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DISSERTATION SUMMARIES

Photoprotection difficulty in D1 protein mutant of *Solanum nigrum*

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Plants are usually exposed to wide ranges of variable and fluctuating light intensities. The excess light energy may damage the photosynthetic apparatus. One of the most important and rapid photoprotective mechanisms is the thermal dissipation of light energy, referred to in the literature as non-photochemical quenching (NPQ) of chlorophyll fluorescence. The major component of NPQ is ΔpH-dependent, rapidly reversible, and called qE quenching. Its formation requires low thylakoid lumen pH, de-epoxidized xanthophylls (Demmig-Adams et al. 1996; Horton 1996; Eskliing et al. 1997), and PsbS protein (Li et al. 2000).

Naturally selected atrazine-resistant (AR) weeds in crop cultivation have a modified D1 protein structure, with a $Ser_{264} \rightarrow$ Gly (S264G) mutation near the Q_B niche (Hirsberg and McIntosh 1983). This mutation greatly reduces the affinity of Q_B and atrazine for Q_B-binding pocket, and causes higher susceptibility to photoinhibition in several weed species (Hart and Stemler, 1990; Váradi et al. 2003).

This summary addresses the question of whether the conserved chloroplastic DNA encoded D1 protein is required for normal photoprotection of plants or not. In order to ascertain how the photoprotective thermal dissipation functions are influenced by the S264G D1 protein mutation (which is related to atrazine resistance), experiments were performed on whether qE is controlled only by nuclear, or by both nuclear and cytoplasmic factors. Photosynthetic properties, chlorophyll fluorescence quenching-related parameters and xantophyll cycle activity were compared in different S. *nigrum* lines.

We used two inbred lines of *Solanum nigrum*: Wild type, atrazine sensitive (AS) and atrazine resistant (AR); *i.e.* carrying the S264G D1 mutation in its chloroplastic DNA. To generate F1 hybrids between the two parental lines, we set up reciprocal crosses between AS and AR parents. The heterozygous F1 plants were allowed to self-pollinate to generate F2 seeds, and designated then as ARF2 and ASF2. The presence of S264G mutation of different *S. nigrum* lines was monitored by chlorophyll fluorescence induction of intact leaves by using the characteristic F_i/F_m fluorescence parameter. The modulated chlorophyll *a* fluorescence was measured on 30-min dark-adapted attached leaves, using a Dual Channel Modulated Fluorimeter (Hansatech, England). Xanthophyll cycle components were determined by means of HPLC according to Váradi et al. (1992).

The fast chlorophyll fluorescence induction kinetics of parents and hybrid lines of *S. nigrum* in principle showed similar induction transients. As expected however, the leaves containing AR chloroplastic genome (AR, ARF1 and ARF2 plants) were always characterized by significantly higher intermediate F_i chlorophyll fluorescence level as compared to the AS, ASF1 and ASF2 wild types. Light-induced gross NPQ for the wild biotypes normally reaches a value of 2.5 to 3, but it remained between 1 and 1.5 (mainly around 1) in the AR biotypes, *i.e.* decreased by about 50%. The capacity of the xanthophyll cycle is lower by 20% in the mutant biotypes. The comparison of Zea and pH dependent qE non-photochemical quenching, determined as the rapidly relaxing component of NPQ showed more definite differences between the wild and D1 protein mutant weeds. The investigated S264G D1 protein mutant lines exhibited 60-70% reduced values of qE. The results of this research clearly show that the PsbA gene-encoded highly conserved D1 protein structure, found in the wild type plants, is also essential for qE photoprotective chlorophyll fluorescence quenching, as revealed by maternal inheritance of D1 protein mutation and reduced qE level.

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Identification of Hox2, the second NAD+-reducing NiFe hydrogenase in the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina* BBS

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Hydrogenases are metalloenzymes catalyzing the oxidation of molecular hydrogen and also the reverse reaction, the evolution of hydrogen. Since, hydrogen has been reported as a good energy carrier and Sun as an environmentally friendly energy source, the study of these enzymes in photosynthetic microorganisms is a hot subject nowadays.

Thiocapsa roseopersicina BBS is a purple sulfur photosynthetic proteobacterium belonging to the Chromatiaceae family. This preferentially anaerobic bacterium, capable to grow under several different conditions (anaerobic, micoraaerobic conditions, ligth, dark, nitrogen fixing), is able to use different electron donors as reduced sulfur compounds and simple organic substrates.

The presence of at least three functional hydrogenases (HupSL, HynSL and HoxEFUYH) (1,2,3) have been reported in this bacterium. Deletion of all the mentioned H_2ases led to a strain still having hydrogenase activity. *T. roseopersicina* genome project is going on. Part of a contig of the sequenced gDNA showed homology to *hoxH* genes encoding for the large hydrogenase subunit of the bidirectional heteromultimeric cytoplasmic [NiFe] hydrogenases. Since a Hox type hydrogenase has already been identified in *T. roseopersicina* the new gene was named *hoxH2*.

A 6 kb DNA fragment, containing the *hoxH2* gene, was cloned and further *hox2* genes were identified. The *hoxY2* encodes for the hydrogenase small subunit, *hoxF2* and *hoxU2* code for diaphorase subunits. In the genome of *Thiocapsa roseopersicina* BBS a region showing homology to *hoxW* genes was also identified, which likely encode for endoproteases responsible for the cleavage of the C-terminal extension of the large subunit as the final step of the processing.

Hydrogenase activity measurements were done using the GB112131 (*hynSL*, *hupSL* and *hoxH* mutant) strain grown on various media and i*n vivo* uptake and evolution activity were detected under certain conditions. These results suggest the presence of a heterotetrameric NAD^+-reducing bidirectional soluble NiFe hydrogenase, Hox2 in *T. roseopersicina*. The existence of two Hox enzymes in a single cell is unique for this bacterium and may disclose further diversification of the Hox enzymes.

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Effect of heavy metal stress at molecular, cellular and whole plant level and the stress responses in plants

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Heavy metals are important environmental pollutants with high toxicity to animals and plants. Cadmium, copper and zinc in high concentrations damage plants by causing oxidative stress, modulating the uptake and distribution of other essential macro elements, inactivate or denature proteins by binding to sulfhydryl groups and degrading the photosynthetic apparatus (Schützendübel and Polle 2002). Higher plants exhibit considerable variation in their ability to tolerate bivalent cation contamination in their environment. Several tolerant species can accumulate significant levels of these metal ions, while another sensitive species can be impaired by lower metal concentrations. Heavy metals can be detoxified by different mechanisms: conjugation with phytochelatins, by scavengers or using the antioxidant enzyme capacity of plants. In this enzymatic way the catalase (CAT), guaiacol peroxidase (GPX), superoxid dismutase (SOD), and glutation reductase (GR) are involved (Zenk 1996).

In our experiments two plant species were investigated: the heavy metal tolerant- and accumulator Indian mustard (*Brassica juncea* L. Czern.) and the sensitive agricultural species pea (*Pisum sativum* L.).

We compared the different physiological changes in the two plant species such as heavy metal accumulation, enzymatic responses and growth parameters. Our aim is to reveal the characteristic properties of metal tolerant plants which can be useful in biological soil cleaning technologies such as phytoremediation (Salt et. al. 1995).

Plants were grown in Hoagland nutrient solution under controlled conditions. Cd, Cu or Zn salts were added in different concentrations to the nutrient solution. We measured the capacity of the heavy metal uptake and accumulation, the root-shoot translocation, and the changes of the macro element distribution. All plants accumulated significant levels of the applied metal ions in their roots, however,

Brassica accumulated Zn ions preferentially in the leaf tissue. Copper treatment had a particularly drastic effect on the root system of both species. The effects were plant- and heavy metal specific: in general in Indian mustard the responses for heavy metal stress were the moderate decreases of oxidative enzyme activities while in pea the activities showed slight increases. The most significant changes in enzyme activities occurred in the second and third days after treatment in both species.

Literature data suggest that there is a causal relationship between NO and metal ion metabolism (Neill et. al. 2002). Our aim is to demonstrate the possible role of NO in the plant response to heavy metals. After treatments, the NO appearance was measured in the root tips with fluorescent method, using a very specific dye to nitric oxide, diaminofluorescein-diacetate (DAF-2 DA). We obtained different NO levels with different heavy metal loads: the most effective metals were copper and cadmium, in this case the NO production became double after one week treatment. In case of copper load, we found a fast NO burst in the first six hours (Bartha et. al. 2005). This fast appearance and the good mobility of NO suggest that it can be a signal molecule in plants under heavy metal stress.

Compared the two species we found basic differences in their cadmium detoxification mechanisms and oxidative stress defense responses. These experiments can help us to find the physiological background of the heavy-metal tolerance. On the basis of our results phytoremediation technology will be worked out. Our results are already applied in pilot experiments on polluted environment and our further aim is to work out a complete phytoremediation technology (Vashegyi et. al. 2005).

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Comparative analysis of 10th -11th century populations in the southern part of the Great Hungarian Plain. A preliminary report

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The regional anthropological characteristics of the Carpathian Basin have never been uniformal, so the biological effects of the Hungarian Conquest were versatile, too. However, in some regions of the country late Avar populations show quite a lot of similarities with early Árpádian Age populations in general (Szathmáry 1996). This fact might be explained by the possible survival of autochthonous groups from the 9th century.

Compared to other regions of the country, Late Avar Age populations and ethnic groups of the Age of the Hungarian Conquest in the southern part of the Great Hungarian Plain show less differences (Barabás et al. 1996), although, new settlers are known to have arrived here during the Conquest. For this reason, the area seems to be a best investigation area concerning survival of certain populations.

The aim of our study is to perform a complete comparative analysis of cemeteries from the 10th and 11th century in the southern part of the area east of river Tisza using both classical methods of historical anthropology and statistical analyses of metrical data. The following series were included in our study: Békés-Povádzug 10th-12th c., Biharkeresztes-Ártánd-Nagyfarkasdomb 10th-11th c., Eperjes-Ifjú Gárda Tsz. 11th c., Gyula-Fövenyes 11th-16th c., Hódmezővásárhely-Nagysziget 10th-11 th. c., Kiszombor-B 10th-11th c., Magyarhomorog-Kónyadomb 10th-12th c., Orosháza-Rákóczitelep 10th-12th c., Sarkadkeresztúr-Csapháti-legelő 10th-11th c., Sárrétudvari-Hízóföld 10th c., Szegvár-Oromdűlő 10th-11th c., Szegvár-Szőlőkalja 10th c. The sample consists of the remains of 2090 individuals (668 male, 620 female, 215 undetermined, 587 subadult). In order to facilitate our biostatistical examinations in the future, we had to choose series with as many cases as possible. Thus, only cemeteries containing at least 50 graves were included in our study. These series usually belonged to the common people of the era and do not represent the whole society. Another concern of sampling was not to leave out any important 10th or 11th century skeletal population. This condition forced us to include series containing lot of burials from the Árpádian Age.

