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Review

Mating-type genes and hyphal fusions in filamentous basidiomycetes☆

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ABSTRACT

In the filamentous basidiomycetes *Coprinopsis cinerea* and *Schizophyllum commune*, mating is regulated by the tetrapolar mating-type system consisting of two unlinked genetic complexes, named *A* and *B*. In the nineties, the molecular structure of *A* and *B* mating type loci and genes was revealed side by side in *C. cinerea* and *S. commune*, first the *A* complex and quite soon thereafter the *B* complex genes. The clear molecular structure of *C. cinerea* mating type genes has led to their use as models for genomic approaches to investigate several other filamentous basidiomycetes. In filamentous fungi, hyphal fusions are important for the distribution of available nutrients in a fungal colony. In ascomycetes and basidiomycetes they are also important for sexual reproduction. These aspects have been approached, especially in filamentous ascomycetes, but in filamentous basidiomycetes the role of fusions in the fungal life cycle has received less attention. Several proteins encoded by the genes required for hyphal fusion in filamentous ascomycetes show homology with proteins forming the striatin-interacting phosphatase and kinase (STRIPAK) complex in eukaryotic cells. Homologs to the genes encoding STRIPAK complex proteins can be identified in *C. cinerea* and *S. commune* genomes suggesting that a STRIPAK-like complex could also regulate hyphal fusions of filamentous basidiomycetes. The STRIPAK complex is a conserved signaling complex also homologous to the *Saccharomyces cerevisiae* Far complex involved in cell cycle arrest at yeast mating, while the fission yeast *Schizosaccharomyces pombe* SIP complex is involved in transition signaling from mitosis to cytokinesis. In filamentous basidiomycetes the signaling pathway regulated by the *B* mating type genes, the pheromone response pathway, is assumed to follow the same pattern as in the yeast *S. cerevisiae*. This poses an interesting question as to the relationship between the STRIPAK complex proteins and the proteins in cellular processes such as cell cycle, septal dissolution, nuclear migration, clamp cell development and fusion, known to take place after hyphal fusions. All these processes are also dependent on the activation of the *B* and *A* mating type pathways in *C. cinerea* and *S. commune*.

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1. Introduction

The molecular mechanism behind the sexual reproduction in filamentous basidiomycetes is presently known in several species due to the analyses of the available total genomic sequences of these fungi. In filamentous basidiomycetes the sexual reproduction starts with hyphal fusion and is regulated by mating type genes (Raper, 1966). The hyphal fusions are important not only for the distribution of available nutrients in a fungal colony, but also for connecting hyphae and thus allowing the interaction of gene products regulated by mating type genes. In *Agaricomycetes*, to which the filamentous basidiomycetes belong, the hyphal fusions bring together haploid nuclei with different mating type genes. When the mating type genes are compatible, a dikaryotic mycelium develops with fruiting bodies. The dikaryotic phase ends in the fusion of the two nuclei with different mating type genes in basidia developing on the fruiting bodies, and the fusion is soon followed by meiosis and occurrence of the haploid progeny (Fig 1).

In spite of the importance of the hyphal fusion process, it has gained little attention in filamentous basidiomycetes. On the other hand, in filamentous ascomycetes, especially in *Neurospora crassa*, the fusions between germinating conidia and vegetative hyphae have received extensively attention during last years (Glass *et al.*, 2004; Roca *et al.*, 2005; Leeder *et al.*, 2011; Riquelme *et al.*, 2011; Read *et al.*, 2012). The genes regulating hyphal fusions have been identified and the proteins encoded by these genes have led to the exciting discovery of the striatin-interacting phosphatase and kinase (STRIPAK) complex (Hwang and Pallas, 2014; Teichert *et al.*, 2014a). The present review discusses the relationship between hyphal fusions and mating type genes in two filamentous basidiomycetes, *Coprinopsis cinerea* and *Schizophyllum commune*, in which the genetics and molecular bases of sexual reproduction are well known (Casselton and Olesnicky, 1998; Kües, 2000; Casselton, 2008; Fowler and Vaillancourt, 2007; Raudaskoski and Kothe, 2010) and have activated research on sexual reproduction in other filamentous basidiomycetes. In addition, similarities between genes and proteins known to play a central role in filamentous ascomycetes at hyphal fusions and sexual reproduction are sought in *C. cinerea* and *S. commune*.

2. Mating-type genes in Agaricomycetes

In *C. cinerea* and *S. commune*, mating is regulated by a tetrapolar mating-type system consisting of two unlinked genetic complexes, named *A* and *B*. The term tetrapolar is due to the occurrence of four possible interactions in matings between haploid strains originating from spores formed on the fruiting bodies of the same parent dikaryon. A fully compatible interaction occurs when both *A* and *B* between the mates are of different specificity, an incompatible interaction when both complexes have the same specificity, and two semicompatible interactions, when either *A*- or *B*-regulated development is turned on due to differences in either *A* or *B* complexes (Fig 1). In both fungi, genetic studies indicated that the *A* complex in *C. cinerea* and the *A* and *B* complexes in *S. commune* consisted of two linked subloci, α and β, tightly linked in the *C. cinerea A* complex (Day, 1960) and more loosely in *S. commune* (Raper, 1966). In both species, *A* and *B* are multiallelic and

