Monday 2 April

Parallel session 9: The Fungal Cell Wall

PS9.1

Advances in fungal cell wall proteomics

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The cell walls surrounding pathogenic fungi represent the first point of contact with their hosts and play an important role in the infection process. Electron microscopy studies on ascomycetous yeasts as well as some other species have shown that two different layers can be distinguished in fungal walls. The inner part of the wall is electron-transparent and mainly consists of carbohydrates. This is surrounded by an electron-dense layer, which is packed with a diversity of covalently-bound cell wall proteins. The protein composition of fungal walls is dynamic and depends on the growth environment. We are interested in studying the role of fungal wall proteins in pathogenesis. As a first step, we perform (comparative) genome-wide *in silico* analyses to identify putative cell wall proteins (and other cell surface proteins). The majority of known fungal cell wall proteins are so-called GPI proteins, which are covalently linked to cell wall β-1,6-glucan through a remnant of their GPI lipid anchor. In a different approach, we have developed techniques to identify and quantify covalently-bound cell wall proteins using advanced mass spectrometry technology. This allows us to identify cell wall proteins whose expression and incorporation is triggered under infection-relevant conditions and therefore may be important for the infection process.

Here, we will discuss our advances in mass spectrometric identification of cell wall proteins, and we will present our recently launched web server named ProFASTA, which facilitates fast genome-wide predictions of fungal cell surface proteins. In addition, we will present examples of functional characterizations of identified infection-relevant proteins in the human pathogen *Candida albicans*.

PS9.2

Interaction of cell wall polysaccharides with amyloid forming proteins

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Fungi secrete a variety of proteins that form amyloid-like fibrils at the cell wall surface. In this way, they fulfill a structural role in growth and development. Hydrophobins are such a class of proteins. In vitro experiments showed that the class I hydrophobin SC3 of *Schizophyllum commune* spontaneously forms amyloid-like fibrils at the water-air interface. In contrast, SC3 is arrested in an intermediate conformation at the interface between water and a hydrophobic solid such as Teflon. This finding prompted us to study conditions that promote assembly of SC3 into amyloid-fibrils. We have shown that amyloid formation at a hydrophobic surface does take place at high concentration (300 µg ml^{-1}) and prolonged incubation (16 h). The concentration of hydrophobin needed for amyloid fibril formation was much lower in the presence of the cell wall components schizophyllan (β-(1-3)β-(1-6)-glucan) and β-(1-3)-glucan. From this it is concluded that SC3 will not only assemble into amyloid-like fibrils at the cell wall of aerial hyphae but also of hyphae in contact with a hydrophobic solid such as the surface of a plant. Experimental data have shown that the resulting amyloid layer forms a molecular sieve and is also an insulating layer that does not allow transfer of electrons unless a mediator is present. The consequences of these properties for fungal growth and development will be discussed.

PS9.3

Microarray analysis of antifungal synergy between inhibitors of chitin synthases and beta-(1,3)-glucan synthase

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Fungi are major agents causing diseases in crops with a strong yield reduction and an alteration of the quality as main consequences. Further knowledge of the infection process can bring new insights to develop novel antifungal strategies. The cell wall of fungi is quite specific and essential for their viability and their aggressiveness towards their hosts. How plant-pathogenic fungi cope with cell wall alterations was explored in *Botrytis cinerea* as a model of necrotrophic and phytopathogenic fungus. Inhibition of the cell wall biosynthesis pathways was assessed using two commercial inhibitors, Nikkomycin Z and Caspofungin, targeting respectively chitin synthases and b-(1,3)-glucan synthase, responsible for the synthesis of the two major polysaccharides of the fungal cell wall. Each compound alone inhibits the *in vitro* growth of *B. cinerea*, and the combination of both inhibitors shows a significant synergistic effect. It was previously reported that inhibition of the synthesis of b-(1,3)-glucans and chitin leads to a compensation phenomenon in several fungi. Our data suggest that, in the case of synergy, *B. cinerea* was not able to compensate all the cell wall deficiencies induced by the application of both inhibitors together. Compensation phenomena are known to involve transcriptional regulations of cell wall related genes or several signalling pathways in other fungi.The effect of both compounds alone or in combination on the *B. cinerea* gene expression are studied using a microarray transcriptomic approach (*B. cinerea*, Nimblegen chip), and results will be presented in the communication.

PS9.4

Cell Wall Stress Affects Chitin Synthase Delivery And Secretion In The Pathogen *Ustilago maydis*

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Chitin is an essential component of the fungal cell wall, whose composition and structural organization changes in response to the environmental conditions. Chitin synthase-containing vesicles are taken to the growth region by molecular motors, which transport their cargo along the fibres of the cytoskeleton. It was shown that chemical-induced cell wall stress increases the expression of chitin synthases in *Candida albicans*, which increases the amount of chitin in the cell wall and allows survival of the cell (Munro *et al*. (2007) *Mol Microbiol* 63: 1399–1413). In this study we investigate the effect of cell wall stress on the actual delivery of chitin synthase containing vesicles. Using the basidiomycete *Ustilago maydis* we show that Calcofluor White and Caspofungin treatments increase the chitin content in this plant pathogen. The effect on polar delivery, secretion and transport rates of the four polar localized chitin synthases (Chs5, Chs6, Chs7 and Mcs1; Weber *et al*. (2006) *Plant Cell* 18: 225–242) will be presented and wider implications discussed.

