Volume 61(1):105-118, 2017
Acta Biologica Szegediensis
http://www2.sci.u-szeged.hu/ABS

DISSERTATION SUMMARIES

H2S confers colonoprotection against TNBS-induced colitis by HO-1 upregulation in rats

Amin Al-awar

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Ulcerative colitis (UC) is a chronic debilitating disease requiring patient hospitalization; however, despite rapidly developing new therapies there are unanswered questions regarding causes, course, treatments and outcomes. The standard approach to treatment is administration of intravenous steroids, but about 40% of patients fail to respond to this. The conventional UC therapy including aminosalicylates, corticosteroids, immune modulators, are associated with a limited response, loss of response, or specific side effects and ~ 25% of patients may undergo colectomy due to the failure of treatment. The advances in understanding the pathogenesis and drug response of UC led to multiple efforts to develop specific therapies, which could target unique elements mediating disease-related immunopathology.

Hydrogen sulfide (H_2S) is an endogenous mediator that contributes to many important physiological processes including vasodilation and vascular smooth muscle relaxation; in turn, preventing tissue damage and reducing inflammation. In the gastrointestinal tract, H_2S production is vital for the maintenance of mucosal integrity and for promoting the resolution of inflammation and healing of ulcers. Heme oxygenase (HO) enzymes, of which HO-1 is inducible to harmful stimuli, was found to regulate intestinal inflammation in experimental animal models. Thus, our aim was to investigate the protective effects of H_2S against 2,4,6-trinitrobenzenesulfonic acid (TNBS) - induced colitis in rats, and whether HO enzyme system is involved in the H_2S- induced colonic cytoprotection. Male Wistar rats were treated with TNBS to induce colitis, and H_2S donor (Lawesson's reagent) was prepared 2 times/day at different concentrations, and delivered per os (from day 1 to 3). Colon samples were collected after 72 hours of TNBS treatment, to measure the extent of inflammation, myeloperoxidase (MPO) level, HO activity, tumor necrosis factor-α (TNF-α) content, protein expression, CBS and CSE enzyme activities. In addition, separate experiments were conducted to test the inhibitory effect of HO, parallel to TNBS intracolonic (i.c.) administration and co-treatment with H_2S donor. Daily treatment (2 times/day) with H_2S donor could significantly decrease the extent of colonic inflammation compared to vehicle-treatment, and the most effective daily dose of H_2S donor against inflammation was 18.75 µM/kg/day. Per os administration of H_2S donor, reduced TNBS-provoked MPO activity and TNF-α level, while increased the colonic HO enzyme activity. In contrary, the protective effect of H_2S was abolished by the co-treatment with HO inhibitor. CBS and CSE enzyme activity decreased insignificantly in EtOH and TNBS treated groups, while significant increase was shown on the protein level. Our findings suggest that H_2S confers colonoprotection, probably by modulation of inflammatory parameters and HO enzyme activity.

Supervisor: Krisztina Kupai
E-mail: amin@expbio.bio.u-szeged.hu

In vitro somatic embryogenesis on *Arabidopsis* root explants

Dóra Bernula

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

In higher plants, several developmental pathways can lead to embryo formation. Somatic embryogenesis (SE) can be induced from vegetative (sporophytic) plant cells *in vitro* by exposing explants to plant hormones (*e.g.,* auxins) and/or stress treatments. *Arabidopsis thaliana* is the most widely used model of plant developmental biology including *in vitro* plant regeneration. However, until now SE research in *Arabidopsis* was mostly limited to direct or indirect SE from immature/mature zygotic embryos due to the absence of an efficient and easy system using more differentiated tissues. Our aim was firstly to establish an efficient experimental system for studying SE *in vitro Arabidopsis* root cultures. In this system the emergence of the lateral root primordia was induced by auxin and the transdifferentiation of this primordia to shoot meristem could be induced on cytokinin-containing medium. We observed that if cytokinin-induced roots were transferred onto hormone-free medium at appropriate time points, somatic embryos appeared on the root surface instead of shoots. To characterize this system, molecular (gene expression measurement by RT-qPCR for the genes *ARABIDOPSIS THALIANA SEED GENE 1* (*ATS1), FUSCA 3 (FUS3), WUSCHEL (WUS) and LEAFY COTYLEDON 1 (LEC1)*) and cellular techniques (stereomicroscope, scanning electron microscope) were used. Furthermore, several embryogenesis marker lines and mutants (*lec1* and *fus3*) were created or collected for functional studies. Our results indicated that this *in vitro* root system can indeed be used for SE induction in a predictable way. In many

in vitro SE systems, the most common inducer is the auxin, however, in our system SE was induced by cytokinin. Therefore, we examined the effect of endogenous auxin transported from the shoot to the root on cytokinin-mediated SE formation on the roots of whole seedlings. We could show that blocking the auxin transport by the application of the auxin transport inhibitor TIBA (2,4,6-triiodobenzoic acid) or removing the shoot resulted more efficient cytokinin-induced embryo formation on induced *Arabidopsis* seedlings. Our results highlighted the importance of proper cytokinin/auxin balance for the parallel induction of shoot and root meristems during SE.

Supervisors: Attila Fehér, Katalin Gémes
E-mail: bernulad@gmail.com

Microbial degradation of hydrophobic organic compounds in polluted environments

Attila Bodor

Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Pollution of soils and waters by petroleum products or other oil-related compounds are still among the major environmental concerns due to accidental oil-spills or reckless human activity. These hydrophobic, organic compounds may represent serious risks to natural communities or human health. Several physicochemical and biological technologies are available in order, to remove these pollutants from the environment, but these methods still need further developments. Bacterial communities occur in aqueous and even in oil phases so isolation and examination of bacterial strains with the ability of hydrocarbon degradation from these oily environments can provide a promising tool for biological remediation and better understanding of the impact on structure and composition of microbial communities in oil-polluted niches. Aiming the bioremediation of a railway station area contaminated with engine oil containing long chain compounds, hydrocarbon-degrading bacterial strains were isolated from mazut, which is the bottom product of the atmospheric distillation process of crude oil. These new isolates were compared to other oil-degraders can be found in our departmental strain collection. Further small-scale biodegradation experiments were performed in the oil-contaminated soil to model and monitor a bioremediation process. Although, the optimal conditions of biodegradation are still barely revealed and need development, our results represent a targeted tool for bioconversion of petroleum products and on the other hand, brings us closer to our final goal, which is the complete bioremediation of an oil-polluted railway station area.