The data of the formerly published series we took over from the references. In case of our own examinations (Eperjes-Ifjú Gárda Tsz., Hódmezővásárhely-Nagysziget (Bereczki et al. 2003a, 2003b), Sarkadkeresztúr-Csapháti-legelő (Bereczki and Marcsik 2005), Szegvár–Szőlőkalja) we used commonly accepted methods of historical anthropology. In some cases X-ray pictures were taken to support pathological diagnoses. All bone material and documentation is housed at the Department of Anthropology, University of Szeged.

Our investigation is long not completed yet, at the current state of the process we can only present our preliminary results. Having successfully finished the examinations we will aim to give answers to questions like e. g. what kind of biological relation exists between these

populations not far from each other in space and time; in which area was the survival of late Avar populations possible; and what kind of biological impact did the time period before and after the Foundation of State have. Besides, our study provides important new data to the prevalence of certain trephination techniques and metastatic tumors.

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Cyclin C and the development of mice

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Cyclins are a group of proteins in eukaryotic cells involved mainly in the regulation of cell cycle. Mammalian and *Drosophila melanogaster* cyclin C genes (Katona and Lahti 2006) were originally identified by rescuing a *Saccharomyces cerevisiae* strain that carried mutation in all known G1-type cyclins (CLN1, 2 and 3). Later studies did not support the assumption that cyclin C could have been a G1-type cyclin. In further experiments it was shown that cyclin C was a regulator of mRNA transcription through different mechanisms (Loyer et al. 2005). In higher eukaryotes cyclin C and Cdk8 together are responsible for the phosphorylation of proteins associated directly or indirectly with mRNA synthesis. Cdk8 with cyclin C attached phosphorylates cyclin H inhibiting its transcriptional and cell cycle function through Cdk7 and TFIIH. Moreover, together cyclin C and Cdk8 phosphorylate the carboxy terminal domain (CTD) of the large subunit of RNA polymerase II and they hyperphosphorylate and inactivate Notch negatively regulating its effect on mRNA transcription. Cyclin C and Cdk8 proteins are also part of one of the mammalian Mediator complexes. These complexes were demonstrated to promote transcription *in vitro*. It has also been revealed that cyclin C cooperates with Cdk3. This molecule complex has been thought to play a role in the exit from G0 phase by phosphorylating specific residues of Retinoblastoma protein (pRb) (Ren and Rollins 2004). Cyclin C was demonstrated to interact functionally with c-myc, which promotes cell division. Overexpression of cyclin C elevates the level of cdc2 mRNA, a key player in G2/M transition (Liu et al. 1998). Growing number of other evidences suggest that cyclin C acts not only in mRNA transcription but in cell cycle regulation as well in mammalian cells.

Preliminary experiments pointed out that cyclin C may have a vital role in early embryogenesis of mice (*Mus musculus*). Deletion of the transcription, translation start point and the first four exons of cyclin C gene results in an embryonic lethal phenotype. Cyclin $C^{-/-}$ mice die before 10th day (E10) of embryonic development (Katona et al. unpublished data). Body sizes are significantly smaller in cyclin C knock-out embryos than in their heterozygous or wild-type littermates. Cyclin C null mutants suffer from serious abnormalities. The cranial end remains split. Heart is large relative to body and unstructured. Forelimbs are usually vestigial and no rear limbs can be found. Generally, the caudal part of the body does not develop at all. Serious retardation can be seen in the differentiation and growth of many other tissues as well. The placental labyrinthine layer, yolk sac and potentially the embryo itself are poorly vascularized.

These findings raise the following questions. What is the tissue localization of cyclin C in early embryonic development? How does the cyclin C expression pattern correlates with the experienced knock-out phenotype?

In order to address these questions we generated transgenic mice carrying a hrGFP reporter gene construction driven by an 3,5 kb upstream region (putative promoter) of the mouse cyclin C gene.

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Integrative approach of Photosystem II repair in Synechocystis PCC6803 under UV-B damage: proteases, prohibitins and sHSP

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Cyanobacteria, the most widespread and abundant oxygenic photosynthetic prokaryotes, are exposed to various types of environmental stresses. With the recent thinning of the ozon layer, the UV-B component of solar radiation is of particular importance. Its targets inside the PSII are the water oxidizing complex (Vass 1999) and the D1 and D2 proteins (Friso et al. 1994; Friso et al. 1995). In intact photosynthetic organism the structure and function of photodamaged PSII centers can be repaired. The critical step of this repair process is the removal of damaged D1 and D2 proteins, followed by the novo synthesis of the D1 and D2 subunits and reassembly and reactivation of the PSII complex.

We have investigated the role of FtsH and Deg proteases in the degradation of UV-B damaged PSII reaction centers proteins D1 and D2 in Synechocystis 6803. We analyzed also the possible involvement of prohibitins in the repair cycle.

PSII activity in a ΔFtsH (slr0228) strain, showed increased sensitivity to UV-B radiation and impaired recovery of activity in visible light after UV-B exposure.

In contrast, in ΔDeg cells, in which all the three deg genes were inactivated, the damage and recovery kinetics were the same as in WT. Immunoblotting showed that the loss of both the D1 and D2 protein was retarded in FtsH (slr0228) during UV-B exposure, and the extent of their restoration during the recovery period was decreased relative to the WT. However, in the Deg cells the damage and recovery kinetics of D1 and D2 were the same as in the WT. These data demonstrate a key role of FtsH (slr0228), but not Deg proteases, for the repair of PSII during and following UV-B radiation at the step of degrading both the UV-B damaged D1 and D2 reaction center subunits.

The genome of Synechocystis contains 5 genes for putative prohibitin proteins. Due to the formation of protein complexes containing FtsH protease and prohibitins in mithocondria of S. cerevisiae and E. coli, a similar interaction was investigated in Synechocystis. Silva and Nixon (unpublished) showed that His-tagged PSII contain both FtsH and prohitin (PhB1).

PSII activity in a triple prohibitin mutant (ΔT) and a quadruple one (ΔQ) measured as the rate of oxygen evolution, under UV-B alone or combined illumination: UV-B and VIS, did not show a significant difference as compared with the WT cells.

Synechocystis PCC 6803 has one small heat shock protein Hsp17 that is known to protect thylakoid membrane against heat and photoinhibitory effects of light exposure (Balogi et al. 2005). Two point mutations in Hsp17, L9P and Q16R resulted in dramatic changes in the cellular distribution of Hsp17 in heat/light acclimated cells: an important fraction of L9P was found in the cytoplasm, while Q16R was exclusively associated with the thylakoid membrane (Balogi, unpublished results). Under UV-B stress the heat/light hardened cells behaved differently: WT and L9P cells lost their photosynthetic activity and the Q16R mutant protected almost completely the photosynthetic function (measured as the rate of oxygen evolution).

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Regulation of translesion synthesis

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Cellular DNA is continually damaged by a variety of sources, including UV light from the sun and reactive oxygen species resulting from aerobic respiration. Although cells posses a variety of repair processes to remove DNA lesions, lesions that escape repair can block the replicational machinery. Eukaryotic cells overcome such blocks and promote the continuity of the newly replicated DNA strand through translesion DNA synthesis.

Genetic studies in the yeast Saccharomyces cerevisiae have indicated that Rad6 an ubiquitin-conjugating enzyme together with Rad18 a DNA-binding protein controls the bypass of UV-damaged DNA via at least three separate pathways: an error-free pathway dependent on the RAD5, MMS2 and UBC13 genes, another error free pathway dependent on the RAD30 gene, and a third pathway that is mutagenic and dependent on the REV1, REV3 and REV7 genes.

Since Rad6-Rad18 complex is indispensable for the function of all three DNA damage pathways the question was what would be the mechanism through which Rad6-Rad18 regulates these pathways.

It has been published that Rad6 together with Rad18 monoubiquitylates PCNA, which serves as a sliding clamp for the replicative polymerases. Two other members of the Rad6 group, Ubc13 and Mms2, form a heterodimeric ubiquitin-conjugating enzyme, which together with the RING-finger-containing protein Rad5 are responsible for the polyubiquitylation of PCNA.

We wanted to identify other possible substrates Rad6-Rad18 might have. First we checked whether Rad5 could be a substrate of Rad6-Rad18. Using purified proteins we have performed in vitro ubiquitylation of Rad5 using Rad6-Rad18. Our results showed that Rad6-Rad18 efficiently ubiquitylates Rad5 in vitro. To check the in vivo significance of Rad5 ubiquitylation we chromosomally tagged Rad5 with 6His3Ha tag. We treated the cells with the DNA damaging agent MMS and we prepared whole cell extract and performed western blot using a-HA antibody to detect Rad5 and any modified forms of Rad5. Indeed we could detect two higher molecular weight forms of Rad5. According to the molecular weight one could be the ubiquitilated form of Rad5. Experiments using specific anti ubiquitin antibody to verify our result are still in progress.

We also want to determine which Lysine residue is involved in the ubiquitylation of Rad5. We have sent in vitro ubiquitilated Rad5 to mass spectroscopy analysis, but unfortunately we could not get back any positive results. To overcome this problem we have created C terminal deletion mutants of Rad5 to map the region that involves the ubiquitilated lysine residue. We have cloned, overexpressed and purified these mutants and presently we are checking which one of them can be ubiquitylated in vitro. Once the target lysine is found genetic analysis will be performed to see the effect of this mutation on DNA damage bypass.

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Study of the mitochondrial DNA polymorphism among Candida species

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Recently the number of infections caused by Candida species increased significantly. Localized, invasive or systemic infections (candidiasis) are frequently associated with immune deficiencies, antibiotic treatment, immunosuppressive therapy and various invasive medical procedures (Nosek et. al. 2002, Deák et. al. 2004). The most frequent species isolated from Candida-infected humans is *C. albicans,* however, the occurrence of other species e.g. *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis* increased as well.

The size, organization and gene content of the mitochondrial DNA in yeasts display high degree of variety. Therefore study of the mitochondrial genomes may give deeper insight into the genetic relatedness of the species. However mitochondrial DNA is typically described as a circular molecule, there are examples in the genera Candida, which have linear mtDNA, with telomeres on the ends of the DNA. Moreover the form of mitochondrial genome may differ within the same species, like in *C. metapsilosis*. Some experiments suggest that, under some circumstances, the linearity and/or the presence of telomeres provides a competitive advantage over a circular-mapping mitochondrial genome (Nosek et. al. 2004).

C. albicans has a circular mitochondrial genome with 40420 bp as confirmed by DNA sequencing. In the present study we analysed the mtDNA polymorphism among 44 clinical isolates of *C. albicans*. All the strains with one exception derived from hemo-culture. Four types of mtDNA were detected on the basis of the RFLP pattern of the *Hinf*I-digested total DNA. The most frequented type was labelled as number I. The group number IV was represented only by one isolate. The restriction profile of the purified mtDNA confirmed the existence of these types. Interestingly the *Pvu*II digestion revealed the same RFLP pattern in each type. Other enzymes (*Eco*RV, *Bgl*II) gave distinct result what means that the different mtDNA types derived from point mutations or recombination.