PS9.5

Self-assembly at air/water interfaces and chitin-binding properties of the small cell wall protein EPL1 from *Trichoderma atroviride*

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The protein EPL1 from *Trichoderma atroviride* belongs to the cerato-platanin family (IPR010829). Members of this family from other fungi appear to be readily recognized by other organisms, and therefore are associated with the induction of defense responses in plants and allergic reactions in humans. However, also in non-pathogenic fungi the genes of cerato-platanin family members are abundantly transcribed under various growth conditions, but the primary function of this protein family has not been elucidated yet. EPL1 is the major secreted protein in submerged cultivations with glucose as carbon source. The *epl1* gene is expressed under all so far tested growth conditions. We were now for the first time able to show that, similar to hydrophobins, the cerato-platanin protein EPL1 self-assembles at air/water interfaces and forms a protein layer. In contrast to hydrophobins, protein layers of EPL1 can be easily re-dissolved in water. Further, for EPL1 no statistically significant alteration of the surface tension of aqueous solutions was detected. In other fungi it was shown that cerato-platanin proteins are not only secreted into the medium, but also partially cell-wall localized. A potential chitin-oligomer binding site was recently found in structural NMR studies of the cerato-platanin protein CP from *Ceratocystis fimbriata* (de Oliveira *et al.* 2011, JBC). Carbohydrate-binding experiments with EPL1 now yielded biochemical evidence this protein indeed binds to different chitin-preparations, but interestingly not to complex fungal cell wall preparations of *T. atroviride*. This shows that this protein has lectin-like properties and advances our knowledge toward understanding the functional roles of cerato-platanin proteins. *Trichoderma* species have three *ep*l-genes. Transcript analysis revealed that only two of these genes - *epl1* and *epl2* - are expressed under most growth conditions. Single and double gene knockout strains were created in order to analyze the function of these genes in fungal growth and development.

PS9.6

Analysis of the cell wall integrity (CWI) pathway in *Ashbya gossypii*.

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Fungal cells are constantly exposed to rapidly changing environmental conditions, particularly considering their osmotic potential. The fungal cell wall takes on an important function in protecting the cell from external stresses and controlling intracellular osmolarity, but it is also required to maintain regular cell shape. However, cells must still be able to remodel the rigid structure of the cell wall to guarantee cell expansion during cell differentiation processes, but also in response to external cell stresses. While several signaling pathways contribute to the maintenance of the cell wall, it is the cell wall integrity (CWI) pathway that is most important in regulating changes made in the cell wall structure during vegetative growth, morphogenesis or in response to osmotic stress.

To characterize the CWI pathway in the filamentous ascomycete *Ashbya gossypii* we generated deletion mutants of several genes encoding for the most important components of the CWI pathway including potential cell surface sensors (e.g. *AgWSC1*), the coupled downstream protein kinases including a MAPK signaling module (*AgPKC1*, *AgBCK1*, *AgMKK1* and *AgMPK1*), but also downstream effector genes (e.g. *AgRLM1*). An initial characterization of the corresponding mutants is presented. While a mutant in *Agpkc1* shows a strong general growth defect found similarly in corresponding mutants in other fungi, mutants in several other components of the CWI pathway, in particular in the MAPK module, show a noticeable colony lysis phenotype. In addition, preliminary experiments showed that riboflavine production, a typical feature of *A. gossypii*, may be affected in some of the CWI mutants.

PS9.7

Efg1 Shows a Haploinsufficiency Phenotype in Modulating Cell Wall Architecture and Immunogenicity of *Candida albicans*

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The *Candida albicans* transcription factor Efg1 is known to be involved in many different cellular processes, including morphogenesis, general metabolism and virulence. Here, we show that Efg1 besides its manifold roles, also has a prominent effect on cell wall structure and composition, affecting strongly its structural glucan part. Deletion of only one allele of *EFG1* already results in severe phenotypes for cell wall biogenesis, comparable to deletion of both alleles, indicative of a severe haploinsufficiency for *EFG1*. The observed defects in structural setup of the cell wall together with the previously reported alterations in expression of cell surface proteins, result in altered immunogenic properties of strains with compromised Efg1 function. This is shown by interaction studies with macrophages and primary dendritic cells. The structural changes in cell wall carbohydrate meshwork presented here, together with the manifold changes in cell wall protein composition and metabolism reported in other studies, contribute to the altered immune response mounted by innate immune cells and the altered virulence phenotypes observed for strains lacking *EFG1*.