Supervisor: Katalin Perei
E-mail: bodor.attila@gmail.com

Salt stress-induced changes in ROS metabolism in wild type and ethylene receptor mutant *Never ripe* tomato: response of plants to exogenous ethylene precursor ACC

Péter Gábor Borbély

Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

The synthesis of the gaseous plant hormone ethylene (ET) is readily activated when plant tissues are encountered with supra optimal salt concentrations. To reveal the effects of excess ET without stress, we investigated the most important physiological functions in wild type (WT) tomato (*Solanum lycopersicum* cv. Rio Fuego) exposed to immediate precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), in hydroponic culture. ACC is oxidised to ET by ACC oxidase that maintains elevated ethylene levels in treated plants. ACC applied at 0.01-100 µM concentrations caused significant changes in dry mass, ion accumulation and photosynthetic activity of plants during 7 days. In other words, ET generated from exogenous ACC may interfere with both ionic and osmotic components of salt stress. In order to reveal the physiological action of salt stress-induced ET, the effects of 100 mM, sublethal and 250 mM, lethal NaCl concentrations were investigated in WT and *Never ripe* (*Nr*) mutants of tomato (*S. lycopersicum* cv. Ailsa Craig). *Nr* mutants have a non-functional ET receptor; thus, they significantly lost the capacity to respond to ET in all tissues. The salt stress induced ET production both in WT and *Nr* plants. In contrast to WT plants, reduced K^+/Na^+ ratio was observed in *Nr* roots under sublethal salt stress, suggesting that the inhibition of ethylene signalling increased ionic stress in tomato exposed to high salinity. The nitro-oxidative stress was stronger in *Nr* roots, which

led to the programmed death of cells in root tips. In the leaves, $O_2^{\cdot-}$ accumulation was also significantly higher under sublethal salt stress in *Nr* mutants. Since $O_2^{\cdot-}$ production is mainly associated with the activity of photosystem I (PSI) in photosynthesizing tissues, PSI activities of *Nr* and WT leaves were determined. In *Nr* plants we found higher expression of FeSOD and Cu/ZnSOD localized to chloroplast and that of MnSOD localized to mitochondria, while the activity of the chloroplastic enzymes was also higher in the mutants. However, ET elevated artificially by 10 μM ACC (applied simultaneously with NaCl) caused significant changes in $O_2^{\cdot-}$ production in the leaves of WT plants and altered ROS, NO and $ONOO^-$ levels under salt stress.

Supervisor: Irma Tari
E-mail: borbely.peter01@gmail.com

How plants connect nutrient and energy status to growth regulatory mechanisms: regulatory links between energy sensor SnRK1 and growth regulatory E2F-RBR pathway

Márta Deli

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Coupling growth and cell proliferation with the available nutrient and energy supply is fundamental for cellular homeostasis, but in plants the mechanisms are little understood. Based on our data together with recent development in this field, RBR, the single retinoblastoma-related protein in *Arabidopsis* is in the focal point of many signalling events involving nutrients, energy and light for fine-tuning the rate of growth and proliferation. Similarly, to animal Rb proteins, the plant RBR is predominantly regulated on a post-translational level, mostly by phosphorylation. Previously we have shown that sucrose can stimulate RBR phosphorylation through a well-conserved cell cycle regulatory mechanism including cyclin-dependent kinases as the major players. Here we found that light can rapidly stimulate RBR phosphorylation, but only in the presence of functional chloroplasts. We suggest that the energy sensor SnRK1 could be involved in this regulation, but whether directly or indirectly is yet unknown. We demonstrated that SnRK1 is present in different protein complexes to regulate diverse processes in young developing seedlings. In nutrient limited conditions, we identified II class trehalose phosphate synthase (TPS) proteins as integral parts of the SnRK1 complex. In contrast to the stress-related SnRK1, RBR was found to associate with SnRK1 in non-stressed conditions. We suggest that RBR with SnRK1 could regulate normal developmental processes such as meristem maintenance. The RBR protein was sensitive to carbon and energy starvation and AKIN10 could regulate the protein abundance of RBR. Altogether our data indicates that RBR and SnRK1 are intimately connected in many effective ways.

Supervisor: Zoltán Magyar
E-mail: delimarti@hotmail.com

ELONGATED HYPOCOTYL 5 mediates light signaling to the plant circadian clock

Orsolya Katalin Dobos

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Circadian clocks are timing mechanisms providing daily rhythms for a wide range of molecular and physiological processes. Eukaryotic clocks are built on gene networks, where clock genes and the encoded clock proteins form interlocked regulatory loops capable of autonomous 24-h oscillations at the level of gene expression. Importantly, this primary oscillation is synchronized with the environmental light/dark cycles by light signals, which are perceived by wavelength-specific photoreceptors and eventually cause a change in the abundance or the activity of selected clock components. In plants, several clock genes are light-responsive offering entry points for synchronizing light stimuli. However, the final molecular steps of this process are poorly understood. ELONGATED HYPOCOTYL 5 (HY5), a bZIP transcription factor functions downstream of several photoreceptors and regulates the expression of more than a thousand responsive genes. Here we show that the function of the clock is impaired in hy5 mutants displaying short period rhythms in a blue light-dependent manner. We demonstrate that the phenotype arises from blue light-induced accumulation of HY5 in wild-type plants that is mediated by transcriptional and post-transcriptional mechanisms. We demonstrate that the blue light-induced change in HY5 levels modulates its binding to specific promoter elements of selected clock genes, activating or repressing their transcription. The HY5-dependent change in the expression level

of these genes is consistent with the period phenotype of hy5 mutants. Taken together, we identify HY5 as a component of the light-quality signaling pathway to the circadian oscillator in plants.