Despite *C. albicans, C. parapsilosis* has linear mitochondrial DNA with tandem repeats at the termini (Nosek et. al. 2004). Earlier study demonstrated the very stable RFLP pattern of the mtDNA within group I isolates of *C. parapsilosis*. Our survey on Hungarian clinical isolates confirmed these results (Pfeiffer et al. 2006).

An international project was established to study the taxonomic relationship and the evolution of the mitochondrial genome in the clade involving *C. parapsilosis* (Diezmann et al. 2004) and some closely related species, such as *C. metapsilosis, C. orthopsilosis* (Kosa et al. 2006), *C. albicans* (Anderson et al. 2001), *C. tropicalis, C. dubliniensis, C. sojae* and *C. maltosa*. In the frame of this work our aim was to get the whole sequence of the mtDNA of *C. maltosa*. Firstly, we have made the restriction map of the mtDNA of *C. maltosa* using the restriction endonucleases *Eco*RV and *Pst*I. We have made random sequencing after physical breaking (nebulization) of the mtDNA. The sequences were aligned by software generating contigs by overlapping regions of the sequences. We already have the 70 percentage of the mitochondrial genome, involving genes of nad1, nad4, nad5, cox1, cox3a, cox3b, cob, rnl, atp6 and some tRNA. The remaining gaps will be filled with primer walking in the near future.

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Children growth in the past

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Children growth is a separate area in physiology and anthropology. Growth rate can be examined using auxology studies. The main aim of auxology is to recognize the principles of growth on the base of longitudinal or mixed sampling (Farkas 2000).

Complications can occur during studying archaeological samples, especially when working with children samples. Very often bones are fragile, and there are difficulties with determining age at death and sex.

Chronological age can be estimated by using biological age-markers at their developmental stage.

One of these age-markers is the development of the skeleton that can be estimated by epiphyseal growth or long-bone measures. In the case of children, whose epiphysis has not closed yet, measurements were used as suggested by Fazekas and Kósa (1978). If the epiphysis was closed, measurements were used as suggested by Knussmann and Martin (1988).

Another method for estimating age is detecting teeth-eruption through different developmental stages (Schour and Massler, 1941).

In both cases it should be taken into consideration that environmental stresses (nutrition, diseases, etc.) have certain effect on bone-measurements and teeth-eruption time. Bones are much more affected then teeth.

Visser (Visser 1998) created regression equation for stature estimation on the base of radiographs made by Maresch in 1943. These equations need the measurements of the humerus, femur and tibia.

The main aim of this study was to get information on children growth by the examination of length and diameter of the long bone's diaphysis in the age categories defined by teeth eruption-time. After mathematical-statistical analysis conclusions were to be reached about children growth in the Avarian age. As a last step the Avarian data were compared with contemporary children data.

As material the Bélmegyer-Csömöki domb cemetery from the Avarian age was studied. Archaeologists suppose that between 670-800 A.D. an Avarian population settled down and lived here. The excavation started in 1985 under the leadership of Medgyesi Pál and was finished in 1989. The total number of individuals found in graves is 243. Among them 64 belongs to the infantia I, infantia II and juvenis categories in which measures were taken: 15 measurements on the cranium and 17 on the postcranial skeleton taking laterality into account.

As a result of the mathematical-statistical analysis it can be stated that the long bones and the stature grew regularly in all age-groups, and that the mean of the age-groups could be clearly separated which confirms the correct principle of creating them. As expected, comparing the stature of the Avarian children with contemporary individuals shows that contemporary children are taller.

This study is an introductory to a medieval cemetery called Bátmonostor-Pusztafalu, which is the largest medieval cemetery in Hungary. The total number of individuals found in graves and reduction areas is 3783; among them 1510 skeletons belong to the infantia I and infantia II categories. The number of juvenis individuals is 153 (Józsa et al. 2004).

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The role of FnrT, an oxygen dependent regulator, in the photosynthetic purple sulfur bacterium, *Thiocapsa roseopersicina* BBS

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In facultative anaerobic bacteria the availability of O_2 is one of the most important regulatory signals (Kovács ÁT et al. 2005a). In the presence or absence of oxygen different metabolic pathways are switched on or off. The FNR protein is an oxygen responsive transcription regulator functioning as a “switch” between the anaerobic and aerobic metabolic pathways. The FNR contains Fe-S clusters which are oxygen sensitive and the *E. coli* FNR was shown to regulate the expression of around 110 operons directly or indirectly (Constantinidou et al. 2006).

Thiocapsa roseopersicina BBS is a Gram-negative, purple sulfur photosynthetic bacterium belonging to the Chromatiaceae family in the γ-subdivision of proteobacteria. In addition to the anaerobic photosynthetic growth, the strain is capable to grow aerobically, chemolithotrophically in the dark.

My work focuses on the FNR analogue, the FnrT recently identified in *Thiocapsa roseopersicina.*

T. roseopersicina BBS has two sets of membrane-associated [NiFe] hydrogenase genes: the HynSL and HupSL and a third, soluble hydrogenase HoxYH (Kovács KL et al. 2005). In our previous report it was demonstrated that the *hyn* enzyme was anaerobically induced and the upregulation was mediated by the FnrT (Kovács ÁT et al. 2005b). In contrast, the *fnrT* mutation had no effect on the expression of HupSL and HoxYH hydrogenases. Using reporter genes, a slight, negative autoregulation in the expression of the FnrT could be noticed under anaerobic conditions in this bacterium. From these observations an interesting question arose: what is the role of an oxygen sensing protein in a preferentially anaerobic bacterium. Since, the FNR had effect on the expression of more that one hundred genes in *E. coli* we assumed the similar global effect of FnrT in *T. roseopersicina*, as well.

To answer these questions an *fnrT* mutant (FNRTM) strain was prepared. Using proteomic approach, genes, metabolic pathways being under the control of FnrT were/are looked for.

The protein patterns of the wild type and the mutant strain were compared on 2-D gel electrophoresis. On the silver stained gels several spots with distinct intensities were found suggesting a global role of *fnrT* in an - especially anaerobic - photosynthetic sulfur bacterium. In addition, other staining methods are tested to increase the resolution of the protein quantitation, which would allow reliable detection of relatively small differences in the protein level.

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Changes in water relations, stress hormone biosynthesis and glutathione S-transferase activities and expression levels in *Triticum aestivum* cultivars under osmotic stress

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We investigated the drought tolerance of two wheat cultivars *Triticum aestivum* cv. Kobomugi, a near isohydric landrace, and *Triticum aestivum* cv. Öthalom, a dehydration tolerating genotype. Drought tolerant wheat cultivars exposed to low water potential can be characterized by changes in water relations, production of stress hormones such as abscisic acid (ABA), production of antioxidative enzymes and other signal molecules under osmotic stress. Osmotic stress treatment was applied gradually reaching 400 mOsm polyethylene glycol (PEG 6000) treatment (-0.976 MPa) on one-week-old *Triticum aestivum* plants under controlled conditions as it was published earlier in Erdei (2002). In ABA production, aldehyde oxidase (AO) plays a key role by catalysing the last, rate limiting step of the biosynthesis. After the osmotic stress treatment, the activity of AO increased earlier in the near isohydric cultivar than in the dehydration tolerating Öthalom. Simultaneously, the cv. Kobomugi accumulated significantly more ABA in the leaf tissue than well-watered control plants, while the ABA

content did not increase in cv. Öthalom. In association with water potential measurements two strategies of acclimation to drought stress have been found. Plants using the first strategy, save tissue water content by a fast decrease of stomatal conductance with fast raise in ABA production and aldehyde oxidase activity. In the second group the closure of stomata occurs later resulting in an intensive loss of water and a fast decrease of water potential in the leaves and tissues restore their turgor after a relatively long acclimation phase. According to our measurements, cv. Kobomugi belongs to the first group, cv. Öthalom into the second group. (Gallé 2002)

Glutathione S-transferase (GST) isoenzymes represents a large and variable group of antioxidative enzymes, with several different activities and sequence patterns. Phylogenetic analysis of wheat *GSTs* was performed *in silico* and using the tentative consensus sequences (TC) a dendogram was composed. According to the conserved sequences used for classification of GST proteins, we could identify four groups of wheat GSTs (*phi, zeta, theta* and *tau*). Homology was found between the osmotic stress upregulated sequences and the GST coding TCs were identified. Real Time PCR analysis with two group-specific primer showed a significant increase in the amount of GST transcripts after plants were exposed to 400 mOsm PEG treatment for two days (Gallé 2005). The changes in the transcripts were compared with the GST activities measuring in the same times. According to our results the members of *phi* GST can be responsible for the fast response after the osmotic stress, while the *tau* GST group for the later enhancement of GST activity.

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Identification and characterization of *Trichoderma* strains causing mushroom green mould disease in Hungary

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World-wide mushroom cultivation is dominated by the production of *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* (Chang 1999). Producers of champignon (Agaricus bisporus) and oyster mushroom (*Pleurotus ostreatus*) are facing recent incidents of green mould epidemics in Hungary.

We examined 66 *Trichoderma* strains isolated from *Agaricus* compost and *Pleurotus* substrate samples from three Hungarian mushroom producing companies by a PCR-based, diagnostic test for *T. aggressivum* (Chen et al. 1999), sequence analysis of the internal transcribed spacer regions 1 and 2 (Druzhinina et al. 2005) and (selectively) of the 4th and 5th intron of translation elongation factor 1α (4), and RFLP of mitochondrial DNA.

Seven *Trichoderma* species were identified [numbers of isolates given in brackets]: *T. aggressivum f. europapeum* [17], *T. harzianum* [3], *T. longibrachiatum* [4], *T. ghanense* [1], *T. asperellum* [4], *T. atroviride* [9], and a still undescribed phylogenetic species: *Trichoderma* sp. DAOM 175924 [28]. *T. aggressivum f. europaeum* was exclusively derived from A. bisporus compost, whereas *Trichoderma* sp. DAOM 175924 exclusively occurred in the substrate for *Pleurotus* cultivation. Sequences of the latter strains were cospecific with those for *Trichoderma* pathogens of *P. ostreatus* in Korea. The isolates of T. sp. DAOM 175924 can be divided into two types on the basis of an A/C transversion at position 447 in ITS2. The two types of *T*. sp. DAOM 175924 isolates differ from each other based on morphological features as well, and they are currently being described as new species, *T. fulvidum* sp. nov. and *T. pleurotophilum* sp. nov., respectively.

The widespread occurence of this new species raises the questions, why infections by it have just only recently been observed. Our data document that (a) green mould disease by *T. aggressivum f. europaeum* has geographically expanded to Central Europe; (b) the green mould disease of *P. ostreatus* in Hungary is due to the same *Trichoderma* species as in Korea and the world-wide distribution of the new species indicates the possibility of spreading epidemics; and (c) on mushroom farms, the two species are specialized on their different substrates.

