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DISSERTATION SUMMARIES

Monitoring the biogas producing microbes

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Nowadays, biogas is one of the most important renewable energy carrier. It is produced in many countries and many facilities to treat biological wastes and to produce heat, biofuel and electricity from it. There is significant potential in replacing fossil fuels with biogas in various areas of our energy consumption, particularly as it combines the benefits of organic waste treatment with renewable energy production.

Biogas is produced by a special microbial population, which can be classified into three groups. The first one is the hydrolyzing bacteria; they cut the long biopolymers into smaller pieces. The acetogenic bacteria comprise the second group. They use mono- or oligosaccharides, lipids and amino acids to produce volatile fatty acids and hydrogen. Finally, the methanogenic archeabacteria utilize the volatile fatty acids and the product is biogas, *i.e.*, a mixture of CH_4 and CO_2. In order to increase biogas production and to improve the economical viability of this technology, it is very important to understand the relationship between these microbial populations and the rate limiting molecular events. This information can be collected via molecular biological techniques. Several approaches are employed. First, we developed a method for quantitative identification of a single bacterium in the biogas generating microbial population that invokes Real-Time PCR. The target microbe was the thermophilic bacterium, *Caldicellulosiruptor saccharolyticus.* Two unique genes, which code for proteins characteristic of this organism were selected. These were Ech (similar to *Escherichia coli* hydrogenase-3), and the Cel (cellulase). Successful experiments were carried out with both targeted genes from samples, originated from biogas fermentors. The other bacterium for our studies was the mesophilic eubacterium, *Enterobacter cloacae*. In this case the target gene was coding for one of the large subunit hydrogenase of this microbe, HycE. The detection of this bacterium was also possible, using whole extracted DNA from the liquid samples.

We have also shown that T-RFLP in capillary gel electrophoresis, combined with the conventional cloning-sequencing is a promising way for quantitative and qualitative monitoring of the biogas producing consortia.

Metagenomic methods are used for the identification of novel genes and pathways implicated in biomass degradation and biogas formation. In order to achieve a high yield of prokaryotic DNA, bacteria are extracted from the anaerobic fermentation using methods already available. The DNA samples are independently pooled and used for DNA sequencing and for the construction of metagenomic libraries. DNA sequences are used to identify the biodiversity of genes involved in organic substrate degradation. Metagenomic mass sequencing also lowers the amount of sequencing of clones isolated from metagenomic libraries. For sequencing we use a strategy based on pyrosequencing in order to obtain long (average 400-500) nucleotides, combined with sequencing using SOLiD and Solexa platforms that yield a huge number of short high-quality sequences. Beside the sequence based searches, we will also perform functional screening. Metagenomic sequencing will result in a large database that will include genes and pathways interesting for other biotechnological application. These databases will be screened to search for genes encoding esterases, lipases, proteases, phytases, cellulases, lignolytic enzymes involved in the decomposition of organic waste streams.

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Expression analyis of the *hup* genes encoded a NiFe hydrogenase in *Thiocapsa roseopersicina*

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Thiocapsa roseopersicina is Gram-negative, phototrophic purple sulphur bacterium, which belongs to the family of Chromatiaceae. There are four active [NiFe] hydrogenases in the cells, which differ in their function, localization and stability. Two of them are membrane-associated [NiFe] hydrogenases (Hyn and Hup), while the other two are soluble hydrogenases (Hox1, Hox2). HynSL shows extraordinary stability and it catalyzes either H_2 uptake or H_2 evolution. The other membrane-associated hydrogenase (Hup) plays a role in hydrogen uptake (hup=hydrogen uptake) exclusively. The soluble hydrogenases of *Thiocapsa roseopersicina*, Hox1 and Hox2 are bidirectional NAD^+

reducing hydrogenases (Kovács et al. 2005; Maróti et al. – in press). Furthermore the bacterium possesses nitrogenase activity, and the atmospheric N_2 fixing is accompanied by H_2 evolution.

The *hup* locus consists of seven genes (*hupSLCDHIR*). Some of these genes are well characterized, *hupC* encodes a cytochrome b-type protein involved in electron transfer (Palágyi-Mészáros et al. 2009), while *hupD* codes for an endopeptidase which plays a role in the maturation of the large subunit (HupL). HupR is a regulatory protein, a part of a transcriptional regulatory system. However little is known about the transcriptional organization and regulation of *hup* gene, function of *hupH* and *hupI* gene products.

According to the literature, HupH protein is required for the translocation of the H_2ase stuctural protein to the membrane by bonding to the small subunit, while HupI is a rubredoxin-type protein plays a role in the electron transfer (Manyani et al. 2005).

In order to investigate the role of *hupH* and *hupI* genes in frame deletion mutants were created and the phenotypical effects of the mutations were analyzed by measuring *in vivo* and *in vitro* hydrogenase enzyme activity. Results showed that the absence neither of HupI nor HupH cause a significant decrease in Hup uptake activity.

The transcription of *hup* genes was investigated by reverse transcription coupled PCR, the results showed that *hupSLCDHIR* genes transcribe as a whole transcript.

Expression level of the *hup* genes was measured by quantitative real-time PCR in cells grown on various medium. Under nitrogen fixing conditions an enhanced *hup* mRNA level was observed, which indicates that the physiological function of Hup is somehow linked to the activity of nitrogenase enzyme complex. In standard non-nitrogen fixing growth conditions the *hupSL* transcription downregulated by both thiosulfate and succinate and upregulated by the inactivation of HupC. Therefore it was hypothesized that the redox status of the membrane/quinone pool controls the expression level of Hup hydrogenase.

To identify regulatory proteins which control the *hup* expression, mini Tn5 transposon based mutagenesis was carried out. A screening procedure was developed for identification of strains having Hup hydrogenase activity when the quinone pool is overreduced. *In vitro* hydrogenase uptake activity measurments were showed an appreciably increased Hup activity in the mutant and this points to the fact that the insertion of the transposon inactivated a gene which encodes a protein likely involved in the redox control of the expression of Hup hydrogenase.

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Clarifying the mechanism of T-cell apoptosis induced by cell-derived or low and high concentration of soluble recombinant galectin-1

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Galectin-1 (Gal-1) is a mammalian lectin with β-galactoside binding activity. It is expressed by numerous cell types and binds to cells and extracellular matrix components presenting glycoconjugates of N-acetyl-lactosamine. The most prominent biological function of Gal-1 is its anti-inflammatory effect which is predominantly exerted by induction of apoptosis of Th1 cells (1). Many studies have emerged analyzing Gal-1 signal transduction mechanism during T-cell apoptosis. However these data have resulted confusing knowledge due to using soluble recombinant protein although Gal-1 exerts its physiological function bound to the producing or neighboring cells or extracellular matrix components.

We have aimed to resolve this controversy by comparing cell death induced by low (1.8 μM, lowGal-1) and high (18 μM, highGal-1) concentration of soluble Gal-1. We show that lowGal-1 and highGal-1 trigger phosphatidylserine exposure, generation of rafts and mitochondrial membrane depolarization. In contrast, lowGal-1 but not highGal-1 are dependent on the presence of p56lck and ZAP70 and activates caspase cascade. The results allow the conclusion that the cell-death mechanism strictly depends on the concentration of Gal-1 (2).