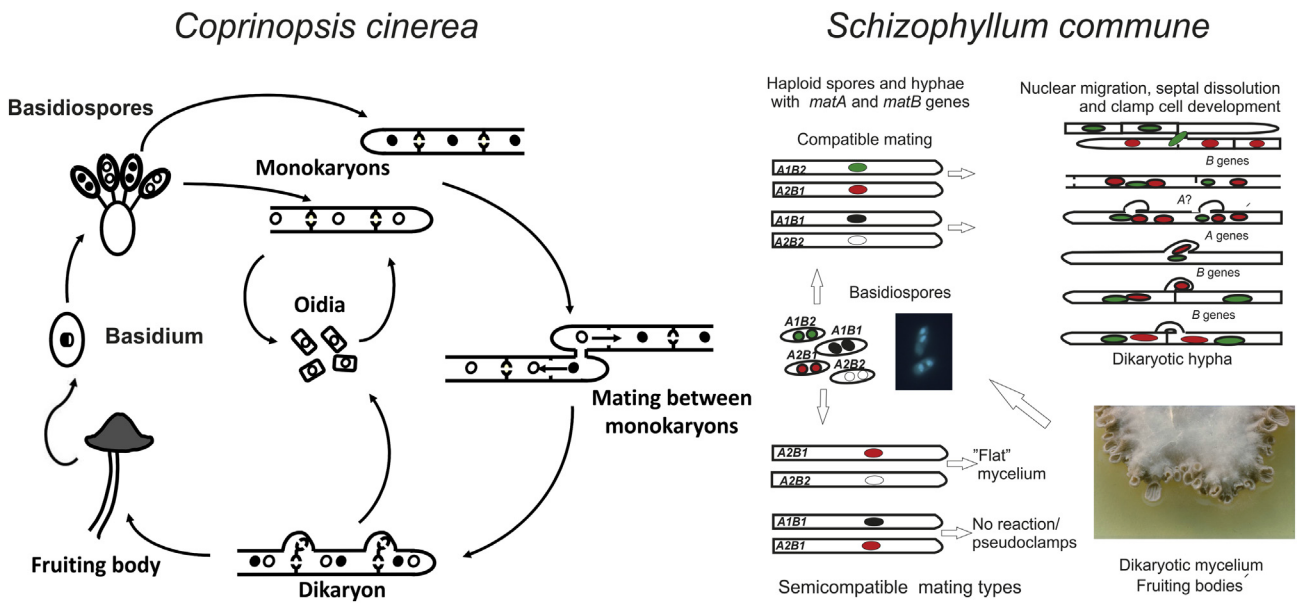

Fig. 1 – Life cycles of *Coprinopsis cinerea* (left) and *Schizophyllum commune* (right). Note the asexual spores, oidia, produced constitutively in abundant numbers by haploid (monokaryotic, homokaryotic) hyphae and light-induced in low numbers by dikaryotic hyphae in the *C. cinerea* life cycle (Kües, 2000). Asexual spores are not common to filamentous basidiomycetes. The role of *matA* and *matB* mating-type genes regulating the sexual reproduction in both species is emphasized in the *S. commune* life cycle.

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the mates are compatible if one of the two subloci in *A* and *B* complexes has different specificities.

In the nineties, the molecular structure of the *A* and *B* mating type genes was revealed side by side in *C. cinerea* and *S. commune*, first the *A* complex and quite soon thereafter the *B* complex genes. The successful cloning of regions including the *A* mating-type genes was facilitated by chromosome walking from auxotrophic marker genes known from previous genetic studies to be located close to the *A* mating type genes (Giasson *et al.*, 1989; Mutasa *et al.*,1990). The *B* mating type genes of *S. commune* were detected by transformation with DNA from a plasmid library with a specific *S. commune B* mating-type into a *B* compatible strain to active *Bα*- and *Bβ* regions, which was observed by occurrence of *Bon* hyphal morphology typical to *S. commune* (Specht, 1995). A modified system including the use of genomic subtraction technique and mating type assays led to discovery of *B* mating type genes in *C. cinerea* (O'Shea *et al.*, 1998).

Molecular structure of B *mating type genes*

The *B* mating-type genes are presented here first, since their activity initiates mating, although both historically and genetically the structure and function of *A* mating type genes was discovered first. In *C. cinerea* and *S. commune* the *B* mating-type gene complex was shown to consist of genes encoding G protein-coupled receptors (GPCRs) and small pheromone peptides (Wendland *et al.*, 1995; Vaillancourt *et al.*, 1997; O'Shea *et al.*, 1998; Fowler *et al.*, 1999; Riquelme *et al.*, 2005). The GPCR consists of seven transmembrane domains with short extracellular and long intracellular parts able to associate with the α-subunit of a heterotrimeric G-protein. This receptor type shares homology to the *Saccharomyces cerevisiae* Ste3 mating type receptor. Close to the receptor encoding sequences in *C. cinerea* and *S. commune* occur small open reading frames encoding polypeptides of 53–72 amino acids in length. Each polypeptide appears to end with a terminal CAAX (cysteine, aliphatic, aliphatic and any residue) motif. From the precursor amino acid sequences of yeast **a**-factor and *Ustilago maydis* pheromones (Bölker *et al.*, 1992) it was known that fungal pheromones end with a CAAX motif. At pheromone maturation, the CAAX motif is post-translationally modified by cleavage of the terminal three amino acids and by farnesylation and carboxymethylation of the terminal cysteine. In pheromone precursors, there are two highly conserved amino acids ER/DR, 14–16 residues from the C terminus which represents a proteolytic cleavage site in pheromone maturation leading to lipopeptides of 11–13 amino acids in size, as in yeast and other fungal species (Caldwell *et al.*, 1995). It is noteworthy that mating interaction can only take place between pheromones and receptors from different *B* loci (Brown and Casselton, 2001).

The organization of *B* mating type gene complexes differs in *C. cinerea* and *S. commune*. The sequencing data from the 13 *B* complexes known from genetic analyses indicated that the *C. cinerea B* mating-type locus consists of three closely linked gene groups. Each group of genes comprises a receptor gene and one to four pheromone genes and is represented by several alleles, group 1 by two, group 2 by five and group 3 by seven alleles (Halsall *et al.*, 2000; Riquelme *et al.*, 2005). The sequencing of individual *S. commune Bα1* (Wendland *et al.*, 1995) and *Bβ1* (Vaillancourt *et al.*, 1997) subloci and the total *S. commune* genome (Ohm *et al.*, 2010a) have revealed that each sublocus (locus) consists of a receptor gene and several pheromone genes. In the sequenced H4-8 strain the distance between the *Bα3* and *Bβ2* loci is about 7 kb. In addition, the sequencing revealed three pheromone receptor-like genes in the surrounding of the *Bα3* and *Bβ2* loci and one elsewhere in the genome.

The knowledge of the *B* locus structure in *C. cinerea* and *S. commune* has facilitated its identification in tetrapolar basidiomycetes *Laccaria bicolor* (Niculita-Hirzel *et al.*, 2008), *Flammulina velutipes* (van Peer *et al.*, 2011), *Lentinula edodes* (Wu *et al.*, 2013) and *Pleurotus eryngii* (Kim *et al.*, 2014). In all these fungi, receptor and pheromone genes were identified in the *B* mating type locus. In *F. velutipes* and *L. edodes*, the *B* mating type genes are suggested to be in two subloci similarly to *S. commune*. In *F. velutipes*, one sublocus consists of a receptor and a pheromone gene and the other, at a distance of 177 kb, of a receptor and two pheromone genes. Similarly, in *L. edodes*, one receptor gene with two pheromone genes and another receptor gene and a pheromone gene 4.5 kb apart are interpreted to form the two subloci. In *P. eryngii*, the four identified genes for receptors and four pheromone genes are included in the same locus as are the three pheromone receptor genes and pheromone genes in *L. bicolor*, resembling the organization of the *C. cinerea B* locus. In all discussed species except for *P. eryngii*, genes encoding pheromone receptor like proteins without a pheromone encoding sequence in proximity were detected. These genes occur either in or at close proximity to the *B* locus as in *L. edodes* or in distance from the *B* locus as in *F. velutipes* and *L. bicolor*.

