be separated into two evolutionarily distinct groups. In humans, Group I includes Nme1-Nme4 and Group II includes Nme5-Nme9 proteins. Nme 1 and 2 exhibit 89% amino acid (aa) sequence homology and possess many identical/similar but also some distinct properties. A good example is the Nme1/2 affinity towards telomeres and telomeric proteins in humans. Sponges (Porifera) are simple metazoans without tissues, closest to the common ancestor of all animals. They changed little during evolution and probably provide the best insight into the metazoan ancestor's genomic features. The sponge possesses one Nme Group I protein, NmeGp1Sd. Here we report new results on the metastasis suppressor gene/protein homolog from the marine sponge *Suberites domuncula*. Our research will broaden the knowledge on Nme1/2-telomere interactions in humans, and since the sponge homolog is an ancestral-type protein that emerged before the duplication and diversification of the Group I Nme family it represents an ideal model protein. The purpose of this study was to investigate the properties of the sponge Group I Nme gene and protein, and compare it to its human homolog in order to elucidate the evolution of the structure and function of Nme.

PI-21

EPIGENETIC REGULATION OF O-6-METHYLGUANINE-DNA METHYLTRANSFERASE IN GLIOMAS

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Gliomas are the most common and most aggressive primary brain tumors in humans. Therapeutic regimen usually includes surgical removal, radiation and chemotherapy. Alkylating agents, such as Temozolomide, are the most frequently use chemotherapy drugs for gliomas. These reagents alkylate the guanine residues, triggering the death of tumor cells. The effectiveness of the treatment is highly dependent on the activity of the O-6-methylguanine-DNA methyltransferase (MGMT), because this enzyme reverses the DNA modifications of the alkylating agent. Previously it was shown that hypermethylation of the MGMT promoter silences its transcription, decreasing its activity, thus the patient will respond to the chemotherapy.
Our goal was to establish a method to measure the methylation status of the MGMT promoter in different gliomas.
The formalin fixed, paraffin embedded samples were obtained from brain biopsies of patients with astrocytoma or oligodendroglioma. After DNA isolation, we converted the DNA with bisulphite reaction, which changes the unmethylated cytosines into uracils, thus converting the epigenetic information into changes of the DNA sequence. We determined the methylation status of the MGMT promoter region by High Resolution Melting Curve analysis.
We compared the methylation status of the MGMT promoter with the histological type of the tumor, clinical grade, survival time and response to the chemotherapy. The level of methylation shows significant differences among patients with different gliomas, but the group with glioblastoma multiforme has hypomethylated MGMT promoter. The methylation analysis of the MGMT promoter is a promising marker in patients with gliomas providing diagnostic and prognostic value. In the future, it might be part of the routine diagnostics as the first step of personalized treatment

PI-22

A PROTEOMIC APPROACH TO UNRAVEL MOLECULAR ALTERATIONS UNDERLYING LARYNGEAL CANCER

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Laryngeal cancer is the most common neoplasm of the upper aerodigestive tract, whose incidence is closely correlated with tobacco and alcohol consumption. Although multiple protein species with potential biomedical significance were detected in plasma and tissues from laryngeal carcinoma patients, none of them have been implemented yet into clinical practice as valid diagnostic biomarkers for monitoring disease progression and prediction of treatment response. Identification of novel molecular and cellular determinants of laryngeal carcinogenesis will open avenues for new, more effective therapies. In the present study, we investigated global proteome profiles of tumour tissues from patients with metastatic laryngeal cancer *versus* patient-matched, histologically normal tissues. Importantly, we applied novel, mass spectrometry-based approaches to analyse the glycan composition of tissue samples, and found that tumour tissues had lower number of glycan signals in comparison with unaffected tissues, as inferred from the ratio of product ions of N-acetyl-hexosamines (HexNAc), sialic acid (NeuAc (-H_2O)) and hexose-N-acetyl-hexosamine (HexHexNAc) in HCD spectra. Although preliminary, these results indicate that aberrant glycosylation might, at least partially, account for development and progression of laryngeal cancer. Furthermore, obtained data revealed 21 and 7 up- and down-regulated proteins, respectively, in tumour tissues that play important roles in diverse cellular processes including nucleo-cytoplasmic transport, protein biosynthesis and degradation, cytokine signalling, mRNA processing and splicing, remodelling of extracellular matrix, regulation of actin cytoskeleton structure and dynamics, immune response, cell-cell adhesion, control of cell proliferation and cellular aging, calcium homeostasis and signalling, drug resistance, regulation of transcription, iron metabolism and transport, blood coagulation and cellular metabolism. In addition to confirming the alterations in the expression level of proteins shown previously to correlate with head and neck cancer progression such as esophagin, filamin B and gelsolin, we also identified several proteins that are novel to laryngeal cancer pathogenesis including heterogeneous nuclear ribonucleoproteins R and Q, receptor-type tyrosine-protein phosphatase C and plastin-3. Functional roles of these newly identified proteins in laryngeal cancer and their diagnostic potentials have yet to be established.

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PI-23

INHIBITION OF $C_{17,20}$-LYASE ACTIVITY BY NEW 17β-OXAZOLIDONYL ANDROSTENE COMPOUNDS

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17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a key regulator enzyme of the steroid hormone biosynthesis in both the adrenals and the testes. Inhibition of this enzyme can block androgen synthesis in an early step, and may thereby be useful in the treatment of prostatic carcinoma, which is androgen-dependent in the majority of cases. Abiraterone and its analogues have been found strong inhibitors of $P450_{17\alpha}$ suggesting that steroid derivatives with heterocyclic substituent on the C-17 position may bear such potential.
We investigated inhibitory effect on $C_{17,20}$-lyase exhibited by our novel 17β-(2-oxazolidon-5-yl)-androstene compounds bearing hydrogen or various derivatized phenyl substituents on the nitrogen of the heterocyclic moiety. Compounds were tested both in the Δ^5-3β-hydroxy and Δ^4-3-keto series. $C_{17,20}$-lyase inhibition was tested by *in vitro* radioincubations, via conversion of 17α-hydroxyprogesterone to androst-4-en-3,17-dione, and full homogenate of rat testis was applied as enzyme source. Results revealed that derivatives with various aryl-substituted oxalidones were weak inhibitors. The Δ^5-3β-hydroxy compound bearing non-substituted oxazolidone ring exerted no inhibition, whereas its Δ^4-3oxo counterpart proved to be a potent inhibitor of rat $C_{17,20}$-lyase (IC_{50}=3.0 μM).
Observations concerning the $C_{17,20}$-lyase inhibitory potential of 17β-oxazolidonyl androstene compounds may provide interesting data for the development of new antiandrogens acting on an enzyme level.
(Supported by the Talentum Fund of Richter Gedeon Plc., the Hungarian Scientific Research Fund OTKA K101659. The Project named "TÁMOP 4.2.1/B-09/1/KONV-2010-0005 - Creating the Center of Excellence at the University of Szeged" is supported by the European Union and co-financed by the European Regional Fund.)

PI-24

CHARACTERIZATION OF THE BIOCHEMICAL PROPERTIES AND BIOLOGICAL FUNCTION OF THE FORMIN HOMOLOGY DOMAINS OF DROSOPHILA DAAM

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We characterised the properties of Drosophila melanogaster DAAM-FH2 and DAAM-FH1-FH2 fragments and their interactions with actin and profilin using various biophysical methods and in vivo experiments. The results show that, while the DAAM-FH2 fragment does not have any conspicuous effect on actin assembly in vivo, in cells expressing the DAAM-FH1-FH2 fragment a profilin-dependent increase in the formation of actin structures is observed. The trachea specific expression of DAAM-FH1-FH2 also induces phenotypic effects leading to the collapse of the tracheal tube and lethality in the larval stages.
In vitro both DAAM fragments catalyze actin nucleation, but severely decrease both the elongation and depolymerisation rate of the filaments. Profilin acts as a molecular switch in DAAM function. DAAM-FH1-FH2, remaining bound to barbed ends drives processive assembly of profilin-actin, while DAAM-FH2 forms an abortive complex with barbed ends that does not support profilin-actin assembly. Both DAAM fragments also bind to the sides of the actin filaments and induce actin bundling. These observations show that the Drosophila melanogaster DAAM formin represents an extreme class of barbed end regulators gated by profilin.

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PI-25

DIFFERENT TRYPSIN-INHIBITING SEQUENCE PATTERNS OF THREE UNRELATED PHAGE-EVOLVED INHIBITORS SUGGEST THAT THE SCAFFOLD HAS AN IMPORTANT ROLE IN DEFINING INHIBITOR SPECIFICITY

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Interaction of reversible serine protease inhibitors with their target enzymes is one of the best characterized examples of protein-protein interactions. There are at least 18 independently evolved such inhibitor families having very different scaffolds but sharing a common surface loop, which is in the same i.e. canonical conformation. This canonical loop is the most important interaction site for the cognate enzymes. Laskowski and his co-workers stated that the binding specificity of the inhibitor is determined by the sequence of the canonical loop therefore the effect of the loop-stabilizing scaffold is negligible. This theory, termed inter-scaffolding additivity, dictates that the optimal inhibitory loop residues at enzyme-contacting positions should be the same for a given enzyme regardless of the inhibitor scaffold. We aim to test this theory by evolving optimal loop sequences on different inhibitors for a given enzyme using phage display. In this study we displayed SPINK1 (serine protease inhibitor Kazal-type 1), SGPI-2 (*Schistocerca gregaria* protease inhibitor-2) and SFTI (sunflower trypsin inhibitor) on M13 phage. Using combinatorial mutagenesis we fully randomized the canonical loop positions on these inhibitors. The three libraries were selected on trypsin. Sequences of large numbers of selected clones were analysed to determine the characteristic sequence patterns in the form of sequence logos. The three sequence logos have characteristic differences. In a separate poster we show a similar analysis comparing chymotrypsin selected logos of loop sequences evolved on SPINK1, SGPI-2 and ecotin. That analysis also locates important differences in the corresponding logos. The two analyses support each other and suggest that the model of inter-scaffolding additivity cannot be general. We conclude that while the scaffold stabilizes the canonical loop conformation, besides this passive role it also contributes to the binding specificity of the inhibitor.
This work was supported by the Hungarian Scientific Research Fund (OTKA) NK81950, K68408, NK100769 as well as by the European Union and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003.

PI-26

LIMK2, A NOVEL PP1 INHIBITORY PHOSPHOPROTEIN

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Protein phosphatase 1 (PP1) is involved in the mediation of cell proliferation via dephosphorylation of the tumor suppressor retinoblastoma protein (pRb). The activity of PP1 is tighly regulated by the presence of different regulatory subunits or inhibitory proteins. In THP-1 cells the basal level of pRb phosphorylation is relatively high, which may reflect increased effectiveness of PP1 inhibitory proteins. Hence, we tested the presence and phosphorylation of CPI-17, a PP1 (and myosin phosphatase) inhibitory phosphoprotein in these cells. Surprisingly, we found relatively low level of phospho-CPI-17, but identified a few other proteins in 20-100 kDa molecular mass range that cross-reacted with anti-phospho-CPI17 antibody. A search in the protein database based on CPI-17 sequence highlighted already known related proteins such as PHI and KEPI. In addition, LIM-kinase 1 and 2 (LIMK1 and LIMK2), and a protein termed as “similar to serologically defined breast cancer antigen” included significant sequence similarity with CPI-17.

Among these phosphorylated proteins LIMK2 was identified by immunoprecipitation in THP-1 cell lysate and was significantly phosphorylated at the sequence motif similar to the CPI-17 phosphorylation site. LIMK2 phopsphorylation was completely diminished upon treatment of the cells with daunorubicin, a chemotherapy drug. Full-length Flag-LIMK2 was expressed in tsA201 cells and purified on Flag-affinity column. Unphosphorylated Flag-LIMK2 inhibited the myosin light chain phosphatase activity of PP1 slightly, whereas phosphorylation by PKC at the inhibitory site (verified using anti-phospho-CPI-17 antibody) increased profoundly the PP1 inhibitory potency.

Our results suggest that LIMK2 is a novel inhibitor of PP1 and it may mediate the phosphorylation level of proteins important in the regulation of the chemosensitivity of malignant cells. (Supported by OTKA CNK 80709 grant.)

PI-27

BIOCHEMICAL SPECIFICITY OF MAP KINASE BINDING LINEAR MOTIFS

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Mitogen activated protein kinases (MAPK) are main organizers of signal transduction in eukaryotes. MAPKs contain a docking groove that determines their partner profile with other proteins via interactions involving linear binding motifs. These motifs (docking peptides or D-motifs) play a pivotal role in mediating specific signaling links within MAPK cascades. In the current study, we have probed the biochemical specificity of fifteen docking peptides directly binding to ERK2, p38α and JNK1 docking grooves in vitro so that to compare this to the physiological connections observed in known MAPK networks. We show that classical D-motifs can mediate highly specific binding only to JNK1, whereas only motifs binding in fundamentally different binding modes are capable to discriminate the topographically more similar ERK and p38 docking grooves. We also solved the crystal structure of four MAPK-docking peptide complexes representing new JNK-specific, ERK-specific or ERK/p38 selective binding modes. These revealed a great deal of main-chain conformational diversity for regions located in-between docking motif consensus sites. While these latter serve as anchor points that tap common MAPK surface features and mostly contribute to docking in a non-discriminatory fashion, specificity is determined mainly by the conformation of an intervening region connecting two anchor points. These insights enabled the successful design of peptides with tailored MAPK binding profiles, which indeed required rationally changing the length and amino acid composition of this formerly unappreciated motif region. We present a coherent structural model underlying MAPK docking specificity. This reveals how short linear motifs binding to a common kinase docking groove can mediate diverse interaction patterns and contribute to correct MAPK partner selection in signaling networks.

PI-28

IDENTIFICATION OF POTENTIAL INTERACTING PARTNERS OF TRANSGLUTAMINASE 2

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Transglutaminase 2 (TG2) is a ubiquitously expressed Ca^{2+} dependent crosslinking enzyme in the cellular compartments, which is known to be involved in diverse cellular functions such as cell differentiation, adhesion and migration, inflammation and apoptosis etc. However the molecular interactions between TG2 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 TG2 in different cellular compartments as it may have an impact on the signaling pathways functioning in these cellular processes Some well-known binding partners of TG2 which are known till date are only integrin, fibronectin and syndecan, functionally important for cell adhesion and migration. However, the complete molecular pathways are still unexplored. Thus the present study aims to identify novel interacting partners of TG2 and finally explore its functional significance.

To achieve this we use NB4 cell line as a model because TG2 expression is undetectable in wild type NB4 cell line but upon ATRA (all *trans* retinoic acid) treatment TG2 expression increases several folds. The differential gene expression analysis of NB4 cell line and TG2 knocked down NB4 cell line after ATRA treatment revealed that TG2 was involved in expression of large number of ATRA-regulated genes.

Further to identify the proteins interacting with TG2 we employed GST and Histidine pull down assays and subsequent mass spectrometry analysis. The TG2 was tagged with GST or Hexa-histidine and tagged recombinant TG2 was purified from the *E. coli* and immobilized to the columns containing agarose beads. Upon passing the NB4 wild type and NB4 knock down cell lysate through the column, the proteins interacting with TG2 bind to the column and TG2-protein complex is eluted and further analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and mass spectrometry. We obtained various novel TG2 binding candidates namely Tubulin α, Histone H2A and heat shock protein 40 (HSP 40) and in addition some known interacting partners such as h Glutathione S Transferase (hGST-P1) validating the experimental approach. Further co–immunoprecipitation and immunofluorescence studies are ongoing to validate these interactions.

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PI-29

REGULATION OF NEUROTRANSMITTER RELEASE BY PROTEIN PHOSPHATASE-1 AND RHO A-ACTIVATED KINASE MEDIATING PHOSPHORYLATION OF SYNAPTIC PROTEINS

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Neurotransmitter release and vesicle recycling are mediated by an extensive array of protein-protein interactions and protein modifications including protein phosphorylation. Protein phosphatase-1M (PP1M, or myosin phosphatase) and the Rho-A-activated kinase (ROK) distributes widely in the rat brain, however their functions in synaptic transmission are not clearly understood. Specific inhibition of PP1 by tautomycetin decreased the exocytosis of rat cortical synaptosomes induced by depolarization with KCl, while the inhibition of ROK by Y27632 had opposite effects. PP2A inhibition by okadaic acid, or inhibition of conventional and novel PKCs by pharmacological inhibitors were without effect. Mass spectrometry analysis identified several myosin phosphatase target subunit-1 (MYPT1) interacting synaptosomal proteins such as synapsin-I, syntaxin-1, SNAP-25, calcineurin and CaM-kinase II, and these interactions were confirmed by reciprocal immunoprecipitation and pull-down assays. Interaction between the C-terminal of MYPT1 and SNAP-25 was also revealed by surface plasmon resonance binding studies. In B50 neurobalstoma cells as well as in synaptosomes, ROK inhibits PP1M activity by phosphorylation of MYPT1 at Thr696. In addition, ROK and PP1M mediate not only the phosphorylation of myosin-II regulatory light chain but synapsin-ISer9, syntaxin-1S14 and SNAP25Ser187. Our data imply that PP1M and ROK interact with SNARE-complex associated proteins and mediate the phosphorylation level of these proteins, thereby regulating synaptic transmission. This work was supported by grants from HSRF OTKA K68416 and CNK 80709, and by the TÁMOP 4.2.2.-08/1-2008-0019 DERMINOVA project and Bolyai Fellowship (BL).

PI-30

IN SILICO DESIGN OF HIGH AFFINITY ACETYLCHOLINESTERASE INHIBITORS

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Role of acetylcholinesterase (AChE) in pathology of neurodegenerative diseases as myasthenia gravis, Parnkinson or Alzheimer disease initiates development of reversible AChE inhibitors. Active site of AChE, 20 Å deep and 5 Å wide, defines interaction site for substrate and inhibitors. Catalytic triade is located at the bottom of an active site (Ser203, His447, Glu334) with an oxyanion hole (Gly121, Gly122, Ala 204), a choline binding site by Trp86, Tyr337 and Phe338, and a peripheral binding site by Tyr72, Tyr124 and Trp286. Crystallographic analysis of various AChE inhibitor complexes show that most of inhibitors form interaction with peripheral and/or choline binding site of AChE. We studied possibility of third binding site for high affinity inhibitors designed *in silico*. These new inhibitors form interaction with peripheral and choline binding site and additional interactions with residues Ser203, Gly121 and Gly122. These glycines are members of the oxyanion hole. Affinity of novel inhibitors were estimated using following scoring functions: PLP2, PMF, PMF04, Jain and LigScore1_Dreiding. By comparing scoring of known high affinity AChE inhibitors and those tested here we conclude that AChE may have higher affinity for new inhibitors due to additional stabilisation with residues from oxyanion hole via hydrogen bonds.

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PI-31

A DUAL ROLE FOR RAC1A GTPASE IN THE REGULATION OF CELL MOTILITY

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Rac proteins are the only canonical Rho family GTPases in *Dictyostelium*, where they act as key regulators of the actin cytoskeleton. In order to monitor the dynamics of activated Rac1 in *Dictyostelium* cells, a fluorescent probe was developed that specifically binds to GTP-bound form of Rac1. The probe is based on the GTPase-binding domain (GBD) from PAK1 kinase, and was selected on the basis of yeast two-hybrid and GST pull-down screens. An interaction between PAK1_GBD and activated Rac1 was corroborated in living cells by fluorescence resonance energy transfer (FRET). In moving *Dictyostelium* cells, PAK1_GBD is strongly enriched at the leading edge where it co-localizes with F-actin, and it also localizes to endocytotic cups during phagocytosis and macropinocytosis. As in vertebrates, activated Rac1 therefore appears to participate in signalling pathways that control de novo actin polymerization at protruding regions of the cell. Additionally, the IQGAP-related protein DGAP1 sequesters active Rac1 into a quaternary complex with the actin-binding proteins cortexillin I and II and, notably, this complex localizes to the trailing, retracting regions of migrating cells. As assessed by latrunculin B treatment, cortical localization of PAK1_GBD strictly depends on the integrity of the actin cytoskeleton, whereas cortical localization of DGAP1 does not. Taken together, these results imply that Rac1 GTPases play a dual role, both at the front and in the back, in migrating *Dictyostelium* cells.

PI-32
STRAIGHTFORWARD METHOD FOR PROTEIN KINASE SUBSTRATE IDENTIFICATION

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Reversible protein phosphorylation takes part in numerous signal transduction pathways; thus, its proper functioning is inevitable for all phenomenon of life. Accordingly to their versatile role, dysfunction of protein kinases can manifest in various diseases; therefore, deciphering protein kinase regulated pathwaysis one of the primary interests of medical sciences and pharmaceutical industry. The number of the identified protein kinases is continuously increasing, more than 500 kinases have been described in the human proteome and the plant kinase family is even more extended. In comparison to the huge number of known protein kinases, hardly any of their substrates have been identified. Generally, biochemical analysis of substrates for eukaryotic protein kinases is a challenging task for two reasons. First, most of them are low abundance proteins in physiological conditions, additionally with weak affinity of protein-substrate interactions. Due to these limitations, isolation of sufficient amount of protein from their original sources often an impractical approach for further analysis. Second, production of functional eukaryotic multidomian proteins cannot be efficiently accomplished by bacterial overexpressing system. We present a generally applicable method for identification of protein kinase substrates, which relies on combination of cell-free protein translation and phosphoprotein specific fluorescent staining. The wheat germ protein extract based in vitro protein translation system provides the properly folded, catalically active kinases and their putative molecular targets. The synthesized proteins can be efficiently purified in single step by magnetic affinity beads. The cell-free system produced kinases and their putative substrates are applied in in vitro kinase assay. Following the kinase assay, the substrates are separated on PAGE and the phosphorylated proteins are visualized by sensitive fluorescent phosphoprotein staining. Fluorescent stained PAGE is also suitable for further analysis by mass spectrometry to determine the specific phosphorylation sites. The main advantages of our substrate identification protocol are no special laboratory requirements, radioactive-free, implementable in general molecular biology, biochemistry laboratories, short analysis time, and cost-effectiveness.

This work was supported by the Hungarian Scientific Fund (OTKA K69187) and New Hungary Development Plan (TÁMOP-4.2.1/B-09/1/KMR-2010-0001).

PI-33
ANALYSIS OF HAND2 EXPRESSION IN UTERUS DURING EARLY PREGNANCY

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The basic helix-loop-helix transcription factor termed heart and neural crest derivatives-expressed protein 2 (Hand2) was previously identified as a regulator of morphogenesis in a variety of tissues. There are reports that, in the uterus, Hand2 is induced by progesterone. Embryo implantation and decidualization of stromal cells are critical events in the establishment of successful pregnancy. Correct spatio-temporal activity of steroid hormones, estrogen (E) and progesterone (P) is vital for these events. The physiological effects of P are mediated by interaction with two progesterone receptors (PRs), PR-A and PR-B. PRs are members of the nuclear receptor superfamily of transcription factors. This study was undertaken to analyze the effects of E and P on Hand2 expression in the uterus. To examine the role of PRs in Hand2 expression, ovariectomised wild type and genetically modified mice lacking both PRs and with only the PR-A isoform were treated with oil, E, EP and P. Uterine tissue was then analyzed for PR and Hand2 expression. Our results indicate that Hand2 expression is regulated by E and P. Hand2 expression was also studied during early pregnancy. Double labeling immunofluorescence and quantitative PCR were used to analyze spatio-temporal expression of PR and Hand2 in the mouse uterus on day 2.5, 4.5 and 7.5 of pregnancy. Although high levels of PRs were present on day 2.5 of pregnancy, we did not detect Hand2 before implantation. On day 4.5 of pregnancy Hand2 co-localized with PR-expressing cells. At this stage, Hand2-expressing cells were localized mainly in the uterine stroma underlying the luminal epithelium (LE). Decidualisation is prominent at day 7.5 of pregnancy and in decidual cells PRs and Hand2 remained co-localized. The Hand2 positive cells were spatially segregated mainly in antimesometrial side.

PI-34

RIBOSOMAL A SITE BINDING PATTERN DIFFERS BETWEEN ARM METHYLTRANSFERASES FROM CLINICAL PATHOGENS AND A NATURAL PRODUCER OF AMINOGLYCOSIDES

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One of the self-protecting systems that evolved within the aminoglycoside-producing bacteria was the employment of enzymes that add a methyl group to specific ribonucleotides in antibiotic-binding sites of the ribosome, thereby disrupting the antibiotic binding. In our previous research we have extensively studied biochemical and functional properties of one such enzyme, Sgm methyltransferase from the natural producer of aminoglycoside, *Micromonospora* zionensis, that belongs to the Arm (aminoglycoside resistance methyltransferase) family of enzymes. Recently, members of Arm family of enzymes were found to be spreading by horizontal transfer in growing number of clinical strains, which poses a serious threat for the successful treatment of severe bacterial infections.

In this work, we compared the ribosomal A site binding pattern of the Sgm methyltransferase with the Arm members isolated from clinical pathogens, RmtA, RmtB, RmtC and RmtD. We used a specialized *E. coli* system, in which all *rrn* operons were inactivated, and ribosomal RNA was transcribed from a vector-based *rrn* operon. We constructed single nucleotide mutations in the part of the operon corresponding to the A site of 16S rRNA. We determined generation time and investigated the ability of these cells to grow in the presence of various concentrations of aminoglycoside kanamycin. We then introduced actively expressing Arm methyltransferases into these cells and monitored the impact of the mutations on the enzyme activity by determining minimal inhibitory concentration of kanamycin and analyzing the target nucleotide methylation with primer extension.

Our results show that the recognition motif of Arm enzymes on the bacterial ribosome differs for the Sgm enzyme versus the enzymes from clinical pathogens. We confirmed that the Arm enzymes from clinical isolates can efficiently methylate the target nucleotide despite the individual A site mutations. However, Sgm methyltransferase cannot methylate the target nucleotide for some of the mutations introduced. This suggests that even though Arm enzymes from clinical strains and a natural producer of aminoglycosides methylate the same target nucleotide, their mode of action is slightly different. In order to successfuly fight the aminoglycoside resistance, it is therefore of great importance to analyze these differences in more detail and consider them for the design of effective inhibitors that would block the action of all the members of Arm family.

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PI-35

LIGAND BINDING INDUCED POLYMERIZATION OF THE LC8 DYNEIN LIGHT CHAIN (DYNLL)

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Amyloidoses are degenerative diseases with diverse and severe symptoms, caused by the formation of protein aggregates inside or outside cells. Disease related protein aggregates show a large diversity in size and morphology from small oligomers to protofibrils and mature amyloid fibrils. However, their common feature is that they are rich in intermolecular beta-sheet structure. Regular beta-sheets in proteins usually have edge strands evolutionarily protected against aggregation by burial, formation of beta bulges, or placing proline or charged residues in the sequence. However, controlled interchain beta-sheet interactions have important roles in living organisms, from the formation and stabilization of many quaternary protein structures to protein-ligand binding, where “ligand” is an unstructured segment of a polypeptide chain and complex formation is coupled with the adaptation of beta-strand conformation. The highly conserved LC8 dynein light chain (DYNLL) is a eukaryotic hub protein, involved in diverse cellular processes, like apoptosis, virus infection, intracellular transport, etc. It has more than 40 experimentally verified binding partners. DYNLL forms homodimers by a beta-chain swapping mechanism. Two accessible beta-edges are hidden at the bottom of deep, hidrophobic grooves formed on the opposite sides of the subunit interface. The unstructured DYNLL binding linear motifs of partner proteins lie into these grooves by extending the beta-sheets of DYNLL dimers. The new beta-edges protrude, giving chance to form new beta-sheet mediated interactions. Here we describe the *in vitro* polymerization of DYNLL, induced by the addition of a short peptide corresponding to the binding sequence of EML3 (Uniprot: Q32P44) a microtubule-binding protein involved in mitosis. Evidences from x-ray crystallography support the hypothesis, that these polymers are stabilized by beta-sheet mediated interactions between the bound peptides. This phenomenon of peptide ligand-induced polymerization we report for the first time, occurs under near-physiological conditions. Considering the high number of binding partners this suggests that similar mechanisms may have roles *in vivo* in the interaction network of DYNLL.

PI-36

REFINEMENT OF LC8 DYNEIN LIGHT CHAIN BINDING PARTNER PREDICTION BY A DIRECTED EVOLUTION METHOD

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The highly conserved homodimer LC8 dynein light chain is a eukaryotic hub protein. It is a micromolar binder of dozens of proteins involved in pleiotropic cellular events from apoptosis to virus infection. LC8 has two symmetric binding grooves and it is considered as a universal "molecular velcro" promoting dimerization or stabilization of its mostly dimeric partners. The interaction is mediated by short linear motif having loose consensus sequence, $[DS]_{-4}K_{-3}X_{-2}[TVI]_{-1}Q_0[TV]_1[DE]_2$. The most conserved Gln residue (position 0) is often flanked by Thr, Val or Ile. These TQT or IQV motifs are good hallmarks for LC8 binding. However, a few partners have been identified without this canonical TQT/IQV pattern (e.g. myosin 5a ($T_{-1}M_0T_1$). We have determined the thermodynamically driven pattern of LC8 binding motif using *in vitro* directed evolution, phage display. An $X_{-5}X_{-4}X_{-3}X_{-2}X_{-1}Q_0X_1X_2$ naïve library was displayed where X presents all of 20 amino acids while the central Gln was fixed. Based on the resulting pattern we have used a bootstrap algorithm and a statistically determined threshold level to predict 242 different sequences of 219 proteins from the human proteome as high probability LC8 partners. Among these we have found 18 sequences of 8 proteins that had already been known validated as LC8 partners.