Recombinant Gal-1 is always manipulated during purification and in apoptosis assays since it has to be in reduced form for functional conformation. To avoid this process we analyzed the role and mechanism of cell-derived Gal-1 in the apoptotic process. In co-culture system Gal-1 remains as a native, functional protein without any chemical modification and the apoptosis assay also avoids addition of reducing agent. We applied co-cultures of various cell lines producing Gal-1 as effectors and T-cells (activated peripheral blood cells or

Jurkat lymphoblasts) as targets. Both Jurkat and activated peripheral T-cells died when co-cultured with various Gal-1 expressing cells, but HeLa, a Gal-1 non-expressing cervix carcinoma cell line did not affect T-cell viability. Removing cell surface Gal-1 with lactose or knocking down Gal-1 expression in Gal-1 producing tumor cells resulted in the diminution of the cytotoxic effect of these cell lines. Moreover, transgenic expression of Gal-1 in HeLa cells or treating HeLa cells with recombinant Gal-1 (rGal-1) converted these cells cytotoxic. T-cell apoptosis required intimate interaction between the effector tumor and target T-cells since neither conditioned supernatant harvested from the tumor cells, nor physical separation of tumor and T-cells in the same medium triggered T-cell death. Mechanism of apoptosis by cell-bound Gal-1 was comparable to that of low concentration of soluble recombinant Gal-1. Requirement for p56lck and ZAP70 has been proved and both the decrease of mitochondrial membrane potential and caspase activation was detected in T-cell apoptosis triggered by tumor cell-derived Gal-1 (3).

Our results show that cell-derived Gal-1 and low concentration of the soluble lectin triggers identical pathway of T-cell apoptosis in contrast to high concentration soluble Gal-1 which act on a different fashion.

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Evolutionary and functional analysis of *Medicago truncatula* symbiotic genes on nodulating and non-nodulating plant species

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The availability of soil nutrients for plants is a major limiting factor regarding growth and productivity at many agronomically important areas. For this demand an evolutionary solution is the existence of symbiotic associations between plants and soil microbes that provide valuable macronutrients (e.g. P or N) for photosynthates as an exchange material from the plant. An ancient type of coexistence is the symbiosis with arbuscular mycorrhiza fungi. It is originated back to the appearance of the first land plants and present in the majority of land plant families. However, another, more recent type of symbiosis exists between nitrogen fixing soil bacteria and a narrow range of plants consisting of phylogenetically closely related species. During this symbiosis a new organ, the root nodule is formed that is specific for nitrogen fixation. Research on genes involved in the formation of these types of symbiotic associations showed that the two systems share genes supporting the idea that already existing elements of the more ancient program were recruited during the evolution of root nodule symbioses.

The mutual recognition of partners is a key process during the establishment of the symbiotic association. Specific molecules have been identified on both sides that are essential for triggering the symbiotic process, e.g. flavonoids secreted by the plants and the so-called Nod factors produced by the symbiotic bacteria. Due to the intensive ongoing research on symbiotic nitrogen fixation there is a rapid increase in the number of identified plant genes involved in these signaling events. LysM type receptor kinases (*MtLYK3*, *MtNFP*) together with the LRR receptor like kinase *MtDMI2* are needed to promote the most characteristic phenomenon of the early symbiotic signaling process: the perinuclear calcium level oscillation via nuclear pore complex elements (*LjNUP85*, *LjNUP133*) and a putative potassium ion channel *LjCASTOR* and *LjPOLLUX*/ *MtDMI1*. The signature of this so called calcium spiking is decoded and forwarded by a calcium-calmodulin dependent protein kinase (*MtDMI3*) via its phosphorylation substrate (*MtIPD3*) towards transcription factors (*MtNSP1*, *MtNSP2*, *MtERN*, *MtNIN*). Moreover, *NIN* and a cytokinin receptor needed for symbiotic nitrogen fixation (*MtCRE1*) are elements of the pathway that allows crosstalk between Nod factors of the symbiotic bacteria and plant cytokinins during nodulation. Living the days of the genomic era more and more `whole genome sequences' are accessible in the databases including plant genomes as well. Searching these databases makes possible to identify genes homologous to known symbiotic genes with high significance not only from legume genomes but also from non-nodulating plants. However, there are only a few papers published so far on evolutionary relationships of particular symbiotic genes and their homologues in different plant species (most recently Chen et al. 2009 on *LjCASTOR*/ *LjPOLLUX*/ *MtDMI1*). We have done systematic searches using the protein sequences of *M. truncatula* symbiotic genes as query and could identify new homologous genes from non nodulating plants highlighting some interesting aspects of their evolutionarily recruited functions. We have selected a few homologous proteins to explore for their possible function, with special regard on their possible ability of fulfilling symbiotic function as well.

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EcoTILLING analysis of candidate genes for drought tolerance in barley

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The development of new barley varieties with improved drought tolerance is one of the main breeding objectives in Hungary, because drought is a main factor limiting the yield of cereals including barley. The development of stress-tolerant varieties with yield stability will help to reduce the risk in barley cultivation. The probability of a successful breeding for drought tolerance is largely dependent on the understanding and knowing of the genetical factors that regulate this highly quantitative trait.

In this project drought tolerance related candidate genes were analyzed by using the EcoTILLING (Comai et al. 2004) technology. EcoTILLING is a high throughput, low cost technique for rapid discovery of polymorphisms in natural populations. It is a variant of TILLING (Targeting Induced Local Lesions IN Genomes), (Colbert et al. 2001) is based on certain PCR steps, such as the formation of heteroduplexes and a nuclease cutting DNA mismatches. It allows both SNP discovery and haplotyping through the sequencing of unique haplotypes.

We have established the EcoTILLING technology in order to identify putative SNPs and small INDELs in a set of 96 barley cultivars and wild germplasm containing drought tolerant and sensitive genotypes (cultivars and landraces and wild relatives) collected worldwide. Target genes were selected based on studies dealing with drought tolerance. Candidate genes are dedicated as potentially involved in the variation of key agronomic traits. The identification/determination of natural genetic variation in candidate genes can provide valuable information about gene function.

In this pilot study 7 drought related barley candidate genes were screened. In the case of 4 genes overlapping amplicons were designed, trying to cover the whole gene in the genetic diversity screens. For these 4 genes also more easily detectable markers were created after the evaluation of the obtained haplotypes sequences allowing distinguishing the main haplotypes. In the case of 3 candidate genes only one primer pair was planned based on the available mRNA sequences.

EcoTILLING reactions were performed in one-well format using fluorescently labeled nucleotides and after heteroduplex formation ENDO-1 and Cel-1 treated products were visualized on an ABI PRISM 377 sequencer.

Untill now more than one hundred unique haplotypes identified for 9 genes (HvARH1, HvDREB1, HvDRF1, HVA1, HvNHX1, HVP1, HvPPD-H1, HvNUD and HvPRPX) in 18 EcoTILLING screens. It's including more than 1.5 million base pairs sequence. The number of haplotypes identified for screened amplicons ranged from 2 to 9. Overall, 185 single nucleotide polymorphisms and 46 insertions/deletions were finded with a mean of 1SNP/92 bp and 1INDEL/372 bp genomic sequence.

In four candidate genes (HvARH1, HVA1, HvDRF1, HvSRG6) a set of informative polymorphisms were converted into easily detectable genetic markers, which are useful for marker assisted selection.

The obtained sequence/haplotype information will be used for development of further easily detectable genetic markers (potential „within gene marker") useful for linkage mapping and Marker Assisted Selection. Functional alleles can be directly integrated in barley breeding programs for improvement of drought tolerance.