Supervisor: László Kozma-Bognár
E-mail: orsolya.dobos91@gmail.com

Characterization of regulatory genes controlling abiotic stress responses in higher plants

Dóra Faragó

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Extremophile plants are valuable sources of genes conferring tolerance traits, which can be explored to improve stress tolerance of crops. *Lepidium crassifolium* is a halophytic relative of the model plant *Arabidopsis thaliana*, and displays tolerance to salt, osmotic and oxidative stresses. During the identification of stress tolerance genes, the large-scale phenomics screen is inescapable process and requires an extremely high throughput. To identify stress tolerance genes from an extremophile plant, we have employed the modified Conditional cDNA Overexpression System and transferred a cDNA library from the halophyte *L. crassifolium* to the glycophyte *A. thaliana*. By screening for salt, osmotic and oxidative stress tolerance through *in vitro* growth assays and non-destructive chlorophyll fluorescence imaging, 20 *Arabidopsis* lines were identified with superior performance under restrictive conditions. Several cDNA inserts were cloned and confirmed to be responsible for the enhanced tolerance by analysing independent transgenic lines (Rigó et al. 2016). One of these insertion, rendered remarkable tolerance to paraquat and encoded a small protein (69 amino acid), with closest similarity to the predicted gene product of *AT3G52105* (92% identity), a gene with unknown function. For detailed characterization we introduced and overexpressed this ortholog gene in transgenic *Arabidopsis* plants. Our result showed this hypothetical protein resulted the same paraquat tolerance. To validate stress tolerance of the transgenic plants overexpressing the identified *Lepidium* gene growth tests were employed and rosette growth, changes of chlorophyll and anthocyanin contents were monitored by a Matlab-based image analysis software. The developed protocol permitted the non-destructive monitoring of growth and stress-related parameters of young *Arabidopsis* plants (Faragó et al. 2017).

Our future plan is to unfold those molecular differences and diversity between *Lepidium crassifolium* and *Arabidopsis thaliana* clones, which responsible for higher abiotic stress responses.

Supervisor: László Szabados
E-mail: faragod@brc.hu

Dissecting the main regulator of DNA damage tolerance

Orsolya Frittmann

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

Cancer is one of the major causes of death in the present world. However, a growing body of evidence supports the idea that the roots of cancers lie in mutations in DNA, the genetic material of cells. DNA damage caused by extrinsic or intrinsic agents are usually removed from DNA and repaired by one of the several DNA repair systems of the cell preserving the genetic information. However, high exposure to DNA damaging agents can lead to the accumulation of unrepaired DNA damages that can block the replication machinery leading to cell death. To ensure survival, cells have evolved mechanisms that can sustain DNA replication on damaged DNA. These so-called damage tolerance or DNA damage bypass processes allow replication to continue on damaged DNA without removing the damaged bases. In human, increased error-prone bypass of DNA lesions causes increased mutagenesis and a rise in the incidence of cancers, whereas error-free replication of damaged DNA contributes to genetic stability. Our research focuses on Rad18, which is a key regulator of DNA damage tolerance (DDT) pathway. Based on yeast genetic experiments DDT can be divided into three different sub-pathways.

(I). The mutagenic translesion polymerase sub-pathway, which includes Polζ and Rev1.

(II). The error-free tolerance sub-pathway, which includes Polη.

(III). The error-free fork regression sub-pathway, which includes Rad5, Mms2-Ubc13.

The above mentioned sub-pathways cannot function without Rad18. In the cells Rad18 (E3 ubiquitin ligase) and Rad6 (E2 ubiquitin conjugase) form a complex, which ubiquitinates the proliferating cell nuclear antigen (PCNA) to initiate the above sub-pathways. The

Rad18 protein has 5 identified domains. These are the Ring-finger, the Zn-finger, and the SAP domains, and the ATP and Rad6 binding domains. The function of some of these domains in DDT is well-described, whereas not much is known about the others. Also, beyond these domains the other parts of Rad18 protein have not been characterized. Our goal is to investigate the exact function of the known domains and the uncharacterized regions of Rad18 using yeast as model organism, with the hope of better understanding the regulation of DNA damage tolerance.

Supervisor: Ildikó Unk
E-mail: forsolya@brc.hu

Identification of a new protein in the preservation of genome integrity

Lili Hegedűs

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

DNA damage can occur as the result of several exogenous and endogenous factors. The lack of removal of these modifications may have serious consequences: replication may be blocked and the replication fork may break resulting in genomic rearrangements. The preservation of genome integrity is essential for cells therefore, several DNA repair pathways have evolved to remove the modified regions. Inappropriate operation of these processes may lead to the accumulation of mutations, which manifests in cancer predisposition, developmental disorders, and progeroid syndromes. Progeria is a rare genetic disorder with the early appearance of aging-related phenotypes. In Cocayne-, Bloom-, or Werner syndromes, non-functional DNA repair genes are the causative agents of the symptoms, but in several cases the mutated gene has not been described. Besides DNA repair genes, other factors have been identified as the origins of progeria. Mutations of the lamin genes may also lead to the acceleration of aging processes. The connection between DNA repair proteins and the nuclear lamina is poorly understood. Recently, several publications have examined the topic at the epigenetic level. It has been described that the Werner protein, together with the nuclear lamina, can form a complex with heterochromatinization proteins. In the absence or non-functional operation of these proteins, the heterochromatinization pattern of the cells may be altered, and thus genes that accelerate aging may be switched on. During our work, we have identified and characterized an unknown protein that can regulate these processes. It has both DNA repair and nuclear lamina-like phenotypes can influence the heterochromatinization state of the cell. It can also regulate the Werner protein. Based on our work, we suggest that its mutation may lead to a yet undescribed progeroid syndrome. This protein may be the missing link in our understanding of the processes that lead to aging.

Supervisor: Lajos Haracska
E-mail: lilidome@gmail.com

Functional characterization of nodule-specific cysteine-rich (NCR) peptides

Zsolt Csaba Horváth

Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Medicago truncatula - like other legume plant in nitrogen starving conditions - forms symbiotic relationship with rhizobia leading to the formation of nodules, novel plant organs specialized for nitrogen fixation, where symbiotic form of bacteria called bacteroids reside.

The *dnf4* (Deficient in Nitrogen Fixation 4) mutant plants - generated with fast neutron irradiation - are incapable to complete the symbiotic development resulting in Fix^- nodules that stay small, poorly developed and white, and where bacteroid development is arrested. Due to this deficiency, the plants suffer from growth retardation and chlorotic discoloration of the leaves in growth medium not containing enough amount of exogenous nitrogen.

Wang et al. (2015) found that a deletion in the *dnf4* plant removed two neighbouring genes coding for very similar nodule specific cysteine rich peptides, NCR211 and NCR178 that share high amino acid sequence similarity. Interestingly, only the NCR211 gene can restore the symbiotic efficiency in the mutant. The *M. truncatula* genome contains >700 *NCR(-like)* genes of which, >600 genes are expressed exclusively in the (infected) nodule cells. The NCR peptides are characterized by conserved secretory signal peptides (Mergaert et al. 2003) targeting the peptides to the bacteroids through the peribacteroid membrane (Dürgő et al. 2015) and by 4-6 cysteines located in conserved positions. The mature peptides have very low sequence similarity that is mirrored in their isoelectric point ranging from 3.5 to 10.5. The mature NCR211 and NCR178 peptides are composed of 34 amino acids of which, 21 are identical.

