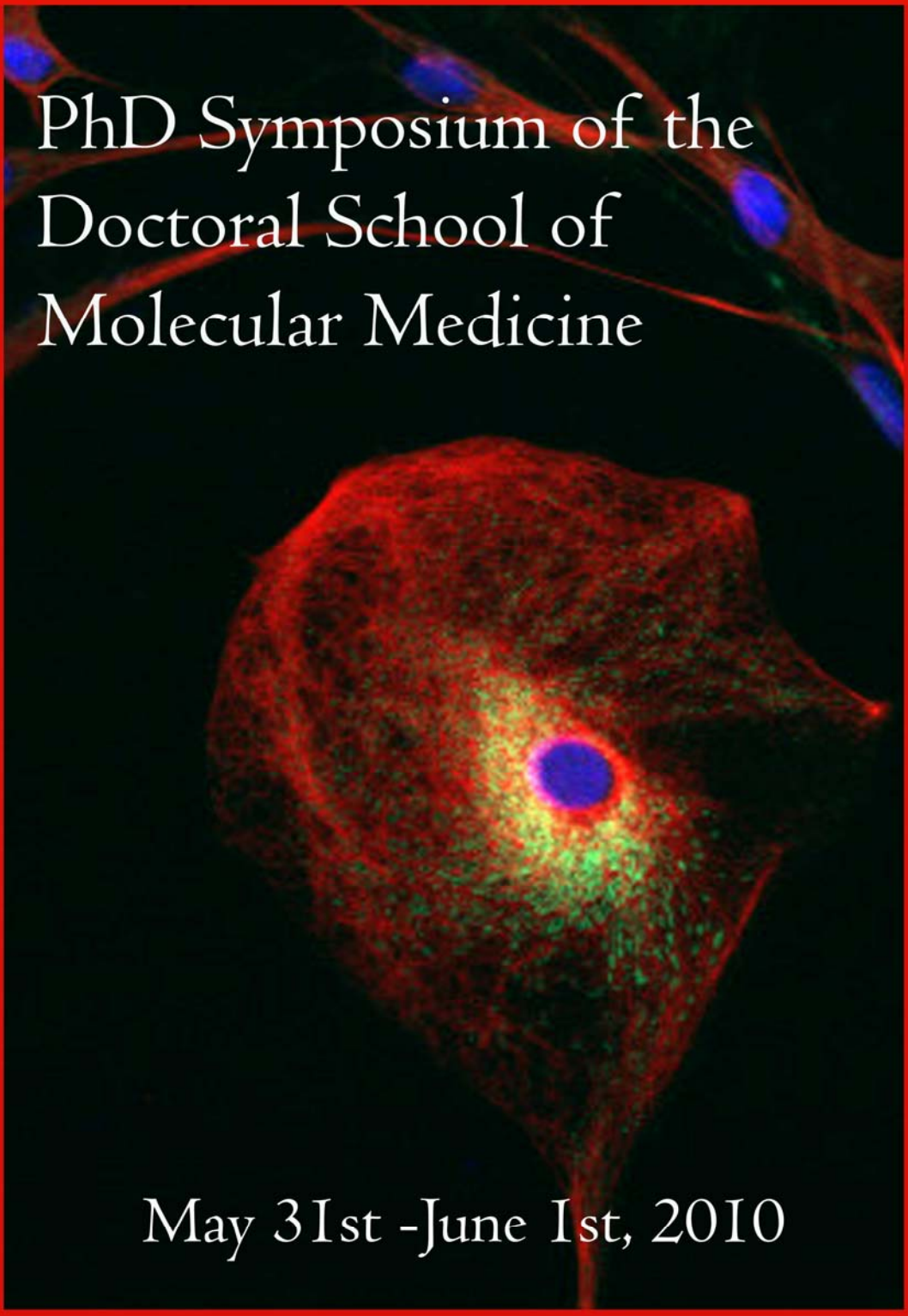
PhD Symposium of the Doctoral School of Molecular Medicine



May 31st -June 1st, 2010

PhD Symposium
of the
Doctoral School of Molecular Medicine
2010

Structure of the Doctoral School of Molecular Medicine

Head of the Doctoral School of Molecular Medicine

László Csernoch DSc, full professor

Head of the Department of Physiology, University of Debrecen, Medical and Health Science Center (UD, MHSC)

Secretary of the Doctoral School of Molecular Medicine

Andrea Dóczy-Bodnár PhD,

Department of Biophysics and Cell Biology, Cell Biology and Signaling Research Group of Hungarian Academy of Sciences, University of Debrecen, Medical and Health Science Center (UD, MHSC)

Doctoral programmes	Head of programmes	Staff of programmes	Supervisors and tutors in programmes
I. Cell and molecular biology of signal transduction	**László Virág**	Csilla Csortos, Viktor Dombrádi, Ferenc Erdődi, Pál Gergely	Péter Bai, Éva Bakó, Ilona Farkas, Endre Kókai, Beáta Lontay, Zsigmond Fehér, András Guttmann, András Penyige, Gábor Somogyi, Éva Szabó, Mária Szabó, György Vargha, György Vereb, Róza Zákány
II. Membrane biophysical questions and research methods	**János Szöllősi**	Sándor Damjanovich, Rezső Gáspár, Zoltán Krasznai, Teréz Márián, László Mátyus, Péter Nagy, György Panyi	László Balkay, László Bene, Andrea Dóczy-Bodnár, Miklós Emri, Zsolt Fazekas, Péter Hajdú, Lajos Trón, György Vámosi, Zoltán Varga, Zsuzsa Varga
III. Physiology and neurobiology	**László Csernoch**	Tamás Bányász, Tamás Bíró, László Galuska, László Kovács, János Magyar, Zoltán Rusznák, Géza Szűcs	Ervin Berényi, Gabriella Czifra, Julianna Cseri, István Jóna, Sándor Sárközi, András Szatmári, Norbert Szentandrássy, Péter Szentesi

PhD Symposium of the Doctoral School of Molecular Medicine

May 31st -June 1st,2010, Life Science Building F.015-016
Duration of lectures: 10 min + 5 min discussion.

May 31st, 2010

12:15	**Arrival** (coffe, cake)
12:45	**Welcome address –** László Csernoch, Head of the Doctoral School of Molecular Medicine
	Moderators **János Magyar** doctoral programme: Physiology and neurobiology **Viktor Dombrádi** doctoral programme: Cell and molecular biology of signal transduction **Péter Nagy** doctoral programme: Membrane biophysical questions and research methods
12:45-14:00	**Section I.** *Chairman* **László Csernoch** Head of the Doctoral School, Director of the Department of Physiology, Head of the doctoral programme Physiology and neurobiology
	Speakers
12:45-12:55	**Áron Kőszeghy 3rd year PhD student** supervisor: Zoltán Rusznák Ca^{2+} transients evoked by the activation of muscarinic cholinergic receptors expressed by the small neurones situated in thin slices prepared from the dorsal cochlear nucleus of the rat.
13:00-13:10	**Dénes Nagy 2nd year PhD student** supervisor: Zoltán Rusznák Cytoplasmic Ca^{2+} concentration changes evoked by muscarinic cholinergic stimulation in primary and metastatic melanoma cell lines.