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Application of synthetic antisense oligodeoxynucleotides in higher plants

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Antisense oligonucleotides *i.e.* short, synthetic strands of DNA or analogs that are complementary to a target DNA or RNA along with short interfering RNAs (siRNAs), 21-25 bp dsRNA with dinucleotide 3' overhangs became a powerful tool for the functional genetics. These structures are designed to interfere with nucleic acid metabolism, most preferentially with transcription, translation or splicing. Sequence-selective inhibition of gene expression is applied extensively for elucidation of complex gene expression patterns or validation of results gained from high throughput genomic experiments such as DNA-arrays. Common and attracting features of both antisense oligo and siRNA are that they act in a dose-dependent reversible manner, while no genetic transformation is required.

Though the sequence-selective gene-silencing by these synthetic oligonucleotides is quite general phenomenon for all organisms, only few applications are described for plant systems. We elaborated several methods for the introduction of oligonucleotides into monocot and dicot plants. By fluorescent labeling, we examined the uptake efficiency and inner traffic of these molecules, and determined the optimal conditions of treatment.

To demonstrate the antisense inhibition, we chose phytoene desaturase (pds) as a model gene which is a key-enzyme of the carotenoid biosynthesis in Triticum aestivum and Nicotiana benthamiana. Selection of the antisense target sites was made by a multistep optimization process which raises the targeting efficiency significantly. By means of quantitative RT-PCR method, we demonstrated sequence-specific knock-down of pds mRNA level. We followed the phenotypical changes of the plants by chlorophyll fluorometry and carotenoid content measurement, thus significant loss PDS function was demonstrated. at a significant level. Our experiences open the way for applying the antisense oligonucleotide technique for elucidation of real genetic problems.

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Epigenetic changes in tumor-associated myofibroblast

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The tumor microenvironment is an important factor in cancer development and progression.

In the stroma of various epithelial tumors the predominant cell types are myofibroblasts. These spindle-shaped cells were originally described in skin wounds where they facilitate wound healing. Myofibroblasts are differenciated fibroblasts expressing specific markers like alpha smooth muscle actin, vimentin and secreting several extracellular matrix proteins (Desmoulière et al. 2004). Compared to normal tissue, the number of myofibroblasts is increased in the tumor stroma and the shape and distribution of the cells are altered as well. The observed morphological changes are believed to be partially due to epigenetic effects, which cause an altered gene expression profile without influencing the DNA sequence. The epigenetic changes occuring in tumor- associated myofibroblasts are poorly understood (Jiang et al. 2008).

In order to compare epigenetic characteristics and gene expression pattern of tumor-associated myofibroblasts with that of normal myofibroblasts in molecular level, we used primary myofibroblast cultures obtained from the gastrointestinal tract. Cells isolated from tumor stroma or from healthy tissues near the tumor margins were provided by Dr. Peter Hegyi (Dept. of Internal Medicine, Faculty of Medicine, University of Szeged). We studied epigenetic changes such as histone H3 and H4 acetylation and methylation in tumor-associated myofibroblasts by immunocytochemistry. The results indicated lower levels of histone H4 acetylated on lysine 8, 12, 16 and H3 dimethylated on lysine 9 in tumor-associated myofibroblast compared to normal cells. Semi quantitative determination of the level of particular histone modifications by immunoblots using modified histone specific antibodies supported and validated the observed epigenetic alterations. The expression profile of subunits of histone acetyltransferase (HAT) complexes were determined by quantitative RT-PCR. The analysis indicated that the mRNA levels corresponding to the *ada2a, ada3* and *gcn5* genes, which code subunits of several HAT complexes, such as SAGA and ATAC, were lower in tumor-associated samples, then in their wild type counterparts.

The role of myofibroblasts in cancer metastasis is also suspected (De Wever et al. 2008). They can secrete many proteolytic enzymes, which digest extracellular matrix in order to promote cancer cell invasion. Therefore we were interested in studying the expression, secretion and activity of the gelatinase enzymes, matrix metalloproteinase 2 and 9 (MMP-2, 9) in the myofibroblast cultures. Based on quantitative RT-PCR data we performed, we concluded that the expression of MMP-2 was elevated in tumor-associated myofibroblasts, while the messenger of MMP-9 was detectable neither in the tumor-associated nor the control cells. We have also performed a gelatin zymography to detect the activity of MMP-2 and 9. For this protein extracts from tumor associated and normal cells, and as well secreted protein samples obtained from the culture media were loaded onto polyacrylamide gels co-polymerized with gelatin and resolved under nondenaturing conditions. Development of the gels with protein specific stain indicated strong MMP-2 activities in both samples, while the tumor-associated myofibroblasts secreted more MMP-2 enzymes to the extracellular space.

In the forthcoming months we plan to further investigate the differences in the gene expression profile of tumor-associated versus control myofibroblast using microarray. We expect that the results will broaden and refine our understanding on the role of myofibroblasts in tumor formation and invasion.

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In memoriam Pál Széchényi. Paleoradiological study of a three-hundred-year-old mummy from Nagycenk

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The study of ancient mummies has contributed greatly to the development of paleoradiology. Many results of radiological examinations of human remains have been published since 1896, when the first study was made on an Egyptian mummy (Böni et al. 2004). As there are only few paleoradiological methodological references, it is necessary to develop new methods for X-ray examinations. On the other hand, we have only scanty information about the technical parameters, settings and values or positioning (Aufderheide 2003; Chhem 2008). Since 2001 in co-operation with the researchers of many radiology departments, I have managed to identify define appropriate technical parameters to be used in paleoradiology (Kristóf et al. 2004). Anthropological research of mummies in Hungary has been carried out in multidisciplinary framework (Pap et al. 1997; Pálfi et al. 2009). One of these case-studies, included in my PhD-research, is that of the three-hundred-year-old mummy of Pál Széchényi.

Archbishop Pál Széchényi's name appeared in the Hungarian history several times. The scientific study of Archbishop's mummy to be found in Nagycenk was carried out by our research team composed of the members of several institutions based in Budapest, Győr and Szeged in 2007 (Kristóf et al. 2010). The scientific examinations represented a milestone, since up till now it was unclear whether it was a natural or artificial mummy and the century-old question of whether Pál Széchényi was in fact a victim of arsenic poisoning in 1710 or this story was only a legend could also be answered.

The non-invasive examinations were carried out with multislice CT, traditional X-ray, biopsy, toxicology, energy-dispersive X-ray, X-ray fluorescent analysis, endoscope and 3D rapid-prototyping printing.

17 conventional X-ray radiographs have been made of the skull, trunk and extremities with computerized radiography. The CT examination was carried out by a 16-slices MSCT equipment. 277 and 557 slices of 2,0 and 0,8 mm thickness respectively were taken the skull. In the course of the examination of the whole body 576 and 1440 slices of 5 and 2mm thickness respectively were taken.

Except for his scull and extremities Pál Széchényi's mummy is in poor condition. The corpse was mummified artificially. There is no trace of removal of the brain. The small amount of brain remnants raise several questions. The Archbishop suffered from diffuse idiopathic skeletal hyperostosis (DISH). The small oval ring-like particles of calc density disclosed in muscles have raised suspicion of helminthiasis, e.g. trichinellosis. X-ray-fluorescency (XRF) analysis detected small amount of arsenic only on the surface of skin and buccal mucosa, but it was traceable neither in nails nor hair. The myth about arsenic poisoning of the Archbishop proved to be false. We also got a replica made of the mummy's scull with 3D printing from the MSCT data.