Molecular structure of A *mating type genes*

In *C. cinerea* the *A* mating type complex is divided into two tightly linked subloci consisting of dissimilar homeodomain transcription factor genes *HD1* and *HD2*, one pair in the *α*-sublocus and two pairs in the *β*-sublocus (Kües *et al.*, 1992, 1994; Casselton and Olesnicky, 1998). With sequencing data available, the terms *Aα* and *Aß* were replaced with a terminology of *a*, *b*, and *d* gene pairs within *A*. Presently, the *A* locus has been visualized to be formed by three groups of genes, each consisting of a dissimilar homeodomain transcription factor gene pair *HD1* and HD2 (Brown and Casselton, 2001). Not all genes are present in the different *A* loci (Casselton and Olesnicky, 1998), only one *A* locus has all six genes (Pardo *et al.*, 1996). In the sequenced *S. commune* H4-8 strain (Ohm *et al.*, 2010a) the *Aα4* locus consists of a *HD1* and *HD2* pair, encoded by genes *aaz4* and *aay4*, respectively (Specht *et al.*, 1992; Stankis *et al.*, 1992). The *Aβ6* locus (Shen *et al.*, 1996), 550 kb apart from *Aα4*, contains at least six homeodomain transcription factor genes, four *HD1* and two *HD2* genes (Ohm *et al.*, 2010a). The sequencing data shows that the *A* mating-type locus in the model fungi *C. cinerea* and *S. commune* is far more complex than predicted by classical genetics.

As with the receptors and pheromones of the *B* mating type locus, the *A* mating type genes show similarities with the yeast *S. cerevisiae* mating-type genes. The HD1 and HD2

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proteins resemble the α2 and **a**1 mating type proteins of yeast, respectively (Casselton and Olesnicky, 1998). In addition to the DNA binding domains in both proteins, HD1 carries nuclear localization signals and an activation domain. The N-terminal parts of HD1 and HD2 carry dimerization motifs and heterodimerization of HD1 and HD2 from different *A* loci is suggested to lead to a transcription factor complex that regulates the genes in the *A* mating type pathway (Banham *et al.*, 1995; Asante-Owusu *et al.*, 1996; Asada *et al.*, 1997; Spit *et al.*, 1998; Brown and Casselton, 2001).

The screening of the *L. bicolor* genome with *C. cinerea* homeodomain transcription factor encoding genes led to the detection of respective HD1 and HD2 protein encoding genes, which are divergently transcribed and with products of about the same domain structure as in *C. cinerea* HD1 and HD2 proteins (Niculita-Hirzel *et al.*, 2008). This already suggested that the *A* mating type genes have a conserved structure in basidiomycetes. This view has recently been confirmed by very similar organization and structure of the genes in *L. edodes A* mating type loci (Au *et al.*, 2014). In *F. velutipes*, the *A* locus structure is less clear and can only be explained by reorganizations in the locus. Three homeodomain transcription factor genes were detected in two subloci (van Peer *et al.*, 2011). One sublocus contains the genes for a conserved HD1-1 and HD2-2 protein pair while another gene for an HD2-1 protein is present at the end of a 70 kb fragment. Around the retained *HD1-1* and *HD2-2* gene pair a high number of genes, including *mip-1* and *betafg* genes usually recognized next to the *A* locus, show synteny with genes in *C. cinerea*, *L. bicolor* and *S. commune* when a 70 kb inversion on both sides of the retained homeodomain transcription factor gene pair is taken in account. The inversion on the right side has probably transferred the homeodomain transcription factor gene *HD2-1* to a distance of 70 kb from the retained *HD2-2 HD1-1* homeodomain transcription factor gene pair.

Evidence for mating type gene functions

Transformation and screening the transformants by mating interactions has played a central role in confirming that cloned *C. cinerea* and *S. commune A* and *B* mating type genes are involved in regulating the mating (Kües *et al.*, 1994; Wendland *et al.*, 1995; Shen *et al.*, 1996; Vaillancourt *et al.*, 1997; O'Shea *et al.*, 1998; Riquelme *et al.*, 2005). *Agrobacterium*-mediated transformation was recently applied for screening the *B* mating type genes in *P. eryngii* (Kim *et al.*, 2014). Furthermore, the detailed analysis of well-known *C. cinerea* and *S. commune* mutant strains with self-activated *B* pathway demonstrated that mutations in receptor encoding genes in *C. cinerea* (Olesnicky *et al.*, 1999, 2000) and in *S. commune* pheromone encoding genes (Fowler *et al.*, 2001) were the reason for the activation of the *B* pathway and the exceptional mating behavior. A very useful strain for testing pheromone and receptor specificities and interactions has been the *S. commune B*-null mutant with no *Bα*- or *Bβ*-specific genes (Fowler *et al.*, 2004).

The idea that the function of the multiallelic *B* locus with receptor and pheromone genes is related to the well-characterized yeast pheromone response pathway was confirmed by expressing pheromones and receptors from *C. cinerea* (Olesnicky *et al.*, 1999, 2000) and from *S. commune* (Fowler *et al.*, 1999, Hegner *et al.*, 1999) in the yeast *S. cerevisiae*. The interaction of a *S. commune* pheromone produced by **a**-factor-deficient yeast MAT**a** cells with *ste3* mutant MATα cells expressing a *S. commune* receptor gene elicited the activation of the reporter systems for the endogenous pheromone response pathway in the yeast (Fowler *et al.*, 1999). The incubation of a haploid yeast strain carrying a wild type *C. cinerea* receptor with synthetic pheromone peptides of different lengths, and with farnesylated and carboxymethylated C terminus gave the best result when the synthetic pheromone was 12 amino acids long and had the ER motif, the site of assumed proteolytic cleavage, at the N-terminus (Olesnicky *et al.*, 1999). In *C. cinerea*, the experiments with fungal wild type and mutant receptors and synthetic wild-type and mutant pheromones in yeast and in fungal matings indicated that a single amino acid change in either the pheromone or receptor belonging to a specific gene group of the *B* locus can lead to self-compatibility and to the expression of the *B* pathway in a homokaryotic strain (Olesnicky *et al.*, 2000). The same result was reached with screening different *Bβ2* mutant strains and chimeric receptors in *S. commune* (Gola *et al.*, 2000; Fowler *et al.*, 2001).

