Annual Symposium of the Doctoral School of Molecular Medicine (Academic Year 2016-2017)

University of Debrecen
September 7, 2017

Program

Date: September 7, 2017

Location: F008-009 Lecture Hall, Life Science Building, University of Debrecen

8:15 Arrival (Coffee, cakes)

8:25 Prof. László Csernoch: Welcome address

Session 1
Chair: Prof. László Csernoch
Head of the Doctoral School and the "Physiology and Neurobiology" doctoral program

08:30 Dána Al-Gaadi (1st year PhD student)
STORE-OPERATED CALCIUM ENTRY IS IMPORTANT FOR MAINTAINING SARCOPLASMIC CALCIUM CONTENT AND RELEASE IN MAMMALIAN SKELETAL MUSCLE FIBERS
Supervisor: Péter Szentesi

08:45 Shahrzad Alimohammadi (2nd year PhD student)
THE ROLE OF LANGERHANS CELLS IN PRURITUS - TRIALS AND TRIBULATIONS OF IN VITRO MODELS
Supervisor: Tamás Bíró, Attila Gábor Szöllősi

09:00 Csaba Aranyi (3rd year PhD student)
MODEL SEARCH METHODS OF BRAIN EFFECTIVE CONNECTIVITY MODELS AND AUTOMATIC IDENTIFICATION OF RESTING NETWORKS
Supervisor: Miklós Emri

09:15 Brigitta Baksa (1st year PhD student)
FUNCTIONAL CHARACTERIZATION OF THE GLUTAMATERIG NEURONS IN THE PEDUNCULOPONTINE NUCLEUS
Supervisor: Balázs Pál

09:30 Beáta Borza (1st year PhD student)
ANALYSIS AND COMPARISON OF BIOSIMILAR AND ITS INNOVATOR PRODUCT'S GLYCAN STRUCTURE WITH CAPILLARY ELECTROPHORESIS
Supervisor: András Guttman

09:45 Karolina Cseri (2nd year PhD student)
ROLE OF SEPTIN7 IN THE DEVELOPMENT OF SKELETAL MUSCLE
Supervisor: László Csernoch

10:00 Tamás Czirják (3rd year PhD student)
CHARACTERIZATION OF CALCIUM EVENTS IN THE SKELETAL MUSCLE OF THE CAV1.1ΔE29 MOUSE
Supervisor: Beatrix Dienes

10:15 Madhura De (1st year PhD student)
SINGLE MOLECULE FRET STUDIES OF THE CHROMATOSOME
Supervisor: Katalin Tóth

Break (15 min) Coffee and drinks

Session 2
Chair: Prof. Viktor Dombrádi
Department of Medical Chemistry, UD

10:45 Gyula Diszházi (2nd year PhD student)
INVESTIGATION OF TOXIN-ION CHANNEL INTERACTIONS
Supervisor: János Almássy

11:00 Abdennour Douida (1st year PhD student)
BALANCING THE MITOCHONDRIA BY THE PA200-PROTEASOMES
Supervisor: Krisztina Tar

11:15 Lina Fadel (1st year PhD student)
THE MODULATION OF NUCLEAR RECEPTOR DIMERIZATION UPON LIGAND BINDING FOLLOWED BY LOCALIZATION
Supervisor: György Vámosi, Gábor Szabó

11:30 Tímea Hajdu (2nd year PhD student)
THE EFFECT OF THE MEMBRANE ENVIRONMENT AND PROTEIN CONFORMATION ON THE LIGAND BINDING OF EPIDERMAL GROWTH FACTOR RECEPTOR
Supervisor: Péter Nagy

11:45 Laura Jankó (1st year PhD student)
THE DEPLETION OF PARP2 ALTERS MITOCHONDRIAL MORPHOLOGY
Supervisor: Péter Bay

12:00 Balázs Kelemen (2nd year PhD student)
VOLATILE ANAESTHETICS INHIBIT TRMP3 ION CHANNEL
Supervisor: István Balázs Tóth

12:15 Éva Kókai (2nd year PhD student)
EXAMINATION OF AXON COLLATERALS OF LAMINA III NEURONS WITH LOW-THRESHOLD INPUTS IN RATS
Supervisor: Péter Szűcs

12:30-13:15 Buffet Lunch

Session 3
Chair: Prof. László Virág
Head of the "Cell and molecular biology of signal transduction" doctoral program

13:15 Brigitta Mészáros (1st year PhD student)
DEVELOPMENT OF A HIGH-RESOLUTION SDS SEPARATION GEL FOR PROTEIN ANALYSIS BY CGE-MS
Supervisor: András Guttman

13:30 Noémi Miltner (3rd year PhD student)
ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECTS OF NOVEL SEMI-SYNTHETIC PHYTOCANNABINOIDS IN HUMAN IN VITRO PRO-INFLAMMATORY KERATINOCYTE MODEL SYSTEMS
Supervisor: Tamás Bíró, Johanna Mihály

13:45 Marianna Nagy (2nd year PhD student)
INVESTIGATION OF THE APPLICABILITY OF THE BRAIN ATLAS TECHNIQUE IN SPACE OCCUPYING LESIONS – AS A PRECONDITION TO CALCULATE REGIONAL HEMODYNAMIC PARAMETERS AND GENERATE PARAMETRIC MAPS
Supervisor: Miklós Emri

14:00 Zsanett Sári (1st year PhD student)
EXAMINATION OF THE RELATIONSHIP BETWEEN HUMAN MICROBIOME AND BREAST CANCER CELLS
Supervisor: Péter Bay

14:15 Ádám Sipos (1st year PhD student)
NEW TARGET FOR GLYCOGEN PHOSPHORYLASE INHIBITORS: SODIUM-GLUCOSE CO-TRANSPORTER OF KIDNEY
Supervisor: Tibor Docsa

14:30 Adrienn Skopál (1st year PhD student)
THE ROLE OF ADENOSINE 2A RECEPTOR IN THE VESICULAR TRAFFICKING OF MACROPHAGES
Supervisor: Endre Kókai

14:45 Krisztina Szabó (1st year PhD student)
PROTEOMIC ANALYSIS OF PROTEIN PHOSPHATASE Z1 FROM CANDIDA ALBICANS
Supervisor: Viktor Béla Dombrádi

15:00 Máté Szarka (2nd year PhD student)
ONBOARD ANALYSIS OF ASTRONAUT'S SERUM IMMUNOGLOBULINS AS A MARKER OF AGING ON DEEP SPACE MISSIONS; A NEW FRONTIER IN CAPILLARY ELECTROPHORESIS
Supervisor: András Guttman

Break (15 min) Coffee and drinks

Session 4
Chair: Prof. János Szöllősi
Head of the "Membrane biophysical questions and research methods" doctoral program

15:30 Emese Tóth (3rd year PhD student)
REGULATION OF MYOSIN PHOSPHATASE HOLOENZYME BY NUCLEOCYTOPLASMIC TRAFFICKING OF THE MYPT1 REGULATORY SUBUNIT
Supervisor: Andrea Kiss

15:45 Kinga Fanni Tóth (1st year PhD student)
THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR FLUOXETINE EXERTS ANTI-INFLAMMATORY ACTIONS ON HUMAN EPIDERMAL KERATINOCYTES
Supervisor: Tamás Bíró

16:00 Tamás Ungvári (2nd year PhD student)
PERRIN AND FÖRSTER UNIFIED: DUAL-LASER TRIPLE-POLARIZATION FRET (3POLFRET) FOR INTERACTIONS AT THE FÖRSTER-DISTANCE AND BEYOND
Supervisor: László Bene

16:15 Judit Vágó (2nd year PhD student)
INVESTIGATING THE FUNCTIONAL AND MORPHOLOGICAL CONNECTIONS BETWEEN SEPTINS AND PRIMARY CILIA IN CHONDRIFYING EXPERIMENTAL MODELS
Supervisor: Róza Zákány

16:30 Roland Veress (2nd year PhD student)
THE ROLE OF INA,L IN ACTION POTENTIAL DEVELOPMENT IN VENTRICULAR MYOCARDIAL CELLS
Supervisor: Balázs Horváth

