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Faculté de Pharmacie Nancy

International Symposium

Glutathione and related thiols in microorganisms and plants

BOOK OF ABSTRACTS

Faculté de Pharmacie de Nancy

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International Symposium

Glutathione and related thiols in microorganisms and plants

Table of contents

Preface

Glutathione (GSH) was identified more than a century ago as a major non protein thiol (NPT) playing a cardinal role in cell metabolism. Research efforts on microbial and plant NPT but also redoxins (thioredoxins, glutaredoxins, peroxiredoxins) increased in the last ten years, largely because these thiols were identified as important components of cell defence lines against numerous stresses generated by oxidants, metals, xenobiotics, nutritional or osmotic imbalance, etc... Moreover NPT and redoxins are now recognized as involved in signal transduction mechanisms and as playing major roles in cell cycle, from differentiation to apoptosis, and cell organisation. They are also involved in aggregation, biofilm formation and also in interaction between microorganisms and plants. The recent discovery that GSH and glutaredoxins are involved in iron sulfur assembly both in mitochondria and in chloroplasts has also renewed interest in the field. Cellular thiols are also of interest for applications in food, pharmaceutical, cosmetic industry and environmental technology, especially waste water treatment.

Few scientific meetings devoted specifically to this topic have been organized, particularly covering microorganisms and plants. A very successful workshop on GSH, bringing together over 70 participants, was organized in Luxembourg in 1992 by the "Société luxembourgeoise de biologie clinique". Therefore, the organization of a new symposium covering various aspects mentioned above obviously responded to a need expressed by the scientific community. It has to be mentioned that more than 150 participants have booked for the present meeting in Nancy, including 31 invited lectures, 18 oral communications and 46 posters. We hope that this meeting will be a nice opportunity to create a platform for exchanging ideas, and assembling investigators coming from different horizons. The organizers hope that this symposium is not a unique event, but the first in a long series to allow the scientific community to monitor progress expected in the field of cellular thiols. We therefore expect from the participants any initiative along these lines.

The organising committee wishes to thank all the partners and auspices mentioned below and individuals whose hard work has enabled the setting up of this meeting. Special thanks are addressed to Mrs. Monique Braconot for her invaluable contribution to the administrative aspects of the symposium.

Organizing and scientific committee

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Scientific programme

- Lectures and oral communications will be held in Amphitheatre Bruntz.
- Posters will be exhibited in Halls 2 and 3 during the full time of the symposium.
- Coffee breaks, lunches, welcome reception and banquet will be held in Rooms Kayser and Cordebard.

Tuesday August 26th	**Wednesday August 27th**	**Thursday August 28th**	**Friday August 29th**
	8h45-9h45: HOLMGREN ***L6***	8h45-9h30: HERRERO ***L16***	8h45-9h30: GRANT ***L24***
	9h45-10h15: YIN LI ***L7*** 10h15-10h45: LOVE ***L8***	9h30-10h00: BACHHAWAT ***L17*** 10h00-10h30: DAWES ***L18***	9h30-10h00: TOLEDANO ***L25*** 10h00-10h30: WINTHER ***L26***
	10h45-11h15: Coffee break	10h30-11h00: Coffee break	10h30-11h00: Coffee break
	11h15-11h45: ROUHIER ***L9*** 11h45-12h15: RASPOR ***L10*** 12h15-12h45: PAWLICK ***L11***	11h00-11h30: FOYER ***L19*** 11h30-12h00: MULLINEAUX ***L20*** 12h00-12h30: JACOB ***L31***	11h00-11h30: POCSI ***L27*** 11h30-12h00: BARTOSZ ***L28*** 12h00-12h30: ZIMMERMANN ***L29***
12h-18h: Registration	12h45-14h00: Lunch + poster session	12h30-14h00: Lunch + poster session	12h30-13h00: Closing address
14h-14h50: Opening ceremony	14H0014h30 : LEMAIRE ***L30*** 14h30-15h10: Oral communications : COBETT ***OC2*** MEYER ***OC8***	14h00-14h30: SIBIRNY ***L22*** 14h30-15h00: LABARRE ***L23***	GDR REDOXINES SEMINAR (Room Richard)
14h50-15h50: NEWTON ***L1*** 15h50-16h20: CONRAD ***L2*** 16h20-16h50: GHEZZI ***L3***	15h10-15h40: NOCTOR ***L12*** 15h40-16h10: HELL ***L13*** 16h10-16h40: FRENDO ***L14*** 16h40-17h10: REICHHELD ***L15***	15h00-17h10: Oral communications : GULLNER ***OC4*** MASI ***OC6*** RIEDIGER ***OC11*** SUZUKI ***OC12*** HERRMANN ***OC18***	
16h50-17h15: Coffee break	17h10-17h30: Coffee break	17h10-17h30: Coffee break	
17h15-17h45: FAIRLAMB ***L4*** 17h45-18h15: BECKER ***L5*** 18h15-18h45: POLLE ***L21***	17h30-19h30: Oral communications : CORTICEIRO ***OC3*** FRELIN ***OC17*** KAUR ***OC5*** MESSENS ***OC7*** MICHELON ***OC9*** ZECHMANN ***OC16***	17h30-19h10: Oral communications : TAMAS ***OC13*** TARRAGO ***OC14*** WESENBERG ***OC15*** BERTAU ***OC1*** MOREL ***OC10***	
18h45-20h30: Welcome reception		20h-23h: Buffet-banquet	

LECTURES

L1 - Mycothiol Biosynthesis and Functions

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Cysteine is required for protein and CoA synthesis by all living organisms, but it is a hazard to aerobic cells due to its rapid metal-catalyzed autoxidation and to all cells as a result of facile *S*-acyl transfer reactions with CoA thioesters. Cysteine is rarely found at high concentration in cells, but stabilized forms of cysteine are found at millimolar levels. These include glutathione in gram-negative bacteria and eucaryotes, γ-glutamylcysteine in halobacteria, and mycothiol in Actinobacteria. The unifying property of these cysteine-containing thiols is a unique bond, such as the γ-glutamyl-cysteine bond in glutathione which resists unwanted autoxidation and degradation by proteases but allows the organism to control the release of cysteine using the unique catabolic enzyme, γ-glutamyl transpeptidase. Most gram-positive bacteria lack glutathione but produce other protective thiols. The Actinobacteria produce mycothiol in place of glutathione. Mycothiol is also resistant to autoxidation and degradation and its unusual cysteinyl-glucosamine amide bond is specifically cleaved by the enzyme mycothiol *S*-conjugate amidase.

Mycothiol is a low molecular weight thiol composed of *N*-acetylcysteine amide linked to the pseudodisaccharide glucosamine-α(1-1)-*myo*-inositol first identified in 1994. Mycothiol is found in nearly all Actinobacteria at cellular levels of 1-10 millimolar and with mycothiol disulfide reductase forms the redox buffer for these bacteria. Actinobacteria include the causative agents of diphtheria, leprosy and tuberculosis as well as the *Streptomyces*, which produce most of the known antibiotics. The mycothiol biosynthetic pathway and metabolic enzymes were initially identified in mycobacteria. The biosynthetic pathway involves 5 enzymes two of which, MshA and MshC, are essential for the production of mycothiol and have no compensating activites in mycobacteria. Efforts toward targeting these enzymes for treatment of multidrug resistant tuberculosis will be discussed.

Mycothiol metabolic enzymes are also essential in mycobacteria and are considered antitubercular drug targets. Mycothiol is maintained in the reduced state by an essential flavin containing reductase homologous to glutathione reductase. An alcohol dehydrogenase, adhE2, responsible for the mycothiol catalyzed detoxification of formaldehyde and nitrous oxide (NO) was also shown to be essential for growth in *Mycobacterium tuberculosis*. The early work on mycothiol-catalyzed detoxification focused on the unique activity catalyzed by mycothiol *S*-conjugate amidase. This enzyme cleaves mycothiol *S*-conjugates produced by reaction of electrophiles with mycothiol, and the resulting mercapturic acid is excreted within minutes. The parallel detoxification pathway in mammals was shown to involve hepatic glutathione, several tissues, and requires days for the excretion of the mercapturic acids in urine. A recent study has shown that mycothiol *S*-conjugate amidase catalyzes the hydrolysis of mycothiol itself to provide cellular access to the stored cysteine. To date the search for mycothiol metabolic functions has been guided by those functions found in glutathione-containing organisms. Future studies in the larger genome Actinobacteria used for bioremediation, such as *Rhodococcus jostii* RHA-1 will likely identify more novel mycothiol functions.

L2 - Transgenic mouse models: unique tools to study the role of mammalian GSH metabolism

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Glutathione peroxidase 4 (GPx4; also termed phospholipid hydroperoxide glutathione peroxidase) is emerging as one of the most important redox enzymes in mammals. GPx4 is one out of 7 glutathione dependent peroxidases, but significantly differs from its family members for its structural and enzymatic peculiarities. Three distinct forms of GPx4, a cytosolic, a mitochondrial and a nuclear, are expressed from one gene, each showing a distinct tissue-specific expression pattern. To dissect the individual roles of GPx4 variants in physiology and pathophysiology, we created transgenic mice with targeted disruption of either nuclear or mitochondrial GPx4 and mice with tissue-specific inactivation of GPx4. Our in vivo studies finally establish that nuclear and mitochondrial GPx4 confer major functions only in male gametogenesis, while cytosolic GPx4 emerges being vital for embryonic development and cell and tissue function.

L3 **- Thiol regulation in innate immunity and inflammation**

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Several studies indicate that glutathione (GSH) is essential for optimal immune response to infections. GSH is often viewed as an anti-inflammatory molecule, including inhibition of inflammatory cell infiltrate, but inflammation is a two-edged sword and also has host defense functions. We used a mouse model of polymicrobial sepsis to investigate the role of GSH in immunity. Administering GSH depletors or GSH precursor (N-acetylcysteine) we found that GSH has a positive role in regulating neutrophil (PMN) migration to the site of infection (host defense), and a negative one in regulating PMN infiltration to distant organs (resulting in organ failure). These data show that GSH has a regulatory role that may not simply be explained with its being a free radical scavenger. In fact, GSH can regulate several cytokines and chemokines possibly through protein glutathionylation, and regulates expression of surface thiols and should thus be considered also as a signaling molecule in immunity, not only an antioxidant defense one.

One major signaling mechanism implicating GSH is formation of mixed disulfides between proteins' cysteines and glutathione. Several proteins were shown to undergo glutathionylation. Using “redox proteomics” to detect proteins undergoing glutathionylation in human T-cells we identified several proteins targets of this posttranslational modification. One of the proteins was cyclophilin A, the endogenous ligand of the immunosuppressor cyclosporin. Glutathionylated cyclophilin A shows a reduced ability to inhibit, complexed with cyclosporin A, the protein phosphatase activity of calcineurin (PP2B) suggesting that glutathionylation can affect signal transduction pathways.

Analysis of the gene expression profile associated with oxidative stress in human monocytic cells in the presence and absence of inhibitors of glutathione synthesis also supports a role of glutathionylation in signal transduction, with several cytokines and chemokines being redox-regulated.

In conclusion, not only reduced glutathione can act as an antioxidant and a free radical scavenger, but glutathione (both reduced and oxidized) is also a signaling molecule that acts as a sensor of the redox state of the cell.

L4 **- BIOSYNTHESIS AND FUNCTIONS OF TRYPANOTHIONE – A KEY DRUG TARGET IN TRYPANOSOMATIDS**

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New drugs are required for neglected parasitic diseases of the developing world such as human African trypanosomiasis, Chagas' disease and the leishmaniases. The causative organisms (*Trypanosoma brucei sspp*, *Trypanosoma cruzi* and *Leishmania spp*) differ markedly from their human host in using trypanothione (N^1,N^8-*bis*(glutathionyl)spermidine) in place of glutathione for many essential functions such as regulation of intracellular thiol redox balance and defence against chemical, heavy metal and oxidant stress. Several of the existing unsatisfactory drugs interact directly or tangentially with these pathways (e.g. eflornithine, antimonials and nifurtimox). Consequently trypanothione-dependent enzymes present attractive targets for drug discovery. Candidates include trypanothione synthetase, trypanothione reductase, type I and type II tryparedoxin peroxidases and the trypanothione-dependent glyoxalase system. Current data on their essentiality, druggability, assay feasibility, potential for selective inhibition, potential for resistance and structural and mechanistic information will be discussed. Current progress in the Drug Discovery Unit at Dundee will be presented.

***L5* - Redox metabolism in malarial parasites and its potential as target for novel chemotherapeutic interventions**

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Malaria is caused by the multiplication of the protozoan parasite *Plasmodium falciparum* in human red blood cells. Currently the disease leads to almost 3 million human deaths annually. The detoxification of reactive oxygen and nitrogen species is a challenge for the parasite-host unit. As a result of (i) the high metabolic rate of the rapidly growing and multiplying parasite, (ii) the host immune response to the parasite infection, and (iii) the degradation of hemoglobin by the parasite, large quantities of toxic redox-active by-products are generated. Thus parasite enzymes involved in antioxidant defence, redox regulation or glutathione related detoxification processes represent interesting targets for rational drug development. Over the last years the redox network in *Plasmodium falciparum* comprising a glutathione and a thioredoxin system has been studied in detail.
There is a number of structural and functional features which define a protein as a promising drug target. Ideally the respective parasite pathway or protein does not occur in the (human) host and can thus be specifically targeted – a strategy decreasing the risk of side effects. Examples of redox-related proteins in *P. falciparum* would be (i) the chimeric enzyme exhibiting both glucose 6-phosphate dehydrogenase and 6-phosphogluconolactonase activity, (ii) plasmoredoxin, a redox-active protein with similarities to thioredoxins, (iii) a glyoxalase I which has evolved via a gene duplication and fusion event, (iv) a glyoxalase I-like protein of yet unknown function, (v) the peroxiredoxin "antioxidant protein", (vi) at least four very recently identified putatively redox-active selenoproteins, and (vii) a glutathione *S*-transferase (GST) which does not belong to any of the known GST classes.
However, also redox-active parasite proteins which do have an ortholog in man but exhibit structural features that might be exploited for drug development, are interesting target candidates. These proteins include glutathione reductase and thioredoxin reductase. For both proteins specific inhibitors have been identified/developed and studied over the last years. The PfGR specific inhibitor methylene blue has recently been shown to act highly synergistically with artemisinins.
The currently available data on structural, biochemical, and functional properties of the most important redox-active proteins in malarial parasites will be summarized in an attempt to evaluate and compare their potential as drug targets.

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L6 **- Life with and without selenium and glutathione**

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Thioredoxin (Trx) together with thioredoxin reductase (TrxR) and NADPH comprise the universal thioredoxin system (1). Human cytosolic and mitochondrial thioredoxins have the conserved CGPC active site and are reduced by TrxR having a high molecular weight TrxR with a C-terminal Gly-Cys-Sec-Gly active site. In contrast bacteria have a small specific cysteine containing TrxR of an entirely different structure and mechanism. Recently, we found that the selenazol drug ebselen, used as an antiflammatory agent is highly reactive with both human Trx and TrxR (2, 3). Ebselen is rapidly reduced by NADPH and TrxR and a very fast oxidant of reduced Trx (2, 3), strongly suggesting that the mammalian target of ebselen is the thioredoxin system. We recently discovered that ebselen is a strong competitive inhibitor of bacterial thioredoxin reductase (4). Gram negative bacteria like *E. coli* have a high content of GSH and glutaredoxin, which can supply electrons to ribonucleotide reductase in the absence of Trx and TrxR. However, we observed that the reason for gram positive bacteria like *Staphylococcus aureus* to be highly sensitive to ebselen is mainly due to the lack of GSH and consequently of glutaredoxins. At present, we are developing use of ebselen and analogs to inhibit bacterial growth targeting specifically pathogenic bacteria, which lack GSH.

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L7 **- Glutathione Protects *Lactococcus lactis* against Multiple Stresses.**

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The physiological role of glutathione (reduced form GSH) in Gram-positive bacteria remains largely unknown. This is mainly due to the fact that most of the Gram-positive bacteria are unable to synthesize this important tripeptide. Using *Lactococcus lactis* as a model organism of Gram-positive bacteria, we demonstrate that GSH can protect the host against multiple environmental stresses. *L. lactis* is widely used in dairy industries as starter cultures, some *L. lactis* strains (such as *L. lactis* SK11) can take up GSH from the environment. SK11 cells containing GSH showed significantly high survival rate as compared to the control upon oxidative stress (H_2O_2, 5 mM), acid stress (lactic acid, pH 4.0), and osmotic stress (NaCl, 6 M). GSH also plays physiological roles in *L. lactis* strains which can not take up GSH from the environment. This is observed by introducing GSH biosynthetic capability into *L. lactis* NZ9000, which can not take up GSH. When genes encoding γ-

glutamylcysteine synthase and glutathione synthase were cloned and expressed in strain NZ9000, the engineered strain NZ9000 (pNZ3203) produces GSH upon nisin induction. Strain NZ9000 (pNZ3203) producing GSH showed significantly high survival rate as compared to the wild type strain upon oxidative stress and acid stress. These results suggest that the robustness and fitness (in particular responses to various environmental stresses) of starter culture can be improved by selecting *L. lactis* strains capable of producing or importing GSH.

