Poster Category 2: Sex and Sexual Development

PR2.1

The General Transcriptional Repressor Tup1 Is Required For Dimorphism And Virulence In A Fungal Plant Pathogen.

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The transition between a yeast-like growth and the formation of polar filaments is a critical step in the life cycle of many fungal pathogens. This morphological shift, known as dimorphism, is triggered by multiple environmental signals and controlled by complex genetic pathways that ensure successful pathogenic development. The transcriptional repressor Tup1 is one of the best known regulators of dimorphism in animal pathogenic fungi. However, the role of Tup1 in plant pathogens remained unknown until now. Here we show that Tup1 plays a key role in the dimorphic transition of the maize pathogen *Ustilago maydis.* Deletion of *tup1* compromises the mating and filamentation capacities of the fungus, leading to a reduce virulence phenotype. In *U. maydi*s, such processes are controlled by the Prf1 transcription factor through *a* and *b* mating-type loci genes. Interestingly, Δ*tup1* strains show a significant reduction in the expression level of *prf1* and that of Prf1 target genes at both loci. We have observed that Tup1 seems to control Prf1 activity by regulating the expression of the *prf1* transcriptional activators, *rop1* and *hap2*. In addition, we have found a putative novel *prf1* repressor, named Pac2, which seems to be an important target of Tup1 in the control of dimorphism and virulence. Our findings establish Tup1 as a key factor coordinating dimorphism in the phytopathogen *U. maydis*, and support a conserved role for Tup1 in the control of hypha-specific genes among animal and plant fungal pathogens.

PR2.2

Plant Specific Activation Of The Unfolded Protein Response Is Necessary For Biotrophic Development Of *Ustilago maydis*

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In the smut fungus *Ustilago maydis*, pathogenic development is controlled by the *b*-mating type locus, orchestrating a regulatory network consisting of different transcription factors. A key factor for the regulation of this circuit is the Clp1 protein. Via physical interactions with two of the key regulators of the *b*-network, bW and Rbf1, the Clp1 protein promotes the cell cycle release and the progression of fungal development at the onset of biotrophic development. In addition to bW and Rbf1 we identified Cib1, a previously undiscovered bZIP-transcription factor to interact with Clp1. Cib1 protein expression is restricted to the biotrophic development via regulated splicing. In accordance with this we observed that deletion of *cib1* leads to a plant specific phenotype: while filamentous growth and formation of appressoria is not affected, mutant strains fail to colonize the plant tissue. We discovered Cib1 to be the homologue of yeast Hac1p in *U. maydis*. Hac1p represents the central regulator of the unfolded protein response (UPR), a highly conserved signalling pathway to remodel and to align cellular physiology to the demands imposed by enhanced protein synthesis and protein secretion. An enhanced capacity for protein secretion is expected to be of crucial importance for biotrophic growth *U. maydis* and required to establish a compatible interaction with the host plant. Hence, these data shed light on a novel strategy by which UPR signalling is coupled to mating-type controlled development in order to promote the parasitic lifestyle of a biotrophic pathogen.

PR2.3

Characterization of the mating type (MAT) locus in the *Grosmannia clavigera* (Ophiostomatales, Ascomycetes) and related species revealed the footprint of a homothallic ancestor