3. A and B regulated morphogenetic pathways

In a compatible mating, hyphal fusions (Fig 2b–f, g–i) are established along with the reciprocal nuclear exchange and migration from one haploid mate into the other. Disruption of septa (Fig 2j) facilitates the reciprocal nuclear migration which changes the haploid sterile mates with uninucleate cells (monokaryons or homokaryons) into a fertile dikaryon with the two haploid nuclear genotypes in each binucleate hyphal cell (Raudaskoski and Kothe, 2010). The dikaryotic condition is maintained by conjugate division associated with a clamp connection formation at each division (Figs 3 and 4). It is noteworthy, that the conjugate division alone without the clamp connection formation is sufficient to maintain the dikaryotic condition in several basidiomycetes (Salo *et al.*, 1989). By comparing the semicompatible matings it is possible to conclude that the different pathways are regulated by genes in *A* and *B* complexes.

After hyphal fusions, septal disruption and reciprocal nuclear exchange and migration is observed in *S. commune* at matings with dissimilar *B* but similar *A* specificities. The function of the *B* pathway is observed as "flat" morphology suggesting that the interaction of *B* genes, pheromones with receptors, regulates these events. The *Bon* mutant with a single amino acid mutation in a pheromone gene (Fowler *et al.*, 2001) is also characterized by the "flat" phenotype including septal breakdown and nuclear movements from one hyphal compartment to the other as in a mating with different *B* specificities (Koltin and Flexer, 1969; Raudaskoski,1984, 1998). In addition the *Bon* mutant has unilateral mating behavior with wild-type strains only donating but not accepting nuclei. It is noteworthy, that in *C. cinerea* matings with different *B* specificities or mutations in the *B* locus (Casselton *et al.*, 1971; Haylock *et al.*, 1980; Olesnicky *et al.*, 1999, 2000) do not cause such a dramatic "flat" phenotype as observed in *S. commune* matings

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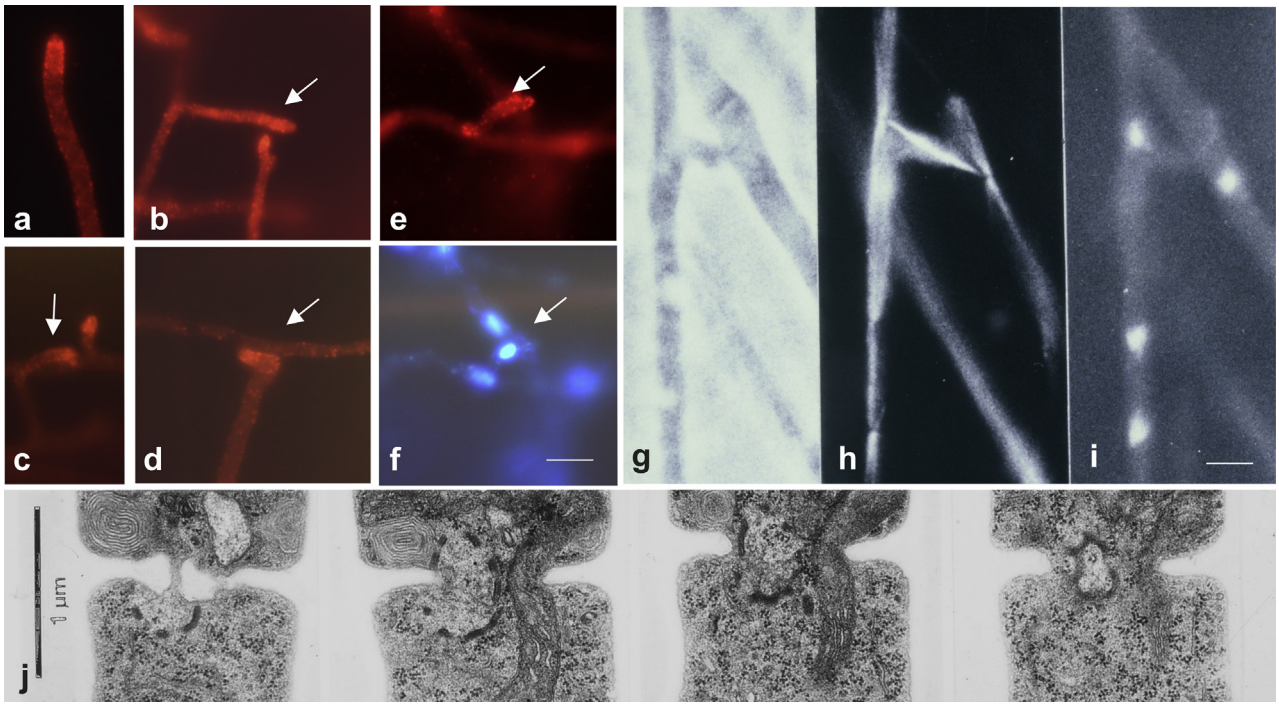

Fig. 2 – Hyphal fusions between compatible mates (b–i) and septal break down in a migration hypha (j) visualized by indirect immunofluorescence (IIF) microscopy of actin (a–e), and tubulin (h), DAPI (4′,6-diamidino-2-phenylindole)-staining of the nuclei (f and i) and with light (g) and electron microscopy (j) in *Schizophyllum commune*. (a) Homokaryotic hypha with strong actin signal at the tip. (b–e) Arrow points to the strong actin signal at hyphal tips either growing towards (b–c) or along (d) a hypha originating from a compatible mate. (e–f) A hyphal fusion by a contacting branch (arrows) containing a nucleus (f). (g–i) Phase contrast microscopy of a hyphal fusion (g) with the spindles and astral microtubules of a dividing nucleus in the fusion bridge and in the hypha at left, visualized with tubulin IIF microscopy (h), and DAPI staining of the telophase nuclei in the same hyphae (i). (j) The ultrastructure of a dissolved septum in a migration hypha shown by four serial sections. Bars 10 μm in (f) and (i) for images (a–f) and (g–i), respectively.

with different *B* specificities or in the *Bon* mutant. *C. cinerea B* mutants have uninucleate hyphal compartments and exhibit normal bilateral mating (Haylock *et al.*, 1980). Only at the last step in dikaryotization, at the clamp cell fusion, a mutant *B* gene is expressed allowing the clamp cell fusion to take place in a mating with different *A* specificities and the *B* mutant gene in both partners (Haylock *et al.*, 1980). This proves that the pheromone response pathway controls the clamp cell fusion, but it also suggests that the *B* pathway in *S. commune* could activate a cellular metabolism which is not typical for other basidiomycetes (Haylock *et al.*, 1980). More recently it has been observed that the transformation of a *C. cinerea* homokaryon with cloned *B* mating type genes of different specificity than of the genes residing in the homokaryon activates the *B* pathway causing different degrees of "flat" morphology depending on the strain and culture conditions used. Moreover, the acceptance of nuclei is blocked in transformants (Kües *et al.*, 2002).