The paleoradiological examinations resulted in important findings about the condition of the mummy. In the future I would like to study more Hungarian mummies from the baroque era, especially of the presumed conservation method of Pauline monks and their burial customs.

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Characterization of the genus *Bipolaris* based on molecular, morphological and physiological features

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Members of the genus *Bipolaris* (*Ascomycota, Pleosporales, Pleosporaceae*) are imperfect filamentous fungi. Most of them have economical significance as plant pathogens infecting mainly cereals and other graminaceous hosts. However, three species are frequently recorded as human pathogens. *B. australiensis*, *B. hawaiiensis* and *B. spicifera* are agents of phaehyphomycoses (infection caused by melanin

producing filamentous fungi). They cause invasive infections in immunsupressed patients, but they are also able to cause local infections through external injuries (first of all on eye and on skin) in immuncompetent patients (Pfaller et al. 2004). Identification and discrimination of *Bipolaris* species and members of some closely related genera using morphological markers are really difficult, because of the great similarity of their conidia. The present taxonomy, which is based on the morphology of the conidia, is fuzzy, incoherent and it does not fit the real phylogenetical relationships.

Our work had three major aims: (i) phylogenetic analysis of the genus *Bipolaris* to clarify their taxonomical relationships using molecular, physiological and biological methods; (ii) to elaborate a reliable molecular methodology for the detection of the human pathogenic strains; and (iii) to examine the biological activity of the sesterterpene-type secondary metabolites produced by the members of this fungal group.

Twenty-five strains isolated from human keratomycosis and 15 isolates obtained from international strain collections were involved in the study. The ITS region of the ribosomal DNA and fragments of the calmodulin, the β-tubulin and the transcriptional elongation factor-1α genes were sequenced and compared to infer phylogenies and investigate the taxonomic position of the involved strains.

Currently, identification of *Bipolaris* strains isolated from clinical samples is carried out by the examination of the conidial morphology (*i.e.* determining the numbers of the conidial septa). Our preliminary examinations suggested that the three human pathogenic species cannot be distinguished merely on the basis of their conidial septation. In the molecular phylogenies inferred from the analysis of the abovementioned genes and also from RAPD-PCR data, only *B. hawaiiensis* could be clearly distinguished from *B. australiensis* and *B. spicifera*, while these two species formed a more or less uniform group in each resulting trees suggesting that they may belong to the same species. Carbon source assimilation tests (utilization of 68 compounds as a single carbon source was tested in the study) and morphological examinations also confirmed the results of the phylogenetic studies.

Sequence data were analysed to test their applicability as markers for molecular identification. As a result, an effective and rapid PCR-based method was developed to identify the members of the two human pathogenic groups (*i.e. B. hawaiiensis* and *B. australiensis* - *spicifera*).

Sensitivity of the clinical isolates against several generally used antifungal agents was also investigated. Itraconazole, clotrimazole and ketoconazole proved to be the most effective against the *Bipolaris* species. Interestingly, all of the investigated strains were resistant to amphotericin B, one of the most frequently used antifungal agents against filamentous fungi.

Bipolaris species often produce ophiobolins, secondary metabolite compounds of the family of sesterterpens. The phytotoxic, antimicrobial and nematocide effects of these compounds are well-known (Li et al. 1995; Au et al 2000) . In our study, effect of different ophiobolins against opportunistic pathogen Zygomycetes fungi was investigated in a broth microdilution assay. We also started to study the background of this antifungal effect in the case of ophiobolin A. This compound induced apoptotic-like changes in *Mucor* and *Rhizopus* strains presumably through the inhibition of the calmodulin. Further investigations are in progress to prove this hypothesis.

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Identification of fusarium resistance QTLs in the Ságvári/Nobeoka Bozu//Mini Manó/Sumai3 prebreeded wheat population

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Fusarium head blight (FHB) is one of the most serious diseases of wheat worldwide, caused by *Fusarium* species complex. Epidemics of FHB can cause severe yield losses and decreasing quality. During pathogenesis harmful levels of mycotoxins can be accumulated, jeopardizing food and feed safety. The most cost-effective way to control the disease is breeding and cultivation of genetically resistant cultivars.

The FHB resistance in wheat is inherited by quantitative trait locus (QTLs). Many QTLs, with different effectiveness, are found on all wheat chromosomes except 7D from different resistant genotypes (Buerstmayr et al. 2009) Identification, effectiveness, inheritance, usage (marker assisted selection, MAS) and pyramiding of different QTLs is a powerful tool to help breeding varieties with enhanced Fusarium resistance.

105 recombinant inbred lines (RIL) of a double cross population (Ságvári/Nobeoka Bozu//Mini Manó/Sumai3) which contains two resistance sources from Asia – *Nobeoka Bouzu* (NB) a Japanese landrace and *Sumai 3* (Sum3) a Chinese variety - and two Hungarian genotypes - *GK Sagvari* (Sgv) and *GK Mini Mano* (MM) - were tested for *Fusarium* resistance.

Phenotyping was made in field trials during 2008 and 2009. Wheat ears were inoculated artificially with two isolates (one *Fusarium graminearum* and one *F. culmorum*) in 2008 and with four isolates (three *F. graminearum* and one *F. culmorum*) in 2009. Suspension of the

fungus was sprayed directly onto a bunch of ears in full flowering stage. Afterwards 48 hours polyethylene bag coverage was maintained to allow moisture condition for growing fungus. Head blight symptoms were evaluated visually as the percentage of scabby spikelets from 10 days after inoculation (dai) on every 4th day till the 26 dai. Inoculated ears are harvested and trashed so as to evaluate the percentage of Fusarium damaged kernels (FDK) and deoxynivalenol content (DON) by HPLC. Flowering time, plant height, ear type, present of awns, thousand kernel weight (TKW) was evaluated also during the experiment. Analysis of the data was made by two-way ANOVA and Pearson's-correlation.

Genotyping was carried out with microsatellite (SSR) markers, linked to FHB resistance QTLs, collected from literature. At the present time four QTL regions, located on the 2D, 3BS, 5A and the 6B chromosomes was mapped with 40 SSR markers. Since in resistant genotypes Sumai3 alleles dominated the tested regions, alleles were scored as derived from Sumai3 and others non-Sumai3. Linkage analysis and QTL mapping were done with JoinMap and MapQTL. Reduced hegiht (Rht) genes Rht-B1b, Rht-D1b and Rht8 were also detected.

Significant (P = 0,1%) positive correlations were found between FHB, FDK, DON, and TKW data. Plant height was in significant (P = 10%) negative correlation with disease traits. This means that taller plant and plants with lower TKW had lower disease level. Highly effective QTL, originated from Sumai3, found on the 3BS chromosome, explained the 60% of the phenotypic variance (p.v.) of FHB with 21 LOD value and the 45% of p.v. of FDK with 14 LOD value. It is flanked by 3 SSR markers on a 3 cM distance. A Sumai3 originated medium effective QTL found on the 5A (LOD 6-8), and small or medium effective QTL on 2D (LOD 3-7) and 6B (LOD 4-6) chromosomes. The mapped regions had significant effect on TKW also. Sumai3 alleles are linked to lower TKW mainly on 5A and 6B chromosomes which is a negative effect in breeding. The distribution of QTLs according to head type showed significant differences. The ratio of QTL carrying genotypes in the group of tapered and spindle headed plants was larger than in the group of square or semi-butt headed plants.