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Study of experimental inflammatory bowel disease in the rat

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The pathogenesis of the inflammatory bowel diseases is still incompletely understood. It is likely that local release of reactive oxygen species (ROS) may be involved, creating epithelial injury in the colon (Simmonds et al. 1993; McKenzie et al. 1996; Dryden et al. 2005). It is therefore possible that endogenous protective antioxidant systems could be evoked in order to attenuate colonic tissue injury. ROS may be modulated by endogenous anti-oxidant products of heme oxygenase-1 (HO-1). In the present work, HO-1 expression in trinitrobenzene sulphonic acid (TNBS)-induced colitis in the rat and the effects of HO-1 modulation, particularly by the HO-1 inducer, heme, were further evaluated. Colitis was induced by intracolonic challenge with TNBS and assessed macroscopically and by myeloperoxidase (MPO) assay. TNBS challenge led to an early and substantial induction of HO-1 protein expression and HO activity in the colon that peaked after 48–72 h and declined over 10 days. Heme (30 μmol/kg/day, s.c) increased colonic HO-1 protein expression and HO enzyme activity and decreased colonic damage and MPO activity. In contrast, the HO-1 inhibitor, zinc protoporphyrin (ZnPP; 50 μmol/kg/day, s.c) significantly increased the colonic damage and MPO activity over 10 days, as did tin protoporphyrin (30 μmol/kg/day, s.c). These results support the proposal that induction of HO-1 provides a protective mechanism in this model under both acute and more-chronic conditions, and that its selective up-regulation could thus be of therapeutic potential in colitis.

The mechanism of action of 5-aminosalicylic acid (5-ASA), the active therapeutic moiety of a number of clinically used anti-colitic agents, has long been considered to include its direct anti-oxidant and radical scavenging activity. The present study also investigates whether this effect *in vivo* could also involve induction of HO-1, known to provide endogenous anti-oxidant and anti-inflammatory moieties which can modulate colonic inflammation. However, an enduring concept is that at least part of the beneficial activity of 5-ASA reflects its actions as an antioxidant and free radical scavenger (Simmonds et al. 1999; Reifen et al. 2004).

The effects of 5-ASA on the colonic expression and activity of HO-1 along with its effect on the inflammatory damage have been evaluated in the colitis provoked by instillation of TNBS over 48 hours. Intra-colonic administration of 5-ASA (8, 25 and 75 mg $kg^{-1}day^{-1}$) dose-dependently reduced the TNBS-provoked macroscopic colonic inflammatory injury and MPO levels, while also dose-dependently increasing colonic heme oxygenase enzyme activity (by 42%, 46% and 77% respectively at the highest dose). Colonic HO-1 activity expression was likewise further induced by 5-ASA. Moreover, intra-colonic administration of 5-ASA alone under unchallenged conditions induced colonic HO-1 expression and stimulated HO activity. Administration of ZnPP (30 μmol/kg/day, s.c.), which prevented the increase in colonic HO activity, abolished the anti-colitic effect of 5-ASA.

These results suggest that 5-ASA may exert its colonic anti-oxidant and anti-inflammatory effects *in vivo* in part through the up-regulation of HO-1 enzyme expression and HO activity.

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Analysis and identification of pathogen *Candida* species based on molecular variability and DNA sequences

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Candida species are members of the normal human flora and can be easily recovered from our environment. Infections caused by *Candida* species are widespread throughout the world. Although *Candida albicans* is the most common *Candida* species encountered as a cause of human infections, other *Candida* species have been increasingly associated with disseminated disease since the 1990s. Among them, *Candida parapsilosis* has been the second most common yeast species isolated from bloodstream infections in several surveys (Messer et al. 2006). This species has emerged as an important nosocomial pathogen, with clinical manifestations including fungemia, endocarditis, endophthalmitis, septic arthritis, and peritonitis, usually occur in association with invasive procedures or prosthetic devices This species is

more frequent in bloodstream infections of neonates, in transplant recipients, and in patients who received parenteral nutrition or previous antifungal therapy.

There is no completely reliable method for detecting *Candida* infections. Although there are good methods for identifying *Candida* species, they are often time- and cost-consuming. Our aim was to develop a rapid and reliable molecular method to identify clinically important *Candida* species from bloodstream infections, and to examine the genetic variability of *Candida parapsilosis*.

For the development of the *Candida* specific primer pairs, we used DNA sequences of interest avaiable in the National Center for Biotechnology Information (NCBI) database. Missing sequences were supplied by direct sequencing of the adequate fragments. Eight primer pairs were developed, capable of specifically identifying *Candida glabrata, C. guilliermondii, C. krusei, C. lusitaniae, C. albicans, C. dubliniensis, C. tropicalis* and *C. parapsilosis*.

To analyse the genetic variability of *Candida parapsilosis* isolates, 209 *Candida* isolates from blood samples from two Hungarian hospitals located in Debrecen and Pécs were examined. The samples came from different patients and identified by standard morphological and physiological methods within the hospitals.

Previous studies clarified that *C. parapsilosis* isolates can be divided into three groups which could be distinguished based on several criteria including randomly amplified polymorphic DNA (RAPD) analysis, sequences of the internal transcribed spacer (ITS) region of the rRNA gene cluster, DNA relatedness, morphotyping, electrophoretic karyotypes, single nucleotide polymorphisms, mitochondrial DNA sequence differences and biofilm producing abilities. Recently, Tavanti et al. (2005) recognized *C. parapsilosis* groups II and III as separate species, *C. orthopsilosis* and *C. metapsilosis*, respectively, based on multilocus sequence typing studies. The two latter species can be recovered relatively rarely in clinical samples. Besides, *C. parapsilosis* group IV has also been found recently among Brazilian clinical *Candida* isolates by Iida et al. (2005). We examined the occurrence of *C. parapsilosis* isolates among *Candida* isolates collected in Hungarian hospitals, and examined the genetic variability of these isolates using sequence analysis of the ITS region, and RAPD technique. Two isolates were found to belong to the recently described *C. metapsilosis* species (*C. parapsilosis* group 3) based on molecular and phenotypic data. This is the first report on the identification of *C. metapsilosis* from bloodstream infection.

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The physiological role of a NAD+-reducing cytoplasmic hydrogenase in a phototrophic bacterium, *Thiocapsa roseopersicina* BBS

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In the near future, the supply of fossil fuels will be likely exhausted and other alternative energy sources and carriers have to be found. Hydrogen seems to be one of the best energy carriers, since its burning leads to water, therefore a hydrogen economy would substantially contribute to the reduction of CO_2 emission and – consequently - global warming. Biological systems, as renewable catalysts, are ideal tools for production of hydrogen, named as biohydrogen. There are few ways for production of biohydrogen, and - in most cases - the key enzyme in the process is the hydrogenase.

Our model organism, *Thiocapsa roseopersicina* BBS is a phototrophic purple sulfur bacterium, which harbours several [NiFe] hydrogenases. The HynSL and HupSL hydrogenases are associated to the membrane, while the HoxEFUYH, a NAD^+-reducing enzyme – being responsible for the light dependent H_2 evolution of the cells - could be identified in the soluble fraction. The *hox* gene products showed the highest similarity to the corresponding subunits of the cyanobacterial bidirectional hydrogenases (HoxEFUYH), which form a separate subfamily of the NAD^+ reducing hydrogenases [1].

There is an obvious question to be answered: why a single cell needs so many isoenzymes, what are their physiological roles. Here, I focused on the physiological characterization of the *hox* genes and enzymes.

Downstream from the *hoxH* gene the *hoxW* gene was indentified, which was likely responsible for the posttranslational cleavage of the HoxH subunit. Reverse transcription coupled PCR experiments revealed that all five structural genes together with the *hoxW* gene were localized on a single transcript. This indicated concerted regulation of the gene cluster.

Very little is known on the the expression pattern of the Hox enzyme. So far, environmental factor effecting its expression level could not be identified. However, using the *lacZ* reporter gene fused to a deletion series of the upstream regulatory region of the *hox* operon, a section having negative impact on the expression of the *hox* gene cluster could be perceived. The fine mapping of this *cis* regulatory element and identification of the corresponding transcription factor(s) are in progress.

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The presence of the HoxE subunit is exclusively characteristic for this subfamily of the NAD^+-reducing [NiFe] hydrogenases. *In frame* deletion of the *hoxE* gene abolished the hydrogen evolution derived from the Hox enzyme *in vivo*, although it had no effect on the activity *in vitro*. This suggested, that HoxE had a hydrogenase-related role: probably it took part in the electron transfer processes.

With the aid of a C-terminal double-tagged construct [2] we could purify the HoxE protein together with the HoxFUYH subunits of the Hox hydrogenase. This confirmed the fact that the HoxE protein is part of the multisubunit hydrogenase-complex. Similar approach is used to identify electron donor/acceptor proteins interacting with the HoxE subunit and linking the Hox enzyme to the respiratory and/or photosynthetic complexes.

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Effect of cell density on lipid composition and heat shock protein response

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Life requires the presence of a barrier around cells, but the cell membrane is not merely a barrier that must be traversed; rather, the membrane and its constituent lipids are also indispensable participants in many events of signal transduction. The field of signal transduction is important because of its fundamental role in cellular communication and regulation of cellular responses to various stresses (Kathleen M. 2007). It was suggested, that the extent of membrane damage and cellular tolerance limits during heat stress depends on lipid composition, fatty acid saturation and membrane fluidity/microdomain organization of the cell membrane (Horváth et al. 1998; Vígh et al. 1998; Vígh and Maresca 2002; Vígh et al. 2005).

When cells are exposed to temperatures that exceed their normal growth temperature, they respond by inducing the synthesis of several polypeptides referred to as heat shock proteins (HSPs). We have demonstrated, that subtle membrane perturbations are critically involved in the conversion of signals from the environment into the transcriptional activation of various hsp genes (Nagy et al. 2007). A specific set of HSPs, like HSP25 and HSP70 are expressed in response to a wide variety of physiological and environmental insults, thus allowing the cells to survive lethal conditions. Several mechanisms account for the cytoprotective effect of HSP25 and HSP70. Both proteins are powerful molecular chaperones. They both inhibit key effectors of the apoptotic machinery at the pre- and post-mitochondrial level and participate in the proteasome-mediated degradation of proteins under stress conditions.

In cancer cells, the expression of HSP25 and/or HSP70 is typically abnormally high, and both HSPs may participate in oncogenesis and in resistance to chemotherapy. In rodent models, overexpression of HSP25 and/or HSP70 increases tumor growth and metastatic potential. By contrary, silencing the hsp25 gene eliminates completely the migration capability of certain highly metastatic tumor cells. In addition, it was recently shown, that cellular distribution and especially surface membrane expression of HSP25 and HSP70 differentially regulates tumor growth and metastasis (Bausero et al. 2004). Therefore, understanding the key factors contributing to the control of the expression and cellular localization of these HSPs has become a novel strategy of cancer therapy.