As an extension of this study we have applied a second phage selection cycle using an $X_{-7}X_{-6}V_{-5}S_{-4}R_{-3}G_{-2}X_{-1}X_0X_1E_2X_3$ library. Based on the first selection, residues having the highest occurrence were fixed at positions -5, -4, -3, -2 and 1. The previously fixed Gln and flanking positions were fully randomized, which allowed us to identify non-canonical motifs (others than IQV or TQT) and to estimate their frequency. 6 out of 52 identical clones were found containing Met and in one case Asn at position 0. Moreover, the library was expanded by two residues toward the N- and one toward the C-terminus thereby lengthening the analyzed segment. Using data derived from both selection cycles we have refined the consensus pattern and also our partner prediction. We have found that the known partners have higher enrichment above the threshold level than in the previous prediction. However, no partner was identified having Met at position 0 above the threshold level. Validation of ten potential partners using biochemical and cellular approaches is in progress.

Supported by OTKA NK81950, the European Union and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003.

PI-37

NUCLEAR LOCALIZATION, FUNCTION AND REGULATION OF MYOSIN PHOSPHATASE

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The myosin phosphatase (PP1M) holoenzyme is a serine/threonine specific protein phosphatase. It consists of a 38 kDa type 1 protein phosphatase catalytic subunit (PP1c), a 130/133 kDa MYPT (myosin phosphatase targeting subunit) and 20 kDa subunits with unknown function. PP1M regulates contractility through the dephosphorylation of myosin light chain. Apart from the myosin, which is a classical cytoskeletal substrate of PP1M, other non-muscle substrates have also been identified. It draws the attention to the complex function of PP1M in different tissues and cellular processes. MYPT was found to be localized not only in the cytosol and cytoskeleton but in the nucleuses of rat aortic smooth muscle cells, primary cultures of neuronal cells as well as of human hepatocarcinoma (HepG2) cells. Our goal is to investigate the nuclear functions of PP1M by determining the subnuclear localization and the interacting proteins of MYPT. Subnuclear fractions of HepG2 cells were analysed by Western blotting and by protein phoshatase enzyme activity assays in the presence of specific PP1 inhibitors such as okadaic acid and tautomycin. The dominant nuclear protein phosphatase was found to be the PP1 in the nuclear fractions. Flag-MYPT pull down assays were carried out using nuclear fractions of HepG2 cells. The eluates were subjected to silver staining and the proteins were identified by mass spectrometry. Numerous potential nuclear MYPT1-interacting proteins were identified such as histone 1, splicing factor proteins, possible enzyme regulators of PP1M and members of the methylosome complex, f. i. the protein arginine methyltransferase 5 (PRMT5). PP1cδ was detected from the nuclear pull down eluate by Western blotting as a partner of MYPT1. We also confirmed the nuclear colocalization of MYPT1 and PP1cδ suggesting that the delta and not the alpha/ gamma isoform is the member of the holoenzyme in the nucleus. MYPT1 showed colocalization with histone 1 and presented distinct localization in the spliceosomes (nuclear splicing factor compartments of cell) by confocal microscopy suggesting that PP1M may play a role in mRNA splicing. We plan to investigate the physiological role of PP1M in the nuclear dephosphorylation processes related to the regulation of transcription, RNA splicing and the functions of the methylosome complex. This work was supported by grants from HSRF OTKA K68416 and CNK 80709 and Bolyai Fellowship (BL).

PI-38

DIRECTED EVOLUTION OF SERINE PROTEASE INHIBITORS DETECTS FUNCTIONAL COUPLING BETWEEN THE REACTIVE LOOP AND THE INHIBITOR SCAFFOLD

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There are at least 18 independently evolved families of reversible serine protease inhibitors that interact with their target enzyme in a substrate-like manner. Each family has a unique fold but all carry a common protease-binding loop, which is always essentially in the same i.e. canonical conformation. Based on this notion and a limited number of experiments it has been widely accepted that affinity and specificity-profile of these inhibitors are dictated solely by the sequence of this reactive loop. In this model called interscaffolding additivity the diverse scaffolds do not modulate specificity. The common function of the scaffolds is to stabilize the reactive loop in optimal conformation. To test this model in a high throughput manner, we determined the optimal inhibitor loop sequences of three unrelated inhibitors, hSPINK1 (human Serine Protease Inhibitor Kazal-type 1), ecotin and SGPI-2 (*Schistocerca gregaria* protease inhibitor 2) against chymotrypsin**.** hSPINK1 is expressed in the acinar cells of the human pancreas, ecotin is an inhibitor first discovered in *E. coli*, while the protease inhibitor SGPI-2 was isolated from the desert locust *Schistocerca gregaria*. The three inhibitors have completely unrelated scaffolds, but their reactive loop conformation and the fine molecular details of loop-stabilization are almost identical, although SPINK1 has an unusually long interacting site. We randomized six functionally analogous reactive loop positions in the three inhibitors and 3 additional ones in SPINK1 and displayed the libraries on M13 phage. Phage display evolution was performed by selecting the libraries for binding to chymotrypsin. The three parallel evolutions resulted in significantly different optimal loop sequences. Some of the differences can be explained based on the 3D structures of the enzyme-inhibitor complexes, but many differences are less trivial. In summary, we find that the optimal inhibitor loop sequence strongly depends on the scaffold therefore the existing model of interscaffolding additivity cannot be universal. Rather, it might apply only to a small subset of canonical inhibitors. This finding should be important for better understanding the mechanism of action of inhibitors, for bioinformatic prediction of their properties and for developing new inhibitor variants.

This work was supported by the Hungarian Scientific Research Fund (OTKA) NK81950, K68408, NK100769 as well as by the European Union and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003.

PI-39

SELECTION OF CARDIAC SPECIFIC TROPONIN SPIEGELMERS

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Cardiac specific troponins are standard markers of myocardial infarction; thus, various systems have been developed for fast and sensitive detection of cTnI and cTnT. Antibodies, the most generally applied receptor molecules in protein detecting devices, have been rivaled by appearance of aptamers, short single stranded oligonucleotides with high molecular recognition and binding capacity. Although aptamers, unlike antibodies, are resistant to chemical and physical conditions, their application is hampered due to their susceptibility to enzymatic degradations. Spiegelmers can be seen as biostable version of aptmers, because in terms of selectivity and affinity towards their target molecules are equal to aptamers, but insensitive to nuclease degradation. Spiegelmers consist of L-sugar instead of naturally occurring D enantiomers, consequently the enantiomer of the final target molecule have to be used during the selection procedure. We aimed at producing cTnI specific Spiegelmers to provide alternative receptors for biosensor development. A peptide sequence with 9 amino acids from the full-length protein was identified as a suitable selection target. The D-peptide was immobilized on magnetic beads, challenged with the initial pool of oligonucleotides, which was designed with a randomized segment in the middle section of its sequence. The specifically bound oligonucleotides were amplified by PCR and denaturated to convert dsDNA to ssDNA. The selection cycle was repeated nine times with gradually decreasing peptide concentration and more vigorous washing conditions to increase the affinity of selected oligonucleotides. The selectivity of oligonucleotides was further enhanced by counter-selection steps that is the selected DNA pool were incubated with peptide-free magnetic beads to eliminate the oligonucleotides which are non-specific for the D-peptide but the matrix of magnetic beads. Following the last selection cycle, the oligonucleotides were inserted into TOPO cloning vector and sequenced by capillary electrophoresis sequencing. According to the obtained data, the presented protocol resulted in isolation of few oligonucleotide sequences indicating the success of selection procedure. Presently, the putative cTNNI specific spiegelmers are evaluated by different approaches.

This work was supported by the Hungarian Scientific Fund (OTKA K816) ENIAC CAJA4EU, and New Hungary Development Plan (TÁMOP-4.2.1/B-09/1/KMR-2010-0001).

PI-40

ANALYSIS OF BCHE STEREOSELECTIVITY DURING CARBAMOYLATION VIA TRANSITION STATE MODELLING

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BChE and related AChE show stereoselectivity in reaction with chiral ligands or esterification compounds as pesticides or carbamates. Chirality is important property especially in drug design due to regulation of target property or minimizing drug side effects. Enantiomers of pharmacological relevant compounds may have different potency or toxic effects. Here we analyse molecular basis of BChE enantioselectivity in carbamoylation. We studied carbamate derivate of following bronhodilatators: terbutalin, methaproterenol and isoproterenol. Carbamate form of terbutaline is known as bambuterol which is used as pro-drug of terbutaline in racemic form. During reaction between BChE and carbamate derivate transition state is formed prior carbamoylation of catalytic serine. We use this conjugate to study interaction between active site residues and chiral carbamate derivate of tested bronhodilatators. Results show similar binding pattern for enantiomers within BChE active site, but interaction between bronhodilatator hydroxyl group, located on asymmetrical carbon atom, and Glu202 near catalytic serine may be the cause of stereoselectivity. Comparison of free energies of carbamoylation between corresponding enantiomers can be related to difference in conjugate stabilisation due to formation of hydrogen bond with Glu202.

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PI-41

SEARCHING FOR NEW MAP KINASE SUBSTRATES WITH A NOVEL *IN SILICO* METHOD

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Mitogen-activated protein kinases (MAPKs) play a central role in the intracellular signaling of mammalian cells. They direct responses to various extracellular stimuli, ranging from proliferation to differentiation, immune responses and cell death. Short, dedicated peptide motifs (D-motifs) are found in most MAPK binding partners and have a crucial role in directing protein-protein interactions of MAPKs. Upstream kinases as well as phosphatases, many scaffold proteins and most substrates all use these flexible peptide motifs to bind to a conserved surface of classical MAPKs. A handful of D-motif-containing proteins have been known for a decade, mostly from MAPK interactors discovered by serendipity. Here we present the first in silico approach that makes use of the structural classification of D-motifs to systematically identify novel, specific MAPK substrates from proteomics databases. Our method is based on the filtering of disordered protein regions capable of forming biologically relevant protein-protein interactions (via the ANCHOR software), a consensus-submotif selective search (that is predictive of the MAPK-selectivity profile of the motif) and ranking of the hits by force field based docking and energy calculations (using the FOLDX package). The method can be shown to identify most of the known D-motifs, with relevantly high scores. It also helped us to find new D-motifs either serving as feedback elements (e.g. in the the KSR2 scaffold) or involved in downstream MAPK signaling (e.g. in the ATG4D protease). This algorithm might be a helpful tool to unravel the true complexity of MAPK signaling with hundreds if not thousands of potentially novel substrates compared to the few dozen currently known examples.

PI-42

DETECTION 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 on 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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PI-43

EFFECT OF DIETS ENRICHED WITH PUMPKIN-SEED, OLIVE AND FISH OIL OR LARD ON MICE LIVER NONPOLAR LIPIDS AND EXPRESSION OF LIPIN AND PPARα AFTER PARTIAL HEPATECTOMY

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The best experimental model for the study of liver regeneration is partial hepatectomy (pHx), in which part of the liver is surgically removed. Fast liver growth, following pHx is a well defined process, that involves the concerted action of extra and intracellular factors resulting in cell replication and its inhibition at the time when the entire liver mass is restored. During regeneration, liver transiently accumulates large amounts of triglyceride fat. One of the factors involved in hepatocellular fat accumulation following pHx could be lipin 1. This protein is defined as the bridge between hepatic glycerophospholipid biosynthesis and lipoprotein metabolism. It activates expression of many genes involved in mitochondrial fatty acid oxidative metabolism, among other, via transcriptional activation of the gene encoding PPARα. In this study, the changes in the expression level of mice hepatic lipin 1 and PPARα were evaluated and compared with the changes in the content of nonpolar liver lipids. As nutritional factors influence the process of liver regeneration, effect of diets enriched with different oils or lard and their impact to gene expression was also evaluated.
For that purpose, male C57/Bl6 mice, aged 8-10 weeks, were fed by diets enriched either with pumpkin-seed, olive or fish oil or lard (5% addition to standard pellet, w/w) during the 3 weeks and afterwards subjected to 1/3 pHx. 2, 4, 24, 48 and 168 hours later mice were sacrificed and the remaining liver analysed. Total liver lipids were extracted and then separated into fractions by solid-phase extraction. Real-time reverse transcription-PCR was performed to quantitate total lipin and PPARα expression levels.
Each of the experimental diets affected liver regeneration differently. In all diets, except in fish oil enriched diets, regenerating liver accumulated extra fat (increment of neutral lipid fraction) in first few days after pHx, with the highest value at 24 hours after pHx. Fish oil enriched diet provoked the fastest liver regeneration (after 48 hours >90% of the original mass) and simultaneously, reflected itself in the lowest total liver lipid content. Lipin expression levels correlates with PPARα expression levels, although each of the diet provokes different response. Besides, we demonstrate that the expression of lipin 1 can be induced by pHx.

PI-44

DEVOLPMENT AND RESOLUTION OF COLITIS IN MICE WITH TARGET DELETION OF DIPEPTIDYL PEPTIDASE IV

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Dipeptidyl peptidase IV (DPP IV/CD26) as a multifunctional serine protease and T cell costimulatory molecule has a significant and complex role in the regulation of various physiological processes, including regulation of immune response in immune-mediated diseases. Considering the immunomodulating role of DPP IV/CD26 and previously established higher T lymphocyte DPP IV/CD26 expression, along with a decreased serum DPP IV/CD26 activity in patients with inflammatory bowel disease (IBD), an association between IBD pathogenesis and DPP IV/CD26 has been suggested. The aim of the study was to investigate the influence of DPP IV/CD26 deficiency on development and resolution of dextran sulfate sodium (DSS) induced colitis in CD26 deficient (CD26-/-) and wild-type (C57BL/6) mice. Colitis development and severity in both mouse strains were monitored by clinical, histological and biochemical changes at systemic and local level. The grade of intestinal mucosa damage was determined by microscopic damage index. In the acute phase of colitis, loss of body mass and disease activity in C57BL/6 mice was more intensive than in CD26-/- mice, in spite of similar histopathological changes at the local level. In the acute phase of colitis, colon DPP IV/CD26 activity was significantly decreased in C57BL/6 mice compared to healthy animals (0.19 ± 0.05 vs 0.69 ± 0.09 nkatal/mg of protein, $P < 0.05$). The results of our study reveal that DPP IV/CD26 deficiency reflects on the onset of clinical symptoms and histological changes at the site of inflammation in CD26-/- animals, suggesting a pathophysiological role of DPP IV/CD26 and providing new insights into the nature of DSS colitis.

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PI-45

QUANTITATIVE CHARACTERIZATION OF THE AUTOACTIVATION STEPS OF MASP-1 AND MASP-2, SERINE PROTEASES OF THE COMPLEMENT LECTIN PATHWAY

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MASPs circulate in the blood in the zymogen (proenzyme) form in complex with pattern recognition molecules: MBL and ficolins. MASPs autoactivate when the complexes bind to pathogen-associated molecular patterns (PAMPs), which triggers the complement system. Autoactivation of complement proteases - MASP-1 and MASP-2 of the lectin pathway, and C1r of the classical pathway - takes place in two steps: (I) cleavage of a zymogen by another zymogen molecule ("zymogen autoactivation"), (II) cleavage of a zymogen by the activated enzyme in an autocatalytic fashion ("autocatalytic activation").
Utilizing recombinant catalytic fragments (CCP1-CCP2-SP), we made an uncleavable proenzyme form of MASP-1 (R448Q) and demonstrated that it can cleave another type of cleavable, but inactive mutant, in which the catalytic Ser was replaced by Ala (S646A). Using these mutants and the wild-type enzyme we could kinetically separate the two autoactivation steps. Analogous mutants were used to determine kinetic constants for MASP-2. Cleavage was followed by SDS-PAGE and quantified by densitometry. The proenzyme variant of MASP-1 (R448Q) was also crystallized and the structure was solved. The structure of zymogen MASP-1 catalytic region gives us an insight into the structural changes during MASP-1 autoactivation.
For MASP-1 the catalytic efficiency (kcat/Km) of zymogen autoactivation was 7.0x103 M-1s-1, whereas that of the autocatalytic activation was 1.7x105 M-1s-1. In the case of MASP-2 autocatalytic activation is much slower (kcat/Km=5.7x102 M-1s-1), whereas zymogen autoactivation is extremely slow (kcat/Km=0.15 M-1s-1). Active MASP-1 can efficiently activate MASP-2 in vitro (kcat/Km=1.1x104 M-1s-1), however activation is less efficient the other way around (kcat/Km=6.0x102 M-1s-1). Based on the kinetic data a model of the lectin pathway activation can be outlined, which includes fast autoactivation of MASP-1 and activation of MASP-2 by MASP-1.
This work was supported by OTKA grants NK77978 and NK100834.

PI-46

EFFECT OF NANOSELENIUM TREATMENT IN FATTY LIVER

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Introduction: Fatty liver can be considered low-grade systemic inflammation. The past several years have shown a growing interest for adjuvant antiinflammatory antioxidant therapy in fatty liver. In this study nanoselenium was used because as trace element is important cofactor in several antioxidant proteins and enzymes. Through this effect can moderate redox-homeostasis, redox sensitive caspase activity and prostaglandine biosynthesis.
Aims: Our aim was to investigate the effect of nanoselenium in alimentary induced fatty liver.
Material and methods: Male Wistar rats (200-250 bwg) were fed with or without high-fat diet (control) for 10 days. High fat diet contained 2% cholesterol, 0.5% cholic acid, 20% sunflower oil mixed in their chow. After 3-day fatty liver induction i.p. nanoselenium treatment started in 2,33µg/bwkg dose for 7 days. Routine laboratory and global antioxidant parameters (induced chemiluminescence intensity, H-donating ability, free SH-group concentration and reducing power), transmethylating abilty, fatty acid analysis, caspase 3/7 activity as well as histopathological examinations were carried out.
Results: Compared the control with fatty liver group higher induced chemiluminescence levels were measured which justified low grade inflammation. At the same time induced free radical level in high fat diet group decreased significantly (180,34 vs. 85,54) by nanoseleium treatment but in group with normal diet free radical level increased significantly (3,34% vs. 45,96). In both treated groups reducing power decreased and level of bounded HCHO as a marker of transmethylating ability increased, but in level of free SH-groups were measured reverse effect of treatment. Caspase 3/7 activity was modified moderately by nanoselenium treatment (control: 3,79; fatty liver: 2,36; fatty liver with nanoSe: 3,26). In routine laboratory parameters showed decreased LDL, cholesterol, ALP, GGT and increased GOT level in treated fatty liver group. In both treated groups level of arachidonate-, linol-, α-linolenic-, γ-linolenic acid decreased. Histopathological examinations showed moderately beneficial alterations in the treated animals already during 7 day-treatment.
Conclusion: On the basis of our results nanoselenium may be potent liver protecting agent in fatty liver.
Supported by ETT 002/02.

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PI-47

MONOSPECIFIC MASP INHIBITORS IDENTIFY MASP-1 AS THE ACTIVATOR OF THE LECTIN PATHWAY AND PROVIDE THE FIRST MICHAELIS-COMPLEX STRUCTURE OF A MASP PROTEASE

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Lectin pathway is an antibody-independent activation route of the complement system. It provides immediate defense against pathogens and altered self-cells, but also causes severe tissue damage after stroke, heart attack and other ischemia reperfusion injuries. The pathway is triggered by target-binding of pattern recognition molecules leading to activation of associated zymogen MASPs (mannose-binding lectin-associated serine proteases). Until now the autoactivating MASP-2 has been considered as the autonomous initiator of the proteolytic cascade. The role of the much more abundant MASP-1 protease was controversial. We evolved unique, monospecific inhibitors against MASP-1 and MASP-2 by phage display. These inhibitors were used as unique reagents to reveal a completely novel mechanism of lectin pathway activation. In normal human serum MASP-2 activation strictly depends on MASP-1. MASP-1 activates MASP-2 and moreover, inhibition of MASP-1 prevents autoactivation of MASP-2. Furthermore we demonstrated that MASP-1 produces 60% of C2a responsible for C3 convertase formation. To understand structural basis of the highly selective interaction between MASP-2 and its physiological substrates we provide a complex structure of MASP-2 and our novel substrate-like inhibitor at a resolution of 1.28 Å. It reveals significant structural plasticity for MASP-2 suggesting that induced fit should contribute to the extreme specificity of the enzyme.

PI-48

THE ROLE OF CATHEPSIN C AND ITS INHIBITOR CYSTATIN F IN INACTIVATION OF NK CELL CYTOTOXIC FUNCTION FOLLOWING TREATMENT WITH ANTI-CD16 ANTIBODY

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Natural killer (NK) cells participate in the immune response against variety of tumor and infected cells. The predominant mechanism of NK cell-mediated cytotoxicity is via induction of apoptosis through the perforin/granzyme pathway which involves the action of cysteine protease cathepsin C. Freshly isolated NK cells lose their cytotoxic function upon the addition of anti-CD16 antibody. Loss of NK cell cytotoxic function can be seen against K562 and UCLA-2 oral tumor cells when either added immediately in the co-cultures or after pre-treatment of NK cells with the antibody before their addition to the tumor cells. Treatment of NK cells with anti-CD16 antibody resulted in decreased expression of mature cathepsin C and accumulation of pro-cathepsin C, which is unable to activate effector serine proteases and mediate cytotoxicity. Thus, binding and triggering of CD16 receptor on NK cells may enhance oral tumor survival and growth by decreased ability of cathepsin C to activate granzymes and mediate cytotoxicity. Cystatin F, a cysteine protease inhibitor, colocalizes with cathepsin C and cathepsin L, an activator of pro-cathepsin C, in the lysosomes of NK cells. Furthermore, cystatin F was found to be present in N- terminally truncated form, which is able to inhibit cathepsins C and L and thus could regulate activity of cathepsin C and/or L in NK cells.

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PI-49

ATORVASTATIN MODULATES LIPOPOLYSACCHARIDE INDUCED TNF-α SECRETION FROM PRECURSORS OF HUMAN SKELETAL MUSCLE

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Pro-inflammatory cytokines are important mediators in chronic diseases and cachexia development. Skeletal muscle actively participates in cytokine production. Tumor necrosis factor (TNF)-α is one of the major inflammation promoters with a central role in sepsis development and chronic diseases progression. Statins have beneficial anti-inflammatory effects and are widely prescribed. We examined TNF-α production in human myotubes and the effect of atorvastatin (AT) on constitutive and lipopolysaccharide (LPS) stimulated TNF-α secretion with regard to AT concentration and time-of-exposure.
Human myotubes were exposed to different AT concentrations ranging from sub- to supratherapeutic (0.1 μM, 1 μM, 10 μM, 100 μM). AT exposure was combined with time-dependant LPS (100 ng/mL) exposure (no exposure, 48 hour co-exposure, 24 hour pre-exposure, 12 hour post-exposure) to evaluate for time of exposure effects. Constitutive and LPS induced TNF-α production was observed. TNF-α concentration was measured using ELISA. Constitutive TNF-α levels were 9.78 ± 1.03 pg/10.000 nuclei. After exposing myotube cultures to increasing AT concentrations no effect on TNF-α secretion was observed. LPS stimulated TNF-α secretion (9.8 vs. 24.5 pg/10.000 nuclei; $p<0.01$). After co-exposing myotube cultures to LPS and AT inhibitory effect of AT on LPS-induced TNF-α secretion was observed, as well as in cultures pre-exposed to LPS before treatment with AT. However, when myotube cultures were first treated with AT and followed by LPS-exposure controversial stimulatory dose dependent effect of AT on TNF-α secretion was observed.
AT does not affect constitutive TNF-α secretion in cultured human myotubes, but inhibits LPS stimulated secretion. Controversial pro-inflammatory AT effect was observed in pre-treatment prior to LPS, suggesting a complex AT effects and involvement of different molecular pathways. Concentration and time-of-exposure seem to be of great importance when considering statin induced effects on TNF-α production.

PI-50

DISTRIBUTION OF TISSUE METALS IN THE BRAIN OF RAT STRAINS WITH GENETICALLY DIFFERENT SUSCEPTIBILITY TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Albino Oxford (AO) rats, compared to the Dark Agouti (DA) strain, exhibit lower susceptibility to the induction of experimental autoimmune encephalomyelitis (EAE). Mechanisms include the differences in peripheral response to immunization and those linked with the CNS milieu, which contribute to limit the injury. In the search for factors related to these differences, previously we found that these strains significantly differ in the constitutive gene and protein expression of the cysteine rich proteins-metallothioneins I and II (MTs) that maintain the metal ion homeostasis and have marked anti-inflammatory and neuroprotective properties. In attempt to enlarge these findings, in this study we correlated the protein expression of MTs with tissue concentrations of Zn^{+2}, Cu^{+2} and Fe^{+2} in the brain and its regions, which were highly sensitive to autoimmune attack - i.e. in hippocampus, cerebellum and lumbar spinal cord.
Rats were immunized with bovine brain homogenate (BBH) emulsified in Freund's Complete Adjuvant (CFA) or with CFA only. On days 7 and 12 after immunization the tissue concentrations of Zn^{2+}, Cu^{2+} and Fe^{2+} were estimated in the whole brain and its regions, and in the spinal cord by inductively coupled plasma spectrometry.
AO rats did not show any clinical signs of EAE after immunization with BBH+CFA, but MTs protein expression were upregulated in some regions of CNS. This strain of rats had, however, greater basal levels of Zn^{+2} and Cu^{+2} in hippocampus and lower concentrations of Zn^{+2}, Cu^{+2} and Fe^{+2} in spinal cord. Besides, in comparison to EAE-susceptible DA rats they were able to better maintain the metal homeostasis in the brain and spinal cord tissue after immunization. The data point to genetic differences in the mechanisms that during the autoimmune attack through activation of MTs and metal uptake or release ensure protection of the most sensitive brain regions against oxidative and nitrosative injuries.

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PI-51

IMMORTALIZATION OF PRIMARY HUVECS TO IDENTIFY GENETIC FACTORS CONTRIBUTE TO CELIAC DISEASE DEVELOPMENT

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Human umbilical vein endothelial cells (HUVECs) derived from high risk celiac person are expected to serve as an excellent source of unaffected endothel cells and open the possibility to use these cells to identify genetic or environmental factors contributing to development of celiac disease. However, primary human endothel cells in culture have a finite proliferative lifespan before they undergo permanent growth arrest, known as replicative senescence. This can hinder to establish standardized experimental conditions to gain relevant and reproducible results.
Our study aimed to immortalize primary HUVECs derived from high risk celiac patients and uneffected control people, and than characterization of these immortalized cells by the comparison to untransfected parental cells.
We have expressed hTERT (the catalytic subunit of telomerase enzyme) in HUVECs by retroviral mediated stable transfection. Ectopic expression of hTERT in HUVECs extended the replicative lifespan of the cells (appr.:150PDL, population doubling) already to over twice that of primary parental controls (appr.:60PDL), technically defining these hTERT(+) HUVECs lines as immortalized. During the characterization of these immortal cells we wanted the confirm the following phenomena: (i) hTERT enzyme expressed (westrn blot), (ii) hTERT expression extends the cell lifespan (growth pattern line), (iii) hTERT(+) HUVEC lines retain endothelial characteristics (Immunocytochemistry for Von Willebrand factor), (iv) maintain angiogenic potential (matrigel), (v) and normal karyotypes (vi) but don't show tumorigenic potential (soft agar assay).
Our data suggest that the introduction of telomerase into normal primary HUVECs in vitro does not lead abnormal growth patterns, cell transformation or functional changes, however hTERT HUVECs show genomic instability, which call further investigation.

PI-52

NEW INSIGHT IN CELL LOCALIZATION OF OAT3 IN THE MOUSE KIDNEY; DIFFERENT SEX-DEPENDENT EXPRESSION OF OAT3 AND OAT1

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Immunolocalization of the organic anion transporter 3 in the mouse kidney (mOAT3, Slc22a8) was previously studied with antibodies against the rat (rOAT3-Ab) and mouse (mOAT3-Ab) proteins, and detected in both species in the basolateral membrane (BLM) of various nephron segments, including proximal tubule (PT), thick ascending limb of Henle, cortical collecting duct, and distal tubule. The *mOat3* mRNA, and mRNA of the functionally-similar transporter *mOAT1* (Slc22a6), were detected in the whole mouse kidney, where they exhibited the female (F)-dominant and male (M)-dominant expression, respectively. The sex-dependent expression of these transporters at the protein level is poorly documented. In our preliminary experiments we observed some discrepancies in immunolocalization of OAT3 in the mouse kidney with previously used antibodies. Here we performed detailed studies with both antibodies in order to define correct localization of mOAT3 and sex-dependency of mOAT3 and mOAT1 proteins. In these experiments we used adult wild-type (WT) intact and gonadectomised mice and *mOat3* knockout (KO) mice of both sexes, and studied: a) cell localization of mOAT3 (with rOAT3-Ab and mOAT3-Ab) and mOAT1 (with rOAT1-Ab) proteins by immunocytochemistry (IC) in kidney cryosections, b) abundance of both proteins by Western blotting (WB) of total cell membranes (TCM) isolated from the whole kidney, and c) effect of castration in M mice on protein expression by IC and WB. In accordance with previous studies, rOAT3-Ab stained the BLM of various nephron segments in WT mice, but the same staining was noted along the nephron of *Oat3* KO mice, proving the nonspecificity of rOAT3-Ab in mouse organs. On the contrary, mOAT3-Ab exclusively stained the BLM of cortical PT, and no staining was noted in the kidneys of *mOat3* KO mice. The latter data were confirmed by the absence of mOAT3-related protein band of ~70 kDa in TCM from the *Oat3* KO mice. In addition, the expression in WT mice of mOAT3 protein was F>M, and upregulated by castration, while the expression of mOAT1 protein was M>F, and downregulated by castration. In conclusion, our results indicate that in the mouse nephron: a) mOAT3 and mOAT1 proteins are exclusively localized in the PT BLM, and b) both proteins exhibit the sex-dependent expression, however, with an opposite pattern; the mOAT3 expression is F-dominant due to androgen inhibition, while the expression of mOAT1 is M-dominant due to androgen stimulation.