Fusarium resistance QTLs are identified and their effectiveness and agronomic relation are investigated in this study. However this research program wasn't finished, members of this population with good agronomic characters and Fusarium resistance (supported by known QTLs) are able to start to do marker assisted selection.

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The role of the *Drosophila* DAAM in the development of the Indirect Flight Muscle

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In the past few years our group has working on to study of a new *Drosophila* formin protein called DAAM (Dishevelled associated activator of morphogenesis). This protein has many interesting tissue-specific functions. For instance, it regulates the tracheal cuticle pattern (Matusek et al. 2006), plays a role during axon growth (Matusek and Gombos et al. 2008), and participates in the process of genitalia rotation.

In collaboration with a research group from the University of York (UK) we showed that the *Drosophila* DAAM (dDAAM) is localized in the Indirect Flight Muscle (IFM) of both the pupae and adult flies.

In non-muscle cells, generally there are two major actin nucleating-polymerizing systems, the formins and the Arp2/3 complex. Formins are producing long straight actin filaments and the Arp2/3 complex is producing branched actin network (Pollard 2007; Chhabra and Higgs 2007).

Because unbranched straight actin filament is the major form in striated muscle cells, it is possible that a formin family protein serves as the key regulator of actin dynamics in myofibrils (Taniguchi et al. 2009). These straight actin filaments are organized into the contractile unit called sarcomere but little is known about the regulation of actin assembly in muscle cells.

Our aim was to reveal the function of *Drosophila* DAAM insight the striated muscle's sarcomere.

With the use of different approaches, like muscle functional tests, immunohistochemistry, biophysical essays, we confirmed that dDAAM plays role in the nucleation of actin filaments and sarcomere assembly.

In the 6% of the dDAAM loss of function hypomorph mutants, called Ex1, about 16% reduction in the sarcomere length was observed compared to the wild type. These flightless mutants carrying the full length protein construct could rescue the phenotype at 100% both in sarcomere level and functionally as well.

With the overexpression of the tagged full length protein, we found that it is partially localized in the expected region, namely in the M-line of the sarcomere.

The RNA interference-mediated depletion of dDAAM resulted in a marked reduction in sarcomere length and disruption of the sarcomeric structure.

These findings suggested that actin dynamics regulated by dDAAM are critical for sarcomere organization in striated muscle cells.

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Some neuroprotective approaches in focal and global ischaemia on *in vivo* and *in vitro* rat models

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Glutamate (Glu) is the major excitatory amino acid neurotransmitter in the central nervous system. It mediates a number of physiological processes, but it is involved in the pathological processes of excitotoxicity too. Traumatic brain injury, focal brain lesion or global hypoperfusion are followed by acute excitotoxicity caused by the presence of abnormally high Glu levels in the cerebrospinal and interstitial fluids.

It has recently been demonstrated that this excess Glu in the brain can be eliminated by the intravenous administration of oxaloacetate (OxAc), which, by scavenging the blood Glu, induces an enhanced and neuroprotective brain-to-blood Glu efflux.

In this study, we subjected rats to a photothrombotic lesion and treated them after the illumination with a single 30-min long administration of OxAc (1.2 mg/100 g, i.v.). Following induction of the lesion, we measured the infarct size by Fluoro-Jade B (FJB)-staining. FJB binds sensitively and specifically to damaged neurons, with increased contrast during acute neuronal stress. Coronal sections (30μm) were cut with a freezing microtome and the sections were stained with FJB. The sections were subsequently analyzed with a fluorescent microscope. The volume of the hemispheric lesion and the number of FJB-positive cells were calculated for each animal. The administration of OxAc resulted in a reduction in the volume of the ischemia-induced cortical damage.

We also examined the functional consequences of the photothrombotic lesion by measuring the amplitudes of the somatosensory evoked potentials (SEPs). SEPs were induced in the contralateral primary somatosensory cortex by electrical stimulation of the right whisker pad and were transcranially recorded. The photothrombotic lesion resulted in appreciably decreased amplitudes of SEPs, but OxAc administration significantly attenuated this reduction.

We suggest that the neuroprotective effects of OxAc are due to its blood Glu scavenging activity, which, by increasing the brain-to-blood Glu efflux, reduces the excess Glu in the brain. This limits the size of the penumbra, improves the tissue perfusion and oxygenation and reduces the ischemia-related functional damage.

Ischemic postconditioning is referred to preventing ischaemia/reperfusion injury in both myocardial and cerebral infarction. The next study was undertaken to evaluate possible neuroprotective effects of kainate postconditioning against delayed neuronal death in hippocampal CA1 neurons if applied two days after hypoperfusion.

Transient global hypoperfusion was induced in male Wistar rats by two-vessel occlusion (2VO) for 30 min. 2VO causes inhibition of protein synthesis in selectively vulnerable brain regions such as CA1 and leads to the decrease of dendritic spine number and resulted in an impaired long-term potentiation (LTP) function in the hippocampal CA1 region.

In order to determine the number of apical dendritic spines we used Golgi-Cox staining. When the impregnation was ready coronal brain sections were cut by vibratome. The clear Golgi sections have been evaluated by light microscopical stereology.

For electrophysiological recordings we prepared coronal slices from the middle part of hippocampi. Field excitatory postsynaptic potencials (fEPSPs) were monitored and after a control period, LTP of the Schaffer collateral-CA1 synaptic response was induced by high-frequency stimulation (HFS). After the HFS the fEPSPs were recorded for at least a further 60 min-long period. If we apply the kainate (5 mg/kg) 48 hours after the 2VO, the loss of hippocampal dendritic spines and dysfunction of LTP could be significantly averted.

These results suggest that a sublethal second post-ischaemic event can be considered as a trigger for the start of protein synthesis activity in post-ischaemic cells. Postconditioning probably causes a re-modulation of protective protein (hsp70, hsp72, Bcl-2) synthesis leading to a switch from pro-apoptotic to anti-apoptotic pathways.

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Investigation of protein phosphorylation and protein kinases in prokaryotes

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Methylococcus capsulatus (Bath) is a Gram-negative, coccoid, methanotrophic bacterium. For the utilization of methane *M. capsulatus* is able to express two methane monooxigenases (MMO): in the presence of copper ions the particulate MMO (pMMO) and its accessory and transport proteins, responsible for copper uptake, are expressed. In the absence of copper the soluble MMO (sMMO) is expressed. sMMO can oxidize a wide range of compounds, from alkanes, alkenes, ethers and haloalkanes to aromatic and even heterocyclic hydrocarbons (Hakemian et al. 2007). Many biodegradation and biotransformation applications for sMMO are currently being investigated.

Although the existence of protein phosphorylation on S, T and Y residues in prokaryotes was first demonstrated in 1978 (Wang et al. 1978), our knowledge about S, T and Y phosphorylation in prokaryotes is very limited. In this recent work the copper regulation of MMO enzymes is studied by comparing the phospoproteome of two cultures grown under distinct conditions and screening for proteins of which's phosphorylation state changes depending on the available copper.