In our study we investigated the effect of different plating densities on the membrane fatty acid (FA) composition of B16-F10 melanoma cells, an established cellular model of melanoma. We have shown that small variations in the initial cell density could profoundly influence the FA composition of membrane lipids. With increasing cell number we observed a strong enrichment of oleic acid content paralleled with the decline of the level of specific polyene fatty acids (mainly the arachidonic acid). Even the saturated FA-s were lessened presumably by being substrates of delta-9 desaturase. Changes in cholesterol level and the accumulation of desmosterol, a percursor of cholesterol were also detected. We could conclude, that cell density is a major determinant of lipid composition of our model system.

In B16-F10 cells the elevated mRNA product for HSP25 and HSP70 were reliably observed at 41ºC and strongly increased upon exposure to 42ºC. We have shown, that parallel with causing different lipid composition variation of initial cell density profoundly altered both the amplitude and ratio of mRNA levels corresponding to HSP25 and HSP70 under mild, physiologically relevant heat stress conditions. Suprisingly, the synthesis of these major heat shock proteins does not necessarily correlate with mRNA levels. Based on these results we could hypothesize that transcription and translation processes are not directly coupled events under conditions investigated.

Taken together, our studies first identify an important role of culture conditions and especially the initial cell densities for controlling the expression of HSP25 and HSP70 during mild heat stress conditions which might be helpful in the future design of antitumor therapies.

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Toxin - antitoxin modules affects the stress response and metabolism in Rhizobia

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The recent expansion of microbial DNA and protein databases that followed the sequencing of a large number of prokaryotic genomes has promoted the identification of numerous toxin-antitoxin modules present in the bacterial plasmids and chromosomes. Chromosomal TA modules are characterized by conserved structural organization, transcriptional autoregulation and a similar function, although their targets in cells are different (2). The two adjacent genes of the operon partly overlap at the stop/start codons. The two proteins form a complex, thus the antitoxin prevents the lethal or bacteriostatic effect of the toxin (3, 5). The generally accepted idea about the function of these chromosomally located systems is that they act as bacterial metabolic stress managers, being associated with the modulation of the global level of translation under conditions of nutrient limitation, or under stress conditions (2).

Rhizobia are soil bacteria capable of eliciting specialized root organs, known as nodules on the roots of leguminous plants, in which they reduce dinitrogen. In this unique association between eukaryotes and prokaryotes, the plant provides the source of energy for the bacterium, which in return synthesizes ammonia for the host plant. Rhizobia as symbiotic bacteria have to face various stresses: different environmental conditions in the soil and inside the root nodule and different nutrition circumstances in free-living and symbiotic state. The TA systems may be involved in helping these bacteria to cope with these transitions. The previously identified *ntrPR* operon in *Sinorhizobium meliloti,* the microsymbiont of alfalfa, represents such a chromosomally located TA module, where the *ntrP* is the antitoxin and the *ntrR* is the toxin component of the module (2). As was demonstrated earlier, a Tn5 insertion in the *ntrR* gene resulted in increased nodulation and more efficient nitrogen fixation capacity (6). When the gene expression patterns of the entire genomes of the wild type and *ntrR* mutant strains were compared, an unexpectedly large number of genes exhibited altered expression in the mutant strain, suggesting a general modulating function for NtrR (7). Among the genes with altered expression, genes coding for several chaperones were identified. Chaperones play an important role in minimizing the cellular damage caused by stress conditions such as heat shock, infection, oxygen deprivation, exposure to high salt (4). A multigene family of chaperonins was identified in *S. meliloti* (*groESL1, groESL2, groESL3, groEL4, groEL5*).

To determine the involvement of chaperonines in the protection of cells from the stress-induced damage, wild type and *ntrR* mutant bacteria containing a *groEL5::lacZ* translational fusion were grown at high temperature and at high salt concentrations. The *groEL5 – lacZ* fusion measurements revealed that the expression of this stress related gene is under the control of the *ntrR* gene and the signals for induction of GroEL5 synthesis involve high temperatures and oxygen limitation. An apparent "salt – tolerance" of *groEL5* in the *ntrR* mutant was also determined.

Using a biocomputational approach we also identified a TA like module in *Bradyrhizobium japonicum,* the microsymbiont of soybean, in which one gene of the operon shows a high degree of homology at the level of aminoacid sequence with the *ntrR* toxin of *S. meliloti*. The chromosomal operon bsl2435/bll2434 (designated as *bat/bto* operon) of the symbiotic bacterium *B. japonicum* encodes a protein pair, which forms a possible toxin-antitoxin module. In order to determine the functional role of this module we constructed a *B. japonicum* mutant in which the whole *bat/bto* operon was deleted. The effect of this mutation was a boost of the growth of these bacteria. *B. japonicum* belongs to the so-called slow growing rhizobia with a doubling time of approximately 14 hours compared to a doubling time of 3-4 hours of *S. meliloti*. The deletion mutant constructed by us has a faster metabolic rate with a doubling time of 1.5-2.5 hours depending on the composition of the media.

In order to develop symbiotically more efficient and stress tolerant strains, the involvement of toxin – antitoxin modules in stress response and metabolism of bacteria belonging to Rhizobiaceae is under further investigation.

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Analysis of the transcriptional regulation of the matrilin-1 gene in transgenic mice

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Matrilin-1 is a non-collagenous protein which functions in the organization of the extracellular matrix. It has the unique feature among cartilage-specific genes that its expression is restricted to distinct zones of the growth plate *in vivo*. In tissue cultures it also shows developmental stage-specific expression. Previous analysis of the matrilin-1 control regions in transgenic mice revealed that the presence of both promoter upstream and intronic elements was necessary for the high-level transgene activity in all chondrogenic tissues and for the extraskeletal transgene expression pattern resembling the most to that of the chicken matrilin-1 gene. We also found that the long promoter alone and the short promoter in combination with the intronic enhancer restricted the transgene expression to the zones of proliferative chondroblasts and prehypertrophic chondrocytes in the growth-plate cartilage (Karcagi et al. 2004). Since these transgenes shared only the short promoter, we raised the question whether the short promoter was responsible for the zonal and tissue-specific expression pattern. Based on computer analysis conserved sequence blocks were found within this region. Previous experiments also showed that these elements can bind to a cartilage-specific transcription factor (Rentsendorj et al. 2005) which plays key role in chondrogenesis. To test the contribution of these elements to the regulation of the gene, we generated *luciferase* and *LacZ* fusion constructs with the short promoter – or its mutant forms – and different combinations of the distal promoter elements. Apart from matrilin-1 regulatory regions, we also tested the promoter activity under the control of heterologous regulatory elements. Activity of the luciferase fusion constructs was measured in transient expression assays in different cell cultures. High density mesenchyme and chicken embryonic chondrocyte consisted mostly of early proliferative and late proliferative chondrocytes, respectively, whereas chicken embryonic fibroblast represented the non-expressing cell type. Those combinations of regulatory regions which showed appropriate activity in transient expression assays, were inserted into a *LacZ* reporter containing plasmid. These constructs were microinjected into the male pronuclei of fertilized mouse eggs. The transgene expression was monitored by whole-mount X-gal staining and histological analysis in the growth plate of G0 founder embryos. We found that the short promoter alone exhibited relatively low activity with preference to zones of proliferating chondroblasts and prehypertrophic chodrocytes of the growth plate (Rentsendorj et al. 2005). Insertion of upstream elements to the short promoter raised the activity of the transgenes. Analysis of the effect of homologous and heterologous enhancer elements and the mutant versions provided further insight into the transcriptional regulation of the matrilin-1 gene.

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Systems biology analysis of a novel microfibrillar network associated human fibrotic disorder

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A female patient was diagnosed with a novel connective tissue disorder at the age of 5. Her symptoms, now at her age of 19, include severe, progressive contractures of the leg and hand joints, areas of her skin appear fragile, thin, and translucent with fibrotic papulae, that resemble hypertrophic scars. These papulae along with collagenous bundles are prevalent at areas of skin under tension from growth or joint flexion.

Histopathology of a skin biopsy showed normal epidermis, preserved skin appendages, and extensive fibrosis within the dermis characterized by dense hypocellular bands and nodules of collagen. Ultrastructural analysis of a skin biopsy revealed a slight variation in the size of the collagen fibers; in addition to normal, 50 nm diameter fibers, abnormally small, 32 nm fibers were also detected. The genetic cause of the disorder is unknown.

The aim of our study was to identify the molecular background of this disorder. Increased collagen fiber accumulation suggested lysyl oxidase (LOX) involvement. LOX is an amine oxidase that plays an essential role in the catalysis of lysine-derived crosslinks in extracellular matrix (ECM) proteins including collagens and elastin (Kagan and Li 2003). We, indeed, detected elevated LOX, and LOX-like (LOXL) mRNA, protein, and catalytic activity levels in dermal fibroblast cultures derived from the patient's skin biopsy.

We performed whole genome expression array analysis that revealed that in addition to *LOX* and *LOXL*, other important ECM genes were also differentially expressed. Notably, the majority of genes encoding microfibrillar network associated proteins, including fibrillin-2 (*FBN2*), microfibril associated glycoprotein-2 (*MAGP2*), fibulin-1 and -3 (*FBLN1,3*) were significantly downregulated. Microfibrillar network is the template and regulator of collagen and elastin fibrillogenesis. Immunofluorescent staining of fibrillin-1 (FBN1) and elastin demonstrated that altered microfibrillar network in the patient's cultured dermal fibroblasts resulted in disorganized collagen and elastin fiber assembly that might contribute to the development of the severe dermal phenotype in the patient.

The patient's clinical symptoms have overlapping features with Marfan syndrome, Congenital Contractural Arachnodactyly (CCA) and scleroderma. Mutational analysis of *FBN1, FBN2* (genes possessing mutations causing Marfan syndrome and CCA, respectively), and *MAGP1*, *MAGP2* (genes whose products interact with FBN1, and FBN2) did not detect any mutations. Therefore, Marfan syndrome and CCA can probably be excluded as a causative for this phenotype. In contrast, the comparison of the transcriptional profile of our patient to the transcription profile of scleroderma patients identified partial overlap in differentially expressed ECM genes (9 out of 18; Tan et al. 2005). However, the mechanism of fibrosis is most probably different in this patient, since in fibrotic disorders, including scleroderma, TGF-β plays an important role in the development of the condition and it causes the upregulation of *lox* mRNA, but in our case microarray analysis and qRT-PCR results revealed that no TGF-β genes, TGF-β receptors or ECM genes known to be regulated by TGF-β were upregulated (Denton and Abraham 2001).

These data suggest that this fibrotic disorder might be a new type of severe, early onset scleroderma or a yet uncharacterized microfibrillar network associated fibrotic disorder, where elevated LOX and decreased microfibrillar protein levels are regulated independently of the TGF-β pathway.