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The mating type (MAT) gene evolution from eight heterothallic fungal species in the order Ophiostomatales, including conifer pathogens associated with mountain pine beetles in the genera *Grosmannia* and its the asexual form *Leptographium* has been investigated. We characterized a MAT1-2 idiomorph from the assembled and annotated genome of *G. clavigera*. The MAT locus is flanked by genes of cytoskeleton protein (SLA) and DNA lyase (APN). The synteny of these genes is conserved and consistent to other members in Sordariomycetes. We also identified a truncated MAT1-1-1 gene adjacent to the MAT1-2-1 gene in the MAT1-2 idiomorph. The truncated MAT1-1-1 is homologous to the '*complete*' MAT1-1-1 gene in the opposite mating type, except that the alpha-box domain had been deleted or removed. The MAT genes determined from additional isolates of *G. clavigera* and seven closely related species were shown to have the same pattern, suggesting that the presence of the truncated MAT1-1-1 gene is ancient. We hypothesize that the ancestor of *G. clavigera* and related species was homothallic and retained both *'complete'* MAT1-2-1 and MAT1-1-1 genes at the MAT locus, and the heterothallic species evolved from this ancestor when MAT1-1-1 and MAT1-2-1 genes were translocated and rearranged from the locus. We also suggest that the deletion of alpha-box domain to maintaining a heterothallic life style, and the 1:1 MAT ratio determined in *G. clavigera* populations, can confer an evolutionary benefit by promoting outcrossing and increasing genetic variability within a species.

PR2.4

Mannitol-1-phosphate dehydrogenase is essential for the development of extreme stress resistant fungal ascospores

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Ascospores of *Neosartorya fischeri* exhibit extreme stress resistance. They survive extreme drought (down to 0.5% relative humidity), high temperature (20 minutes at 85°C), high pressure (6000 Bar) and various chemical stresses (*e.g.* pH and salt stress). The spores are constitutively dormant and can survive several years in a dormant state. Exit of dormancy, and subsequent germination, can be realized by a short "heat flash" at 85°C. While much research has been performed on the characterization of spores, not a lot is known about the process of ascospore development. During maturation ascospores become more heat resistant; this is accompanied with an increase of micro-viscosity and an increase of compatible solutes (e.g. trehalose). A remarkable observation is the high concentration of mannitol in young spores, which slowly decreases during maturation of the spores. To evaluate the role of mannitol in development of ascospores, two genes involved in the mannitol metabolism (mannitol-1-phosphate dehydrogenase (MPD) and mannitol dehydrogenase (MDH)), are deleted within *N. fischeri*. The MPD mutant is not producing fully developed ascospores, while the formation of ascomata and asci is not affected. Within conidia (asexual spores), mannitol is thought to play a role in stress resistance and dormancy. We hypothesize a different role of mannitol. High mannitol concentration could result in an osmotic pressure, attracting water and nutrients to the ascocarp needed for the formation of functional spores. Ongoing research on the promoter of the two mannitol synthesis genes and qPCR will give us more information about when and where MPD is transcribed.

PR2.5

Evidence for sexual recombination in *Penicillium chrysogenum*

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Penicillium chrysogenum is the major industrial producer of the β-Lactam antibiotic penicillin with a world market value of about 600 million € per year. Although no sexual propagation has been reported so far for this filamentous fungus, we recently were able to detect mating type loci in different strains, indicating a sexual lifecycle. Isolates, carrying opposite mating types, were found in near-equal proportion in nature and we observed transcriptional expression of mating type loci as well as pheromone and pheromone receptor genes [1]. Thus *P. chrysogenum* possesses the genetic requirements for heterothallic breeding. For induction of a sexual cycle we performed crossing experiments resulting in the production of cleistothecia and ascospores, which were similar to those described recently for *Eupenicillium crustaceum* [2]. Here we provide evidence for sexual recombination in ascospore progeny. A sexual cycle provides an invaluable tool for classical genetic analyses and extends an insight into the evolution of fungal sexual development.

[1] Hoff B, Pöggeler S, Kück U (2008) Eighty years after its discovery, Fleming`s *Penicillium* strain discloses the secret of its sex. Eukaryot Cell 7: 465-47.

[2] Pöggeler S, O´Gorman CM, Hoff B, Kück U (2011) Molecular organization of the mating-type loci in the homothallic ascomycete *Eupenicillium crustaceum*. Fungal Biol. 115: 615-624.

PR2.6

Cdc42 causes gasteromycete-like basidial anatomy in *Schizophyllum commune*

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Gasteroid fungi are a morphologically defined group of the Agaricomycetidae, characterised by spore formation within enclosed basidiomata and by statismosporic basidia. The underlying genetic processes are widely unknown so far, since gasteroid fungi have not been subject of genetic studies for decades.