13:15-13:25	**Eszter Kosaras 3rd year PhD student** supervisor: Zsuzsa Varga Effect of new flavonoid concentrate on oxidative stress markers and total antioxidant capacity in human plasma: results of 8-week supplementation.
13:30-13:40	**Petra Lakatos 1st year PhD student** supervisor: László Virág Role of poly(ADP-ribosyl)ation in the regulation of UV-induced cell death pathways in human epidermal keratinocytes.
13:45-13:55	**István Kovács 1st year PhD student** supervisor: László Virág Poly(ADP-ribosyl)ation in differentiating mesenchymal stem cells: implications for regenerative medicine.
14:00-14:15	**Coffee break**
14:15-15:30	**Section II.** *Chairman* **Ferenc Erdődi** doctoral programme: Cell and molecular biology of signal transduction *Speakers*
14:15-14:25	**Olga Ruzsnavszky 2nd year PhD student** supervisor: László Csernoch Examination of hormone receptors on appendix testis and other human tissue.
14:30-14:40	**Bálint Bécsi 2nd year PhD student** supervisor: Ferenc Erdődi Interaction of protein phosphatase and endothelial nitric oxide synthase inhibitors with lipid micelles from the membrane of different tissues.

14:45-14:55	**István Czikora Predoctor** supervisor: Csilla Csortos The role of TIMAP in the regulation of endothelial barrier function.
15:00-15:10	**Tamás Oláh 3rd year PhD student** supervisor: László Csernoch The alterations of store-operated calcium entry in TRPC1-overexpressing C2C12 myotubes.
15:15-15:25	**Anita Kása 3rd year PhD student** supervisor: Csilla Csortos The role of protein phosphatise 2A (PP2A) regulatory subunits in the regulation of pulmonary endothel cell (EC) cytoskeleton structure.
15:30-15:45	**Coffee break**
15:45-17:00	**Section III.** *Chairman* **János Szöllősi** Head of the Department of Biophysics and Cell Biology; Head of the doctoral programme Membrane biophysical questions and research methods. *Speakers*
15:45-15:55	**Julianna Volkó 2nd year PhD student** supervisor: György Vámosi / László Damjanovich Influence of MHC I gene silencing on interactions of membrane proteins and IL-2R signaling in human lymphocytes.
16:00-16:10	**Fabian Bartz 3rd year PhD student** supervisor: Heiko Runz Identification of Cholesterol-Regulating Genes by Targeted RNAi Screening.

16:15-16:25	**Vera Böhm 3rd year PhD student** supervisor: Jörg Langowski How does the nucleosome break apart? Direct evidence through single molecule fluorescence.
16:30-16:40	**Jan Krieger 2nd year PhD student** supervisor: Jörg Langowski Brownian Dynamics Simulations of FCS Measurements on Single Fluorophore-Labeled Superhelical DNA.
16:45-16:55	**Gábor Mocsár 3rd year PhD student** supervisor: György Vámosi Protein clusters in intact membranes and blebs of T lymphoma cells.

June 01, 2010

13:00	**Arrival** (coffe, cake)
	Moderators **Zoltán Rusznák** doctoral programme: Physiology and neurobiology **Zsigmond Fehér** doctoral programme: Cell and molecular biology of signal transduction **György Vámosi** doctoral programme: Membrane biophysical questions and research methods
13:30-15:00	**Section IV.** *Chairman* **László Kovács** doctoral programme Physiology and neurobiology

	Speakers
13:30-13:40	**Dóra Dedinszki 3rd year PhD student** supervisor: Ferenc Erdődi The role of phosphorylable inhibitor proteins of protein phosphatase-1 in the survival of malignant cells.
13:45-13:55	**Róbert Bátori 3rd year PhD student** supervisor: Ferenc Erdődi Investigation of the interaction between myosine phosphatase and endothelial nitric oxide synthase.
14:00-14:10	**Gábor Harmati 2nd year PhD student** supervisor: János Magyar Effects of β-adrenergic stimulation on delayed rectifier potassium currents in canine ventricular cardiomyocytes.
14:15-14:25	**Adrienn Sipos 1st year PhD student** supervisor: Beáta Lontay The localization of myosin phosphatase int he nucleus and its regulation by SMTNL1.
14:30-14:40	**Attila Szöllősi 3rd year PhD student** supervisor: Tamás Bíró Endocannabinoids regulate proliferation, differentiation, and survival of human sweat gland epithelial cells.
14:45-14:55	**Attila Oláh 2nd year PhD student** supervisor: Tamás Bíró Cannabidiol: A new, promising anti-acne agent.
15:00-15:15	**Coffee break**

15:15-16:30	**Section V.** *Chairman* **Pál Gergely** doctoral programme: Cell and molecular biology of signal transduction
15:15-15:25	*Speakers* **Tímea Váradi 2nd year PhD student** supervisor: Péter Nagy c-erbB receptors are indirectly affected by severe cell membrane modifications induced by Irvalec (PM02734) treatment.
15:30-15:40	**Zoltán Palicz 2nd year PhD student** supervisor: Péter Szentesi In vivo test of a highly stable antifungal protein (PAF) against lung aspergillosis.
15:45-15:55	**András László Kovács 2nd year PhD student** supervisor: András Guttman Generation and characterization of a comprehensive analyte library representing the human plasma proteome.
16:00-16:10	**Csilla Somogyi 1st year PhD student** supervisor: Róza Zákány The role of Protein Phosphatase 2A (PP2A) in HT168 and WM35 human melanoma cell lines.
16:15-16:25	**Bernadett Kolozsvári Predoctor** supervisor: Éva Bakó The role of calcineurin in the regulation of myosin phosphatase in endothelial cells.
16:30	**Conclusion**

Abstracts

Ca2+ transients evoked by the activation of muscarinic cholinergic receptors expressed by the small neurones situated in thin slices prepared from the dorsal cochlear nucleus of the rat

Áron Kőszeghy 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: Zoltán Rusznák

We have shown in a previous study that the cholinergic agonist carbachol (CCh) increased the spontaneous activity of the giant neurons of the dorsal cochlear nucleus (DCN). This effect was (at least partially) mediated via muscarinic receptors expressed by the giant neurons, but cholinergic modulation of the synaptic inputs reaching the giant cells was also evident. To investigate the exact mechanisms of cholinergic modulation in the DCN further, "multi-neuronal calcium imaging" has been employed in the present work using Oregon Green-AM loaded DCN slices.

At the beginning of the experiments, the action potential-coupled nature of the fast calcium-events (Ca2+ transients) was demonstrated, followed by the investigation of the effects of CCh on the Ca2+ transients. In these experiments all synaptic transmissions were inhibited using a neurotransmission blocking "cocktail". Since under these circumstances the usual synaptic pathways were not functional, it could be concluded that the Ca2+ transients seen in the presence of CCh were mediated via cholinergic receptors situated directly on the surface of the investigated cells themselves. It was noted that in the presence of CCh the frequency of Ca2+ transients significantly increased. The average diameter of the "carbachol-responding" cells was about 7 μm, suggesting that they most likely corresponded to the granule cells of the DCN. Since the observed effects of CCh could be effectively inhibited by atropine, it was concluded that the cholinergic effect was mediated via muscarinic receptors.

Our results point out a significant effect of cholinergic stimulation on the granule neurones of the DCN. Considering that the granule cells are synaptically connected to a number of projection neurones in the DCN, cholinergic modulation of them may have a major importance in determining the firing behaviour of the projection cells.