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Identification and characterization of genes involved in abiotic stress responses in *Arabidopsis*

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Land plants struggle with adverse environmental factors such as drought, salinity, and cold during their lifetimes (Bohnert et al. 1995). In response to these factors changes occur in physiological and molecular level controlled by cell signaling networks (Xiong et al. 2002).

The major plant stress hormone abscisic acid has a complex regulation. A number of genes have been indentified as positive or negative regulators of ABA signaling (Finkelstein and Rock 2002). One of the most important aspect of understanding plant stress regulation is elucidation of ABA dependent and independent processes.

To indentify *trans*-acting regulators of the well characterized ABA inducible gene *alcohol dehydrogenase 1 (adh1)* we have constructed luciferase reporter gene fusions with the promoter of *ADH1* gene, and tested the applicability in genetic screen experiments.

We have developed a new genetic technology to identify yet unknown stress related factors. A full-length cDNA expression library has been constructed using RNA templates isolated from different tissues of *Arabidopsis* seedlings under salt stress conditions. It has been cloned into pER8 - an estradiol inducible plant expression vector (Zuo et al. 2000), and transformed into *Arabidopsis* plants that contain the *ADH1-luc* reporter construct. We have indentified an *Arabidopsis* transformant line, in which estradiol induction of cDNA expression *trans*-activates the *Arabidopsis ADH1* promoter in the absence of stress signals. We determined that cDNA is a transcripton factor Related to Apetala 2.12 (RAP2.12) belongs to the AP2/EREBP gene family. In further experiments we revealed that the induced RAP2.12 expression and the hormone ABA have a synergistic effects on *ADH1* promoter. The cDNA of RAP2.12 has been re-cloned into the estradiol inducible and constitutive expression vector and retransformed into the tester *Arabidopsis* line to confirm its functional effects.

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Drosophila p53- a model for the better understanding of human p53 function

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The p53 protein plays a vital role in safeguarding the integrity of the genome by inhibiting the cell cycle, or bringing about apoptosis in case of different cellular stresses. In accordance with this role mutations of the tumor suppressor gene *p53* are frequently found in different types of cancer. Identification of the *Drosophila melanogaster* homologue of p53 (Dmp53) opens up possibilities to apply a combination of biochemical and genetical tools for studying p53 functions in flies.

We analysed several interacting partners of the Dmp53 and determined the interacting regions of the protein using the yeast two-hybrid assay. Two main aspects of this work is reported here.

Several of the identified Dmp53-interacting proteins (dUba2, lwr, dPias) are involved in the process of sumoylation. Sumoylation is a posttranslational modification in which a small polypeptide is attached to a target protein resulting in a great variety of changes in the life of the modified protein (changes in nuclear transport, transcriptional activity etc…). The human p53 has been shown to be sumoylated. We found a putative sumoylation site at the C-terminus of the Dmp53 protein as well. Taken together, we suggest that the Dmp53 protein, like its human counterpart, can be sumoylated. Using the yeast two-hybrid assay we characterized the interactions between dUba2, lwr and dPias. Based on these data we propose that sumoylation has a role on Dmp53 function.

Another Dmp53-interacting partner is the *Daxx-like protein* (*DLP*), the homologue of the human Daxx protein, which plays a role in Fas-mediated apoptosis and transcriptional repression. Daxx has been shown to bind p53, but the effects of this interaction are controversial. To gather more information about *DLP*, we generated loss of function mutants by P element remobilization. We found that *DLP* is not essential as homozygous mutants are viable and fertile, although their longevity is reduced. Moreover, we observed the effect of Dmp53 overexpression in flies on DLP mutant and wild-type background and found that the DLP mutant animals die earlier indicating that DLP acts as a repressor of p53 functions.

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Heavy metal-induced genes in Synechocystis sp. PCC 6803. Applications for biosensor development

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The aim of the study is to characterize the transcriptional response of *Synechocystis* sp. PCC 6803 to metalloid and heavy metal stress, to find potential candidates for biosensing applications and to develop recombinant whole-cell sensors by coupling the metal-specific genes with bacterial luciferase genes.

A series of small-scale studies were conducted with a set of selected ORFs: slr1457, slr0944, sll1957, slr1950, sll1920, ssr2857, slr0798, slr0797, slr0793. The cells were incubated with biologically relevant concentrations of Co II (3 μM), Zn II (6 μM), Ni II (15 μM), Cd II (1.5 μM), Cr III (50 μM), Cr VI (50 μM), As III (1mM). As V (1mM) and Cu II (1μM) and the gene expression was assessed by quantitative RT-PCR.

ORF slr0798 (*ziaA*) that encodes a putative Zn II efflux P-type ATPase (Thelwell et al. 1998) showed a markedly increase in mRNA level after 15 min incubation with Cd II (49x) and arsenic ions (50x-As III and 18x-AsV), besides the known induction by Zn II (30x). ORF slr0797 (*coaT*) encodes a putative Co II translocating P-type ATPase (Rutherford et al.1999) and was strongly induced in our experiments by Co II and Zn II (≈ 15x), moderately induced by As III (5x), and weekly induced by Cd II (3x). Expression of ORF slr0793 (*nrsB*) that is involved in Ni II and Co II efflux (Lopez-Maury et al. 2002) was highly induced by Ni II (350x) and to a low extent by Co II and Zn II (≈8x).

Synechocystis sp PCC 6803 contains an arsenic and antimony resistance operon *arsBHC*, under the control of a transcription repressor encoded by a distinctly located *arsR* (Lopez-Maury et al. 2003). We showed that ORF *slr0944* (*arsB*) was highly induced after 15 min incubation with arsenic ions (1800x-As III and 700x-As V) and ORF sll1957 (*arsR*) displayed no significant modification of its transcript level. ORFs *atx1* (ssr2857), *ctaA* (slr1950) and *pacS* (sll1920) encode copper-trafficking determinants. The Atx1 copper metallochaperone interacts with P(1)-type copper ATPases CtaA and PacS to supply copper proteins within intracellular compartments (Tottey et al. 2002). We could detect no significant transcript level modifications for these ORFs after exposure to the previously mentioned metal concentrations.

The predicted protein product of ORF slr1457 (*chrA*) belongs to the CHR family of prokaryotic transporters probably encoding chromate/sulfate antiporters. In our experiments *chrA* transcript level was not substantially modified by 7 µM, 20 µM and 50 µM Cr III or Cr VI added to BG-11 medium.

Two recombinant strains were constructed in our laboratory: strain *nrsB::luxAB* that showed specificity for Ni II and the bioluminescent signal was proportional to the metal concentration within the range 0.5-17 µM and strain *coaT::luxAB* that responded to both Co II and Zn II, proportional to the metal concentration between 0.2 and 4 µM, with a higher response for Co II. The recombinant strains activity is to be tested on environmental samples containing known concentration of Ni II, Zn II or Co II.

ORF slr0944 (*arsB*) is specifically induced by arsenic ions and represent another good candidate for biosensing applications.

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New approaches in microbial degradation of mycotoxins

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Mycotoxin contamination of agricultural products is a serious health hazard throughout the world. One of the most important mycotoxins is ochratoxin A (OTA), which is produced by several *Aspergillus* and some *Penicillium* species. The occurrence of OTA in several commodities (feeds, foods and beverages) is considered as a serious health hazard in view of its nephrotoxic, teratogenic, hepatotoxic and carcinogenic properties.

Several strategies are available for the detoxification of mycotoxins. These can be classified as physical, chemical, physicochemical and (micro)biological approaches. Microbes or their enzymes could be applied for mycotoxin detoxification; such biological approaches are now being widely studied. An adsorption mechanism has also been suggested for OTA removal by lactic acid bacteria, yeasts (Bejaoui et al. 2004) and conidia of black aspergilli (Bejaoui et al. 2005). We examined *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates for their ability to degrade and/or adsorb ochratoxin A in a liquid medium (Péteri et al. 2006). *Phaffia rhodozyma* is a red-pigmented fermentative yeast. Besides producing astaxanthin, *P. rhodozyma* is also able both to detoxify and adsorb OTA at temperatures well above the temperature optimum for growth of *Phaffia* cells. We also examined several filamentous fungi representing the genera *Aspergillus*, *Rhizopus* and *Mucor* for their ability to degrade ochratoxin A in a liquid medium (Varga et al. 2005).

The kinetics of OTA degradation of *P. rhodozyma* CBS 5905 has been examined at two cell concentrations at 20°C in a liquid medium. The *Phaffia* isolates could degrade more than 90% of OTA in about 7 days at 20°C. Previously, an *A. niger* isolate CBS 120.49 and *Actinomucor elegans* NRRL 3104, *Rhizopus stolonifer* TJM 8A8 were found to be able to degrade more than 90% of OTA after 4 and 10 days incubation. Interestingly, a significant amount of OTA was found to be bound by the cells after two days, indicating that OTA is also adsorbed by the cells.

When the effect of temperature was examined, the temperature optimum of this enzyme was found to be above 30°C, which is much higher than the temperature optimum for growth of *P. rhodozyma* cells, which is around 20°C, and the cells are unable to grow at higher temperatures. When the temperature range of the OTA degrading enzyme was further examined, it was found that the enzyme remains active at up to 60°C. Above this temperature, OTA adsorption only could took place.

We hypothesized that a carboxypeptidase enzyme could be responsible for OTA degradation as observed previously in other fungi. To prove this hypothesis, the effect of various carboxypeptidase inhibitors was tested on OTA degradation activities of *P. rhodozyma* cells. Two of these inhibitors, the chelating agents EDTA and 1,10-phenanthroline inhibited significantly OTA degrading activities of the *P. rhodozyma* cells, indicating that the enzyme responsible for OTA degradation is a metalloprotease.

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Further studies are in progress to identify the enzymes and genes responsible for ochratoxin detoxification (for example carboxypeptidase A), and to transfer these genes to yeast and other fungal isolates.

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The interaction of lysyl oxidase with the hormone placental lactogen and their effect on mammary epithelial cell proliferation and migration

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Lysyl oxidase (LOX), a copper-dependent amine oxidase, contributes to the assembly and maintenance of the extracellular matrix (ECM) by initiating the formation of covalent cross-links in collagen and elastin (Smith-Mungo et al. 1998). Besides its matrix-stabilizing function, LOX has recently been shown to play a role in cell motility, transcriptional regulation, embryonic development and pathological conditions such as breast cancer (Csiszar et al. 2001). While the ECM cross-linking activity of LOX is well studied, the mechanisms of the novel functions are not known. To identify protein interactions that determine and/or regulate these LOX activities, we performed a yeast two-hybrid screen using a human placental cDNA library and both full-length and mature LOX as baits, and identified placental lactogen (PL) as a possible interacting partner. PL, a member of the growth hormone (GH)-prolactin (PRL) hormone family, stimulates mammary gland development, lactogenesis and the growth and metabolism of the foetus (Walker et al. 1991). PL is only expressed in the placental syntitiotrophoblasts, but its expression was shown in 77% of invasive ductal carcinomas, and the amplification of the PL genes has been reported in 22% of the cases (Latham et al. 2001). While PL was reported to promote epithelial cell proliferation in breast carcinomas, its role in breast tumours is not fully understood.