There are multiple explanations, why the NCR178 peptide cannot replace the biological function of NCR211. One possibility is that, there is a significant, approximately ten-fold difference in the expression levels of the genes, thus, not enough *NCR178* is produced in the nodule cells. Another possibility is that, despite the high sequence conservation, the NCR178 cannot interact with those bacterial proteins that are interacting with and affected by NCR211. To investigate these possibilities, we have created shuffled genes by exchanging between the two genes different parts, such as the promoter, the promoter and the first exon or parts of the second exon of different length. Using these constructs in complementation experiments with *dnf4* plants, we concluded that the differences in the C-terminal half of the mature NCR178 render the peptide inactive during the symbiotic interaction.

In the future, we plan to further delimit the amino acids important for the activity and try to isolate the bacterial targets of the NCR211 peptide.

Supervisor: Attila Kereszt
E-mail: horvathzs1984@gmail.com

The role of a chloroperoxidase enzyme in the ochratoxin A biosynthetic pathway

Noémi Kiss

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Ochratoxin A (OTA) is a mycotoxin first characterized by van der Merwe and co-workers (1965) from a South African *Aspergillus ochraceus* isolate. Several other *Aspergillus* and some *Penicillium* species able to produce this secondary metabolite. This mycotoxin frequently contaminates various food and feed products, such as cereals, spices, nuts, coffee, rice, olives, grapes, wine and other beverages. OTA is a nephrotoxic, hepatotoxic, immunosuppressive, teratogenic and carcinogenic compound, classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer. Ochratoxin A is composed of a chlorinated polyketide dihydroisocoumarin ring linked via an amide bond to L-β-phenylalanine. Among Aspergilli, the biosynthetic pathway of OTA has not been elucidated completely yet. There are enzymatic reactions suggested to be responsible for the synthesis of OTA, but the order of reactions has not well-defined yet. In this pathway, a chloroperoxidase enzyme catalyses the addition of a chloride to the dihydroisocoumarin derivative. Harris and Mantle (2001) suggested that chlorination is the penultimate step in the synthesis. However, Gallo et al. (2012) disproved it and they established a new OTA biosynthesis pathway scheme. In this model, the attachment of phenylalanine and dihydroisocoumarin catalysed by a non-ribosomal peptide synthetase (NRPS) precedes the chlorination step in *A. carbonarius*.

In our work, we used *Aspergillus carbonarius* (SZMC 2020) to establish a chloroperoxidase knockout mutants. A plasmid was constructed based on the known genome sequence of *A. carbonarius* ITEM 5010 including a hygromycin B phosphotransferase from pCSN43 as dominant marker gene for selection. We isolated possible transformants, checked them with PCR and examined their toxin production. Further works are in progress to elucidate the exact role and the mechanism of action of this enzyme in the biosynthetic pathway of OTA.

Supervisors: Sándor Kocsubé, Csaba Vágvölgyi
E-mail: kissnoemi621@gmail.com

Molecular and functional markers of different neocortical interneuron types: combined electrophysiological, imaging and single-cell PCR analysis *in vitro* and *in vivo*

Balázs Kovács

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

In the human neocortex, several pathways are still unknown, for instance the neuronal circuits, the cell functions and so on. For this reason, the focus of our study is the somatosensory cortex, more specifically, the molecular mechanisms underlying in the function of inhibitory cells like neurogliaform (NGF) and axo-axonic cells (AAC). These kinds of GABAerg inhibitory interneurons (NGF, AAC) located in the somatosensory cortex layer 1 and 2 in rats. Moreover, it is interesting to highlight the human brain single cell functions like in the cases of layer 2-3 pyramidal cells and layer 1 interneuron-, and rosehip cells. Our first aim was to collect NGF cell cytoplasms to

measure the difference in RNA level for molecular identity. For this experiment, low (0.5 mM) and high (10 mM) level glucose ACSF (Artificial Cerebro-Spinal Fluid) were used as well. The collected cytoplasms were used for cDNA-chip. Furthermore, we focused on the more efficient separation of AACs from the basket cells. For this reason, we use immune-cytological and PCR techniques to identify the AAC via Nav1.6 channel, which is an important element to separate both neuronal type. Finally, we are going to compare different human neuron cell types (pyramidal cells, interneurons, rosehip cells). However, in the case of NGF cells, we did not find any difference in the electrophysiological parameters, but we did find in the molecular level, which means several genes. These results may lead us to get better knowledge in some serious brain diseases. In the future, we perform *in vitro* single cell human experiments to collect pyramidal-, rosehip cells and any other layer 1 interneuron cytoplasm to analyse and separate them with NGS (Next Generation Sequencing) method, real-time PCR and pathway analysis.

Supervisor: Gábor Tamás
E-mail: rameiosmage@gmail.com

Genetic dissection of legume-rhizobia symbiosis via *Tnt1*-insertion mutagenesis in *Medicago truncatula*

Szilárd Kovács

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

Legumes are special among flowering plants in their ability to establish symbiotic associations with nitrogen-fixing bacteria, collectively known as rhizobia. Revealing and understanding the functions of genes and proteins involved in the legume-rhizobia symbiosis have a great importance, not just in the basic, but also in the applied research. A very efficient way of identifying important genes in model plants is the forward and reverse genetic analyses of mutants. The use of tagged mutant collections has already proved to be successful in revealing plant genes that function in the nitrogen-fixing symbiosis. In this work, we have used the *Tnt1* insertion mutant collection of the model legume *M. truncatula* cv. Jemalong that was produced during the EU GLIP project in parallel to the one already existing at the Noble Foundation for ecotype R108 (http://bioinfo4.noble.org/mutant/). In the case of the GLIP collection, during the EU project only the construction of the mutant lines was financed. Consequently, only a limited number of mutants were characterized in this collection, despite the value of such characterized collections for the community. Now, we have done a large scale symbiotic screen using this mutant collection and those lines were selected, in which individuals with impaired symbiotic phenotype appeared. We focused on those mutants that indicated defects in different steps of the symbiotic process, thus 24 lines were chosen for back-cross and further genetic analyses. From these lines segregating populations were produced for 11 mutants. In the meantime, FST sequences belonging to these lines were also carefully analyzed to use candidate gene approach. Thorough phenotype characterization and genotype determination of the candidate genes resulted in the identification of the mutated gene responsible for the symbiotic phenotype in two different mutant lines. One of them is a novel gene, playing role in the early bacterial invasion of the nodule and the other is a new allele of the recently cloned NAD1. The characterization of these two genes and their protein products revealed their roles during the early symbiotic stages.