16:45 Orsolya Vörös (3rd year PhD student)
THE C-TERMINAL HRET SEQUENCE OF KV1.3 REGULATES GATING RATHER THAN TARGETING OF KV1.3 TO THE PLASMA MEMBRANE
Supervisor: Péter Hajdu

17:00 Florina Zákány (3rd year PhD student)
CHARACTERIZING THE EFFECTS OF CHOLESTEROL AND 7-DEHYDROCHOLESTEROL ON THE GATING OF KV1.3 AND KV10.1 ION CHANNELS USING VOLTAGE-CLAMP FUORIMETRY TECHNIQUE
Supervisor: György Panyi, Zoltán Varga

17:15 Closing remarks

ABSTRACTS

Dána Al-Gaadi (1st year PhD student)

STORE-OPERATED CALCIUM ENTRY IS IMPORTANT FOR MAINTAINING SARCOPLASMIC CALCIUM CONTENT AND RELEASE IN MAMMALIAN SKELETAL MUSCLE FIBERS

Store-operated Ca2+-entry (SOCE) is a Ca2+ entry process activated by the depletion of intracellular stores and has an important role in many cell types. In skeletal muscle, however, its role during physiological muscle activation has been controversial. To address this question, sarcoplasmic reticulum (SR) calcium release in a mouse strain with a naturally occurring mutation in the myostatin gene (Compact, Cmpt) leading to a hypermuscular yet reduced muscle force phenotype was compared to that in wild type (WT) mice. To elicit Ca2+-release from the SR of flexor digitorum brevis (FDB) fibers either a Ryanodine receptor agonist (4-chloro-meta-cresol, 4-CmC) or depolarizing pulses were used. In muscles from Cmpt mice endogenous protein levels of STIM1 and Orai1 were reduced and, consequently, SOCE following 4-CmC-induced store depletion was suppressed. While the voltage dependence of SR calcium release was not statistically different between WT and Cmpt fibers, the amount of releasable calcium was significantly reduced in the latter, indicating smaller SR content. To assess the immediate role of SOCE in replenishing the SR calcium store, the evolution of intracellular calcium ([Ca2+]i) during a train of long-lasting depolarizations to a maximally activating voltage were monitored. Cmpt mice exhibited a faster decline in calcium release suggesting a compromised ability to refill the SR. A simple model that incorporates a reduced SOCE as an important partner in regulating immediate calcium influx through the surface membrane readily accounts for the steady-state reduction and the more pronounced decline following calcium release in SR calcium content.

Our results help resolve the long-standing question whether or not SOCE has any role during normal skeletal muscle activation. They favor the idea that SOCE is immediately activated upon voltage-dependent SR calcium release. By doing so it plays an important role in regulating SR calcium content both on the long run and also during a contraction-relaxation cycle. The data also suggest that reduced function and/or expression of SOCE partners (STIM1 and/or Orai1) could play an important role in muscle weakness associated to certain pathologies and aging. Tackling this calcium influx pathway could thus open novel and safe strategies to alleviate the symptoms of sarcopenia.

Supervisor: Péter Szentesi

Shahrzad Alimohammadi (2nd year PhD student)

THE ROLE OF LANGERHANS CELLS IN PRURITUS - TRIALS AND TRIBULATIONS OF IN VITRO MODELS

Human skin contains multiple subsets of antigen presenting cells capable of responding to both exogenous and endogenous factors. The two main subsets of these cells, Langerhans cells (LC) and dendritic cells, emigrate from the skin upon their activation, and initiate a wide range of T cell responses. Of the two cell types dendritic cells are more accessible for research since they can be easily and reliably differentiated from monocyte precursors. LC on the other hand have only been investigated from mouse models or from primary human samples. The latter approach produces a very limited quantity of viable cells, and the isolation process itself has been shown to activate these cells. It is therefore not surprising that little is known, however, about their role locally in the skin, especially in the steady-state. While there have been studies aimed at developing methods to differentiate LC from more commonly accessible precursors, there is no widely used and accepted protocol to date. LC are epidermal antigen presenting cells characterized by the expression of Langerin (CD207) as well as a host of other markers (e. g.: CD1a, CD14, CD33, CD34, CD40, CD44, CD80).
In our work we have tried to reproduce previously published results that reported relatively high CD207 and CD1a expression (commonly used to discriminate LC from other dendritic cell subsets, which might express CD1a but not CD207), by culturing CD14+ monocytes with various combinations of cytokines (GM-CSF, IL-4, TGFB1, TNFa), as well as using cocultures with epidermal keratinocytes. These first experiments did not produce a sufficient ratio of double positive cells, unfortunately, so in subsequent experiments we attempted to combine these techniques. Since E-cadherin interactions were shown to be essential for LC differentiation we also determined the expression of the protein on keratinocytes used for our cocultures, and found that E-cadherin is uniformly present on the cells in all stages of differentiation.
We hope that with further optimization a reliable protocol is just around the corner, and that the resulting cells will help us shed light on the role of LC in health and common skin diseases.

Supervisor: Tamás Bíró, Attila Gábor Szöllősi

Csaba Aranyi (3rd year PhD student)

MODEL SEARCH METHODS OF BRAIN EFFECTIVE CONNECTIVITY MODELS AND AUTOMATIC IDENTIFICATION OF RESTING NETWORKS

Effective connectivity analysis of a brain network comprises several distinct steps: a) defining regions in the brain to indirectly extract time-varying activation in them, b) building connectivity models between network nodes, c) fitting the regional time-series to the measured data based on the model structure and d) selecting the best model / group of models for the problem. Our work is centered around model selection and resting state region identification based on functional MRI.

For network modeling and effective connectivity computations we used Dynamic Causal Modeling (DCM). After defining a model space of 531441 different models we evaluated them with DCM. Then, we applied model search algorithms to compare their efficiency in approximating the best effective connectivity model. The 'Attention to motion' served as experimental fMRI dataset, which is available in the SPM software. It is designed to investigate a visual network stimulation modulated by attention on moving objects. Four model search algorithms were adapted: greedy search (GS), Hamming-distance based search (HD), a genetic algorithm (GA) and random search (RND). We found that these methods can approximate, or in some cases, can reach the best model using the GA. However, it is not as reliable as the deterministic GS or HD algorithms and requires much more models to be estimated.

Localization of network regions in resting state analysis is usually done using independent component analysis (ICA). There are several known resting state networks (RSN) with similar neural fluctuations (e.g. Default Mode Network or Executive Control Network). We created a method to automatically identify ICA components corresponding to RSNs based on network templates. We found in our examples that the most robust measure for component selection was the Jaccard-index between the component and the template. For template creation, we developed a web based software using the R Shiny language. This method was already used in a multimodal epilepsy diagnostic project in Kaposvár to examine the effects of hypometabolic areas measured by FDG-PET on resting state networks.

Supervisor: Miklós Emri

Brigitta Baksa (1st year PhD student)

FUNCTIONAL CHARACTERIZATION OF THE GLUTAMATERIG NEURONS IN THE PEDUNCULOPONTINE NUCLEUS

The pedunculopontine nucleus (PPN) is a structure belonging to the reticular activating system (RAS), thus it has a role in regulation of sleep and wakefulness. Although it is best known as a cholinergic member of the RAS, it consists of non-cholinergic (i.e. GABAergic and glutamatergic) neurons, as well. As post hoc immunohistochemical identification of glutamatergic neurons is more complicated as for cholinergic or GABAergic neurons, less is known about the functional properties of these neurons. In the present work, we aimed to characterize membrane properties of this neuronal type of the PPN.

To achieve our aims, mice expressing tdTomato fluorescent marker or channelrhodopsin-2 in a vesicular glutamate transporter type 2 (Vglut2)-dependent way were employed, and slice electrophysiology and morphological reconstruction were used.

We revealed that the input resistance of the glutamatergic neurons was significantly greater than of the cholinergic and GABAergic ones. Similar to the GABAergic neurons and in contrast with the cholinergic ones, almost all glutamatergic neurons lacked M-current.

Based on the changes of spike frequency adaptation, action potential amplitude and width elicited by current injections with increasing amplitude, 3 functional subtypes of glutamatergic neurons are suggested: in the first group, there is minimal change in the parameters above with increasing depolarization. In the second group, the spike frequency adaptation increases, whereas the action potentials become smaller and slower at the end of the train. In the third group, increasing depolarization shortens the duration of the train, which is restricted to the first half –one-tenth of the depolarizing current injection.