Schizophyllum commune is a well studied model fungus for the sexual development of basidiomycete fungi, due to its ability to fulfil its life cycle in two weeks. Despite its unusual cyphelloid fruiting body anatomy, its hymenium and ballistosporic basidia develop in the same fashion as most other representatives of Agaricales. A number of small GTPases such as Cdc42 are known to influence sexual development of many fungi, including *S. commune*. The Rho-protein Cdc42 regulates elongation and adhesive capacities of hyphal cells. It is also responsible for branch site selection and branch development by proper actin cytoskeleton orientation in monokaryotic hyphae, during mating and in clamp formation of dikaryotic mycelium.

Basidiomata of heterozygotic dikaryons of *S. commune*-mutants ectopically expressing constitutively active Cdc-42 are developmentally disturbed. Although later stages of spore formation are lacking, dysfunctional spores are produced. These are borne on divergent sterigmata, which are thick-walled and longer than in the wildtype, and obviously incapable of active spore discharge. In gasteroids, sterigmata are also often likewise divergent, as seen in the exceptionally long, sclerified pedicels of some Lycoperdaceae, or the pleurocarpous basidia of Tulostoma. These features are hypothesized as evolutionary disturbances in the order and magnitude of developmental gene expression, including Cdc42 and its downstream factors.

PR2.7

The forkhead gene *fkhA* positively regulates sexual development in *Aspergillus nidulans*

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In a homothallic filamentous fungus *Aspergillus nidulans*, sexual developmental process is largely affected by the genetic and environmental factors. To regulate the corresponding genes necessary for the sexual development, tight regulations of transcription factors are required. Here, we identified the *fkhA* gene which encodes a putative forkhead transcription factor homologous to the yeast *FKH1* gene that is involved in sexual development. The *fkhA* deletion resulted in the complete loss of fruiting body formation under all conditions favoring sexual development, indicating that the *fkhA* gene is required for normal sexual development in *A. nidulans*. Furthermore, overexpression of *fkhA* showed enhanced production of fruiting bodies under induction condition not only in the normal condition but also in the inhibiting condition of sexual development. These results suggest that the *fkhA* gene is necessary and sufficient for regulating sexual development in *A. nidulans*. [This work was supported by the NRF grant 2011-0027448]

PR2.8

Evidence of Sexual recombination in the phytopathogen, *Ramularia collo-cygni*

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Ramularia collo-cygni is the main causal agent of the major barley disease, Ramularia Leaf Spot (RLS). Control of this disease is based on chemical control, due to the lack of varietal resistance. The appearance of resistance in this fungus to Quinone outside Inhibitor (QoI) fungicides was observed in 2001. This rapid evolution has now been confirmed by the detection of the G143A mutation, which confers resistance, in a number of *R collo-cygni* isolates from a number of geographically diverse locations. The life cycle of this fungus is only being slowly elucidated. An asteromella stage has been observed on senescent leaf material. The precise nature and function of this structure is now being investigated. The successful transformation of *R. collo-cygni* using Agrobacterium has allowed detailed microscopic examination of the fungus, including the formation of sexual structures *in vitro.* Preliminary experiments indicate that the precursors of spermagonia and ascogonia form *in vitro* and compatable isolates form sclerotial like structures when growing together. In addition to microscopic evaluation, mating type loci are being characterised using information derived from the sequencing of *R. collo-cygni.*

PR2.9

B-Regulated Sexual Development And The Sugar Transporter Sts1 In The Mushroom *Schizophyllum Commune*