Cytoplasmic Ca2+ concentration changes evoked by muscarinic cholinergic stimulation in primary and metastatic melanoma cell lines

Dénes Nagy 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: Zoltán Rusznák

Experiments were carried out to explore differences existing between cultured primary and metastatic melanoma cell lines regarding their muscarinic acetyl-choline receptor (MR) related intracellular Ca2+ signalization. The expression of type 1 and type 3 specific MR (MR1, MR3) proteins was noted using both immunocytochemistry and Western blotting. The functionality of the MRs was tested by applying carbamyl-choline (CCh, a cholinergic agonist; 1 mM) and recording the resulted increases in cytoplasmic [Ca2+] using a Ca2+ imaging technique (fluo-4). Our experimental results indicated that the expression levels of the receptor proteins did not show significant differences in the cell lines employed. CCh was capable of evoking Ca2+ transients in all cell lines, but the proportion of the CCh-responding cells was smaller in the WM35 cell line. The Ca2+ transients had heterogenous time course and they could be effectively blocked by atropine (0.1 mM). If present, the late (plateau-like) component of the transients depended on the presence of extracellular Ca2+; when the extracellular Ca2+ concentration was reduced, the transients became considerably shorter. This shortening of the transients was more pronounced in the metastatic cell lines. Our results indicate that there are no fundamental differences in the MR-related Ca2+ signalization of the primary and metastatic melanoma cell lines. However, some quantitative differences could be noted that may eventually contribute to the increased malignancy and migratory potential of the metastatic cell lines.

Effect of new flavonoid concentrate on oxidative stress markers and total antioxidant capacity in human plasma: results of 8-week supplementation.

Eszter Kosaras 3rd year PhD student
University of Debrecen, Medical and Health Science Center, First Department of Medicine
Supervisor: Zsuzsa Varga

Previously, we have demonstrated that structural requirements are different in the case of antioxidants (such as flavonolignans, flavonoids and tocopherols) for inhibition of enzyme activities responsible for initiation of oxidative stress (PKCα and NADPH oxidase in human neutrophils, or xanthine oxidase), scavenge free radicals, and reduction of transition metals, in vitro; therefore, antioxidant efficacy of a given compound depends on source of ROS. Human supplementation studies with a single antioxidant (e.g. Vitamin E or C, beta-carotene) gave contradictory results suggesting that mixture of antioxidants should be effective.

We studied effect of a new antioxidant mixture (Kombuflavonoid) on plasma antioxidant capacity and oxidative stress in 20 healthy volunteers after 8-week supplementation (10 ml/day). Blood was taken before and after eight weeks supplementation.

Total phenol and flavonoid content in plasma increased and was concomitant with significant enhancement in antioxidant activity of plasma characterized by ORAC assay (91.2±11.7 vs. 77.10±9.3 μmolTE/L, $p<0.0001$). Electron donor activity (FRAP: 3.58±1.08 vs. 3.37±1.01 μgTE/ml, $p<0.05$), and free radical stabilization capacity of plasma also increased (DPPH: 0.647±0.63 vs. 0.450±0.132 μgTE/ml, $p<0.0001$). Significant decrease in oxidative stress parameters were observed (TBARS: 0.132±0.046 vs. 0.417±0.103 nmol/L, $p<0.0001$, AOPP: 98.7±45.6 vs. 119.9±56.5 nmol/L CT equivalent, $p<0.001$). Closed relationship between total phenol and flavonoid content of plasma and oxidative stress parameters (TBARS $r=-0.262$, $p<0.02$; AOPP $r=-0.200$, $p<0.04$) and antioxidant capacity (ORAC $r=0.457$, $p<0.001$; FRAP $r=0.311$, $p<0.01$) confirmed that changes were due to increased flavonoid consumption.

In conclusion, short-term administration of Kombuflavonoid proved to be efficient to decrease oxidative stress and increase antioxidant capacity of body.

Role of poly(ADP-ribosyl)ation in the regulation of UV-induced cell death pathways in human epidermal keratinocytes

Petra Lakatos 1st year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: László Virág

Poly(ADP-ribose) polymerases (PAPRs) use nicotineamide adenine dinucleotide (NAD) as a substrate to synthesize a branched biopolymer poly(ADP-ribose) (PAR) covalently attached to proteins. The degradation of PAR is catalyzed by PAR glycohydrolase (PARG).

Human skin, unlike all other organs, is continuously and directly exposed to environmental influences. Solar UV radiation is divided into three regions depending on wavelength, short-wave UV-C (200-280 nm), mid-wave UVB (280-320 nm), and long-wave UVA (320-400 nm). UVA radiation is responsible for various skin disorders including photoaging and skin cancer. UV radiation, in particular UVB is known to alter cellular function via DNA damage, generation of reactive oxygen species (ROS), and the consecutive alterations in a large variety of signaling events.

The aim of our project is to investigate the role of PARylation in UV-induced dysfunction and death of normal human epidermal keratinocytes (NHEK).

First we determined cell viability of NHEK following irradiation with different doses of UVA and UVB in order to determine the effective doses of UV in our model. We found that both UVA and UVB caused a dose-dependent decrease in cell viability as determined with the colorimetic MTT assay. UVB also induced PARP activition as determined with the PARP activity assay based on the incorporation of isotope from 3H-NAD+ into TCA precipitable proteins. (Testing the effect of UVA on PARP activation is ongoing.) PARP activation is also proved by the detection of the enzyme's product PAR with Western blotting. UV induced morphological alterations (apoptotic morphology) was visualized with May-Grünwald-Giemsa staining. Nitric oxide production was determined with the chemiluminescent NO analyzer. The PARP inhibitor 3-aminobenzamide (3-AB) but not the more potent and specific inhibitor PJ34 provided protection from UV-induced cytotoxicity indicating that the effect of 3-AB might not be related to its PARP inhibitory effect. Neither of the inhibitors affected production of NO.

We plan to characterize in detail the regulatory effects of PARylation in UV-induced keratinocyte dysfunction and cell death.

Poly(ADP-ribosyl)ation in differentiating mesenchymal stem cells: implications for regenerative medicine

István Kovács 1st year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: László Virág

Regenerative medicine focuses on the restoration of lost or damaged tissues and organs. Mesenchymal stem cells (MSC, also called mesenchymal stromal cells) are one of the cell types being intensively studied as a source to improve tissue repair.

Although more than a hundred clinical trials are running with MSCs in the world, our knowledge on the MSC differentiation signaling mechanisms is poor.

The role of poly(ADP-ribosyl)ation, a posttranslational protein modification, catalyzed by poly(ADP-ribose) polymerases (PARPs) in MSC differentiation pathways is unknown. We propose that poly(ADP-ribosyl)ation may be required for regulating differentiation pathways mainly at the transcriptional level but possibly also interfering with upstream signaling events.

In order to support our hypothesis we set up isolation protocols to obtain MSCs from placenta and umbilical cord, evaluated the surface molecules expression patterns of MSC by flow cytometry and differentiated the cells into osteo-, chondro- and adipogenic lineages. We examined the effect of the specific PARP inhibitor PJ34 on the MSC differentiation. To follow the differentiation events we used different qualitative and quantitative methods, like Alizarin Red staining, alkaline phosphatase activity measurement, calcium concentration measurement, real time quantitative PCR to assess osteogenesis, Nile Red staining, Oil Red O staining, enzymatic triglyceride measurement, real time quantitative PCR – to characterize adipogenesis and dimethyl methylene blue staining – to monitor chondrogenesis.

Our preliminary results indicate that poly(ADP-ribosyl)ation regulates the adipogenic, chondrogenic and osteogenic differentiation process in MSC. We plan to investigate the molecular mechanisms underlying the differentiation-modulating effects of poly(ADP-ribosyl)ation.

Examination of hormone receptors on appendix testis and other human tissues

Olga Ruzsnavszky 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: László Csernoch

In our work we examined the mRNA expression of androgen receptor, estrogen receptor type 1 and 2 and anti-Müllerian hormone receptor on appendix testis and other urological samples, like appendix epididimidis, bag of inguinal hernia and musculus cremaster.