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***L8* - Glutathione as an in vivo Indicator of Chemical Stress in Complex Biological Systems**

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Oxidative stress-inducing chemical contamination in the environment is a significant concern for public and environmental health. This work focused on characterizing a cell-based biological indicator for detecting bioavailable forms of oxidative stressors in water samples. Our early studies demonstrated the relevance of the glutathione-gated K^+ efflux (GGKE) response characteristic of heterotrophic Gram negative bacteria to the performance of biological wastewater reactor systems that utilize gravity settling where we correlated K^+ efflux with severe deflocculation of bacterial flocs when exposed to thio-reactive oxidative stressors. As glutathione is an important global antioxidant involved in cellular defense against oxidative stress, we elected to evaluate the relevance of using bacterial GGKE as an indicator of bioavailable oxidative stressors pertinent to a broad range of biological systems. Recently, we compared the specific oxidative stress responses in two distantly related cell types: the bacterial GGKE mechanism; and mitochondrial dysfunction in rat brain cells. Our results show that the GGKE response in *Pseudomonas aeruginosa* cells was dose-dependent. Similarly, mitochondrial dysfunction in a mixed culture of rat brain cells to a model oxidative electrophilic chemical, *N*-ethylmaleimide, was also dose-dependent. The dose-response patterns for the two cell types corresponded well to each other. We also observed that both responses were accompanied by the depletion of intracellular glutathione, as expected because glutathione oxidation precedes the GGKE response in *P. aeruginosa* as well as mitochondrial damage in rat brain cells. This study result suggests that the quantitative bacterial GGKE response to oxidative stress could potentially be used as an early warning sensor to predict the presence of bioavailable oxidative chemicals that can induce oxidative stress in prokaryotic and eukaryotic systems.

***L9* – Incorporation of bridged [2Fe-2S] clusters into dimeric monothiol and dithiol glutaredoxins: evidence for two chloroplast monothiol glutaredoxins acting as scaffold proteins**

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Several lines of evidence indicate that glutathione (GSH) and glutaredoxins (Grxs), the oxidoreductase associated, are implicated in the response to oxidative stress, especially through the regeneration of enzymes involved in peroxide and methionine sulfoxide reduction. On the other hand, emerging functions for plant Grxs and GSH concern the regulation of protein activity via glutathionylation and the capacity of some glutaredoxins to bind iron sulfur centers and/or to transfer it into apoproteins (Rouhier et al, Annu Rev Plant Biol. 2008). More than 30 distinct grx genes are expressed in higher plants, but little is currently known concerning their functional diversity. Recent biochemical, spectroscopic and structural data indicate that heterologously expressed Grxs, a cytosolic Grx C1 (CGYC active site) and two chloroplastic Grxs, GrxS14 and GrxS16 (CGFS active sites), are able to incorporate a [2Fe-2S] cluster using the catalytic cysteine of two monomers and two glutathione molecules (Rouhier et al., PNAS 2007, Bandyopadhyay et al, EMBO J. 2008). In addition, *in vitro* cysteine desulphurase-mediated assembly allowed the incorporation of an identical [2Fe-2S] cluster in apo-GrxS14 (Bandyopadhyay et al, EMBO J. 2008). *In vitro* kinetic studies monitored by CD spectroscopy indicate that [2Fe-2S] clusters on GrxS14 are rapidly and quantitatively transferred to apo chloroplast ferredoxin (Bandyopadhyay et al, EMBO 2008). Both monothiol CGFS Grx, but not GrxC1, were able to complement a yeast *grx5* mutant defective in Fe-S cluster assembly. These data demonstrate that chloroplast CGFS Grxs have the potential to function as scaffold proteins for the assembly of [2Fe-2S] clusters that can be transferred intact to physiologically relevant acceptor proteins. Alternatively, they may function in the storage and/or delivery of preformed Fe-S clusters or in the regulation of the chloroplastic Fe-S cluster assembly machinery.

***L10* – ANTIOXIDATION vs. OXIDATION PROCESSES IN YEAST: How much can be elucidated with cellular and proteomic approach?**

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Yeasts like other organisms living in aerobic conditions are continuously exposed to reactive oxygen species (ROS) formed as byproducts during normal cellular metabolism. Under normal physiological conditions cell defense systems are able to avoid molecular damages. Different stress conditions (oxidants, heat shock, ethanol, metal ions) increase the levels of ROS leading to induction of endogenous antioxidant defense systems – oxidative stress response. When ROS levels exceed the antioxidant capacity of the cell, an oxidative stress appears. The connection of different ions such as Cr(III), Cr(VI), Fe(III), Se(VI) and I(V) with ROS production and oxidative stress response induction was studied *in vitro* and *in vivo*. This can be serious problem in any metabolism including humans. For this reason the role of exogenous antioxidants (Trolox, ascorbic acid, royal jelly) on prevention of oxidative cellular damage and oxidative stress was studied. Currently there are many methods to study oxidative stress response, however they are not easily comparable because of different mode of action. For that reason still new methods have been evolved. Among current methods we can see also advanced methods like proteomics, metabolomics, which can cover this issue on more comprehensive level. We expect that battery tests which are under development will be able to elucidate mode of action of pro-oxidants and antioxidants in the cell. Both, cellular and proteomic approach was used to better understand oxidation and antioxidant processes in the yeast cell, which was used as a model organism in our studies.

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L11 – PHYTOCHELATINS IN ALGAE AND LICHENS

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Phytochelatins (PCs) are thiol peptides synthesized from glutathione by PC-synthase, genes of which were recently reported in all eukaryotic kingdoms, but not in all species. PCs due to complexing capability of heavy metal(loid)s represent first line of their intracellular detoxification. In algae and lichens PC synthesis was induced by surplus of some essential (Cu, Co, Ni, Zn) and non-essential trace elements (Cd, Pb, Hg, Ag, As, Sb), but not by Cr. PC production was found in several classes (Bacillariophyceae, Chlorophyceae, Euglenophyceae, Eustigmatophyceae, Phaeophyceae, Prymnesiophyceae, Rhodophyceae, Trebouxiophyceae, Xanthophyceae) of common marine, freshwater and terrestrial algae, both the free-living and symbiotic ones. No PC synthesis was found in cyanobacteria and dinoflagellates. Metal-exposed apo-symbiotically grown eukaryotic photobionts (Trebouxiophyceae) of epiphytic lichens synthesized phytochelatins as well, in contrast to mycobionts, in which PCs were not detected. Oxidized PCs found in a Cu-tolerant lichen suggest also their role in Cu^{2+} reduction. Production of PC molecules of different chain lengths and molecular weights is dependent on species/ecotype, metal, its concentration, bioavailability and exposure time. Hence, PC contents may be very variable in the metal(loid)-exposed cells. For example, Cd-resistant diatoms produced mainly longer-chain peptides (PC_{4-9}) of higher metal complexing capacity, however, in the Zn-resistant green algae short-chain molecules (PC_2) predominated. Some Zn-tolerant freshwater

green algae and metal-loaded marine fucoids produced high amounts of PCs and related peptides in contrast to a Cu-resistant lichen photobiont and some Zn/Pb- or Cu-resistant terrestrial algae. The role of PCs in metal(loid)-resistance/tolerance is very controversial, due to involvement of other sulfur-rich compounds (cysteine, glutathione, metallothioneins) as well as mechanisms of metal avoidance.

***L12* – Glutathione in plant responses to H_2O_2**

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H2O2-triggered redox signalling is a central part of plant responses to the environment [1]. To study these signalling processes, we are using a catalase-deficient Arabidopsis knockout mutant (*cat2*) as a model stress-mimic system. Because the function of the knocked-out catalase isoform in *cat2* is tightly linked to metabolism of H2O2 produced in photorespiration, altering external factors that change the rate of this metabolic process allows the H2O2 signal to be readily controlled in this line. In conditions where photorespiration is occurring at moderate rates, and no oxidative stress is apparent in the wild-type, cellular redox perturbation in *cat2* is clearly evident from a marked drop in the GSH/GSSG ratio and accumulation of leaf glutathione to several times wild-type contents. Though associated with slower growth, this dramatic redox perturbation does not necessarily lead to cell death, for which an additional daylength-dependent signal is required [2]. To explore the factors regulating H2O2-activated glutathione synthesis and the significance of glutathione concentration and redox state in oxidative stress responses, we have engineered constitutive changes in glutathione synthesis and GSSG reduction capacity into the *cat2* background. Results of some of these experiments, as well as related work, will be presented.

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L13* – GLUTATHIONE REDUCTION AND COMPARTMENTATION IN *ARABIDOPSIS THALIANA

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Glutathione is present in many compartments of the plant cell, including the organelles, cytosol, ER and possibly cell wall and vacuole. Exchange of glutathione between the compartments is implicated by the sole presence of glutathione reductase (GR) in plastids, mitochondria and cytosol, unless other proteins would mediate electron transfer to oxidized glutathione. The membrane transport mechanisms are still largely elusive but may occur as reduced, oxidized, conjugated or by way of the glutathione cycle found in animals. To investigate the role of GRs in the three compartments, T-DNA insertion lines of the two *GR* genes of Arabidopsis were characterized. GR1 encodes plastid and mitochondrial GR by way of a dual target sequence and a null allel of *gr1* was lethal. Inactivation of *gr2* encoding cytosolic GR2 caused no visible phenotype, but resulted in 60% reduction of overall GR activity and significantly increased contents of oxidized glutathione and consequently of glutathione redox potential in the cytosol. To dissect the roles of both organelles the *gr1* mutant was complemented by plastid or mitochondria-specific constructs. Expression of GR1 only in plastids was sufficient for survival while targeting only to mitochondria was not. Thus, exchange of reduced or oxidized glutathione or of precursors across the plastid membrane was insufficient to meet the demands inside

the organelle. This demand for plastidic glutathione reduction capacity is already essential during early embryo development long before initiation of chlorophyll biosynthesis.

L14* – Glutathione regulates the symbiosis between *Medicago truncatula* and *Sinorhizobium melitoti.

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Legumes can have a symbiotic relationship with ground bacteria *Rhizobium* to produce a particular root organ, the nodule, able to realize the fixation of atmospheric N_2. We have examined the importance of glutathione (GSH) and homoglutathione (hGSH) during symbiosis between *Medicago truncatula* and *Sinorhizobium meliloti*. (h)GSH is essential for nodule development as a deficiency inhibits the formation of this organ. To characterize the genes regulated by (h)GSH during the nodulation process, the transcriptomes of control and (h)GSH-depleted plants inoculated by *S. meliloti* were compared. Transcripts of genes involved in meristem formation accumulated at higher levels in control plants than in (h)GSH-depleted plants. An upregulation of the expression of genes belonging to the Defense and Cell Rescue class was also observed in (h)GSH-depleted plants suggesting that (h)GSH deficiency modulates the host defense response occurring after *Rhizobium* inoculation.

As numerous microsymbionts are present in the nodule infected cells, we have tested the involvement of the bacterial GSH pool in the nodulation process. γ–glutamylcysteine synthetase and glutathione synthetase defective mutant strains (SmgshA and SmgshB respectively) derived from wild type *S. meliloti* Rm1021 were constructed. SmgshA mutant strain was unable to grow under nonstress conditions, precluding any nodulation. The SmgshB strain showed a delayed-nodulation phenotype coupled to a 75% reduction in the nitrogen fixation capacity. This phenotype was associated to an early senescence process. Taken together, these results show that the bacterial GSH pool plays a critical role in the interaction of *S. meliloti* with the plant partner.

***L15* – Interplay between thioredoxin and glutathione pathways**

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NADPH-dependent thioredoxin reductases (NTRs) are key regulatory enzymes that determine the redox state of the thioredoxin system. Two genes coding for NTRs (/NTRA/ and /NTRB/) are found in the genome of /Arabidopsis thaliana/. Both encode a mitochondrial and a cytosolic isoform (1). In order to characterize the role of NTRs in plants, we have isolated a double /ntra ntrb/ knock-out mutant. Surprisingly, plants devoided of NTRs are still viable and fertile demonstrating that, in contrast to mammals, neither cytosolic nor mitochondrial NTRs are essential in plants. Interestingly, we found that in the /ntra ntrb/ mutant plant growth is hypersensitive to buthionine sulfoximine (BSO) a specific inhibitor of glutathione biosynthesis. Moreover, we found an alternative thioredoxin reduction pathway involving the GR/GSH/GRX pathway. In order to decipher the interplays between thioredoxin and glutathione pathways, we generated multiple mutants in the two pathways. Strikingly, crossing the /ntra ntrb/ mutant with /rootmeristemless1 (rml1)/, a mutant blocked in root growth due to strongly reduced glutathione synthesis, led to complete inhibition of both shoot and root growth (2).

This indicates that at least one of the NTR or the glutathione pathways is required to allow the post-embryonic activity of the apical meristem. Data on other mutants will be presented and potential functions of thioredoxin and glutathione pathways will be discussed.

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L16 – THE STRUCTURAL AND FUNCTIONAL COMPLEXITY OF MONOTHIOL GLUTAREDOXINS

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Monothiol glutaredoxins (Grxs) with the active site CGFS sequence exist in a wide variety of prokaryotic and eukaryotic organisms. Two subclasses exist, those with a single Grx domain and those with a thioredoxin-like domain followed by one or more Grx domains. *Saccharomyces cerevisiae* has a mitochondrial Grx (Grx5) which is involved in the last steps of the formation of Fe/S clusters at this organelle. Homologues of Grx5 in higher organisms seem to have a conserved function, restoring the defects caused by the absence of Grx5 when expressed in yeast. The absence of the human Grx5 protein leads to anemia-like diseases. In addition, yeast cells contain two nucleocytoplasmic monothiol Grxs with the thioredoxin-like extension (Grx3 and Grx4), acting as modulators of the transcriptional activator Aft1, which regulates iron uptake. Overall, the three monothiol Grxs in yeast participate in iron homeostasis and metabolism. The human PICOT protein is a Grx3/Grx4 homologue with the same hybrid primary structure, which regulates protein kinase C activity and may participate in physiological processes such as control of cardiac function. Fungal cells contain another group of monothiol Grxs with the active site sequence CP/SYS. Contrary to the CGFS-type molecules, these Grxs are active in the standard thiol oxidoreductase assays, and contain an N-terminal transmembrane domain. *S. cerevisiae* has two members of this group (Grx6 and Grx7), which are located at the endoplasmic reticulum and Golgi, and could act as regulators of the response against the oxidative stress generated during protein folding at the secretory apparatus.

L17 – A novel pathway for glutathione utilization in yeasts

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Degradation of glutathione in all living systems has been thought to be mediated by a single enzyme, gamma-glutamyl-transpeptidase. We have recently shown that an alternative pathway of glutathione degradation exists in the yeast *Saccharomyces cerevisiae* that is mediated by three proteins, Dug1p, Dug2p and Dug3p that function together in glutathione degradation in a complex. Together with the yeast glutathione transporter, Hgt1p (ScOPT1), also isolated as Dug4p, these proteins form the glutathione utilization pathway in *S. cerevisiae*. We have initiated studies on the Dug1p-Dug2p-Dug3p protein complex to identify possible mechanisms by which this complex may come together in the cell, and also how the complex may function in glutathione degradation. In addition we have also begun to investigate these pathways in other yeasts to see if this might throw some additional light on the evolution of this pathway.

L18 – Cellular adaptation to oxidative stress and regulation of cellular redox in yeast

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Maintenance of redox balance in cells is likely to be important in prevention of apoptosis and reducing the effects of oxidative damage on ageing. Part of the cellular response to oxidative stress, induced at low doses of reactive oxygen species involves the adaptation of cells leading to increased resistance to higher doses. A screening of H_2O_2-sensitive *Saccharomyces cerevisiae* deletion mutants identified genes that are important for adaptation to H_2O_2, and subsequent analysis provided an interesting insight in to the way cells regulate their normal cellular redox potential. The mutants unable to adapt fell into two groups based on their responses to a brief acute dose of H_2O_2 and to chronic exposure to H_2O_2.Transcription factors Yap1p, Skn7p and Gal11p were important for both acute and chronic responses to H_2O_2. Yap1p and Skn7p were acting in concert for adaptation, which indicates that up-regulation of anti-oxidant functions rather than generation of NADPH or glutathione is important for adaptation. Deletion of *GPX3* and *YBP1* involved in sensing H_2O_2 and activating Yap1p affected adaptation, but to a lesser extent than *YAP1* deletion. NADPH generation was also required for adaptation. *RPE1, TKL1* or *IDP1* deletants affected in NADPH production were chronically sensitive to H_2O_2, but resistant to an acute dose and other mutants affected in NADPH generation tested were similarly affected in adaptation. These mutants overproduced reduced glutathione (GSH) but maintained normal cellular redox homeostasis. This over-production of GSH was not regulated at transcription of the gene encoding γ-glutamyl-cysteine synthetase.

L19 – COMPARTMENTATION OF GLUTATHIONE IN ANIMAL AND PLANT CELLS

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The hypothesis that the intracellular distribution of glutathione between the different cellular compartments is important in its metabolic and signalling functions is widely accepted but little information is available on the how glutathione is compartmented in response to metabolic and developmental cues. It has recently been shown that glutathione movement from the cytosol to the nucleus is important in the control of progression through the mammalian cell cycle (1). In the case of proliferating fibroblasts, GSH was located mainly in the nucleus when the nearly half of the cells were in the S and G2/M phase of the cell cycle. The nucleus/cytoplasm fluorescence ratio reached a maximal mean value of 4.2 six hours after cell plating. When the fibroblasts reached confluence (G0/G1 phase), GSH was localized only in the cytosol. We set out to determine whether a similar pattern of movement of glutathione to the nucleus could be observed during the plant cell cycle in Arabidopsis roots and in cell cultures. The maximum rate of cell division in Arabidopsis cell cultures was co-incident with the maximal glutathione accumulation. We will report on the intracellular compartmentation of glutathione upon exposure to various mitotic triggers in relation to cell division and expansion.

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L20– Is there two roles for reduced glutathione in abiotic and biotic stress signalling in *Arabidopsis*?