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Mushroom development in the basidiomycete *Schizophyllum* commune is normally the end result of a sexual interaction between two individuals differing at what are termed the *A* and *B* mating type loci. *sts1* is a putative sugar transporter gene also implicated in the regulation of mushroom development. Null (Δ) mutant strains lacking functional copies of *sts1* displayed severely attenuated mushroom production. When *Δsts1* strains were outcrossed, some of the *Δsts1* null haploid progeny displayed a “flat” phenotype, suggestive of an inappropriately activated *B* mating type pathway. Test matings and genetic analysis of these haploid “flats” confirmed that this was indeed the case. A complicating factor was the observation that the original *Δsts1* strain did not exhibit a “flat” phenotype. These and other data indicated that the absence of *sts1* is a necessary, but not sufficient condition to activate the *B* mating pathway in these homokaryotic individuals. Fluorescent microscopy of these B-On *sts1* null haploids stained with DAPI/Calcofluor revealed the presence of multiple hook cells at inappropriate locations, with nuclei frequently found within. We have exploited the recent availability of the sequenced *S. commune* genome and have employed a transcriptome analysis approach to identify likely gene targets regulated in the B-On *sts1* null strains. The preliminary results of this analysis will be discussed at the conference.

PR2.10

Analysis of Ras Proteins as Signal Transduction Elements in *Schizophyllum commune*

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The white rot basidiomycete *Schizophyllum commune* has been used as a model organism to study mating and sexual development as well as analysis of cell development.

Subsequent to pheromone recognition, intracellular signal transduction leads to a specific phenotype involving nuclear migration and clamp cell fusion. The *S. commune* genome encodes more than 30 putative signal transduction proteins of the Ras superfamily containing the Ras, Rho, Rab, Ran and Arf subfamilies. The comparison of both proper *S. commune* Ras proteins reveal a low sequence identity of 44 %. Phylogenetic investigation of Ras proteins from various basidiomycetes show that they cluster in two main groups. High sequence similarities between these proteins in basidiomycetes suggesting an ancient duplication event. The role of the small G-proteins Ras1 and Ras2 have been postulated in pheromone response in addition to MAPK signalling.

To investigate the role of Ras1 mutants with constitutively active ras alleles as well as a ΔRasGap1 mutant were analyzed. They show phenotypes with disorientated growth pattern, reduced growth rates and hyperbranching effects. The fungal cytoskeleton, composed of actin and microtubules has been investigated by immunofluorescence microscopy to reveal whether Ras signaling influences the formation of cytoskeleton. The second Ras protein, Ras2, was detected by genome analysis. Its function is analysed in current studies.

PR2.11

Proteins expressed during hyphal aggregation for fruiting body formation in basidiomycetes

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The first visible step in fruiting body development in basidiomycetes is the formation of small hyphal knot by localized intense branching of hyphae of restricted length followed by hyphal aggregation. In *Coprinopsis cinerea*, the first not yet fruiting-specific step of hyphal branching occurs in the dark, the second step requires a light signal. Hyphal aggregation implies cell-cell contacts and protein interactions on the outer cell walls are anticipated. Few protein candidates were identified and discussed in the past for such function, amongst were the galectins in *C. cinerea* and the Aa-Pri1 protein (aegerolysin) in *Agrocybe aegerita* that are specifically expressed during the step of hyphal aggregation as well as during subsequent primordia development. In this study we follow up the distribution of such genes in the steadily growing number of available genomes of basidiomycetes. Neither galectin genes nor *Aa-pri1*-like genes are present in all mushroom species, making an essential role in hyphal aggregation unlikely.

R2.12

Differential Regulation of Laccases in *Schizophyllum commune*

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Schizophyllum commune, a saprophytic white rot fungus, is involved in the degradation of complex organic molecules including lignin. Previous reports say that this fungus can degrade refractory organic matter from black slate with the help of different exoenzymes. Laccases are multicopper glycoproteins which are able to oxidize a broad spectrum of organic compounds including xenobiotics, synthetic dyes, pesticides and polycyclic aromatic hydrocarbons by a radical catalyzed reaction mechanism using molecular oxygen. *S. commune* able to produce laccases and laccase-like enzymes.