PI-53

EXPRESSION OF AQUAPORIN 1 (AQP1) ALONG THE MAMMALIAN NEPHRON; SEX AND SPECIES DIFFERENCES

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In the mammalian kidneys, AQP1 is supposed to be a constitutive water channel located in the apical and basolateral domain of proximal tubule (PT) and descending thin limb (DTL) epithelium. In the membrane it exists in two, nonglycosylated (NG, ~28 kDa) and glycosylated (G, 40-50 kDa) forms, both being water permeable. Factors influencing renal AQP1 expression in (patho)physiological conditions are poorly known; thus far only angiotensin II and hypertension were found to upregulate its protein and mRNA expression in the rat PT (*Am J Physiol Renal Physiol* 297:F1575, 2009). In order to investigate possible sex and species differences in the expression of renal AQP1, we used an anti-AQP1 polyclonal antibody and performed immunocytochemistry on tissue cryosections and Western blotting (WB) of cell membranes isolated from various kidney zones of adult male (M) and female (F) rats, mice, pigs and humans. Effects of sex hormones on AQP1 expression were studied more thoroughly in prepubertal and adult, gonadectomized and sex hormone-treated gonadectomized rats. In rats and mice, the AQP1-related immunostaining in various kidney zones in M was stronger than in F, whereas the expression (immunostaining intensity) of AQP1 in the pig and human kidneys of both sexes was similar. These results were confirmed by WB of total cell membranes (TCM), and brush-border and basolateral membranes isolated from the respective kidney zones. The observed sex differences in expression were comparable for both NG and G forms of AQP1. In the adult rats, castration had no effect, while ovariectomy increased the abundance of AQP1 in the renal TCM. Furthermore, treatment of castrated animals with testosterone upregulated, whereas treatment with estradiol and progesterone had no significant effect on NG and G forms of AQP1. Strong, but sex-independent AQP1 expression was detected in red blood cell membranes isolated from adult rats, whereas in TCM isolated from the kidneys of prepubertal rats, the AQP1 expression was weak and similar in both sexes. We conclude that: a) sex differences exist in the expression of AQP1 along the nephron of adult rats and mice (M>F), which result from both upregulating effects of androgens in M and downregulating effects of estrogens in F after puberty, and b) similar sex differences are absent in the pig and human kidneys, thus indicating the presence of species differences in the expression of renal AQP1.

PI-54

FUNCTIONAL CHARACTERIZATION OF A NOVEL UPTAKE TRANSPORTER OAT2A (SLC22A7) IN ZEBRAFISH (*DANIO RERIO*)

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Organic anion transporter 2 (OAT2) belongs to SLC22 family of multi-specific transport proteins (solute carriers SLC22; Organic anion transporters) and is responsible for uptake of various endogenous and exogenous compounds across the plasma membrane. Previous phylogenetic and expression analyses showed that OAT2 is present in human, rat and mouse with the highest gene expression in liver and kidney. However, tissue distribution and cellular localization differ among species and genders. Physiological role of human OAT2 is based on sodium-independent uptake of hormones: estrone-3-sulfate (E3S) and dehydroepiandrosterone sulfate (DHEAS); eicosanoids: prostaglandin E2 (PGE2) and F2α (PGF2α); bile salts: taurochenodeoxycholate (TCDC) and cholate; citric acid cycle intermediate α-ketoglutarate; fatty acid propionate and signal molecules cAMP and cGMP. Considering lack of data on non-mammalian OAT2 co-orthologs, the goal of our study was to determine phylogenetic relationships, tissue distribution and substrate specificities of zebrafish Oat2a. Phylogenetic analysis showed that zebrafish Oat2a is one of five OAT2 co-orthologs and the only direct OAT2 ortholog. Our qPCR tissue expression profiling pointed out different expression pattern of Oat2a compared with OAT2 and confirmed sex dependant differences in tissue distribution. In zebrafish males, Oat2a showed the highest expression in testes, gills and brain, while in females it is predominantly found in brain. Interestingly, Oat2a showed low to negligible expression in liver and kidney, the main sites of mammalian expression. Using the transiently transfected HEK293 cells and fluorescent substrate lucifer yellow, we carried out functional characterization of Oat2a. Among the 33 compounds tested, we have found that physiological substrates of Oat2a are E3S, DHEAS, TCDC, cGMP and bilirubin, model substrates of OAT2. We also showed that Oat2a transports exogenous compounds such as furosemide, a loop diuretic, and diclofenac, a non-steroidal anti-imflammatory drug (NSAID). In contrast to OAT2, Oat2a does not show interaction with physiological OAT2 substrates α-ketoglutarate, glutarate, p-aminohippurate (PAH), PGE2 as well as xenobiotics erythromycin and NSAIDs salicylate and indomethacin. In conclusion, the first functional characterization of zebrafish Oat2a revealed substantial differences in tissue distribution pattern and partial similarities in substrate specificity, compared to its direct ortholog OAT2.

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PI-55

RENAL EXPRESSION AND LOCALIZATION OF SODIUM-D-GLUCOSE COTRANSPORTER 1 (SGLT1) IS DIFFERENT IN RATS AND MICE

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SGLT1 is a high affinity/low capacity transporter of glucose (G) in the mammalian small intestine and kidneys. In the small intestine, SGLT1 is responsible for the entire G absorption, whereas in kidneys, it contributes to ~10% of G reabsorption, the bulk being handled by the low affinity/high capacity SGLT2. In our recent studies in rat kidneys (*Am J Physiol Renal Physiol* 290:F913, 2006 *& Am J Physiol Cell Physiol* 295:C475, 2008), the SGLT1 protein was characterized with the rat-specific polyclonal antibody, and immunolocalized to the proximal tubule (PT) brush border membrane (BBM) and intracellular organelles, exhibiting segmental (S1<S2<S3), zonal (cortex<outer stripe), and sex (females (F)>males (M)) differences in expression. Specific immunoreactivity was also observed in the luminal membrane of cortical thick ascending limb of Henle (TALH) and macula densa. However, previous studies in mice did not reveal clear expression and localization of SGLT1 in their kidneys and other organs due to lack of specific antibody. In order to characterize the SGLT1 protein in mouse organs, we have generated a novel polyclonal antibody against the mouse SGLT1 (mSGLT1-ab). Specificity of the antibody was confirmed by Western blotting (WB) of BBM isolated from the mouse small intestine and kidneys, and by immunostaining of tissue cryosections using wild type (WT) and *Sglt1* knockout (KO) mice. In WT mice, mSGLT1-ab labeled the ~75 kDa protein band in the BBM from the small intestine and kidneys, and stained the brush-border of epithelial cells in both organs, whereas in KO mice, both the protein band and immunostaining were absent. In the kidneys of WT mice, the antibody strongly stained the BBM of PT S2 and S3 segments (S1 was negative), exhibiting segmental (S2>S3) and zonal (cortex>outer stripe) differences in staining intensity, similar in both sexes. The SGLT1-ab further stained the apical domain of TALH in the kidney cortex and outer stripe, liver bile ducts, and pancreatic ducts. In these organs, as well as in the small intestine, similar staining intensity in F and M was observed. The cells of macula densa remained unstained. Other tested extrarenal organs, such as brain, spleen, skeletal and heart muscles, and eyes were negative. Therefore, comparison of the data in rats and mice indicates the presence of species differences in renal expression and localization of SGLT1 protein.

PI-56

FUNCTION AND STRUCTURE OF THE TANDEM EXTRACYTOPLASMATIC SUBSTRATE-BINDING DOMAINS OF THE ABC TRANSPORTER GlnPQ

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The glutamine/glutamic acid ABC transporter GlnPQ from *Lactococcus lactis* has two substrate-binding domains (SBD1 and SBD2) linked in tandem and fused to the transmembrane domain (NBD). The functional GlnPQ complex has four SBDs in total. Here, we present the crystal structures of the tandem SBDs and individual domains with and without ligand. Moreover, we used isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC) to characterize the ligand binding thermodynamically. We show that SBD1 only binds glutamine, whereas SBD2 binds glutamine and glutamic acid. Glutamine binding to the tandem SBD is non-cooperative even though the protein domains are covalently linked. Guided by the crystal structures, we have designed mutations in and around the binding-site of SBD1 and SBD2 to assess the role of the individual receptor domains in the full-length transporter. We show that either SBD1 or SBD2 is sufficient for glutamine uptake, whereas SBD2 is essential for the transport of glutamic acid. Thus, both SBDs are capable of docking independent of each other onto the TMD and deliver glutamine for translocation by the ABC transporter.

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PII-57

THE REGULATORY ROLE OF MYOSIN PHOSPHATASE IN THE ACTIVATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE

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Endothelial nitric oxide synthase (eNOS) produces nitric oxide (NO) that is involved in the regulation of smooth muscle relaxation and many other physiological processes. eNOS activity is enhanced by phosphorylation at Ser1177, while phosphorylation of Thr495 decreases the activity of the enzyme. It has been shown that protein phosphatase-1 (PP1) catalytic subunit (PP1c) dephosphorylates eNOS at Thr495, however, little is known about the regulatory (targeting) subunit(s) involved.

HEK293 cells, transfected with eNOS plasmid, expressed myc-eNOS and exhibited higher NO level as revealed by chemiluminescence nitrite measurements. Upon PMA plus calyculin-A (CLA) treatment phosphorylation of Thr495 was stimulated by 3-4-fold and this was accompanied with decreased NO production. We investigated whether myosin phosphatase (MP) consisting of PP1cδ and myosin phosphatase target subunit-1 (MYPT1), is involved in the dephosphorylation of eNOS at Thr495. We demonstrated the interaction of MYPT1 and eNOS in endothelial cells (EC) by immunoprecipitation, by pull-down assays, in HEK293 cells co-expressing both Flag-MYPT1 and myc-eNOS, furthermore, by surface plasmon resonance (SPR) based binding experiments. Purified myc-eNOS phosphorylated by Rho-kinase at Thr495 was dephosphorylated by the PP1c-MYPT1 complex. Confocal fluorescent microscopic images showed co-localization of MYPT1 and eNOS in dividing EC's. Treatment of purified Flag-MYPT1 with peroxynitrite resulted nitration of the regulatory subunit which leads to lower affinity to PP1c.

Our results demonstrate that MP and eNOS interacts in endothelial cells and MP is identified as the phosphatase holoenzyme which dephosphorylates phospho-Thr495 in eNOS, therefore it may be involved in the activation of this enzyme.

This work was supported by grants from the Hungarian Scientific Research Fund OTKA K68416 and CNK 80709, TÁMOP-4.2.2/B-10/1-2010-0024 and by the TÁMOP 4.2.2.-08/1-2008-0019 DERMINOVA project.

PII-58

CHARACTERIZATION OF NHERF1/NHERF2 PROTEINS IN ENDOTHELIAL CELLS

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The Na+/H+ exchanger regulatory factor (NHERF) family consists of four related PDZ domain containing scaffolding proteins. Within the family NHERF1 and NHERF2 share the highest sequence homology and have C-terminal ezrin-radixin-moesin (ERM)-binding domains. It is likely that the primary function of NHERFs is to act as scaffold proteins and to form bridges among plasma-membrane and cytoskeletal proteins. Their roles were studied in epithelial cells by several aspects but not in the endothelium. In our study BPAE (Bovine Pulmonary Artery Endothelial) cells were used to characterize the localization and potential role of these scaffolding proteins. We found that endogenous NHERF1 has unusual nuclear localization compared to epithelial cells, in contrast to cytoplasmic appearance of NHERF2. NHERF1 and NHERF2 were amplified from bovine cDNA and cloned into pCMV mammalian expression vectors. Immunofluorescent staining of the over-expressed proteins in BPAEC showed the same localization as endogenous NHERF1 and -2. It was known from studies made on epithelial cells that during mitosis NHERF1, but not NHERF2, became phosphorylated by CDK1. We detected phosphorylation dependent localization change of NHERF1 during the cell cycle. Furthermore, the A regulatory and the C catalytic subunits of PP2A co-immunoprecipitated with NHERF1, but no specific interaction was found with the PP1c isoforms. Interaction of NHERF1 with the B55alpha subunit containing PP2A holoenzyme was also detected by pull-down experiments. By electric cell-substrate impedance sensing (ECIS) we found that the phosphomimic mutant form of NHERF1 supports wound healing, suggesting its role in cell migration. Because ERM proteins can be affected during these events, we initiated experiments to investigate the specificity of NHERF1 and NHERF2 towards ERM proteins. We created GST-tagged bacterial constructs of each protein. Different binding affinity was found by pull-down experiments between NHERF1 or -2 and ezrin, radixin, moesin. Our goal is to investigate the role of NHERF1 and NHERF2 during the cell cycle with special emphasis on ERM binding aspects.

This work was supported by the grant CNK80709 (GP) from the Hungarian Science Research Fund.

PII-59

Tks4 SCAFFOLD PROTEIN REGULATES EGF-DEPENDENT CELL MIGRATION

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Tks4 and Tks5 belong to a family of scaffolding proteins recently shown to be involved in podosome formation and cell invasion. In this study, we show that Tks4 is also involved in EGF signaling. It has been found that upon EGF treatment Tks4 translocates to the cell membrane where it associates with the activated EGF receptor and becomes tyrosine phosphorylated. We identified members of the Src family to be the most likely kinases responsible for this phosphorylation. The association between the EGFR and Tks4 is not direct and requires the presence of Src. Since we found prominent Tks4 redistribution to lamellipodia after EGF stimulation we also investigated if Tks4 may play a role in cell migration. Silencing of Tks4 was shown to markedly inhibit HeLa cell migration in a Boyden chamber assay in response to EGF or serum. Our results therefore reveal a new function for Tks4 in the regulation of growth factor-dependent cell migration.

PII-60

OLIGOMERIZATION AND CELL-CYCLE DEPENDENT PHOSPHORYLATION GOVERNS NUCLEAR TRANSPORT OF dUTPases

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The genomic integrity of living organisms is guarded by several physiological processes, among which is the maintenance of low uracil levels. The main effector in this process is the evolutionarily highly conserved dUTPase enzyme, responsible for the cleavage of dUTP, resulting in low dUTP/dTTP ratio. Most dUTPases are homotrimeric enzymes which require an active mechanism for their transport into the nucleus, where they are known to reside. This energy dependent import is done through the recognition of their NLSs (Nuclear Localization Signals) by importin-α, a common mediator of nuclear import. In our studies we have characterized two distinct but likely to be coexisting and cooperating mechanisms in the regulation of the dUTPase's nucleocytoplasmic shuttle. Phosphorylation of nuclear human dUTPase in the vicinity of its NLS is already confirmed but its role has not yet been described. Using hyperphosphorylation and hypophosphorylation mimicking mutants we revealed that this phosphorylation blocks the nuclear localization of the dUTPase. Our video microscopic and protein transfection studies showed that after cell division, a phosphorylated wild type dUTPase form may re-enter the nucleus but in a delayed manner, putatively due to a time taking dephosphorylation event. Further we defined that the kinase in this process is CDK1. Our in vitro experiments (native-PAGE, gel filtration, Thermofulor, CD spectroscopy) with purified proteins showed that importin-α binds the phosphorylation mimicking mutants to a much lower extent. We also revealed the structural background for this lower affinity using X-ray crystallography. For investigating the role of oligomerization in nuclear transport, we used modified forms of the Drosophila virilis dUTPase as a model protein. In ITC and native-PAGE experiments, we examined the importin-α's complexation with dUTPases bearing different numbers of NLSs. We confirmed that a lower stoichiometry that means less importins bound to a dUTPase trimer, results in reduced nuclear transport. We verified the in vivo relevance of our findings with immunohisto-, immunocytochemistry and with fluorescent reporter systems. We propose that phosphorylation of only some of the three NLSs on a dUTPase trimer can lead to lower functional NLS numbers. This could result in an altered, dynamic stoichiometry of the cargo-importin-α complex. Taken together oligomerization and phosphorylation might serve as a fine tuning mechanism of the dUTPase's subcellular localization.

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INTERACTION OF GLI1, SUFU AND GSK3β IN CENTROSOMES OF HEK293 CELLS

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The Hedgehog (Hh) signaling pathway is a developmental pathway, mostly inactivated in adult tissues. Aberrant activation has been found in various tumor types, such as lung, breast, ovarian and colon. The pathway is activated by the ligand Hedgehog that causes its receptor Patched to release its repression over the coreceptor Smoothened. This triggers a cascade of events in the cytoplasm leading to activation of transcription factor Gli1. The interactions between the transcription factor Gli1 and its regulators Suppressor of Fused (SuFu) and GSK3β or the role of GSK3β in activated cells are not fully understood yet. Recently the primary cilium was shown to play an important role in signal transduction.

The human embryonic kidney cells, HEK293 show an interesting pattern of Gli1, SuFu and GSK3β accumulation in the centrosome, which gives rise to the basal body of the primary cilium. Since the primary cilia of these cells were undetectable we wanted to investigate if it is possible for these proteins to interact in the centrosome in the absence of a primary cilium. Exogenous Shh protein treatment causes a shift in protein localization, Gli1 translocates to the nucleus and SuFu remains in the cytoplasm. The amount of cells with visible accumulations of these proteins in the centrosome decreases from 80% to 19%. This suggests that the pathway is fully active and functioning properly. Preliminary results reveal that Gli1 and SuFu form a complex in these cells, suggesting that their interaction is independent of the primary cilium. Gli2 is undetectable in these cells, while Gli3 localizes to vesicles in the cytoplasm. Therefore it is likely that Gli1 is the main mediator of signal transduction.

To examine the effect of GSK3β inhibition on protein localization and interactions, we treated the cells with a GSK3β inhibitor (LiCl). Treatment elevates the pathway activity, increases expression of *GLI1* and *PTCH1* and also causes a shift in protein localization consistent with pathway activation.

Our results propose that HEK293 cells have an active Hh signaling pathway, with the regulatory processes between Gli1, SuFu and GSK3β taking place in the centrosome.

PII-62

CAN BACTERIAL HANKS-TYPE KINASES CONSTITUTE SIGNAL TRANSDUCTION CASCADES?

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Signal transduction in bacteria most often includes phosphorylation of histidine and aspartate residues, within relatively simple two-component systems. Some bacteria also possess serine/threonine-specific protein kinases, which belong to a large superfamily of Hanks-type kinases, together with the homologous eukaryotic enzymes. Aim of this research was to find out whether bacterial Hanks-type kinases constitute a phosphorylation cascade. Signal transduction through protein kinase cascades enables amplification of the signal and integration of different signalling pathways. This type of signal transduction is well studied and an important phenomenon in eukaryotes, where it regulates various cellular processes, but it hasn't been associated with bacteria. A model Gram-positive bacterium *Bacillus subtilis* possesses a few Hanks-type kinases, two of them being membrane proteins. To determine whether these kinases phosphorylate each other, it was necessary to abolish their autophosphorylation activity. This was accomplished by site-directed mutagenesis of the active site. After expression and purification of recombinant proteins, *in vitro* phosphorylation assays with ^{32}P-γ-ATP were performed. Wild-type and mutant forms of different kinases were combined. It was determined that both membrane-linked kinases, PrkC and YabT, phosphorylate a citosolic kinase YbdM. Also, intermolecular kinase activity of PrkC was confirmed. Structural resemblance of PrkC to eukaryotic receptor kinases and its ability to transphosphorylate, make this membrane kinase a good candidate for the first step of the newly characterized signal transduction cascade in bacteria.

PII-63

RETINOIDS PRODUCED BY MACROPHAGES ENGULFING APOPTOTIC CELLS CONTRIBUTE TO THE APPEARANCE OF TRANSGLUTAMINASE 2 IN APOPTOTIC THYMOCYTES

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Transglutaminase 2 (TG2) has been known for a long time to be associated with the *in vivo* apoptosis program of various cell types including T cells. Though the expression of the enzyme was strongly induced in mouse thymocytes following apoptosis induction *in vivo,* no significant induction of TG2 could be detected, when thymocytes were induced to die by the same stimuli *in vitro* indicating that signals arriving from the tissue environment are required for the *in vivo* induction of the enzyme in apoptotic thymocytes. Previous studies have shown that one of these signals is TGF-β which is released by macrophages engulfing apoptotic cells. Besides TGF-β the TG2 promoter contains retinoic acid response elements as well. Here we show that *in vitro* retinoic acids, or TGF-β and retinoic acids together can significantly enhance the TG2 mRNA expression in dying thymocytes, and the apoptotic signal contributes to the TG2 induction. Inhibition of retinoic acid synthesis either by aldehyde or retinaldehyde dehydrogenases significantly attenuates the *in vivo* induction of TG2 following apoptosis induction indicating that retinoids indeed might contribute *in vivo* to the apoptosis-related TG2 expression. What is more, the *in vivo* apoptosis induction in the thymus is accompanied by an enhanced retinoid dependent transcriptional activity possibly due to the enhanced retinoid synthesis by macrophages engulfing apoptotic cells. Our data reveal a new crosstalk between macrophages and apoptotic cells, in which apoptotic cell uptake-induced retinoid synthesis in macrophages enhances TG2 expression in the dying thymocytes.

PII-64

ADENOSINE AS A MEDIATOR OF THE ANTIINFLAMMATORY EFFECTS OF APOPTOTIC CELL UPTAKE

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It is well known that the apoptotic cell uptake inhibits inflammatory response in macrophages. This inhibition is mediated directly via cell surface receptors or by soluble mediators release by macrophages engulfing apoptotic cells. Adenosine is an endogenous anti-inflammatory mediator. The different adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, A_3) - which all can be found on macrophages - are all seven transmembrane and G-protein coupled receptor. These are further classified based on their ability to either stimulate or inhibit the adenylate cyclase activity. While the A_{2A} and A_{2B} receptors are coupled to Gs and mediate the stimulation of adenylate cyclase; the A_1 and A_3 are coupled to Gi and inhibit the adenylate cyclase activity. Previous studies in our laboratory have shown that adenosine is released by macrophages engulfing apoptotic cells and inhibits the secretion of neutrophil chemoattractant factors (i.e. MIP-2, KC) which otherwise would be produced following apoptotic cell exposure by stimulating the A_{2A} receptor – adenylate cyclase pathway. (Köröskényi et. al. *The Journal of Immunology*, 2011).
The aim of the present study was to test the effect of the loss of adenosine A_3 receptor on the proinflammatory cytokine production of macrophages following apoptotic cell uptake.

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RETINOIDS INDUCE A NUR77-DEPENDENT APOPTOSIS IN MOUSE THYMOCYTES

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Nur77 is a transcription factor, which plays a determinant role in mediating T cell receptor-induced cell death of thymocytes. In addition to regulation of transcription, Nur77 contributes to apoptosis induction by targeting mitochondria, where it can convert Bcl-2, an anti-apoptotic protein into a proapoptotic molecule. Previous studies have demonstrated that retinoids are actively produced in the mouse thymus and can induce a transcription-dependent apoptosis in mouse thymocytes. Here we show that similar to TCR stimulation, retinoic acids also induce the expression of Nur77 in a dose-dependent manner, 9-cis retinoic acid being more effective than all-trans retinoic acid. Retinoid-induced apoptosis was found to be completely dependent on Nur77, as retinoids were unable to induce apoptosis in Nur77 null thymocytes. In wild-type thymocytes retinoids induced the expression of five apoptosis-related genes, FasL, TRAIL, NDG-1, Gpr65 and Bid, all of them in a Nur77-dependent manner. The combined action of these proteins led to Caspase 8-dependent Bid cleavage in the mitochondria. In addition, we could demonstrate the mitochondrial translocation of Nur77 leading to the exposure of the Bcl-2/BH3 domain. The retinoid-induced apoptosis was dependent on both Caspase 8 and 9. Our data together indicate that retinoids induce a Nur77-dependent cell death program in thymocytes activating the mitochondrial pathway of apoptosis.

PII-66

CALCINEURIN REGULATES ENDOTHELIAL BARRIER FUNCTION BY INTERACTION WITH AND DEPHOSPHORYLATION OF MYOSIN PHOSPHATASE

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Endothelial cells (EC) lining the inner surface of blood vessels form a selective permeable barrier between blood and the interstitial space. Thrombin is a potent inducer of hyperpermeability of cultured monolayers of EC resulting in cell contraction by myosin light chain (MLC) phosphorylation. MLC phosphorylation level is determined by the balanced activities of MLC kinase and myosin phosphatase (MP). In the structure of MP holoenzyme, the catalytic subunit (PP1c) is associated with a regulatory subunit, termed myosin phosphatase target subunit-1 (MYPT1). Phosphorylation of Thr695 in MYPT1 by Rho-kinase causes inhibition of PP1c activity.

We have studied the role of calcineurin (CN) in the cytoskeletal rearrangements of bovine pulmonary artery endothelial cells (BPAEC) in response to different agonists. CN is a Ca^{2+}/CaM-dependent phosho-Ser/Thr specific protein phosphatase. Cyclosporine-A (CsA), a CN inhibitor caused prolonged thrombin-induced stress fiber formation and increased the phosphorylation level of MYPT1 at Thr695 residue. Transient phosphorylation of MYPT1 was observed by thrombin treatment. Inhibition of CN with CsA in the presence of thrombin led to sustained phosphorylation of MYPT1. These phosphorylation events might correlate with changes in endothelial permeability since CsA slows down the recovery of transendothelial electrical resistance of BPAECs reduced by thrombin. Interaction of MYPT1 with CN was also revealed by co-localization using confocal microscopy and surface plasmon resonance (SPR) based binding experiments. SPR studies with full-length and truncated mutants of MYPT1 localized the CN binding sites at the N-terminal half of MYPT1, in accordance with the presence of a putative motif (PxIxIT) in MYPT1. These results suggest that CN is involved in the recovery of EC from thrombin-induced dysfunction, presumably via regulation of MP activity by dephosphorylation of MYPT1.

This work was supported by grants: OTKA: CNK 80709 (GP) and K68416 (EF) from the Hungarian Science Research Fund, TÁMOP4.2.2.-08/1-2008-0019 DERMINOVA, TÁMOP 4.2.1./B-09/1/KONV-2010-0007.

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HOG SIGNALING PATHWAY IN EXTREMELY HALOPHILIC FUNGUS *WALLEMIA ICHTHYOPHAGA*

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All living cells must maintain optimal water potential and turgor to assure normal physical and chemical environment for cellular processes. Sensing and reacting to changes in osmolarity is of essential importance to organisms, especially if they inhabit environments with either fluctuating or high osmolyte concentrations. Signaling pathway sensing osmolarity in *Saccharomyces cerevisiae* is known as High-Osmolarity Glycerol (HOG) and has been studied extensively in the past decades. However, similar signaling pathways with homologous proteins are also present in other fungi, among them in extremely halotolerant fungus *Hortaea werneckii.* Our research is aimed at HOG components in extremely halophilic basidiomycetous fungus *Wallemia ichthyophaga*, which is the most halophilic eukaryote known to date. Compared to *H. werneckii,* which does not require salt to remain viable, *W. ichthyophaga* needs at least 10% NaCl (w/v) in the media and is metabolically active even at saturated salt concentration. In this extermophilic fungus we successfully identified three sequential MAP kinases and a putative homologue of Sho1 transmembrane protein, which is considered as part of sensory complex of HOG pathway. Comparison of *S. cerevisiae*, *H. werneckii* and *W. ichthyophaga* protein sequences revealed high conservation of key motifs and domains, which are responsible for their structure and function. However, in contrast with *H. werneckii*, where duplications of *HwSte11*, *HwPbs2* and *HwSho1* genes were observed, we noticed only duplication of the *WiHog1* kinase gene in *W. ichthyophaga*. Expression of *W. ichthyophaga* kinases in *S. cerevisiae* deletion mutants successfully rescued osmosensitivity of the mutant strains. Their localization pattern was monitored by microscopic observation of GFP fusion constructs. On the other hand, when WiSho1 was expressed in functional complementation experiments we got discordant results. By identifying HOG pathway MAP kinase module (WiSte11 (MAPKKK), WiPbs2 (MAPKK) and two paralogous WiHog1 (MAPK)) and WiSho1 transmembrane protein we confirmed the existence of HOG signaling pathway as well as its putative role in osmosensing in *W. ichthyophaga*.