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Two roles can be envisaged for reduced glutathione (GSH) in the modulation of gene expression in response to both high light and in the induction of defences against pathogen infection. The first, and most likely, of these roles is to act as an antioxidant in modulating the levels of hydrogen peroxide (H_2O_2) engaging in signalling. It has been proposed that the containment of H_2O_2 at its sites of production is an important feature of its role in signalling processes that lead to acclimation or are involved in development, as opposed to the initiation of cell death processes. Consequently, GSH levels, especially in the cytosol, may be important in maintaining the spatial integrity of H_2O_2 signalling initiated in, for example, the chloroplast. However, is there a second, H_2O_2-independent role for GSH as a signalling molecule? There may be, since a number pathogen- and high light responsive genes are induced in a NPR1-dependent manner by GSH infiltration of leaves. In contrast, in transgenic plants with elevated steady state GSH levels neither high light nor pathogen responsive genes were significantly altered in their expression. This suggests that it is a *change* in GSH levels which influences the expression of these genes. Microarray experiments has revealed that only a low number of genes (34) were consistently affected in the GSH elevated line compared with controls. Of the up-regulated group (21), 7 encode genes involved in flavonoid biosynthesis and metabolism, consistent with previous reports.

***L21*– Antioxidative systems in symbiotic fungi and stress protection of host plants**

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Most terrestrial plant form symbioses with mycorrhizal fungi at their roots. The function of the fungi is to supply nutrients and water to the host plant in exchange for plant-derived carbohydrates. It is also known that that these associations provide protection of the plant against various abiotic and biotic stresses. We have characterized the antioxidative system of the ectomycorrhiza-building basidiomycete, Paxillus involutus (strain MAJ), and shown that the fungus contains glutathione but not ascorbate as major soluble antioxidant. Heavy metal stress led to increased thiol biosynthesis and accumulation of the heavy metal in electron-dense particles in association with sulfur containing compounds. When P. involutus (MAJ) formed a functional mycorrhiza with poplar roots, the hyphal mantle accumulated high concentrations of H2O2 but not the Hartig net or the plant cells. Since this response was not present in free-living mycelium or in Paxillus strains that were not able to build a functional mycorrhizal structure, we suggest the H2O2 plays a central role in EM formation and maintenance. To test whether functional EM can also protect from other stresses, mycorrhizal and non-mycorrhizal poplar trees were exposed to excess salinity. Transcriptional profiling of non-stressed and stressed as well as mycorrhizal and non-mycorrhizal poplar roots indicates that P. involutus attenuates the host stress response.

L22* – GENETIC CONTROL OF GLUTATHIONE METABOLISM IN THE METHYLOTROPHIC YEASTS *HANSENULA POLYMORPHA

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Glutathione (GSH) plays pivotal role in defense of yeast cell against different stress factors. In methylotrophic yeasts, role of GSH is especially important as metabolism of each molecule of methanol gives equimolecular quantities of two extremely toxic compounds, formaldehyde and hydrogen peroxide, which detoxification depend significantly on GSH. We are interesting in studying mechanisms of GSH involvement in metabolic stress defense of the biotechnologically important methylotrophic yeast *Hansenula polymorpha* which is used for production of heterologous proteins and is promising organism for high-temperature production of ethanol from lignocellulosic sugars.

Using conventional and insertional mutagenesis, *H. polymorpha* mutants defective in GSH synthesis were isolated. Two defective genes have been identified, *GSH2* (ortholog of *Saccharomyces cerevisiae* gene *GSH1* responsible for first reaction of GSH biosynthesis) and *GSH2* homologous to *S. cerevisiae* gene *MET1* involved in sulfur metabolism. Gene *GGT1* coding for γ-glutamyl transpeptidase, responsible for GSH catabolism has also been identified. Resistance of the corresponding mutants to metabolic stress induced by methanol, heavy metals and other agents has been studied.

Transformant with multicopy insertion of *GSH2* gene has been isolated which is characterized by increase in cellular content of GSH. It was found that such strain accumulates more ethanol during glucose fermentation relative to the *H. polymorpha* wild-type strain. Thus, GSH content is important factor for improvement yeast alcoholic fermentation. *H. polymorpha* mutants with elevated content of intracellular GSH have also been isolated by insertional mutagenesis. Corresponding genes are under isolation. Perspectives of isolation of GSH superproducers in *H. polymorpha* will be discussed.

L23 **– Induction of glutathione synthesis pathway in response to cadmium in yeast**

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In response to cadmium (Cd) intoxication, yeast cells induce the synthesis of strong amounts of glutathione (GSH) to detoxify the metal: GSH forms complexes with Cd (Cd-GS_2) which are transported into the vacuole through the ABC pump Ycf1.

We used comparative proteome and metabolome approaches associated to a mathematical modelisation of the sulfur pathway to get insights into the mechanisms and regulation of the metabolic response. The analyses showed that the responses could be classified according to the Cd dose used in the study. We distinguished 3 types of Cd concentration: (1) weak doses (from 0.2 to 1 µM), (2) medium doses (from 1 to 10 µM) and (3) high doses (e. g. 50 µM).

At high concentration, most enzymes of the GSH pathway were induced. The metabolite pools and flux in the pathway were also strongly increased, particularly γ-glutamyl-cysteine. A kinetic analysis showed sequencial and dramatic changes in intermediate sulfur metabolite pools within the first hours of the treatment. This metabolic response was concomitant with a marked decrease in sulfur incorporation in proteins due to both a global decrease of protein synthesis and a reduced utilization of sulfur amino acid in the proteome expressed under cadmium conditions. At this concentration, enzyme expression, metabolite pools and fluxes were all increased showing a high correlation. Interestingly, we identified at this dose a novel metabolite, γ-glutamyl-cystathionine specifically produced under Cd conditions that we showed synthesized through Cys3 enzyme activity.

At low concentrations, a significant increase of GSH production is observed (factor 2 to 3) whereas no modification of the proteome is evidenced. The primary effect of low doses of Cd is a 20%/30% increase of sulfate assimilation, leading to increased sulfur available for GSH production. At intermediate doses, the sulfate assimilation remains high and Cd slightly inhibits protein synthesis,

thus enhancing the amount of sulfur available for GSH synthesis. Consistently, a further increase in GSH production is observed (factor 3 to 5). The proteome analysis shows that only Cys3 enzyme is significantly induced under these conditions. It then appears that at low concentrations, the increase of GSH synthesis does not seem to be linked to enhanced enzyme amounts but rather to sulfur availability as confirmed by a simple model of the pathway.

The results highlight the importance of combining metabolome, proteome and modeling approaches for the comprehensive and integrative description of cellular metabolism.

L24 – REGULATION OF REDOX HOMEOSTASIS IN YEAST

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All organisms contain complex regulatory machinery to maintain the redox status of -SH groups in proteins and low molecular weight sulphydryls. Glutaredoxins and thioredoxins are small heat-stable oxidoreductases which contain two conserved cysteine residues in their active sites. They were originally identified as hydrogen donors for ribonucleotide reductase, but are also required for a number of antioxidant and metabolic enzymes that form a disulphide as part of their catalytic cycle. Yeast contains two gene pairs encoding cytoplasmic glutaredoxins (*GRX1, GRX2*) and thioredoxins (*TRX1, TRX2*). Oxidised thioredoxins are reduced directly by NADPH in a reaction catalysed by thioredoxin reductase (Trr1), whereas, glutaredoxins are reduced by glutathione (GSH), and oxidised glutathione (GSSG) is in turn reduced in an NADPH-dependent reaction catalysed by glutathione reductase (Glr1). Analysis of the redox state of thioredoxins and glutaredoxins has revealed that thioredoxins are maintained independently of the GSH/glutaredoxin system. In contrast, there is a strong correlation between the redox state of glutaredoxins and the oxidation state of the GSSG/2GSH redox couple. In addition to the cytoplasmic thioredoxin system, yeast contains a complete mitochondrial thioredoxin system, comprising a thioredoxin (*TRX3*) and a thioredoxin reductase (*TRR2*). Analysis of the redox state of the mitochondrial Trx3 revealed that it is buffered by both the mitochondrial thioredoxin reductase (Trr2) and the glutathione (GSSG/2GSH) redox couple. I will present our recent data describing the antioxidant roles of the mitochondrial redox systems.

L25 – Revisiting the cellular functions of GSH in eukaryotes

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The tripeptide GSH is generally considered as having a unique cellular redox buffering function, capable of maintaining reduced the thiol-redox cellular balance, and also to eliminate electrophilic compounds including ROS and RNS species. This function is in keeping with the characteristics of GSH of being the most abundant low molecular thiol of eukaryotic cells and having a relatively low redox potential. Nevertheless, redox buffering might not explain all GSH cellular functions, and in particular its essential nature in both yeast and mammals. We have used *S. cerevisiae* as a model eukaryote to explore the essential function(s) of GSH, exploring the cellular defects ensuing (i) the deletion of the GSH biosynthetic gene γ-glutamyl cysteine synthase (*GSH1*) that leads to GSH depletion and cell death, and (ii) an artificial increase in its concentration > 10 mM, which also eventually leads to cell death. Data gathered using a combination of global and cell biological approaches lead to the conclusion that the essential requirement of GSH is due to its role in the maturation of Fe-S clusters, and can be explained by its ability of assembling with monothiol glutaredoxins (Grxs) Fe-S cluster that are important for such process. Toxicity of GSH at elevated concentrations also associates with a major alteration of iron metabolism, which is also due to

defective assembly of Fe-S at monothiol Grxs, and to a major block of secretion due to ER reductive stress. Surprisingly, both GSH cellular depletion and its presence at toxic concentrations do not appear to alter thiol-redox control in the cytoplasm. We will discuss the respective cellular functions of the GSH and thioredoxin pathways in eukaryotes.

***L26* – A GLOBAL CELLULAR INVENTORY OF THIOLS AND DISULFIDES**

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We have developed and applied a method for quantitative determination of total pools of thiols and disulfides on all cellular proteins (including membrane proteins) in cultured mammalian cells. We have combined these data with determinations of glutathione, glutathione disulfide and glutathione in mixed disulfides with protein in the same cells. The main conclusions from these studies is that steady state levels of protein glutathionylation is very low (less than 0.1 % of total protein cysteine is glutathionylated) but that this level can increase to 25% after treatment with sub-lethal pulse of diamide. Comparing the numbers shows that the available redox-active protein thiol pool is larger than that of GSH and that cells have an extraordinary ability to recover for disulfide stress.

***L27* – Glutathione metabolism of filamentous fungi with industrial significance**

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Glutathione may influence the production of important secondary metabolites at several points including (i) enzyme level inhibition of the synthesis of intermediates like δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine and isopenicillin N in the β-lactam biosynthetic pathway, (ii) maintenance of the redox stability of O_2-consuming and reactive oxygen species sensitive biosynthetic enzymes, (iii) uptake, intracellular transport and activation of metabolite precursors, (iv) supply of metabolic machinery with sulphur, (v) appearance of yeast-like cell morphology and (vi) glutathione/glutathione disulphide-dependent (GSH/GSSG) redox regulation of biosynthetic genes, *e.g.* in the case of mycotoxins like sterigmatocystin and aflatoxins. According to some more recent observations, FadA/FlbA and GanB/RgsA heterotrimeric G-protein signalling pathways contribute to the regulation of GSH-degradation in the filamentous fungus model *Aspergillus nidulans*, *i.e.* the regulations of vegetative growth, asexual development, secondary metabolite production and glutathione metabolism seem to be inherently interconnected in filamentous fungi. The modulation of intracellular GSH pool and GSH/GSSG ratio *via* elements and regulation of GSH-biosynthetic and GSH-utilising metabolic pathways is possible and can be important in the metabolic engineering of filamentous fungi, when alterations in either metabolite production or cell morphology are considered.

***L28* – Antioxidants in the protection of *Saccharomyces cerevisiae* against oxidative stress**

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Saccharomyces cerevisiae can be a useful model for studies of the efficacy of antioxidants at the cellular level. We tested protection by antioxidants of *S. cerevisiae* cells against exogenous oxidative stress and against endogenous oxidative stress induces by disruption of genes coding for key antioxidant proteins. Yeast disruptants in SOD1 show lysine and methionine auxotrophy which is abolished by hypoxic/anoxic atmosphere and some antioxidants including glutathione, cysteine, N-acetylcysteine and ascorbate. Only these compounds out of a range of antioxidants tested protect *Δsod1* cells against osmotic stress and *Δsod1, Δgrx5 and Δtrx1Δtrx2* cells against exogenous oxidants and aldehyde toxicity. These results point to significant differences between the *in vivo* and *in vitro* effects of antioxidants. The selectivity of action of antioxidants at the level of yeast cells cannot be accounted for by rates of their reactions with superoxide but correlates with their ability to maintain reduced protein thiol groups, as exemplified by protection of thiol-dependent activity of aldehyde dehydrogenase exposed to oxidants (especially hydrogen peroxide) and prevention of activation of Yap1p induced by oxidants and aldehydes, and with their effects on the redox potential of the medium. Another important aspect of cellular action of antioxidants is their effect on the level of glutathione and redox state of the glutathione couple, and on the expression of genes coding for antioxidant proteins and enzymes involved in glutathione metabolism.

L29* – Differences of GSH metabolism in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe

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1.) Both yeasts can take up GSH from the medium and use it as a sulfur source. In *S. cerevisiae* at least two transporters can take up GSH, as the disruption of a high affinity GSH-transporter does not abolish the ability to grow with GSH as a sole sulfur source.
In *Sz. pombe* the disruption of a single putative GSH-transporter abolishes the use of GSH as sulfur source completely. Localization studies with a GFP-tagged transporter show that it is in the plasma-membrane.
Crossing of the strain disrupted in the transporter with a strain bearing a disruption in the gsh2-gene and subsequent tetrad analysis show that the combination of both disruptions is lethal. No tetrads of the nonparental-ditype or tetratype were obtained.
2.) The gsh1-gene encoding the catalytic subunit of the γglutamyl-cystein-synthetase was disrupted. This leads to GSH auxotrophy in both yeasts, in *S. cerevisiae* mutants bearing a suppression of the GSH-auxotrophy arise with a high frequency, in *Sz. pombe* the auxotrophy is stable.
In *Sz. pombe* an ORF encoding a putative regulatory subunit of the γglutamyl-cystein-synthetase is found, there is no homolog in *S. cerevisiae*.
3.) The gsh2-gene encoding the glutathione-synthetase was disrupted, this disruption leads to GSH auxotrophy in *Sz. pombe*, but *S. cerevisiae* can grow without GSH supply. The dipeptide γGlu-Cys can fulfil the tasks of GSH well enough.
4.) Upon exposure to cadmium *Sz. pombe* makes phytochelatins from GSH, *S. cerevisiae* does not. Overexpression of gsh1 and the putative regulatory subunit of GSH1p leads to a higher concentration of GSH and phytochelatins but not to an increase in cadmium resistance.
Strains selected for higher Cd-resistance do not contain more GSH or PCs than the wild type.

***L30* – Redox regulation by glutathionylation and glutaredoxins in Chlamydomonas**

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Glutathione plays multiple roles in the physiological response of plants to oxidative stress. Besides its contribution to reactive oxygen species (ROS) scavenging, glutathione has been recently shown to be implicated in a reversible post-translational modification, named S-glutathionylation, consisting of the formation of a mixed disulfide between a protein thiol and a molecule of glutathione. Studies on animals have shown that this modification can protect proteins from irreversible oxidation and/or modulate enzyme activity under conditions of enhanced production of ROS. On the contrary very little is known about glutathionylation in plants. We have recently shown that thioredoxin f (1) and A_4-glyceraldehyde-3-phosphate dehydrogenase (2) from Arabidopsis, two chloroplastic proteins playing a major role in carbon assimilation, can be regulated by glutathionylation in vitro.

In order to get more insight into the proteins regulated by glutathionylation *in vivo*, we developed a proteomic approach based on [^{35}S]-cysteine labeling of the glutathione pool in the unicellular green alga *Chlamydomonas reinhardtii*. Proteins radiolabeled after oxidative stress treatments were visualized on 2D-gels and identified by mass spectrometry. This method allowed identification of a number of glutathionylated proteins, most of which are located in chloroplasts. Glutathionylation of three of these targets was confirmed *in vitro* on recombinant proteins.

In non photosynthetic organisms, deglutathionylation reactions are considered to be catalyzed much more efficiently by glutaredoxins (GRX) than by thioredoxins. We have examined the ability of cytosolic and chloroplastic thioredoxins and glutaredoxins from photosynthetic organisms to catalyze this reaction. Cytosolic GRX1 exhibited properties similar to those previously described for GRX from non-photosynthetic organisms. On the other hand, chloroplastic GRX3 exhibited unique properties, notably, the ability to be very efficiently reduced in the light by ferredoxin thioredoxin reductase (3).

(1) Michelet et al. (2005) **Proc. Natl. Acad. Sci. USA**, 102, 16478-16483.
(2) Zaffagnini et al. (2007) **FEBS J.,** 274, 212-226.
(3) Zaffagnini et al. (2008) **J. Biol. Chem.,** in press.

L31 – Plant sulfur species and their role in redox regulation with possible applications in Medicine and Agriculture

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Many plants contain sulfur compounds which occur naturally in a range of different oxidation states and chemotypes [1]. Most of these sulfur species are redox active and therefore able to interfere with various biochemical pathways, such as redox signalling pathways, phosphorylation and apoptotic pathways. Not surprisingly, such sulfur species may also have an impact on human health and disease. Recent research has therefore explored the various chemical and biochemical properties of a number of naturally occurring sulfur species and has considered their biological activity in a range of different assays indicative of antioxidative, cancer preventive, anticancer, antibacterial, fungicidal, nematocidal and pesticidal activity.
Together, these studies indicate that compounds belonging to the group of Reactive Sulfur Species (RSS), many of which are found in household plants such as garlic, onions, asparagus, mustard and broccoli, exhibit a wide spectrum of biological activity [1]. These compounds include various thiols (most of which are antioxidants); thiones (*e.g.* ergothioneine from ergot), disulfides, thiosulfinates (*e.g.* allicin from garlic), mono-, di- and polysulfides (*e.g.* dialkyl- and diallylpolysulfides found in onions and garlic, Shiitake mushrooms) and even transient sulfenic acids. The presence of RSS in edible plants and fungi has, of course, raised a strong interest in their use as 'natural medicines' and – in the agricultural sector – as 'green' pesticides.