The comparison of the purified phosphoproteomes on 2D ELFO revealed that two subunits of sMMO (smmoB and smmoC) are phosphorylated proteins and unstable elongation factor (EfTU) is only phosphorylated when the media contains no copper. In case of smmoB and EfTU exact phosphorylation sites (smmoB:ser2, EfTU ser144) were determined by mass spectrometry. After changing potential phosphorylation site on smmoB from ser2 to ala by directed mutagenesis the whole enzyme preserved its full activity and smmoB still remained phosphorylated. Furthermore even smmoB heterologously expressed in *E. coli* proved to be phosphorylated by host protein kinases. In order to identify the protein kines(es) that is(are) responsible for the phosphorylation of smmoB a set of kinase deletion mutants were prepared in *E. coli*. After deletion of all known ser/thr and tyr kinases (*yihE*, *argK*, *aceK, etk*, *wzc, hipA, yeaG, yniA*) *E. coli* still preserved its capability to perform protein phosphorylation and smmoB was still phosphorylated, furthermore the deletion of these kinases hardly affected the protein pattern of the whole phosphoproteome of the host bacterium. Although *E. coli* is one of the most studied organisms, its genome is known and well characterized these results suggest that it still may possesses at least one unknown functional protein kinase that is responsible for the phophorylation of the majority of phosphpoproteins including overxpressed smmoB.

During amino acid starvation bacteria activate stringent control elements that result in adaptation to the amino acid shortage by increased amino acid synthesis, restricted protein translation and intensive protein degradation (Chatterji et al. 2001). In *Methylococcus capsulatus* activation of stringent control cascade results in the activation of smmo operon even in copper rich media (unpublished results). Promoting amino acid starvation in *M. capsulatus* grown in copper rich medium also resulted the phosphorylation of EfTU suggesting that phosphorylation of this protein may restrict protein sinthesys *via* direct or indirect inhibition of the translation.

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A novel genetic approach for Identifying genes involved in abscisic acid regulation

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Abscisic acid is the main stress response hormone in higher plants. In the past few decades many stress regulatory factors were identified which are involved in ABA dependent stress regulation. In order to understand the complicated regulatory web of ABA signaling the Controlled cDNA Overexpression System have been developed (COS, Papdi et al., 2008). We have transformed the *Arabidopsis* Col-0 wild type plants with the COS library and screened progenies of infiltrated plants for ABA insensitivity in the presence and absence of estradiol in germination assays. Screening one million seeds (aproximately 25,000 transformed seeds), of T1 generation resulted 156 plants, which were selected based on their germination capacity on high concentration ABA supplemented media. By testing of T2 generation, estradiol dependent ABA insensitivity was confirmed in 32 lines. Estradiol dependent ABA insenstitive germination was most notable in A26 and A44 lines, which were able to germinate in the presence of 5µM ABA, which otherwise completely inhibited the germination of wild type seeds. Insertions were identified in both lines and corresponded to full-length cDNA encoding the small heat-shock protein HSP17.6A-cII (A26) and a previously unknown zinc-finger domain containing transcription factor protein (A44). GFP fusion and HA-tagging experiments showed nuclear localization of the A44-derived transcription factor. While constitutive overexpression of this transcription factor reduced

fertility, insertion mutants, where transcription of the corresponding gene was abolished, were hypersensitive to ABA. Our results show, that the COS system is suitable for the identification of novel ABA regulatory factors.

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Expression and epigenetic studies of MDR1 genes in drug-resistant rat cells

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The overexpression of multidrug resistance 1 protein (MDR1, Abcb1 or P-glycoprotein), a member of the ABC (ATP Binding Cassette) transporter superfamily, can be responsible for the decreased efficiency of chemotherapeutic drugs in tumour cells. MDR1 is an energy-dependent transporter that is able to extrude cytotoxic agents from the cell. In the presence of these drugs MDR1 expression is up-regulated by different mechanisms, though the molecular background of increased MDR expression is mostly unknown. Recent studies suggested that epigenetic modifications (e.g. histone acetylation, methylation) might play an important role in this process.

The aim of our study was to reveal epigenetic modifications responsible for the increased MDR1 level in multidrug resistant cell lines.

We studied the MDR expression in drug resistant rat hepatoma cells kindly provided by A. Venetianer. The cell lines we used in our experiments were a drug sensitive parental rat hepatoma cell line (D12), a medium (col500) and a highly (col1000) drug-resistant variant of it, selected using increasing concentrations of colchicine (Pirity 1996).

In contrast to humans, rodents have two MDR1 isoforms: Abcb1a and Abcb1b. First, we determined the expression of these genes and found that the mRNA levels of both Abcb1a and Abcb1b were increased in the drug resistant cell lines compared to the parental D12. A potential reason for the elevated expression of the Abcb1 genes is gene amplification. Indeed, we observed an increase in the copies of the Abcb1 genes in the col1000 cell line, however our data suggested that gene amplification was not the (only) reason for the overexpression of Abcb1 genes in the resistant cells.

Next we studied the possible role of histone acetylation in the increased expression of Abcb1 genes. For this, we treated the cells with histone deacetylase inhibitors (Na-butirate and trichostatin A) to maintain the acetylated state of histones. As a consequence of the treatment, the acetylation of H3 and H4 histones increased. Surprisingly, Abcb1a and Abcb1b genes responded to the treatment in an opposite way: the expression of Abcb1a was decreased, while the expression of Abcb1b was increased in cells treated with histone deacetylase inhibitors. Since acetylation of histone 3 lysin 9 and 14 (H3K9ac and H3K14ac) have been shown to play key roles in the regulation of chromatin structure and function, and are linked to transcriptional activation, next we focused on these modifications in order to determine whether they play a role in the differential expression of Abc1a and b genes. Using chromatin immunoprecipitation we determined the H3K9ac and H3K14ac levels at the transcriptional start sites and at upstream regulatory regions of both genes. We found elevated H3K9 and H3K14 acetylation in the col500 resistant cell line in all tested Abcb1 regions. In contrast with that, the acetylation levels of these histones were comparable in the parental D12 and in the other resistant (col1000) cell lines. After histone deacetylase inhibitor treatment, H3K9 and H3K14 acetylation increased in all tested regions of both genes, contrary that, their expression changed in opposite directions.

Since HDAC inhibitors changed the expression levels of Abcb1 genes, we wondered whether this treatment affected the drug efflux capacity of the cells. To answer this question we compared the accumulation of a fluorescent cytotoxin, a substrate of MDR1, in treated and untreated cells. As expected, we detected an increased efflux activity in the drug resistant col500 and col1000 cells; however, TSA-treatment did not influence significantly this process.

In conclusion, our data suggest that elevated Abcb1 gene expression is not always coupled to histone acetylation changes and conversely, the H3K9 and H3K14 acetylation levels do not necessarily predict the expression level of the Abcb1 genes. Thus, further histone acetylation sites and other histone modifications need to be examined to understand the complex regulation of MDR by mechanisms affecting chromatin structure.

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Involvement of carotenoids in the synthesis and in the assembly of protein subunits of photosynthetic reaction centers of *Synechocystis* sp. PCC 6803

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The *crtB* gene of *Synechocystis* sp. PCC 6803, encoding phytoene synthase, was inactivated in the Δ*crtH* mutant. Thus, a carotenoid-less mutant, Δ*crtH/B*, was produced. Cells of the mutant were light sensitive and could grow only under light-activated heterotrophic growth conditions in the presence of glucose. Carotenoid deficiency did not significantly affect the cellular content of phycobiliproteins while the chlorophyll content of the mutant cells decreased. The mutant cells exhibited no oxygen-evolving activity suggesting the absence of photochemically active PSII complexes. This was confirmed by 2D electrophoresis of photosynthetic membrane complexes. Analyses identified only a small amount of a non-functional PSII core complex lacking CP43, while the monomeric and dimeric PSII core complexes were absent. On the other hand, carotenoid deficiency did not prevent formation of Cyt b_6f complex and PSI, which predominantly accumulated in the monomeric form. Radioactive labeling revealed very limited synthesis of inner PSII antennae, CP47, and especially CP43. Thus, carotenoids are indispensable constituents of the photosynthetic apparatus being essential not only for the anti-oxidative protection, but also for the efficient synthesis and accumulation of photosynthetic proteins and especially that of PSII antenna subunits.