Supervisor: Gabriella Endre
E-mail: kovacssz@brc.hu

Phylogenetic analysis of *Propionibacterium acnes* by newly developed methods and the determination of antibiotic sensitivity and pathogenic potential of commensal vs. pathogenic strains

Márta Magyari

Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

The human skin is the first line of defense of our body. As such, it gets in contact with numerous microorganisms, both commensal and pathogenic. *Propionibacterium acnes* (*P. acnes*) is a Gram-positive, coryneform, non-spore forming, anaerobic, opportunistic bacteria that have been associated with the emergence of many diseases. It predominantly plays a role in the pathogenesis of acne vulgaris, a skin condition that has multifactorial origin and is characterized by chronic inflammation of the pilosebaceous follicles. In an attempt to better understand, the pathogenesis of acne vulgarism we have developed techniques - such as Touchdown Multiplex PCR and eMLST - which

can quickly, effectively and reliably identify and classify the different isolates of *P. acnes*. We have developed a workflow - follicle-specific sampling followed by Multiplex PCR and eMLST characterization of the cultivated strains - that efficiently classifies the given *P. acnes* strain into phylotypes. Moreover, by using eBURST analysis, we have subdivided the isolates derived from healthy and acneic skin into eST-s, which precisely reflects their pathogenic potential. In recent decades, treatment of acne occurred by widely used antibiotics, but their effects seem to fade, because their improper use leads to the formation of resistant strains. To determine if antibiotic resistance plays a role in the pathogenic potential, antibiotic sensitivity of the isolated *P. acnes* strains was determined and antibiotic resistant strains were typed accordingly. We have determined, that eST-s consisting of antibiotic resistant strains indeed has greater pathogenic potential. Finally, full genome sequences of isolated belonging to different eST-s highlighted numerous regions responsible for morphological differences, pathogenicity and antibiotic resistance.

Supervisor: István Nagy
E-mail: magyari.marta@gmail.com

Ubiquitin quantification in *Drosophila*

Ágota Nagy

Department of Genetics, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Ubiquitination is a reversible posttranslational modification of proteins that plays critical roles in the regulation of many cellular functions. In my PhD project, I explore the mechanisms behind this process by focusing my research on the regulation of ubiquitin dependent protein degradation, the role of specific ubiquitinating and deubiquitinating enzymes, and on the balance between different ubiquitin pools. The ubiquitin pool in all eukaryotic cells is divided to distinct fractions that include free mono- and polyubiquitins as well as covalently linked mono- and polyubiquitin-protein conjugates. These ubiquitin forms thought to reach a dynamic intracellular equilibrium, in which the availability of free monoubiquitin appears to be essential for normal cell physiology. Precise measurement of the ubiquitin pool, and the ratio of free versus conjugated ubiquitin forms or their cycle dynamics an important step in the study of the ubiquitylation machinery and to better understand ubiquitin regulated intracellular processes. In this talk I present my results on ubiquitin quantification in different developmental stages and tissues of *Drosophila*.

To analyze ubiquitin pool dynamics, we adapted a simple and reliable Western blot based method to *Drosophila melanogaster*, which allowed us to quantify the different forms of ubiquitin. In this assay, endogenous DUBs present in the lysates process all conjugated ubiquitins to monoubiquitins therefore, the total ubiquitin content of cell lysates is determined in the form of monoubiquitins. The free monoubiquitin fraction in turn is determined from similar lysates supplemented with a strong DUB inhibitor. Appropriate samples of these lysates are Western blotted together with ubiquitin standards that permit the quantification of the different ubiquitin fractions by densitometric analysis. We determined the total, free monoubiquitin and conjugated ubiquitin concentrations in different developmental stages and in various tissues of *Drosophila melanogaster*. Our data demonstrate the highly dynamic nature of the ubiquitin equilibrium.

Supervisor: Péter Deák
E-mail: nagyagota.na@gmail.com

Measuring the activity of inhibitory neocortical cells with patch clamp technique *in vivo*

Gáspár Oláh

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

To understand how the brain works it is essential to investigate it on cellular level. In the last few decades patch clamp technique was developed to measure physiological parameters of neurons. The use of this technique on brain slice preparations helps us to understand how neurons make local microcircuits, but we cannot get any information from long range connections due to the slicing, and it is impossible to associate physiological phenomenon with behavioral events. Our aim is to develop a pipeline, in which we can perform patch clamp recordings in anaesthetized mice, to investigate how inhibitory neocortical cells take part in brain state transitions. For visualize the inhibitory cells in the neocortex we use Ai9xvGATcre mouse line. In these animals GABAergic cells express red fluorescent protein (tdTomato)

so we can perform two-photon targeted whole cell patch clamp recordings from interneurons. It is crucial to fix the head of the animal during recording. This step is very stressful for mice, but it is possible to train them to concede it. We developed an automatic home cage for mice, in which they get water only in a head fixation frame. The frame detects the animal and the head fixation and water apply sequence run automatically. The time of the head fixation increases day by day. Within a week, the animals trained to head fixation and we can use them for *in vivo* recordings. A well-trained animal is able to rest under the two-photon microscope up to 4 hours, which contains sleeping periods. In this period, we are able to perform cellular electrophysiological measurements or two-photon Ca^{2+} imaging to investigate the function of inhibitory cells in brain state transitions. After the recording session, the animals are anesthetized and transcardially perfused with fixative solution and the brains are stored for further anatomical investigations.

Supervisors: Gábor Tamás
E-mail: olah.gaspar@gmail.com

Summation of metabotropic $GABA_B$ receptor mediated postsynaptic potentials in the supragranular layer of the neocortex