In matings with different *A* and *B* complexes, clamp cell formation takes place with synchronous division of two nuclei with different genotypes (Fig 3a–h). A septum is formed at the base of the clamp and in the hypha below the clamp (Raper,1966; Brown and Casselton, 2001; Raudaskoski and Kothe, 2010, Fig 3h). HD1 and HD2 proteins encoded by different allelic versions of a gene group in the *A* locus are suggested to form through heterodimerization transcription factors to regulate target genes in the pathway of clamp cell formation (Brown and Casselton 2001). At matings with only different *A* complexes, hyphae with clamp cells are formed at the site of junction of the mated strains. In a fully compatible mating with the septal dissolution and extensive mobility of nuclei the *A* gene products may also be necessary for repressing the activity of the *B* pathway on septal dissolution and nuclear migration. The last step of clamp connection development requires the activity of *B* genes for the fusion of the clamp cell tip with the subapical cell and the movement of the nucleus from the clamp into the subapical cell both in *S. commune* and *C. cinerea* (see above).

4. Hyphal fusions

It has been long known, that in *C. cinerea* (Smythe, 1973) and *S. commune* (Ahmad and Miles, 1970) hyphal fusions (anastomoses), occur in homokaryptic and dikaryotic colonies as well as in colonies with the same *A* or *B* mating type specificities, therefore the mating-type genes are not considered

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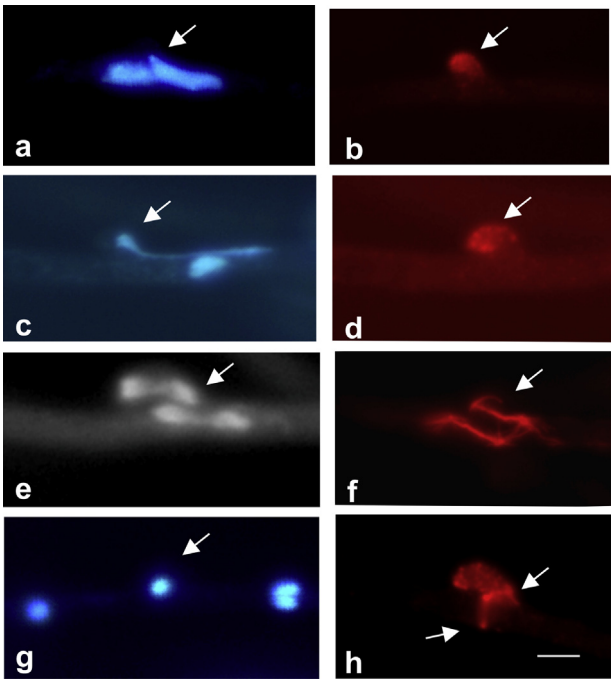

Fig. 3 – Nuclei visualized by DAPI staining (a, c, e, g), and with IIF microscopy of actin (b, d, h) and tubulin (f) at developing clamp connections in *Schizophyllum commune*. (a and c) Two examples of a differently stretched nucleus moving from the hypha into a developing clamp cell (arrows), the other nucleus of the dikaryotic pair is seen in both cases in the hypha. (b and d) Actin localization (arrows) at the tips of the developing clamp cells in (a) and (c). (e, f) Conjugate division. Anaphase nuclei (e) and the spindels (f). (g) Four nuclei are seen after conjugate division, one nucleus in the subapical cell, one in the clamp cell (arrow) and two close to each other in the apical cell. (h) The same hypha as in (g) with actin fluorescence in the developing septa in the hypha (arrow at left) and at the base of the clamp cell (arrow at right). Parallel images of clamp cell development in *C. cinerea* are presented in Kamada *et al.*, (1993) and in Tanabe and Kamada (1994, 1996). Bar 10 μm in (h) applies for all images.

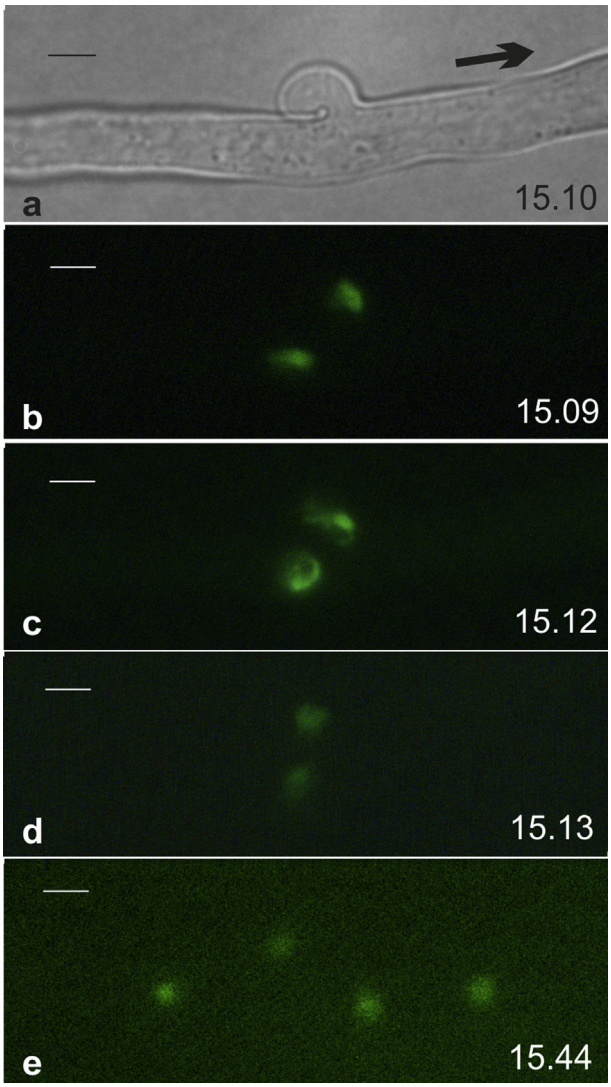

Fig. 4 – Light microscopy of a dikaryotic hypha (a) and fluorescence microscopy of histone 2B-EGFP expression in nuclei (b–e) during conjugate division in *Schizophyllum commune*. (a) Light microscopy of the hypha with a developing clamp cell. The black arrow points to the growth direction of the hypha. (b–d) Images at minute intervals of the dividing nuclei in the clamp cell and hypha. (e) Four telophase nuclei. The phase contrast microscopy image (a) was taken between fluorescence images in (b) and (c). Bar 10 μm.

to play a role before hyphal fusions (Vaillancourt *et al.*, 1997; Casselton and Olesnicky, 1998; Olesnicky *et al.*, 1999). However, in wild type strains hyphal fusions are indispensable for allowing the interaction of mating type genes with different specificities. Several different types of hyphal fusions have been described in *S. commune* compatible, semi-compatible and incompatible mating interactions (Raudaskoski, 1973, 1998). A distinguishable feature among the different types was the tighter association between hyphae when strains with different *B* mating type genes came in contact with each other (Fig 2c–f). In the same matings, less tight hyphal attachments occurred at fusions as well. The continuation of microtubules through the contact site or the presence of a spindle (Fig 2g–i) in the bridge between the hyphae proved that there was an opening between the confronted hyphae (Raudaskoski, 1998).