Taken together, functional subgroups of glutamatergic PPN neurons can be defined based on their functional characteristics; which might serve as a background of the heterogeneity of their previously observed in vivo properties. The potential significance of these observations are that these neurons are the potential targets of deep brain stimulation of the PPN.

Supervisor: Balázs Pál

Beáta Borza (1st year PhD student)

ANALYSIS AND COMPARISON OF BIOSIMILAR AND ITS INNOVATOR PRODUCT'S GLYCAN STRUCTURE WITH CAPILLARY ELECTROPHORESIS

There is a growing demand in the biopharmaceutical industry for recombinant protein pharmaceuticals such as monoclonal antibody-based therapeutics, hormones, growth factors, blood products, and recombinant vaccines. These biologics have played a progressively significant role in modern pharmaceuticals. Due to the expiration of several patents and regulatory data protection of original biotherapeutics mostly the recombinant monoclonal antibodies, competing companies have the opportunity to evolve their own biosimilar replica. Biosimilars are similar but not accurately the same as the innovator products. Because of the limited information on manufacturing procedures, the developed process may contain some differences such as employed cell line, advanced cell culture conditions as well purification processes. Consequently, there is crucial to find an appropriate analytical method, which is able to compare the final and the original therapeutics.

Structural differences could affect on their clinical performance. Thus, the comparison of the reference product and the biosimilar medicine based on the following criteria: 1) primary structures; 2) higher-order structures (HOS); 3) posttranslational modifications; 4) degradation hotspots; 5) chemical modifications. To the comprehensive description is recommended using orthogonal analytical methods such as capillary electrophoresis (CE).

Major of recombinant proteins (e.g. monoclonal antibodies) and their biosimilar versions are glycosylated. Glycosylation of biosimilar products could have a considerable influence on the pharmacokinetic (PK) profile, biological activity, serum half-life, and effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and immunogenicity of the drug products. Thus, glycosylation is a critical quality attribute, what could be investigated with capillary electrophoresis.

Supervisor: András Guttman

Karolina Cseri (2nd year PhD student)

ROLE OF SEPTIN7 IN THE DEVELOPMENT OF SKELETAL MUSCLE

Septins are conserved GTP-binding proteins with 13 subtypes and associate with cellular membranes, actin filaments and microtubules. They polymerize to form filaments, rings that act as diffusion barriers for biochemical processes in the cell and as molecular scaffolds for proteins. The role of septins in skeletal muscle has not yet been investigated.
We examined the interaction between Septin7 and the key proteins involved in calcium homeostasis of skeletal muscle (ryanodine receptor type 1: RYR1, calcium release-activated calcium channel protein 1; Orai1). The colocalization of Septin7 with these proteins was investigated with immunofluorescent staining on single flexor digitorum brevis (FDB) muscle fibers isolated from 5-month-old C57BL6 mice and C2C12 mouse myoblast cell line.
On C2C12 cell line Septin7 pattern changes with the state of differentiation. At the beginning of the proliferation Septin7 appears as parallel lines and in certain cells as granules and small circles around the nucleus. During differentiation a diffuse, grainy cytoplasmic pattern replaces this structure. Initially Orai1 shows an asymmetric, cap-like arrangement around the nucleus and as the differentiation progresses it surrounds the nucleus like a ring. Sometimes it forms larger granules in the cytoplasm farther on the nucleus. RYR1 receptor originally shows a weak homogenous pattern in the cytoplasm and later it forms larger grains in the myotubes.
Based on our experiments Septin7 localizes in the area of I-band and colocalizes with RYR1 in adult mouse muscle. Similarly Orai1 has a totally overlapping colocalization with Septin7.
Septin7, Orai1 and RYR1 colocalization in adult skeletal muscle fibers suggests that septins may play a role in the regulation of calcium homeostasis. Based on the patterns found on C2C12 cells, it is likely that the dynamic rearrangement of Septin7 is a cell cycle-sensitive process. The role of Septin7 in the regulation of calcium homeostasis requires further functional experiments.

Research support: NKFIH NK-115461, GINOP-2.3.2-15-2016-00044.

Supervisor: László Csernoch

Tamás Czirják (3rd year PhD student)

CHARACTERIZATION OF CALCIUM EVENTS IN THE SKELETAL MUSCLE OF THE CAV1.1ΔE29 MOUSE

During the excitation contraction coupling the activated nerve fiber releases the neurotransmitter acetilcholine, which depolarises the membrane of the skeletal muscle and generates an action potential. This rapidly spreading stimulus reaches the transversal tubule, where the voltage sensor L-type Ca2+ channel (Cav1.1) or in other name the dihydropyridine receptor (DHPR) will have a conformational change by which the ryanodine-receptor (RyR) will open on the surface of the sarcoplasmic reticulum (SR) resulting a Ca2+ release from the SR. The synchronous activity of this small Ca2+ releasing events will develop the muscle contraction.
In the Cav1.1ΔE29 mouse the embriotic isoform of the Cav1.1 is expressed, where it's acting as a Ca2+ channel, but also has retained its role as a voltage sensor. This isoform (Cav1.1e) shows altered gating properties and shifted voltage-dependence of activation and significantly contributes the formation of the Ca2+ transients during the depolarization. Moreover there are spontaneous Ca2+ events can be observed on isolated muscle fibers. The expression of the embriotic isoform is strongly correlates with the miotinic dystrophy disease.
During our work we characterized this spontaneous events based on their intensity profiles, distribution and their pharmacological response. There are three main groups based on their intensity characteristics on a timescale. The sparks are the short (46,0±1,5 ms), the embers are the long events (473±14 ms) and there were complex events. Furthermore, their distribution inside the muscle fiber didn't show any random distribution, the events appeared at specific locations. With raised Ca2+ content (5mM) of the external Tyrode solution several spontaneous events were observed compared with the normal Tyrode solution (1.8 mM), which represents normal physiological conditions. The Cav1.1 and RyR channel blockers are also abolished the likelihood of events development. We also examined the alterations in the gene expression where we found small changes in the level of the Ca2+-binding calsequestrin protein, located inside of the SR.

Supervisor: Beatrix Dienes

Madhura De (1st year PhD student)

SINGLE MOLECULE FRET STUDIES OF THE CHROMATOSOME

The nucleosome comprises of an octamer of histone proteins, surrounded by 146 base pairs of double helical DNA in 1.65 turns. The linker arms of DNA that extends out of this central nucleosome are associated with linker histone, a 200 amino acid protein. The nucleosome together with the linker histone is the chromatosome, the smallest unit of chromatin. Although, recent structural (X-ray crystallographic and cryo-EM) studies have shed light on the localisation of the linker histone, it is still unclear as to how the linker histone associates with the linker DNA arms. Do the conformation of the disordered C-terminal domain have a DNA sequence or linker length specificity? Our preliminary results show that the linker histone mediates a salt dependent compaction of the chromatosome. Employing single-pair FRET spectroscopy and EMSA we show that the linker histone compacts chromatosome at a salt range of 5 to 150mM, with the compaction gradually decreasing with increasing salt. By fluorescent labelling of the DNA linker arms and the linker histone, we will try to study the salt-dependent dissociation pathway of linker histone. We also plan to explore possible influence that the linker histone might have on nucleosomes bearing a mutation K27M on histone H3, that is found in paediatric glioblastomas. Course grained and atomistic simulations (unrestrained and FRET-restrained) will accompany the experiments.