Attila Ozsvár

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

In the mammal neocortex GABAerg interneurons forms diverse classes based on morphology and physiological properties. Among them the neurogliaform cell has the exclusive feature as capable to activate extrasynaptic $GABA_B$ receptors with a single action potential. The neurogliaforms cell forms uniquely dense axonal arborization, that contains presynaptic boutons very closely. However, the neurogliaform cell forms not only traditional synaptic connections. During synaptic transmission beside activating of the synaptic ionotropic $GABA_A$ receptor the released neurotransmitter reach the metabotropic extrasynaptic $GABA_B$ receptors through a molecular diffusion-based volume transmission of GABA neurotrasmitter. $GABA_B$ receptor activation is a key functional modulator in cortical microcircuits affecting basic properties like motor integration in the somatosensory cortex, sensory input control, interhemispheric inhibition, and initial changes in persistent cortical activity. Our aim was to characterize the quantal properties of the volume transmission, to have a better understanding what is the possible coverage of GABA release by a single action potential from neurogliaform cells in layer 1. Upon cooperative neurogliaform activation what is the possible effect on the postsynaptic cell, since spatial summation of ionotropic synaptic inputs is well-known, nevertheless to date there is no experimental analysis how neurons integrate metabotropic receptor mediated signals. We performed paired and triple whole cell patch clamp recordings on layer 2 pyramidal and layer 1 neurogliaform cells to measure neurogliaform cell induced postsynaptic potentials. We characterized the parameters of the neurogliaform synaptic transmission with Bayesian quantal analysis. With this approach, we were able to estimate the quantal parameters of the synaptic transmission: (1) probability of vesicular release, (2) number of functional release sites, (3) size of postsynaptic potentials mediated by single vesicle release. Our anatomical studies provided us to reconstruct neurogliaform cells axonal and bouton distribution in layer 1 and construct model of neurogliaform cell network. Using this model, we found that ~30% of the neurogliaform-cell outputs are overlapping. Therefore the co-activation of converging neurogliaform outputs summating on the dendrite region of the postsynaptic cell. With direct measurements of converging neurogliaform inputs we revealed linear summation of unitary $GABA_B$ mediated postsynaptic potentials.

Supervisor: Gábor Tamás, Gábor Molnár
E-mail: ozsat1990@gmail.com

Biotechnological route for hydrogenotrophic methanogenesis

Márk Szuhaj

Department of Biotechnology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Growing human population and activities increase the utilization of fossil energy resources, which in turn leads to the deterioration of the climate. Replacing the fossil fuels with renewable energy carriers is the promising solution. The rapidly increasing renewable capacities are wind and photovoltaics based electricity production, but these technologies operate inherently in fluctuating mode. Finding a solution to the problem of the storage of the surplus electricity generated by these renewables is indispensable. The excess power can be employed in

splitting water in an electrolyzer to H_2 and O_2. The technologies to store and transport the H_2 are not cost-effective and handling is complicated, therefore conversion H_2 to CH_4 is preferable. CH_4 can be transported and stored easily via the existing natural gas grid. The chemical methods to reduce CO_2 with H_2 are well-developed, but the same results can be reached in an environmentally friendly and economically feasible way with the help of biological systems. Hydrogenotrophic methanogens catalyze the conversion of H_2 and CO_2 to CH_4. These microbes are present in the biogas producing natural and man-made systems. An inexpensive source for hydrogenotrophic methanogens is the fermentation effluent of any industrial biogas plant.

Our aims were to determine the optimal conditions of a laboratory scale fermentation system, which operates well in the presence of H_2. The fermentation effluent from a mesophilic biogas plant was used directly as catalyst. The limiting factor in this system was the efficiency of gas/liquid mass transfer. Several operational conditions were tested and efficient CH_4 evolution was developed. The addition of stoichiometric combination of CO_2 and H_2 resulted stable and sustained CH_4 production. The proposed novel strategy suggests the utilization of the biogas effluent reservoir, which is part of most industrial-scale biogas facilities and stores the digested material until its utilization as organic fertilizer. The microbial community of the biogas effluent transforms green electricity-derived H_2 into bio CH_4, and thus acquire an entirely new function for the biogas plant. Based on our results, we also examined thermophilic biogas effluents in order, to achieve more effective methane production, although the thermophilic anaerobic microbial community may be more susceptible to instability.

Supervisor: Zoltán Bagi
E-mail: szuhaj@bio.u-szeged.hu

Pathogenicity of *Curvularia* species causing opportunistic phaeohyphomycoses

Eszter Judit Tóth

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Members of the genera *Curvularia* and *Bipolaris* are closely related melanin producing filamentous fungi. While *Bipolaris* species infect only plants and may cause serious agriculture damages, some *Curvularia* species has been recovered from opportunistic human infections. The human pathogenic species typically cause phaeohyphomycoses, *i.e.* mould infections caused by melanised fungi, which can manifest as invasive mycoses with frequent involvement of the central nervous system in immunocompromised patients or as local infections (*e.g.,* keratitis, sinusitis, and cutaneous lesions) in immunocompetent people. Although, their plant-fungal interactions have been intensively studied, there is only little information available about the human pathogenic feature of these fungi. During our study, interaction of human monocytes and neutrophil granulocytes with *Curvularia* and *Bipolaris* species were examined. *Curvularia* strains isolated from human infections were characterized also.

In case of monocytes, we analyzed the relative transcription level of certain activation related and cytokine or chemokine coding genes and measured the amount certain cytokines after interaction with the conidia and the hyphae of the fungi. Monocytes responded only to the hyphal form of *C. lunata*, while *C. spicifera* or *C. hawaiiensis* did not induced these immune cells. Role of the melanin in lack of recognizing the conidia by monocytes was examined by blocking the melanin biosynthesis during the production of the conidia. Responses of neutrophil granulocytes to *C. lunata*, *B. zeicola* and *Aspergillus fumigatus* were compared. Activation of neutrophils, production of hydrogen peroxide and superoxide anion was measured after co-incubation with un-opsonized or serum opsonized fungi. It was shown, that recognition of all investigated fungi is dependent of serum opsonisation. We analysed the appearance of a certain extracellular matrix on the surface of *C. lunata*. It seems, the production of this matrix is dependent on reduced oxygen tension and affect the interaction of the hyphae with the cellular components of the immune systems. In further studies, we would like to examine the interaction of other cell types of the innate immune system with *Curvularia* strains to identify the immune cell groups taking part in defence against these opportunistic human pathogenic fungi.

