Poster Category 6: ROS, Autophagy and Apoptosis

PR6.1

The role of the NADPH oxidase complex in the biotrophic interaction of *Claviceps purpurea* and *Secale cereale*

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Claviceps purpurea is an obligate biotrophic pathogen on diverse monocots. We are interested in the impact of the Nox complex on the interaction of the fungus and its host *Secale cereale*.

C. purpurea encodes two homologues of the mammalian gp91^{phox}, *cpnox1* and *cpnox2*. Cpnox1 is a virulence factor in *C. purpurea*: the knockout mutant shows drastically reduced infection rates compared to the wild type. Formation of honeydew, the first macroscopic sign of infection, is strongly retarded and mature sclerotia, typical fungal resting structures, have never been observed[1]. In contrast, the knockout mutant Δcpnox2 is not affected in early colonization stages as it shows significantly enhanced and prolonged production of honeydew compared to the wild type, while sclerotia are even less developed than in the Cpnox1 deletion strain. These data indicate that both NADPH oxidase catalytic subunits have impact on the biotrophic interaction of *C. purpurea* and rye. Cpnox1 plays a major role in early colonization of plant tissue while Cpnox2 is involved in the metabolic switch leading to development of sclerotia.

Recently, we were able to obtain a deletion mutant of the regulatory subunit CpnoxR. In pathogenicity assays on rye it shows strong production of honeydew, but sclerotia are very small and not fully mature, comparable to the Cpnox1 deletion mutant.

We are also interested in the composition and recruitment of the Nox complexes. Yeast two-hybrid experiments already showed that CpnoxR interacts with the small GTPase Rac, suggesting that Rac is involved in regulation of the complexes.

PR6.2

New aspects of the regulatory functions of the MAP kinase BcSak1 of *Botrytis cinerea* during stress and pathogenesis

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The mitogen-activated protein kinase (MAPK) BcSak1 of *Botrytis cinerea* is activated upon exposure to H_2O_2 and hence might be involved in coping with oxidative stress during infection. However, beside osmotic and oxidative stress sensitivity Δbcsak1 mutants have a pleiotropic phenotype as they do not produce conidia and are unable to penetrate unwounded host tissue.

In this study the role of BcSak1 was investigated in the stress response and during infection of French beans by *Botrytis cinerea*. Using a macroarray approach it was shown that BcSak1 is only marginally involved in the specific oxidative stress response. In fact, the induction of several genes after oxidative stress treatment is BcSak1-dependent, but most of these genes are also induced under conditions of osmotic stress. The majority of genes regulated by BcSak1 are not involved in the stress response at all. Using a translational fusion of BcSak1 to GFP, it was shown clearly that the localization of this MAPK depends on the type of stress being applied: it associates rapidly to the nucleus only under osmotic stress. Interestingly, the MAPK is also involved in the regulation of secondary metabolism, as the major phytotoxins secreted by this fungus are reduced in the Δbcsak1 deletion mutant. Experiments done *in planta* underlined the essential role of BcSak1 in the early stages of infection when it translocates to the nucleus and then changes to cytosolic distribution during hyphal growth within the tissue.

PR6.3

Identical ROS production of sterile nox and pro mutants in *Sordaria macrospora*.

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The ascomycete *Sordaria macrospora* is an excellent model organism to study the complex cell differentiation process in sexual development [1]. In this process, simple structured, vegetative hyphae change their direction of growth and begin to form three dimensional, sexual structures. The regulation of this differentiation is dependent on multiple signaling factors like pheromones, nutrient sufficiency and reactive oxygen species (ROS). To date only few data are available, that explain the interplay and regulation of different signaling factors.

In the last few years, we identified important players involved in sexual development. Among them are the pro mutants, which show an arrest in sexual development after protoperithecia formation and a severe defect in hyphal fusion. Here we present data on knockout mutants carrying a deletion of the NAD(P)H oxidase genes *noxA* and their regulator *noxR,* which correspond to the two above described phenotypes. Interestingly, both the nox and pro mutants show also a strong increase of ROS production in distinct cellular structures. Regarding this data we hypothesize coherence between hyphal fusion, fertility and ROS production.