During last years the cell and molecular biology of hyphal fusions in filamentous ascomycetes *N. crassa* and *Sordaria macrospora* have been investigated during both vegetative growth and sexual reproduction (Read *et al.*, 2012; Bloemendal *et al.*, 2012; Dettmann *et al.*, 2013). Genes regulating hyphal fusions have been identified and the proteins encoded by these genes have led to the exciting discovery of the striatin-interacting phosphatase and kinase (STRIPAK) complex previously known from human cells (Goudreault *et al.*, 2009; Kean *et al.*, 2011; Hwang and Pallas, 2014). The possibility that STRIPAK complex proteins are involved in intercolonial hyphal fusions and mating related hyphal fusions in filamentous basidiomycetes deserves attention.

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Regulation of hyphal fusions in filamentous ascomycetes

In *N. crassa* two types of vegetative hyphal fusions occur, conidial anastomoses tubes (CATs) consisting of specialized hyphae that develop between genetically identical germlings (Roca *et al.*, 2005; Read *et al.*, 2012) and intracolonial cell-to cell fusions in the subapical part of vegetative colonies (Xiang *et al.*, 2002; Fu *et al.*, 2011). Fusions between genetically identical germinating conidia and in/or between colonies identical in heterokaryon incompatibility (*het*) loci create an interconnected hyphal network, while hyphal fusions between "heterokaryon incompatible" isolates leads to self-destruction (Marek *et al.*, 2003). Chemotrophic interaction, different from pheromone receptor interaction in sexual reproduction, has been shown to direct the growth of CAT tips towards each other and to activate MAP kinase pathways. The conserved mitogen-activated protein kinase (MAPK) cascade, consisting of NRC-1 (a MAPK kinase kinase), MEK-2 (a MAPK kinase) and MAK-2 (a MAPK), is essential for the formation of CATs. During chemotropic interactions, MAK-2 and SO (SOFT,HAM-1) proteins show a highly coordinated, oscillatory expression at CAT tips (Pandey *et al.*, 2004; Fleißner *et al.*, 2005, 2009; Leeder *et al.*, 2011; Read *et al.*, 2012). The mutant *soft* (*so/ham-1*) gene, which encodes a conserved protein of unknown molecular function, is deficient in chemotropic interactions. In the filamentous ascomycete *S. macrospora*, closely related to *N. crassa*, the protein PRO40 homologous to the *N. crassa* SOFT (SO/HAM-1) protein was shown to function as a scaffold protein for kinases PKC1, MIK1 and MEK1 in the MAK-1 pathway regulating the cell wall integrity (CWI) (Teichert *et al.*, 2014b). The deletion of the genes in the MAK-1 pathway in *N. crassa* leads to the absence of CATs (Maerz *et al.*, 2008). In screening the genome-wide deletion library of *N. crassa* for hyphal fusion mutants, fourteen genes required for hyphal fusions but also for CATs were identified (Fu *et al.*, 2011a). Six of the fourteen genes belonged to MAP pheromone response (MAK-2) and cell wall integrity (MAK-1) pathways known to be required for CATs but also for the vegetative hyphal fusions (Maerz *et al.*, 2008). In addition, the deletion mutants of the *ham-1/so* to *ham-5* (hyphal anastomoses) genes and the *mob3* gene also previously identified as inhibitors of CAT fusions (Read *et al.*, 2012) were shown to inhibit hyphal fusions as well. The screening of the deletion library led to include *rac-1* and *pp2A* genes among the ones necessary for both CATs and vegetative hyphal fusions (Fu *et al.*, 2011a). Recently, it was shown that RAC-1 is specifically required for CAT formation in *N. crassa* (Lichius *et al.*, 2014).

Striatin-interacting phosphatase and kinase (STRIPAK) complex

Several proteins encoded by the genes required for CATs and vegetative hyphal fusion in *N. crassa* showed homology with proteins forming the striatin-interacting phosphatase and kinase (STRIPAK) complex in the ascomycete *S. macrospora* (Bloemendal *et al.*, 2012), in the ascomycetous yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* and in animal cells (Goudreault *et al.*, 2009; Kean *et al.*, 2011; Hwang and Pallas, 2014, Teichert *et al.*, 2014a). HAM-2 represents STRIP1/2, and HAM-3 striatin (phosphatase B‴ subunit) proteins, HAM-4/SLMAP is a protein with fork head domain and MOB3 a phocein. These proteins were identified to form a STRIPAK complex in *S. macrospora* together with protein phosphatase PP2A scaffold protein A and catalytic subunit PP2A-C (Bloemendal *et al.*, 2012). In *N. crassa* GFP-trap affinity purification coupled to mass spectrometry experiments with HAM-2, HAM-3 and HAM-4 proteins as well as with MOB3 and PPG-1 (PP2Ac) showed that *N. crassa* proteins also associate into a STRIPAK complex (Dettmann *et al.*, 2013). Strong interactions existed between HAM-3/HAM-2 and HAM-3/MOB3 and less tight between HAM-3/HAM-4, HAM-3/PP2A-A and HAM-3/PPG-1 (PP2Ac). Different combinations of STRIPAK complex proteins marked with fluorescence reporter proteins visualized the STRIPAK complex assembled at the nuclear envelope. The analysis of the STRIPAK complex in human HeLa cells also suggested that the complex was bound to the nuclear envelope at interphase with the hydrophobic part of the HAM-4/SLMAP protein (Frost *et al.*, 2012).