Unfortunately, most RSS seem to exhibit a rather complicated chemical and biochemical (redox) behaviour *in vivo*, which is still only partially understood [2]. This lack of knowledge limits the practical application of most of these sulfur compounds to date. Current research into the biological chemistry of RSS has uncovered conventional oxidation and reduction reactions, but also (superoxide) radical formation, sulfur radical chemistry, release of inorganic sulfur species and interactions with metal ions. This 'chemistry' appears to form the basis for the pro- and antioxidant activities of natural sulfur compounds which are frequently observed *in vitro* and *in vivo*. It also sets RSS apart from other classes of (bioactive) compounds. As they are slowly emerging, the chemistry, biochemistry and biology of RSS provide ample opportunities for multidisciplinary research, the development of natural medicines, 'green' antibiotics, fungicides and pesticides and the development of synthetic drugs.

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ORAL COMMUNICATIONS

OC1 - Effects of cell stress protectant glutathione on the whole-cell biocatalytic conversion of ethyl 4-chloroacetoacetate with *Saccharomyces cerevisiae*

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Ethyl (*R*)-4-chloro-3-hydroxybutyrate is an important chiral building block for pharmaceutically active compounds. Of the variety of synthetic methods the biocatalytic approach is superior to classical chemical alternatives which produce the chiral product in insufficient stereoselectivity.
For these purposes the whole-cell biocatalytic approach to ethyl (*R*)-4-chloro-3-hydroxybutyrate with *Saccharomyces cerevisiae* was investigated. Due to the xenobiotic stress exerted by the alkylating substrate, several competing reactions were encountered at the activated positions of the substrate molecule which are mediated by cytosolic glutathione (GSH): (i) reductive dehalogenation which proceeds along a thus far novel mechanism, (ii) ester hydrolysis and (iii) a previously non-described cleavage of the C_4-backbone (*retro*-Claisen condensation). The catalytic effect of a glutathione *S*-transferase accelerates these processes, but is not necessarily required for the reactions to occur. Ester hydrolysis product chloro-acetone and *retro*-Claisen product ethyl chloro-acetate significantly affect stereoselectivity of the whole-cell reduction. For dehalogenation product ethyl (*S*)-3-hydroxybutyrate no effects on the biotransformation were observed.

OC2 - A NOVEL FAMILY OF PLASTID TRANSPORTERS INVOLVED IN GLUTATHIONE METABOLISM

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Using mutants of Arabidopsis with increased resistance to the glutathione biosynthetic inhibitor, buthionine sulfoximine (BSO), we have identified a gene, *CLT1*, and its paralogues, *CLT2* and *CLT3*. *clt1,clt2,clt3* triple mutants (and *clt1,clt3* double mutants) exhibit increased sensitivity to heavy metals such as Cd and arsenate. The *clt1,clt2,clt3* mutant has about 10-fold less glutathione in roots compared to the wildtype although no significant difference in shoot glutathione levels was observed. In an alternative approach we used redox-sensitive GFP (roGFP) as an indicator of glutathione levels in sub-cellular compartments. Comparisons of roGFP activity in the cytosolic and plastidic compartments of the *clt1,clt2,clt3* mutant and the wildtype indicate no difference between mutant and wildtype in the plastids. The roGFP is significantly more oxidized in the cytosol of the mutant compared to wildtype in both roots and leaves suggesting a lower cytosolic glutathione concentration in both roots and leaves of the *clt1,clt2,clt3* mutant. The *CLT* genes encode predicted integral membrane transport proteins which have been shown to be targeted to the plastids by using GFP fusions. Xenopus oocytes expressing CLT1 show increased intracellular levels of GSH when exposed to either exogenous

glutathione or the pathway intermediate, γ-glutamylcysteine (γEC). We propose that the CLT's transport γEC and or glutathione from the plastids to the cytosol.

OC3* - Glutathione mediating cadmium sequestration in *R. Leguminosarum

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In a previous report, we demonstrated the existence of an important intracellular cadmium (Cd) detoxification mechanism, involving GSH, in Rhizobium leguminosarum cells. GSH–Cd complexes were extracted and analysed in two R. leguminosarum strains with different Cd tolerance levels, through a modified protocol, originally developed for metal–phytochelatins complexes. In the present work, we aim to illustrate the dynamic of that process during the growth of Rhizobium cells. We try to explain Cd mobility in the cells, GSH synthesis, the formation of the GSH-Cd complexes during the 72h of Rhizobium's growth and the efficiency of this mechanism in tolerant and sensitive strains. Cells were grown in the presence of 1 mM Cd (tolerant strain) and 0.25 mM Cd (sensitive strain), and harvested at 0h, 6h, 12h, 18h, 24h, 48h and 72h and analysed. Different sub-cellular Cd fractions were extracted: loosely bound Cd was extracted with water, in an ultrasonic bath; intracellular Cd was sequentially extracted with HEPES buffer and wall-bound Cd was extracted with acid. Intracellular Cd was separated through size exclusion chromatography, and the amount of Cd in collected fractions was analysed. Peptide peaks containing the higher Cd concentrations were analysed by RP-HPLC analysis with pre-column derivatisation, in order to quantify GSH and/or other thiols present in Rhizobium cells.

***OC4* - TRANSCRIPTION OF GLUTATHIONE S-TRANSFERASE GENES ARE MARKEDLY INDUCED IN RESISTANT TOBACCO LEAVES AFTER TOBACCO MOSAIC VIRUS INFECTION**

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Oxidative stress was observed in several incompatible plant-virus interactions including Xanthi-nc tobacco - *Tobacco mosaic virus* (TMV). This oxidative stress initiates lipid peroxidation processes resulting in membrane destruction, cell death and in the appearance of visible necrotic lesions on inoculated leaves (hypersensitive reaction). Increased glutathione (GSH) levels and considerably elevated glutathione S-transferase (GST, E.C. 2.5.1.18.) activities were observed in TMV-infected resistant Xanthi-nc tobacco leaves. GST isoenzymes catalyze the covalent conjugation of GSH to various toxic compounds or to their electrophilic metabolites to produce less toxic and more water-soluble conjugates, which are transported to the vacuoles. It is supposed that in infected plants GSTs contribute to the protection against oxidative membrane damage by catalyzing the breakdown of toxic lipid hydroperoxides. Thus, GSTs participate in the suppression of necrotic disease symptoms.

In our studies, resistant and susceptible tobacco (*Nicotiana tabacum* L. cv. Xanthi-nc and cv. Samsun, respectively) plants were inoculated with TMV. Transcription of GST genes was studied in virus-infected and mock-inoculated leaves by RT-PCR with degenerated primers and by real-time RT-qPCR. PCR amplicons were cloned and sequenced, and several novel full length and partial GST genes were identified. A marked but transient induction of GST gene transcription was observed in resistant Xanthi-nc leaves at 2 days after TMV inoculation. However, in susceptible Samsun leaves, TMV infection resulted in a negligible activation of GST gene transcription. The considerable upregulation of GST gene transcription in resistant leaves by TMV is probably necessary to remove toxic substances, which derive from membrane damaging oxidative processes.

OC5 - STRUCTURE-FUNCTION ANALYSIS OF THE HIGH AFFINITY GLUTATHIONE TRANSPORTER FROM THE YEAST *SACCHAROMYCES CEREVISIAE*

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The high affinity glutathione transporter, Hgt1p, in *Saccharomyces cerevisiae* is the only high affinity glutathione-specific transporter to be cloned and characterized in any system so far. Hgt1p belongs to a relatively novel Oligopeptide Transporter Family. We have initiated a study on structure-function characterization of Hgt1p, to identify the substrate-binding motifs and other signature sequences that are crucial for glutathione transport. Two independent approaches have been adopted.

Firstly, a cysteine-free Hgt1p molecule was generated for cysteine-scanning based structural studies on Hg1tp. The cysteine-free Hgt1p was, however, non-functional. A genetic approach to isolate functional revertants of cysteine-free Hgt1p always picked functional molecules with double reversion bearing cysteines at positions 622 and 632 of Hgt1p, which retained 10-15% of the functional activity. A sequence alignment of the OPT family revealed that these two cysteines though not absolutely conserved, are co-conserved, in majority of the members of the OPT cluster to which Hgt1p belongs, prompting us to undertake a detailed investigation on the role of native cysteines in Hgt1p. The two cysteines C622 and C632 are probably covalently linked via a disulfide bond, which is crucial for protein trafficking to plasma membrane. In contrast, the remaining cysteines, though not individually essential, contribute additively to the stability and functionality of Hgt1p. In a second approach, an alanine scanning mutagenesis across the transmembrane domains has been undertaken, to identify residues involved in forming salt bridges required for the structural stability of the protein or binding to the substrate.

OC6 - SIGNIFICANCE OF THE GAMMA-GLUTAMYL CYCLE IN THE RECOVERY OF EXTRACELLULAR GLUTATHIONE IN PLANT CELLS.

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The gamma-glutamyl cycle is a process by which extracellular glutathione is recovered by means of the enzyme gamma-glutamyl transferase (GGT).
Studies on the properties and localization of this enzyme in plants have been recently reported, pointing to its subcellular localization in the apoplast and vacuole. More in details, we have described by immunocytochemical analysis that GGT is almost exclusively localized in the cell wall of barley roots, and by modelling studies we suggest that a net surface charge may be responsible for anchoring the protein to cell wall components. We also show enzymehistochemically that GGT activity is high in the root tip and cortex.
By the combined use of enzyme activity inhibitors and diamide treatment, we describe variations in thiol content in roots and growth solution that are compatible with the existence of a gamma-glutamyl cycle in roots which assists the recovery of extracellular glutathione and may participate to the control of the apoplastic redox state.
Our results obtained using different approaches including *in vivo* activity measurements, enzymehistochemical analysis, in gel activity and gene expression analysis performed on *A. thaliana* wild type vs. knockout mutants lacking apoplastic GGTs, grown under different treatments, highlight

the existence of compensatory and regulatory mechanisms that further confirm a role for GGTs in the recovery of extracellular glutathione.

***OC7* - Arsenate reductase of *Corynebacterium glutamicum* depends on mycothiol for electron transport**

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The frequent abundance of arsenic in the environment has guided the evolution of enzymes for the reduction of arsenate. The arsenate reductases (ArsC) from different sources have unrelated sequences and structural folds and can be divided in different classes on the basis of their folds, reduction mechanisms and the locations of catalytic cysteine residues. So far, two major classes have been identified: the thioredoxin-coupled arsenate reductase class and the glutathione/glutaredoxin-linked class. Arsenate reductases belonging to these classes are small cytoplasmic redox enzymes that reduce arsenate to arsenite by the sequential involvement of three different thiolate nucleophiles that function as a redox cascade.
Recently, we unravelled the mechanism of a single cysteine containing arsenate reductase from *Corynebacterium glutamicum,* a member of the *Actinobacteria* group, which depends for the reduction of arsenate to arsenite on an electron transfer cascade in which mycothiol is involved. During my presentation, I will guide you through this novel mechanism and show you that this enzyme is the first representative of a novel class of mycothiol-coupled arsenate reductases.

***OC8* - DYNAMIC IMAGING OF THE GLUTATHIONE REDOX POTENTIAL AT SUBCELLULAR LEVEL**

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Redox-mediated signalling in response to adverse environmental conditions is an important pathway leading to alterations in metabolism and gene expression. The cellular glutathione pool has long been suggested to participate in co-ordination of some of the key re-adjustments underlying the physiological and developmental plasticity in response to biotic and abiotic stress, but so far time resolved measurements have been hampered by the lack of *in vivo* probes for dynamic redox measurements. Ratiometric redox-sensitive GFP (roGFP) preferentially acts as a sensor for the glutathione redox potential due to specific interaction with glutaredoxins, which mediate the reversible transfer of electrons between the glutathione pool and roGFP. Specificity of roGFP for the glutathione redox potential is further emphasized by altered fluorescence readouts when roGFP2 is expressed in several Arabidopsis mutants affected in glutathione homeostasis, both through altered total levels of glutathione and the reduction status of the glutathione pool. In all compartments tested roGFP2 reversibly responded to perfusion with H_2O_2 and DTT emphasizing that roGFP2 is a reliable probe for dynamic redox imaging *in planta*. Ratiometric analysis of roGFP2 fluorescence shows that in most compartments the cellular glutathione buffer is far more reduced than previously thought. The only

oxidizing compartment is the endoplasmic reticulum, in which roGFP2 is almost completely oxidized. The glutathione redox buffer is highly sensitive to redox changes triggered by external stress factors suggesting that redox signals in living cells are channelled through the glutathione redox buffer at subcellular level.

OC9 **- Implication of exofacial sulfhydryl-groups in reducing activity of *Lactococcus lactis***

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Lactococcus lactis is the most used cheese starter. These lactic acid bacteria (LAB) are especially chosen for their acidifying, proteolytic and lipolytic properties. However, the redox potential (Eh) is a major parameter although it is rarely taken into account (Cachon et al., 2002). The mechanism of the Eh reduction has not been fully established yet. This reaction allows to obtain an anaerobic system, essential for the optimal growth of anaerobic microflora. In addition, the Eh value influences the production of aroma compounds (Kieronczyk et al., 2006) and protects these compounds from oxidation. Among the LAB, *Lactococcus lactis* is the species with the best reducing capacity(Brasca et al., 2007). Many studies suggest the role played by the SH-groups in the micro-organism reduction (Oktyabrskii & Smirnova, 1988, Bagramyan et al., 2000)

For this purpose, *L. lactis* was cultured in MRS medium. After reduction, a filtration was realized or a specific thiol oxidant was added. In these two experiments, the initial Eh was restored in filtrate and in culture where an oxidant compound was added. To validate these results, confocal microscopy observation with a specific probe able to bind with exofacial free thiols has been realized. Finally, the exofacial thiols were quantified with a specific non-permeant oxidant compounds. 15µM of thiols were found at the end of the growth.

Thus, the reducing properties are carried out by the whole cell and these reactive exofacial sulfhydryl-groups were involved on this redox phenomenon.

Keywords: *Lactococcus lactis*, Eh, exofacial free thiols, cheese

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OC10 **- New fungal classes of glutathione-S-transferases: Role in oxidative stress and wood degradation process in *Phanerochaete chrysosporium***

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Taking advantage of the recent release of several fungal genome sequences, we report here an overview of fungal glutathione transferases (Gsts) focusing mainly on the basidiomycete *Phanerochaete chrysosporium*. A phylogenetic analysis has revealed the occurrence of new fungal specific Gst classes, in particular a class related to Omega one (Gto) and a class (Gte), whose members exhibit homology with bacterial etherases. The characterization of proteins belonging to these groups has been performed at biochemical level and the expression of the corresponding genes has been studied in relation with Lips expression and wood colonization. We have identified proteins of putative interest in wood decay and oxidative stress response, these processes being apparently linked. In particular, Gtt2 and Gte3 could be directly involved in lignin degradation and indirectly, thanks to their peroxidase properties, in the oxidative stress response resulting from the release of toxic compounds during this process.

OC11 **- A new role for glutathione in modulating plant/fungal interactions by the regulation of protein nitrosylation**

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Besides its function as an important antioxidant, recent studies in plant/pathogen interactions reveal a new role for glutathione in plant cells. One important signalling molecule in plant/pathogen interactions indicating pathogen invasion and leading to plant defence responses is nitric oxide (NO). NO is very reactive and transport of this molecule is assumed to be performed by stable delivery and storage forms. S-nitrosoglutathione, the nitrosylated form of glutathione is a candidate, as recent studies indicate important functions of S-nitrosoglutathione concerning the delivery of nitric oxide in the regulation of protein activity.

The role of glutathione and S-nitrosoglutathione as well as the activity of the nitrosoglutathione -reductase, releasing NO from glutathione, and the amount of nitrosylated proteins in barley and oilseed rape were investigated during the interaction with biotrophic (*Blumeria graminis*) and pertotrophic (*Verticillium longisporum*) pathogens. As there are no *V. longisporum* resistant oilseed rape genotypes available, the non-host system *V. dahliae/B. napus* was used as a control. While in both *Verticillium*/oilseed rape interactions the activity of the S-nitrosoglutathione-reductase was induced, only in the pertotrophic interaction an increase in nitrosylated proteins was detected. In the barley powdery mildew interaction the susceptible barley line Ingrid and the tolerant backcross Ingrid-*mlo5* have been used to compare differences regarding the role of glutathione and S-nitrosoglutathione. The results indicate that susceptibility against *B graminis* is related to quantitative changes in glutathione content, S-nitrosoglutathione and pool of nitrosylated proteins. Both interactions will be discussed regarding this role of glutathione in the regulation of pathogen interactions.

OC12 **- MOLECULAR MECHANISM OF ENZYMATIC AND PROCESSING REACTIONS OF γ-GLUTAMYLTRANSPEPTIDASE OF *ESCHERICHIA COLI***

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γ-Glutamyltranspeptidase (GGT) (EC 2.3.2.2) is the key enzyme of glutathione metabolism and works as a glutathionase in cells. We showed that the precursor GGT is autocatalytically processed into two subunits and that the catalytic nucleophile for this proteolytic processing of *E. coli* GGT is the oxygen

atom of the side chain of Thr-391, which becomes the newly generated N-terminal residue of the small subunit. The crystal structures of the wild-type GGT and a mutant GGT^{T391A}, which cannot autocatalyze this process, were compared. The core regions were not changed, but marked differences were found near the active site. Upon processing, the newly generated C-terminus (residues 375-390) flipped out and the loop (residues 438-449) formed the lid over the γ-glutamyl group-binding pocket. By the biochemical study using a novel affinity-labeling agent, we identified that the oxygen atom of the side chain of Thr-391 also acts as the catalytic nucleophile for the enzymatic reaction. The three-dimensional structure of γ-glutamyl-enzyme intermediate trapped by flash cooling the GGT crystal soaked in glutathione solution clearly showed that the side chain of Thr-391 is γ-glutamylated. Although the wild-type *E. coli* GGT has no detectable glutaryl-7-aminocephalosporanic acid acylase activity, our previous study showed that it acquired the activity by a single amino acid substitution, D433N. Based on the three-dimensional structure, residues involved in substrate recognition were mutagenized followed by a novel screening method and a mutant GGT, which has almost 50-fold higher activity than the D433N mutant, was obtained.