References:
[1] Engh I, Nowrousian M, Kück U (2010) Eur J Cell Biol 89(12):864-72

PR6.4

Protective role of thiamine (vitamin B_1) in baker's yeast cells exposed to hydrogen peroxide.

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Thiamine diphosphate (TDP) serves as a cofactor for main metabolic pathways in all cells. Moreover, numerous recent reports have suggested other biological functions of thiamine independent of its cofactor role, such as involvement in the response of plants and bacteria to stress conditions. However, similar data on *Saccharomyces cerevisiae*, a model eukaryotic organism which is also able to synthesize vitamin B_1 *de novo* are scarce.

The aim of this study was to analyze thiamine biosynthesis in a baker's yeast wild type strain under oxidative stress conditions and to compare its response to that of mutants with disrupted thiamine biosynthesis (*thi4Δ*, *thi6Δ*) or transport (*thi7Δ*). Additionally, we studied a strain with damaged *YAP1*-dependent stress response system which is based on hydrogen peroxide sensing.

Our results showed that thiamine biosynthesis was up-regulated in the wild type strain under oxidative stress. The *thi4Δ* mutated strain was characterized by elevated activity of superoxide dismutase and thiamine pyrophosphokinase under control conditions, while under hydrogen-peroxide treatment the process of thiamine activation to TDP was accelerated 2 fold stronger. The *thi6Δ* mutant with catalytically defective thiamine monophosphate synthase showed a decreased stress response and rate of TDP-dependent pentose phosphate pathway. On the other hand, the *YAP1* mutant presented almost 3 fold higher level of thiamine activation and utilization, suggesting some compensation of disrupted defence system by thiamine biosynthesis, thus confirming a hypothesis of a protective role of thiamine in baker's yeast against oxidative stress.

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PR6.5

***Aspergillus fumigatus* counteracts nitric oxide stress**

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Aspergillus fumigatus is a saprophytic living mold that can cause life-threatening infections in immunocompromised patients. To improve diagnosis and therapy a detailed knowledge of the processes related to host-pathogen interaction is required. In the lung, inhaled conidia are confronted with immune effector cells. After recognition of the conidia are phagocytosed and attacked by host-derived reactive oxygen species (ROS) and antimicrobial proteins. In recent studies it was found that macrophages and neutrophil granulocytes can also form nitric oxide intermediates (RNI) that are putatively involved in killing of the fungus. Because both radicals are present in infected tissue it is very likely that they interact to form highly reactive intermediates like peroxynitrite. *A. fumigatus* produces several enzymes potentially involved in RNI detoxification. Two flavohemoglobins, FhpA and FhpB, convert NO to nitrate, and the S-nitrosoglutathion (GSNO) reductase, GnoA, reduces GSNO to ammonium and glutathion disulphide (GSSG). To elucidate the role of these enzymes in detoxification of RNI single and double deletion mutants of FhpA, FhpB and GnoA encoding genes were generated. Mutant strains revealed enhanced sensitivity against the NO donor DETA-NO. Furthermore, Δ*gnoA* mutants were negatively affected in germination. To investigate the role of RNI and its detoxification in fungal pathogenicity virulence of the D*gnoA* mutant was analysed in a cortisone acetate murine infection model for invasive aspergillosis. However, no difference in pathogenicity was detectable compared to the wild type and complemented strains. Therefore, the ability to detoxify host-derived RNI does not have a major influence on virulence of the human pathogenic fungus *A. fumigatus*.