Furthermore, the *N. crassa* STRIPAK complex was shown to interact with the MAK-1and MAK-2 protein kinase pathways (Dettmann *et al.*, 2013). Importantly, MAK-2 kinase was shown to phosphorylate the N-terminus of MOB-3, which could lead, with the help of HAM-2 and HAM-3 associations to the transport and accumulation of MAK-1 kinase into the nucleus to activate the CWI pathway regulating transcription factors. *N. crassa* hyphal fusion mutants also include the genes encoding transcription factors ADV-1, ADA-3, RCO-1, SNF5 and PP1 (Fu *et al.*, 2011a, 2014; Leeder *et al.*, 2013). These transcription factors are possibly needed to activate genes involved in cell communication and hyphal growth in CATs and hyphal fusions (Glass *et al.*, 2004). Presently about 50 proteins are known to be involved in cell to cell communication and fusion in *N. crassa*, but the three major signaling modules regulating the development of CATs and hyphal fusions appear to be MAK-1 and MAK-2 kinase pathways and the STRIPAK complex (Fu *et al.*, 2014).

STRIPAK complex and mating related hyphal fusions

The HAM-2, HAM-3, HAM-4 and MOB-3 proteins as well as the catalytic sub-unit protein PP2Ac and the scaffold protein PP2A-A have their homologues in the *C. cinerea and S. commune* genomes (Table 1). Only for the SOFT/HAM-1/SO protein no homologous protein is detected. The six homologs to STRIPAK complex proteins are consistent with the idea that a STRIPAK-like complex could also regulate hyphal fusions of filamentous basidiomycetes. The role of mating related hyphal fusions, in the absence of specialized structures for sexual reproduction, would then be to bring the hyphae together for the receptor—pheromone interaction of the *B* pathway. An interesting question is, then, what is the relationship between the STRIPAK complex and the pheromone response signaling pathway in filamentous basidiomycetes?

Dissecting the interrelationship between the STRIPAK pathway and the sexual reproduction in *N. crassa* may provide some help, although the early stage of sexual reproduction in the heterothallic *N. crassa* takes place between specific hyphal structures and is clearly separated from the hyphal fusions. Mating is dependent on pheromones and receptors produced by *matA* and *mata* cells (Kim and Borkovich, 2006; Kim *et al.*,

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Table 1 – Putative STRIPAK homologues in the filamentous basidiomycetes *S. commune*, *C. cinerea* and ascomycetes. The STRIPAK complex proteins of ascomycetes used in the screening for the homologous proteins in *S. commune* and *C. cinerea* are shown. The model for Table 1 is from Bloemendal *et al.* (2012). The protein identity numbers are from the *S. commune* genome sequence H4-8 v3.0 (http://genome.jgi-psf.org/Schco3/Schco3.home.html) and the *C. cinerea* genome sequence (http://www.broadinstitute.org/annotation/genome/coprinus_cinereus/MultiHome.html).

S. commune	*C. cinerea*	*N. crassa*	*S. macrospora*	*S. cerevisiae*	*S. pombe*
Striatin Schco3_81259	Striatin CC1G_05176.3	Ham-3	PRO11	Far8	Csc3p
Strip1/2 Schco3_2599392	Strip1/2 CC1G_00049.3	HAM-2	PRO22	Far11	Csc2p
PP2A-A Schco3_2611649	PP2A-A CC1G_11311.3	PP2AA	SmPP2AA	Tpd3	Paa1p
PP2Ac Schco3_2739215	PP2A CC1G_03234	PP2Ac	SmPP2Ac	Ppg1	Ppa3p
Mob?/phocein Schco3_2625253	Mob?/phocein CC1G_09564.3	MOB-3	SmMOB3	–	–
SLMAP Schco3_2645915, Schco3_2642385	SLAMP CC1G_07331.3 CC1G_12024.3	HAM-4	PRO45	Far9/10	Csc1p

2012). Cell fusion occurs between a specific filamentous hypha, the trichogyne, originating from the protoperithecium, the female reproductive structure and the very early stage in fruiting body development, and a microconidium. The latter represents the male part and attracts the trichogyne probably by secreting a pheromone. The SOFT/HAM-1/SO protein was clearly shown to be unnecessary for fertilization but the development of fruiting bodies (perithecia) was disturbed in strains carrying the mutant gene (Fleißner *et al.*, 2005). The mutations in the members of MAK-2 and MAK-1 kinase pathways cause also female sterility (Pandey *et al.*, 2004; Maerz *et al.*, 2008) and the deletion mutants of STRIPAK complex proteins also impair the perithecium development in *S. macrospora* (Bloemendal *et al.*, 2012) perhaps due to the requirement of cell fusions at this developmental stage (Fu *et al.*, 2014). These observations suggest that the same proteins are functioning at hyphal fusions and at the late stage of the sexual reproduction in filamentous ascomycetes.

Pheromone response pathway proteins

The pheromone response pathway in filamentous basidiomycetes is suggested to consist of a heterotrimeric G protein, Ste20 kinase, MAK-2 pathway kinases Ste11, Ste7 and Fus3/Kss2 and the Ste12 transcription factor (Raudaskoski and Kothe, 2010). The pheromone response pathway is assumed to follow the pattern in yeast *S. cerevisiae*. Support to this idea is not only coming from structural similarity in receptor and pheromone structures to those in yeast, but also from activation of the pheromone response pathway by expressing *C. cinerea* and *S. commune* pheromone and receptor genes in *S. cerevisiae* (Fowler *et al.*, 1999; Hegner *et al.*, 1999; Olesnicky *et al.*,1999) as discussed earlier.

The first down-stream step in the activation of the pheromone response pathway is the interaction of the ligand binding receptor with the Gα subunit of the heterotrimeric G protein (Raudaskoski and Kothe, 2010). Filamentous fungi have several Gα subunits and in *N. crassa*, the pheromone receptors signal through the Gα protein GNA-1 (Kim and Borkovich, 2004; Kim *et al.*, 2012). Mutations in the *N. crassa* heterotrimeric G protein, necessary for sexual development had no effect on vegetative fusions while they effectively blocked the sexual development (Fleißner *et al.*, 2008). With regard to filamentous basidiomycetes, it is still unclear which of the putative Gα subunits is involved in the pheromone response pathway. In the smut *U. maydis* it has clearly been shown that the Gα subunit Gpa3 is involved in the mating response (Regenfelder *et al.*, 1997) and the binding of five C-terminal amino acids from this subunit to the yeast GPA1 protein improved the *C. cinerea* pheromone-receptor interaction in yeast (Olesnicky *et al.*, 1999). These results are consistent with a requirement for the appropriate Gα subunit to interact with the ligand-bound receptor. Dissociation of the receptor-associated heterotrimeric G protein leads to release of Gβγ for further activation of the signaling pathway leading, in *S. cerevisiae*, to a requirement of scaffold protein Ste5 to the plasma membrane for interaction with the Ste20 kinase and MAP kinase cascade activation (Merlini *et al.*, 2013).