In this study, relative expression of the laccase and laccase-like genes (LO family) in *S. commune* were analysed using quantitative real time PCR (qRT-PCR) under different conditions. Also, laccase enzyme activity was measured using ABTS (2,2´-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)) as a substrate. Two laccases and four laccase-like genes all of which are well conserved in *S.commune* were selected for studying differential regulation during fungal morphogenesis and in different sexual phases. Regulation of laccases during substrate utilization was tested using powdered black slate and artificial lignin as the core C- sources, both of which can be used by *S. commune* at low growth rates. Higher laccase enzyme activity was seen in black slate cultures.

PR2.13

PRO45, a potential membrane associated protein is a component of the conserved fungal STRIPAK complex

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Sordaria macrospora is an important model organism for developmental biology and permits insights into the complex formation of three-dimensional fruiting bodies. This is mediated by an interaction between developmental proteins and conserved signaling cascades. A prominent role in sexual development of *Sordaria macrospora* plays a complex of striatin-interacting phosphatases and kinases (STRIPAK), which is associated with the vacuolar membrane. This complex contains homologs of striatin, the striatin-interacting protein, PP2A A, Mob3 (monopolar spindle-one-binder) and SLMAP (sarcolemmal membrane-associated protein).

PRO11 and PRO22, important developmental proteins of *Sordaria macrospora*, represent homologs of striatin and striatin interacting protein, respectively [1, 2]. For *Sordaria macrospora* a direct interaction of PRO22 with PRO11 and PP2A A was shown recently. This raises the question whether a STRIPAK-like complex exists in filamentous fungi and whether homologs exist to components of the human STRIPAK comlex.

Here we present the characterization of another component of the fungal STRIPAK complex: PRO45 in *Sordaria macrospora* is a homolog of SLMAP containing both a FHA- and a transmembrane domain. Deletion strains show sterility together with a severe defect in hyphal fusion. Tandem affinity purification (TAP) followed by mass spectrometry and yeast-2-hybrid analysis showed subunits of the STRIPAK-complex as interaction partners. Further, proteins were identified which are taking part in ubiquitination and Golgi-organization. We will present data of localization experiments to approve a relation between STRIPAK and regulatory effectors.

[1] Pöggeler S, Kück U. 2004. Eukaryot. Cell 3: 232-240
[2] Bloemendal S, Lord KM, Rech C, Hoff B, Engh I, Read ND, Kück U. 2010. Eukaryot. Cell 9: 1856–1866

PR2.14

How Sex Influences Carotene Metabolism in Zygomycetes Fungi?

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In Blakeslea trispora, the mating partners develop zygophores that exchange (pro)hormones to co-ordinate the process of sexual reproduction. The hormones comprise a unique cocktail of compounds known as trisporic acids. Apocarotenoids are the bioactive oxidative degradation products of Beta-carotene. We focus on the "chemical interaction" between (+) and (-) zygophores during sexual cycle and how these chemical signals influence the transcription of genes involved in apocarotenogenesis. Dynamics of transcriptional regulation in liquid cultures of individual and mated Blakeslea were analysed for 144 hours by real time quantitative reverse - transcription PCR. Relative quantification of m-RNA for carRA(Phytoene synthase and lycopene cyclase) and tsp3(carotenoid cleavage dioxygenase) were done using act-1(actin) as reference gene. Individual strains were treated with C-13 apocarotenone and the prohormone methyl trisporic acid-C for 60 hours. In vivo co-expression experiments using Beta-carotene overproducing plasmid and tsp3 gene insert in E.Coli, were carried out to identify the apocarotenoid product. Gene expression kinetics pinpoint a trend of upregulation in both carRA and tsp3 from 72-144 hours in mated culture. Methyl trisporic acid C is more active in (+) with a 3.5 fold upregulation over C-13 apocarotenone concerning tsp3 expression. Interestingly, Methyl trisporic acid C had no significant impact on carRA expression either in (+) or (-) strain. C-13 apocarotenone had a 2 fold impact over methyl trisporic acid C with tsp3 expression in (-). LC-MS analysis for the co-expression experiments in E.coli resulted in identification of 12'-apo carotenal as the apocarotenoid product formed by tsp3 in Blakeslea trispora.