***OC13* - Glutathione export as an extracellular arsenite detoxification mechanism**

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Arsenic is ubiquitously present in nature, and various mechanisms have evolved enabling cells to evade toxicity and acquire tolerance. We have previously shown that arsenite-exposed yeast cells (*Saccharomyces cerevisiae*) channel a large part of assimilated sulfur into glutathione biosynthesis and that this response is an important step in cellular arsenite tolerance acquisition (Thorsen *et al* (2007) *Physiol Genomics)*. Besides accumulating glutathione in the cytosol, yeast cells also export glutathione during persistent arsenite exposure. The exported glutathione conjugates with arsenite outside of the cells, which results in reduced arsenite influx. Further, our data indicate that exofacial plasma membrane thiols are reduced during arsenite exposure. Hence, glutathione export may serve two functions during arsenite exposure: extracellular detoxification and redox regulation of exofacial thiols.

***OC14* – REDUCTION OF ARABIDOPSIS PLASTIDIAL METHIONINE SULFOXIDE REDUCTASE B1 BY GLUTAREDOXINS.**

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Reactive oxygen species are able to damage macromolecules such as lipids, DNA and proteins. Methionine (Met) can be converted into methionine sulfoxide (MetSO) resulting in altered conformation and activity for many proteins (1). MetSO is reduced by two types of Methionine Sulfoxide Reductases (MSRs), A and B, specific to the *S*- and *R*-diastereomers of MetSO, respectively (2-3). Thioredoxins (Trxs) are common reducers of MSRBs, but thionein and some selenocompounds are also potential physiological reducing agents for these enzymes (4-5). Arabidopsis possess two plastidial MSRBs, termed MSRB1 and MSRB2 (6). Both possess the C-terminal catalytic cysteine (Cys) present in all MSRBs, but only MSRB2 contains a resolving Cys (6). We showed a strong specificity among plastidial Trxs towards MSRB2 (7). Using recombinant proteins and a site-directed

mutagenesis strategy, we demonstrated that MSRB2 reduction by Trxs is achieved through a classical disulfide exchange. In contrast, MSRB1 could be reduced only by the peculiar bimodular Trx CDSP32 (Chloroplastic Drought-induced Stress Protein of 32 kDa) and by various monothiol and dithiol Grxs (7), revealing a new type of reducer for MSRBs that possess only one redox-active Cys. To determine whether the resolving Cys in the active site or the extra C-terminal Cys in CDSP32 and in Grxs, participate in the reduction of MSRB1, we performed MSRB1 activity assays using recombinant mutated forms of CDSP32 and Grxs. Our results show that CDSP32 and Grxs are able to directly reduce the MSRB1 sulfenic acid form using only the conserved catalytic Cys.

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OC15 **- Glutathione pools in *Physcomitrella patens* exposed to cadmium**

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The terrestrial moss *Physcomitrella patens* responds to cadmium stress with a strong increase in its intracellular thiol pool (cysteine, y-glutamyl cysteine and glutathione (GSH)). No glutathione-derived phytochelatins were detectable. Addition of 10 µM Cd to the culture liquid resulted in an intracellular content of 1.5 µmol Cd per g fw. After 3 days of Cd exposure the total GSH pool increased up to three fold. Therefore, we hypothesized that Cd is detoxified by formation of Cd bis-glutathionato complexes in this plant.
Differentiation of intracellular GSH pools was achieved (i) by *in vivo* labeling with monochlorobimane (MCB) which was visualized by confocal laser scanning microscopy, and (ii) by in vitro quantification of GSH via monobromobimane (MBB) derivatization. Each was quantified using RP-HPLC followed by fluorescence detection. In contrast to non-stressed plants, total intracellular glutathione in Cd exposed cells increased remarkable. Surprisingly, in vivo labeling demonstrated significantly diminished GSH in stressed plants.
These results suggest the accumulation of intracellular Cd-$(GS)_2$ complexes as a novel way of Cd detoxification in most cells.

OC16 **- Subcellular Distribution of Glutathione in Plants**

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Glutathione is an important antioxidant in plants and plays key (protective) roles in cell metabolism through activating defense genes, by sensing reactive oxygen species (ROS) and by participating in ROS-signaling pathways that can control the fate of plants. Inter- and intracellular glutathione contents and the ratio of glutathione levels between certain cell compartments are therefore important

measurements of the plants ability to sense and fight oxidative stress and can give key information about the physiological condition of the plant.
Here we present a method that allows the visualization of glutathione in all cell compartments simultaneously in one experiment at a high level of resolution. This method is based on immunogold cytochemistry with anti-glutathione antisera and computer-supported transmission electron microscopy. By applying this method on several different transgenic and non-transgenic plant species (*Arabidopsis thaliana, Nicotiana tabacum, Cucurbita pepo* and *Beta vulgaris*), it was possible to gain thorough knowledge about the subcellular distribution of glutathione in plants and on the importance of glutathione in certain cell compartments during abiotic and biotic stress situations.
In the here presented study, these results are summarized and discussed in respect to the importance of compartment specific glutathione in plant cell metabolism, defense, growth and development. Additionally, future perspectives on how the subcellular distribution of glutathione correlates with the subcellular distribution of its precursors are presented.

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OC17 **– REGULATION OF SULFUR METABOLISM IN THE RICE BLAST FUNGUS MAGNAPORTHE GRISEA**

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The ability of the pathogens to sense and respond to their environment is crucial. The low concentration of several amino acids such as cysteine (Cys) and methionine (Met) in the leaf apoplast may not be sufficient to fulfil the parasitic growth. Thus phytopathogenic fungi may first mobilize their storage compounds during the early stages of the infection process, and then redirect their metabolism during the plant colonization for both structural and defence purposes, as they have to face the host reactions (ROS production).
In this context, our studies focus on the characterization of the regulation of the Cys and Met metabolism in the hemibiotrophic rice blast fungus *Magnaporthe grisea*. The expression of the genes encoding the sulfate reduction pathway leading to Cys, Met and glutathione (GSH) biosynthesis is under the control of the bZip transcription factor MetR, the ortholog of the yeast Met4p.
The deletion strain *M. grisea* Δ*metR* is unable to grow on minimal medium containing inorganic sulfur sources. The growth can be restored by the addition of organic sulfur sources such as Cys, Met or homocysteine. Metabolite profiling of the Δ*metR* performed by reverse chromatography (HPLC) reveals a drastic drop in the GSH content (up to 80%) even in presence of Met in the medium. The identification of the genes under the control of MetR and of the fungal defences raised in response to GSH reduction is performed using a global transcriptomic approach. The results are discussed with respect to the model established in *Saccharomyces cerevisiae*.

OC18 **- The zinc-binding protein Hot13 is a component of the mitochondrial disulfide relay system**

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In the bacterial periplasm and in the ER of eukaryotic cells, sulfhydryl oxidases catalyze the formation of disulfide bridges between cysteine residues in order to induce or stabilize protein folding. In contrast, other cellular compartments are assumed to be generally counteracting the formation of disulfide bridges to maintain proteins in a reduced state.

It therefore was completely unexpected when recently a machinery was identified in the intermembrane space of mitochondria - the compartment that phylogenetically originated from the bacterial periplasm - that catalyzes the oxidative folding of proteins. This machinery is vital for protein translocation of certain cysteine-containing precursor proteins into mitochondria. It consists of two highly conserved components: the import Mia40 and the sulfhydryl oxidase Erv1. Here we present evidence that the intermembrane space protein Hot13 represents as a third critical component of this machinery. Hot13 is a zinc-binding protein which interacts with Mia40. It strongly improves the Erv1-dependent oxidation of Mia40 presumably by maintaining Mia40 in a zinc-free state. Our observations propose a direct interplay of redox processes and metal homeostasis in the intermembrane space.
Thus, the principle to oxidize proteins was obviously conserved from the bacterial periplasm to the intermembrane space of mitochondria, but this system was adapted during evolution so that it now mediates the vectorial translocation of proteins from the cytosol into the organelle.

POSTERS

P1 **- Glutathione-dependent redox status of two yeast models of Friedreich's ataxia: *Saccharomyces cerevisiae* and *Candida albicans*.**

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Friedreich's ataxia is a neurodegenerative disease caused by reduced expression of the mitochondrial protein frataxin. The main phenotypic features of frataxin-deficient human and yeast cells include iron accumulation in mitochondria, iron-sulphur cluster defects and high sensitivity to oxidative stress. Glutathione is a major protective agent against oxidative damage and glutathione-related systems participate in maintaining the cellular thiol/disulfide status and the reduced environment of the cell. Here, we present the first detailed biochemical study of the glutathione-dependent redox status of wild-type and frataxin-deficient cells in two different yeast models of the disease.
There was five times less total glutathione (GSH + GSSG) in frataxin-deficient cells, imbalanced GSH/GSSG pools and higher glutathione peroxidase activity. The pentose phosphate pathway was stimulated in frataxin-deficient cells, glucose-6-phosphate dehydrogenase activity was three times higher than in wild-type cells, and this was coupled to a defect in the $NADPH/NADP^+$ pool. Moreover, analysis of gene expression confirms the adaptative response of mutant cells to stress conditions and we bring evidence for a strong relation between the glutathione-dependent redox status of the cells and iron homeostasis.
Dynamic studies show that intracellular glutathione levels reflect an adaptation of cells to iron stress conditions, and allow to distinguish constitutive stress observed in frataxin-deficient cells from the acute response of wild-type cells. In conclusion, our findings provide evidence for an impairment of glutathione homeostasis in a yeast model of Friedreich's ataxia and identify glutathione as a valuable indicator of the redox status of frataxin-deficient cells.

P2 **- THE EFFECTS OF ANTIOXIDANTS ON THE REDOX STATUS OF *SACCHAROMYCES CEREVISIAE* CELLS**

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It has been shown previously [Koziol et al., *Free Radic. Res*. 39 (2005) 365-371; Zyracka et al., *J. Biotechnol*. 115 (2005) 271-278] that only a limited number of antioxidants is effective in protection of yeast *Saccharomyces cerevisiae* cells against various types of oxidative stress.
To highlight this selectivity of protective action we examined the influence of 16 compounds commonly considered as antioxidants on the glutathione content of the yeast cells. As a model yeast strain we used strain defective in Sod1p. The intracellular redox potential of glutathione in *Δsod1* strain is known to be considerably more oxidizing in comparison with the respective wild-type strain [Lopez-Mirabal et al., *FEMS Yeast Res.* 7 (2007) 391-403]. We found that cysteine, dithiothreitol and N-acetylcysteine increased the level of GSH in *Δsod1* strain as well as wild-type strain and quercetin,

Tempo considerably decreased the amount of GSH. The ability of these compounds to alter the GSH content in yeast cells was consistent with the protective effect against the toxicity of thiol-depleting agent 4, 4'-dithiopyridine. We found that the increase of GSH level after 3 h incubation of the cells with cysteine correlates with the down-regulation of *GSH1* and *GLR1* genes and the decrease of γ-glutamylcysteine synthetase (γ-GCS) and glutathione reductase (GR) in *Δsod1* strain. On the contrary, Tempo and quercetin caused up-regulation of *GSH1* and *GLR1* genes and increased the activity of γ-GCS and GR.
Our data suggest that alteration of the intracellular redox potential is an important aspect of antioxidants action in yeast cells.

P3- IDENTIFICATION OF GENES INVOLVED IN GLUTATHIONE HOMEOSTASIS OF METHYLOTROPHIC YEASTS *HANSENULA POLYMORPHA*

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Regulation of glutathione (GSH) homeostasis in biotechnologically important strains of *Hansenula polymorpha* with naturally high resistance to different kinds of stress is not studied well. We've developed a system for selection of *H. polymorpha* strains with changed regulation of GSH homeostasis. Such mutants were obtained by electrotransformation of *H. polymorpha* strain NCYC 495 *leu 1-1* with pL2 linearized plasmid (gifted by Dr. K. Dmytruk) contained *Saccharomyces cerevisiae LEU2* marker gene. Inserted mutants were selected by their resistance to N-metyl-N'-nitro-N-nitrozoguanidine ($MNNG^R$). Among 100 $MNNG^R$ transformants there were selected only 10 mutants with additional sensitivity to cadmium ions and hydrogen peroxide. During growth experiments (120 hours) these mutants with $MNNG^R H_2O_2{}^S Cd^S$ phenotype demonstrated decreasing of extra- and intracellular levels of GSH (4-30 times) compared to wild type strain. Insertion cassette with flanking fragments of damaged gene was isolated from the one of mutants. Sequencing of genomic DNA fragments showed that homologue to *S. cerevisiae UBP1* was disrupted. Possible mechanisms of its GSH homeostasis regulation are under discussion.

Also 108 inserted mutants with resistance to different prooxidant and toxic agents (hydrogen peroxide, Cd^{2+}, 1-chloro-2,4-dinitrobenzene, formaldehyde and methylglyoxal) were isolated. Only 4 of them with additional resistance to formaldehyde demonstrated the increasing of GSH production during growth experiments.

P4 - GLUTATHIONE-DEFICIENCY MODULATES HOST GENE EXPRESSION DURING THE SYMBIOTIC INTERACTION BETWEEN *MEDICAGO TRUNCATULA* AND *SINORHIZOBIUM MELILOTI.*

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Glutathione (GSH) and its legume-specific homologue, homoglutathione (hGSH), are major antioxidant molecules which are involved in plant defence against biotic and abiotic stresses, and redox state control of the cell.
We have evidenced that (h)GSH plays an important role in the nodulation process of *M. truncatula*/*S. meliloti* (Frendo *et al*.(2005) Mol. Plant Microbe Interact., 18: 254-259). In this framework, our laboratories are characterizing the gene targets of the (h)GSH during the nodulation process using cDNA-Amplified Fragment Length Polymorphism (AFLP). The analysis of the transcriptome of control and (h)GSH-deficient plants enabled the collection of 181 genes showing GSH/hGSH-dependent expression changes.
The cDNA-AFLP analysis showed that transcripts of genes related to Chromatin and DNA metabolism, cytoskeleton and cell wall formation accumulate at higher levels in control in comparison to (h)GSH-depleted plants, confirming our previous observation that meristem formation is impaired under (h)GSH deficiency.
A modulation of the expression of genes belonging to the Defense and Cell Rescue functional class was also observed in (h)GSH depleted plants. Several genes belonging to other metabolic classes but involved in multiple aspects of the plant defense exhibited a similar behavior. These results suggest that (h)GSH deficiency affects the early plant defense response which occurs after *rhizobium* inoculation.
In conclusion, our results suggest that (h)GSH content is crucial for the correct expression of genes involved in key steps of nodule differentiation and in the modulation of the host defense.

P6 - CHARACTERIZATION OF TWO GLUTAREDOXINS IN *TRYPANOSOMA BRUCEI*, AN ORGANISM LACKING GLUTATHIONE REDUCTASE

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Trypanosoma brucei, the causative agent of African sleeping sickness, has a unique thiol metabolism where the GSH/glutathione reductase system is replaced by a trypanothione/trypanothione reductase system.
The parasites encode two putative glutaredoxins (Grx). Grx1, located on chromosome 11, has the classical CPYC active-site motif but the overall protein sequence is more similar to human Grx2 (40 % identity) than to human Grx1 (31%). The protein contains an additional cysteine in the C-terminal part that is conserved in human Grx1 but not in human Grx2. Grx2, located on chromosome 1, has a CQFC active-site, lacks additional cysteines and is only distantly related with the parasite Grx1 and other glutaredoxins.
Both *T. brucei* genes were cloned and overexpressed in *E. coli*. In the classical HEDS (hydroxyethyl-disulfide)-assay, recombinant *T. brucei* Grx1 and Grx2 possess 30 % and 3 %, respectively, of the activity of *E. coli* Grx1. In contrast, *T. brucei* Grx2 – but not Grx1 – reduces protein disulfides.
Both parasite proteins catalyze the reduction of dehydroascorbate to ascorbate by glutathione with 13 % (Grx1) and 11 % (Grx2) activity compared to *E. coli* Grx1. However, the spontaneous reduction of dehydroascorbate by trypanothione is three orders of magnitude faster than by glutathione and the reaction is not further accelerated by glutaredoxins.
Western blot analyses revealed that Grx1 is expressed in procyclic cells which multiply in the tsetse fly as well as in the blood-stream parasites of the mammalian host. Work is in progress to evaluate if the glutaredoxins are essential for *T. brucei*.

P7 - CHARACTERIZATION OF THE *T. BRUCEI* GLUTATHIONE-TYPE TRYPAREDOXIN PEROXIDASE

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Trypanosoma brucei, the causative agent of African sleeping sickness, encodes three cysteine-homologues (Px I to III) of classical selenocysteine-containing glutathione peroxidases (Hillebrand *et al*., 2003). The enzymes obtain their reducing equivalents from the unique trypanothione [bis(glutathionyl)spermidine]/tryparedoxin system. During catalysis these tryparedoxin peroxidases cycle between an oxidized form with an intramolecular disulfide between Cys^{47} and Cys^{95} and the reduced peroxidase with both residues in the thiol state (Schlecker *et al*., 2007).
Here we report on the 3-dimensional structure of oxidized *T. brucei* Px III at 1.4 Å resolution obtained by X-ray crystallography and of both the oxidized and the reduced protein analyzed by NMR spectroscopy. Px III is a monomeric protein and 60% of all residues are located in extended loop structures. The structures of the oxidized and reduced protein species do not markedly differ in contrast to what was recently found for a related plant thioredoxin peroxidase (Koh *et al*., 2007). In Px III, Cys^{47}, Gln^{82}, and Trp^{137} do not form the catalytic triad observed in the selenoenzymes and related proteins. Moreover, both residues are unaffected by the redox state of the protein. Gln^{82} is probably involved in a network of hydrogen bonds that stabilize the loop containg Cys^{95}. The mutational analysis of three conserved lysine residues in the vicinity of the catalytic cysteines revealed that exchange of Lys^{107} against glutamate abrogates the reduction of hydrogen peroxide. In contrast, Lys^{97} and Lys^{99} play a crucial role in the interaction with tryparedoxin.