Our direct interaction studies, Far-Western analysis and solid phase binding assays supported LOX binding to PL and suggested binding to GH as well. In addition, *in vitro* amine oxidase activity assays showed that PL is neither a substrate nor an inhibitor of LOX. Since increased expression and enzyme activity of LOX have been reported in highly invasive and metastatic breast cancer cell lines as well as in metastatic breast tumours, and PL has been shown to be expressed in breast carcinomas, we tested their expression in tissue sections of mammary carcinomas and breast cancer cell lines. Using fluorescence-labelled immunostaining on a tissue macroarray, we detected LOX and PL expression in and around tumour cells. Subsequently, we tested protein expression of breast cancer cell lines, and found elevated PL expression in highly invasive MDA-MB231 and Hs578T cell lines, where LOX expression was also increased. Furthermore, we showed PL expression in poorly invasive MCF-7 and T47D cells at elevated and low levels, respectively. Since the highly invasive and metastatic breast cancer cell lines express both PL and LOX, we decided to study their individual and combined effects on cell behaviour by overexpressing and coexpressing these proteins in immortalized normal breast epithelial cells. LOX plays a role in promoting cell migration, while PL was shown to induce cell proliferation, thus we tested these processes. Stably transduced MCF-10A normal mammary epithelial cells coexpressing PL and LOX had significantly increased proliferation rates compared to the parental and the PL-expressing cells, while LOX alone had no effect on proliferation. Therefore, the coexpression of LOX with PL appears to enhance the proliferation-inducing effect of PL. Coexpressing cells in addition showed a significantly higher migratory rate compared to cells expressing either or none of these proteins. Our results demonstrated that LOX, in addition to promoting tumour cell invasion through a H_2O_2-induced FAK/Src activation (Kirschmann et al. 2002), may further induce tumour cell migration in interaction with PL by activating independent signalling.

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Genetic and molecular analysis of the *Drosophila* CalpA and CalpB

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Calpains are Ca2+ -activated cytoplasmatic SH-proteases, that through a limited proteolysis of their substrate proteins, regulate apoptosis, the cell cycle and many cellular pathways. From the four calpain genes found in the Drosophila genome, only two (CalpA and CalpB) code for canonical and functional calpain proteins (Friedrich et al. 2004). Our aim was to study the functions of these two genes, by creatind mutants with the remobilization of the P-elements found in their vicinity.

Based on our and other groups (Grabbe et. al 2004) results, we concluded that the CalpA gene is not essential and its alteration does not lead to a lethal fenotype. Based on this, we remobilized the EY10816 P-element (Voelker RA et al. 1984), and screened the resulting CalpA mutant candidates for homozigous viable mutants. Using Anti-CalpA primary antibody we examined the expression of the CalpA protein during the different developmental stages of the Drosophila ontogenesis. In the embryos the CalpA protein is mainly expressed in the CNS, while in the later larval stages the expression of the protein is reduced to only a few neurons. In the Drosophila ovaries, the CalpA protein is located at the membranes and in the nuclei of the germ- and follicular cells. The protein is also present in the testes, where it is located only at the membranes of the spermatogonial cells, while in the spermatocytes and spermatids it can be found both at the membranes and nuclei of the cells. In the ring gland and larval fat body, the CalpA protein exibits the same nuclear and membranar expression pattern.

With the help of EP972 P-element we created homozigous lethal CalpB mutants and analysed the molecular breakpoints of five candidates, but regretably all of them were double mutants, which means that the adjacent Taf2 gene was also affected by the deletions. Our group performed another remobilization using a different P-element, the EY08042. This time we created five homozigous viable CalpB mutants, in which the deletions did not affect the adjacent Taf2 gene.

These findigs suggest that the Drosophila CalpA and CalpB do not play an essential role. We were curious what would happen, if we alterd their function in the same time, and we found, that combining the different CalpA and CalpB homozigous viable mutants resulted in lethality and sterility, which indicates the redundancy of these genes.

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Role of the estrogen caused up-regulation of heme-oxygenase enzyme in the protective mechanism of cardiovascular system

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Gender-based differences in the incidence of hypertensive and coronary artery disease, the development of atherosclerosis, and myocardial remodelling after infarction are attributable to the direct effect of estrogen on the myocardium, vascular smooth muscle and endothelium. In the heart and vasculature, these mediate rapid vasodilatation (White et al. 1997), reduces both myocardial infarct size and the occurrence of ischemia- and reperfusion-induced ventricular arrhythmias in hearts (Node et al. 1997).

Since the effects of hormone replacement therapy on the risk of cardiovascular events are controversial, and treatment with estrogen in high dose can cause breast and endometrial carcinoma, selective estrogen receptor modulators (SERMs) have come to the focus of attention. Raloxifene (RAL), a second generation selective estrogen receptor modulator (SERM), is of use for the treatment of osteoporosis. RAL acts as an estrogen agonist in cardiovascular system but as an estrogen antagonist in breast and endometrium. RAL is currently being assessed for menopausal women at risk of ischemic heart disease (Raloxifene Use for the Heart Trial). According to several reports, nitric oxide (NO) plays an important role in mediating the beneficial effects of estrogen and raloxifene in the vascular system. NO is a well established effector molecule of estrogen and SERM mediated vasoprotection (Pávó et al. 2000)

Heme-oxygenase (HO) is the rate-limiting enzyme for heme degradation in mammals. To date, three isoforms of HO have been characterized: HO1 is widely expressed and is inducible by a range of stimuli that produce oxidative stress. HO2 is non inducible form, it occurs in neuronal populations and vascular endothelial cells, while HO3 is newly recognized form. We attempted to clarify the effects of estrogen and raloxifene modulated HO1 and HO2 enzymes in rat cardiac.

Hypothesis: Treatment with 17-Ðestradiol or SERM raloxifene increases the activity of heme- oxigenase (HO) and the expression of HO2 in the ventricle and decreases the arginin vasopressin (AVP) induced increase of blood pressure and heart perfusion in experimental menopause.

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Methods: In our experiments intact females in oestrus cycle, ovariectomized (OVX), RAL or 17-β oestradiol treated OVX female Wistar rats were used. HO enzyme system was inhibited by tin-protoporphyrin (SnPP; 30 mg/kg, s.c.). In all groups we examined 1. / the activity of HO and expression of HO1 and HO2 in the ventricle, 2./ the ST depression (standard lead II surface ECG) *in vivo*, 3./ the increase in blood pressure *in vivo* and heart perfusion *ex vivo* induced by AVP.

Results: Ovariectomy 1. / decreased HO activity (from 2.2 nmol bilirubin/h/mg protein to 0,9 nmol bilirubin/h/mg protein); HO1 and HO2 expression (46 ± 4% and 47 ±3 % respectively) in the ventricle, 2. / increased the tendency of the heart ischemia (ST segment change: from -0,02 mV- -0,13 mV) , increased the response of blood pressure (15-25%) and heart perfusion (10-30%) to AVP, as compared to intact females. 17-β oestradiol and RAL replacement restored the difference induced by OVX to the level observed in the ovary-intact females. SnPP treatment intensified the response of blood pressure (intact female 25-30%; OVX 10-12%; 17- β oestradiol 20-25%; RAL 23-28%) and heart perfusion (intact female 10%; OVX 5%; 17- β oestradiol 12%) to AVP.

Discussion: Presumably raloxifene can also diminish the increase in blood pressure induced by menopause by the augmentation of HO synthesis. In the system applied, raloxifene had estrogen agonistic effect.

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Positional cloning of the rough coat mice; molecular analysis of a novel predicted adhesion molecule

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The rough coat (*rc*) phenotype arose spontaneously as a recessive trait in C57BL/6J mice. Homozygous *rc* mice were characterized with an unkempt-looking hair coat by weaning age, cyclic and progressive hair loss and sebaceous gland hypertrophy. Previously, the *rc* locus was mapped to a 4-centimorgan region (at 32.0 centimorgan) on chromosome 9, close to the *Mpi-1* gene at 57 Mb (Ensembl Mouse Genome Database v38) but the gene mutation remained unidentified.

To understand the genetic basis of the rc phenotype, we carried out positional cloning in backcross mice. Our research group outcrossed B6J-*rc/rc* mice with both CAST/Ei mice and Balb/cJ mice to obtain F_1 hybrids (+/*rc*) on two mixed strain backgrounds to compensate for a potential low rate of recombination within the *rc* region. Female F_1 hybrids were backcrossed with male B6J-*rc/rc* mice to obtain F_2 hybrids. We analyzed linkage between the rc locus and published microsatellite polymorphisms (Mouse Genome Informatics "Strains and Polymorphisms" database (www.informatics.jax.org) or the Ensembl Mouse Genome Database (www.ensembl.org/Mus_musculus)) between the parental strains, within the 4-centimorgan region on chromosome nine using 700 B6J-Balb/cJ F_2 hybrids. We reduced the rc interval to a 1.54 Mb region, between D9Mit228 (44.13Mb) and D9Mit192 (45.67Mb). Within this interval, we identified 18 novel microsatellite polymorphisms between B6J and Balb/cJ strains. Using 361 B6J-CAST/Ei F2 hybrids, my collaborators reduced the rc interval to 246 kb, between 44.83Mb and 45.0796 Mb. Within this 246-kb region, there are 11 candidate genes (Ensembl Mouse Genome Database v38). After analyzing all the coding exons and flanking splice sites by sequence analysis of PCR products using wild type B6J and B6J-*rc/rc* genomic DNA as templates, a G→A transition was identified in the coding sequence of a novel gene ENSMUSG00000070305 (44.989~ 45.009Mb).

The highest homology of this gene are to Myelin Protein Zero (MPZ) and Myelin Protein Zero-like 2 (MPZL2, also called Epithelial V-like Antigen or EVA1). We therefore named this gene *Mpzl3* (Myelin Protein Zero-like 3).

The *Mpzl3* gene contains at least six exons, and gives rise to at least two transcripts through alternative splicing. A two-exon transcript encodes a 91 amino acid polypeptide, and a six-exon transcript encodes a 237 amino acid polypeptide.

RT-PCR based expression pattern confirmed that both *Mpzl3* transcripts are expressed in a variety of organs with high levels in the brain, heart, liver and the skin.

Bioinformatical analysis of the predicted MPZL3 protein revealed a cell adhesion molecule with signal peptide, two transmembrane domains and a highly conserved immunoglobulin domain, in which the point mutation was found to affect a conserved residue.

To begin to identify tissue expression and localization of this protein, we carried out bioinformatical analyses and indirect immunofluorescence assay. The results confirmed our hypothesis that this protein might be a membrane protein expressed in multiple tissues.