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The role of bone marrow derived mesenchymal stem cells and their galectin-1 expression in the progression of mouse tumors in models of 4T1 breast carcinoma and B16F10 melanoma

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Galectin-1 (Gal-1) has a powerful anti-inflammatory effect (Kiss et al. 2007; Veronika et al. 2008) dominantly due to the induction of apoptosis of activated T cells (Ion et al. 2005, 2006). Gal-1 expression or overexpression in a tumor or in the tumor associated stroma must be considered as a sign of a poor prognosis for patients (Camby et al. 2006). Recent literature data report about the role of bone marrow derived mesenchymal stem cells (bmMSCs) in the process of tumorigenesis. It has been shown in these studies that bmMSCs selectively migrate into the tumor sites and are engrafted in the tumor stroma (Berber et al. 2009). Moreover bmMSCs may contribute to the formation of tumor associated stroma supporting the progression of cancerous cells, may regulate the neoangiogenesis and prevent the tumor specific immune response (Lazennec 2008). Several proteomic studies revealed the Gal-1 expression in mesenchymal stem cells (Silva et al. 2003; Panepucci et al. 2004; Kadri 2005; Lepelletier et al. 2009) however its role in MSC has to be elucidated.

Our purpose was to examine the role and influence of bmMSCs and bmMSC derived Gal-1 in the course of primary tumor development and metastasis.

We examined the Gal-1 production in MSCs in Western blot and FACS experiments. Balb/C or C57Bl/6 mice were subcutaneously injected with 4T1 breast carcinoma or B16F10 melanoma cells, respectively with or without bmMSCs. Primary tumor size was regularly measured. After sacrificing the animals, weight of the lung and number of metastatic nodules were analyzed. Histochemical analysis was also carried out on different tissues isolated from treated mice. We established Gal-1 knock-down MSCs in order to investigate their effect in tumor progression, neovascularization and metastasis.

Co-injection of bmMSCs with 4T1 breast carcinoma or B16F10 melanoma tumor cells induced larger primary tumor size and increased necrotic lesions on the 3rd week after treatment compared to these parameters in animals treated with the tumor cells alone. Metastatic phenotype characterized by the lung mass and the number of metastatic nodules is also more pronounced in animals injected with combination of tumor cells and bmMSCs. Histopathology also confirms the participation of bmMSCs in the pathogenesis of cancer, since bmMSCs enhance the number of micrometastasis in lungs and in lymph nodes. Moreover we detected CM-DiI labeled MSCs in the tumor samples on the 3rd week after treatment. Co-transplantation of Gal-1 knock-down MSCs with 4T1 cells slowed down the 4T1 tumor growth and vascularization compared to that of the effect of wild type MSCs.

In this study we show that bmMSCs enhance the growth kinetics of the primary orthotropic 4T1 mouse breast carcinoma and B16F10 melanoma. Also they contribute to progression of metastatic phenotype of the investigated tumor models. Supportive effect of bmMSCs to the tumor progression prevails on the 3rd week of treatment, since co-injection of bmMSCs with tumor cells do not modify the survival period of the animals. MSC derived Gal-1 could play an important role in the tumor promoting effect of MSCs.

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Studying the chromatin structure of MDR1 gene in drug-sensitive and drug-resistant human cells

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The reason for failure of chemotherapy is often the development of multidrug resistance, which is caused by the elevated level of ABC (ATP binding cassette) type transporters. One of the most often described ABC transporter is encoded by the MDR1 (multidrug resistance 1) gene. It was earlier described that drug induced upregulation of MDR1 is associated with increased H3 acetylation level in discrete region of MDR1 locus. Therefore exploration of the epigenetic mechanisms that contribute to the development of multidrug resistance has great importance. Several human diseases may originate from impaired function of histone acetyl transferases (HATs); therefore, these enzymes will serve as novel molecular targets for therapy in the future.

We aimed to study the changes in specific histone acetylation upon MDR1 gene induction and to analyze the histone acetyl transferase complexes that are responsible for these modifications.

We characterized a drug resistant, MCF7-derived, breast carcinoma cell line named MCF-KCR. It was generated via long term treatment of MCF7 cells with doxorubicin. We examined the level of MDR1 gene and mRNA in these cell lines. We found that the MDR1 gene level is 17 fold while the mRNA level 23000 fold elevated in the drug-resistant cells compared to the parental cells. These data suggest that epigenetic upregulation of MDR1 transcription is more important in developing the drug resistance than gene amplification. Importantly, the level of MRP1 (multidrug resistance protein 1) mRNA was not elevated in the drug resistant cells.

We showed by immunoblotting that the global H3 acetylation is elevated in the MCF-KCR cells. In addition, we examined the histone acetylation pattern in the regulatory regions (two promoters) and the coding region of the MDR1 gene by employing chromatin immunoprecipitation. Our data reveal an interesting acetylation map. With the use of acetylated residue specific antibodies we found that the acetylation level of H3K9 is about 100 fold elevated in the downstream promoter region and in the first exon in the drug resistant subline compared to the drug sensitive cells. H3K4, H3K14 and H4K12 are also slightly increased in the downstream promoter region and in the first exon of the MDR1 gene in those cells that overexpress MDR1 mRNA (Toth et al. 2009).

When we treated the cells with trichostatin A (TSA), a histone deacetylase inhibitor, MDR1 expression increased, while that of the other genes examined did not change in the drug sensitive cells. In contrast, MDR1 mRNA level did not change in the drug resistant cells upon TSA treatment. To try to down-regulate the acetylation and, along with that, the expression of the MDR1 gene, we treated the cells with a novel HAT inhibitor (HATi II) that strongly inhibits p300 and CBP (CREB binding protein) and weakly inhibits PCAF (p300/CBP associated factor) and GCN5 (homolog of yeast general control nonderepressable). Importantly, expression of MDR1 was further increased in the drug resistant MCF-KCR cells, while it did not change in the parental MCF7 cells. Next, we knocked down the level of PCAF, GCN5 and Ada2b (homolog of yeast alteration/deficiency in activation 2b) mRNA by transfecting specific siRNAs. The latter is a component of GCN5 containing multisubunit HAT complexes, such as hTFTC/hSTAGA. Interestingly, PCAF downregulation resulted in a reduction of the MDR1 mRNA level in MCF7 cells but not in the MCF-KCR cells. MDR1 mRNA level did not change in the drug resistant subline and decreased only slightly in drug sensitive cells upon GCN5 or Ada2b knockdown. These data suggest that MDR1 expression can not be easily reduced by simple inhibition of HATs in the drug resistant cells, probably because histone acetylation is highly deregulated in these cells.