P8 - WHY THE *ARABIDOPSIS* GLUTATHIONE DEFICIENT MUTANT *PAD2* IS MORE SUSCEPTIBLE TO PATHOGENS?

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The *Arabidopsis pad2* mutant is highly susceptible to many pathogens such as *Pseudomonas syringae*, *Botrytis cinerea* and *Phytophthora brassicae*. Positional cloning revealed that *pad2* is a S298N mutation in γ-glutamylcysteine synthetase, the first enzyme of glutathione (GSH) biosynthesis. As a consequence, *pad2* plants contain only about 25% of wild-type GSH. In response to *P. brassicae, pad2* mutant showed a lower hypersensitive response (HR) compared to wild-type. HR is a plant programmed cell death triggered during resistance involving notably reactive oxygen species (ROS). We compared the H_2O_2 accumulation in response to the elicitor oligogalacturonides (OG) or after *P. brassicae* infection in wild-type and *pad2*. Surprisingly, *pad2* mutant showed a lower H_2O_2 production, totally absent in the *rbohD* mutant, suggesting that GSH could interfere with the regulation of RbohD at the gene or protein level. The corresponding gene expression has been checked and the possibility that upper signalling events modulating the RbohD activity (plasma membrane depolarization, phosphorylation...) could be modified by GSH has been tested. Preliminary results indicated that depolarization in *pad2* is lower than in wild-type cells in response to OG. Moreover, Meyer *et al*. (2007) have recently demonstrated that the oxidation state in the GSH mutant *cad2* (an allele of *pad2*) is significantly higher than in wild type plants using a redox-sensitive GFP. Taken together, these results suggest that modification of the redox state in GSH mutants could interfere with ion fluxes across plasma membrane, themselves determinant for ROS production and plant resistance via the RbohD regulation.

P9 - PHYTOCHELATINS INDUCTION AFTER HEAVY METAL EXPOSURE IN *VICIA FABA* ROOTS

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Higher plants are very sensitive to variations in their environment and have been identified as potentially accurate bioindicators of environmental pollution. In some higher plants, toxic action of cadmium was reported by many authors, and several defence mechanisms have been described. One mechanism of chelation and sequestration of Cd involves the production of phytochelatins (PCs). These small cystein-rich peptides (general structure: $[\gamma\text{-GluCys}]_n\text{-Gly}$; n = 2 to 11) are able to bind heavy metal cations *via* their sulfhydryl groups. They are synthesized from glutathione by a specific γ-glutamylcysteine dipeptidyl transferase, also named phytochelatin synthase, activated by heavy metals, especially Cd.

This study aims at investigating different effects of cadmium exposure in *Vicia faba* (broad bean) grown in hydroponical conditions. Genotoxic and cytotoxic effects are assessed using the well-known *Vicia* root tip micronucleus assay (AFNOR T90-327), and the mitotic index in root tip cells, respectively. In parallel, glutathione and phytochelatins (PCs) concentrations are measured by HPLC. Antioxidative enzymes activities are also observed: APX (ascorbate peroxydase), catalase, and SOD (superoxyde dismutase). The link between induction of PCs, genotoxic effects and oxidative stress observed in *Vicia faba* after cadmium exposure will be discussed for a better understanding of mechanisms of action of Cd in plants.

P10 - Physiological role of glutathione reductase in the respiratory yeast *Kluyveromyces lactis*

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Glutathione is a molecule that works as an antioxidant factor, playing an important role in the neutralization of reactive oxygen species (ROS) in the cell. The *Kluyveromyces lactis* gene *KlGLR1* encodes for the protein glutathione reductase (GLR), which reduces the oxidated form of gluthatione, using NADPH as a cofactor and regenerating $NADP^+$. The *K. lactis* phosphoglucose isomerase mutant (rag2) is forced to metabolize all the glucose through the oxidative reactions of the pentose phosphate pathway (PPP), and produces higher levels of ROS and NADPH than the wild type strain. We have previously proved that the rag2 mutant is more resistant to oxidative stress (OS) and that the activities of GLR and catalase are increased. To study the role of GLR in the defense against OS, we created knock out mutants in three strains of *K. lactis*, namely, rag2, the wild type NRRL-Y1140 and PM5-3c. We have shown that resistance to OS is decreased in the mutants, and this phenotype is reinforced when catalase activity is also inhibited. We have also studied the respiratory rates of the mutants at different glucose concentrations and the relationship with the relative flux of NADPH oxidation by GLR or external mitochondrial dehydrogenases. An approach to determine the subcellular localization of the enzyme, by measuring GLR activity in cytosol and mitochondria, was also performed.

P11 - TOLERANCE TO CR (VI) IN SULFUR-STARVED UNICELLULAR GREEN ALGA *SCENEDESMUS ACUTUS*: INTERACTIONS WITH CYSTEINE AND REDUCED GLUTATHIONE BIOSYNTHETIC PATHWAYS

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Sulfur starvation enhances tolerance to Cr(VI) in two strains of *Scenedesmus acutus*, the wild-type and a laboratory selected Cr-tolerant strain. The increase in tolerance is specifically due to sulfur deprivation and ascribable to phenotypic plasticity. To identify which physiological/biochemical alterations could be responsible for the tolerance increase, the time course of changes in free Cys and GSH pool (end-products of sulfate assimilatory process involved in counteracting oxidative stress) was studied. Both strains were cultured in presence (unstarved) or in absence of sulfate (S-starved) and then maintained in standard medium (S-sufficient and S-replete cells respectively) with or without Cr(VI). Cys, GSH and total sulfur were determined after 1, 24, 48 and 72 h, as well as in unstarved and S-starved cells. S-starved cells underwent a drastic reduction of Cys, GSH and sulfur content, but were able to rapidly increase the levels upon sulfate re-supply. Despite the low amounts of Cys and GSH, S-starved cells of both strains were able to grow at Cr(VI) concentrations much higher than the LOEC of unstarved cells. Cys and sulfur content in S-replete tolerant strain increased to levels which were double those of the wild-type and S-sufficient cells. Cys increase was not observed when S-replete cells were treated with Cr(VI). These results suggest that the higher tolerance to Cr(VI) after starvation is linked to the up-regulation of sulfate uptake/assimilatory process rather than to intracellular Cys and GSH pool and that S-starved Cr-tolerant strain synthesizes a new sulphate uptake/assimilatory system with a different interaction with Cr(VI).

P12 - GLUTATHIONE: AN IMPORTANT MODULATOR OF ANTIBIOTIC SUSCEPTIBILITY IN *E. coli*

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Antibiotics are chemicals interfering with diverse cellular processes thereby acting as either bacteriostatic or bactericidal compounds. Bacterial exposure to antibiotics leads to the activation of antibiotic specific stress response pathway. Glutathione (GSH), which plays a vital role in protection against diverse environmental stresses (i.e. oxidative stress, osmotic stress and chlorine compounds) acts as an important modulator for antibiotic susceptibility as well in *Escherichia coli.* We have shown that the presence of glutathione in the medium protects bacteria against fluroquinolone (Goswami, M. et al. 2006. Antimicrobial Agents & Chemotherapy 50:949-954) and aminoglycoside groups of antibiotics (Goswami, M. et al. 2007. Antimicrobial Agents & Chemotherapy. 51:1119-1122). We have demonstrated that reduced fluoroquinolone susceptibility of *E. coli* cells is due to glutathione mediated scavenging of reactive oxygen species. However the mechanism of glutathione mediated protection against aminoglycoside antibiotics is other than ROS scavenging. Interestingly we have found that glutathione, unlike the above-mentioned protection against two groups of antibiotics, substantiates the effect of β-lactam antibiotics. Our recent results on the mechanism by which glutathione gives protection against aminoglycosides will be presented. However irrespective of the mechanisms operating behind the above mentioned phenotypes our observations reveal that glutathione can act as a differential modulator of antibacterial activity for various groups of antibiotics which might be an important factor in determining the extent of antibacterial effect for a given class of antibiotics.

P13 - INTERACTIONS BETWEEN GLUTATHIONE METABOLISM AND ALCOHOLIC FERMENTATION IN THE METHYLOTROPHIC YEASTS *HANSENULA POLYMORPHA.*

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Glutathione (GSH) is a non-protein thiol, which plays a key role in protection of cells against various stress conditions. It is known, that metabolic stress is induced also during industrial alcoholic fermentation, which could impair ethanol productivity.

To check correlation between the GSH level and alcohol fermentation, we tested *H. polymorpha* strains with multicopy expression of genes involved in glutathione biosynthesis: *GSH2* (homologue of *Saccharomyces cerevisiae GSH1* gene) and *MET4* (gene involved in regulation of sulfur metabolism) as well as in mutants defective in genes involved in GSH synthesis (*Δgsh1* and *Δgsh2*).

Ethanol concetration was measured during glucose fermentation in synthetic minimal medium at conditions of weak aeration.

Strains with additional copies of *H. polymorpha GSH2* and *MET4* genes with elevated cellular GSH concentration, accumulated ethanol in almost two times higher concentration than the appropriate wild type. Mutants *Δgsh1* and *Δgsh2* didn't differ in respect of alcoholic fermentation from the wild-type strain.

We have also checked resistance of mentioned strains to exogenous ethanol.Those strains with higher ethanol productivity were sensitive to 8% ethanol on YPD solid medium, whereas wild type strain grew normally at 10% ethanol.

It seems that multicopy integration genes involved in glutathione metabolism in *Hansenula polymorpha* strains influences on better glucose fermentation and accumulation of ethanol. We can assume that elevated GSH concentration is very important for improvement of alcoholic fermentation in yeasts.

P14 - A SYSTEMATIC ANALYSIS OF THE *IN VIVO* CONTRIBUTION OF YEAST ANTIOXIDANTS IN H_2O_2 SCAVENGING.

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Although wealth of information exists on the enzymology of antioxidants, the *in vivo* function of these enzymes has only been studied superficially. A major difficulty exists in the possible functional redundancy within and between the antioxidant families that are present in most organisms. It is important to establish their exact contribution to the control of the intracellular concentration of hydrogen peroxide.

We have taken a systematic genetic approach in the single-celled eukaryote *S. cerevisae*. We have established multiple strains carrying deletions of antioxidant genes, individually and in combination. These strains were assayed for how efficiently they regulate H_2O_2 homeostasis using: **(i)** classical sensitivity assays on plates and liquid medium; **(ii)** a careful measure of total antioxidant scavenging capacity as deduced from the rate of H_2O_2 consumption after a single bolus of the oxidant (200 μM); (**iii**) the use of a biochemical and chemical monitors of the intracellular H_2O_2 concentration; (**iv**) a

careful measure of the mutator phenotype of each strain using a genetic reporter. Some of the most striking results indicate that in the H_2O_2 consumption of log phase growing cells in fermentative medium mitochondria and the mitochondrial cytochrome c peroxidase (Ccp1) enzyme have a major importance, whereas peroxiredoxins (Prx) do not have any significant effect, as shown by both single and multiple Prx gene deletions. In contrast, in the mutator reporter assay, Prx are the most important, whereas Ccp1 has no contribution. In all the assays used, catalases became important only when deleted with another antioxidant gene, such as Ccp1.

P15 - Grx6 AND Grx7 ARE TWO MONOTHIOL GLUTAREDOXINS ASSOCIATED TO THE PROTEIN SECRETORY APPARATUS IN *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae Grx6 and Grx7 proteins have a glutaredoxin (Grx) module with an N-terminal extension containing a putative transmembrane domain. The Grx module has the active site sequences CSYS (for Grx6) and CPYS (for Grx7), which differ from the active sites of classical dithiol Grxs and lead to include Grx6 and Grx7 into the monothiol Grxs group. A recent study (Mesecke et al., Biochemistry 47:1452-63) demonstrates that both proteins are active in an *in vitro* thiol oxidoreductase assay, contrary to other monothiol Grxs such as yeast Grx5. In this study, we show (by sucrose gradient centrifugation and immunomicroscopy) that Grx6 locates at the endoplasmic reticulum and Golgi membranes, while Grx7 locates at Golgi, and that these locations depend on the transmembrane region. Membrane topology studies using proteinase K digestion indicate that Grx6 and Grx7 are integral membrane proteins that face the lumen of the membranes. *GRX6* expression is upregulated by calcium, sodium and oxidative stresses, under the control of the calcineurin pathway, while *GRX7* expression is induced by sodium and oxidative stresses under the control of the Msn2/4 transcription factor. The respective protein levels parallel gene induction patterns, and in the case of Grx6 temporary modification by hyper-O-glycosylation occurs under such stresses. Grx6 and Grx7 also participate in the tunicamycin-mediated unfolded protein response, and single and double *grx6 grx7* mutants exhibit a modest resistance to the growth inhibitory action of tunicamycin. These observations point to a relationship between Grx6 and Grx7 and the response to endoplasmic reticulum stress.

P16 - ROLE OF TRANSPORTERS IN GLUTATHIONE EXPORT DURING ARSENITE EXPOSURE

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Yeast cells (*Saccharomyces cerevisiae*) strongly increase glutathione biosynthesis during arsenite exposure (Thorsen *et al., Physiol Genomics,* 2007*)*. Moreover, we have evidence that part of the glutathione is exported as part of an extracellular detoxification mechanism. In this work, we analyse the role and contribution of various transporters in arsenite-induced glutathione export. Pittman *et al.* (*J Biol Chem,* 2005) and C. L. Hammon *et al. (J Biol Chem,* 2007) previously showed that proteins involved in glutathione export in bacteria and in human cells belong to the family of ABC-transporters.
Therefore, we are currently investigating whether (a) member(s) from this family is responsible for glutathione export also in yeast. A number of transporters are studied by gene deletion and over-expression and the effects obtained in terms of glutathione export and arsenite tolerance are evaluated. The results of this analysis will be presented.

P17 - Spatial distribution of glutathione in tobacco plants (*Nicotiana tabacum*)

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Plants have evolved various ways of coping with their changing surroundings. Adaptive responses are directly regulated by genetic and biochemical characteristics. An understanding of these biochemical changes involved in plant stress responses will enable the selection of plant material with enhanced resistance to biotic and abiotic stresses. Environmental stress cause an increased formation of reactive oxygen species and the role of glutathione in the antioxidative defence system provides a rationale for its use as a stress marker.
In this preliminary study, we quantified the spatial distribution of total glutathione (TG: GSH + GSSG) in tobacco using HPLC-PAD. Plants were grown in a greenhouse (up to 11-14 leaf stage) at 20°C.
It was shown that TG content ranged from 80 to 450 pmol/mg (FW) related to leaf number (from base to top). In order to understand if this gradient was dependent on the light received by plants or by intrinsic plant factors, light quality and quantity on the TG spatial distribution were evaluated. No significant differences were observed. The location of the light source had no significant influence on the TG gradient.
In contrast, this gradient was affected when plants were submitted to darkness. Moreover, quantifying separately GSH and GSSG, revealed that (1) in the dark, TG corresponded to the reduced forms, (2) when plants were submitted to light, the glutathione gradient was mainly due to the oxidized form.
These results show that plant developmental and physiological stages have to be considered cautiously for the interpretation of TG variation and molecular defence responses when plants are experimentally submitted to stresses.

P19 - CHARACTERIZATION OF DUG1P, A NOVEL PEPTIDASE INVOLVED IN THE ALTERNATIVE PATHWAY OF GLUTATHIONE DEGRADATION IN *S.CEREVISIAE.*

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Dug1p (Yfr044cp) has been recently shown to be involved in the alternative pathway of Glutathione degradation in *S.cerevisiae* as part of the "Dug complex" (Ganguli *et. al.* 2007). However, Dug1p has also been predicted to independently function as a peptidase. Site directed mutagenesis of the putative active site residue to alanine renders the protein nonfunctional, as seen in *in vivo* complementation assays. We have been interested in investigating, if Dug1p is a general di- or tripeptidase, or if it is specific for glutathione-derived peptides. To investigate these possibilities, we have purified recombinant Dug1p from *E.coli* to apparent homogeneity by using C-terminal His tag. The purified protein is shown to exist as a dimer. The kinetics of purified Dug1p is being investigated using Cys-Gly and other dipeptides and tripeptides as substrates. Initial studies using Cys-Gly as a substrate reveal that Dug1p has an optimum pH of 8.0 and optimum temperature between 30^0C-37^0C for activity. Peptidase activity is shown to be inhibited by EDTA and recovered by Zn^{++} and Mn^{++} ions, thereby showing the metallopeptidase character of the protein. The Km and Vmax for different peptides will be presented.

P20 - THIOL PEPTIDE RESPONSE OF THE AQUATIC FUNGUS *HELISCUS LUGDUNENSIS* TO CD CD EXPOSURE.

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Fungal cells have evolved sophisticated mechanisms to buffer toxic concentrations of heavy metals in their environment and to regulate intracellular metal concentrations. In heavy metal exposed fungi thiols are essential agents in cellular redox signalling and control. Some aquatic fungi respond to cadmium (Cd) by increased synthesis of SH - containing compounds. The glutathione level together along with the ratio of reduced to oxidized glutathione (GSH) provide insights into the cellular redox state as well as the assumed metal binding potential of GSH amongst other thiol peptides. Cd stress response was measured in two strains of the aquatic hyphomycete Heliscus lugdunensis isolated from polluted sites. Under 100 µM Cd exposure the content of GSH and its precursors (cysteine, y-glutamyl-cysteine) increased significantly. The response was dependend on fungal strain used and the heavy metal concentration applied. The increase of the GSH pool is accompanied by the simultaneous induction of phytochelatin 2 (PC2) and a novel metallothionein (MT). The metallothionein has high homology to family 8 MTs (http://www.expasy.ch/cgi-bin/lists?metallo.txt). Using integrated methods for separation and structural analysis the structure and metal binding capacity of the novel MT was elucidated. Studies underway will explore the potential role of the thiol peptides GSH, PC2 and MT1_NECLU as intracellular agents for heavy metal detoxification by aquatic fungi.