PII-68

INVOLVEMENT OF ADENOSINE A_{2A} RECEPTORS IN ENGULFMENT-DEPENDENT APOPTOTIC CELL SUPPRESSION OF INFLAMMATION

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Efficient execution of apoptotic cell death followed by efficient clearance mediated by professional macrophages is a key mechanism in maintaining tissue homeostasis. Removal of apoptotic cells usually involves three central elements: (1) attraction of phagocytes via soluble 'find me' signals, (2) recognition and phagocytosis via cell surface presenting 'eat me' signals, and (3) suppression or initiation of inflammatory responses depending on additional innate immune stimuli. Suppression of inflammation involves both direct inhibition of pro-inflammatory cytokine production and release of anti-inflammatory factors, which all contribute to the resolution of inflammation. In the present study, using wild type and adenosine A_{2A} receptor ($A_{2A}R$) null mice, we investigated whether $A_{2A}Rs$, known to mediate anti-inflammatory signals in macrophages, participate in the apoptotic cell-mediated immunosuppression. We found that macrophages engulfing apoptotic cells release adenosine in sufficient amount to trigger $A_{2A}Rs$, and simultaneously increase the expression of $A_{2A}Rs$, as a result of possible activation of liver X receptor and peroxisome proliferators activated receptor δ. In macrophages engulfing apoptotic cells, stimulation of $A_{2A}Rs$ suppresses the NO-dependent formation of neutrophil migration factors, such as macrophage inflammatory protein-2, using the adenylate cyclase / protein kinase A pathway. As a result, loss of $A_{2A}Rs$ results in elevated chemoattractant secretion. This was evident as pronounced neutrophil migration upon exposure of macrophages to apoptotic cells in an in vivo peritonitis model. Altogether our data indicate that adenosine is one of the soluble mediators released by macrophages that mediate engulfment-dependent apoptotic cell suppression of inflammation.

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GLUTAMYL-LYSINE ISODIPEPTIDE PRODUCED BY TRANSGLUTAMINASE INHIBITS THE PROINFLAMMATORY CYTOKINE PRODUCTION OF LPS STIMULATED MACROPHAGES

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Transglutaminase is a multi-functional enzyme that post-translationally modifies proteins by catalyzing the formation of intermolecular ε-(γ-glutamyl)-lysyl bonds between glutamine and lysine side chains.
In apoptotic cells tissue transglutaminase (TG2) is upregulated and activated. The enzyme can stabilize the apoptotic bodies by forming glutamyl-lysyl isopeptide bonds between its target proteins. When apoptotic cells are phagocytosed, their content is degraded in phagolysosomes. The γ-glutamyl-lysyl bonds are resistant to lysosomal proteases and the phagocytes secrete ε-(γ-glutamyl)-lysine isodipeptides to their environment. Increased plasma level of isodipeptide molecule indicates the rate of apoptosis though the possible physiological effects of the isodipeptide haven't been investigated yet.
In our experiments we found, that this isodipeptide has potential anti-inflammatory effect. It can decrease the expression of LPS-induced TNF-α, IL-6, MIP-2 and iNOS in primer mouse macrophages and RAW264 mouse macrophage cell line.
In the future we would like to characterize the effect of the molecule on inflammation, migration and phagocytotic capacity.

PII-70

REGULATION OF CASKIN1 SCAFFOLD PROTEIN BY EPHB1 TYROSINE KINASE

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Scaffold proteins have an important role in the regulation of signal propagation. These proteins do not possess any enzymatic activity but can contribute to the formation of multiprotein complexes. Although scaffold proteins are present virtually in all cell types, the nervous system contain them in the largest amount.
Caskin proteins are typically present in neuronal cells, particularly, in the post synaptic density (PSD). Caskin1 is able to form a complex with the Cask proteins; therefore it is referred to as Cask-interacting protein. By the means of yeast two hybrid screening we have identified earlier a number of interacting protein partners of Caskin1, including Abi2 and Nck adaptor proteins.
Here we demonstrated that EphB1 receptor tyrosine kinase can recruit Caskin1 through the adaptor protein Nck. Upon activation of the receptor kinase, Nck SH2 domain binds to one of its tyrosine residues, while Nck SH3 domain interacts with the proline-rich domain of Caskin1. Complex formation of the receptor kinase, adaptor and scaffolds proteins results in the tyrosine phosphorylation of Caskin1 on its SH3 domain. The phosphorylation sites were identified by mass-spectrometry as tyrosines 296 and 336. To reveal the physiological consequence of this phosphorylation, CD spectroscopy was performed. This measurement suggests that upon tyrosine phosphorylation the structure of the Caskin1 SH3 domain changes dramatically.
Taken together, we show here that the scaffold protein Caskin1 can form a complex with the EphB1 tyrosine kinase via the Nck protein as a linker. Translocation of Caskin1 to the plasma membrane leads to its tyrosine phosphorylation on its SH3 domain. Although we were not able to identify any physiological partner of the SH3 domain so far, we could demonstrate that phosphorylation on conserved tyrosine residues results in marked changes in the structure of the SH3 domain.

PII-71

THE FUNGUS SPECIFIC PROTEIN PHOSPHATASE Z1 PLAYS IMPORTANT ROLES IN SALT TOLERANCE, CELL WALL INTEGRITY, GERM TUBE GROWTH, AND VIRULENCE OF THE HUMAN PATHOGEN *CANDIDA ALBICANS*

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C. albicans is a common opportunistic human pathogen. Its genome contains a single protein phosphatase Z gene termed *CaPPZ1*. The primary structure and especially the catalytic domain of the gene product CaPpz1 is similar to that of *Saccharomyces cerevisiae* Ppz1 and *Schizosaccharomyces pombe* Pzh1 enzymes. We demonstrated that the bacterially expressed and purified recombinant CaPpz1 protein dephosphorylates p-nitrophenylphosphate, and this phosphatase activity can be inhibited by Hal3, a known inhibitor of *S. cerevisiae* Ppz1. By site-directed mutagenesis experiments we identified three amino acid residues inside the catalytic domain that affect either the activity or stability of the enzyme. With the expression of *CaPPZ1* in *ppz1 S. cerevisiae* and *pzh1 S. pombe* cells that lack their authentic protein phosphatase Z we proved that the heterologous C. albicans protein was able to complement the salt and caffeine phenotypes of the deletion mutants. Moreover, the CaPpz1 rescued the *slt2 S. cerevisiae* mutant in which the MAP kinase activity mediating the cell wall integrity signalling pathway was deleted. To identify the physiological functions of CaPpz1 in *C. albicans,* we disrupted both copies of gene in the diploid organism. We proved that *ppz1 C. albicans* mutants were sensitive to salts, caffeine, Calcofluor White, and Congo Red, but were tolerant against spermine and hygromycin B. We also established that the reintegration of the *CaPPZ1* into the deletion mutant compensated the typical mutant phenotypes. From the result we concluded that CaPpz1 is involved in cation homeostasis, cell wall integrity and the regulation of the membrane potential of *C. albicans*. These results indicate that the orthologous PPZ enzymes have similar but not identical functions in different fungi. In addition, we tested the germ tube growth rate and the virulence of the deletion mutant strain and found that both of them were reduced relative to the wild type strain. Our findings indicate that the protein phosphatase gene Z is involved in the virulence, and suggesting a novel function for CaPpz1 in the yeast to hypha transition of the pathogenic fungus.
This work was supported by OTKA K 68765, TÁMOP 4.2.1/B-09/1/KONV-2010-0007, BFU2008-04188-C03-01, HH2008-0026, and ES-22/2008 grants.

PII-72

A NEW MECHANISM FOR CYTOPLASMIC EFFECT OF PARP-1

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Previously, it was suggested that release of nuclearly-formed ADP-ribose polymers (PAR) or ADP-ribosylated proteins could be responsible for the cytosolic and mitochondrial effects of poly(ADP-ribose) polymerase (PARP)-1 activation in oxidative stress. In the present report, we provided a novel alternative mechanism. We found that reactive oxygen species (ROS)-activated PARP-1 regulated the activation of JNK and p38 MAP kinases since inhibition of PARP-1 by pharmacons, small interfering RNA silencing of PARP-1 expression or the transdominant expression of enzymatically inactive PARP-1 resulted in the inactivation of these mitogen-activated protein kinases (MAPKs). This regulation was achieved by increased expression and enlarged cytoplasmic localization of MAPK phosphatase-1 (MKP-1) upon PARP-1 inhibition in oxidative stress since changes in MKP-1 expression were reflected in the phosphorylation states of JNK and p38. Furthermore, we found that in MKP-1-silenced cells, PARP inhibition was unable to exert its protective effect indicating the pivotal roles of JNK and p38 in mediating the oxidative-stress-induced cell death as well as that of increased MKP-1 expression in mediating protective effect of PARP inhibition. We suggested that regulation of a protein which can directly influence cytoplasmic signaling cascades at the expression level represents a novel mechanism for the cytoplasmic action of PARP-1 inhibition.

PII-73

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.

PII-74

INTERACTION BETWEEN THE HEDGEHOG SIGNALING PATHWAY AND ESTROGEN RECEPTOR ALPHA

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Aberrant activation of the Hedgehog (Hh) signaling pathway in adult tissues has been implicated in the development of various cancers, including breast cancer. There are several reports on links between Hh signaling and estrogen receptor alpha (ER) in breast cancer. Some of them report a negative correlation between the expression of ER and Hh pathway genes (Ptch1 and Gli1) in estrogen-dependent cells, while others report upregulation of Shh expression after ER activation. Our previous results on combined treatment with cyclopamine (the Hedgehog pathway inhibitor) and tamoxifen (breast cancer therapeutic ,estrogen receptor inhibitor) in ER+ cell line showed dramatically increased survival compared to either treatment alone. We investigated the effect of Hh signaling on ER in estrogen-dependent breast cancer cells (MCF7 cells). Treatment with exogenous Shh protein causes an increase in pathway gene expression and an increase in ER expression after a shorter treatment period. To investigate whether this effect on ER is mediated through the transcription factor Gli1, we transfected the cells with Gli1, but observed no change in ER expression. Only after the subsequent addition of Shh protein did the expression of ER increase. This suggests a non-canonical mechanism, mediated by an upstream component. Mature Shh protein is small and contains two lipid modifictions which led us to a hypothesis that these modifications might enable Shh protein to bind to ER and activate it. Immunofluorescent staining revealed that after the addition of exogenous Shh protein, ER colocalizes with Shh protein in the cytoplasm. The estrogen-independent cell line SkBr3 did not show this upregulation in ER expression after treatment with Shh protein, probably because it only has a basal level of ER expression. We show a potentially interesting link between the Hh signaling pathway and ER, where Shh protein binds to and activates ER directly without the need for other Hedgehog pathway components.

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RETINOIDS ENHANCE GLUCOCORTICOID-INDUCED APOPTOSIS OF T CELLS BY FACILITATING GLUCOCORTICOID RECEPTOR-MEDIATED TRANSCRIPTION

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Glucocorticoid-induced apoptosis of thymocytes is one of the first recognized forms of programmed cell death. It was shown to require gene activation induced by the glucocorticoid receptor (GR) translocated into the nucleus following ligand binding. In addition, the necessity of the glucocorticoid-induced, but transcription-independent phosphorylation of phosphatidylinositol-specific phospholipase C (PI-PLC) has also been shown. Here we report that retinoic acids, physiological ligands for the nuclear retinoid receptors, enhance glucocorticoid-induced death of mouse thymocytes both in vitro and in vivo. The effect is mediated by retinoic acid receptor (RAR) alpha/retinoid X receptor (RXR) heterodimers, and occurs when both RARα and RXR are ligated by retinoic acids. We show that the ligated RARα/RXR interacts with the ligated GR, resulting in an enhanced transcriptional activity of the GR. The mechanism through which this interaction promotes GR-mediated transcription does not require DNA binding of the retinoid receptors and does not alter the phosphorylation status of Ser232, known to regulate the transcriptional activity of GR. Phosphorylation of PI-PLC was not affected. Besides thymocytes, retinoids also promoted glucocorticoid-induced apoptosis of various T-cell lines, suggesting that they could be used in the therapy of glucocorticoid-sensitive T-cell malignancies.

PII-76

THE INVOLVEMENT OF HEME OXYGENASE AND CYP4A14 IN SEX-RELATED RESISTANCE TO HYPEROXIA IN LIVER OF CBA MICE

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It has been observed that coping with oxidative stress is often sex-and age-related. Since the response to oxidative stress involves not only the classical antioxidant enzyme system (superoxide dismutase, catalase and glutathion peroxidase) but also heme-oxygenase (HO) and CYP450 enzymes, we wanted to define which and to what extent these particular parts of the oxidative stress defences are involved in age and sex-related response to hyperoxia in liver of CBA mice. Hyperoxia induced oxidative stress only in young male mice as demonstrated by increased lipid peroxidation and higher catalase (CAT) activity. In females, no sign of oxidative stress was demonstrated in any group. These responses were mostly in association with CYP4a14 level, which was found to be downregulated only in young male mice. The absence of oxidative stress in females might be the consequence of a) significantly upregulated CYP4A14 in females of all ages b) upregulated level of HO-1 gene expression in young females and c) significantly higher basal HO activity in 1 month old females than in males of the same age.

PII-77

DEVELOPMENT OF A NEW SURFACE DISPLAY SYSTEM FOR *LACTOCOCCUS LACTIS*

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Lactococcus lactis is a safe lactic acid bacterium which has been suggested as a delivery vehicle to human intestine. For majority of such applications, the surface display of recombinant proteins is required e.g. for the delivery of antigens or binding molecules. For this purpose, new approaches for surface display are being sought.
We have analysed the bacterial surface proteome of *L. lactis* MG1363 to identify new candidate carrier proteins for surface display. We have made two different predictions of surface-associated proteins using Augur and LocateP software, which yielded 666 and 648 proteins respectively. Surface proteins of *L. lactis* NZ9000, a derivative of MG1363, were identified using a proteomics approach. Surface proteins were cleaved from intact bacteria and the resulting peptides were identified by mass spectrometry. The latter approach yielded 80 proteins, 34 of which were not predicted by either software.
Seven of the 80 proteins were selected for further study. They were cloned in frame with a C-terminal hexa-histidine tag and over-expressed in *L. lactis* NZ9000, using nisin-controlled expression. Proteins of correct molecular weight carrying a hexa-histidine tag were detected. Their surface localization was confirmed with flow cytometry. Basic membrane protein (BmpA) was exposed at the highest level.
To test BmpA as a candidate carrier protein, the B domain of staphylococcal protein A was displayed on the surface with BmpA as a carrier. Surface display system was further optimized by minimizing the BmpA molecule and by inserting a 15 amino-acid spacer between BmpA and B domain. A total of 18 variants of BmpA protein were cloned and tested for the ability to display B domain on the bacterial surface. A BmpA variant, termed Bmp1, which lacks 44 amino-acids on the C-terminal, improved surface display for more than 6-fold in comparison to the whole molecule. Comparison of the models of the 3D structure of Bmp1and BmpA revealed that the B domain is probably fused to the equivalent sites of the two opposite domains.
Bmp1 display is comparable to established AcmA display system, but has the advantage of irreversible covalent binding. This could make it an attractive system for the delivery applications in *L. lactis*.

PII-78

IMPORTANCE OF p38 MAPK PATHWAY IN DEVELOPMENT OF DUPUYTREN'S DISEASE

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Dupuytren's disease (DD) is a fibroproliferative disorder of the palmar fascia described as irreversible permanent contracture of fingers resulting with the loss of hand function. The hallmarks of DD are intensive fibroblasts/myofibroblasts proliferation, excessive collagen and extra-cellular matrix deposition and myofibroblasts driven contraction. Myofibroblasts are thus central to DD pathogenesis and may even be considered an active part of the innate immune system that displays many functions and receptors in common with macrophages. Due to such features, myofibroblasts can recruit immunomodulatory molecules and stimulate the immune response thus amplifying fibrotic processes.

Among known fibrogenic cytokines, TGF-β has been implicated as a key stimulator of myofibroblasts activity and fascial contraction in Dupuytren's disease. TGF-β exerts its activities through the Smad proteins or alternatively through Ras, Erk, Rho GTPase, JNK or p38 MAP kinases. The inhibiton of the p38 MAPK pathway has indeed, been reported to reduce some fibroses such as pulmonary and renal fibroses in animal models.

We have therefore, developed a primary cell culture model to assess the role of p38 MAPK pathway in DD. For that purpose, the cells were grown from unaffected Dupuytren's disease patients fascia obtained from the edge of primary incision. Cells were treated with TGF-β alone and together with the p38 phosphorylation inhibitor. Upon treatment of cells, we assessed the (1) activation status of a kinase phosphorylated solely by p38, namely the MK2 kinase by Western blotting (2) measured the expression levels of fibrotic-related genes and genes involved in human autoimmune and inflammatory immune responses by quantitative real-time PCR and (3) the effect on fibroblast/myofibroblast contractility by use of three-dimensional collagen gel contraction assay. Obtained results reveal that treatment of cells by TGF-β successfully induced activation of various fibrotic genes*, i.e*. α-SMA, FN, PALLD, COL1A1, ARHGDIA, IGFR-1, THBS-1, PAI-1, TIMP-1, CCL11 and IL-6. Moreover, inhibition of p38 phosphorylation inhibited phosphorilation of MK2 kinase that led to decreased expression of fibrotic genes THBS-1, COL1A1, FN, PAI-1 and CCL11. In line with these results, we managed to measure increased fibroblast contractility upon TGF-β treatment and decreased contractility of cells upon inhibition of p38 phosphorylation. In conclusion, inhibition of p38 MAPK signalling pathway may provide a novel therapeutic avenue for the treatment of Dupuytren's disease.

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IMPACT OF FUMONISIN B_1 ON GLUTAMATE TOXICITY IN NEURONAL PRIMARY CULTURE

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Fumonisin B_1 (FB_1) is a mycotoxin, produced by *Fusarium* spp. mould that contaminates maize and maize-based food world-wide. Although FB_1 neurodegenerative potential is well established, mechanism of its cytotoxicity is still unknown. In our previous study on cells of neuronal origin we demonstrated that FB_1 inhibits mitochondrial complex I, depolarises mitochondrial membrane and deregulates calcium signalling. In the brain several pathological conditions, as stroke, are characterised with mitochondrial membrane depolarization and calcium deregulation. The aim of this study was to check possible impact of FB_1 on calcium level and mitochondrial membrane depolarization in such conditions.

Primary hippocampal neuronal co-culture were pre-treated with FB_1 (0.5 μM or 10 μM) and changes in calcium signal and mitochondrial membrane potential in three different models were followed. The first model, referred as the physiological model, was induced by treatment of neurons with low glutamate concentration (5 μM) that generated physiological calcium signal. The second model, the glutamate excitotoxicity model, was produced by treatment of neurons with high concentration of glutamate (100 μM) mimicking glutamate overload as seen in stroke that induce delayed rise of calcium signal to the higher plateau. The third model was the well established low magnesium model of epilepsy characterised by oscillatory synchronized release of endogenous glutamate that induce repetitive calcium oscillations.

FB_1 increased or changed cytosolic calcium level simultaneously with mitochondrial membrane depolarisation in all three tested models. However, FB_1 had no impact on delayed calcium deregulation observed after treatment with high glutamate concentration suggesting that high glutamate concentration is possibly already too toxic. In the low magnesium model FB_1 increased the calcium peaks in neurons and also changed the shape of the signal implying that FB_1 can significantly enhance the epilepsy seizures. Since FB_1 alone induced mitochondrial depolarisation and limited mitochondrial ability to uptake calcium, the higher calcium signal in neurons after FB_1 pre-treatment in tested models can be explained by lower calcium uptake in mitochondria.

Taken together our results indicate that FB_1, even in very low concentrations that humans can be exposed to, made neurons more vulnerable to glutamate toxicity and epiteloform conditions indicating that FB_1 can act as a trigger to cell death.

PII-80

LOOKING FOR COMBINATORIAL BIOMARKERS IN THE ALZHEIMER'S DISEASE

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Alzheimer's disease costs more than 1% of the GDP globally, and it hurts more people than cancer or heart disease. By the time the disease appears clinically, large part of the neurons are irreversibly lost [1]. Therefore it is an important task to find early biomarkers for the disease.

By using the database of the Tucson, Arizona based Critical Path Institute, we analyzed laboratory data and cognitive tests of more than 6000 Alzheimer's patients with advanced data-mining tools, developed by us, and found new combinatorial relations in the data, that may lead to novel combinatorial biomarkers.

Reference
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IDENTIFICATION OF A CYTOPROTECTIVE DIBENZOYLMETHANE DERIVATIVE COMPOUND BY HIGH-THROUGHPUT SCREENING

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The compound library of the University of Debrecen was tested in a high-throughput viability assay. 1863 compounds were tested for cytoprotective effect against hydrogen peroxide toxicity in Jurkat cells. 26 compounds were identified as cytoprotective. Antioxidant – and poly(ADP-ribose) polymerase (PARP) inhibitory activities were also determined. Four compounds – three flavanone-derivatives and one dibenzoylmethane derivative - that were neither antioxidants nor PARP inhibitors were further investigated as potentially novel cytoprotective structures. The cytoprotective effects were confirmed in manual assays. Since the flavanone compounds provided protection from both apoptotic and necrotic cell death indicating general and non specific mechanism and two of them proved to be antioxidants despite their lack of antioxidant effect in the screening assay, therefore they were not further investigated. The dibenzoylmethane-derivative inhibited propidium-iodide-uptake – a measure of necrotic cell death – but has not affected apoptotic death. Its cytoprotective effect is not cell-type-specific as it could be observed in A549 lung epithelial cells. Dibenzoylmethane derivatives are used in sunscreens and our data suggest that their UV protective effect may be due to interfering with the cytotoxic pathway elicited by reactive oxygen species such as hydrogen-peroxide.

This work was supported by the following grants: OTKA K73003, K82009, PD83473, TAMOP-4.2.2-08/1-2008-0019 and TÁMOP 4.2.1./B-09/1/KONV-2010-0007, the National Innovation Office (Baross program Seahorse grant).

PII-82

THE MYCOBACTERIAL dUTPase: BIOCHEMISTRY, PHYSIOLOGY AND MOLECULAR INTERVENTION

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Thymidine biosynthesis is an essential metabolic pathway, as dTTP is one of the nucleotide building blocks of DNA. Three major pathways exist for dTTP synthesis in humans, while in mycobacteria only one of these is present. This one involves the dUTPase reaction. In addition, the constantly produced dUTP needs to be eliminated to prevent DNA uracilation. Therefore, dUTPase is also required to eliminate excess dUTP.
We aimed to investigate the significance of the dUTPase pathway in mycobacteria. More specifically, we wish to relate the previously deciphered *in vitro* reaction mechanism of dUTPase to physiology in the living mycobacterial cell.
Mycobacterium smegmatis was used as a fast growing model for mycobacterial thymidine biosynthesis. Marked allelic replacement, growth assays and *in vitro* enzyme assays were used.
We found that mycobacterial dUTPase genes (*dut)* exhibit over 85% sequence identity and thimidylate biosynthesis is highly conserved among mycobacterial species. Interestingly, mycobacterial dut has a genus-specific surface loop absent in the human dUTPase. The knock-out of *dut* resulted in lethality in *M. smegmatis*, which could be reverted by complementation with the wild-type *dut*. We assayed complementation with four dUTPase mutants with different *in vitro* characterized enzyme activity. Importantly, mutant *dut* lacking the genus-specific loop was enzimatically unaffected, but was unable to complement the lethal knock-out phenotype. However, partially or fully inactive mutants having the genus-specific loop could revert lethality. Growth assays are in progress to reveal the effect of decreased dUTPase activity on various stress conditions.
Our results prove that *dut* is essential in *M. smegmatis* and that essentiality is brought about by the mycobacterium-specific dUTPase motif and not by the enzymatic activity. Therefore, we propose that targeting the mycobacterium-specific motif will potentially yield an efficient, specific antimycobacterial treatment.

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CHANGES IN EXPRESSION AND ACTIVITY OF 5HT-REGULATING PROTEINS IN BRAINS OF HYPERSEROTONEMIC RATS

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Serotonin (5-hydroxytryptamine, 5HT) has two important roles in the mammalian brain: it regulates serotonergic outgrowth and maturation of the target regions in the developing brain, and modulates function and plasticity of the adult brain. Several lines of evidence suggest that alterations in the serotonergic neurotransmitter system might represent one of the biological substrates of Autism. The most consistent 5HT-related finding in Autism is hyperserotonemia (elevated blood 5HT levels) paralleled with altered brain serotonin synthesis, but the relationship between the 5HT disturbances in the two compartments is not understood. The aim of this study was to pharmacologically induce hyperserotonemia in rats during the period of most intensive development of 5HT neurons - from gestational day 13 to post-natal day (PND) 21 - using either the immediate 5HT precursor, 5-hydroxytryptophan (5HTP, 25 mg/kg), or the non-selective irreversible MAO inhibitor tranylcypromine (TCP, 2 mg/kg). The treatment was successful in inducing permanent hyperserotonemia that lasted into adulthood. In the raphe nuclei of adult rats, 5HT and 5-hydroxyindol acetic acid (5HIAA) concentrations were measured by high-performance liquid chromatography with electrochemical detection and expressed per g of wet tissue; and gene expression analyses were made with qRT-PCR using Taq-man chemistry. Although the 5HTP treated group had a significant increase in monoamine oxidase (MAO) A gene expression, no significant changes were observed in midbrain serotonin levels or metabolism. In TCP treated rats, gene expression of MAO A and B was very significantly increased in comparison to the control group, and this was biochemically reflected in a strong decrease in 5HT concentrations and a great increase in 5HIAA/5HT ratio indicating the level of 5HT metabolism. We suppose that the perinatal inhibition of MAO A and B induced compensatory changes in the expression of their genes leading to a permanently disturbed 5HT homeostasis in rats. These results speak in favor of the theory that possible alterations in the expression of one or more of the 5HT elements could lead to the dysregulation of 5HT transmission in the brain (affecting so its early development and resulting in autistic behavioral symptoms), while it is at the same time reflected in the periphery as hyperserotonemia.

PII-84

TNF-α BASED PROTEIN NANOPARTICLES AND THEIR USE IN BIOMEDICAL APPLICATIONS

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Universal technology for protein nanoparticle (NP) formation was developed based on metal coordination of specifically designed protein analogs. TNF-α analogs with surface exposed clusters of histidines (LK801) or histidine tags attached to N-terminus (His10-TNF and H7dN6TNF) were designed as model proteins. Since TNF-α is a pleiotropic protein we prepared various TNF-α analog based NPs for different biomedical applications.
Different approaches were used to form protein NPs, either by binding of protein molecules to inorganic NPs or by self-assembly of NPs. Here we will focus mainly on self-assembled NPs comprised of different polyfunctional biocompatible chelators or dendrimers with chelating function in combination with histidine rich TNF-α analogs and zinc ions.
In all cases metal coordinative binding was found to be reversible, enabling gradual release of individual protein molecules upon suitably changed environmental conditions. Size, stability, *in vitro* biological activity and release profiles of different protein NPs were determined.
NPs containing TNF-α analog LK801 were analyzed on mouse tumor model. Anti-cancer therapy, where slow release of active molecules from NPs was achieved, resulted in tumor growth delay and lower systemic toxicity effects. Distribution of LK801 analog in tumor bearing animals also proved prolonged localization of NPs in tumor tissue.
Additionally we prepared PEGylated analog LK801. Via binding of polyethylene glycol (PEG) on protein molecule its half life can be extended several fold. Various modified analogs with different number of PEG chains bound to the protein were prepared with on-column PEGylation. The most promising analog was used on mouse tumor model and prolonged anti-tumor effects due to increased half life were observed for PEGylated analog alone and for self-assembled protein NPs.
On the other hand, TNF-α NPs containing analog with reduced biological activity (H7dN6TNF) could be used for different application. As it is known, TNF-α plays a central role in chronic inflammatory diseases so anti-TNF therapy is an effective way for tackling them. A principle of active immunization and formation of anti-TNF antibodies could serve as a basis for developing new drugs for chronic diseases associated with pathogenically elevated TNF-α levels (rheumatoid arthritis, inflammatory bowel disease...). We were able to trigger formation of anti-TNF-α antibodies after administration of H7dN6TNF containing NPs to the testing animals.