Supervisor: Tamás Papp
E-mail: scedobipo@gmail.com

Volume 61(1):105-118, 2017
Acta Biologica Szegediensis
http://www2.sci.u-szeged.hu/ABS

Traditional ecological knowledge of non-domestic animals in the Carpathian Basin

Viktor Ulicsni

Department of Ecology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Local people may possess rich knowledge of their environment that can be used for sustainable resource and nature conservation management. This widening of knowledge sources echoed in the policy arena of IPBES that declared the use of different knowledge systems. The understanding and use of traditional ecological knowledge (TEK) by academic researchers is, however, difficult due to different world views. Research on TK has mainly focused on boreal areas and the tropics, while the temperate continental areas were less examined. Previously, there has been no extensive data collection on TEK on wild animals in Central Europe, notably on the wild invertebrate fauna. We have documented such folk knowledge in seven regions in Hungary, Romania, Slovakia and Croatia using methods of social sciences such as semi-structured interviews, conducted picture sorts and participatory data collection. We documented ca. 412 wild folk taxa from which, 216 are invertebrates; discuss folk biological classification and nomenclature, morphologically, ecologically and culturally salient features, uses, economic impacts of these species and conservation. Besides the necessarily exploratory basic research, we analyzed sociocultural aspects of conservation management priority species and keystone species of ecosystems. We examined the development of new ecological knowledge and perceptions-knowledge-generation, hybrid knowledge in the case of the reintroduced Eurasian beaver and the sustainable elements in resource management of the European ground squirrel in Hungary. We also examined how well zoologists and a trait-based model can predict the level of knowledge among local people. We argue that if zoologists value, respect and seek for TEK and ethnobiologists help them locate target species for knowledge co-production, efficiency of cooperation between Western Science and traditional knowledge systems could be increased. This way traditional knowledge holders and their knowledge could participate more effectively in zoological and conservational knowledge co-production and thus contribute to a better understanding and conservation of biodiversity and ecosystem services.

Supervisors: Attila Gyula Torma, Zsolt Molnár
E-mail: ulicsni.viktor@t-online.hu

Role of carotenoids in the structure and function of cyanobacterial photosynthetic protein complexes

Sindhujaa Vajravel

Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Carotenoids are critical for photoprotection, regulation of the membrane properties, but less information available about their structural roles. These pigments are classified into subgroups of xanthophylls and carotenes. The photosynthetic organisms contain especially high amount of carotenoids. On a global scale, the cyanobacteria represent an ecologically and biotechnologically important group of oxygenic photosynthetic organisms. In order to utilize their advantages, it is essential to understand the factors determining photosynthetic efficiency. Cyanobacterial photosynthesis is mediated by membrane embedded photosystem I (PSI) and photosystem II (PSII). The light harvesting efficiency is enhanced by the peripheral antennae complex, the phycobilisome (PBS). Our aim is to clarify the influence of carotenoids on the major photosynthetic protein complexes using *Synechocystis* sp. PCC 6803. Although, the PBS does not contain carotenoids, the absence or low level of β-carotene lead to the co-existence of unconnected peripheral rod units of PBS and assembled PBS with shorter rods. The presences of unconnected rods were independent from the presence of PSs or the level of reactive oxygen species. Enzymatic PBS proteolysis induced by nitrogen starvation in the carotenoid mutant cells revealed a retarded degradation of the unconnected rod units suggesting a distorted PBS metabolism. We also investigated the function of xanthophylls in the organization and structure of the trimeric PSI complex. We found echinenone and zeaxanthin molecules in the isolated PSI trimers and used various carotenoid biosynthesis mutants to study the specific role of these xanthophylls. Our spectroscopic results revealed specific structural changes of PSI complex in the absence of zeaxanthin and echinenone resulting in destabilization of the PSI trimer. Hence, these xanthophylls are important for the PSI trimer structure and they could be part of the complex or present in the vicinity of PSI. Currently, I am studying the possible correlation between xanthophylls and supramolecular organization of thylakoid membranes.

Supervisor: Zoltán Gombos
E-mail: vajravel.sindhujaa@brc.mta.hu

Volume 61(1):105-118, 2017
Acta Biologica Szegediensis
http://www2.sci.u-szeged.hu/ABS

Calorie restriction, high triglyceride diet and physical exercise in experimental menopause

Médea Veszelka

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Premenopausal women have a lower risk of developing cardiovascular disease (CVD) compared to age-matched men, however, this sex advantage for women gradually disappears after the onset of menopause, suggesting that sexual hormones have a strong influence on cardiometabolic and inflammatory parameters. It is widely accepted that physical exercise has become a non-pharmacological therapeutic option in the prevention and treatment of CVD. Furthermore, exercise-mediated cardioprotection has been linked to the induction of antioxidant defense mechanism and the reduction of metabolic risk factors. We hypothesize that 12-week voluntary exercise and calorie restriction (CR) are effective strategies to modify the cardiovascular, metabolic anti-inflammatory processes. Ovariectomized (OVX) and sham operated (SO) female Wistar rats were randomized to running (R) and non-running groups. The types of diet were standard chow (CTRL), high triglyceride diet (HT) and CR for 12 weeks. The levels of glucose, insulin, triglyceride (TG), leptin as well as the concentration of cardiac and aortic heme oxygenase (HO-1) enzymes, the concentrations of plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in serum were detected by ELISA method. We measured the enzymatic activity of HO, myeloperoxidase (MPO) via spectrophotometric assay and detected the matrix metalloproteinases (MMP-2) activity by gelatin zymography. The HO activity and HO-1 concentration, the collagenase MMP-2 level, the metabolic and inflammatory were significantly decreased in the non-running groups, however, the 12-week physical exercise combined CR diet improve the parameters cased, by high triglyceride diet and "negative-effects" of estrogen depletion.

In this study, we revealed that moderate exercise training can attenuate the OVX induced heart fibrosis via MMP-2/HO/MPO regulation. This project supported by: ÚNKP-ÚNKP-16-4, (Pósa Anikó) and ÚNKP-ÚNKP-16-3 (Szabó Renáta) New National Excellence Program of the Ministry of Human Capacities and GINOP-2.3.2-15-2016-00062.

Supervisors: Csaba Varga, Anikó Pósa
E-mail: veszmed@gmail.com

CARD9 and Syk: dispensable for the control of systemic *Candida parapsilosis* infections?

Erik Zajta

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

An important antimicrobial intracellular pathway initiated by C-type lectin receptors leads through Syk and CARD9. Several major fungal pathogens activate this route. *Candida parapsilosis* is a regular cause of candidemia and threatens especially neonates. Despite its clinical relevance, little is known about the immunological processes during *C. parapsilosis* infections. Our goal was to examine the role of CARD9 and Syk in *C. parapsilosis* infections.

We generated bone marrow chimeras with wild type, Syk- or CARD9-deficient hematopoietic systems. Mice were infected intravenously with *C. parapsilosis* or *C. albicans* and fungal burden was determined from organs and blood. Peritoneal macrophages and bone marrow derived macrophages were cultured and infected with *C. parapsilosis* or *C. albicans* and supernatants were analysed for cytokine content and LDH activity. Phagocytosis of *C. parapsilosis* by macrophages and fungal survival in co-cultures were assessed.