Supervisor: Katalin Tóth

Gyula Diszházi (2nd year PhD student)

INVESTIGATION OF TOXIN-ION CHANNEL INTERACTIONS

Maurocalcine (MCA) is a 33-amino acid residue peptide that was initially isolated from the venom of Scorpion Maurus palmatus. The toxin is heavily charged (net charge of +12) and the charge distribution of the peptide is strongly asymmetric, creating an important dipole moment. Single-channel measurements revealed that MCA acts as a pore blocker on the sarcoplasmic reticulum (SR) calcium channel (ryanodine receptor (RyR)) and causes a long-lasting, semi-conducting state. In this process the key amino acid is the arginine in position 24, which is located in the center of the positively charged face of the toxin, because the 24 alanine mutant has not been effective. The effect of MCA resembles the pore blocking mechanism of charybdotoxin (CHTX) on KCa1.1 (BK) channel. Because there is a high structural homology between MCA and CHTX, we investigated the effect of CHTX on RyR. We found that CHTX blocked RyR in a close state in 80 nM concentration, suggesting a common binding site of the two toxins in the channels vestibule. As toxin-channel interactions are very specific, we investigated the structure homology of RyR and BK channel vestibules by testing MCA on BK channels using outside-out patch-clamp electrophysiology. However, MCA did not affect RyR function.
In silico analysis will be performed to map BK channel vestibular structure by using the high resolution structure of RyR and the toxins.

Supervisor: János Almássy

Abdennour Douida (1st year PhD student)

BALANCING THE MITOCHONDRIA BY THE PA200-PROTEASOMES

The proteasome is the major proteolytic system in the cytoplasm and nuclei of eukaryotic cells. It is required for the maintenance of protein homeostasis through the ability to degrade unfolded proteins (e.g., proteins damaged by oxidation) generated in response to environmental stressors in cells.
The activity of the proteasome depends on the interaction of the proteasome-20S core particle with distinct proteasome regulators/activators. One of these activators is the Blm10/PA200 activator family in yeast and mammals, respectively. Our previous data showed that Blm10 is required for the maintenance of functional mitochondria through its involvement of the regulated degradation of the fission protein Dnm1.
Assuming that the impact of Blm10 on Dnm1 turnover is conserved in mammals, we proposed that the upregulation of its mammalian ortholog PA200 might provide a cytoprotective function through the regulated degradation of Drp1 and is involved in the altered mitochondrial dynamics and activity.
We generated PA200 knock-down SH SY5Y human neuroblastoma cell lines (shPA200) using shRNA lentiviral technology. We looked at Drp1 turnover by cycloheximide (CHX) chase assay and have shown that PA200 is required for the correct degradation of Drp1 by the proteasome. Furthermore, we have observed that loss of PA200 leads to dramatic alteration of mitochondrial morphology and to significant changes in mRNA expression of key fission and fusion genes.
Our preliminary data by Seahorse XF analyzer have shown that cells lacking PA200 exhibit increased spare respiratory capacity, reduced coupling efficiency and ATP production. FACS data analysis indicate altered membrane potential in shPA200 cells, especially upon oligomycin treatment, but no changes were observed in mitochondrial content compared to control cells.
Taken together our data indicate that PA200 regulates mitochondrial dynamics and homeostasis by a still unknown mechanism and that loss of PA200 leads to enhanced mitochondrial dysfunction.

Supervisor: Krisztina Tar

Lina Fadel (1st year PhD student)

THE MODULATION OF NUCLEAR RECEPTOR DIMERIZATION UPON LIGAND BINDING FOLLOWED BY LOCALIZATION

Nuclear receptors are one of the most abundant classes of transcriptional regulators in metazoans, controlling the development, homeostasis and metabolism of the organism. They have the ability to directly bind to DNA and regulate the expression of adjacent genes, hence these receptors are classified as transcription factors. Receptors bind corepressors and actively repress target gene expression in the absence of ligand. Corepressors are found within multicomponent complexes that contain histone deacetylase activity. Deacetylation leads to chromatin compaction and transcriptional repression. Upon ligand binding, the receptors undergo a conformational change that allows the recruitment of multiple coactivator complexes. Some of these proteins are chromatin remodelling factors or possess histone acetylase activity, whereas others may interact directly with the basic transcriptional machinery. Recruitment of coactivator complexes to the target promoter causes chromatin decompaction and transcriptional activation. The characterization of corepressor and coactivator complexes, in concert with the identification of the specific interaction motifs in the receptors, has demonstrated the existence of a general molecular mechanism by which different receptors elicit their transcriptional responses in target genes.
In our study we examine Retinoid X Receptor and its dimerization partners RAR, PPARγ, VDR and LXR. Our objective is to show the effect of ligand binding on nuclear receptor dimerization, if it increases the stability of the dimers or leads to more dimerization, using a translocation assay, imaging fluorescence cross-correlation microscopy and FRET. For our studies we are using fluorescently labelled nuclear receptors that compete for binding to RXR. We also plan to determine the dissociation constants of the receptor dimers by FRET titration experiments. Our results may help to understand how the promiscuous RXR molecule behaves in the presence of several potential heterodimeric partners and ligands.

Supervisor: György Vámosi, Gábor Szabó

Tímea Hajdu (2nd year PhD student)

THE EFFECT OF THE MEMBRANE ENVIRONMENT AND PROTEIN CONFORMATION ON THE LIGAND BINDING OF EPIDERMAL GROWTH FACTOR RECEPTOR

The ErbB/HER family of transmembrane receptor tyrosine kinases (RTKs) takes part in various cellular responses activated by EGF and other peptide growth factors. Our aim was to find out if there is some kind of cooperativity between the two active sites of an EGFR dimer and what it depends on.
For the experiments fluorescently labelled EGF was used (TAMRA-EGF) and cells were labelled with it for 1 hour. Fluorescence intensities were measured using a FACS Aria flow cytometer. Result were fitted with the Hill-equation, in which the Hill coefficient (n) characterizes the nature of cooperativity: if it is above 1 the cooperativity is positive, if it is below 1 the cooperativity is negative. Most cell lines show positive EGF-binding cooperativity: A431 cells and EGFR-GFP-transfected A431 cells are characterized by a Hill coefficient of n=1.26 and n=1.46 respectively, while EGFR-GFP-transfected CHO cells have a coefficient of 0.98. Latrunculin that inhibits the polymerisation of actin filaments reduced the Hill coefficient to 1.04 in A431 cells. In order to examine the effect of sufficient protein glycosylation on EGF-binding, tunicamycin, known for inhibiting N-glycosylation of proteins was used leading to a decrease of the Hill coefficient to 0.71 in A431 cells.
In order to find out if the amount of EGFR expressed on cells influences the cooperativity of ligand binding, the expression level of EGFR was decreased by RNA interference. As A431 expressing GFP-labelled ErbB1 was used for these experiments, it was possible to track the decrease of expression from the intensity decrease of GFP. Populations showing lower GFP intensity were characterized by a reduced Hill coefficient compared to non-transfected cells. The effect of ligand internalisation on the apparent cooperativity of EGF-binding was also examined. Cells were labelled with fluorescent-EGF for 1 and for 24 hours on ice. One day incubation resulted in a decrease in the Hill coefficient to 1.02 in A431 cells. This reduction is most likely attributable to contamination of the membrane-bound EGF signal with intracellular fluorescence as suggested by confocal microscopic experiments.
According to our result we can conclude that proper glycosylation of EGFR and its anchoring to actin-filaments are required for the positively cooperative ligand binding. In addition, the expression level of EGFR also plays a key role in the nature of EGF-binding.

Supervisor: Péter Nagy

Laura Jankó (1st year PhD student)

THE DEPLETION OF PARP2 ALTERS MITOCHONDRIAL MORPHOLOGY

The family of poly(ADP-ribose) polymerases comprise 17 enzymes, among them poly(ADP-ribose) polymerase-2 (PARP2). The role of PARP2 was first identified in DNA repair, however, later several other functions of PARP2 were identified in different tissues. In skeletal muscle, in liver and in pancreas PARP2 is repressor of the SIRT1 promoter, so upon PARP2 deficiency SIRT1 level and activity increases and induces the mitochondrial biogenesis. We continued this track of research by assessing changes in mitochondrial morphology upon the ablation of PARP2.

In our work we examined the structure of the mitochondrial network in control and PARP2 knockdown C2C12 myoblast, in HepG2 hepatocarcinoma and in Min6 insulinoma cells. We examined changes in gene expression in case of 5 genes which are responsible for mitochondrial fusion or fission.