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ADIPONECTIN GENE (ADIPOQ) AS PREDICTOR OF EARLY ABDOMINAL OBESITY AND ELEVATED BLOOD PRESSURE

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Human obesity is accepted as an important risk factor for development of certain syndromes and many chronic diseases. Visceral fat accumulation is associated not only with quantitative and qualitative changes in serum lipids and lipoproteins but also with elevated blood pressure and dysregulation in the secretion of adipocytokines. The genetics of obesity is complex and involves interactions between genes and gene and environment. Adiponectin is the most abundant adipose tissue-derived cytokine which was linked to central obesity and ADIPOQ variants are promising markers for understanding the genetic base of obesity. Clear contribution of ADIPOQ gene variants for the development of obesity has not yet been fully understood due to differences in age and genetic or ethnic background of study populations. We aimed to perform analysis of the possible associations of adiponectin concentrations and ADIPOQ gene variants with abdominal obesity and elevated blood pressure in young subjects of Croatian origin.
A total of 149 unrelated young subjects were chosen during routine medical check-up. Central obesity and elevated blood pressure were defined according to the criteria of the IDF. Blood pressure and waist circumference were measured by standard procedures. Adiponectin levels were measured by ELISA assay. ADIPOQ -11391G>A and -11377C>G were genotyped by real-time PCR.
Central obesity was found in 46.6% of women and in 44.6% of men. Elevated systolic and diastolic blood pressure were detected in 15.4% and 19.5% of subjects, respectively. Waist circumference, systolic and diastolic blood pressure showed inverse correlation with adiponectin concentrations. For central obesity, we found association with -11377C>G and with -11391G>A polymorphisms. ADIPOQ -11377GG and -11391GA significantly increased the risk for the development of central obesity (OR 5.57 and OR 3.37, respectively). The test of overall association showed significant correlation of central obesity with -11377C>G and -11391G>A haplotypes ($p<0.001$). In relation to elevated blood pressure significant association was found with -11377CG variant ($p=0.035$). Each mutation -11377C>G significantly increased the risk for development of elevated blood pressure in a young population (OR 2.74). Analysis of adiponectin concentration and ADIPOQ -11391G>A and -11377C>G promoter gene variants could be clinically meaningful for estimation of obesity and obesity-related syndrome risk in young adult population.

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POLY(ADP-RIBOSE) SIGNALLING REGULATES CIGARETTE SMOKE-INDUCED CELL DYSFUNCTION AND DEATH IN A549 LUNG EPITHELIAL CELLS

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Cigarette smoking can contribute to the development of many human diseases such as cardiovascular disease, lung cancer, asthma and chronic obstructive pulmonary disease. Thousands of compounds are present in cigarette smoke including a large number of free radicals which can cause DNA damage. When DNA damage occurs, poly(ADP-ribose) polymerase-1 (PARP-1) becomes activated and enhances DNA repair and cell survival. Excessive PARylation, however, may also cause necrotic cell death. Here we investigated the effect of cigarette smoke extract (CSE) on A549 human lung epithelial cells. CSE induced poly(ADP-ribose) accumulation as detected by immunofluorescence and immunoblotting. CSE also caused a concentration dependent decrease in viability and plasma membrane injury (as assessed by propidium iodide uptake). Decreased viability was also determined with ECIS (Electric Cell-substrate Impedance Sensing) instrument. CSE-induced cell death was also characterized by mitochondrial depolarization but massive translocation of apoptosis inducing factor (AIF) could not be observed. In order to investigate the role of RARylation in CSE-induced oxidative stress, PARP-1- and PARG-silenced A549 cells were used (shPARP-1 and shPARG cells, respectively). Silencing of both PARP-1 and PARG sensitized cells to CSE-induced toxicity. PARP-1 and PARG silenced cell lines exhibited reduced clonogenic survival and displayed a delayed repair of DNA breaks. Using selective fluorescent probes, mitochondrial superoxide and intracellular hydrogen peroxide production was detected following CSE exposure. Addition of cell permeable form of superoxide dismutase (PEG-SOD) significantly increased the level of hydrogen peroxide production and PAR accumulation in cells, and reduced cell viability. PEGylated catalase partially attenuated the effects of PEG-SOD indicating that CSE induces mitochondrial superoxide production which contributes to cell death via conversion to hydrogen peroxide. Hydrogen peroxide-induced DNA breakage activates PARylation which serves as a survival mechanism in CSE-exposed cells.

This work was supported by the following grants: OTKA K73003, K82009, PD83473, TAMOP-4.2.2-08/1-2008-0019 and TÁMOP 4.2.1./B-09/1/KONV-2010-0007, the National Innovation Office (Baross program Seahorse grant).

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ROLE OF POLY(ADP-RIBOSYL)ATION IN THE REGULATION OF UVA-INDUCED CELL DEATH OF PRIMARY HUMAN KERATINOCYTES

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UV radiation in sunlight is divided into three regions depending on the wavelength, UV-C (200-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). While UV-C is filtered out by the ozone layer, both UV-B and, to a lesser extent, UV-A radiation are responsible for various skin disorders including photoaging and skin cancer. UV radiation alters cellular function, generates radical oxygen species (ROS), alters signaling events and causes DNA damage. UVA mainly generates free radicals causing damage in biomolecules. Here we set out to investigate the role of poly(ADP-ribosyl)ation (PARylation) in the UVA response of human primary keratinocytes. PARylation by PAR polymerase (PARP) enzymes can be induced by DNA damage, therefore we hypothesized that UVA-induced ROS production may cause PARP activation via DNA damage and may affect cell fates (survival or cell death). We used three different photosensitizing agents (8-methoxypsoralen, enoxacin and chlorpromazine) and two different PARP inhibitors (PJ34 and 3-aminobenzamide, 3-AB). Both with or without photosensitizers, UVA caused PARP activation as verified by immunocytochemical detection of PAR polymer. Surprisingly, the potent and specific PARP inhibitor PJ34 sensitized keratinocytes to cell death caused by UVA+photosenzitizers. 3-AB, however, had a protective effect. PJ34 increased the number of cells displaying apoptotic morphology and showing signs of caspase activation (PARP-1 cleavage) and also aggravated plasma membrane injury (as assessed by LDH release). On the contrary, 3-AB provided protection both from apoptotic and necrotic cell death as indicated by right shifts in the dose response curves. Our data indicate that UVA stimulates PARP activation that contributes to keratinocyte survival. 3-AB protects human keratinocytes from UVA-induced toxicity by a PARP independent mechanism.

This work was supported by the following grants: OTKA K75864, K82009, PD83473, TAMOP-4.2.2-08/1-2008-0019 and TÁMOP 4.2.1./B-09/1/KONV-2010-0007, the National Innovation Office (Baross program Seahorse grant).

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INITIAL CHARACTERIZATION OF THE *CYP51* LIVER CONDITIONAL KNOCKOUT MICE REVEALS SEX-SPECIFIC AND AGE-DEPENDENT HEPATIC DEFECTS

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Despite being one of the most investigated molecules in organisms, certain aspects of cholesterol synthesis and homeostasis are still poorly understood. Lanosterol 14α-demethylase (CYP51) is a key regulatory enzyme in the late stage of cholesterol synthesis. Complete disruption of *Cyp51* causes embryonic lethality in mice (Keber et al., JBC 2011). To gain further insights into the *in vivo* role of CYP51, we generated two conditional knockout models: liver-specific *Cyp51* knockout (*Cyp51*$^{lox/lox}$;Alb-Cre^{+}) mice and mice with one *Cyp51* allele absent in the entire organism and the other allele absent only in the liver (*Cyp51*$^{-/lox}$;Alb-Cre^{+}). Both transgenic strains are viable and normal in outer appearance. Approximately 4 % of the progeny (almost exclusively males of both knockout genotypes) experience growth arrest with jaundice and hepatomegaly between 6 and 9 weeks of age. Initial histological studies of the liver revealed proliferation of small bile ducts accompanied by singular cases of mitosis and apoptosis of hepatocytes. Biochemical analyses of the plasma indicate disrupted cholesterol and lipid homeostasis with higher LDL-cholesterol and lower HDL-cholesterol and triglycerides compared to liver knockouts that develop normally as well as to wild types. Sex-specific and age-dependent aggravation of cholangiocyte proliferation and hepatomegaly is also observed in *Cyp51*$^{lox/lox}$;Alb-Cre^{+} and *Cyp51*$^{-/lox}$;Alb-Cre^{+} mice without jaundice. Immunohistochemical staining of the liver with pan-cytokeratin antibody confirmed liver stem (oval) cell response that provide precursor cells for either hepatocytes or cholangiocytes. Challenging *Cyp51*$^{lox/lox}$;Alb-Cre^{+} and *Cyp51*$^{-/lox}$;Alb-Cre^{+} mice with different diets showed lower body weight and increased liver-to-body weight ratio compared to the wild types on standard chow and on high-fat diet without cholesterol but not on high-fat diet with added cholesterol. These initial data indicate that the hepatic disruption of *Cyp51* and consequently the disruption of cholesterol synthesis causes oval cell response with cholangiocyte proliferation and can in male mice lead to early growth arrest with severe jaundice. Intact cholesterol biosynthesis is thus crucial for normal liver development. Molecular mechanisms leading to sex-specific and age-dependent liver defects are under investigation.

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POLYMORPHISMS OF *TLR2* (ARG753GLN) AND *TLR4* (ASP299GLY) GENES AND COPD: THE CROATIAN STUDY

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Chronic obstructive pulmonary disease (COPD) is a disease defined by progressive development of irreversible airflow limitation typically determined by reduction of quantitative spirometric parameters like forced expiratory volume at 1s (FEV_1) and the ratio of FEV_1 to forced vital capacity (FVC, FEV_1/FVC). The current understanding of pathogenesis includes complex interaction between environmental factors and genetic predisposition. The major environmental risk factor is cigarette smoke, while candidate genes that may influence a person's risk for COPD development are still under study.

Toll like receptors are members of transmembrane proteins that recognize conserved molecular motifs of viral and bacterial origin and initiate innate immune response. In COPD patients, recurrent infections of the lower bronchial tree with different bacteria and viruses, are present, suggesting a defect in immune response initiation. Therefore, the aim of this study was to investigate the potential association of polymorphisms in TLR2 (Arg753Gln) and TLR4 (Asp299Gly) genes and risk of COPD.

The study group comprised of patients with COPD (n=106) and healthy controls (n=48). COPD was diagnosed by pulmonology specialist according to clinical examination and spirometry results FEV_1/FVC < 0.70 and FEV_1 <80% predicted. DNA was isolated from whole blood and subjected to genotyping with TaqMan SNP Genotyping Assays of interest, using AB 7500 Real Time PCR System (Applied Biosystems).

In this study, only two genotypes for each of the polymorphism were determined, homozygote with major allele (GG for TLR2; AA for TLR4) and heterozygote. Homozygous carriers of the minor alleles were not detected. No significant deviations from the Hardy Weinberg equilibrium for either TLR2 (Arg753Gln) or TLR4 (Asp299Gly) polymorphisms were observed. There were no statistical significant differences in allele (TLR2: X^2=0.003; P=0.955; OR=1.83; 95%CI=0.20-16.57; TLR4: X^2=0.011; P=0.914; OR=0.82; 95%CI=0.29-2.89) or genotype frequency distribution (TLR2: X^2=0.003; P=0.954; TLR4: X^2=0.013; P=0.911;) of the tested polymorphisms between COPD patients and healthy controls.

According to obtained results, it seems that polymorphisms in TLR2 (Arg753Gln) and TLR4 (Asp299Gly) genes are not associated with development of COPD in the population of Croatia. Our results suggest that further studies based on a larger number of samples are needed to confirm or reject the reported conclusion.

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FREQUENCY OF Q192R, L55M AND -108C>T POLYMORPHISMS OF *PON1* AND S311C POLYMORPHISM OF *PON2* GENE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: THE CROATIAN STUDY

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic local and systemic inflammation, and increased oxidative stress. The paraoxonase (PON) gene family includes three members (*pon*1, *pon*2, *pon*3). Paraoxonase 1 (PON1) is a HDL-associated enzyme which participates in lipid metabolism. It has been shown that polymorphisms in coding (Q192R, L55M) and promoter regions (-108C>T) of *pon*1 gene affect PON1 activity. Paraoxonase 2 (PON2) is ubiquitously expressed in nearly all human tissues and acts as cellular antioxidant. S311C is a common polymorphism of *pon*2 gene and it has been reported to be associated with the high risk of atherosclerosis.

The aim of this study was to determine the frequency of Q192R, L55M and -108C>T polymorphisms of *pon*1 gene and S311C polymorphism of *pon*2 gene as well as to assess the association of polymorphisms of *pon*1 gene and the level of PON1 activity in COPD.

The study was carried out on 107 COPD patients (32 smokers, 28 ex-smokers, 47 non-smokers) and 45 healthy volunteers (16 smokers, 13 ex-smokers, 16 non-smokers). Polymorphisms were determined by PCR-RFLP procedure. PON1 activity was assayed with paraoxon (in absence and in the presence of NaCl) and phenylacetate as substrates.

Basal and salt-stimulated paraoxonase PON1 activity alone and standardized with HDL concentration were significantly reduced in COPD patients as compared with controls ($P<0.05$). In addition, arylesterase PON1 activity alone and standardized with HDL or apoAI concentration was also significantly lower in COPD patients ($P<0.001$).

The analysis of *pon*1 gene polymorphisms in COPD patients showed following distribution of genotypes: 71% QQ, 25% QR and 4% RR for Q192R; 45% LL, 42% LM and 13% MM for L55M; 15% CC, 42% CT and 43% TT for -108 C>T. In the control group we found different distribution of genotypes for Q192R: 89% QQ, 7% QR, 4% RR ($P<0.05$) and for -108 C>T: 38% CC, 49% CT and 13% TT ($P<0.001$), while distribution of genotypes for L55M was similar to COPD group (60% LL, 35% LM and 5% MM). The analysis of *pon*2 gene S311C polymorphism showed following distribution of genotypes in COPD patients: 55% SS, 32% SC and 13% CC, which was similar to distribution of genotypes in control group (61% SS, 18% SC and 21% CC). Our results suggest that Q192R and -108C>T *pon*1 gene polymorphisms may be associated with lower PON1 paraoxonase and arylesterase activity in COPD patients in Croatian population.

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ELASTIN-LIKE POLYPEPTIDE AS AN TARGETED ANTICANCER DRUG MACROMOLECULAR CARRIER

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A standard approach to the antineoplastic therapy involves administration of more than one highly toxic compound. The major limitations of this approach are poor efficacy and/or severe side-effects. Recently, therapeutic peptides (TP) have emerged as a new and promising class of anticancer drugs. TP's are specific for their targets, enabling reduction of the administered dose and, consequently, the unwanted side effects. In addition, TP's can be easily designed to modulate specific protein function in cancer cells. The biggest obstacle in the utilization of peptides drugs, however, is their poor pharmacokinetic parameters. Peptides are often degraded in circulation, poorly deposited in tumor tissue, and inefficiently internalized by tumor cells due to poor translocation. A thermally responsive delivery vector, based on Elastin-like polypeptide (ELP), has previously been utilized to deliver bioactive peptides into cells. ELP is soluble in aqueous solutions below a characteristic transition temperature (T_t), but undergoes an inverse phase transition when the solution temperature is raised above the T_t. The ELP drug delivery system can be further enhanced by modification of the ELP sequence with cell penetrating peptides (CPP) to efficiently translocate various cargoes into the cells. ELP is advantageous as a drug carrier because it is a macromolecule, which confers the advantages of increased drug solubility, extended plasma half life, passive tumor accumulation, and reduced drug toxicity. Because it is thermally responsive, there is the additional advantage - it can be actively targeted to the tumor. In previous studies, it has been shown that different CPP-ELP-TP molecules were able to efficiently cause inhibition of various cancer cell line proliferation as well as induce tumor reduction in various *in vivo* models. In addition to TPs, ELP molecules have been shown to be able to efficiently internalize small molecule drugs. ELP macromolecular carrier was used for targeted delivery of paclitaxel, a member of the taxane family and doxorubic*in* in *in vitro* and *in vivo* models. In the end, ELP carrier represents a viable alternative approach for targeted therapeutics against cancer that can be used for the tumor specific delivery of small molecule drugs and therapeutic peptides.

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MOLECULAR ANALYISIS OF c-KIT AND PDGFRA MUTATIONS IN GASTROINTESTINAL STROMAL TUMOURS

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Gastrointestinal stromal tumors (GIST) are low-grade sarcomas characterized by activating mutations in the tyrosine kinase genes c-KIT or platelet derived growth factor receptor α (PDGFRA) gene. Most patients with GISTs respond well to imatinib, and other tyrosine kinase inhibitors (TKI). Mutational analysis of c-KIT and PDGFRA genes has predictive and prognostic value, so that it is strongly recommended in the diagnostic work-up of all GISTs.
We analyzed tumour samples from 15 patients treated at University Hospital Split, for c-KIT (exons 9 and 11) and PDGFRA (exon 12 and 18) gene mutations by direct sequencing.

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PROTEIN CONJUGATES STUDIED BY ASYMMETRICAL-FLOW FIELD-FLOW FRACTIONATION (AF4) AND MULTI-DETECTION SYSTEM

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Protein molecules applied as drugs are often covalently modified with poly(ethylene glycol) in order to prepare protein conjugates with larger hydrodynamic volume as compared to that of the pure protein. In this way, a plasma clearance of the protein from the treated organism is considerably reduced. A detailed analysis of protein conjugates was proven difficult in the past. The main limitations appeared to be the complex analytical mixtures, which may contain the un-reacted protein and/or modifier, conjugates of different degree of conjugation, the presence of high molar mass aggregates in solution as well as the lack of calibration standards in the case of conjugate analysis by column chromatographic techniques.
In this study asymmetric-flow field-flow fractionation (AF4) technique was used for high-resolution separation of protein conjugate samples in solution. The AF4 separation system was combined with a multi-detection system, i.e., UV detector, multi-angle light-scattering detector, dynamic light-scattering detector, and refractive-index detector (UV-MALS-QELS-RI) to define individual sample constituents, the presence of aggregates and provide the following information about the conjugate: absolute molar-mass averages, molar-mass distribution, radius of gyration, hydrodynamic radius and chemical composition of the conjugate. The results of AF4 were compared to these obtained by a size-exclusion chromatography (SEC) coupled to the same detection system. The matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was also used for the analysis of protein conjugate samples.
With careful analysis of protein conjugates stored in different solvent compositions we determined the most suitable storage conditions for our conjugate product. Furthermore, by the simultaneous use of two concentration detectors (UV and RI) with UV detector sensitive only to a protein moiety, we determined the precise chemical composition of the conjugate, and its molar mass stoichiometry.

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PREVENTIVE AND THERAPEUTIC EFFECTS OF OLEUROPEIN AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN MICE

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Olives and olive products, an inevitable part of the Mediterranean diet, possess various beneficial effects, such as a decreased risk of cardiovascular disease and cancer. Oleuropein is a non-toxic secoiridoid found in the leaves and fruits of olive (*Olea europaea* L.). In this study, we have investigated the hepatoprotective activity of oleuropein in carbon tetrachloride (CCl_4)-induced liver injury in male BALB/cN mice. Oleuropein in doses of 100 and 200 mg/kg was administered intraperitoneally (ip) once daily for 3 consecutive days, prior to CCl_4 administration (the preventive treatment), or once daily for 2 consecutive days 6h after CCl_4 intoxication (the curative treatment). CCl_4 intoxication resulted in a massive hepatic necrosis and increased plasma transaminases. Liver injury was associated with oxidative/nitrosative stress evidenced by increased nitrotyrosine formation as well as a significant decrease in Cu/Zn superoxide dismutase activity (Cu/Zn SOD) and glutathione (GSH) levels. CCl_4 administration triggered inflammatory response in mice livers by inducing expression of nuclear factor-kappaB (NF-κB), which coincided with the induction of tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). In both treatment protocols, oleuropein significantly attenuated oxidative/nitrosative stress and inflammatory response and improved histological and plasma markers of liver damage. Additionally, in the curative regimen, oleuropein prevented tumor necrosis factor-beta1 (TGF-β1)-mediated activation of hepatic stellate cells, as well as the activation of caspase-3. The hepatoprotective activity of oleuropein was, at least in part, achieved through the NF-E2-related factor 2 (Nfr2)-mediated induction of heme oxygenase-1 (HO-1). The present study demonstrates antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic activity of oleuropein, with more pronounced therapeutic than prophylactic effects.

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DIFFERENTIAL HEPATOPROTECTIVE MECHANISMS OF RUTIN AND QUERCETIN IN CARBON TETRACHLORIDE-INTOXICATED BALB/CN MICE

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The aim of this study was to investigate the hepatoprotective mechanisms of flavonoid rutin against carbon tetrachloride CCl_4-induced liver damage in mice and compare it with its aglycone quercetin. Rutin at doses of 10, 50, and 150 mg/kg and quercetin at 50 mg/kg were administered intraperitoneally into BALB/cN mice once daily for 5 consecutive days before administration of CCl_4. The animals were sacrificed 24 h later. CCl_4-intoxication increased the activity of plasma transaminases and induced severe liver necrosis. In addition, the hepatic activity of Cu/Zn superoxide dismutase (Cu/Zn SOD) and glutathione (GSH) levels were reduced, whereas nitrotyrosine (3-NT) formation increased. Pretreatment with rutin, and to the less extent, quercetin, significantly reduced the activity of plasma transaminases and improved histological signs of acute liver damage. Quercetin prevented the decrease in Cu/Zn SOD activity more potently than rutin, however, it was less effective in the suppression of 3-NT formation. Quercetin, and to the less extent, rutin, significantly attenuated inflammation in the liver by down-regulating CCl_4-induced activation of nuclear factor kappaB (NF-κB), tumor necrosis factor-alpha (TNF-α), and cyclooxygenase (COX-2). The expression of inducible nitric oxide synthase (iNOS) was more potently suppressed by rutin than quercetin. Treatment with both flavonoids significantly increased NF-E2-related factor 2 (Nrf2) and heme oxygenase (HO-1) expression in injured livers, although quercetin was less effective than rutin at the equivalent dose. The results of this study suggest that rutin provides stronger protection against hepatocellular damage and nitrosative stress but has weaker antioxidant and anti-inflammatory activity than quercetin.

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STRESS TYPE DEPENDENT ACTIN-RELATED CYTOSKELETAL TRANSCRIPTIONAL ALTERATIONS IN THE RAT BRAIN

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Stress is present during our entire life, allostatic load or overload could critical however, depending on the type stressors. The major aim of our study was to compare the effect of the most frequently used stress models on transcription of the major actin related cytoskeletal genes.
Male Wistar (200-300g) rats were exposed to different types of stress conditions such as electric foot-shock (EFS), forced swimming stress (FSS) and psychosocial stress (PSS) at four different time points during a three week interval. The total RNA was purified from hippocampal and cortical samples (n=6-10/group). β-actin and cofilin relative RNA expression was determined by means of quantitative real time PCR.
Acute (3 days), chronic (7 and 21 days) EFS caused significant increases in hippocampal β-actin mRNA expression only. The cofilin mRNA level was not modified by the EFS. In the case of FSS, significant changes were detected in the β-actin mRNA expression by the 3rd and 7th days, in both examined brain areas and a significant decrease was found by the 7th day in the cortical cofilin mRNA expression. On the other hand, PSS caused a significant decrease in hippocampal β-actin mRNA levels by the 21st day. The amounts of cofilin mRNA are not altered neither in hippocampal nor in cortical samples.
These findings indicate a very delicate, stress type dependent regulation of neuronal cytoskeletal components with a particular relevance to stress-related human disorders such as anxiety, depression and Alzheimer's dementia.
This study was supported by OTKA (grant Nos 60589, 83667), ETT (grant No 052-07/2/2009), and by The Ministry of Education and Culture (grant No TÁMOP 4.2.2-08/1-2008-0002, TÁMOP 4.2.1./B-09/1/KONV-2010-0005-3).

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MMP-9 CONCENTRATION AND *MMP-9* GENE POLYMORPHISMS IN PATIENS WITH CRONIC OBSTRUCTIVE PULMONARY DISEASE

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Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent endopeptidase capable of cleaving several constituents of extracellular matrix, such as collagen, gelatin, elastin, etc. Therefore, MPP-9 plays an important physiological role in lung extracellular matrix remodeling and repair. In the lungs of patients with chronic obstructive pulmonary disease (COPD) increased degradation of extracellular matrix and airway remodeling are present. Several studies showed increased levels of MMP-9 in alveolar macrophages, neutrophils and sputum of COPD patients. In this study, we measured MMP-9 concentration in plasma samples of COPD patients using a commercially available ELISA kit. We found statistically significant increase in the median value of MMP-9 concentration in COPD patients (204.1 (115.7-351.2) ng/mL; N=59) comparing to healthy controls (70.2 (52.4-104.9) ng/mL; N=21), P<0.001. Previous studies suggested that presence of genetic variations in MMP-9 gene might be associated with changes in MMP-9 activity. However, no consistent results were found in COPD patients. Hence, we analyzed 2 polymorphisms in promotor region (-1562 C/T polymorphism and number of CA repeats) and a +2679 A/G (Gln279Arg) polymorphism in exon 6 of MMP-9 gene. The results show no statistically significant differences in distribution of genotype or allele frequencies for -1562 C/T (P=0.199 and P=1.000, respectively) and +2679 A/G (P=0.223 and P=1.000, respectively) polymorphisms between patients with COPD (N=137) and control subjects (N=43). Likewise, there were no statistically significant differences in either genotypes or number of CA repeats (P=0.199 and P=1.000, respectively). These results indicate no association of selected genetic variations in promotor region and exon 6 of MMP-9 gene with increased MMP-9 concentration in plasma of COPD patients.

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THE EFFECTS OF THE SMALL HEAT SHOCK PROTEIN, HSP27 ON ALZHEIMER'S DISEASE RELATED PHENOTYPES IN TRANSGENIC MICE

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Alzheimer's disease (AD) is one of the most common neurodegenerative diseases. AD is characterized by senile plaque deposition and neurofibrillary tangle formation. Aggregation of β-amyloid and the disruption of the microtubular network lead to congnitive dysfunctions and eventually to neuronal cell death. Heat shock proteins (Hsps) are ubiquitously expressed evolutionary conserved proteins. Hsp27 belongs to the small heat shock protein family, which are ATP-independent chaperones. The most important function of Hsp27 is that it can bind to non-native proteins and inhibits the aggregation of incorrectly folded proteins. Moreover, it also has anti-apoptotic, antioxidant activities and several reports demonstrated that heat shock proteins might play a protective role in various neurodegenerative diseases.

To study the effect of the small heat shock protein, Hsp27 on Aβ accumulation and related pathological features we generated transgenic mice overexpressing Hsp27. Then, Hsp27 transgenic strain was crossed with APPswe/PS1dE9 mice, a validated model of the Alzheimer disease. This mouse line expresses the mutant forms of amyloid precursor protein and presenilin-1 and develops several AD related phenotypes by the age of 6 months. Spatial learning and memory were studied in triple transgenic mice (Hsp27 x APPswe x Pse1dE9) using Morris water maze and Barnes maze tests. We found that spatial learning was impaired in AD model mice however, it was comparable to wild-type mice in triple transgenic mice. Amyloid deposition was investigated using immunohistochemistry. We counted significantly less amyloid plaques in the brain of APPswe/PS1dE9/Hsp27 animals compared to AD model mice. Presynaptic function was investigated using electrophysiological recordings (long-term potentiation (LTP) and paired pulse facilitation (PPF) on hippocampal slices. Excitability of neurons was significantly increased in AD model mice whereas it was normalized in triple transgenic mice. Furthermore, LTP was impaired in APPswe/PS1dE9 mice but this was restored in triple transgenic mice. These results suggest that the overexpression of Hsp27 protein might ameliorate symptoms of Alzheimer disease.

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IDENTIFICATION OF ESTERASE ISOENZYMES RELATED TO CELL DIFFERENTIATION IN *ARMORACIA LAPATHIFOLIA* GILIB. TISSUE CULTURE BY GEL ELECTROPHORESIS AND MASS SPECTROMETRY TECHNIQUES

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Biochemical markers, like peroxidase, acid phosphatase and other enzymes, may be useful in predicting developmental events, which are caused by modification of gene expression patterns. Esterases, a group of enzymes that hydrolyse ester bonds, are present in many isoforms in plant and animal cells. Isoesterases have also been studied as markers of embryogenic potential and of embryo development. Some isoesterases are related to tissue transformation of different plant species by the Ti plasmid of *Agrobacterium tumefaciens*. The goal of the present study was to isolate and identify esterases related to cell differentiation in horseradish (*Armoracia lapathifolia* Gilib.) tissues propagated *in vitro*.

Crown-gall tumours were induced on the leaf fragments with a wild octopine strain B6S3 of *A. tumefaciens*. Two lines of transformed tissues were established: unorganised tumour without any morphogenic capacity and teratoma capable of shoot formation. The esterase isoenzyme patterns of horseradish leaf, teratoma and tumour tissues were compared. Isoenzymes were separated electrophoretically in polyacrylamide gels (8-18 % native gels and 12.5 % SDS gels) and by isoelectric focusing (IEF, pH 3-9). Two esterase substrates, 1- and 2-naphthylacetate, were applied for isoenzyme detection.

Esterases from horseradish tissue were purified by classical chromatography techniques – ion exchange and size exclusion. After separation by IEF, proteins active towards esterase substrates were excised from gels and subjected to in-gel digestion with trypsin. Analysis of tryptic peptides with mass spectrometry (MS) and MS/MS of certain peptides, combined with database searching (databases SwissProt and NCBInr, via programs mMass and Mascot), allowed identification of several horseradish esterases.