P21 - STUDY OF THE γ- GLUTAMYL CYCLE IN YEAST

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Glutathione is the major low-molecular-weight thiol compound present in almost all eukaryotic cells at intracellular concentrations ranging from 0.1 to 10 mM. The γ-glutamyl cycle carries out the synthesis and degradation of glutathione through six enzymatic steps. This cycle is also involved in transport of amino acids in mammalian cells. In *Saccharomyces cerevisiae*, two enzymes of the γ-glutamyl cycle namely γ-glutamyl cyclotransferase and 5-oxoprolinase, the last two enzymatic steps in the cycle could not be detected, and thus this cycle has been thought to function as a truncated cycle in yeasts. We are investigating theγ-glutamyl cycle in yeast, and to understand why yeasts might have evolved a truncated cycle. The possibility that the alternative pathway of glutathione degradation in *S. cerevisiae* that has recently been discovered in our lab might be playing a compensatory role leading to a truncated pathway is being investigated. These results and hypothesis on the functioning of the γ-glutamyl cycle in yeast will be presented.

P23 - THE PHYSIOLOGICAL AND PHENOTYPIC EFFECTS OF MANIPULATING THE GLUTATHIONE BIOSYNTHETIC PATHWAY

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Glutathione (GSH) is synthesized via a two-step pathway. The first step is catalyzed by glutamylcysteine synthetase (GSH1) which forms the dipeptide, γ-glutamylcysteine (γ-EC) and, in the second step, glycine is added by glutathione synthetase (GSH2). It has been demonstrated using reporter fusions and immunolocalization that in Arabidopsis GSH1 is localized exclusively to the plastid whereas GSH2 is found in both the plastid and cytosol. Thus, the biosynthesis of GSH occurs in two sub-cellular compartments, the cytosol and the chloroplasts. Yet little is understood regarding the mechanisms that control and interconnect these two pools and the extent to which they independently influence gene expression. We aim to study the mechanisms by which GSH in plants is distributed in sub-cellular pools and the effects of the different pools of glutathione on gene expression and stress responses. We will attempt to undertake a systematic analysis on the effects of changing the plastid and cytosolic GSH pools on gene expression by constructing strains in which GSH biosynthesis is directed exclusively to the cytosol or plastid. This can be achieved by directing GSH1 and/or GSH2 exclusively to the cytosol in *gsh1* and *gsh2* knock-out mutants. These transgenic lines are being characterized for their responses to stress, for their status and to identify effects on gene expression. This will offer new insights into the roles of GSH pools in stress responses.

P25 **- Deletion of cytosolic glutathione reductase reveals functional plasticity of glutathione redox metabolism in Arabidopsis**

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Tight control of cellular redox homeostasis is essential for maintenance of normal metabolism and development. Especially under stress conditions redox homeostasis is often challenged through increased production of reactive oxygen species (ROS). ROS may be exploited as a trigger for signal transduction cascades which ultimately lead to stress adaptation, but at the same time need to be quickly removed to avoid deleterious effects. The tripeptide glutathione is a key component of the cellular antioxidant defence system and it has been shown recently that nanomolar changes in the concentration of oxidized glutathione disulfide (GSSG) are sufficient to switch the reduction state of intramolecular protein disulfides. In non-stressed cells, glutathione is maintained in almost completely reduced form by glutathione reductases (GRs) at the expense of NADPH. In Arabidopsis, knockout of organellar GR is lethal. Deletion of cytosolic GR, in contrast, results in no obvious phenotype. In vivo measurements with redox-sensitive GFP (roGFP) showed that the glutathione redox potential in these mutants is significantly shifted to more oxidizing conditions. Moreover, time-resolved measurements clearly showed that the mutants lack the ability to appropriately detoxify exogenously applied H_2O_2. The fact that lack of cytosolic GR does not lead to complete over-oxidation of the glutathione pool indicates that alternative mechanisms for GSSG reduction are in place. This hypothesis is strongly supported by biochemical and genetic evidence, which also shows that these alternative pathways are less efficient than GR.

P26 **- Stress-related thiol metabolism in *S. cerevisiae***

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Glutahione (GSH, L-γ-glutamyl-L-cysteinyl glycine), the most abundant thiol compound in yeast cells, has attracted a lot of interest by several fields of food and biotechnology, mainly because of its antioxidative properties. There is an abundance of information available on the stress related transcriptional regulation of GSH metabolism in yeast, however, most of this information is based on experiments carried out in simple flask experiments, where the physiological states of the cells are remarkably different from those usually applied in industrial processes. Hence, knowledge about the exact effect of these stresses, whether applied specifically or in combination, on the intermediate level of cellular thiols during steady state growth, is rather scarce. In this work we have analysed the impact of an array of environmental stresses on the content of GSH and related thiols, as well as on their redox status in yeast using continuous cultivations with smooth and rapid stress inductions.

P28 **- Unravelling endogenous redox regulation in the absence of peroxiredoxin Tsa1 and recombinational repair in *Saccharomyces cerevisiae***

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Maintaining genome stability is crucial for cell growth and cell survival. We previously demonstrated that the absence of Tsa1, a key *S. cerevisiae* peroxiredoxin that functions to scavenge H_2O_2, caused the accumulation of a broad spectrum of mutations including gross chromosomal rearrangements (GCRs). Deletion of *TSA1* (*tsa1*) also caused synthetic lethality in combination with mutations in *RAD6* and several key genes involved in DNA double-strand break repair. Anaerobic growth conditions greatly reduced GCR rates of wild-type and *tsa1* mutants, and restored the cellular viability of *tsa1 rad51*, *tsa1 mre11* and *tsa1 rad6* double mutants. These results provide *in vivo* evidence that oxygen metabolism and reactive oxygen species are important sources of DNA damages that lead to spontaneous chromosome rearrangements and lethal deleterious effects in *S. cerevisiae*.
To better understand the intracellular redox regulation and its possible genetic interaction with DNA repair systems, we have performed a genetic study to identify spontaneous genetic alteration(s) that allow cell growth in the absence of Tsa1 and Rad51 under aerobic condition. Charactererization of such suppressor(s) is underway. A model for the mechanism by which the suppressor restores the cellular viability of *tsa1 rad51* double mutants will be presented.

P29 **- Synthesis of glutathione is regulated post-transcriptionally in *Saccharomyces cerevisiae* in response to saline stress.**

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Many organisms synthesize glutathione (GSH) during adaptation to oxidative stress, heavy metals and xenobiotics. The yeast *S. cerevisiae* also accumulates organic osmolytes (e.g., glycerol) in response to hyperosmotic stress. We show that intracellular GSH levels increase 2-fold in response to 1M NaCl and 5-fold in control cells treated with 100 µM $CdSO_4$. Ionic stress specifically stimulates GSH synthesis because sorbitol, ethanol and nystatin do not increase GSH levels. The *GSH1* gene encodes the first enzyme of GSH biosynthesis. We constructed a *GSH1*::*lacZ* reporter plasmid to quantify

GSH1 induction and study its regulation. We find that increasing NaCl concentrations do not increase *lacZ* expression versus media without NaCl, but do cause a 2-fold induction in control cells stressed with 100 µM $CdS0_4$; induction with $CdSO_4$ is ablated in a *yap1*Δ strain. This suggests that GSH synthesis is regulated post-transcriptionally during saline stress. In yeast osmotic responses are controlled by HOG pathway. Therefore we measured the intracellular GSH levels in *hog1*Δ, *hot1*Δ, *skn7*Δ, *gpd1*Δ and *yap1*Δ strains. When stressed with 0.8M NaCl, cellular GSH levels increased 2-fold in the *hog1*Δ, *hot1*Δ and *yap1*Δ strains; thus, osmo-stimulated GSH synthesis is independent of these regulators. GSH levels in *gpd1*Δ and *skn7*Δ strains do not increase in response to saline stress and basal GSH levels are slightly elevated in the *gpd1*Δ strain. Consequently, *GPD1* and *SKN7* could play a role in NaCl-dependent GSH synthesis. We conclude that GSH levels are regulated post-transcriptionally during hyperosmotic stress, but regulated transcriptionally during heavy metal exposure.

P30 **- Disulfide stress requires genome wide involvement in *Mycobacterium smegmatis***

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Diamide selectively oxidizes low molecular weight thiols to their disulfide forms, decreasing the cellular concentrations of reduced thiols; thus, the utilization of diamide provides a useful model of thiol deficiency as a source of oxidative stress. Previously microarray and proteomic studies have examined genes and proteins that are induced/upregulated by diamide treatment in *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG respectively. In order to identify constitutive genes that may not be induced but still play an important role during disulfide stress, a *Mycobacterium smegmatis* transposon mutant library of over 15, 000 mutants was constructed and screened for mutants sensitive to diamide. One hundred and five diamide sensitive mutants were identified and the regions flanking the transposon was cloned and sequenced to identify the disrupted genes. A set of largely constitutive cell processes involved in degradation of protein, lipid, and nucleic acids and biosynthesis of these same macromolecules were affected by disulfide stress. In addition, mutants disrupted in several regulatory genes, genes coding for membrane transporters, genes involved in energy generation and detoxification were sensitive to diamide stress. Surprisingly, none of the diamide sensitive mutants were disrupted in genes involved in mycothiol biosynthesis, although mutants disrupted in genes involved in biosynthesis of molybdpterin and a gene coding for a thioredoxin were identified in this screen. Furthermore, sensitivity assays with hydrogen peroxide, cumyl hydroperoxide, and plumbagin confirmed that the majority of diamide sensitive mutants were also sensitive to other oxidants.

P31 **- APPLICATION OF AN EXPERIMENTAL DESIGN TO IMPROVE EXTRACELLULAR GSH PRODUCTION FROM *S. CEREVISIAE***

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Glutathione (GSH, L-γ-glutamyl-L-cysteinyl-glycine) is the most abundant non-protein thiol compound widely present in living organisms, from prokaryotes to eukaryotes (Anderson 1998). It is synthesised intracellularly by the consecutive actions of γ-glutamylcysteine synthetase, feedback inhibited by GSH, and GSH synthetase. This tripeptide's very low redox potential gives it the properties of a cellular redox buffer (Udeh and Achremowicz 1997). In living tissues, GSH plays a pivotal role in bioreduction, protection against oxidative stress, xenobiotic and endogenous toxic metabolite detoxification, enzyme activity and sulphur and nitrogen metabolism (Penninckx 2002).

These characteristics make this active tripeptide an important aid and/or support for the treatment of numerous diseases, such as HIV infections, liver cirrhosis, pancreatic inflammations and aging (Wu et al., 2004). In addition, GSH is of interest in the food additive industry and sports nutrition (Lomaestro and Malone 1995).
The present research was aimed at obtaining GSH in extracellular form, released from cells, at high levels. A full factorial design, with 3 variables tested at three levels and 1 at two levels (for a total of $3^3 \cdot 2 = 54$ trials), was carried out. Three *S. cerevisiae* strains (MIM 175, L 288 and a baker's yeast in compressed form) were tested comparatively. Cells were comparatively treated employing physical and chemical procedures. The best result (2.9 g/l, 90% of produced GSH in extracellular form) was achieved at 24 h reaction, employing lyophilised cells from compressed baker's yeast. The possibility of obtaining GSH directly in extracellular form, skipping the downstream cell extraction step, represents an interesting opportunity of reducing GSH production cost and furthering the range of application and utilization of this molecule.

P34 - The study of glutathione production in batch and fed-batch cultures of wild type and recombinant strains of methylotrophic yeast *Hansenula polymorpha*

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GSH is the most abundant non-protein thiol compound of almost all living organisms that is used as a strong antioxidant agent in therapeutics, food additive and cosmetic industries. Thermotolerant methylotrophic yeast *Hansenula polymorpha* with naturally high level of GSH and high tolerance to various environmental stresses, well established genetic techniques and tremendous experience on industrial cultivation are considered to be promising microorganism to produce GSH by fermentation of natural or genetically engineered strains.

In the present study we estimated GSH-producing capacity of *H. polymorpha* DL1 dependence on parameters of cultivation (dissolved oxygen tension, DO, pH, stirrer rate), carbon substrate (glucose, methanol) and type of overexpressed genes of GSH biosynthesis and sulfur metabolism in fermenter batch and fed-batch cultures.

It was demonstrated that the highest GSH concentration (w/w) and GSH yield (mg/L medium) for wild type strain were obtained at low oxygen agitation rate, 200 rpm, without pH control in batch process and at DO -30% during fed-batch fermentation on glucose minimal medium using dual control algorithms of carbon source feeding.

Acquisition of additional copies of *H. polymorpha MET4* gene of global sulfur regulation, *H. polymorpha GSH2* gene, homologue of *Saccharomyces cerevisiae GSH1* gene, under native and strong *H. polymorpha MOX* promoters, improved GSH productivity parameters 1.3-2.5 times compared to that of wild type strain. The intracellular GSH yield of the best *H. polymorpha* engineered strain that expressed *HpGSH2* cassette under *MOX* promoter proved to be 2257 mg GSH/L culture medium and GSH concentration of 31 mg/g dry cell weight.

P35 - Thiol protein expression during germination and biological quality of wheat seed

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The quality of the wheat seed corresponds to two realities which are not always compatible. On one side, there is the technological quality which is a function of storage proteins, and we showed for example that there is a negative correlation between the content of glutathione fixed on these polymers proteins and the technological quality since glutathione seems play a crucial role in controlling the degree of polymerization of these polymers and influence their baking performance.
On the other side, there is the biological quality which is usually evaluated by the germination rate. During this last phase, a certain number of thiol proteins genes are expressed intervening in the remobilization of the storage molecules and in the management of the oxygen reactive species resulting from the reactivation of the metabolism and from the soil quality.
In order to better know the expression profile of these genes we subjected wheat seeds (cv. Soissons) to biotic and abiotic stresses during the first phases of germination and follow-up by real-time quantitative PCR the expression redoxins as well as the glutathione reductase and genes of the anti oxidative system.
The results enabled us to determine a window during which the expression of these genes presents the greatest modulability. A diagram of interaction between these genes is proposed and discussed according to each stress.

P36 - THE PERIPLASMIC PILB PROTEIN FROM *NEISSERIA MENINGITIDIS*: REGENERATION MECHANISM OF THE METHIONINE SULFOXIDE REDUCTASE ACTIVITIES

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Neisseria meningitidis is a Gram-negative pathogen that remains a leading cause of bacterial meningitis and septicaemia world-wide. Its pathogenicity is linked to its ability to resist reactive nitrogen and oxygen species, which are produced by phagocytes. *N. meningitidis* have different antioxidants systems including the PilB protein which is described to be specific towards human obligatory pathogenic bacteria of *Neisseria* genus and to be implicated, *in vivo*, in survival in the presence of hydrogen peroxide.

PilB is a periplasmic three-domain protein: the central and C-terminal part displays a methionine sulfoxide reductase activity of class A (MsrA) and B (MsrB), respectively, while the N-terminal domain displays a disulfide oxido-reductase activity. The N-terminal domain recycles efficiently only the oxidized forms of the MsrB domain, as an isolated form, and presents a Trx-fold, with more exactly a DsbE topology. The DsbE family is a typical membrane anchored periplasmic thiol/disulfide oxidoreductase family involved in electron transfer during the maturation of *c*-type cytochromes. So far, studies have been performed only on isolated domains of the PilB protein.

Therefore, the question arises whether the N-terminal domain is operative in reducing the downstream MsrA and MsrB domains in the PilB context, and the nature of the periplasmic disulfide oxidoreductase responsible for the N-terminal domain recycling. A structure-function relationship study has been carried out using site-directed mutagenesis, stopped-flow and fluorescence approaches. Data will be presented which illustrate the exact mechanism of the Msr activity of PilB in the periplasm.

P37 - THE CELLULAR PROCESSES UNDERLYING GLUTATHIONE ESSENTIAL REQUIREMENT FOR GROWTH AND TOXICITY AT HIGH CONCENTRATIONS

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Glutathione (GSH) is considered as the major cellular redox buffer because of its abundance in the mM range and its relatively low redox potential of -260 mV. GSH is essential for eukaryotic life as shown in both *S. cerevisiae* and mice, organisms in which inactivation of the γ-glutamyl cysteine synthase gene leads to an essential GSH requirement for growth. We show here that GSH becomes toxic and induces cell death to yeast cells at a concentration > 10 mM. The molecular mechanisms that underlie the GSH essential requirement and toxicity are elusive. We have carried out a genome-wide transcription analysis to analyze the response of yeast to a lack of GSH and to the presence of the tripeptide at toxic concentrations. Our study reveals that lack of GSH leads to a potent stimulation of the iron uptake and utilization Aft1 regulon and repression of genes involved in respiration, Fe-S proteins and lipid metabolism. In contrast, toxic concentrations of GSH lead to a prolonged and potent stimulation of the ER unfolded protein response (UPR), and more intriguingly also activates the Aft1 regulon. We provide evidence that the UPR triggered by GSH at toxic levels is the result of an ER reductive stress that alters oxidative protein folding, and causes a total block of protein secretion**.** Further, deregulation of iron metabolism under both lack of GSH and its presence at toxic levels appears to be due to defective Fe-S cluster biogenesis and alteration of iron sensing**.** Our results underline the central role of GSH as a redox buffer in ER and an essential function in cellular iron homeostasis, two essential cellular processes.