Mitochondrial structure changed in PARP2 knockdown cells, it became more fragmented as compared to controls. We found that the ablation of PARP2 conferred protection against hydrogen-peroxide induced mitochondrial fragmentation. We observed changes in the expression of mitochondrial fusion and fission genes upon the silencing of PARP2. We found that upon PARP2 depletion the Mitofusin1 gene expression decreases, the Mitofusin2 gene expression increases and they are responsible for the mitochondrial fusion. In case of 3 genes, which are responsible for the mitochondrial fission, we found that gene expression of Dinamin-1-like decreases, while Parkin2 gene expression increases in all cell types. In case of two cell types, Fissin1 gene expression increases, but in case of C2C12 cell type, it decreases.

Supervisor: Péter Bay

Balázs Kelemen (2nd year PhD student)

VOLATILE ANAESTHETICS INHIBIT TRMP3 ION CHANNEL

Volatile anaesthetics (VAs) are the most commonly used compounds to maintain general anaesthesia during operations, both in human therapeutic interventions and animal experiments. Their reversible loss of consciousness effect is probably exerted through some ion channels of the central nervous system, like the GABAA receptor, but VAs also affect the functioning of other channels. In the recent years studies revealed that VAs affect some nociceptive and thermosensitive members (TRPV1, TRPA1, TRPM8) of the transient receptor potential (TRP) ion channel family, which may play role in the development of several side effects of VAs (eg.: respiratory irritation).
TRPM3 is a novel thermosensitive nociceptor TRP channel, expressed by a large subset of primary sensory neurons of the dorsal root and trigeminal ganglia (DRG and TG). Beyond temperature and thermal pain sensation, TRPM3 also contributes to the development of inflammatory heat hyperalgesia and transmits chemical pain sensation evoked by its steroid ligand pregnenolone sulfate (PS).
In our study, we investigated the effect of VAs with various chemical structures (chloroform, halothane, isoflurane, sevoflurane) on recombinant TRPM3 overexpressed by HEK293T cells, and native TRPM3 in primary sensory neurons isolated from mouse DRG. The ion channel functions of TRPM3 were investigated by measuring transmembrane currents using the patch-clamp technique in whole cell configurations and by monitoring changes in intracellular Ca2+ concentration using fluorescent ion sensitive dyes (FURA-2, FLUO-4).
The investigated VAs dose dependently inhibited the transmembrane currents and Ca2+ signals evoked by TRPM3 activators PS and CIM0216 in HEK cells. In accordance with previous literature data, VAs caused Ca2+ transients in some of the sensory neurons which were independent of TRPM3 expression, however, all VAs applied in 1 mM concentration significantly inhibited the activation of TRPM3.
Our results further enhance our knowledge about the mechanism of action and non-anaesthetic effects of volatile anesthetics and provide significant new data about the pharmacological properties of TRPM3.

Supervisor: István Balázs Tóth

Éva Kókai (2nd year PhD student)

EXAMINATION OF AXON COLLATERALS OF LAMINA III NEURONS WITH LOW-THRESHOLD INPUTS IN RATS

Primary afferents terminate with a specific distribution pattern in the spinal cord. The large myelinated Abeta primary afferents with low-threshold convey non-nociceptive information, that projects mainly to laminae III-VI. By contrast, thinly myelinated Adelta and unmyelinated C afferents (nociceptive) project preferentially to more superficial laminae: Adelta fibers to lamina I, IIo and V, and C afferents to laminae I-II. Despite the importance of the dorsal horn in sensory processing under normal and also pathological conditions, we still know little about the how neuronal circuits work in this region. The main limitation is, that while neurochemical characterization of spinal cord neurons is very detailed, at the same time there is very little information on dendritic and almost none about axonal arborization of individual cells. Such information is essential for understanding the wiring inside the dorsal horn.

Recently, together with our Portuguese colleagues, using electrophysiological methods we have described a feed-forward inhibitory circuit that probably involves lamina III inhibitory neurons. The putative neurons receive low-threshold primary afferent input and relay it to lamina I, where projection neurons reside, serving as a gate for nociceptive information flow.

Our aim was to examine the detailed morphology of the recorded lamina III neurons to test the hypothesis that their axon may directly, without further intercalated neurons, reach lamina I. In order to give detailed description I used Neurolucida to do full 3D reconstruction of 9 individually recorded and filled lamina III neurons with low threshold Abeta or Adelta input.

We found that axon collaterals of 4 of the 9 reconstructed lamina III neurons reached lamina I and II providing morphological basis for their feed-forward function. In line with earlier description of dendritic morphology of lamina III neurons our cells also had extensive dendrites suggesting an integrative function. We are planning to perform similar analyses for other dorsal horn neurons to estimate the possible postsynaptic targets of different neurons in the DH circuitry.

Supervisor: Péter Szűcs

Brigitta Mészáros (1st year PhD student)

DEVELOPMENT OF A HIGH-RESOLUTION SDS SEPARATION GEL FOR PROTEIN ANALYSIS BY CGE-MS

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been the method of choice for size-based separations of proteins for over decades and plays a well-established role in protein separations and analyses in most of the biological research laboratories. However, in recent years SDS-capillary gel electrophoresis (SDS-CGE) also gained significant importance in bioanalytics, especially in the biopharmaceutical industry. SDS-CGE can be considered as the automated version of SDS-PAGE, having numerous advantages such as on-column detection, automated operation, high resolving power, and capability of accurate protein quantification as well as molecular mass determination. In the biopharmaceutical industry SDS-CGE has been recognized as an important tool in process development and quality control of proteins and glycoproteins.
SDS-CGE hyphenation with mass spectrometry (MS) analysis offers additional selectivity provided by the MS spectrum. However, SDS-CGE coupling with MS is very challenging since the separation gel and the surfactant (SDS) can contaminate the MS and may compromise its detection system. Our goal was to develop an MS friendly separation buffer and gel system for protein analysis. Major aspects, which have been taken into consideration during the development process were 1) separation gel components have to be compatible with mass spectrometer and 2) avoid non MS compatible compounds getting into the MS. The determination of an optimal protein separation gel-buffer composition was the major focus of my work with special emphasis on MS compatibility.

Supervisor: András Guttman

Noémi Miltner (3rd year PhD student)

ASSESSMENT OF THE ANTI-INFLAMMATORY EFFECTS OF NOVEL SEMI-SYNTHETIC PHYTOCANNABINOIDS IN HUMAN IN VITRO PRO-INFLAMMATORY KERATINOCYTE MODEL SYSTEMS

It is common wisdom in pharmacology that fluorination can significantly increase the efficacy of the active components in pharmaceuticals – actually, ca. 30% of the best-selling drugs worldwide contain fluorinated compounds. The laboratory of Prof. Mechoulam has recently synthesized a series of fluorinated derivatives of cannabidiol (CBD), the major non-psychoactive component of the plant Cannabis sativa. The goal of the current study was to assess the potential cutaneous anti-inflammatory actions of these compounds (F-CBDs).

Effects of CBD and F-CBDs (HUF-101, HUF-103 and HU-559a) were investigated in seven, previously established in vitro human epidermal keratinocyte models (employing the immortalized HaCaT and HPV-KER keratinocyte cell lines) which mimic, as closely as possible, various human inflammatory skin conditions and diseases (e.g. microbial, UVB-induced, allergic, contact, and atopic dermatitis). Gene expression and protein release were assessed by RT-qPCR and ELISA, respectively.

As expected, expressions of certain pro-inflammatory cytokines (e.g. IL-6 and IL-8) were significantly down-regulated upon the administration of CBD and F-CBDs in most models. Of great importance, however, all F-CBDs exhibited significantly higher efficacies in comparison to the non-fluorinated counterpart CBD, with the rank order of efficacy in the in vitro human epidermal keratinocyte models being HUF-103 > HU-559a > HUF-101.

Our study provides the first evidence that F-CBDs exert remarkable anti-inflammatory actions on human epidermal keratinocytes. These intriguing data invite further pre-clinical and clinical studies to exploit the therapeutic potential of certain F-CBDs in a various cutaneous inflammatory conditions.