PII-100

MYRICETIN–FLAVONOL PREVENTS D-GLUCOSE INDUCED DYSFUNCTION AND OXIDATIVE STRESS IN Hep G2 CELLS

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Myricetin is a naturally occurring flavonol with hydroxyl substitutions, and was found to be effective in scavenging radicals generated by both enzymatic and nonenzymatic systems.
An imbalance in the antioxidant protective mechanism leading to oxidative stress in the cells is being identified as a common factor in diabetes mellitus and several other disorders. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins.
The aims of this study were: 1) to investigate the effect of the low concentration range of myricetin on cell viability and activity of lactate dehydogenase (LDH) - as indicator of cell damage and 2) investigate activities of endogenous antioxidative enzymes: glutathione peroxidase (GPx) and glutathione reductase (GR). GPx catalyzes the reduction of hydroperoxides, including hydrogen peroxide, by reduced glutathione and functions to protect the cell from oxidative damage. Hep G2 cells were supplemented with various concentrations of myricetin (10^{-5}M, 10^{-7}M, 10^{-9}M) for 24 h in hyperglicemic conditions (20 mM glucose). Cell viability was assessed by MTT test and GPx and GR activity were determined using Cayman·s Assay Kit. Exposure Hep G2 cells to 20 mM glucose during 24 h resulted in significantly decrease in GPx activity ($p < 0.05$). Myricetin in concentration of 10^{-9}M significantly enhanced GPx activity in Hep G2 cells but didn·t effect on GR activities. Results of the MTT assay showed that myricetin in all low concentrations range significantly enhance viability of the Hep G2 cells.
Concluding, this study shows that myricetin in low concentration range protected Hep G2 cells against D-glucose induced dysfunction and oxidative stress.

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POSSIBILITIES OF INCREASING LOBELINE CONTENT OF INDIAN TOBACCO (*LOBELIA INFLATA* L.) BY APPLYING DIFFERENT FERTILIZER TREATMENTS

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Lobelia inflata L. is a traditional medicinal plant native to North America. It contains more than 20 piperidine alkaloids, among those lobeline as pharmacologically active secondary metabolite. As lobeline is a competitive nicotinic receptor antagonist, it is frequently used in anti-smoking preparations. The role of lobeline has increased in recent years due to their activity on the central nervous system. The pharmaceutical importance could be improved by the polyacetylenes (lobetyol, lobetyolin, lobetyolinin). The purpose of the trial is to show how the different types of fertilizers utilize in the plant. The nutrients were applied in the following methods and quantities in 2010: untreated (control), 50 kg/ha N-, 100 kg/ha Nitrogen ground fertilizers and 50 kg/ha Magnesium ground fertilizer. The nutrients were applied in the following methods and quantities in 2011: untreated (control), 50 kg/ha N-, 100 kg/ha Nitrogen ground fertilizers, 50 kg/ha Mg- and 100 kg/ha Magnesium ground fertilizers. Lobeline content (µg/g) of above ground plant parts were determined. Lobeline content of above ground plant parts varied between 234 µg/g and 294.63 µg/g in 2010. Highest values of lobeline content were recorded in the non-fertilized control (234 µg/g), whereas a 25.9% increment was observed in the 50 kg/ha N ground fertilizer treatment: 294.63 µg/g. The 50 kg/ha Nitrogen ground fertilizer treatment exerted a positive influence on the lobeline content of plants measured. Lobeline content of above ground plant parts varied between 361.55 µg/g and 420.65 µg/g in 2011. The highest values of lobeline content were recorded in the non-fertilized control (389.06 µg/g), whereas a 8.1% increment was observed in the 100 kg/ha N ground fertilizer treatment: 420.65 µg/g. The 100 kg/ha N ground fertilizer proved to be the most successful in the trial accomplished in the second year (2011). Based on our experiments it can be established that applying different fertilizer treatments to *Lobelia inflata* L., the lobeline content of the species can be successfully increased.

PIII-102

QUANTITATIVE PROTEOMICS ANALYSIS OF TEAR FROM PATIENTS WITH ALZHEIMER DISEASE

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Tear is a biological fluid easy to collect by non invasive methods and the diagnosis of diseases based on tear analysis has high diagnostic potential. Alzheimer disease is one of the most abundant neurodegenerative diseases in the elderly population affecting 5% of people above age 65 and 20% of people above 80. Our aim was to collect tear from patients with Alzheimer disease and to compare their protein profile with tear obtained from age and sex matched control volunteers. The collected tears were subjected to electrophoresis and quantitative proteomics analyses. The label free protein quantification of 14 tear samples originated from patients with Alzheimer disease was performed and compared to the control tear pool. In this pilot study the differential expression of some of the proteins could be observed and they can serve as potential biomarkers for Alzheimer disease detection in tears.

PIII-103

EXPLORING THE CONFORMATION AND ACTIVITY OF TRANSGLUTAMINASE 2 BY FLUORESCENCE RESONANCE ENERGY TRANSFER

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Four crystals of the Ca^{2+}-dependent aminoacetyltransferase, transglutaminase 2 (TGM2), have been resolved by X-ray crystallography yielding structures that attest to major domain reorganizations upon allosteric effector binding. Förster resonance energy transfer (FRET) has gained wide acceptance as a method to detect dynamic conformational changes in proteins. A common strategy is to append fluorophores to the ends of the protein domains involved and to monitor changes in FRET efficiency upon conformational transitions. Based on monitoring FRET with the help of an appropriately decorated recombinant TGM2 we aimed to verify that active and inactive TGM2 indeed acquires open and closed conformations as suggested by the crystal structures, and to show how these conformational transitions are affected by the allosteric effectors Ca^{2+} and GTP. We used small, membrane permeable, barely fluorescent biarsenical dyes – an emerging alternative to fluorescent proteins – which bind six amino acid motifs of C-C-X-X-C-C, called tetracystein (TC) tags (X=any amino acid), with picomolar affinity and upon that increase their fluorescence quantum efficiency. We placed two TC-tags at the two ends of TGM2 (TGM2-TC_2). Simultaneous labeling of TGM2-TC_2 with the dyes, ReAsH and FlAsH, results in 50 % of the molecules being heterologously decorated to give FRET when the two termini are close to each other. We expressed the TGM2-TC_2 protein in bacteria, purified it by histidine metal ion affinity chromatography, labeled it with ReASh and FlASh, separated it from the unbound dyes on a desalting column and carried out fluorometric measurements in the presence of allosteric effectors. We describe hypothetical conformational changes inferred on the grounds of FRET between the donor FlASh and the acceptor ReASh and its modulation by TGM2 allosteric effectors and enzyme inhibitors.We intend to characterize the conformers in terms of physical distance between the TC-tags at the termini relying on the strict dependence of the FRET phenomenon on distance.We are also investigating the possibility of using FRET generating constructs in cells as biosensors of TGM2-activity exploiting the membrane permeability of ReASh and FlASh. We hope to obtain a subcellular activity map with spatiotemporal resolution and expect that the probe will reveal intracellular sites and biochemical processes where TGM2 is active as a transamidase.

PIII-104

THE ROLE OF KEX2P AND YAPSINS IN THE PROTEOLYTIC PROCESSING OF SCW4P IN THE SACCHAROMYCES CEREVISIAE CELL WALL

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Yeast cell wall contains proteins that are noncovalently (Scw-proteins) or covalently (Ccw-proteins) bound to β-1,3-glucan, the latter either through GPI-anchors and β-1,6-glucan, or by alkali labile ester linkages between γ-carboxyl groups of glutamic acid and hydroxyl groups of glucoses (Pir-proteins, extracted from the cell wall by mild alkali). It was previously shown that one of the most abundant Scw protein, Scw4p is partly also covalently linked to the cell wall. In this work it was shown that part of Scw4p underwent the proteolytic processing resulting in two forms of the protein in the cell wall. The proteolytic enzymes which might have a role in processing of Scw4p are Kex2p and a family of aspartic proteases called yapsins. To get a better insight in the processing of Scw4p, *kex2* yeast strain and strain with all yapsin genes disrupted (*5ypsΔ*) were used. Scw4p was overproduced in these strains and Scw4p processing was examined.

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EFFICIENCY OF OXIMES IN REACTIVATION OF PHOSPHORYLATED ACHE IS LIMITED BY THEIR INTERACTIONS WITH THE ACHE PERIPHERAL ALOSTERIC SITE

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Acetylcholinesterase (AChE, EC 3.1.1.7) presents an important enzyme in the cholinergic neurotransmission. Its specific active site is the primary target of organophosphorus compounds (OP) like pesticides and nerve agents. Namely, OPs inhibit AChE by phosphorylating its catalytic serine in the active site. Such inactivation of AChE leads to a series of life threatening manifestations which calls for a fast medical response. Currently, compound known as oximes, that have the ability to dephosphorylate AChE, are used as antidotes. Even if there is an ongoing discussion on oximes efficiency, they are still the mainstay of the post OP-exposure treatment. In the search for more efficient oxime an important step presents understanding of its interactions within the active site of phosphorylated AChE. Therefore, AChE site-directed mutants provide a powerful tool for such investigation. In here presented study we evaluated reactivation of five nerve agent tabun phosphorylated AChE mutants by newly developed bispyridinium oximes. We selected site-directed mutants with mutations at the choline binding site (Y337A, F338A), the acyl pocket (F295L) and the peripheral binding site (Y124Q, W286A) to get an overview of possible interactions. Moreover, interactions of oximes with active site amino acids were evaluated using the molecular docking technique. Our results indicated that substitution of the aromatic amino acids with aliphatic ones in the choline binding site and the acyl pocket negatively influenced reactivation by bispyridinium oximes. These changes probably opened up a space for oximes to form stable interactions with other present aromatic residues, which resulted in increased affinity for these oximes but lower reactivation rates. It can be concluded that selected aromatic residues are important for placing bispyridinium oximes in the right position to the phosphorylated active site serine. On the other hand, disruption of the π-π sandwich formed between one of the oxime pyridinium rings and the amino acids of the peripheral site (i.e. Y124Q, W286A), allowed oximes to get into the more favourable position for nucleophilic attack on the phosphylated catalytic serine. In this case, reactivation rates increased 2-5 times compared to w.t. AChE. Therefore, it seems that aromatic amino acids at the AChE peripheral site present limitation in bispyridinium oxime reactivation efficiency.

PIII-106

HUGGING INTERACTION: ASYMMETRIC BINDING OF THE METASTASIS ASSOCIATED PROTEIN S100A4 TO NON-MUSCLE MYOSIN 2A TAIL

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S100A4 is a member of the S100 family of calcium-binding proteins that is directly involved in tumor metastasis. In the cytoplasm it binds to non-muscle myosin IIA (NM2A) near the assembly competence domain (ACD) promoting filament disassembly which could be associated with increasing metastatic potential of tumor cells. In this study we present a structural model of S100A4 – NM2A interaction based on the crystal structure of S100A4 complexed with a myosin IIA fragment, and various solution techniques such as circular dicroism (CD) and NMR spectroscopy, small-angle x-ray scattering (SAXS) and isothermal titration calorimetry (ITC).
According to our ITC measurements a 45-residue-long fragment of myosin tailpiece binds to S100A4 with nanomolar affinity and a stoichiometry of one S100A4 dimer per one myosin peptide. The crystal structure of a mutant S100A4 in complex with the Arg1894-Lys1937 NM2A fragment reveals a novel mode of interaction in the S100 family: a single, predominantly α-helical myosin chain is wrapped around the Ca^{2+}-bound S100A4 dimer occupying both hydrophobic binding pockets. SAXS curve of wild-type S100A4 | NM2A complex fits well the calculated theoretical scattering curve of the crystal structure. Peak doubling in ^{1}H-^{15}N HSQC spectrum of S100A4 upon the titration with the myosin tail fragment supports the asymmetric binding mode in solution.
Thermal denaturation experiments of a coiled-coil forming longer NM2A fragment indicate that the coiled-coil partially unwinds upon S100A4 binding, which affects the stability of the ACD. Titration of NM2A filaments with S100A4 shows that two-fold excess of S100A4 is required for complete filament disassembly. We hypothesize that the N-terminal random coil tailpiece and the C-terminal coiled-coil region of each myosin chain are wrapped around an S100A4 dimer which disrupts the ACD and sterically inhibits the assembly of the myosin rods. To elucidate the detailed mechanism of S100A4 binding to dimeric myosin by kinetic assays are in progress.
Supported by OTKA NK81950 and the European Social Fund (TÁMOP) 4.2.1./B-09/KMR-2010-0003 and the Swedish Research Council.

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STRUCTURAL ANALYSIS OF DUTPASE FROM THE HELPER PHAGE φ11 OF *STAPHYLOCOCCUS AUREUS*

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Recently an interesting derepressing interaction was suggested between the StI SaPIbov1 repressor with dUTPase from the φ11 helper phage. The enzyme family of dUTPases has a major role in preservation of genomic integrity by hydrolyzing dUTP into dUMP and pyrophosphate. On the one hand, dUTPases provide the precursor dUMP for dTTP de novo biosynthesis, on the other hand they also remove dUTP from the DNA polymerase pathway, keeping the cellular ratio of dUTP/dTTP pools on low level. The lack or severe malfunction of this enzyme results in cell death. Staphylococcus aureus superantigen-carrying pathogenicity islands (SaPIs) have a determinant role in spreading virulence genes among bacterial populations, fact that constitutes a major health hazard. There are important functional genes within pathogenicity islands, such as genes for virulence factors and superantigens (for instance sometimes toxins or invasions as well). Repressor (StI) proteins are responsible for transcriptional regulation of pathogenicity island genes. The putative derepressing interaction of dUTPase from the φ11 helper phage raises the interest to elucidate a non-canonical function in the dUTPase family. This study aims at elucidating the molecular mechanism of this interaction approaching by various methods as X-ray crystallography, kinetic measurements and spectroscopy.
Diffracting crystals were obtained by the hanging-drop and sitting-drop vapor-diffusion methods, by the application of a large variation of high-throughput sparse matrix crystallization screens. Crystals were tested at home source diffractometers. X-ray data were collected from one type of crystal at 2.98 A resolution at the ESRF Grenoble synchrotron. Structure solution using PHENIX software of φ11 helper phage dUTPase allows an in-depth investigation of the identity of the derepressor protein and its detailed mechanism of action.

PIII-108

ROLE OF THE C-TERMINAL ARM IN THE dUTPase CATALYTIC MECHANISM

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According to WHO data, tuberculosis is the most deadly infectious disease which causes nearly 2 million deaths each year. The dUTPase enzyme that is in focus in our laboratory is a potential drug target as it is essential for *Mycobacterium tuberculosis*. dUTPase plays a crucial role in DNA integrity, as it catalyses the hydrolysis of dUTP to dUMP and pyrophosphate, thereby it produces the precursor of dTTP biosynthesis and keeps the dUTP:dTTP ratio low. In the absence of dUTPase this ratio will be high, which causes DNA fragmentation and the so-called thymine-less cell death.

To understand the enzymatic mechanism of dUTPase, mutant enzymes were generated, which differ from the wild type at the active site forming C-terminal arm. The C-terminus is considered to play a major role in substrate binding and positioning, as it was identified as a P-loop-like motif similar to ATPases. Interestingly, however, the mutations caused a large decrease in the enzymatic activity, while substrate binding was only slightly affected. The structures of two mutants – one with alanine instead of the conserved histidin that stacks over the uracil ring, and one that lacks the full C-terminal arm – could be resolved and have been deposited in the PDB database with IDs 3LOJ and 3I93.

To compare the substrate hydrolysis mechanism of the wild type and the mutants, hybrid quantum mechanics/molecular mechanics simulations have been performed. The calculated activation energies were found to be in good agreement with the experimentally observed catalytic rate constants. Although, the scissile bond is between P_α and P_β, still the proper position of the P_γ (the last phosphate group of the phosphate chain of dUTP) was found to be required for efficient catalysis. The proper coordination geometry of Mg^{2+} ion was found to be crucial, as well, furthermore this cation is supposed to have similar role and behavior as metal ion B in two-metal ion catalysis of DNA and RNA polymerases and nucleases, where the coordination shell of this ion plays an important role and it stabilizes the leaving group.

Our calculations suggest that the C-terminal arm lowers the activation free energy barrier of the dUTP hydrolysis reaction by stabilizing the transition state. The results might also provide a mechanistic explanation for the role of the C-terminal arm in substrate specificity, as the enzyme is unable to hydrolyze the diphosphate substrate analogue dUDP.

PIII-109

EFFECT OF DIFFERENT BOUND NUCLEOTIDES ON THE SKELETAL ACTIN DYNAMICS

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Actin is one of the main components in the eukaryote cells which plays significant role in many cellular processes, like force-generation, maintenance of the shape of cells, cell-division cycle and transport processes. It has been generally believed that the torsional flexibility of actin filaments and the amount of twisted structure correlate with the biological functions.

Actin monomer, called G-actin, consists of four subdomains. The interaction between the subdomains is dynamic, as derived by several biophysical and structural studies; the subdomains might have different conformational and motional states depending on the bound cations and nucleotides, and on the interactions with small molecules and larger entities as proteins. In this work a spin label was attached to Cys-374 residue in F-actin, and the motional dynamics of the label was measured by EPR in both forms of actin when the bound ATP was exchanged by its non-hydrolyzable analogue AMP.PNP. Additional measurements carried out by DSC, in order to get thermodynamical data which correlate with the domain motions.

Temperature-dependent EPR measurements showed that the hyperfine splitting constant which reflects the rotational motion of the attached label in G-actin (AMP.PNP) increased in the whole temperature range in comparison with G-actin (ATP). Similar tendency was concluded in the case of F-actin as well. Analysis of DSC transitions in samples of AMP.PNP-G-actin and AMP.PNP-F-actin showed that the local conformational changes detected by EPR measurements are coupled with global motions and domain interactions measured by DSC. The data of both measurements showed significant increase in EPR and DSC parameters after nucleotide exchange (AMP.PNP-G-actin melting temperature T_m=65.2 °C, AMP.PNP-F-actin T_m=74.1 °C).

PIII-110

MUTAGENESIS OF ACETYLCHOLINESTERASE ENABLES OXIME-ASSISTED REACTIVATION OF SOMAN-ENZYME CONJUGATE THAT RESISTS AGING

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In the event of poisoning by organophosphorus compounds (OP), immediate therapeutic treatment usually consists of combined administration of an anticholinergic drug, such as atropine, and an oxime-reactivator of acetylcholinesterase (AChE, E.C. 3.1.1.7). However, the treatment is very limiting in case of nerve agent soman poisoning due to extremely rapid aging of phosphorylated enzyme. For now, HI-6 is the best known reactivator of soman inhibited AChE. Today, because of limited reactivation of phosphorylated AChE and fast ageing, researches are pointed towards AChE bioscavangers, human AChE mutants among other enzymes. The mutant of our interest is Y337A/F338A where increased accessibility of the Y337A mutation to oximes is combined with aging resisting the F338A mutation.
We screened 35 oximes (1mM) for the reactivation activity of soman inhibited human AChE mutant, Y337A/F338A. Only 15 oximes were able to restore more than 30% of soman inhibited Y337A/F338A activity. None of the tested oximes restored Y337A/F338A activity fully nor any of those oximes were better than HI-6 despite the fact that some of these oximes have CH_2-O-CH_2 linking chain and/or oxime group in position 2, which is known to be characteristic for the most potent reactivators of soman inhibited AChE. Infact, some of the most potent reactivators among tested oximes have benzene ring in the linking chain so our findings could point the quest for reactivators of soman inhibited AChE and AChE mutants in new direction.

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CHARACTERISATION OF THE RECOMBINANT POTEIN Rny1p/Ccw12p EXPRESSED ON THE CELL WALL OF YEAST *SACCHAROMYCES CEREVISIAE*

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Yeast cell surface systems for the display of heterologous proteins have the advantages of simplicity of genetic manipulation and immobilization of heterologous proteins covalently under mild, physiological conditions without risk of damage by chemical treatment. In the past several years a number of surface – engineered yeasts, displaying different heterologous proteins interesting for biotechnological or medical applications, have been constructed. In this work Ccw12p cell wall protein, that belongs to the group of cell wall proteins bound to the wall through GPI-anchor remnant, is used for C-terminal immobilization of yeast intracellular nuclease Rnyp, to the yeast cell surface. Recombinant protein localization, activity and stability, optimal pH and optimal temperature for the enzymatic reaction were determined.

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CATION-PI INTERACTION DETERMINES EFFICIENCY OF CTP: PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE FROM *PLASMODIUM FALCIPARUM*

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Phospholipid synthesis in *Plasmodium,* the causative agent of malaria, during its intraerythrocytic cycle is essential and constitutes a validated and original pharmacological target. (*1*) The *P. falciparum* CTP: phosphocholine cytidylyltransferase (*Pf*CCT) enzyme has key regulatory function and catalyzes a rate-limiting step of *de novo* phosphatidylcholine biosynthesis. We aimed to decipher the mechanism of action of *Pf*CCT, focusing on its choline binding site, which may serve as an interaction surface for choline analogue antimalarials.(*2*) *Pf*CCT constructs with one of the catalytic domains were successfully optimized for *E coli* expression. Using these constructs, we investigated for the first time the role of a conserved Trp692 residing in the hydrophobic choline binding subsite of the active centre. By performing Trp/Tyr and Trp/Ala mutagenesis, we tuned its cation-pi interaction ability to the choline group. The performed point mutations did not perturb the secondary structure of the enzyme, as reported by far UV circular dichroism spectra. For the Trp692Ala mutant protein, mutation induced conformational changes, potentially within the choline binding site were suggested by a diminished tyrosine signal in near UV circular dichroism spectrum.
Mutants showed dramatic decrease in steady state activity: 50 and 3000-fold, for Trp692Tyr and Trp692Ala mutants, respectively. Nevertheless, the Michaelis constant of the Trp692Tyr mutant for CTP substrate remained the same as for the wild type enzyme, indicating the major perturbation to be present at the choline binding site. This compromised binding ability was also confirmed by 6-fold decrease of CDP-choline product affinity to the Trp692Tyr mutant enzyme, measured by isothermal titration calorimetry. The effect of mutation on ligand binding is currently being investigated by molecular dynamics.
Our results indicate that in addition to its role in the enzyme's functional integrity, tryptophan residue in the choline binding site provides essential cation-pi interaction that has fundamental role in CCT ligand binding and catalysis. A proposed push effect of the residue exerted on choline phosphate substrate during catalysis may contribute to deeper understanding of the dynamics of CCT catalysis.

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Laura Chis thanks POSDRU/88/1.5/S/60185 2007-2013 "Investing in people!" for support.

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BIOLOGICAL ACTIVITY AND PORE FORMATION MECHANISM OF A *PLEUROTUS OSTREATUS* BI-COMPONENT CYTOLYSIN

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Ostreolysin A (OlyA) is a 15 kDa protein from the aegerolysin protein family, found in the oyster mushroom (*Pleurotus ostreatus*). OlyA was previously reported to be cytolytic, forming homo-oligomeric transmembrane pores. In contrast, aegerolysins pleurotolysin A (PlyA) from *P. ostreatus* and erylysin A (EryA) from *P. eryngii* have been functionally characterized as non-lytic, *i.e.* both require a 59 kDa protein (PlyB, EryB) with a MACPF-domain for pore formation. Our results demonstrate that OlyA is a membrane-binding component of the lytic complex, however pore-formation proceeds only after the addition of a larger, MACPF domain-containing protein. Several expression constructs of OlyA (with or without the N- or C-terminal His_6-tag) were tested for cytolytic activity in combination with recombinant pleurotolysin B (with or without the N-terminal His_6-tag) containing the MACPF domain. In parallel, we have isolated native ostreolysin OlyA, separated from the MACPF-domain containing B component. The proteins were biochemically characterized using Edman's amino acid micro-sequencing and ESI-MS. Screening for binding to CFG glycan-arrays showed no interaction of OlyA with saccharides. Testing of the native and recombinant proteins for binding to lipids showed a crucial role of cholesterol/sphingomyelin, however, their stable binding was achieved only if combined with PlyB. Hemolytic activity depended on stoichiometry of the binding component OlyA and PlyB. Biological role of the *P. ostreatus* cytolytic proteins was examined by testing the toxicity of *P. ostreatus* extracts and recombinant proteins to model organisms *Arabidopsis thaliana*, *Drosophila melanogaster*, and *Caenorhabditis elegans*. No effect or little effect on plant germination and insect larvae development was noticed, however, preliminary results showed potent nematocidal activity of the recombinant OlyA and PlyB.

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IDENTIFYING AMINO ACIDS IMPORTANT FOR CHOLESTEROL SENSING AND SUBSTRATE RECOGNITION 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. Human ABCG2 has been shown to require cholesterol for its function with Arg 482 playing an important role in cholesterol sensitivity of the protein. However, the sensing site(s) for cholesterol have not yet been identified. Steroid hormone receptors contain the so-called sterol sensing domain (SSD) (LxxLxxL). ABCG2 contains an SxxLxxL motif corresponding to a shorter LxxL motif that was also shown to be implicated in steroid recognition. In our present work we have characterized the role of aa 482 and the sterol sensing domain in cholesterol sensitivity of human ABCG2. Nine ABCG2-R482 and three SSD mutants were expressed in Sf9 insect cells, containing relatively low amounts of plasma membrane cholesterol (5-8 μg cholesterol/mg membrane protein). ATPase and transport activity of the mutants were investigated at different membrane cholesterol levels to analyze their effects on cholesterol sensitivity of ABCG2. We found, that similarly to the wild-type protein, increase in membrane cholesterol level greatly enhanced the stimulated ATPase activity and transport capacity of R482I, M, K and Y mutants. In case of mutants R482G, T, S, D and N, we found increased baseline ATPase activity upon cholesterol loading, however their stimulation with potential substrates was not altered. In Hoechst33342, PheophorbideA and rhodamine123 uptake experiments these latter R482 mutants proved to be fully active already at low cholesterol levels and cholesterol enrichment did not further activate transport. We demonstrate that mutations in the SSD (L555A, L558A and L555A/L558A) greatly alter the function of ABCG2. Although all SSD mutants were active, their substrate specificity changed, as compared to the wild-type protein. Importantly, stimulated ATPase activity and transport function of the SSD mutants was unaltered upon cholesterol enrichment of the membranes. Our findings reveal that at least two regions (R482 and the SSD) are involved in cholesterol sensing of ABCG2.

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dUTPASE TASK DISTRIBUTION BETWEEN CELL ORGANELLS IN EUKARYOTES

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Faithful maintenance of genomic integrity is indispensable for life. The object of our studies, dUTPase, is essential in a number of organisms due to its crucial role in genome maintenance. dUTPase catalyses the hydrolysis of dUTP into pyrophosphate and dUMP, providing low cellular dUTP/dTTP ratio thus immunity against uracil for the genome. Deficiency in dUTPase function leads to DNA fragmentation and cell death. The therapeutic potential of inducing this thymine-less cell death is a promising approach in the treatment of abundant and deadly diseases such as cancer, tuberculosis and malaria.
Most eukaryotic organisms encode two dUTPase isoforms, a one which is nuclear, and one which is targeted into the mitochondria or into the cytoplasm. dUTPases are mainly homotrimeric enzymes thus harbouring potentially three cognate localization signals. In case of *D. melanogaster*, the existence of two splice variants of dUTPase is already known, one containing a nuclear localization signal (longer isoform) thus targeted into the nucleus while the other one lacks the NLS signal and localises in the cytoplasm. *D. virilis* has, however, unique dUTPase architecture, consisting of three covalently linked none identical monomers, forming a pseudo-heterotrimer containing only one NLS signal, having nucleo-cytoplasmic localization.
Our aim was to identify possible isoforms of dUTPase in *D. virilis* that might also differ in their N terminals which contain their NLS signals, thus determining localization. Applying *in silico* prediction programs we found potential splice sites in the dUTPase gene, however, the probability of these sites were much lower than in the case of *D. melanogaster* splice sites. To investigate existence of more isoforms we wanted to identify possible mRNA transcripts, differing in their 5' end using 5' RACE technique. We also applied western blot experiments to detect possible isoforms at the protein level. Based on our results we concluded that *D.virilis* only encodes one dUTPase isoform unlike D. melanogaster.
It is interesting to investigate whether the two dUTPase isoforms, encoded by *D. melanogaster* to be targeted into two different compartments (nucleus and cytoplasm), could be replaced by only one isoform, through NLS copy number variation in *D. virilis.* We propose that reduction of the NLS copy number in *D. virilis* dUTPase allows for both nuclear and cytoplasmic occurrence whereas *D. melanogaster* requires two respectively dedicated dUTPase isoforms.

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HIGH THROUGHPUT ISOLATION AND GLYCOSYLATION ANALYSIS OF IgG – VARIABILITY AND HERITABILITY OF THE IgG GLYCOME IN THREE ISOLATED HUMAN POPULATIONS

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All immunoglobulin G molecules carry *N*-glycans which modulate their biological activity. Changes in *N*-glycosylation of IgG associate with various diseases and affect the activity of therapeutic antibodies and intravenous immunoglobulins. We have developed a novel 96 well protein G monolithic plate and used it to rapidly isolate IgG from plasma of 2298 individuals from three isolated human populations. *N*-glycans were released by PNGase F, labeled with 2-aminobenzamide and analyzed by hydrophilic interaction chromatography with fluorescence detection. The majority of the structural features of the IgG glycome were consistent with previous studies, but sialylation was somewhat higher than reported previously. Sialylation was particularly prominent in core-fucosylated glycans containing two galactose residues and bisecting GlcNAc where median sialylation level was nearly 80%. Very high variability between individuals was observed, approximately three times higher than in the total plasma glycome. For example, neutral IgG glycans without core fucose varied between 1.3% and 19%, a difference that significantly affects the effector functions of natural antibodies, predisposing or protecting individuals from particular diseases. Heritability of IgG glycans was generally between 30% and 50%. The individual's age was associated with a significant decrease in galactose and increase of bisecting GlcNAc, while other functional elements of IgG glycosylation did not change much with age. Gender was not an important predictor for any IgG glycan. An important observation is that competition between glycosyltransferases which occurs *in vitro* did not appear to be relevant *in vivo,* indicating that the final glycan structures are not a simple result of competing enzymatic activities, but a carefully regulated outcome designed to meet the prevailing physiological needs.