The RT PCR technic was used to detect the mRNA level of the above listed hormon receptors. Our goal was to find the best protocol to izolate RNA from the samples.

First we izolated the RNA with the RNeasy Mini Kit (Qiagen). For this we dissected the samles with scissors but the effectivity of this technic was very low. After we tried the TRIzol reagent to izolate the RNA. In the early experiments we make powder from the samples with liquid nitrogene in a mortar. This technic was better than the first one. After we tried to separate the RNA without dissecting the samples. We used again the TRIzol reagent and we sonicated the samples for 3x10 seconds in it. Combination of these technics (dissecting the samples with scissors and sonicating) give the best results. Before the experiments we didn't know the connection between the disease and the sample (the examinations were blind).

We tried to find any correlation with hormone receptors and diseases. We found that patients with retentio testis the mRNA of androgen receptor was missing from lot of samples. In case of descended testis, the mRNA of androgen receptor was found almost in every cases. Examining the other hormone receptors, we couldn't find any correlation between the mRNA levels and the diseases yet.

In these experiments we had information only from the mRNA expression. In the future we would like to check the protein expression of these receptors with Western –blot and immunohistochemistry.

Interaction of protein phosphatase and endothelial nitric oxide synthase inhibitors with lipid micelles from the membrane of different tissues

Bálint Bécsi 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Ferenc Erdődi

The membrane permeable inhibitors, have to diffuse through the membrane to develop their intracellular influence. In this permeation process the interaction of the inhibitors with the membrane lipids could be a primary determinant process. We used Biacore 3000 instrument with surface plasmon resonance (SPR) based detection to determine the association-dissociation characteristics of the interaction between the inhibitors and lipid micelles. Micelles were prepared from lyophilized total lipid extract of brain, heart or liver. Liposomes (1 mg/ml) were captured on L1 Sensor Chip in isolated flow cells at approximately the same immobilization level (~7500 RU). After immobilization the reference (lipid free) and the three lipid surfaces were blocked with 0.1 mg/ml BSA.

We analysed the binding of protein phosphatase inhibitors, okadaic acid (OA), microcystin-LR (MC), cyclosporin-A (CsA) and epigallocatechin-3-gallate (EGCG), as well as the endothelial nitric-oxide synthase inhibitor nitro-L-arginine methyl-ester (L-NAME) to the lipid surfaces. OA and CsA bound simiarly to the three lipid surfaces with respects to both association and dissociation characteristics. In contrast, MC and EGCG bound with higher affinity to the liver lipids compared to the brain or heart counterparts. Plotting the observed resonance unit maximums against the concentration of the inhibitors indicated that the association of EGCG to the lipid surfaces was highly concentration dependent (1-75 μM), which was less characteristic for MC (1-5 μM). The attachment of L-NAME to the lipid surfaces was characterized by similar sensorgrams, and the calculated equilibrium constants for the dissociation are also close (brain: KD=4,60±2,77×10-5; heart: KD=4,71±2,54×10-5; liver: KD=3,26±1,72×10-5).

In conclusion, our results imply that the memrane association-dissociation of enzyme inhibitors may be modelled by SPR studies using lipid micelles to capture the molecules. This method may also be used to assess the membrane permeation and „lipid solubility" properties of pharmacological drugs.

The role of TIMAP in the regulation of endothelial barrier function

István Czikora Predoctor
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Csilla Csortos

Multiple signal-transduction pathways, regulating EC contraction and barrier function, involve the activity of several protein kinases and protein phosphatases on junctional and cytoskeletal/cytoskeleton-associated proteins. TGF-β-inhibited membrane-associated protein, TIMAP is regarded as a member of the MYPT (myosin phosphatase target subunit) family of protein phosphatase 1 (PP1) regulatory subunits, however, its function in endothelial cells (EC) is not understood yet. Compared to other cell lines the level of expressed TIMAP protein in endothelial cells is high. We have shown earlier that TIMAP has a barrier-protective effect against thrombin-induced EC permeability. The interaction of TIMAP and PP1c was shown by immunoprecipitation and by surface plasmon resonance using recombinant proteins. Immunofluorescent staining of TIMAP in HPAEC shows its localization in the plasma membrane and in the nucleus. Ezrin/radixin/moesin proteins (ERM) are also abundant in the plasma membrane. TIMAP co-immunoprecipitates with moesin, therefore we assumed that TIMAP is involved in moesin dephosphorylation, and that participation of this dephosphorylation in TIMAP-mediated EC barrier protection. When the threonin 567/564/558 residue of the ERM proteins is directly phosphorylated by PKC-θ or Rho kinase, it reveals intramolecular and/or intermolecular head-to-tail association of ERM proteins, which is an important mechanism of regulation of their activity as actin filament/plasma membrane crosslinkers. We performed in vitro phosphatase assays using PP1c catalytic subunit and phosphorylated wild type form of the recombinant moesin as substrate. Addition of Timap has decreased the activity of PP1c, however the phosphorylated form of TIMAP (S333/S337) had no effect on the activity. We speculate that TIMAP might play role in the regulation of PP1c at the plasma membrane, it regulates the dephosphorylation of ERM proteins and beyond that the ERM proteins might be possible targets of TIMAP-mediated barrier function enhancement.

The alterations of store-operated calcium entry in TRPC1-overexpressing C2C12 myotubes

Tamás Oláh 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: László Csernoch

After depletion of the endoplasmic reticulum (ER) calcium store, a Ca2+ influx is activated from the extracellular space to refill the intracellular stores. This well-regulated Ca2+ uptake mechanism, called store-operated Ca2+ entry (SOCE), depends on the cooperation of several proteins as STIM1, Orai1 and TRPC1. The role of STIM1 as the calcium sensor of the ER and Orai1 as the Ca2+ influx channel is well-known from the recent publications, but the function of TRPC1 as a store-operated channel remains elusive.

Here we overexpressed TRPC1 by liposome-mediated transfection in C2C12 mouse skeletal muscle cell line. Overexpression was confirmed at mRNA level by RT-PCR and at protein level by immunostaining and Western-blot. The SOCE mechanism was studied by measuring the changes in intracellular calcium concentration ([Ca2+]i) evoked by the re-addition of 1.8 mM [Ca2+]e following the SERCA-inhibition by thapsigargin. As a result of TRPC1 overexpression, the amplitude and the maximum of the derivative of SOCE was significantly increased. When YM-58483, the antagonist of TRPC1 was used, these differences were eliminated, moreover in TRPC1-overexpressing myotubes the SOCE was slightly but not significantly lower, suggesting the downregulation of the STIM1-Orai1 system. This decrease in the expression level of STIM1 was confirmed by Western-blot together with the downregulation of SERCA. As a consequence a reduction in maximal Ca2+ uptake, and a higher resting [Ca2+]i following the transients evoked by 120 mM KCl were detected. Morphological changes caused by the overexpression of TRPC1 were also observed. The differentiation of the myoblasts started later, and the myotubes were thinner in TRPC1-overexpressing cultures. For these changes the decreased nuclear expression of NFAT1 as a consequence of TRPC1 overexpression could be responsible.

Our results suggest that enhancing the expression level of TRPC1 increases SOCE and has a negative feedback effect on the STIM1-Orai1 system, suggesting a cooperation between these proteins.

The role of protein phosphatise 2A (PP2A) regulatory subunits in the regulation of pulmonary endothel cell (EC) cytoskeleton structure

Anita Kása 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Csilla Csortos

Protein phosphorylation and dephosphorylation is essential in the EC barrier function. Tight and adherent junctions just as the cytoskeletal structure are crucial in EC connections. In cell junctions the phosphorylation of interacting proteins and the performing kinases are known. For example, in adherent junctions (AJ) the Ser/Thr phosphorylation of cadherin is necessary to bind β-catenin. However, the role of phosphatases and their regulation is still unknown.