PR6.6

The Thioredoxin System of *Botrytis cinerea* Has a Severe Impact on Virulence

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In the course of infection the pathogenic grey mould fungus *Botrytis cinerea* triggers an oxidative burst as early plant defense reaction. This leads to an active release of reactive oxygen species (ROS), which are on the one hand known to be responsible for molecular damages of biological molecules, but on the other hand they are also involved in cell signaling pathways. We are particularly interested in the influence of ROS on pathogen-host interaction and development. In order to investigate the maintenance of the fungal intracellular redox state, we focused on the thioredoxin system, which is composed of two enzymes, the thioredoxin (BcTrx) and the thioredoxin reductase (BcTrr). Knock-out and complementation approaches of *bctrx1* and *bctrr1* revealed a severe impact on pathogenicity. The mutants were able to penetrate, but only caused small necrotic lesions that were not able to spread. Furthermore, Δ*bctrr1* and Δ*bctrx1* showed a strong sensitivity to oxidative stress; in addition an enhanced H_2O_2 production of Δ*bctrr1* and generally retarded growth compared to Δ*bctrx1* and the wild-type was striking. Northern analyses showed that oxidative stress response genes were constitutively expressed in the Δ*bctrr1* mutant, while the transcriptional level of these genes was not altered in the Δ*bctrx1* mutant. Consequently, the thioredoxin system seems to be essential for the detoxification of ROS, fungal pathogenesis and the development of *B. cinerea*.

PR6.7

Regulation of cAMP levels during *neurospora crassa* conidiation

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Conidiation involves three morphogenetic transitions: growing hyphae to adhered hyphae, adhered hyphae to aerial hyphae and aerial hyphae to conidia. A hyperoxidant state develops at the start of each of these morphogenetic transitions. The *ras-1^{bd}* and a *Δsod-1* strain exhibit cyclic conidiation that is suppressed by N-acetyl-cysteine. Paraquat shortens its period in both strains. This behavior suggests a cyclic oxidative stress. We investigated how RAS-1 controls the switch between growth and conidiation.

Adenylate cyclase (AC) has a predicted RAS association domain and cAMP is involved in mycelial morphology, aerial hyphae formation and conidia development. Adenylate cyclase null mutant strains do not form aerial hyphae but conidiate profusely. *N. crassa* has a high (PDE_H) and a low affinity (PDE_L) phosphodiesterases. D*pde$_H$* strain grows slow and does not conidiate; there is no evident phenotype for the D*pde$_L$* strain.

We found that oxidative stress and RAS-1 determine cAMP levels during the first two hyperoxidant states of the conidiation process; higher levels than *Wt* were observed in *ras-1^{bd}*. In both strains, a rapid decrease in cAMP at the start of the first two hyperoxidant states was due to activation of PDE_L. PDE_H was important for maintenance of initial cAMP levels, once oxidative stress was compensated. During oxidative stress of the second morphogenetic transition both phosphodiesterases participate in decreasing cAMP and mainly PDE_L is used to restore initial cAMP levels. Thus, RAS influences the level of cAMP, probably though AC activation, but more critical for development is the activation of phosphodiesterases during oxidative stress.

PR6.8

Jasmonic acid and methyl jasmonate induce defense response in grape berries against postharvest gray mould caused by *Botrytis cinerea*.

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Botrytis cinerea, a necrotorphic pathogen, causes serious losses in both yield and quality in grapes (*Vitis vinefera* L.). Jasmonic acid (JA) and its derivatives incuding methyl jasmonate (MeJA) occur naturally in host plant tissues and have signalling roles in defense against necrotrophs and as well as induce systemic resistance (ISR) against disease. The present study investigates the effect of exogenous JA and MeJA, on the suppression of postharvest gray mould in green grape cultivar 'Thompson' and red grape cultivar 'Flame'. The surface sterilized grape bunches (15 grapes/bunch and three replicate treatments) were spray-treated with 0.2 mM of JA or 1mM of MeJA, air dried for 3 hours. Three days after the JA or MeJA treatment, each of the grape berries in the bunch was wounded with a needle and inoculated with 1 x 10^4 spores of *B. cinerea* B05.10 and incubated in the dark at 12 ºC and 85% RH. Control treatment did not receive JA or MeJA. The lesion diameter was recorded at 7 and 14 days after inoculation. Both jasmonic acid and methyl jasmonate induced defense response by significantly suppressing the *Botrytis* gray mould disease in the green grape cultivar 'Thompson', and in the red grape cultivar, 'Flame'. Investigations on the mechanisms of how JA and MeJA induce defenses of grape berries against Botrytis are underway. Postharvest treatment with jasmonic acid or methyl jasmonate may be incorporated as the potential tools in the grape postharvest disease management strategies.