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MEMBRANE BINDING OF OSTREOLYSIN A-mCHERRY FUSION PROTEIN

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Membrane microdomains, such as lipid rafts, are transient, dynamic and unstable membrane entities that are involved in cellular trafficking, signal transduction, pathogen entry, and attachment of various ligands. The increasing amount of experimental evidence on a variety of membrane microdomains and their biological roles urges the development of new techniques and approaches that would allow their structural and functional characterization. In particular, fluorescently labeled cytolytic proteins that interact specifically with molecules enriched in lipid rafts are gaining interest.

Ostreolysin A (OlyA), a 15-kDa protein from the edible oyster mushroom (*Pleurotus ostreatus*), is a representative of aegerolysin protein family. The protein is very similar to pleurotolysin A, a membrane-binding counterpart of fungal bi-component cytolysins (Tomita et al., 2004) using a ~59 kDa B component with a MACPF domain for pore formation. Even when alone, OlyA specifically recognizes cholesterol/sphingomyelin-enriched membrane domains of living cells and artificial lipid bilayers (Sepčić et al., 2004), and therefore, it seems appropriate candidate for development of a cholesterol-specific membrane marker.

For the purpose of live cell imaging we have constructed a His6-tagged fusion protein, OlyA-mCherry, and expressed it in *E. coli*. The purified and biochemically characterized fluorescent fusion protein retained binding properties to vesicles enriched in cholesterol and sphingomyelin. Even more, when combined with recombinant pleurotolysin B the complex was hemolytic. The fused protein has enabled the use of fluorescence microscopy to study protein-membrane interactions without interference of immunolabeling artifacts and membrane microdomain perturbations. The preliminary results have shown that OlyA fused with mCherry labels plasmalemma of MDCK cells in a concentration- and time-dependent manner, and even more, it induces specific remodeling of the cell membrane.

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PIII-118

BUILDING TUBULAR NANOSTRUCTURES FROM FLAGELLIN-XYLANASE A FUSION PROTEINS

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The aim of our work is to furnish enzymes with polymerization ability by creating fusion constructs with the polymerizable protein, flagellin, which is the main component of flagellar filaments. The D3 domain of *Salmonella* flagellin, exposed on the surface of flagellar filaments, is formed by the hypervariable central portion of the polypeptide chain. D3 is not essential for filament formation. The concept in this project is to replace the D3 domain with suitable monomeric enzymes without adversely affecting polymerization ability, and to assemble these chimeric flagellins (flagzymes) into tubular nanostructures. To test the feasibility of this approach, xylanase A (XynA) from *Bacillus subtilis* was chosen as a model enzyme for insertion into the central part of flagellin. With the help of genetic engineering, a fusion construct was created in which the D3 domain was replaced by XynA. The construct was transformated into an IPTG-inducible *Escherichia coli* overexpression strain. The N-terminally His_6-tagged flagellin-XynA flagzyme, purified by nickel-affinity chromatography, exhibited catalytic activity as well as polymerization ability. To improve polymerization properties, a removable GST-tag was conjugated through a TEV protease site to the fusion protein which allows preparation of the untagged flagellin-XynA flagzyme. Furthermore a plasmid, coding untagged flagellin-XynA, was transformed into *Salmonella typhimurium* SJW1103 wild (motile), and SJW2536 flagellin-deficient (non mobile) strain in the expectation of recombinant protein production and secretion by the type III secretion system of *Salmonella* in filamentous form. Our results demonstrate that polymerization ability can be introduced into various proteins, and building blocks for rationally designed assembly of filamentous tubular nanostructures can be created. (This work was supported by the National Development Agency grants TÁMOP-4.2.2/B-10/1-2010-0025 and REG_KD_09-2-2009-0022).

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TRADE-OFF BETWEEN COOPERATIVITY AND SPECIFICITY IN THE dUTPase SUPERFAMILY

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It is an intriguing question with oligomeric enzymes whether allosteric communication exists between their active sites. Members of the homotrimeric dUTPase superfamily (dUTPases and dCTP deaminases (DCD)) eliminate dUTP and produce precursors for dTTP biosynthesis. The DCD enzymes display an interesting dTTP feedback inhibition mechanism, which shed light on the partially hidden homotropic allosteric communication mediated through the central channel of the homotrimer.
dUTPase cannot bind dTTP due to its tight substrate binding pocket. Based on sequence and structural homology it was proposed, however, that eukaryotic dUTPases, similarly to DCD-s, also behave cooperatively. As we previously could not detect cooperativity in the homotrimer, we set out to explore perturbed conformational states potentially mimicking dTTP binding. We therefore created covalently linked human dUTPase heterotrimers in which we could manipulate active sites selectively. In the resulting asymmetric pseudo-heterotrimers one or two monomers ***a,*** were catalytically inactive ***b,*** had reduced activity and substrate affinity ***c,*** or could not bind substrate at all. Using these mutant heterotrimers we investigated whether the interruption of different steps of the enzymatic cycle – and thereby of the putative active-inactive conformational equilibria– affects functioning of the non-mutated active sites. Using a variety of biochemical and enzyme kinetics methods and four different mutants we found no indication of cooperativity in human dUTPase.
To understand the structural basis of the surprising lack of cooperativity, we investigated the central channel of human dUTPase. We found that Mg^{2+} binding to the channel decrease the overall flexibility of human dUTPase which in turn strengthens dUTP binding to the active site. In conclusion, we propose that the central channel of eukaryotic dUTPases lacks the allosteric route that mediates cooperativity in DCDs and instead, acquired increased substrate affinity with Mg^{2+} binding to it. The lack of cooperativity in dUTPases seems to be adaptation to high specificity and catalytic efficiency to break down dUTP. Our hypothesis is that the trade-off between cooperativity and specificity in dUTPases and DCDs represents the adaptation to the distinct roles of dUTP elimination/uracil-DNA avoidance and dUMP production/dTTP synthesis, respectively.

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THE EFFECT OF mDIA1-FH2 ON THE ATPASE ACTIVITY OF ACTIN FILAMENTS

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Formins are conservative proteins with important roles in the regulation of the actin based microfilamental system in eukaryotic cells. They have several domains including FH1, FH2, GPB and DAD. In the interaction between actin and formin the FH2 domain plays a key role. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. These mDia1 formin fragments affect the conformation of the actin filaments in a concentration dependent manner. In the current work we have investigated whether the mDia1-FH2 affects the nucleotide exchange on the actin filaments. Steady-state fluorescence anisotropy and photometric coupled assay measurements showed that the ATP-ADP conversion was accelerated in the presence of formins, and the effect was stronger at greater formin concentrations. These observations indicate that there must be a tight coupling between the rate of nucleotide exchange on actin protomers and the conformational properties of the filaments.

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TROPOMYOSIN ISOFORM-SPECIFIC REGULATION OF ACTIN ASSEMBLY

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In the cohesive structure of the cytoskeleton functionally distinct actin arrays orchestrate fundamental cell functions in a spatiotemporally controlled manner. Emerging evidences emphasize that protein isoforms are essential for the functional polymorphism of the actin cytoskeleton. The generation of diverse actin networks is catalyzed by different assembly factors, like formins and Arp2/3 complex. These actin arrays also exhibit qualitative and quantitative differences in the associated tropomyosin (Tm) isoforms. How the molecular composition and the function of actin networks are coupled is not completely understood.
We investigated the effects of different tropomyosin isoforms (skeletal muscle: sk, cytoskeletal 5NM1 and Br3) on the activity of mDia1 formin and Arp2/3 complex using fluorescence spectroscopic approaches. The results revealed that the Tm isoforms have different effects on the mDia1-, and Arp2/3 complex-mediated actin assembly. The activity of the Arp2/3 complex is inhibited by skTm and Tm5NM1, whereas TmBr3 does not have any effect. All three Tm isoforms inhibited the activity of mDia1.
These results contribute to the understanding of the mechanisms by which tropomyosin isoforms regulate the functional diversity of the actin cytoskeleton.

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CHARACTERIZATION OF THE FIRST PROKARYOTIC M49 METALLOPEPTIDASE REVEALS A REGULATORY CYSTEINE RESIDUE IN THE ACTIVE-SITE MOTIF

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Dipeptidyl peptidase III (DPP III), a member of the metallopeptidase family M49, was considered as an exclusively eukaryotic enzyme involved in intracellular peptide catabolism and pain modulation. New data on genome sequences revealed only in 2003 the first prokaryotic orthologs, which showed low sequence similarity to eukaryotic ones and a cysteine residue in the zinc-binding motif HEXXGH.
We cloned and overexpressed the gene encoding putative DPP III from human gut symbiont *Bacteroides thetaiotaomicron* and biochemically characterized the isolated protein. Substrate specificity and catalytic efficiency of bacterial DPP III for the hydrolysis of preferred synthetic substrate was very similar to that of the human host enzyme. Substitution of Cys^{450} from the active-site motif $H^{448}ECLGH^{453}$ by serine did not substantially change the enzymatic activity. However, this residue was wholly responsible for the inactivation effect of sulfhydryl reagents. Molecular modeling of bacterial DPP III indicated seven basic amino acid residues in the local environment of Cys^{450} as possible cause for its high reactivity. Sequence analysis of 81 bacterial M49 peptidases revealed the conservation of the HECLGH motif in 73 primary structures. Majority of proteins lacking an Cys in the active-site motif originated from aerobic bacteria, and by phylogenetic analysis were found to form separate cluster.

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THE BENZOATE 4-MONOOXYGENASE INHIBITORS AS NEW LEAD STRUCTURES IN ANTIFUNGAL DRUG RESEARCH

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The incidence of invasive fungal infections (IFI) is increasing, particularly among immuno-compromised patients, like individuals infected with HIV, transplant recipients and patients with cancer. Adverse effects, toxicity and resistance to currently available antifungal drugs are limitations, that advance the identification of novel antifungal targets and the development of new antifungal agents for the effective treatment of fungal infections. New methods like comparative genomics in combination with high throughput technologies, combinatorial strategies and large compound libraries, will reveal a host of new lead structures not only for already well-accepted drug targets such as CYP51, but also for CYPs, which have not yet been addressed as targets, like fungal CYP53 protein family. A limited number of substrates, high specificity and absence of homologue(s) in higher eukaryotes designate CYP53 as interesting drug target and provide the opportunity to design more specific, selective and effective inhibitors of filamentous fungi.
In the work presented here, we explored chemical properties of isoeugenol for ligand-based virtual screening and a reliable CYP53A15 homology model[#] for structure-based searching of composite chemical library. Highest scoring compounds were analyzed in spectral binding titration with CYP53A15, assayed for antifungal activity and evaluated as inhibitors of benzoate 4-monooxygenase. Based on potent antifungal activity and good inhibition of CYP53A15 enzyme activity, compounds I26 and I30 were selected as suitable for further screening of compound library and promising new lead structures in antifungal drug research.
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PIII-124

STREAMLINED-GENOME *ESCHERICHIA COLI*: AN IMPROVED CHASSIS FOR SYNTHETIC AND MOLECULAR BIOLOGY APPLICATIONS

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The mobile genetic element-free, streamlined-genome *E. coli* K-12 MDS series (Pósfai et al., 2006, Science) was shown to display advantageous characteristics as a host cell for maintenance and expression of artificial genetic constructs (Sharma et al., 2007, Biotechnol Bioeng; Umenhoffer et al., 2010, Microb Cell Fact; Csörgő et al., 2012, Microb Cell Fact). Additional engineering, including genetic removal of energy-consuming surface structures, deletion of error-prone DNA polymerase genes, implementation of inducible expression constructs, and correction of metabolic defects, further improved the performance of the strains. Extensive analysis revealed: i) improved genetic stability of otherwise unstable and toxic artificial genetic constructs, ii) improved electroporation efficiency, iii) fast growth rate, iv) excellent recombinant protein production with practically zero uninduced background and high level induced expression, and v) improved, high-efficiency host for mutant oligonucleotide recombination-based genome engineering protocols. In addition, we show that removal of up to 22% of the genome neither increased, nor decreased the maintenance energy requirement and yield, but made the cells genetically less adaptable and physiologically more uniform. In conclusion, the MDS series provides an improved platform for fast and efficient genetic manipulations and a stable background for maintenance and expression of synthetic biological constructs.

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THE ACE-DD GENOTYPE DISTRIBUTION DEMONSTRATES POSSIBLE AGE DEPENDENCE AMONG PATIENTS WITH DIABETIC NEPHROPATHY

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Over the past few decades diabetes mellitus has expand to epidemic size. Diabetic nephropathy, being one of the severe complications of diabetes, is the leading cause of end-stage renal disease (ESRD) in developed countries. Evidence for a genetic component to diabetic nephropathy comes from family studies displaying familial aggregation of diabetic nephropathy both in type 1 and in type 2 diabetes mellitus, as well as differences in the prevalence of diabetic nephropathy between ethnic groups. The aim of our study was to investigate the significance of insertion/deletion polymorphism of angiotensin-converting enzyme (ACE) as a possible contributing factor in the development of the diabetic nephropathy. Genotyping was done in a cohort of 100 patients with diabetic nephropathy and 102 diabetic control patients with preserved renal function (urinary protein excretion rate less than 300 mg/day and creatinin clearance level ≥ 80 ml/min) using the LightCycler System (Roche, EU). Statistical analysis was performed using the SPSS 19.0 software (SPSS Inc, Chicago, IL, USA). An excess of DD genotype was found among patients with nephropathy younger than 66 years (45.3%) compared to those of the same age but with preserved renal function (22.9%). The difference is statistically significant (χ^2=6,751, p=0,034). No statistical difference in the genotype distribution was observed among patients older than 65 years. Our results suggest that patients with type 2 diabetes carrying the DD genotype could be susceptible to early development of end-stage renal disease (ESRD).

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OXIDATIVE STRESS IN PATIENTS WITH LIVER AND KIDNEY TRANSPLANTATION

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INTRODUCTION: Oxidative stress as a consequence of increased free radicals production and/or exhaustion of antioxidant defense results in a damage of DNA, proteins, lipids and carbohydrates. As such it is considered to be involved in mechanisms of metabolic disorders and disease development. The aim of this study was to analyze oxidative/antioxidative status in patients subjected to organ transplantation.
METHODS: 61 patients were included in the study, 50 with liver, and 11 with kidney transplantation. Blood samples were collected on day 0 (before transplantation), and again one month after transplantation. In erythrocytes catalytic concentrations of glutathione peroxidase (eGPx) and superoxide dismutase (eSOD) were measured using commercial kits, and expressed in terms of U/g Hb. Hemoglobin concentrations were determined using commercially available kits. As a marker of the degree of peroxidation, the concentration of substances which react to thiobarbituric acid was measured in serum spectrophotometricaly. Results were calculated and expressed as concentration of malondialdehyde (sMDA).
RESULTS: Among 50 liver transplant patients there were 37 males and 13 females in the age from 21 to 69 years. The average body mass index (BMI) was 24.2 kg/m^2 (range 15.6 – 36.6 kg/m^2). In kidney transplant group there were 5 male and 6 female patients in the age from 28 to 68 years with the average BMI 24.7 kg/m^2 (range 18.4 to 35.6 kg/m^2). Catalytic concentrations for eGPx were increased on day 0 in patients awaiting kidney transplantation, but after transplantation they went back to normal. Activities for eSOD and sMDA values were within reference values for both groups. For the whole group a significant negative correlation between eGPx and sMDA was found. Furthermore, higher BMI values were observed in ex-smokers.
CONCLUSIONS: With the exception of increased eGPx activity in patients with kidney transplant, no increase in parameters of oxidative stress was detected in patients with organ transplantation.

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ADVERSE EFFECTS OF NARINGENIN, QUERCETIN, CHRYSIN AND CAFFEIC ACID ON LIPIDS IN MICE ON A HIGH FAT DIET

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Polyphenolic compounds naringenin, quercetin, chrysin and caffeic acid are potent natural antioxidants. Lipids are thus transferred in blood as lipoprotein complexes of lipids and apolipoproteins. They are classified according to size, density, and lipid - apolipoprotein ratio: chylomicrons, very low density lipoprotein (VLDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL) and high density lipoproteins (HDL). Large amounts of LDL are not absorbed in the liver and consumed by macrophages, so they accumulate in the blood stream causing oxidative stress and many pathological conditions. Polyphenolic compounds are alleged antioxidants. In this study we studied the possible antioxidant effects of five polyphenoles on the composition of serum and liver lipids in mice on high-fat diet. The naringenin, quercetin, chrysin and caffeic acid were given orally to mice at dose of 50 mg/kg/bw every 48 h, for 30 days. At the end of the treatment serum total triglyceride, cholesterol, HDL, LDL, serum enzymes and metabolites (AST, ALT, ALP i LDH), liver enzymes (MDH and CAT) and MDA as product of lipid peroxidation were measured. Results sow that quercetin, chrysin and caffeic acid increased the “bad” and decreased “good” cholesterol in serum probably as a result of slight but significant hepatotoxic effects. On the contrary, no adverse effects reported naringenin.

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INVESTIGATIONS ON SOME GLUCOSE METABOLISING ENZYMES DURING DIFFERENTIATION IN HL-60 CELLS

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In our former work we gave a good model why neutropenia is characteristic for Von Gierke's disease 1b. We have shown that in human neutrophils and differentiated HL-60 cells inhibition of the endoplasmic reticulum (ER) glucose-6-phosphate transporter (G6PT) is responsible for early apoptosis of these cells. We investigated the possible factors responsible for this apoptosis evoked by inhibition of the G6PT. Interestingly high amount of cortisol was able to save leukocytes from apoptosis induced by a decreased glucose-6-phosphate influx. Investigations of the cortisol activation system - namely through hexose-6-phosphate dehydrogenase in granulocytes - resulted in a close functional cooperation between glucose-6-phopshate entry, and cortisone reduction. Showing that inhibition of 11-beta-hydroxysteroid dehydrogenase leads to enhanced apoptosis in leukocytes we suggested, that reduced NADPH in the ER lumen has a crucial role saving granulocytes from early apoptosis. Interestingly in Jurkat cells and non differentiated HL-60 cells inhibition of G6PT had no effect on apoptosis.
In the last years it has become evident, that a glucose-6-phosphate hydrolysing enzyme is also present in neutrophil leukocytes, namely glucose-6-phosphatase-beta - a closely related enzyme to the liver type glucose-6-phophatase. Lack of this enzyme is responsible for neutropenia in sever congenital neutropenia type IV.
In our current presentation we try to evaluate why only the mature differentiated HL-60 cells are affected by the inhibition of G6PT. What are the events happening during differentiation regarding the proteins we investigated earlier? If there is any difference, what can be the explanation?
We found that among the mentioned proteins G6PT remains unaltered during differentiation, but hexose-6-phosphate-dehydrogenase is well induced, approximately 15 fold. We also investigated the expression and presence of glucose-6-phosphatase-beta during HL-60 cell differentiation, but the enzyme level is not changing at all.
Our data suggest that not the substrate supply, but the intraluminal metabolism is altering during differentiation in HL-60 cells, and so it is feasible, that maintaining NADPH level is the crucial point during differentiation. This give advance towards why patients having non functional G6PT and patients in severe congenital syndrome type IV are suffering in granulocytopenia.

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EFFECTS OF DIETARY BUTYRATE SUPPLEMENTATION ON HEPATIC MICROSOMAL CYTOCHROME P450 ACTIVITY IN CHICKEN AND RAT

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Butyrate is a short chain fatty acid used as an alternative growth promoter in poultry and pig nutrition, also known as an epigenetically active molecule due to its inhibitory effect on histone deacetylases. As a consequence of its possible epigenetic actions, butyrate in vivo may influence the expression and activity of certain enzymes, such as hepatic drug-metabolizing microsomal cytochrome P450 (**CYP**) monooxygenases, which are involved in the biotransformation of most xenobiotics. The aim of the authors was to investigate, whether the orally added butyrate modulates the activity of hepatic microsomal CYPs in chicken and rat (the latter as a monogastric model animal), to study the possible relevant pharmacokinetic interactions between butyrate and certain drugs. One-day-old Ross 308 broilers and 5-week-old Wistar rats were fed by normal diet with or without sodium butyrate supplementation (1.5 g/kg diet), n=10/group. In addition, ten broilers and ten rats received phenobarbital (PB) injection on the last 3 days to serve as positive control. On day 21 all animals were slaughtered, the liver was flushed by chilled physiological saline solution through the portal vein and microsome fraction was isolated by a multi-step differential centrifugation. CYP2H (chicken) or CYP2B/3A (rat) activity was detected by aminopyrine N-demethylation assay, in which the amount of the produced formaldehyde was measured by the method of Nash. The activity of CYP3A (CYP3A37 in chicken) was tested by testosterone 6β-hydroxylation assay, where the amount of 6β-testosterone was measured by HPLC. Specific enzyme activities were calculated according to the total protein concentration of microsome samples.
No significant difference was observed between the aminopyrine N-demethylation activity of CYP enzymes in butyrate-fed and control broilers nor in rats. PB treatment caused notable enzyme induction with significantly increased specific activity values. Similarly, butyrate did not influence the hepatic microsomal CYP3A activity in both species, tested by the testosterone 6β-hydroxylation assay, while PB treatment resulted in a significantly higher enzyme activity. Dietary butyrate supplementation does not affect the microsomal CYP activity of the liver, so there might be no remarkable pharmacoepigenetic consequences of the butyrate addition. However, possible effects of dietary butyrate on other CYP subfamilies cannot be excluded.

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TACROLIMUS DOSE ADJUSTEMENT TO THE DRUG-METABOLIZING CAPACITY OF A HEART-TRANSPLANT PATIENT

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CYP3A enzymes, which account for almost 30 % of total hepatic P450s, are the main catalysts of many drugs, including immunosuppressive agents. Clinical relevant genetic polymorphism has not been described for the *CYP3A4* gene, whereas CYP3A5 expression is highly polymorphic. CYP3A5*3 mutation is a A>G change in the region of the 3rd intron of the *CYP3A5* gene, causing the absence of the total enzyme. The frequency of defective CYP3A5 allele is 90-93 % in Caucasians. Since CYP3A4 and CYP3A5 have largely overlapping substrate specificities, patients carrying functional CYP3A5 allele are expected to metabolize CYP3A substrates (e.g. immunosuppressive drugs) at higher rates, and they require a higher daily dose. Owing to this and to the narrow therapeutic range, continuous monitoring of immunosuppressive drug levels in blood is essential.An 8 year-old child underwent heart transplantation was orally treated with the daily dose of 8 mg tacrolimus, which was higher than the normal dose. In spite of this extremely high dose, the heart biopsy indicated mild rejection. The blood levels of tacrolimus were determined by LC-MS/MS in 14th, 26th January and 8th February 2011 and the values were 7.91, 6.22, and 6.33 ng/ml, respectively. These blood level values were much lower than the concentration (10-12 ng/ml) required for the therapeutic effect. The patient's *CYP3A5* genotype determined by PCR was heterozygous (CYP3A5*1/*3), resulting in the expression of active CYP3A5 enzyme which is quite rare in the white populations. It means that he can metabolize the CYP3A substrates (including tacrolimus) at higher rates. Since CYP3A5 can metabolize the tacrolimus more intensely than CYP3A4 enzyme, an increase in the dosage of tacrolimus was required (from 8 mg to 8.25 and later to 8.50 mg/day).The other part of the patient's immunosuppressive therapy was methylprednisolone (8 mg/day) according to the protocol. The dose was continuously reduced to 4 mg according to the protocol. It is known that steroids induce the expression of CYP3A4/5 enzymes and the reduction of the steroid dose can diminish the induction rate. Although the dose of tacrolimus was the same, the patient's blood concentration of tacrolimus increased to 13,1 ng/ml, which is considered to be the consequence of the reduction of the methylprednisolone dose.
This case study shows the importance of the determination of patients' drug-metabolizing capacity and to set the personalized immunosuppressive drug therapy.
This study was funded by the European Union, and co-funded by European Regional Development Fund (GOP-1.1.1-09/1-2009-0001).

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OPIORPHIN INCREASES THE SOECIFIC BINDING AND AFFINITY OF MERF AND MEGY

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Endogenously occurring opioid peptides are rapidly metabolized by different ectopeptidases. Human Opiorphin is a recently discovered natural inhibitor of the enkephalin-inactivating neutral endopeptidase (NEP) and the aminopeptidase-N (AP-N) . To date, *in vitro* receptor binding experiments must be performed either in the presence of a mixture of peptidase inhibitors and/or at low temperatures, to block peptidase activity. Here we demonstrate that, compared to classic inhibitor cocktails, opiorphin dramatically increases the binding of [^{3}H] MERF (Met-enkephalin-Arg-Phe) and [^{3}H]MEGY (Met-enkephalin-Gly-Tyr) ligands to rat brain membrane preparations. We found that at 0°C the increase in specific binding is as high as 40-60% and at 24°C this extend was even higher. In contrast, the binding of the control [^{3}H] endomorphin-1, which is relatively slowly degraded in rat brain membrane preparations, was not enhanced by opiorphin compared to other inhibitors. In addition, in homologous binding displacement experiments, the IC_{50} affinity values measured at 24°C were also significantly improved using opiorphin compared to the inhibitor cocktail. In heterologous binding experiments the differences were less obvious, but still pronounced using [^{3}H]MERF and MEGY compared to dynorphin$_{1\text{-}11,}$ or naloxone and DAGO as competitor ligands.

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DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING

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Individual cells exhibit a large degree of variability in their gene expression profile. Whole-cell patch-clamp recording enables detecting electrophysiological signals from neurons, and total RNA can be harvested into the patch pipette from the same cells. Subsequent nucleic acid amplification techniques can provide gene expression measurements on electrophysiologically characterized cells. However, the shortcoming of RNA profiling experiments based on sample amplification protocols on single cells, such as traditional QRT-PCR is the lack of exact quantitation, and experimental variations caused by the limited amount of nucleic acids. We describe a protocol for determining mRNA or miRNA expression in single neurons after patch-clamp recording at a single molecule level by using high-density nanocapillary digital PCR. Specific expression of *gabrd* in neurogliaform cells, and oxidative-stress induction of *hspb1* and *hmox1* expression in pyramidal cells was confirmed in individual neurons. The expression of *mir-132* was determined in three different cell types and found to be equally expressed. The accuracy and sensitivity of the method will enable broad application of single cell transcript analysis and make possible single neuron gene expression measurements in a complex inter-connective neuronal network in a physiological or pathophysiological context.

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RASGAPS IN THE LEARNING AND MEMORY OF *C. ELEGANS*

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RasGAPs, a subfamily of GTPase activating proteins, increase the intrinsic activity of Ras and therefore decrease the downstream signaling in the Ras/MAPK pathway. Their role have been identified mainly in tumorgenesis and development, but was not related to memory and learning so far.
Orthologues of *gap* genes can be found in many species including *C. elegans*, fruit fly, mouse and humans. The nematode, *C. elegans* is the only organism in which all three *gap* isoforms can be studied, and even their double mutants are viable. This worm is an excellent model system due to its quick lifespan, widely explored genome and the wide variety of available and proven experimental methods. Its simple nervous system of 302 neurons can produce behaviors which resemble even the complex human cognitive and psychiatric processes.
We created loss of function single mutants of *gap-1*, *gap-2*, *gap-3* genes and loss of function double mutants of *gap-1/-2*, *gap-1/-3* and *gap-2/-3* genes. The mutant strains were tested to chemotaxis towards volatile compounds and motility to check sensory and motor defects. Negative conditioning tests were also performed to study learning and associative short-term and long-term memory.
While the gap-1 gene plays a role in learning, *gap-2* and *gap-3* genes are required to memory formation. *Gap-1/-2* and *gap-2/-3* double mutants show both learning and memory defect. *Gap-1/-3* has a chemosensory defect.
Our results reveal that RasGAPs are involved in the memory and learning of *C. elegans*. Human orthologues of these genes are known, but their role in cognitive functions has not been studied yet. Our findings may give a novel clue about the evolutionary conserved molecular background of memory and learning.

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Nme6-LIKE GENE/PROTEIN FROM MARINE SPONGE *SUBERITES DOMUNCULA* - STRUCTURE, FUNCTION AND EVOLUTION

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Nucleoside diphosphate kinases (NDPKs) are evolutionary conserved enzymes involved in many biological processes such as metastasis, proliferation, development, differentiation, ciliary functions, vesicle transport and apoptosis in vertebrates. Biochemical mechanisms of these processes are still largely unknown. Sponges (Porifera) are simple metazoans without tissues and therefore are considered to be closest to the common ancestor of all animals. They changed little during evolution and probably provide the best insight into the metazoan ancestors' genomic features. The purpose of this study was to address structural and functional properties of Group II Nme6 gene/protein ortholog from the marine sponge *Suberites domuncula*, Nme6Sd, in order to elucidate its evolutionary history.
Nme6Sd gene and promoter were sequenced and analysed with various bioinformatical tools. Nme6Sd and Nme6SdΔ31 were produced in *E. coli* strain BL21 and NDPK activity was measured using a coupled pyruvate kinase-lactate dehydrogenase assay. Subcellular localization in human tumour cells was examined by confocal scanning microscopy.
Our results show that the sponge Nme6Sd compared to human Nme6 does not possess NDPK activity, does not localize in mitochondria at least in human cells although it has a mitochondrial signalling sequence, lacks two recent introns that comprise miRNAs, and have different transcriptional binding sites in the promoter region. Therefore, we conclude that the structure and function of Nme6 gene/protein changed during metazoan evolution and that complex actions of Nme6 in different biological processes probably correlate with increasing complexity of the organism.