Previously we demonstrated that the PP2A enzyme activity plays an important role in the regulation of the barrier function and the rearrangement of cytoskeletal proteins in EC. The core dimer enzyme consists of a 36 kDa catalytic subunit (PP2Ac) and a structural subunit of molecular mass 65 kDa (PP2Aa). The third, regulatory B subunit (PP2Ab) can be associated with this core structure. Four different families of B subunits have been identified, termed as B, B', B", B'". These different B subunits determine the subcellular localisation and the substrate specificity of the holoenzyme.

In our present work to investigate the role of B subunits both in the organisation of the cytoskeleton and in the regulation of proteins in cell junctions we employed different methods.

Bovine pulmonary artery EC (BPAEC) were transfected with PP2Ab and PP2Ab' constructs. The overexpressed proteins localise in the nucleus, however, this overexpression significantly changes the morphology of the cells, suggesting the critical role of these proteins in the cytoskeletal structure. Immunostaining shows the endogenous PP2Ab and PP2Ab' subunits localize in the cytoplasm, or in the cytoplasm and nucleus, respectively. After thrombin treatment of BPAEC some of the B subunit translocates into the plasmamembrane, while the B' subunit enriches in the nucleus, indicating the role of B subunit in the maintenance of the barrier function. To elucidate the possible connection between β-catenin and the B subunit, Bα depleted cells were studied. The level of β-catenin decreased in the plasmamembrane, thrombin treatment further reduced this level, confirming that the B subunit can be involved in the dephosphorylation of AJ proteins.

Influence of MHC I gene silencing on interactions of membrane proteins and IL-2R signaling in human lymphocytes

Julianna Volkó 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Biophysics and Cell Biology
Supervisor: György Vámosi / László Damjanovich

Major histocompatibility class I and II glycoproteins and interleukin-2 and -15 receptors, which play important roles in immune processes, form supramolecular clusters in lipid rafts of FT7.10 T lymphoma cells. Pair-wise molecular proximities can be mapped by fluorescence resonance energy transfer (FRET), and the mobility of molecules can be characterized by fluorescence correlation spectroscopy (FCS). FRET and electron microscopic data indicate that MHC class I and II build small- and large-scale homo- and hetero-aggregates in the membrane of T lymphoid cells.

We were interested whether protein-protein interactions within the clusters depend on the abundance of their most highly expressed member, MHC I. Therefore we silenced the expression of MHC I by RNA interference. 48 h after transfection the expression of MHC I was reduced by ~90%. FRET data indicated that the homoassociation of MHC I as well as its heteroassociation with IL-2Rα and IL-15Rα was reduced. In addition, the homoassociation of IL-2Rα and the heteroassociation of IL-2Rα with IL-15Rα also weakened. FCS data showed that the mobility of both MHC glycoproteins and interleukin receptor subunits increased upon gene silencing, implying a decrease in cluster size. We studied the effect of MHC I gene silencing on IL-2R signaling.

Our data suggest that MHC I plays an organizing role in maintaining supramolecular receptor clusters, revealing an additional function of MHC I besides its classical role in antigen presentation.

Identification of Cholesterol-Regulating Genes by Targeted RNAi Screening

Fabian Bartz 3rd year PhD student (guest researcher)
Institute of Human Genetics; Molecular Medicine Partnership Unit (MMPU), EMBL
Supervisor: Rainer Pepperkok

Elevated plasma cholesterol levels are considered responsible for excess cardiovascular morbidity and mortality. Cholesterol in plasma is tightly controlled by cholesterol within cells. Here, we developed and applied an integrative functional genomics strategy that allows systematic identification of regulators of cellular cholesterol levels. Candidate genes were identified by genome-wide gene-expression profiling of sterol-depleted cells and systematic literature queries. The role of these genes in cholesterol regulation was then tested by targeted siRNA knockdown experiments quantifying cellular cholesterol levels and the efficiency of low-density lipoprotein (LDL) uptake. With this strategy, 20 genes were identified as functional regulators of cellular cholesterol homeostasis. Of these, we describe TMEM97 as SREBP target gene that under sterol-depleted conditions localizes to endo-/lysosomal compartments and binds to LDL cholesterol transport-regulating protein Niemann-Pick C1 (NPC1). Taken together, TMEM97 and other factors described here are promising to yield further insights into how cells control cholesterol levels.

Participation of guest researchers was also supported by MOLMEDREX EU FP7 project.

How does the nucleosome break apart? Direct evidence through single molecule fluorescence.

Vera Böhm 3rd year PhD student (guest researcher)
German Cancer Research Center (DKFZ), Division: Biophysics of Macromolecules
Supervisor: Jörg Langowski

Access to DNA buried within nucleosomes is regulated by their disassembly and assembly processes. The mechanisms by which the the cellular machinery accomplish this is not yet known. Therefore, using single molecule fluorescence techniques, we have been able to show the mechanism of nucleosome disassembly in vitro through salt-induced disassemboly. Our data suggests the first step in the nucleosome disassembly is opening of the tetramer/dimer interface, followed by dimer release, and lastly tetramer removal. The existence of an initial open state could have significant in vivo consequences such as facilitated dimer dissociation and dimer exchange, as well as increased DNA accessibility. Additionally, we have been able to show nucleosome assembly by in vitro reconstitution follows the reverse pathway of that for salt-induced disassembly.

Participation of guest researchers was also supported by MOLMEDREX EU FP7 project.

Brownian Dynamics Simulations of FCS Measurements on Single Fluorophore-Labeled Superhelical DNA

Jan Krieger 2nd year PhD student (guest researcher)
German Cancer Research Center (DKFZ), Division: Biophysics of Macromolecules
Supervisor: Jörg Langowski

We investigated the dynamics of a single-fluorophore-labeled pUC18 plasmid through a Brownian dynamics algorithm, followed up by a simulation of the Fluorescence Correlation Spectroscopy (FCS) process. Recent experimental FCS measurements indicated a sensitivity of the monomer mean square displacements in DNA circles towards superhelicity. Simulations with homogeneous DNA elasticity and local straight equilibrium are not sufficient to reproduce this observed behavior. But inserting permanently bent sequences into the DNA, which favor end loop formation, caused a dependence of the calculated FCS correlation curves on superhelical density. Furthermore, our simulations allow us to take into account the orientation of the fluorophore in polarized excitation, which might explain the observed appearance of a Rouse-like regime at intermediate time scales.

Participation of guest researchers was also supported by MOLMEDREX EU FP7 project.

Protein clusters in intact membranes and blebs of T lymphoma cells

Gábor Mocsár 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Biophysics and Cell Biology
Supervisor: György Vámosi

Major histocompatibility class I and II (MHC I and II) glycoproteins and interleukin-2 and -15 receptors (IL-2R, IL-15R), which play important roles in immune processes, form supramolecular clusters organized at various hierarchical levels. Beyond the small-scale molecular associates revealed by FRET, they also form larger aggregates (from a few 10 to a few 100 nm-s), which are enriched in specific membrane microdomains (lipid rafts). We were interested whether the higher order organization of the cell membrane and the cytoskeleton were necessary for maintaining protein-protein interactions at the nm-scale. Large blebs formed during apoptosis/necrosis do not possess cytoskeletal connections, and there is no sign of any higher order (microdomain) organization at the resolution of confocal microscopy. Such blebs can thus be considered as almost planar bilayers having a relatively homogeneous distribution.