Supervisor: Tamás Bíró, Johanna Mihály

Marianna Nagy (2nd year PhD student)

INVESTIGATION OF THE APPLICABILITY OF THE BRAIN ATLAS TECHNIQUE IN SPACE OCCUPYING LESIONS – AS A PRECONDITION TO CALCULATE REGIONAL HEMODYNAMIC PARAMETERS AND GENERATE PARAMETRIC MAPS

Objectives: The application of brain atlas technique is not evident in the post processing of MR images of patients with intracranial space occupying processes, since brain structures are shifted due to the mass effect of the lesion. Therefore, the fitting of gray and white matter structures to atlas templates around the affected areas can only be expected to be partially accurate. This work was required for the determination of regional hemodynamic parameters and for the generation of hemodynamic response curves. Our goals were to characterize the accuracy of spatial normalization of T1-weighted MR images of stroke patients and control subjects; to investigate how it is possible to use the Dynamical Causal Modelling (DCM) software to calculate regional hemodynamic parameters and generate hemodynamic parametric maps.
Materials and methods: The accuracy of fitting was measured by the spatial variability in the atlas space of 12 predefined regions drawn on the patient images. Five software packages have been used for the spatial standardization. Functional MRI data from stroke patients were included in this study for the calculation of regional hemodynamic parameters. The Human Connectome Project's data was also used to generate hemodynamic parametric maps from the averaged resting state fMRI images of the previously selected groups.
Results: One of the goals of this study has been completed, the spatial normalization accuracy measuring framework has been developed and tested. We found that the hemodynamic parameters are significantly different in the healthy and the paretic sides. Hemodynamic parameter maps were generated and it can be stated that some areas of the brain the hemodynamic responses are different.
Conclusion: There are three strong points of this work: the extensible image database and the modular and scalable software. The DCM method can be used to calculate the regional hemodynamic parameters by the Balloon model, and the values of these parameters just slightly depend on the network structure. The significant differences of hemodynamic responses will be localized in various intracranial diseases in individual and group levels.

Supervisor: Miklós Emri

Zsanett Sári (1st year PhD student)

EXAMINATION OF THE RELATIONSHIP BETWEEN HUMAN MICROBIOME AND BREAST CANCER CELLS

The human body habors a large number of bacteria, that is referred as the normal flora of the organism and its collective genome as the microbiome. There is a dynamic bidirectional interaction between the host and the microbiome, which has an effect on the microbial composition. Microbiome changes are associated with changes in the microbial metabolome and through that modulate metabolic physiology such as insulin sensitivity, furthermore contributes to the development of various inflammatory and metabolic diseases (obesity, insulin resistance and type 2 diabetes). These changes are individual risk factors of cancers.
Our aim with the current study was to investigate a potential causal link between changes in the microbiome, microbiome derived metabolites and breast cancer.
In my experiments we have examined some of the bacterial secreted metabolites that have known metabolic effects in human. I have tested indole derivatives, for example indoxyl sulfate and indolepropionic acid, as well as the cholesterin derivatives cholestanol on mouse and human breast cancer cell lines.
We investigated the effect of these metabolites on tumor cell proliferation, and determined the ratio of apoptotic and necrotic cells. Changes in the cell to cell interactions were studied with actin staining. Furthermore, we examined the expression pattern of stool samples from breast cancer patients. We found several metabolites that inhibited the proliferation of breast tumor cells but were not toxic. In the proliferation inhibition Mesenchymal-Endothelial Transition had a central role.

Supervisor: Péter Bay

Ádám Sipos (1st year PhD student)

NEW TARGET FOR GLYCOGEN PHOSPHORYLASE INHIBITORS: SODIUM-GLUCOSE CO-TRANSPORTER OF KIDNEY

Diabetes is a prevalent disease, afflicting approximately 6-20% of the adult population in Western industrialized societies. More than 420 million people world-wide are afflicted with type 2 diabetes, accounting for ~95% of cases. Type 2 diabetes is a major public health problem with severe complications, including cardiovascular disease, neuropathy, retinopathy, and nephropathy. Tight control of blood glucose levels prevents and delays the onset of diabetic complications, but such control is often hard to achieve with oral antidiabetic agents. While many drugs are available for the treatment of type 2 diabetes, glycaemic control remains a problem in diabetic patients and the development of novel drugs is necessary.
Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a new class of diabetic medications indicated only for the treatment of type 2 diabetes. SGLT-s are a group of intracellular glucose transporters in mammalian cells, mediates the glucose reabsorption into the blood. Inhibitors of SGLT2 can decrease the amount of reabsorbed glucose and increase the concentration in the urine leading to decrease blood sugar levels in diabetic patients. However, the European Medicines Agency and Federal Drug Administration have confirmed recommendations to minimise the risk of diabetic ketoacidosis in patients taking SGLT2 inhibitors. Thus, there is a need for the development of new SGLT2 inhibitors with less risk of ketoacidosis.
The common scaffold of clinically tested SGLT2 inhibitors (among them six marketed antidiabetic drugs approved in the last four years) consists of a glucose moiety and aromatic rings with alkyl spacers. Several glucose analogue inhibitors for glycogen phosphorylase (GP) synthesized by our collaborators and tested by our group share a similar structural arrangement. Therefore, we would like to investigate whether these structurally related compounds could inhibit both GP and SLGT2. Further glucose-analogues will be synthesized to test their inhibitory capacity for both GP and SLGT2. We hypothesize that dual inhibition of GP and SGLT2 will eliminate the ketoacidosis related to GP inhibition by mobilizing endogenous insulin.

Supervisor: Tibor Docsa

Adrienn Skopál (1st year PhD student)
THE ROLE OF ADENOSINE 2A RECEPTOR IN THE VESICULAR TRAFFICKING OF MACROPHAGES

Macrophages play an important role in the formation of immune responses and they have a prominent function in inflammatory processes. These cells participate in the cellular and humoral immune response. Within the framework of the humoral immune response they also present antigens. They are able to absorb pathogens by phagocytosis as well as take part in receptor-mediated endocytosis. The damage in the vesicular transport mechanism leads to the deficiency of membrane repair mechanism, consequently developing apoptosis, necrosis and lysosomal permeabilization or disruption.
Adenosine regulates macrophage cytokine production in inflammatory processes. It reduces the level of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and enhances the production of anti-inflammatory cytokines (IL-4, IL-10). Macrophages express the adenosine 2A (A2A) receptors which have already demonstrated its role in regulating anti-inflammatory processes. Our work group previously identified the functional interaction between A2A receptor and Cathepsin D (CtsD) lysosomal protease in mouse intraperitoneal macrophages (IPMϕ). The co-localization between them was confirmed in RAW 264.7 cells by confocal microscopy. After that we identified several proteins as putative interacting partners of A2A C-terminal domain by immunoprecipitation which involved in the regulation of vesicular trafficking. Our aim is to investigate the effect of A2A receptor activation on vesicular trafficking mechanism of macrophages.
During my work I also examined and confirmed the co-localization of A2A receptor and CtsD protease in IPMϕ. In the interest of study the changes in localization and expression of A2A receptor I applied aspartyl protease inhibitor treatment. I investigated the appearance of the lysosomal-associated membrane protein-2 (LAMP-2) on the cell surface via A2A receptor activation. LAMP-2 is a marker of membrane repair. It has a role in trafficking, vesicle fusion and cholesterol homeostasis. I detected increased level of LAMP-2 protein on the cell surfaces after the lipopolysaccharide (LPS) activation of macrophages compared to the untreated control cells. A2A receptor agonist treatment decreased the presence of LAMP-2 on the cell surfaces compared to the LPS treated cells. In order to follow the recycling of A2A receptor and its co-localization with putative intracellular interactor proteins we created pmax-GFP-A2A mammalian expression construct and we could express the GFP-A2A fusion proteins in macrophage cell line.

Supervisor: Endre Kókai

Krisztina Szabó (1st year PhD student)

PROTEOMIC ANALYSIS OF PROTEIN PHOSPHATASE Z1 FROM CANDIDA ALBICANS

Protein phosphatase Z1 is a novel type, fungus specific serine/threonine phosphatase. In Candida albicans there is a single CaPPZ1 gene that codes for the CaPpz1 protein, which has important physiological roles according to previous gene deletion studies. In order to reveal the proteomic consequences of the absence of a functionally competent CaPPZ1 gene we adopted a proteomic method and compared the cappz1 deletion mutant with the genetically matching QMY23 control strain. Proteins extracted from the two strains were separated by two dimensional gel electrophoresis and the protein spots were stained with RuBPS and Pro-Q Diamond to visualise the proteome and the phosphoproteome of the samples, respectively. The spots which showed significant alterations were cut out and their protein content was identified by LC-MS/MS mass spectrometry. 15 proteins were found that exhibited alterations in their amounts and 20 proteins were detected whose phosphorylation levels were modified in the deletion mutant strain. RT-qPCR data revealed that the expression of these protein coding genes were not altered significantly by the phosphatase deletion. In agreement with previous findings the affected proteins are involved in protein synthesis, oxidative stress response, regulation of morphology and metabolism. In addition from the proteomic data we deduced a role for the CaPPZ1 gene in biofilm formation. We validated the latter hypothesis with experiments, thus we identified a novel function for the CaPpz1 phosphatase in C. albicans. This work was supported by OTKA grant K108989.