P38 - Redox status in *Saccharomyces cerevisiae* and its role in oxidative stress tolerance of cells growing under biofilm form

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Saccharomyces cerevisiae is able to initiate biofilm formation. The yeast adhered efficiently on plastic surfaces, especially when grown in low-glucose media, and formed mats or colonies of biofilm nature covering a large surface on semi solid gel (Reynolds & Fink, 2001). Recent report on the aggregation of *Kluyveromyces lactis* (Coulon *et al.,* 2006) and biofilm formation in *Candida albicans* have highlighted the role of glutathione (Murillo *et al.,* 2005) Tolerance of *Saccharomyces cerevisiae* biofilms cells to oxidative stress (H_2O_2 and Cd^{2+}) was tested comparatively to planktonic cells, using yeast mutants deleted for genes related to glutathione metabolism and oxidative stress. It was shown that biofilm forming cells occupied predominantly G1 cell cycle stage. This could explain their higher tolerance to oxidative stress and the young replicative age of cells in an old culture. Moreover, the GSH status of *S. cerevisiae* was affected by the growth phase, and has apparently an important role in oxidative stress tolerance for cells growing under biofilm form (Gales *et al*., 2008).

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P39 **- INFLUENCE OF LUMINESCENT THIOLS CAPPED CADMIUM NANOPARTICLES ON THE VIABILITY OF TWO YEAST STRAINS *S. CEREVISIAE* and *C. ALBICANS***

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The purpose of the present studies is to characterize interactions between fluorescent semi-conductor nanoparticles (also called quantum dots, QDs) and yeast cells. Two different viability assays (liquid medium test and MTT assay) were performed to evaluate toxic concentrations of different types of QDs.

QDs are fluorescent nanocrystals that show great promise as an alternative to organic dyes for biological imaging, diagnosis and drug delivery. The main advantages of QDs are narrow, symetrical, and tunable emission spectra [1], strong resistance to photobleaching, and controlable surface characteristics. QDs size, charge, concentration, outer shell bioactivity and oxidative, photolytic or mechanical stress are all factors that, collectively and individually, can determine their cellular toxicity. Various cadmium telluride CdTe nanoparticles capped with thioglycolic acid (TGA), reduced glutathione (GSH) or functionalized with GSH were synthesized using a hydrothermal method (these nanoparticles were respectively noted CdTe@TGA, CdTe@GSH and CdTe@TGA-GSH). Their properties (diameter, molar absorbance, fluorescence, stability) were evaluated using spectrophotometric and fluorimetric methods [2].

The results of the cytotoxcity tests show that CdTe@GSH QDs exhibit a higher cytotoxicity than CdTe@TGA and CdTe@TGA-GSH. Interestingly, the level of cytotoxicity was dependent on the test and the yeast strain used.

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P41 **– Evidence for the formation of a covalent thiosulfinate intermediate with peroxiredoxin in the catalytic mechanism of sulfiredoxin.**

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Reactive oxygen species, including hydrogen peroxide (H_2O_2) originating both from exogen and endogen sources are compounds deeply involved in cellular physiology. They can result in either toxic or beneficial effects through chemical modification of cellular components. Indeed, H_2O_2 can be produced endogenously in response to cell stimuli by e.g. growth factors. Typical 2-cysteine peroxiredoxins (2-Cys Prx) represent a type of peroxidase distinct from catalase and glutathione peroxidase, which are involved both in cell defence against H_2O_2 and in the transduction of H_2O_2-mediated cell signalling. Eukaryotic 2-Cys Prx are susceptible to the overoxidation of the catalytic Cys to the sulfinic acid state, resulting in the transitory inactivation of the peroxidase activity. Indeed, eukaryotic typical 2-Cys Prx are the only known proteins in which the overoxidized sulfinic catalytic cysteine can be reduced, which restores peroxidase activity and terminates the signal mediated by H_2O_2. The enzyme responsible for this reduction is sulfiredoxin (Srx), which thus would regulate both the cell defence and cell signalling functions of 2-Cys Prx.

Srx was originally proposed to operate by covalent catalysis, with formation of a PrxSO-SSrx intermediate linked by a thiosulfinate bond between the catalytic Cys of both partners (1), a hypothesis rejected by a study on the human enzyme (2). The first proposition states that Srx acts both as a phosphotransferase that leads to the activation of the sulfinic acid into a phosphoryl sulfinic intermediate with ADP release, and as a reductase, which would form a thiosulfinate intermediate via the attack of the catalytic cysteine of Srx on the activated sulfinic acid of Prx with concomitant phosphate release. This intermediate would be then reduced by thiols like thioredoxin or glutathione. In the second mechanism, Srx would only act as a phosphotransferase, the thiol reducer attacking directly the sulfinic phosphoryl intermediate. To settle the argument, we investigated the catalytic mechanism of *S. cerevisiae* Srx, by the characterization of the nature and kinetics of formation of the protein species formed between Srx and its overoxidized 2-Cys-Prx substrate in the presence of ATP, using mutants of the non-essential Cys residues of both Srx and of the overoxidized Prx. Our results favour the first hypothesis involving the attack of the catalytic cysteine of Srx on the activated sulfinic acid, implying the formation of a thiosulfinate Prx-Srx intermediate.

P42 - The redox pathway of *c*-type cytochrome maturation (CCM) in plant mitochondria.

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Cytochromes *c* are electron transport proteins characterized by the covalent attachment of haem group to the apoprotein. For this maturation, plant mitochondria follow a pathway inherited from its proteobacteria ancestor, which is completely different from that used in animal mitochondria (1). The CCM machinery consists of an haem delivery (2-3) and a thiol redox pathways (4) which deliver reduced heme and apocytochrome to a putative haem lyase (5). In bacteria, CcmH and the thioredoxin CcmG are components of a periplasmic thio-reduction pathway proposed to maintain the apocytochrome *c* cysteines in a reduced state. In plant CCMH is a protein of the inner mitochondrial membrane with its conserved RCXXC motif facing the intermembrane space. *In vitro* experiments show that CCMH cysteines could interact with different biological disulfide reductants and the haem binding motif of an apocytochrome *c* model peptide suggesting that the RCXXC motif of CCMH can be functionally active in redox processes. Yeast two-hybrid assays show interactions between *A. thaliana* apocytochrome *c* and the intermembrane space domain of AtCCMH. The function of

CCMH RCXXC motif was investigated. Interactions between other CCM proteins and/or with apocytochrome will be presented. A split-ubiquitin yeast two-hybrid library is screened to identify CCMH partners.

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P43 **- Thioredoxin f regulates chloroplastic glucose-6-phosphate dehydrogenase from *Arabidopsis thaliana* and thereby can coordinate reductive (Calvin-Benson cycle) and oxidative pentose phosphate pathways**

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Plants, like all other living organisms, use the reducing power of NADPH in many metabolic processes. In plastids, NADPH is supplied by photosynthesis and the oxidative pentose phosphate pathway which is the major source for reducing power in non-photosynthetic tissues or under non-photosynthetic conditions. The enzyme glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first and committed reaction of this pathway. Previous work has shown that G6PDH plastidial isoforms were redox-regulated via disulfide reduction by thioredoxins. In *Arabidopsis thaliana*, the existence of 9 TRX isoforms (subdivided into 4 types: f, m, x and y) and of 4 G6PDHs in plastids raises the question of specific interactions between different isoforms. In the present work, we have addressed this question by testing the regulatory capacities of 6 TRX isoforms, representative of the 4 plastidial types, towards G6PDH1, both in the inhibitory (reductive) or activatory (oxidative) directions. The results show some specificity of the various TRX isoforms. As previously proposed for potato G6PDH, TRX m were efficient, but, most interestingly, Trx f was a very efficient regulator, and Trx y an efficient activator of G6PDH1 activity. In an *in vitro* reconstituted ferredoxin/thioredoxin system TRX f regulates G6PDH1 both by inhibition (in the light) or by activation (in the dark or in the presence of oxidants). Biochemical evidence and 3-D structure modeling support the hypothesis of a strict TRX-dependence of plastidial G6PDH regulation. A model for the antagonistic co-regulations of the reductive (Calvin cycle) and the oxidative pentose phosphate cycles by TRX f is proposed.

P44 **- A safe procedure for measurement of S- nitrosoglutathione, the central metabolite in S-nitrosothiols formation and bioactivity.**

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S-Nitrosoglutathione (GSNO) plays an important role in the transport and metabolism of nitric oxide (NO), and seems to be involved in many patho-physiological processes. Research and evaluation of GSNO in human diseases has long been hindered by the lack of analytical procedures provided with

adequate sensitivity, specificity and reproducibility. All three requirements are now satisfied at the highest level by our patented protocol.
The procedure is based on the innovative, unprecedented concept of using the commercial enzyme gamma-glutamyltransferase (GGT) coupled to the fast decomposition of its product CysGlyNO (CGNO) by copper ion (ancillary reaction), which give oxidized CysGly and NO. NO then is reacted with 4,5-diaminofluorescein (DAF-2) giving the triazole derivate, detectable by spectrofluorimetry (ex = 480 nm, em = 515 nm) or in black microplates (ex = 485±15 nm, em = 535±25 nm). The limit of quantitation (LOQc) of GSNO in PBS is 20 nM, the precision (CV) 5.5% at 300 nM concentration level, and the dynamic linear range 5-300 nmol/L, depending on the DAF-2 concentration.
The method is environmental-safe (no mercury), and achieves detection limits two orders of magnitude lower than direct UV detection. These features make it suitable for investigating GSNO pathophysiology *in vitro* and *in vivo,* in experimental conditions encompassing animal, microbiology as well as plant studies.

P45 **- Some tea flavonoids are potential antibiotics targeting thioredoxin systems**

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Some flavonoids have exhibited antibacterial activity, however the mechanism is not known. Thioredoxin (Trx) and glutathione systems are required to supply electrons to ribonucleotide reductase (RNR) which is essential for DNA synthesis. The deficiency of glutathione system in some bacteria makes thioredoxin system to be a potential target for antibiotics. Here we point out that the antibacterial spectra of tea flavonoid EGCG are mainly the bacteria deficient in glutathione (GSH) system and test the target strategy in *Escherichia coli* strains. *E. coli* strain deficient in GSH or redox transcription factor OxyR were more sensitive to the tea flavonoids EGCG, GCG and myricetin, comparing to wild type strain. Furthermore, the flavonoids have a time dependent inhibitory effect on E. coli and Helicobacter pylori Trx system via an attack on their active site. These results shed light on the flavonoids antibacterial mechanism and provide a novel target strategy for antibiotics development.

P46**- MOLECULAR DISSECTION OF THE MITOCHONDRIAL SULFHYDRYL OXIDASE ERV1**

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Proteins of the mitochondrial intermembrane space employ an unique import pathway relying on the activity of two proteins, Mia40 and Erv1 which constitute the mitochondrial “disulfide relay” system. Mia40 acts as an import receptor that binds newly imported substrate proteins thereby introducing disulfide bonds and trapping them in the intermembrane space. Erv1 is a sulfhydryl oxidase that maintains Mia40 in an active, oxidized state. The Mia40-Erv1 import machinery is connected to the respiratory chain as the electrons that are taken up from Mia40 are passed on from Erv1 to cytochrome *c*.
To catalyse dithiol–disulfide transfer reactions Erv1 has three cysteine pairs which are essential for its normal function. In its C-terminal domain, Erv1 coordinates an FAD cofactor which allows the transfer of electrons from reduced thiols to the non-thiol electron acceptor FAD. The mechanism by which Erv1 transfers the electrons from its substrates to the FAD cofactor is poorly understood. We employed a mutagenesis approach to identify the genetic and molecular interactions between the different cysteine residues in Erv1. Based on our results we propose a shuttle model according to

which the cysteine pair in the flexible N-terminus interacts with reduced thiols in the substrates and shuffle the electrons to the active site cysteine pair near the bound FAD. On our poster, we will present evidence for this hypothesis.

P47- TRX2 GENE OVEREXPRESSION AFFECTS THE GLUTATHIONE METABOLISM AND IMPROVES THE CELLULAR OXIDATIVE STRESS RESPONSE IN WINE YEAST PROPAGATION PROCESS

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As other industrial uses of yeasts, the process of biomass propagation of wine strains involves a series of stressing conditions that affect the biomass yield and fermentative capacity of the final product [1,2]. By using bench-top experiments to simulate the industrial process of yeast production [3], we have demonstrated the existence of a strong oxidative stress in critical points of metabolic transition from the initial fermentative growth to respiratory metabolism. This metabolic stress causes a molecular response involving the induction of a set of gene markers for oxidative stress, and also changes in several biochemical redox parameters. Northern analysis shows the induction of *TRX2, GSH1, TRR1 and GRX5,*. This transcriptional response is accompanied by changes in the level of glutathione and the degree of lipid peroxidation, both measured by different espectrophotometric assays. In order to study the physiological relevance of this molecular response, overexpression of the *TRX2* gene has been carried out in a model wine yeast strain and the performance of the modified strain has been tested in biomass propagation experiments. An improvement in the biomass yield during the production process is observed for the wine strain overexpressing *TRX2*, and also a higher fermentative capacity of the dry biomass. To better exploit the benefits of an improved oxidative stress response on the technological performance of industrial yeasts, we are characterizing the response in both the overexpressing and control strains. The results obtained during gene expression analysis and biochemical data, such as glutathione content, levels of lipid peroxidation, protein carbonilation and antioxidant enzymatic activities suggest that TRX2 gene overexpression can modify glutathione metabolism and improve the oxidative stress response during wine yeast propagation process.

P48 - ANTIOXIDANT DEFENCE AND THE ROLE OF THIOREDOXINS DURING PEA SEED GERMINATION

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To analyze the impact of heavy metals on the antioxidant potential in germinating pea seeds (*Pisum sativum* L.cv. douce province), the thioredoxin protein levels were quantified by Western-Blot analysis. Using antibodies generated against poplar proteins and pea samples containing identical protein amounts, thioredoxin (Trx) levels were analyzed. Trx h3 and Trxh4 were detected, but Trx h1 and Trx h2 were not using antibodies generated against poplar proteins. The Trx h3 protein levels in cotyledons and embryonic axis rapidly decreased, respectively, after 18 and 48 h after H_2O-imbibition, but they increased strongly following exposure to Cd. In cotyledons, Trx h4 had the same behaviour in

presence and absence of cadmium, while Cd- fluctuations were more pronounced in the protein expression of Trx h4 in embryonic axis. The influence of cadmium stress on enzyme activity was studied. The thioredoxin activities rapidly increased after H_2O-imbibition. These activities were statistically decreased after Cd-imbibition. The disturbances in the resumption of redox metabolism may be a contributory cause of deleterious effect of cadmium on pea seed germination. Our data suggest that Trx might play an important role during pea seed germination in the presence of high Cd concentrations.

P49 **- Structural and functional characterization of a plastidial poplar glutaredoxin with an atypical WCSYS active site**

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Glutaredoxins (Grxs) are small ubiquitous oxidoreductases that belong to the thioredoxin (Trx) superfamily and generally contain a CxxC/S active-site motif. By using a NADPH-dependent glutathione reductase (GR) and reduced glutathione (GSH) as reductants, Grxs are able to reduce disulfide bridges or glutathionylated proteins (Fernandes and Holmgren, 2004). In higher plants, different Grx isoforms can be classified into three distinct subgroups (Rouhier et al., 2004, 2006). The first class, which contains Grxs with C[P/G/S][Y/F][C/S] motifs, is homologous to the classical dithiol Grxs such as *Escherichia coli* Grx1 and Grx3, yeast Grx1 and Grx2, and mammalian Grx1 and Grx2. The second class has a strictly conserved CGFS active-site sequence and includes Grxs homologous to yeast Grx3, Grx4, and Grx5 or *E. coli* Grx4. The third class is specific to higher plants and includes proteins with CC[M/L][C/S] active sites. We report here the characterization of poplar GrxS12 which belongs to the first class of Grxs and possesses a monothiol WCSYS active site. Using GFP fusion, we have shown that the protein is localized in chloroplasts. The purified recombinant protein expressed in *E. coli* is able to reduce *in vitro* in the presence of GSH conventional substrates such as HED and DHA and some putative physiological plastidial target proteins such as methionine sulfoxide reductases (MsrA4 and MsrB1) and peroxiredoxin (PrxIIE) (Vieira dos Santos et al., 2007, Gama et al., 2008). Mass spectrometry analysis and the resolution of the cristallographic 3D structure of the recombinant protein "as purified" demonstrates that it is glutathionylated on the active site cysteine, when produced in *E. coli*. In addition, changing the active site from WCSYS to YCGYC, to mimic the Grx C1 active site, allowed the incorporation of an iron sulphur cluster into Grx S12, similar to the one naturally present in Grx C1. This mutational study shows that the active site sequence is essential in the incorporation of the cluster but only after replacement of the Trp residue by a Tyr.

P50 **- Chloroplast monothiol glutaredoxins act as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters**

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Glutaredoxins (Grxs) are small oxidoreductases able to reduce disulfide bonds or protein-glutathione mixed disulfides and are known to be involved in oxidative stress response. More than 30 grx genes are expressed in higher plants, but little is known about their functional diversity. Recently, we have shown the presence of a [2Fe-2S] cluster in two chloroplastic Grxs, GrxS14 and GrxS16. These Grxs possess the same monothiol CGFS active site as yeast Grx5, which is known to be involved in mitochondrial Fe-S cluster biogenesis, and both were able to complement a yeast *grx5* mutant defective in Fe-S cluster assembly. *In vitro* studies monitored by CD spectroscopy indicate that GrxS14 can quickly transfer its [2Fe-2S] to apo chloroplast ferredoxin from *Synechocystis*. These data demonstrate that chloroplast CGFS Grxs can function as scaffold proteins for the assembly of [2Fe-2S] clusters and they can transfer it to acceptor proteins. Also, they might function in the storage or delivery of Fe-S clusters.

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