Molecular interactions: the homo- and heteroassociation of MHC I, MHC II, IL-2Rα and IL-15Rα as well as the heteroassociation of MHC I with IL-2Rα and IL-15Rα were determined by an intensity based confocal microscopic FRET method. We used fluorescence correlation spectroscopy to assess the size of MHC clusters. Whereas MHC molecules, IL-2R and IL-15R formed largely overlapping patches in the intact membrane of FT7.10 T lymphoma cells, they were evenly distributed in blebs. The characteristic FRET efficiencies measured between the appropriate epitopes were equal within experimental error when measured in intact membranes or in apoptotic blebs, i.e. small scale molecular clusters remained stable.

Our experiments suggest that the interactions stabilizing the studied small-scale molecular clusters persist even after the disappearance of the higher order organization of the T cell plasma membrane, and that these interactions do not depend critically on the cytoskeleton.

Previously our aim was to develop our microscope for measuring single pair FRET.

In the second part of the presentation, the instrumentation and the first results of the Alternate Laser Excitation FRET measurements will be presented.

The role of phosphorylable inhibitor proteins of protein phosphatase-1 in the survival of malignant cells

Dóra Dedinszki 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Ferenc Erdődi

Apoptosis, caused by chemotherapy agents, is influenced by the phosphorylated stages of many proteins. Caliculyn-A, which is a protein phosphatase-1 (PP1) and-2A (PP2A) inhibitor toxin, reduces the effect of Daunorubicin (DNR) as helps to induce the phosphorylation of anti-apoptotic proteins (Erk-1/2, Akt, pRb). In cells, suffering from cancer the phosphorylation level of pRB is originally higher. Maybe it can cause the increased proliferation of these cells. Earlier we showed out that PP1 is able to dephosphorilate the phosphorylated pRb. Because of it PP1 inhibition has an important role in the regulation of the phosphorylation level of pRb and the cell cycle in cells suffering from leukemia. The high phosphorylated level in relaxed cells can indicate that the PP1 is inhibited in the cells and different inhibitor proteins can have role in it. Because of it we examined the presence and the phosphorilation level of a phosphorylation-dependent (17 kDa) inhibitor protein (CPI-17) in THP-1 and MCF-7 cells. Our results show that in THP-1 cells there are many proteins that have the CPI-17 phosphorylation sequence (ARV(phosphoT)VKYDRREL). Out of the CPI-17 like proteins (KEPI, PHI-1) we found other proteins (LIMK, serologically defined breast-cancer antigen) with similar phosphorylation sequence. We transfected FLAG-KEPI plasmid into HEK, MCF-7 and THP-1 cells and expressed the protein in HEK cells. The transfection efficiency was confirmed by immunohistochemistry using anti-FLAG antibody. Later we would like to examine the probable KEPI binding proteins with Biacore 3000 Instrument. We showed out that CLA pre-treatment can cause an increased surviving in THP-1 cells because it increases the phosphorilation level of anti-apoptotic proteins. We also examined if CLA has this „protector" effect on DNR treatment in transfected MCF-7 cells. We found that CLA increased the pRb phosphorilation although we could not confirm yet with MTT assay that this increased phosphorilation helps in cell-surviving. In the future we would like to transfect FLAG-LIMK plasmid to these cells and examine if phospho-LIMK has the same effect on pRb phosphorilation and cell surviving.

Investigation of the interaction between myosine phosphatase and endothelial nitric oxide synthase

Róbert Bátori 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Ferenc Erdődi

Myosine phosphatase (MP) holoenzyme consists of a 38 kDa protein posphatase 1 (PP1) catalytic and a 110/130 kDa regulatory subunit (MYPT1). MP activity is inhibited by phoshorylation of MYPT1 in Thr695 and Thr850 residues. Endothelial nitric oxide synthase (eNOS) is constitutively expressed in different cell lines. Nitric oxide (NO) produced by NOS modulates various cellular events and its activity can be regulated via phosphorylation by several kinases, but the dephosphorylating phosphatase holoenzymes are less known. eNOS activity can be enhanced by phosphorylation at Ser1177 while phosphorylation of Thr495 decreases the enzymic activity.

Our aim was to investigate a possible interaction between MP holoenzyme and eNOS. The experiments was performed on two cell types: human umbilical vein edothelial cells (HUVEC) and HEK-293 cells. We analysed the presence of the eNOS and MYPT1 as well as their phosphorylated forms in the presece and in the absence of serum by immunfluorescence and western blot. We have found that in the presence of sera MYPT1 was distributed between the cytoplasm and the nucleus, but phospho-Thr695-MYPT1 and phospho-Thr850-MYPT1 was present mainly in the nucleus. Phospho-eNOS-Ser1177 and Thr495 was also mainly located in the nucleus. In contrast in the absence of sera eNOS or MYPT were predominantly localized in the cytoplasm. We demonstrated the interaction of eNOS and MYPT1 in HUVEC cells by immunoprecipitation, pull-down and confocal microscopy. In divideing cells phospho-eNOS-Thr495 signal can not be detected, while phosphorylation of Ser1177 is increased especially in the centrioles which suggests increased eNOS activity during mitosis. In further experiments we analysed HEK-293 cells transfected with eNOS plasmid. eNOS was phosphorylated at Ser1177 residue while phosphorylation of Thr495 residue was negligible. Treatment the cells with PKC activator PMA increased phosphorylation of Thr495 and Ser1177 residues. When PP1c inhibitory calyculin-A and PMA were applied together the phosphorylation was further increased.

Our results suggest that MP and eNOS interacts and MP may be involved in the dephosphorylation of eNOS at phospho-Thr495.

Effects of ß-adrenergic stimulation on delayed rectifier potassium currents in canine ventricular cardiomyocytes

Gábor Harmati 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: János Magyar

Background and purpose: While the slow delayed rectifier K+ current (IKs) is known to be enhanced by ß-adrenergic stimulation in several mammalian species, phosphorylationdependent regulation of the rapid delayed rectifier K+ current (IKr) is controversial.

Experimental approach: In the present study, therefore, the effect of isoproterenol (ISO), activators and inhibitors of the PKA and PKC pathways on IKr and IKs were studied in canine ventricular myocytes using the whole cell patch clamp technique.

Key results: IKr was significantly increased (by 30-50 %) following superfusion with ISO, forskolin, or intracellular application of PKA activator cAMP analogues (cAMP,

8- Br-cAMP, 6-Bnz-cAMP), 100 nM isoproterenol from 0,38±0,015 pA/pF to 0,52±0,028 pA/pF, while decreased by inhibition of PKA (in the presence of 20 µM H-89 from 0,38±0,015 pA/pF to 0,12±0,019 pA/pF) and PKC (GF- 109203X, chelerythine- in the presence of 10 µM cheleritrine from 0,37±0,02 pA/pF to 0,04±0,01 pA/pF). The stimulatory effect of ISO on IKr was fully eliminated by H- 89, chelerythine, and by the PKA activator cAMP analogues – but not by the EPAC activator 8-pCPT-2'-O-Me-cAMP. In comparison, IKs was increased threefold by activation of PKA (by ISO or 8-Br-cAMP), and strongly reduced by H-89. Inhibition of PKC (by GF-109203X or chelerythine) failed to alter IKs significantly. The ISO-induced enhancement of IKs was eliminated by H-89 and 8-Br-cAMP, but not by PKC inhibitors.

Conclusions and implications: The results indicate that ß-adrenergic stimulation increases IKr - similarly to IKs - via activation of PKA in canine ventricular cells. In the case of IKr integrity of the PKC system is also required – in contrast to IKs, which is controlled by direct modulation of PKA.