In our future studies we are going to focus on the human homologue of the Mpzl3 gene in order to identify potential mutations and the corresponding human phenotype(s) linked to these gene mutations. Our data provide insight into the role of novel *Mpzl3* gene and help to better understand the molecular mechanism which cause the *rc* phenotype.

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Investigation of neuroprotection in different models

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Trauma in general, and head injury in particular, is the most frequent cause of mortality and morbidity. The outcome of a severe head injury depends on the severity of the primary lesion and the manifestations of secondary brain damage. Brain edema and the secondary growth of traumatic brain tissue, necrosis, are important manifestations of secondary brain damage.

The cold injury model is one of the established models for study of disruption of the blood-brain barrier (BBB) and vasogenic brain edema development. A cold lesion was induced by applying a precooled (-78°C) copper cylinder for 30 s to the intact skull of rats. This cold lesion induces a dysfunction not only of the BBB, but also of the cellular membranes. This may induce a secondary neuronal loss in the perilesional rim, the main target of neuroprotective interventions. The non-intact cells can be detected by markers of apoptosis only hours or even days after injury.

The dye Evans blue (EB) is known to bind to serum albumin after intravenous injection and has been used as a tracer of serum albumin. The early membrane dysfunction allows extravasated serum proteins and their tracers, such as EB, to enter the cells, permitting their early visualization.

The aim of our work was to demonstrate injured cells that take up EB in the perilesional rim. In cold-lesioned animals, the extravasated EB content in the injured hemisphere was highest 0.5 h after EB administration (Murakami et al. 1999). Accordingly, there is hope that we can obtain information on the cortical extent of the area of non-intact cells much earlier than with other immunohistochemical methods. In our study, EB-positive cells were detected in the perilesional rim. These cells emitted bright-red autofluorescence and could easily be counted (Rákos et al. 2007). This method proved a useful tool in pilot experiments performed to test the presumed neuroprotective effects of candidate agents in the cold lesion model (Juhasz-Vedres et al. 2006)

We used another model to produce focal cerebral ischemia. Ischemic stroke is also a leading cause of death and disability. The photothrombotic model for stroke was originally described as a focal cortical infarction resulting from occlusive thrombosis. In this model, through the use of transcranial illumination with a cold light source in combination with the intravenous injection of rose bengal, a potent photosensitive dye, it was possible to produce the thrombosis of small blood vessels. It is important to understand the cellular and molecular responses to cerebral ischemia in order to provide adequate therapeutic strategies for such injury. It is well known that elevated glutamate levels after cerebral ischemia play a key role in the development of neuronal damage.

We used Fluoro Jade, an anionic fluorochrome, to visualize the ischemic neuronal damage. The histochemical application of Fluoro Jade results in a simple and sensitive method for staining degenerating neurons.

In our study, we tested the prediction that oxaloacetate-mediated blood glutamate scavenging causes neuroprotection in the photothrombotic lesion model. The volume of the hemispheric lesion and the number of Fluoro Jade-positive cells were determined. The results demonstrated that the extent of the lesion and the number of Fluoro Jade- labeled cells were significantly smaller in the oxaloacetate-treated group. It is concluded that even a single posttraumatic administration of oxaloacetate may be of substantial therapeutic benefit in the treatment of focal brain injury.

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Growing or escaping: transcriptome analysis during long-term drought stress adaptation in two wheat (*Triticum aestivum L.*) genotypes

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Among environmental stresses, drought is a major abiotic factor that limits agricultural crop production. Nevertheless, water deficit usually coincides with other abiotic stresses, *i.e.* high temperature, high light intensity, salt stress, adversely effecting plant growth.

However, plants can survive these adverse conditions using different strategies. Plant resistance to water deficit may arise from escape, avoidance, or tolerance strategies (Levitt 1972). “Escapers” are able to complete their life-cycle before the onset of severe stress; drought avoidance relies on maintaining high tissue water potential by minimizing water loss and maximizing water uptake (Chaves et al. 2003) while drought tolerance is achieved by co-ordination of physiological and biochemical alterations at the cellular and molecular levels including specific gene expression and accumulation of specific proteins under drought stress. Plants usually apply a combination of these strategies under dry conditions.

To examine long-term drought stress adaptation of two wheat cultivars, both displaying tolerance to water-deficit stress but following distinct strategies, a greenhouse experiment was established including treated and non-treated wheat plants growing in perlite as a soil substitute. Treated samples were irrigated with one sixth the amount of irrigation solution causing a mild water-deficit stress to the plants. The presence and extent of the stress was confirmed by following the expression levels of L- ^{1}-pyrroline-5-carboxylate synthetase (P5CS), the key enzyme of proline biosynthesis, during the four-week-long treatment. Proline accumulation, the consequence of induced expression of P5CS, is a well-described phenomenon in plants subjected to drought stress (Yoshiba et al. 1997).

Furthermore, water deficit alters growth of shoots and roots, resulting in an increased root/shoot ratio. In this study, the adaptive genotype showed a much higher root/shoot ratio under water stress, due to favored root growth to shoot growth, compared to the “escaper” one.

To follow the transcriptional changes under water deficit, cDNA macroarray was hybridized with root samples from both genotypes. As a result, 8.0% of the genes were up-regulated and 8.5% were down-regulated in the adaptive cultivar. In the “escaper” genotype, these ratios were 5.1% and 4.8%, respectively.

Up- and down-regulated genes were clustered into six groups each, based on their temporal expression profiles. The clusters’ functional classification was done based on the predicted function of the encoded proteins, according to a modified version of the categorization described by Yang et al. (2004).

After analyzing the classes of up-regulated genes, three of these groups showed significant differences between the two genotypes, referring to the possible genetic background of their strategies. In the “escaper” genotype, a considerable proportion of the up-regulated genes, encoding proteins endowed with predicted stress- and defense-related functions, was presented. However, in the adaptive genotype, the ratio of genes, encoding proteins involved in signal transduction and cell wall biogenesis, was higher than in the “escaper” one.

These results suggest a hypothetical elucidation of the molecular genetic background of the different tolerance strategies of the two examined wheat genotypes.

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Differential polarization laser scanning microscopy – development and biological applications

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Confocal microscopes are quite often used for 3D imaging of fluorescent samples. But the intensity measurements will give only partial information about the interaction between the material and the light: there are 16 parameter in the Mueller matrix describing the interaction, but the absorbance is only one of them.

We have constructed a differential polarization (DP) attachment for imaging linear dichroism (LD) fluorescence detected linear dichroism (FDLD) and other DP values with our Zeiss 410 laser scanning microscope. The attachment uses high frequency modulation and subsequent demodulation, via lock-in amplifier, of the detected intensity values, and displays pixel-by-pixel the measured DP quantity. LD

carries information on the orientation of the dye molecules, more exactly on the anisotropic distribution of the absorbance transition dipole vectors of the chromophores, in the sample. FDLD carries the same information for fluorescent dyes: the fluorescence intensities excited with orthogonally polarized light are being proportional to the number of the absorbed quanta. Due to the confocal fluorescent measurement, 3D anisotropic distribution of the transition dipoles can also be reconstructed.

Demonstration was performed on two different samples – that LD and FDLD images, specially after the performed 3D reconstruction are able to carry detailed information about the molecular organization: on sections of Convolaria majalis root tissue stained with Acridine Orange provide quantitative information on the anisotropy of cell wall. LD images, in non-confocal mode, appear to indicate that the cell walls exhibit weak anisotropy. However, confocal FDLD images, due to the thin optical sections, reveal that these highly organized fiber-laminate extracellular structures exhibit very strong local anisotropy values. A simple mathematical model shows that the magnitude of FDLD depends on the intercalation angle between the dye molecule and the fiber and the angular distribution of the fibers with respect to their preferential orientation, *i.e.*, with respect to the cell walls

Amyloid fibrils, insoluble protein aggregates, are associated with a large variety of diseases. When stained with an intercalating dye, such as Congo Red, the fibrils exhibit strong linear birefringence due to the highly ordered molecular architecture of the self-assembled bundles. By using the differential polarization laser-scanning microscope, we investigated the spatial distribution of optical anisotropy properties of isolated human amyloid fibrils stained with Congo Red and Rivanol, and investigated the fine structure of the fibrils.

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Diurnal regulation of brassinosteroid biosynthesis and perception

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Brassinosteroids (BRs) are recently recognized polyhydroxylated steroid hormones that are important regulators of plant growth and development. Their physiological functions were determined by using brassinosteroid-deficient mutants. In addition to the role of these phytohormones in promoting growth, fertility, and stress resistance, they also regulate photomorphogenesis (Li et al. 1996; Szekeres et al. 1996). BRs are known to act at, or near, the sites of their synthesis, therefore the regulation of BR biosynthesis can directly control local physiological effects.

BRs are synthesized from phytosterols through multiple, mostly oxidative steps leading to brassinolide (BL), the biologically most active BR. The oxidative reactions are catalyzed by closely related cytochrome P450 monooxygenases of the CYP85 or CYP90 families (Fujioka and Yokota 2003). Recent studies have indicated that in *Arabidopsis* the expression of all BR-biosynthetic P450 genes is under both developmental and organ-specific regulation that takes place primarily at the level of transcription. In addition, all these genes are under negative feedback regulation by active BRs (Mathur et al. 1998; Bancos et al. 2002). Therefore, it seemed likely that the activities of genes encoding rate-limiting enzymes, such as *CPD* (*CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM*) and *CYP85A2* can directly influence the efficiency of biosynthesis and hormone level. In an attempt to monitor the expression of these key biosynthetic genes, we generated transgenic plants carrying promoter:luciferase fusions, and followed gene activities on the basis of luciferase-generated *in vivo* luminescence. We found that *CPD* activity displays a complex diurnal pattern, with maxima following the onset, and coinciding with the end of light periods. This expression profile is determined by a circadian oscillation and a superimposed positive light regulation. Very similar daily expression cycles were observed with *CYP85A2*, the gene encoding the enzyme that produces BL.

To characterize the nature of light signaling, we determined the photoreceptor dependence of light induction. The severely decreased expression level of *CPD* in phytochrome-deficient background and the red light-specific induction in wild type plants suggest that the light regulation of *CPD* is mediated primarily by phytochrome signaling.

The diurnal periodicity of *CPD* activity is maintained in the BR insensitive *bri1* (*brassinosteroid insensitive 1*) mutant, indicating that the underlying regulation is independent of the changes in the endogenous BR level. But we also observed that BR regulation is an important modulator of the diurnal expression pattern, being responsible for the repression of *CPD* in the dark.

In order to find out whether diurnal fluctuations of *CPD* and *CYP85A2* expression is accompanied by changes in the levels of bioactive BRs, we analyzed the BR content of *Arabidopsis* seedlings during the day using gas chromatography-coupled mass spectrometry. We

found a major, transient increase of the BL content in the middle of the light period, in good coincidence with the light activation of the key BR biosynthetic genes. Our results, therefore, suggest that the level of bioactive BRs is dependent on the diurnal changes in *CPD* and *CYP85A2* expression.

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