PR2.15

Parasexual development in the host – parasite-pair *Absidia glauca* – *Parasitella parasitica* and its use as system for genetic manipulation of zygomycete fungi

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Biotrophic fusion parasitism within zygomycete species often leads to structures resembling sexual morphogenesis, including cytoplasmic fusion and transfer of nuclei between the interacting individuals. Microscopy of fixed and sectioned infection structures reveals complex interactions between the partner hyphae, with altered cell wall morphology in contact regions and well defined pores of 2-3 µm in diameter, which will easily allow the transfer of the 1 µm sized nuclei from one compartment to the next. The parasexual interaction between *Parasitella parasitica* and its multiple host species also involves the zygomycete trisporic acid-based sexual recognition system. We are aiming at establishing this fusion reaction as a tool for genetic manipulation within this group of fungi.

Methionine auxotrophy in a strain of *Absidia glauca* was found to be the result of an insertion disrupting the single copy *HAT* gene coding for homoserine acetyltransferase. This strain was infected with methionine prototrophic *P. parasitica* containing two different *HAT* genes but defective in adenine biosynthesis and displaying pink colored mycelium. Spores from the infection plates were screened for the ability to grow on minimal medium, where only progeny with recombinant phenotypes will survive. Primary gene transfer events occured with a frequency at least two orders of magnitude higher than spontaneous reversion of the mutation but were found to be mitotically unstable over the subsequent sporulation cycles. Hybridization analyses revealed that successful transformation and complementation is possible also with the wild type *A. glauca HAT* gene which is maintained as autonomously replicating plasmid in the recipient mutant strain.

PR2.16

Functional Characterization of predicted genes in the Aβ mating-type locus of *Schizophyllum commune*

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Sexual development in the basidiomycete fungus *Schizophyllum commune* is controlled in part by a complex system of interacting homeodomain proteins that function in non-self combinations to regulate the initial events of sexual development. Two functionally redundant "A" mating type loci (Aα and Aβ) encode these interacting proteins. While Aα has been well characterized, little is known about the functional nature of the more complex Aβ locus. The purpose of this investigation is to accomplish a functional characterization of the predicted homeodomain genes encoded in Aβ. Predicted genes (*V6, U6, T6, S6, R6,* and *Q6*) were amplified by PCR and cloned into suitable transformation vectors. Transformants were analyzed for activation of A mating-type developmental events. Our results to date indicate that strains transformed with *V6* show evidence of *V6* specific developmental activity in appropriate mating reactions and that *U6* transformants do not. Analysis of the remaining cloned genes is ongoing. Our initial results indicate that activation of sexual development by the homeodomain genes encoded in the Aβ locus is likely to be complex and may be dependent on the mating-type identity of the transformed strain.

PR2.17

Cross-talk between nitric oxide and light for the regulation of development

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In *Aspergillus nidulans,* light is the main signal that influences the decision if cells undergo asexual (conidiation) or sexual (cleistothecia) development. In all eukaryotes nitric oxide (NO) is an important signalling and defence molecule and we have shown previously that the short-lived nitrogen oxide radical is generated during the nitrate assimilation process, and detoxified by flavohemoglobin proteins FhbA and FhbB.

Here we report that the metabolism of NO is additionally regulated by light. We found that the expression of the flavohemoglobin gene *fhbB* is induced by light and that this regulation depends on the photoreceptor complex. Our data show that conidiation is gradually repressed by increasing NO levels and at the same time formation of cleistothecia is promoted. We also found that other metabolic genes which potentially affect NO formation or consumption are regulated by light and thus may participate in the fine-balanced regulation of developmental decisions in *A. nidulans.*

PR2.18

Mub1 protein regulates mating differentiation and yeast cell morphogenesis in *Cryptococcus neoformans*