The localization of myosin phosphatase in the nucleus and its regulation by SMTNL1

Adrienn Sipos 1st year PhD student
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Beáta Lontay

Myosin phosphatase (MP) is a heterotrimer composed of a 38 kDa type 1 protein serine/threonine phosphatase catalytic subunit (PP1c), a 130/133 kDa MYPT (myosin phosphatase targeting subunit) and 20 kDa subunits. MYPT has several functions such as targeting PP1c to the substrate and activation and regulation of MP activity. MP was characterized as the regulator of contractility through the dephosphorylation of 20 kDa myosin light chain (MLC20). MYPT was found to be localized not only in the cytosol but in the nucleus in rat aortic smooth muscle cells, primary cultures of neuronal cells and in human hepatocarcinoma cells. One of the potential effectors of MP regulation is the smoothelin-like protein 1 (SMTNL1), which is a target of protein kinase A and G (PKA/PKG) and prevents MP activity towards MLC20. After SMTNL1 phosphorylated on Ser301 by PKA/PKG translocates to the nucleus and colocalizes with the nuclear MP. Our goal is to investigate the nuclear functions of MP by determining the subnuclear localization of MYPT and its nuclear interacting partners. We also intend to describe the molecular interaction between SMTNL1 and MYPT and its regulation via PKA/PKG signalling. Our hypothesis that SMTNL1 is responsible not only regulation of MP in contractility but it also acts on MP in the nucleus in a novel mechanism. Nuclear and subnuclear fractions of HepG2 cells were analysed by Western blotting and enzyme activity assays. MYPT1 was localized out of the nucleolus without binding to the nuclear membrane. MYPT1 showed localization in spliceosomes by confocal microscopy suggesting that MP may play a role in mRNA splicing. Chromatin immunoprecipitation showed that MYPT1 is also bound to chromatin through the N-terminal region of MYPT subunit. GST-MYPT pull down assay was applied to identify potential MYPT1 nuclear interacting proteins such as SMTNL1. We plan to determine the region of MYPT is responsible for the interaction with SMTNL1 by surface plasmon resonance-based technology. We also plan to investigate the effect of SMTNL1 on MP activity in the nucleus and to reveal their physiological function in the nucleus.

Endocannabinoids regulate proliferation, differentiation, and survival of human sweat gland epithelial cells

Attila Szöllősi 3rd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: Tamás Bíró

In our present experiments we examined the endocannabinoid system on the human eccrine sweat gland cell line NCL-SG3. Conforming previous data, expression of both cannabinoid receptor subtypes (CB1, CB2) were unambiguously identified, as well as expression of those enzymes which are involved in the synthesis and degradation of the endocannabinoids. In keeping with these results we also found that NCL-SG3 cells produce the prototypic endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG). Since the expression level of both CB1 and CB2 changed in accordance with cell confluence, we tested whether the ECS plays a role in the regulation of growth and differentiation of NCL-SG3 cells. Our findings show that both AEA and 2-AG dose-dependently suppressed the viable cell number and proliferation of NCL-SG3 cells. Further experiments revealed that both endocannabinoids significantly increased apoptotic cell death. Higher concentrations of 2-AG also caused necrosis, while AEA had no such effect. Since in most cell types of human skin and skin appendages the cessation of proliferation and the induction of apoptosis are accompanied by the onset of terminal differentiation we investigated the effect of endocannabinoids on the expression of various epithelial differentiation markers. Both AEA and 2-AG significantly elevated the expressions of the terminal differentiation markers, while markedly suppressing the levels of some of the early differentiation markers as well as increasing the lipid synthesis of NCL-SG3 cells. Interestingly these effects could not be abrogated by either pharmacological or siRNA based inhibition of CB1 and CB2, however, the MAPK inhibitor PD098059 prevented the effect of endocannabinoids, while the general PKC inhibitor GF109203X and the PI-3K inhibitor Wortmannin had no such effect, implicating the role of the MAPK secondary messenger pathway.

Cannabidiol: A new, promising anti-acne agent

Attila Oláh 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: Tamás Bíró

We have previously shown that prototypic endocannabinoids (anandamide, 2-arachidonoyl glycerol) are constitutively produced in human sebaceous glands. Moreover, using human immortalized SZ95 sebocytes we have also demonstrated that these locally produced endocannabinoids induce terminal differentiation of these cells, which is characterized by increased neutral lipid (sebum) production and apoptosis-dominated cell death. Although we have confirmed that human sebocytes have a functionally active endocannabinoid system, we do not possess any data about the potential effect(s) of plant-derived cannabinoids. Therefore, in the current study we have investigated the effects of cannabidiol (CBD) on the viability and lipid synthesis of the SZ95 sebocytes.

First we have demonstrated that non-cytotoxic concentrations (<10 uM) of CBD did not affect the basal lipid synthesis (Nile Red staining) of the sebocytes, however they dose-dependently prevented the lipogenic effect of various substances (anandamide, arachidonic acid, linoleic acid, testosteron). As this action was markedly suppressed by the decrease of [Ca2+]EC, we have investigated how did CBD affect the Ca2+ homeostasis of the sebocytes. Using fluorimetric Ca2+-imaging techniques (Fluo-4 AM - FLIPR) we have demonstrated that CBD increased the [Ca2+]IC of the sebocytes, which was prevented by the non-specific transient receptor potential (TRP) channel blocker ruthenium red. Among the possible TRP channels which are known to be modulated by different cannabinoid substances, we have shown the expression both at mRNA (Q-PCR) and protein (Western blot, immunocytochemistry) levels of TRPV1, TRPV2 and TRPV4. The results of selective “gene silencing” of these channels suggest that the target molecule of the CBD is most probably the TRPV4.

Collectively, our findings suggest that CBD is a novel, very effective sebostatic agent. Therefore, it could be successfully used in the management of such a common skin disorder as acne, which is characterized by pathologically elevated sebum production of the sebaceous glands.

c-erbB receptors are indirectly affected by severe cell membrane modifications induced by Irvalec (PM02734) treatment

Tímea Váradi 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Biophysics and Cell Biology
Supervisor: Péter Nagy

Background: Irvalec® is a marine-derived antitumor agent that is currently undergoing phase II clinical trials. Although little is known about its mechanism of action, it has been reported that the compound induces a necrotic cell death associated with the appearance of membrane blebs and severe cell swelling reflecting a serious membrane damage caused by the drug. Indirect evidence has also suggested a possible role of ErbB proteins, in particular ErbB3, and lipid rafts in conferring sensitivity to Irvalec treatment.

Methods: The homoassociation of ErbB2 and ErbB3 was measured by flow cytometric FRET. The distribution of GFP-, mYPF- or citrine-labeled proteins was examined by confocal microscopy.

Results: In order to assess the role of ErbB protein expression in determining sensitivity to Irvalec, and to measure the effect of drug treatment on the clustering properties of these proteins, we generated CHO cell lines stably transfected with ErbB2 (CHO-ErbB2) or with ErbB2 and ErbB3 (CHO-ErbB2-3). The IC50 values of the three cell lines for Irvalec did not differ significantly from each other. On the other hand treatment with Irvalec induced a significant increase in the binding of a conformational sensitive antibody to ErbB3 (ErbB3.105.5) without modifying the binding of other ErbB3 antibodies or antibodies against ErbB2. In addition, Irvalec treatment decreased the homoassociation of ErbB2 and ErbB3 measured by flow cytometric FRET. A431 cells transfected with GPI-anchored GFP or ErbB3-citrine showed significant Irvalec-induced patching of the GPI-anchor and ErbB3, whereas the distribution of ErbB1 and ErbB2 was not affected in cells transfected with ErbB1-GFP or ErbB2-mYFP.