The absence of Syk or CARD9 in macrophages led to reduced IL-1β, TNFα and KC secretion upon *C. albicans* and *C. parapsilosis* infections. There was no difference in phagocytic activity between WT and CARD9-deficient BMDMs nor in their LDH release. Both Syk- and CARD9-deficient mice were highly susceptible to *C. albicans*. Notably however, Syk- and CARD9-deficient mice were only slightly more sensitive to *C. parapsilosis*.

Our data suggest that both Syk and CARD9 influence *C. parapsilosis* induced host responses *in vitro*. However, unlike in the case of *C. albicans*, these proteins are not key players in the immune-control of systemic *C. parapsilosis* infection.

This work was supported by the National Talent Programme (NTP-NFTÖ-16-0569).

Supervisor: Attila Gácser
E-mail: writetotono@gmail.com

Volume 61(1):105-118, 2017
Acta Biologica Szegediensis
http://www2.sci.u-szeged.hu/ABS

A novel reporter system for hairy root transformation and its application to study the function of NCR peptides

Senlei Zhang

Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Hairy root transformation is widely used method in research of root nodule development and nitrogen fixation in legume plants. Biological reporters are normally used to distinguish transformed roots from adventitious roots, which can also develop from same site. However, those reporters can only be detected via specific methods: GFP with fluorescent microscopy, while GUS with specific staining, making them not convenient to handle. In this research, we are developing a new reporter system for hairy root transformation that visualize transformation events without any specific equipment or chemicals. The overexpression of the *MtLAP1* gene coding for a MYB-R2R3 family transcription factor facilitates anthocyanin accumulation in *Medicago*, *Trifolium* and tobacco cell vacuoles. Hairy roots expressing the constitutive *MtLAP1* gene showed very strong purple color as expected and complementation test with the *dnf7* mutant confirmed that nodules expressing this gene can conduct nitrogen fixation, while accumulate anthocyanin. As nodule-specific expressing genes, NCRs were proved quite essential for function of root nodule in IRLC plant, like *Medicago*. There are more than 700 members of NCRs in *Medicago* making it extremely difficult to discover their function for potential redundancy and function overlapping, however, the *dnf4* and *dnf7* mutants deficient in symbiotic nitrogen fixation were shown to be defective in the *NCR211* and *NCR169* genes, respectively. We use the reporter system described above to identify other essential *NCR* genes in *Medicago* with the help of the CRISPR-CAS gene knockout method.

Supervisor: Attila Kereszt
E-mail: senleizhang@126.com

DNA-dependent protease activity of human Spartan facilitates replication of DNA-protein crosslink-containing DNA

Eszter Zsigmond

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

Our DNA is constantly exposed to different exogenous and endogenous factors that cause DNA damage which if left unrepaired, challenges the movement of the replication machinery. Stalling of the replication fork can lead to strand breaks and chromosomal rearrangements causing genome instability, early onset of aging and eventually cancer. To rescue the stalled replication fork, different DNA damage pathways have evolved. One of them is the DNA damage tolerance pathway, which is activated by the DNA damage-induced monoubiquitylation of the Proliferating Cell Nuclear Antigen (PCNA), the sliding camp of replicative polymerases. A regulatory function of human Spartan has been implicated in this pathway, but the exact function of the protein remained unclear. It is also known that mutations in SPARTAN are associated with early onset hepatocellular carcinoma and progeroid features.

The aim of our study was to reveal the role of human Spartan in facilitating replication of DNA-protein crosslink-containing DNA. We prove that purified Spartan has a DNA-dependent protease activity degrading certain proteins bound to DNA. In concert, Spartan is required for direct DPC removal *in vivo*; we also show that the protease Spartan facilitates repair of formaldehyde-induced DNA-protein crosslinks in later phases of replication using our DNA-Protein Crosslink-specific bromodeoxyuridine (BrdU) comet assay. Moreover, DNA fiber assay indicates that formaldehyde-induced replication stress dramatically decreases the speed of replication fork movement in Spartan-deficient cells, which accumulate in the G2/M cell cycle phase. Finally, epistasis analysis mapped these Spartan functions to the RAD6-RAD18 DNA damage tolerance pathway. Our results reveal that Spartan facilitates replication of DNA-protein crosslink-containing DNA enzymatically, as a protease, which may explain its role in preventing carcinogenesis and aging.

Supervisor: Lajos Haracska
E-mail: zsigmond.eszter@brc.mta.hu

Volume 61(1):105-118, 2017
Acta Biologica Szegediensis
http://www2.sci.u-szeged.hu/ABS

Investigation of the role of fungal prostaglandin like molecules in *Candida parapsilosis* host pathogen interaction

Tanmoy Chakraborty

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

Candida parapsilosis is one of the opportunistic fungi, which cause systemic fungal infection in immunocompromised host. Although, *Candida albicans* is the most prevalent species in candidiasis, the occurrence of non-albicans *Candida* infection has increased significantly in recent decades. *C. parapsilosis*, one of the emerging non-albicans *Candida* species, is of special importance, because of its ability to cause infection primarily to immature infants, compared to adults. Although the virulence properties of *C. albicans* are extensively studied, very little knowledge is available for *C. parapsilosis*.

Prostaglandins are long chain fatty acid molecules that are involved in both pro- and anti-inflammatory response in humans. They also play important role in antifungal immunity with many other lipid mediators. Recently, it has been shown that pathogenic fungi like *C. albicans* and *Cryptococcus neoformans* can produce their own prostaglandin from exogenous arachidonic acid (AA) independent of the host prostaglandins. In *C. albicans*, two genes *OLE2* and *FET3* are involved in prostaglandin biosynthesis. Whereas, it has been shown from our lab that the *OLE2* homologue is not involved in prostaglandin biosynthesis in *C. parapsilosis,* so we are trying to find new genes or pathways involved in prostaglandin production in this pathogenic fungi. To investigate this, RNA sequencing was performed after growing *C. parapsilosis* in presence of exogenous AA and the sequence analysis shows several genes are up-regulated in which 14% are involved in lipid metabolism. We generated knock out (KO) mutants for 6 candidate genes and among them two KO mutants showed significant reduction in prostaglandin production. Further, to prove the role of fungal prostaglandin in inflammation we studied cytokine such as IL-1α, IL-1β, IL-8 and IL-10 production using human THP-1 cell lines. Our next aim is to check the mutants for host pathogen interaction studies by animal survival assay, phagocytosis and killing assay using different *in-vivo* and *in-vitro* model systems.

Supervisor: Attila Gácser
E-mail: tanmoy.microbiology@gmail.com