Supervisor: Viktor Béla Dombrádi

Máté Szarka (2nd year PhD student)

ONBOARD ANALYSIS OF ASTRONAUT'S SERUM IMMUNOGLOBULINS AS A MARKER OF AGING ON DEEP SPACE MISSIONS; A NEW FRONTIER IN CAPILLARY ELECTROPHORESIS

On a roundtrip (~360 days) to Mars, astronauts would be exposed to approximately 0.66 Sv (1) radiation dose, mainly due to Galactic Cosmic Rays (GCR) and Solar Energetic Particles (SEP). This amount of exposure exceeds the lifetime limits here on Earth according to RAD measurements (1). Previous studies have shown that after extensive radiation exposure, inflammatory proteins such as citokines are induced in the human body. In general, radiation modulates the hematopoietic system (2). Under the exposure to strongly ionizing radiation, serum glycoprotein (IgG and IgM) levels in the blood initially drastically decrease even to hypogammaglobulinemic levels but in a long term, a regenerative hypercompensation is observed (3). Specific glycosylation patterns of these immunoglobulins, especially IgG have already been associated with various ailments such as chronic inflammation, autoimmune diseases and malignant transformation just to mention a few. Moreover glycan based biomarkers indicating biological age (4) are of significant interest in the monitoring of age-related diseases known to accelerate in space. The focus of our work is to profile the IgG glycans after medium-energy proton irradiation to reveal the resulting possible glycosylation profile changes. Structural elucidation will be accomplished by our small and lightweight image analysis based LED induced capillary electrophoresis system to decipher the effects and threats of SEP on possible physiological changes of future astronauts during long duration missions beyond the shielding environment of Low Earth Orbit (LEO).

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Supervisor: András Guttman

Emese Tóth (3rd year PhD student)

REGULATION OF MYOSIN PHOSPHATASE HOLOENZYME BY NUCLEOCYTOPLASMIC TRAFFICKING OF THE MYPT1 REGULATORY SUBUNIT

Protein phosphatase 1 (PP1)-type holoenymes consist of a catalytic subunit (called PP1c) complexed with variable regulatory subunits, which determine substrate specificity, phosphatase activity and localisation of the holoenzyme. MYPT1, the regulatory subunit of the PP1-type myosin phosphatase holoenzyme (MP) is generally distributed between the nucleus and the cytoplasm of cells. Besides myosin, MP is implicated in the dephosphorylation of several other cytoplasmic and nuclear substrates, such as retinoblastoma protein, SNAP-25 or eNOS. These diverse functions suppose a dynamic MYPT1, which can shuttle in and out of the nucleus of cells, but the mechanism of its nuclear translocation was not examined yet.
In THP-1 leukemic cells MYPT1 is localized principally in the nucleus and upon phosphatase inhibition by calyculin-A (CLA) it translocates to the cytoplasm. Meanwhile, the subcellular distribution of PP1c remains unchanged, suggesting that nucleocytoplasmic shuttling of MYPT1 requires dissociation from PP1c. To identify the phosphorylation site that may mediate the localization of MYPT1 immunoprecipitation of Flag-MYPT1 from untreated and CLA-treated cells and subsequent phosphoproteomic analysis was performed, which detected Ser20 as the only phosphorylation site in the N-terminal region of MYPT1 where the nuclear localization signal sequence and the PP1c-binding motif are also located. Ser20 was predicted to be phosphorylated by casein kinase 2 (CK2), but the ability of CK2 to phosphorylate MYPT1 was not investigated yet. Recombinant GST-CK2 was able to phosphorylate full-length MYPT1 as well as N-terminal fragments containing Ser20 residue, proving that Ser20 may be a target for CK2. The CK2 inhibitor TBBt decreased the CLA-induced translocation of MYPT1 in THP-1 cells. These data suggest that CK2 might be involved in the regulation of nucleocytoplasmic trafficking of MYPT1 via phosphorylation of Ser20.
Supported by OTKA K109249 and PD111715.

Supervisor: Andrea Kiss

Kinga Fanni Tóth (1st year PhD student)

THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR FLUOXETINE EXERTS ANTI-INFLAMMATORY ACTIONS ON HUMAN EPIDERMAL KERATINOCYTES

By releasing several cytokines and mediators (e.g. endothelin 1 [ET1]), keratinocytes are key players in orchestrating cutaneous inflammation and itch. The selective serotonin reuptake inhibitor (SSRI) fluoxetine (FL), a widely applied antidepressant, has already been shown to exert anti-inflammatory actions in mice; thus, in the current study, we assessed putative anti-inflammatory effects of selected SSRIs (FL, citalopram [CP] and paroxetine [PX]) as well as serotonin (ST) on immortalized (HaCaT) and primary (NHEK) human epidermal keratinocytes.

We found that, although high (>10 μM) concentrations of FL and PX (but not CP or ST) decreased viability (48-72h), non-cytotoxic doses of FL and PX (but again, not CP or ST) suppressed poly-(I:C)-induced elevation of the production of several pro-inflammatory cytokines (HaCaT; Q-PCR and ELISA). Moreover, FL (but not ST) prevented poly-(I:C)-induced ET1 release (HaCaT; ELISA), exerted anti-proliferative actions (HaCaT; CyQUANT assay), and prevented confluence and high extracellular Ca2+ concentration-induced up-regulation of several differentiation markers (HaCaT and NHEK; Q-PCR); however the latter effect exhibited great donor dependence at the protein level (NHEK; Western blot). Finally, we found that both FL and ST inhibited poly-(I:C)-induced activation of p38 MAPK, but had no effect on the NF-κB pathway (HaCaT; Western blot), and our preliminary data suggest that FL (but not ST) may induce Ca2+-influx to the keratinocytes (HaCaT; Fluo-4 AM).

Collectively, FL exerted anti-inflammatory effects, and suppressed the release of ET1 of the keratinocytes in a most probably ST receptor independent way. Considering its excellent safety profile, these data may encourage one to test efficiency of its topical formulations in alleviating cutaneous inflammation and itch in appropriate clinical trials.

Supervisor: Tamás Bíró

Tamás Ungvári (2nd year PhD student)

PERRIN AND FÖRSTER UNIFIED: DUAL-LASER TRIPLE-POLARIZATION FRET (3POLFRET) FOR INTERACTIONS AT THE FÖRSTER-DISTANCE AND BEYOND

Dual laser flow cytometric energy transfer (FCET) – elaborated by Trón et al in 1984 – is an efficient and rapid way of measuring FRET on large cell populations. FRET efficiency and the donor and acceptor concentrations are determined from one donor and two acceptor signals. In this communication this method is extended towards the domain of receptor dynamics by the detection of polarized components of the three intensities. By enabling a complete description of the proximity and dynamics of FRET-systems, the new measuring scheme allows a more refined description of both the structure and dynamics of cell surface receptor clusters at the nano-scale and beyond. Associated donor fraction, limiting anisotropy and rotational correlation time of the donor, and cell-by-cell estimation of the orientation factor for FRET ($\kappa 2$) are available in the steady state on a single FRET sample in a very rapid and statistically efficient way offered by flow cytometry. For a more sensitive detection of conformational changes the "polarized FRET indices" – quantities composed from FRET efficiency and anisotropies – are proposed. The method is illustrated by measurements on a FRET system with changing FRET-fraction and on a two donor-one acceptor-system, when the existence of receptor trimers are proven by the detection of "hetero-FRET induced homo-FRET relief", i.e. the diminishing of homo-FRET between the two donors in the presence of a donor quencher. The method also offers higher sensitivity for assessing conformational changes at the nano-scale, due to its capability for the simultaneous detection of changes of proximity and relative orientations of the FRET donor and acceptor. Although the method has been introduced in the context of FRET, it is more general: It can be used for monitoring triple-anisotropy correlations also in those cases when FRET actually does not occur, e.g. for interactions occuring beyond the Förster-distance R0. Accodingly, interpretation of $\kappa 2$ has also been extended.