Conclusions: Our data do not support a role for ErbB proteins in determining sensitivity to Irvalec, but show that they are indirectly affected by exposing cells to the compound. These alterations should be ascribed to cell membrane modifications induced by Irvalec treatment.

In vivo test of a highly stable antifungal protein (PAF) against lung aspergillosis.

Zoltán Palicz 2nd year PhD student
University of Debrecen, Medical and Health Science Center, Department of Physiology
Supervisor: Péter Szentesi

The Aspergillus fungi can cause a severe lung disease (aspergillosis) in people with an attenuated immune system, so it would be important to find new drugs against this species.

Our research topic was to investigate the effects of the antifungal peptide produced by the Penicillium chrysogenum (PAF) in animal experiments, to find out the toxicity or side effects of this drug on mammalian organisms.

In our experiments adult mice were inoculated intranasally with PAF. Animals were randomly divided to five groups in which they got PAF in 0, 2, 10, 50 and 100 µg/ml concentration once a week for 8 weeks. There were histological examinations from the lungs, livers and the mucous membrane of the nose of the animals. Then a new group got PAF in 1 mg/ml concentration weekly for 5 weeks.

Animals did not die during the experiment because the treatment and no side effects were observed. We did not find any pathological reaction during the histological experiments, even in the 1 mg/ml concentration.

In the next experiment the effect of the drug was examined on the skin in an irritative dermatitis model. PAF was spread on the ears of the mice in an ointment. There were 3 groups – negative control, PAF and positive control (phorbol-12-myristate-13-acetate – PMA). After five treatments the thickness of the ears were measured and a histological experiment was carried out.

The PMA caused a significant expansion of the ears compared to the control while PAF did not. Histological reaction was present only in the case of the positive control.

Consequently we did not find any toxic effect of PAF either in systemic or in local external treatment. In our next experiment the effect of PAF will be investigated on the procession of the disease in infected animals by Aspergillus.

Generation and characterization of a comprehensive analyte library representing the human plasma proteome

<u>András László Kovács</u> 2nd year PhD student
Research Centre for Molecular Medicine, Horváth Laboratory of Bioseparation Sciences
Supervisor: András Guttman

Characterization of the human plasma proteome is a highly challenging task due to the large dynamic range (up to 12 orders of magnitude) in the concentration of the individual proteins it contains. Therefore, methods that increase the detectability of low abundant plasma proteins are of high interest. We are generating a comprehensive set of fractions, representing the entire human plasma proteome, referred to as the analyte library, using combined chromatographic and protein precipitation techniques, expecting hundreds to thousands of well-defined fractions, each containing of some 10-100 intact proteins.

First the large human serum albumin and immunoglobulin content were removed from 500 mL normal pooled human plasma by albumin and IgG specific partitioning methods and the resulting depleted plasma was pre-fractionated by ammonium sulfate precipitation. Each fraction was then further separated by size exclusion chromatography, followed by cation and anion exchange chromatography. The interim and final fractions were first analyzed by SDS-PAGE; however, their further characterization by protein microarray and LC-MS techniques is planned.

The obtained fractions will be used for biomarker screening using ELISA and/or protein microarray techniques with antibodies specific for various diseases. Fractions that prove positive will be used for the identification of the individual protein antigens by LC-MS as part of our biomarker discovery endeavor.

The role of Protein Phosphatase 2A (PP2A) in HT168 and WM35 human melanoma cell lines

Csilla Somogyi 1st year PhD student
University of Debrecen, Medical and Health Science Centre, Department of Anatomy, Histology and Embryology
Supervisor: Róza Zákány

PP2A, which is a member of the serine/threonine phosphoprotein phosphatases (PP), is reported to play an important role in regulating melanosome aggregation in normal human melanocytes. Since little is known about its function in case of malignant melanomas, we aimed to investigate the role of PP2A in two human melanoma cell lines, in the metastasis forming HT168 and in the less malignant, non metastatic WM35. Okadaic acid (OA), a potent pharmacological inhibitor of this PP, was applied on two consecutive days of culturing for four hours at a concentration (20 nM) regarded to specifically inhibit PP2A activity. As a result of the treatment, both cell lines showed a change in cellular morphology: especially cells of the polymorphic HT168 cell line were losing their processes and getting spherical while this phenomenon was less intense in the case of WM35 cell line. When we investigated fibronectin guided chemotaxis in Boyden chamber, OA treatment caused a 40-60% reduction in the number of membrane-passed cells of HT168 cells, while migration of WM35 cells was not altered significantly. The applied OA concentration was not cytotoxic in either cell lines. Proliferation assays showed a dramatic decrease in OA treated HT168 cells while in WM35 cells the decrease of proliferation was less prominent compared to the respective. Although the RNA expression of PP2A detected by RT-PCR was not altered by OA, the protein expression of PP2A decreased in the OA treated WM35 cells, while as a possible compensatory effect, it was elevated in HT168 cells. Enzyme activity assays detected a 2-3 fold higher basal PP2A-activity of HT168 cells compared to WM35 cells and OA caused an approximately 40% reduction of PP2A activity in both cell lines.

According to the literature, PP2A is regarded to be a tumour suppressor, while its inhibitors (OA and calyculin A) have a tumour promoting effect in non-malignant cells. On the contrary, our data suggest that the activity of PP2A may have an important role in maintaining the aggressive migrating character of HT168 cell line, and we suppose that the non metastatic character of WM35 cell line is partly due to its lower PP2A activity.

The role of calcineurin in the regulation of myosin phosphatase in endothelial cells

Bernadett Kolozsvári Predoctor
University of Debrecen, Medical and Health Science Center, Department of Medical Chemistry
Supervisor: Éva Bakó

Intercellular gap formation evoked by bioactive agents (like thrombin) results in increased endothelial permeability, a typical feature of acute inflammatory lung syndrome. The integrity of the endothelial cell (EC) monolayer is essential for the maintenance of the cellular barrier. Thrombin causes EC barrier dysfunction inducing cell contraction by myosin light chain (MLC) phosphorylation. MLC phosphorylation level is determined by the balanced activities of MLC kinase and myosin phosphatase (MP). Dephosphorylation of EC MLC by myosin phosphatase (MP), a type 1 phospho-Ser/Thr specific protein phosphatase (PP1), has been demonstrated. In the structure of MP holoenzyme PP1 catalytic subunit (PP1c) is associated with a regulatory subunit (110-130 kDa), termed myosin phosphatase target subunit-1 (MYPT1). Phosphorylation of Thr 695 and Thr 850 in MYPT1 causes inhibition of PP1c activity. Dephosphorylation of phospho-MYPT1 is less investigated especially in endothelial cells.

We studied the role of calcineurin in the cytoskeletal rearrangements of calf pulmonary artery endothelial cells in response to different agonists. Calcineurin (CN) is a Ca2+/CaM-dependent phosho-Ser/Thr specific protein phosphatase. Cyclosporine-A (CsA), a CN inhibitor prolongs the thrombin-induced stress fiber formation. CsA resulted in increased phosphorylation level of MYPT1 at the inhibitory sites (Thr 695 and Thr 850 residues). Treatment with thrombin alone resulted in transient phosphorylation of MYPT1, while inhibition of CN by CsA before thrombin treatment led to sustained phosphorylation of MYPT1. Interaction of MYPT1 with CN was also revealed by pull-down assays, 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 to the N-terminal regions of MYPT1.

These results suggest that CN is involved in the recovery of Ecs from thrombin-induced dysfunction, presumably via regulation of MP activity by dephosphorylation of MYPT1.