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Cryptococcus neoformans is a heterothallic basidiomycete that grows vegetatively as yeast cells and produces mating filaments in the sexual state. Mating initiates when *MAT***a** and *MAT*α cells conjugate and fuse, and then dikaryotic sexual filaments are subsequently produced. Generation of final fruiting structures basidia and meiotic progeny basidiospores leads to completion of the sexual cycle. Prior studies have revealed that *C. neoformans* Cwc1 and Cwc2 proteins are two central photoregulators which form a complex to inhibit the production of sexual filaments upon blue light irradiation. To reveal the detailed light response networks, a genome wide mutagenesis screen was conducted and components involved in light-mediated filamentation pathway have been identified. In this study, a suppressor mutant EE24 was characterized and T-DNA is found to insert at the upstream regulatory region of *C. neoformans MUB1* gene, a homologue of *Saccharomyces cerevisiae MUB1* gene. In *S. cerevisiae,* Mub1p is a MYND domain-containing protein required for ubiquitination and turnover of Rpn4p, a transcription factor of proteasome genes. *mub1* mutant shows a multiple-budding phenotype. Deletion of *C. neoformans MUB1* gene caused compromised growth at 37°C. *mub1* mutants similarly displayed the multiple-budding phenotype and altered structure of bud scars were observed. Morphogenesis of dikaryotic sexual filaments and generation of basidiospores were defective in the *mub1* bilateral cross. Interestingly, same sex mating was also regulated by *C. neoformans* Mub1. Our studies demonstrate that *C. neoformans MUB1* is an important gene that regulates yeast cell morphogenesis and mating differentiation.

PR2.19

Do you recognize me? – Orphan receptors in the basidiomycete *Schizophyllum commune*

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The filamentous fungus *S. commune* is a model organism for sexual development of basidiomycetes. Numerous studies revealed the importance of two gene loci, *A* and *B*, responsible for mating and sexual development. While *A* codes for homeodomain transcription factors, *B* codes for a pheromone/receptor system. Both verify compatibility or abortion of mating. The *B*-receptors (Ste3-like, seven transmembrane domains, G-protein coupled) recognize pheromones of non-self specificity and induce signal transduction pathways and specific gene regulation. After sequencing of strain H4-8 four new Ste3-like GPCRs, homologous to the known *Bα* and *Bβ* specific ones, were found. Three of the four are located close to the *B* locus. Their function is unknown, because a *B*-locus defective strain without any interactions seen in *B*-dependent development still contains those four GPCRs, which obviously do not respond to any wild type pheromone. However, our results indicate their importance since sequence identity – analyzed by PCR, cloning and sequencing – between unrelated strains was found arguing for conservation of these genes. Gene expression was observed with Reverse Transcriptase PCR and also with quantitative Real Time PCR during mating interaction and in monokaryotic strains, which showed comparable results between gene *brl4* and the mating receptor *bar2*. Overexpression of the gene *brl2* under control of *tef1*-promoter is performed to give insights into the function of this new class of pheromone receptor-like genes. Furthermore, the *Agrobacterium tumefaciens* mediated transformation is established for this basidiomycete, to improve efficiency of transformation and increase the number of homologous recombination events.

PR2.20

Blue light acts a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*)

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Trichoderma reesei, an industrially important cellulolytic filamentous fungus, is an anamorph of the pantropical ascomycete *Hypocrea jecorina*. *H. jecorina* undergoes a heterothallic reproductive cycle, and the mating yields fertilized perithecia imbedded in stroma containing dechexads with 16 linearly arranged ascospores. Here, we investigated the mechanism and environmental regulation of *H. jecorina* sexual development by applying white light or blue light (440-460 nm). We show that visible light is dispensable for *H. jecorina* sexual development. The experiments on mutant analysis revealed that blue-light photoreceptor BLR1 and BLR2 have both positive and negative regulatory roles in stroma formation during early sexual development, and that the photoadaptation protein ENV1 dampens the light-dependent inhibitory effect in response to changes in illumination. Our results suggest that blue light acts a double-edged sword in regulating *H. jecorina* sexual development.