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Functional interplay between factors involved in transcription in *Drosophila melanogaster*

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Regulation of transcription, the synthesis of RNA from a DNA template, is one of the most important steps in control of cell growth and differentiation. Gene regulation occurs in the context of chromatin where recruitment of chromatin remodeling complexes such as ATP-dependent remodeling complexes and histone modifying enzymes (HAT, HDAC, HMT, etc.) represents a crucial step in gene transcription (Svejstrup 2004). We are interested in studying the physical and functional interaction between proteins involved in transcription regulation such as the RNA PolII subunit Rpb4, the transcription factor p53, and adaptor proteins of the chromatin modifier complexes.

The RNA polymerase II is composed of a ten-subunit core and a two-subunit dissociable subcomplex comprising the forth and the seventh largest subunits, Rpb4 and Rpb7. In *Drosophila*, Rpb4 is a product of a bicistronic gene together with the ATAC histone acetyltransferase complex constituent ADA2a (Pankotai et al. 2010). The alignment of Ada2a and Rpb4-related sequences indicated that the two proteins share the same transcription unit. Comparison of the related protein and nucleotide sequences revealed that Ada2a and Rpb4 coding region are present in similar organization in 12 *Drosophila* species, other then *D. melanogaster*; however a similar gene organization in other organisms cannot be identified. We investigated the mechanism by which the two mRNAs are generated. From RT-PCR analysis we concluded that the shift between Ada2a and Rpb4 mRNA formation takes place by splice acceptor site selection. Ada2a protein has another distinct homolog in *Drosophila*, Ada2b which is present in the SAGA complex. Although the two proteins contain the same conserved domains and their interacting partners are rather similar, they are part of two distinct HAT complexes. We questioned what confers the complex specificity of the Ada2 adaptors, more precisely which regions are responsible for their interaction with different transcriptional coactivators. Firstly, different Ada2a/Ada2b or Ada2b/Ada2a hybrids proteins were generated by joining PCR fragments corresponding to functional domains of one and the other ADA2. All the hybrid plasmids were generated using the Gateway system and were successfully tested on western blot for their expression in *Drosophila* S2 cells. Since the chimeric structure of the proteins did not disturb their expression in S2 cells, plasmids suitable for embryo injections were generated. Our goal is to see if their expression will restore one or the other ADA2 function. The coding region of hybrid proteins were inserted into a P-element containing vector allowing site specific insertion and injected into *Drosophila* embryos. Two out of six plasmids are ready for *in vivo* analysis in *Ada2a* and *Ada2b* mutant background.

Previous studies have shown that HATs, beside their ability to relax chromatin, can regulate many other factors through acetylation, including transcription factors (Wang et al. 2001). In mammalian system it was shown that the Gcn5-containing acetyltransferase complex (STAGA) plays a role in p53-dependent gene activation. Ada2b and Gcn5L proteins are identified as direct interacting partners of the p53 transcription factor (Gamper and Roeder 2008). According to previous studies *Drosophila* p53 is a functional homolog of mammalian p53 (Ollmann et al. 2000). We investigated whether the adaptor proteins of the HAT complex have any effect on p53 transcriptional activity. A plasmid containing three copies of *rpr* consensus binding site upstream of the luciferase gene was constructed. The reporter plasmid was cotransfected into S2 cells with different constructs expressing Ada2a, Ada2b or Ada3 proteins and performed luciferase assays. There was no significant change in transcriptional activity of the p53 responsive element. Although it was previously described that hAda3 can increase p53 trancriptional activity (Wang et al. 2001), perhaps in *Drosophila* S2 cells the efficiency of this interaction requires additional factors which can promote a significant response. We also tested plasmid vectors containing *Drosophila melanogaster* p53 (Dmp53) and a point mutant form of it ($Dmp53^{K302R}$) in reporter assays. Expression of either the wild type or the mutant variant increased the activity of the reporter gene 20 fold compared to control. No significant change was observed in the level of transcriptional activation ability of the wild type and the mutant variant of Dmp53. Future *in vivo* studies are planned to understand the mechanism by which p53 is activated.

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Regulation of the phosphorylation status of rice retinoblastoma-related protein by PP2A phosphatase

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The retinoblastoma protein (pRB), a nuclear phosphoprotein, is a general regulator of cell proliferation. The ability of pRB and pRB-related proteins to inhibit cellular proliferation is counterbalanced by the action of Cdks (Nakagami et al. 2002). Sixteen potential sites for Cdk-mediated phosphorylation (Ser/Thr-Pro motifs) exist in pRB, and twelve of these sites have been shown to be phosphorylated *in vivo* (Nakagami et al. 2002). In quiescent and early G1 cells, pRB exists in a predominantly unphosphorylated state. As cells progress towards S phase, pRB becomes phosphorylated. Inactivation of pRB by phosphorylation leads to the dissociation and activation of E2F, allowing the expression of many genes required for cell cycle progression and S phase entry (Inoue et al. 2007; Poznic 2009). The phosphorylated state of pRB is accumulating till the end of mitosis when pRB is dephosphorylated by protein phosphatases (Nakagami et al. 2002; Poznic 2009).

Two retinoblastoma-related (RBR) genes have been found in rice, OsRBR1 and OsRBR2. OsRBR2 is expressed mainly in differentiated cells, but the function of the OsRBR1 gene may be related to cell division or cell cycle progression (Lendvai et al. 2007).

Protein phosphatase 2A (PP2A) is a family of serine-threonine phosphatases implicated in the control of a diverse array of cellular processes. The PP2A core enzyme consists of a catalytic C subunit and a structural A subunit. The AC dimer recruits a third regulatory B subunit that has been predicted to dictate the substrate specificity and function of the PP2A heterotrimeric complex. Four unrelated families of B subunits have been identified in mammals (Groves et al. 1999; Yan and Mumby1999; Janssens and Goris 2001; Cicchillitti et al. 2003). In plants B subunits are also important for cellular localization and substrate specificity. B subunits are classified into at least three distinct groups: B, B' and B'', based on molecular weight and domains (Janssens and Goris 2001). The PP2A was reported to be implicated in the dephosphorylation of RBRs, particularly upon oxidative stress (Cicchillitti 2003). A PP2A regulatory subunit (PR70) was also shown to associate with hyperphosphorylated pRb and mediate its dephosphorylation (Groves et al. 1999).

Yeast-two hybrid interaction results show that the OsPP2A B'' regulatory subunit is a strong interactor of OsRBR1, but has no detectable association with OsRBR2.

All the proteins in the RB family can be divided into three regions: the N-terminal region, the pocket domain (include A, B domain and the spacer between A and B) and the C-terminal region. OsPP2A B'' interacts strongly with the OsRBR1 protein minus the C-terminal version, even more strongly than with the full length of OsRBR1, but does not interact with any version of the protein which has some part of pocket domain missing. In addition, there was no interaction between OsRBR1 and EF-hand truncated OsPP2A B'' protein. It was demonstrated that the interaction between the OsRBR1 and OsPP2A B'' proteins needs an intact pocket domain of the RBR protein and the presence of the EF-hands domains on the B'' regulator subunit.

We used OsPP2A B'' regulatory subunit as a bait to screen the interactor from rice suspension cells and leaves cDNA libraries, but then switched to the OsPP2A B'' one-EF-hand minus version because of the self-activation of the full length B'' subunit. We got 30 more interesting interactors of B'' subunits by screen, which we will use in following research.

We have constructed clone encoding the His_{6-} and GST-tagged OsRBR1, OsRBR2 and OsPP2A B'' proteins using pET-28a and pGEX vectors. Recently, we verified the binding of recombinant His-tagged OsRBR1 protein to GST-PP2A B'' demonstrated that this binding increase in the presence of Ca^{2+}.

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