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TAXONOMY AND DIVERSITY OF THE CALCAREOUS SPONGES IN THE ADRIATIC SEA

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The sponges (Porifera) retain the essential role in studying genome evolution of animals, with the aim to resolve the deep animal phylogeny. Ongoing research in the evolution of development, encouraged by advanced sequencing technology, has brought the sponges into the spotlight.
Calcarean phylogeny play an important role in understanding of the early metazoan evolution, since molecular data showed that calcareous sponges might be the link between lower and higher metazoan phyla.
The calcareous sponges are represented by about 500 exclusively marine species, distributed in all oceans. Class Calcarea Bowerbank, 1864 includes two subclasses: Calcinea and Calcaronea. According to extensive morphological and molecular data, both subclasses are monophyletic. Knowledge of recent Calcarea worldwide is considerably poorer than for other groups of Porifera, regarding their poor fossil record, difficult taxonomy and cryptic life style. The systematics and taxonomy are based on very few morphological characteristics which are often variable or plastic, hence difficult to interpret. There is no consensus as to the level of intraspecific morphological plasticity in calcareous sponges and many so far described species were lumped into a few supposedly very polymorphic species. Recent morphological and molecular analyses showed that highly variable and widely distributed sponge species were not as common as previously thought, as the genetic separation was linked to very small or sometimes undetectable differences in «classical» taxonomic characters. Together with detailed histology, molecular data seem essential to provide substantial evidence for phylogenetic hypotheses.
This study presents an effort to inventory the markedly underestimated calcarean sponge diversity in the Adriatic Sea, thus introducing the species new to science. We combined traditional morphological approach (spicule size, shape and distribution) and molecular (using partial 18S, 28S and ITS1-5.8S-ITS2 rDNA sequences) analysis using 9 individuals of class Calcinea and 7 individuals of class Calcaronea. Our results extended the currently available dataset of calcarean species, showing high biodiversity in the Adriatic Sea. Future studies will hopefully uncover more exceptional features in these remarkable animals. According to our results, the taxonomy of Calcarea is in desperate need of a thorough revision. More taxon sampling and detailed analysis both morphological and molecular is needed.

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CORRELATION BETWEEN POLYMORPHISM -13910C>T IN THE *LCT* GENE PROMOTER WITH DAILY LACTOSE INTAKE AND SYMPTOMS CHARACTERISTICS FOR LACTOSE INTOLERANCE AND RISK FOR INFLAMMATORY BOWEL DISEASE

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Lactose intolerance is an inherited autosomal recessive metabolic characteristic that affects approximately 75% of the adult world population. Individuals with lactose intolerance have a mutation -13910C> T in the LCT gene, which causes reduced activity of the enzyme lactase, which prevents the complete degradation of lactose to glucose and galactose in the small intestine and causes unpleasant symptoms. Research carried out on a Finnish population showed a 100% link between the mutant CC genotype and clinically proven lactose intolerance. In this study we determined the frequency of mutations in the promoter of the LCT gene in the Slovenian population. In the survey we evaluated 607 randomly chosen individuals in which we examined the frequency of individual lactase genotypes. Through the questionnaire, which included 42 questions, we checked the nutritional habits of randomly selected healthy individuals. The prevalence of the CC genotype was 36,6%, CT genotype 46,6% and the TT genotype 16,8%. The daily intake of lactose was among subjects with symptoms and CC genotype higher than in subjects with the CC genotype with no symptoms of lactose intolerance. This suggests dietary lactose avoidance in individuals with CC genotyped without symptoms of LI. We found a statistically significant association ($p = 0.038$, $OR = 0.393$) between CC genotype and individuals who consume large amounts of lactose per day and have frequent digestive problems, that are characteristic for lactose intolerance. We also examined the frequency of individual lactase genotypes in patients with inflammatory bowel disease and control groups. Among patients with ulcerative colitis the prevalence of CC genotype was 31,82%, the prevalence of CT genotype was 50% and of TT 15,91%. Among patients with Crohn's disease the prevalence of CC genotype was 37,08%, of CT genotype was 50,56% and the prevalence of TT genotype was 13,48%. Among patients with refractory Crohn's disease there are 27,1% of CC homozygotes, 50,47 of CT heterozygotes and 22,43% of TT homozygotes. The control group included 41,13% people with genotype CC, 44.91% people with genotype CT and 13.96% people with genotype TT.
Our study is one of the few studies that don't examine only the genotyping, but also the dietary habits of the subjects.

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ANTENNARY FUCOSYLATION OF PLASMA PROTEINS IS A RELIABLE SCREENING TOOL FOR HNF1A-MODY: TRANSLATION OF A GLYCOME-GWAS HIT INTO A CLINICALLY USEFUL DIAGNOSTIC MARKER

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Maturity-onset diabetes of the young (MODY) is a dominantly inherited form of non-insulin dependent diabetes caused by mutations in several genes. A subtype of MODY is caused by mutations in *HNF1A*, a nuclear transcription factor which appears to be one of the key regulators of metabolic genes. Recently we performed the first genome wide association analysis of the human plasma N-glycome and identified *HNF1A* as a master regulator of plasma protein fucosylation. Since even non-coding polymorphisms in the *HNF1A* gene have clearly detectable multiple effects on plasma protein fucosylation, we hypothesized that deleterious coding mutations in *HNF1A* should have even more profound effects and that antennary fucosylation of plasma proteins could be significantly decreased in HNF1A-MODY patients. *HNF1A*-MODY was found to be associated with a significant decrease in several HPLC peaks containing mainly antennary fucosylated glycans and the increase in peaks containing mainly glycans without antennary fucose. The proportion of HPLC peak DG9 in the sum of DG8 and DG9 roughly indicates the level of antennary fucosylation of triantennary glycans in plasma. Low values of this index appeared to be very indicative of HNF1A-MODY. HNF1A-MODY patients could be nearly completely separated from Type 1 diabetes, Type 2 diabetes, GCK-MODY and general population on the basis of the HAFU index with Receiver-Operator Characteristic (ROC) curves approaching 90% specificity at 90% sensitivity. Diabetic patients with DG9/(DG8+DG9) index indicative of HNF1A-MODY were sequenced and in two probands (with clinical labels of T1DM and T2DM respectively) HNF1A mutations consistent with a diagnosis of HNF1A-MODY were found.

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THE GENETIC BACKGROUND OF SALT SENSITIVITY-SENSISALT STUDY

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Excess salt intake is an important environmental risk for the predisposition to essential hypertension. However, blood pressure responses to high salt intake differ between individuals. Salt sensitive people react to salt load with an increase in blood pressure, while salt resistant people with modest or no increase. In clinical practice, salt sensitivity is not easily diagnosed with phenotypic studies although intervention in susceptible individuals by reducing salt intake could prevent development of hypertension, and the increased risk of cardiovascular events. Genetic profile plays a role in blood pressure responses to sodium but to date, only a few genetic variations have been associated with salt sensitivity.
Our study aims to estimate the prevalence of salt sensitivity in a Hungarian population, to identify genetic variants affecting its development and build a genetic model predicting salt sensitivity. 150 volunteers with untreated high–normal blood pressure will be recruited and their salt sensitivity assessed with the standard dietary intervention protocol: participants will consume a low-salt diet (3g NaCl /day) for seven days followed by a high-salt diet (18g NaCl/day) for seven additional days and blood pressure measurement will follow each dietary regimen.
Single nucleotide polymorphisms that were previously reported to be associated with hypertension and their interactions will be tested for associations with salt sensitivity. To discover single –nucleotide polymorphisms in genes that encode proteins involved in an increased sensitivity of blood pressure to salt intake could lead to new diagnostic tools for salt sensitivity.

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5-HIDROXYMETHYL-CITOSINE DETECTION BY MASS SPECTROSCOPY AND IMMUNOFLUORESCENCE IN DIFFERENT GENOMIC CONTEXTS

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5-hidroxy-methyl cytosine (5-hmC) is a recently discovered epigenetic modification, which appears to have an important role during mammalian development, especially within the nervous system. Produced by oxidation of 5-methyl-citosine (5-mC) by the TET family of enzymes, it has been suggested to participate in the process of DNA demethylation. DNA demethylation erases epigenetic marks from the DNA, allowing gene expression reprogramming and remodelling of the chromatin structure. Detection techniques for 5-hmC are quickly developing and there is an increasing body of evidence indicating its biological significance in different organisms at different stages of development.
In our lab, we have set up two different detection systems for 5hmC. On one side, the mass spectrometry (LC-MS/MS) has allowed us to determine total levels of 5-mC and 5-hmC in different cell types under various conditions, compared to adequate standards. This led us to determine the rapid turnover of DNA methylation in human cells. On the other side, immunofluorescent detection of both molecules on fixed cells shows their spatial arrangement on the intact nuclei and chromosomes. These two complementary approaches have provided us information on 5-hmC status of chicken DT-40 cells, human lymphoblast and HepG2 hepatoblastoma cells under various stress conditions.

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METAL-INDUCED ALTERATION IN THE EXPRESSION OF TWO HEME-OXYGENASE GENES

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Introduction: Heme-oxygenases (HOs) are rate-limiting enzymes in the heme catabolic pathway. HOs play role in the heme degradation, and also produce carbon monoxide, a vasoactive dilator agent with important free radical scavenger properties. Rapid upregulation of *ho* genes in response to heavy metal exposure is a protective mechanism preventing free radical accumulation.
Materials and methods: Expression of *ho* genes was assessed by reverse transcription coupled polymerase chain reactions (RT-PCR) in different tissues of untreated and metal (Cd^{2+} and As^{5+}) exposed common carp (*Cyprinus carpio)*. Cd^{2+} and As^{5+} were employed in two concentrations (1 or 10 mg/l). Cd^{2+} accumulation in the tissues was determined with atomic absorption spectrophotometry.
Results: cDNA library was generated using total RNA as a template from carp liver. cDNA clones carrying the coding region of HO-1 and HO-2 were screened for and sequenced. Gene-specific primers were designed and used to measure the *ho-1* and *ho-2* mRNA levels in different tissues (brain, liver, kidney, heart, skin, spleen, blood, gill and muscle). The basal level of the inducible *ho-1* transcript is on the edge of detectability in most of the examined tissues, except in skin, spleen and blood. The *ho-2* gene is constitutively expressed at a relatively high level in all the tissues examined. The highest level was detected in the skin, blood, spleen, heart and brain but it was less expressive in kidney and liver.
Alterations in the level of gene specific mRNAs were followed after exposure to Cd^{2+} and As^{5+}. In the kidney, the two metals had similar effect on the expression of both genes. On the contrary, in the liver only high dose of Cd^{2+} induced *ho-1* expression, while As^{5+} treatment resulted in a similar *ho-1* mRNA level at a low but not at a high concentration.
Conclusion: The expression of *ho* genes are gene- and tissue-specific under physiological condition. Heavy metal (Cd^{2+} and As^{5+}) exposure alters gene expression in a gene- tissue-, dose and metal-specific manner.
The financial support by the TÁMOP-4.2.2/B-10/1-2010-0012 fund is gratefully appreciated.

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DEUBIQUITYLATION AND APOPTOSIS

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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.
From a genome-wide search using bioinformatics techniques, we identified 40 Drosophila genes sharing high sequence homology with known human and yeast DUBs. Analyses of P element insertion mutants and/or transgenic RNA interference (RNAi) knockdown lines suggest that the function of 14 of them is essential in the development of Drosophila, while others show interesting cellular phenotypes. These results can stimulate further functional studies of potential DUB genes in this model organism.
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. Western blot analysis and polyubiquitin specific ELISA assay demonstrate the accumulation of polyubiquitins and polyubiquitylated proteins in DmUsp5 mutants. 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.

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POLYPHENOLS: MODULATING THE ANTIOXIDANT STATUS IN SUPPORT OF THE LIVER REGENERATION IN MICE

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Affecting antioxidant status is one mechanism of interfering with cell proliferation. Some plant constituents modulate redox-signaling pathways and thus may be of value in tissue regeneration. Hence, regulation of antioxidant state in response to olive oil polyphenols extract (PF) pretreatment during liver regeneration (LR) after partial hepatectomy (pH) in mice was exploited.

Changes in the liver antioxidant profile were evaluated in the group of mice: a) subjected only to pH, b) receiving PF prior to 1/3 pH, and c) receiving vehicle alone. Total hepatic glutathione (GSH) concentration, the activities of enzymes involved in GSH utilization (glutathione peroxidase; GP) and recycling (glutathione reductase; GR), along with catalase (KAT) activity were determined spectrophotometrically. Withal, liver mass restoration was calculated, and the quantitative real-time polymerase chain reaction was employed to examine hepatic mRNA levels of NF-*κ*B gene and its targets: antioxidant SOD2 gene, and *γ*-glutamylcystein synthetase (GCS) gene, which regulates GSH biosynthesis.

Mice subjected only to pH exerted induced GP activity during the first 3h of LR, followed by GR activity induction and increase in GSH content 12h after pH. In contrast, in periods up to 3h after pH, PF treatment provoked KAT activity induction and GSH depletion, pointing to the increase in oxidative stress. These changes were accompanied by the NF-*κ*B gene upregulation in all time intervals and concomitantly, by a higher and prolonged expression level of the SOD2 and GCS gene. Consequently, GSH levels increased in periods up to 12h, with reflection of these events in higher liver mass restoration compared to the untreated group.

PF related oxidative stress induction during the early course of regeneration process may function as a signaling pathway supporting liver growth. Such function is likely mediated through the NF-*κ*B induction, which enhances GSH biosynthetic capacity and confers antioxidant protection.

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COPD AS A SYSTEMIC DISEASE

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Chronic obstructive pulmonary disease (COPD) is characterized by a specific pattern of chronic inflammation in small airways and lung parenchyma, but also at the systemic level. The major factor of COPD is chronic oxidative stress (OS) resulting from long-term smoking which causes oxidative damage to a number of different molecules in cellular components including membrane lipids, proteins, carbohydrates and DNA.

Poly-(ADP-ribose) polymerase-1 (PARP-1) is highly activated by reactive oxygen species-induced DNA strand breaks. From its substrate NAD^+, PARP-1 forms poly(ADP-ribose) (PAR) polymers attached to protein acceptors. After release from the DNA degradation of the polymers by poly(ADP-ribose), glycohydrolase takes place within a few minutes. Massive DNA damage might deplete NAD^+ cellular stores, altering glycolysis and mitochondrial respiration, and leading to cell necrosis. Although significant systemic effects are known in COPD, the data on DNA damage, as well as on the activation and mRNA expression levels of PARP-1 have not been sufficiently addressed.

We evaluated the levels of mononuclear DNA damage in peripheral blood of healthy control subjects, non-obstructive smokers, patients with different severity of COPD as defined by Gold, and patients with COPD exacerbation using comet assay method (single cell gel electrophoresis). Also the activation and mRNA expression levels of PARP-1 were investigated using accordingly Biotinylated-NAD incorporation assay and TaqMan-qRT-PCR method. The seven groups had similar sex and age distribution.

Results of comet assays in this study showed strong correlation between DNA damage in peripheral blood mononuclear cells and the severity of the disease. PARP-1 activity increased according to the severity of the disease, which suggests an intensified DNA repair. Spearman's rank correlation coefficient was accordingly p=0,883 and p=0,802. Unlike the PARP-1 activity, no changes in PARP-1 mRNA expression level, according to the severity of the disease, were found. The fact that these changes were present in peripheral blood mononuclear cells firmly indicates that COPD is a major systemic disease.

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PROHEPCIDIN BINDS TO THE HAMP PROMOTER AND AUTOREGULATES ITS OWN EXPRESSION

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Hepcidin is the major regulatory peptide hormone of iron metabolism, encoded by the *HAMP* gene. Hepcidin is expressed mainly in hepatocytes and is secreted to the blood both in mature- and pro-hormone forms. The hormone acts by binding to the iron exporter ferroportin, triggering its internalization and intracellular degradation. Under physiological conditions the expression of the *HAMP* gene in the liver is modulated by numerous factors. The known positive regulators are the hereditary hemochromatosis protein (HFE), transferrin receptor 2 (TfR2), hemojuvelin (HJV) and bone morphogenetic proteins (BMPs). In addition, hepcidin expression can be regulated by factors independent of body iron levels, such as erythroid factors, hypoxia and inflammation. One of the identified negative regulators of liver hepcidin expression is matriptase-2 encoded by the TMPRSS6 gene. SMAD7 was described as another potent inhibitor of *HAMP* gene expression. Although, the function of mature hepcidin and the regulation of the *HAMP* gene have been extensively studied, the intracellular localization and the fate of prohepcidin remain controversial.
In this study, we propose a novel role for prohepcidin in the regulation of its own transcription. Using immunocytochemistry, a portion of prohepcidin was detected in the nucleus of hepatocytes and we assumed that prohepcidin may have a role in gene expression. We studied the prohepcidin-DNA interaction performing a conventional chromatin immunoprecipitation assay and we developed a PCR-based promoter-binding assay. Prohepcidin was found to specifically bind to the STAT3 site in the promoter of *HAMP.* Overexpression of prohepcidin in WRL68 cells decreased *HAMP* promoter activity, whereas decreasing the amount of prohepcidin caused increased promoter activity measured by luciferase reporter-gene assay. Moreover, overexpression of the known prohepcidin binding partner, alpha-1 antitrypsin caused increased *HAMP* promoter activity, suggesting that only the non-alpha-1 antitrypsin-bound prohepcidin affects the expression of its own gene. Our results indicate that prohepcidin can bind to and transcriptionally regulate the expression of *HAMP*, suggesting a novel autoregulatory pathway of hepcidin gene expression in hepatocytes.

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INVESTIGATION OF THE ROLE OF LXR RECEPTOR ON APOPTOTIC CELL UPTAKE OF MOUSE MACROPHAGES

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Effective clearance of apoptotic cells is essential for embryonic development and for maintaining immunological tolerance. Previously it was reported that engulfment of apoptotic cells triggers the LXR receptor in macrophages (MΦ) resulting in the induction of Mertk, retinoic acid receptor alpha (RARα) and tissue transglutaminase (TG2) leading to enhanced phagocytosis of apoptotic cells.
Following treatment with the synthetic LXR agonist GW3965, we detected enhanced apopto-phagocytotic capacity of mouse peritoneal MΦ. Investigation of gene expression revealed that besides Mertk, RARα and TG2 genes, the expression of the PS receptor TIM4 and Stabilin2, the opsonin C1qb, the transcription factor SREBP1c and two of its target genes, the retinoic acid (RA) producing retinal dehydrogenase 1 and 2 enzymes (RALDH1 and 2) were also upregulated in the GW3965 treated MΦs. Since increased RALDH enzyme expression suggests endogenous RA production in MΦs we performed HPLC analysis and detected increased RA production following LXR treatment. To test the role of RA in apoptotic cell uptake we treated MΦs with all-trans RA (ATRA) and measured their phagocytic capacity. According to our results ATRA treatment also increased the phagocytic ability of MΦs. Gene expression analysis following ATRA treatment revealed increased expression of the phagocytosis associated genes Mertk, TG2, TIM4, Stabilin2, C1qb, CD14 and thrombospondin. Ligation of RXR receptors with the synthetic RXR agonist also promoted apopto-phagocytosis in MΦs and resulted in increased Mertk, Stabilin2, TIM4 and TG2 expression. These results indicate that retinoic acids via RAR and RXR receptors can increase the apopto-phagocytic capacity of MΦs. To find out which of the LXR induced genes are dependent on retinoic acid signaling pathway we treated MΦs with RALDH inhibitor and GW3965 together. The LXR induced Mertk, C1qb, RALDH and RAR upregulation was partially reduced but the induction of TG2, TIM4 and Stabilin2 was completely abolished by co-incubation with DEAB.
Our results indicate that LXR activation in MΦs enhances expression of SREBP-1c, RARα and of RALDH enzymes. This leads to endogenous RA production which results in upregulation of retinoid regulated genes such as TG2, TIM4 and Stabilin2. Additionally activated RXR can heterodimerize with LXR receptor facilitating the transcription of Mertk and RARα contributing further to the enhanced phagocytic capacity of apoptotic cell engulfing MΦs.

PIII-146

EXPRESSION OF ABCB1 TRANSPORTERS IS REGULATED BY SEVERAL DIFFERENT MECHANISMS IN DRUG RESISTANT RAT HEPATOMA CELLS

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MDR1 (Abcb1) is an energy-dependent transporter that is able to extrude cytotoxic agents from the cell. In the presence of these drugs MDR1 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 MDR1 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 have two MDR1 isoforms: Abcb1a and Abcb1b. First, we determined the expression of these genes and found that the mRNA levels of both Abcb1a and Abcb1b were increased in the drug resistant cell lines compared to the parental D12.

Next we treated the cells with histone deacetylase inhibitors (HDACi) to maintain the acetylated state of histones. Surprisingly, Abcb1a and Abcb1b genes responded to the treatment in an opposite way: the expression of Abcb1a was decreased, while the expression of Abcb1b was increased in cells treated with HDACi. After the treatment, H3K9 and H3K14 acetylation increased in all tested regions of both genes, contrary that, their expression changed in opposite directions.

In conclusion, our data suggest that elevated Abcb1 gene expression is not always coupled to histone acetylation changes and conversely, the H3K9 and H3K14 acetylation levels do not necessarily predict the expression level of the Abcb1 genes.

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COMPLEMENTATION BETWEEN INACTIVE FRAGMENTS OF SssI DNA METHYLTRANSFERASE

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DNA (cytosine-5) methyltransferases (C5-MTase) transfer a methyl group from S-adenosyl-methionine to carbon 5 of cytosines in specific DNA sequences and typically function as monomers. We have shown that truncated inactive N-terminal fragments of the CG-specific prokaryotic C5-MTase M.SssI can assemble with truncated inactive C-terminal fragments to form active enzyme *in vivo* when produced in the same *E. coli* cell. Overlapping and non-overlapping fragments as well as fragments containing short appended foreign sequences had complementation capacity. In optimal combinations the beginning of C-terminal fragments is between Asn240 and Lys250, in the region located between conserved motif VIII and the predicted target recognizing domain of M.SssI. DNA methyltransferase activity in crude extracts of the clones with the best complementing fragment pairs was 2-3 per cent of the activity in cell extracts containing the full-length enzyme. Fusions of the fragments to 21.6 kDa zinc finger domains only slightly reduced complementation capacity between the fragment pairs. C5-methylation of cytosines in promoters of mammalian genes leads to gene silencing and is ,a key player in epigenetic gene regulation. Silencing mammalian genes by targeted DNA methylation of selected CpG sites in the genome would be a powerful technique to analyse epigenomic information and to study the roles of DNA methylation in health and in pathological states. The phenomenon of fragment complementation shown by M.SssI, which shares the sequence specificity of mammalian DNA methyltransferases (CG), offers a promising approach to use split M.SssI fragments fused to zinc finger proteins for silencing selected mammalian genes by targeted DNA methylation.

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ERYTHROPOIETIN EFFECT ON BREAST CANCER CELL PROLIFERATION AND PROTECTION FROM CISPLATIN INDUCED CYTOTOXICITY IS TIME-DEPENDENT AND MODULATED BY STEROID RECEPTOR AND P53 STATUS

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Background: Human recombinant erythropoietin that is widely used for the treatment of chemotherapy-induced animia has been reported to be questionable treatment choice for solid tumors due to increased adverse effects and reduced survival that is potentially related to rHuEpo-induced disease-progression. In this study, we assessed the effect of rHuEpo treatment on cell proliferation and its potential to synergize with cisplatin (cDDP) in suppression of breast cancer cell growth.
Materials and Methods: MCF-7 and MDA-MB-231 breast cancer cell were cultured with or without rHuEpo for 24 h and 9 weeks and analyzed for their viability and proliferation after exposure to cDDP. Expression of p53-dependent genes and *bcl2*-gene family members was also addressed together with activation of MAPK and PI-3K signaling pathways. Differences in cell response to cDDP and rHuEpo treatments were paralleled with estrogen (ER) and progesterone (PR) receptor expression and *p53* status, which differ in both breast cancer models used.
Results: Short-term exposure of breast cancer cells to rHuEpo reduces proliferation and protects cells from CDDP cytotoxicity. Proliferation and viability of MCF-7 cells, that are ER(+)/PR(+) and express wild-type *p53*, are opposing during short and long-term rHuEpo treatment. Prolonged exposure to rHuEpo increases MCF-7 proliferation and induces sensitivity to cDDP. On the other hand, MDA-MB-231 cells, with ER(+)/PR(-) status and mutated *p53*, are almost irresponsive to rHuEpo but show altered level of ERK phosphorylation, indicating involvement of MAPK signaling pathway. Gene expression analysis of *p53*-dependent genes and *bcl-2* gene family members confirmed differences between long and short-term rHuEpo effects, indicating the most prominent changes in *BCL2* and *BAD* expression.
Conclusions: MCF-7 cells proliferation and viability seem to be reversely influenced by the length of rHuEpo treatment, while MDA-MB-231 cells are almost imperceptible to the long-term rHuEpo. This could be explained in terms of ER/PR and p53 genetic signature. Speculatively, this signature may be used to predict the beneficial or maleficent effect of rHuEpo supportive therapy in the individual patient.

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Addenda et corrigenda

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THE EFFECT OF NM23-H1 EXPRESSION LEVEL ON MIGRATION AND ADHESION OF CAL 27 CELLS

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Nm23-H1 is the first described metastasis suppressor protein and it can inhibit formation of metastasis without affecting primary tumor growth. Decreased expression of Nm23-H1 is often observed in invasive tumors such as breast and ovary cancer, hepatocellular carcinoma, colon cancer and melanoma but not in neuroblastoma, osteosarcoma and blood cell malignancies. Furthermore, Nm23-H1 can reduce metastatic potential of cancer cells both *in vivo* and *in vitro.* Although being extensively studied the mechanism of metastasis suppression is still largely unknown. During metastasis the cancer cell acquires ability to migrate and invade surrounding tissue which includes changes in adhesion properties of cells. In this study we evaluated the migratory and adhesion properties of Cal 27 cells (squamous cell carcinoma of tongue) in relation to the level of Nm23-H1 expression. While moderate Nm23-H1 upregulation decreases migration, very high levels increase migration of Cal 27 cells. Cells expressing high levels of Nm23-H1 also displayed decreased adhesion on vitronectin. Moderate downregulation of Nm23-H1 increased ability of migration of Cal 27 and adhesion on laminin which is in agreement with previously reported Nm23-H1 mediated inhibition of motility and adhesion in other cancer cell lines. However, our results indicate that at least in some cancer cell types high expression of Nm23-H1 can in fact increase migration and at this point we can speculate that Nm23-H1 might have dual role in regulation of cancer cell migration depending on exact quantity of protein and cell context. That hypothesis is currently under further investigation in our laboratory.

FEBS 3+ Meeting
From molecules to life and back
Opatija, Croatia / 13-16 June 2012

PIII-150

MUTANT OF THE DROSOPHILA LONG ABCC PROTEIN, DMRP, GIVES INSIGHT TO 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. The exact mechanism how ATP hydrolysis is coupled to substrate transport is still an open question.
DMRP, the only "long ABCC-type protein" in *Drosophila*, has at least an order of a magnitude higher activity in *in-vitro* assays than its human homologues. Therefore we used DMRP as a model to gain further insight to the mechanism of ABCC transporters.
We characterized mutants of the functionally critical conservative Walker-A motives in the N- and C-terminal NBDs of DMRP. The function of the C-terminal NBD mutant was completely abolished, while the N-terminal mutant partially retained its activity. Thus we detected the same functional non-equivalence of the N- and C terminal NBDs for DMRP, as it was reported previously for human long ABCC transporters.
However, while analogous human ABCC mutants cannot be studied in details due to their low activity, the activity of the N-terminal NBD mutant DMRP was sufficient for detailed kinetic analysis in transport and ATPase activity measurements. While the substrate/inhibitor pattern was fully retained, transport kinetic parameters were strongly altered in the mutant DMRP.
We further investigated the effect of various substrates on the catalytic cycle of DMRP. We detected distinct modulation of the ATPase activity of the wt and the mutant protein for certain substrates. We hypothesize distinct intra-molecular communicatory pathways for the different substrates. We claim that the mutated conservative N-terminal amino acid is involved in the intra-molecular communication between substrate binding sites and ATP hydrolytic sites for some of the substrates, while it is not involved in the communicatory pathway for other substrates. Alternatively, different substrates modulate the catalytic cycle of the protein at different extents, resulting in strict or loose coupling of ATP hydrolysis to substrate transport.