Supervisor: László Bene

Vágó Judit (2nd year PhD student)

INVESTIGATING THE FUNCTIONAL AND MORPHOLOGICAL CONNECTIONS BETWEEN SEPTINS AND PRIMARY CILIA IN CHONDRIFYING EXPERIMENTAL MODELS

Septins are guanosine triphosphatases that belong to a highly conserved family of proteins in eukaryotes. They form hetero-oligomeric complexes and interact with the actin-based cytoskeleton and microtubules. They are involved in forming diffusion barriers for example at the base of primary cilia. Primary cilium is a solitary protrusion of cells which functions as a cellular antenna. It can detect mechanical and chemical signals of the environment and transmit it into the cells. It is already proved that septins are key members of ciliogenesis and also essential in the physical and functional separation of the primary cilium from other cellular compartments. Previous studies have shown that defects in primary cilia can result in skeletal dysplasia. The purpose of our study is to investigate the role of primary cilia in chondrogenesis as well as exploring connections between septins and the integrity and function of primary cilia of developing chondrocytes.
Our experiments were carried out on bone marrow stroma-derived human mesenchymal pluripotent stem cells (hMSC) differentiated as monolayer and high density cultures. First, we examined the expression of different septins at mRNA and protein levels by RT-PCR, Western blot and immunocytochemistry. In order to study the connection between septins and chondrogenesis, high density cultures were treated with the inhibitor of septin remodeling, forchlorfenuron (FCF). We compared chondrogenesis in control to FCF-treated cultures by chondrogenic marker analysis and histological staining. Cell proliferation and viability assays were also carried out. We have started studying the expression of different primary cilium marker genes in hMSC monolayer cultures by qPCR.
In monolayer cultures, hMSCs expressed various kinds of septins. FCF treatment in micromass cultures decreased the expression levels of chondrogenic marker genes, reduced proliferation and mitochondrial activity of differentiating chondrocytes, and affected chondrogenesis in a negative way. Surprisingly, FCF treatment caused either significant increase or decrease in the expression levels of different primary cilium marker genes.

Supervisor: Zákány Róza

Roland Veress (2nd year PhD student)

THE ROLE OF INA,L IN ACTION POTENTIAL DEVELOPMENT IN VENTRICULAR MYOCARDIAL CELLS

Membrane excitation leads to rapid voltage dependent activation and inactivation of Na+ channels and nearly complete “turning off” of the current. A transient, or peak Na current is observed and is chiefly responsible for the rapid action potential upstroke. A second component of Na+ current that persists throughout the duration of the action potential, is termed late INa (INaL). INaL occurs throughout the low conductance phase of the action potential and thus contributes to action potential morphology, plateau potentials, and AP duration in human ventricular myocytes and Na+ buildup in cardiac cells.
In some pathological settings INaL is upregulated, which may disrupt the repolarization phase of the action potential and lead to the development of arrhythmia triggers. INaL, has been linked to disease manifestation in inherited and acquired cardiac diseases including LQT3 syndrome and heart failure.
Our aim was to determine the role of INa,L in AP morphology and to study the current itself using a specific INa,L blocker, GS-458967. We measured AP parameters: short term variability (SV), duration at 90% (APD90), amplitude (APA), resting membrane potential (RMP), peak, maximum velocity (Vmax), plato potential at 20 and 50% (Plato20/50), phase one amplitude and slope.
All electrophysiological measurements performed in canine left ventricular myocytes. During action potential measurements the cells were paced through the recording electrode at steady cycle length of 0.3-2s. Transmembrane ion currents were recorded using action potential voltage clamp technique, at 0.7s cycle length. INa,L was dissected using GS-458967 and presented as IGS-458967.
Our AP measurement results at pacing length 1s: SV decreased by 18%, APD90 by 15%, RMP by 5%, APA by 15%, Vmax by 44%, -Vmax 12%, Phase1 amplitude by 47%, Phase1 slope by 53%, Plato20 by 6 mV, Plato50 by 6 mV, peak by 12mV.
Our results reveal the role of INa,L in AP development in ventricular myocardial cells. They may have important therapeutic implications as they help to better understand cardiac arrhythmias and thus develop new, more effective antiarrhythmic strategies.

Supervisor: Balázs Horváth

Orsolya Vörös (3rd year PhD student)

THE C-TERMINAL HRET SEQUENCE OF KV1.3 REGULATES GATING RATHER THAN TARGETING OF KV1.3 TO THE PLASMA MEMBRANE

Kv1.3 channels are expressed in several cell types including immune cells, such as T lymphocytes. The targeting of Kv1.3 to the plasma membrane is essential for T cell clonal expansion and assumed to be guided by the C-terminus of the channel. Using two point mutants of Kv1.3 with remarkably different features compared to the wild-type Kv1.3 (A413V and H399K having fast inactivation kinetics and tetraethylammonium-insensitivity, respectively) we showed that both Kv1.3 channel variants target to the membrane when the C-terminus was truncated right after the conserved HRET sequence and produce currents identical to those with a full-length C-terminus. The truncation before the HRET sequence (NOHRET channels) resulted in membrane-targeting but non-functional phenotypes. NOHRET channels did not display gating currents, and coexpression with wild-type Kv1.3 did not rescue the NOHRET-A413V phenotype, no heteromeric current was observed. Interestingly, mutants of wild-type Kv1.3 without or with five alanine substituted for the HRET(E) motif expressed current indistinguishable from the wild-type. These results demonstrate that the C-terminal region of Kv1.3 immediately proximal to the S6 helix is required for the activation gating and conduction, whereas distal region of the C terminus is not required for trafficking of Kv1.3 to the plasma membrane.

Supervisor: Péter Hajdu

Florina Zákány (3rd year PhD student)

CHARACTERIZING THE EFFECTS OF CHOLESTEROL AND 7-DEHYDROCHOLESTEROL ON THE GATING OF KV1.3 AND KV10.1 ION CHANNELS USING VOLTAGE-CLAMP FUORIMETRY TECHNIQUE

Voltage gated ion channels contain a voltage sensing (VSD) and a pore forming domain (PD). The movement of the VSD induced by depolarization is transmitted to the PD, and causes the opening of the channel. This mechanical coupling can be tight (Kv1.3) or loose (Kv10.1), which influences the physiological function of the ion channel.
Membrane lipids can affect the gating of voltage sensitive ion channels through different non-specific and specific mechanisms. It has been shown that cholesterol and 7-DHC have remarkable effects on the gating of Kv1.3: loading of the cells with these sterols results in a shift in voltage-dependency of activation and an elongated time course of activation. It is not known whether these effects are mediated by the actions through the VSD or the PD. Our aim was to investigate whether the major target of the action of cholesterol and 7-DHC is on the VSD, PD or the coupling between these two domains. To test the specificity of the effect we carried out our measurements on Kv1.3 and Kv10.1.
The RNAs of the Kv1.3 and Kv10.1 channels were injected into Xenopus laevis oocytes, current recordings were performed by VCF. The main advantage of this technique is that simultaneously with current recordings we can monitor the movement of the VSD by labeling a cysteine residue on the S3-S4 extracellular linker with an MTS-TAMRA dye.
During the electrophysiological measurements we determined the voltage dependency of activation and inactivation, the activation time constant based on current recordings, and the F-V curves determined from the fluorescent signal in control and sterol-loaded cells. In the oocyte expression system we were able to successfully reproduce the shift in voltage-dependency of activation and an elongated time course of activation in the case of Kv1.3 and as a novel finding we obtained similar results with Kv10.1. We found no voltage shifts in F-V curves in either ion channels, but the slopes of the F-V curves were decreased in both cases.
These results suggest that the cholesterol and 7-DHC exert their effects by acting on the PD and/or the coupling mechanism, instead of influencing the voltage sensor.

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