No clear homolog to the Ste5 scaffold protein exists in filamentous fungi, although for another yeast adaptor protein, Ste50, also belonging to the yeast pheromone response pathway (Truckses *et al.*, 2006), orthologs are found. Ste50 protein is detected in filamentous ascomycetes, in *N. crassa* (NCU0045), in the basidiomycete smut *U. maydis* (Klosterman *et al.*, 2008) and in the basidiomycetous yeast *Cryptococcus neoformans* (Jung *et al.*, 2011) as well as in *C. cinerea* (Klosterman *et al.*, 2008; Jung *et al.*, 2011; Nakazawa *et al.*, 2011a) and *S. commune* (Raudaskoski and Kothe, 2010). At the N-terminus of Ste50, there is first a SAM domain for protein–protein interactions and then an RA domain for interaction with Ras proteins (Truckses *et al.*, 2006). Two basidiomycete specific SH3 domains are at the C-terminus of the latter four species. The *U. maydis* yeast two-hybrid analyses proved, that the Ste50 (Ubc2) SAM domain is able to interact with the Ste11 (Ubc4) kinase and that the C-terminal SH3 domains are required for pathogenicity (Klosterman *et al.*, 2008). In *C. neoformans*, Ste50 is required exclusively for mating interaction with no virulence or stress response (Jung *et al.*, 2011). Another study (Fu *et al.*, 2011b) on the *C. neoformans* Ste50 protein indicated interaction between the Ste50 and Ste11 SAM domains and with the N-terminus of Ste20 in the yeast two-hybrid system.

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Genetic studies placed Ste50 as a protein between Ste20 and Ste11 kinases suggesting a central role in the pheromone response pathway, however, leaving unanswered the connection of the Ste7 and Fus3/Kiss2 kinases to this complex. In *C. cinerea*, the Ste50 protein is named Cc.Ubc2 and is suggested to be involved in nuclear migration for dikaryosis and in the fusion of the clamp cell to the subapical cell, since the *Cc.ubc2-1* mutation blocks both these pheromone responsive pathways (Nakazawa *et al.*, 2011a).

Transcription factors Ste12 and MCM1

The target for the mating-type-specific pheromone response signaling cascade in yeast is the transcription factor Ste12. Putative homologues to this yeast protein have been characterized in several filamentous ascomycetes (Wong Sak Hoi and Dumas, 2010) and can be characterized in *C. cinerea* (Ste12alpha, XP_001834471.2), *S. commune* (|Schco3|2638469|, XP_003027994.1) and other basidiomycetes (Chang *et al.*, 2001). The annotated Ste12-like proteins of *C. cinerea* and *S. commune* show about 50 % similarity to each other, and they have an STE domain for DNA binding. At the C-terminus there are two closely located C2H2 zinc finger domains specific for Ste12-like factors of filamentous fungi. In addition, one or two nuclear localization signals can be detected in each basidiomycete Ste12-like protein as well as few putative MAP kinase phosphorylation sites. In the N-terminal DNA-binding part a set of 10 amino acids is previously reported to be specific for fungal Ste12 proteins in ascomycetes (Wong Sak Hoi and Dumas, 2010). An almost identical amino acid sequence is also detected in the DNA binding domain of the *C. cinerea* and *S. commune* Ste12-like proteins.

In *S. cerevisiae* the FUS3/KSS1 phosphorylated Ste12 transcription factor activates, with the help of MCM1 transcription factor, expression of the genes for cell fusion (Park and Bi, 2007). In the ascomycete *S. macrospora* the MCM1 protein was identified to interact with STE12 and mating type SMTA-1 proteins (Nolting and Pöggeler 2006a, b). An MCMA protein has been annotated in *C. cinerea* (XP_001835736.2) and a putative one |Schco3|2492285| is identified in the *S. commune* genome. These proteins show 51 % overall identity but 100 % identity in their MADS box domains also with this part of the *S. macrospora* and *N. crassa* MCM1. Nuclear localization signals are present in the basidomycete proteins (unpublished observations).

The deletion of *ste12* had no influence on the vegetative growth and fruiting body formation but impaired the ascospore production in the self-fertile, homothallic *S. macrospora* (Nolting and Pöggeler, 2006b). Instead, the MCM1 protein deletion led to a pleiotropic phenotype with reduced vegetative growth and sexual sterility. In self-sterile heterokaryotic *N. crassa*, the deletion of *ste12* ortholog *pp1* results in slow growth, lack of hyphal fusions, and the mutant is female sterile, indicating that Ste12 is involved both in the regulation of vegetative growth and sexual reproduction in this fungus (Leeder *et al.*, 2013). No information of *mcm1* deletion is available from *N. crassa*. Out of the genes encoding proteins of the STRIPAK complex, only *mob3* and *pp2A* showed decrease in their expression in a *pp1* deletion strain of *N. crassa* (Leeder *et al.*, 2013). The different functions predicted for the Ste12 and MCM1 proteins in *S. macrospora* and *N. crassa* in vegetative growth and sexual reproduction emphasize the need to sequester the relationship between the pheromone response pathway and STRIPAK complex proteins. Presently, the genome sequence based annotation of homologous proteins is the only support in filamentous basidiomycetes for a functional STRIPAK complex and the pheromone response pathway. No biochemical evidence exists yet. However, the downstream target for these putative signaling pathways in filamentous basidiomycetes must be genes regulating cell cycle, septal dissolution, nuclear divisions and movements.

5. Regulation of cell cycle, septal dissolution, nuclear migration and clamp cell fusion in *C. cinerea* and *S. commune* matings

Cell cycle

Reciprocal nuclear exchange and migration between compatible mates leading to establishment of the dikaryotic mycelium requires new cellular processes not recorded during hyphal fusions. If the *S. cerevisiae* model is followed, these are the activation of the transcription factors Ste12/MCM1 to allow induction of mating-specific genes and cell cycle arrest. In yeast the activated heterotrimeric G protein also controls a second pathway branch that serves to repolarize growth in the direction of the mating partner, leading to shmoo formation (Kemp and Sprague, 2003). Cell cycle arrest has also been suggested to take place as a result of the pheromone response pathway in filamentous basidiomycetes (Fowler and Vaillancourt, 2007). In *S. cerevisiae*, the cell cycle arrest includes Far proteins and two types of cell cycle arrest activities occur, one regulated by the Far1 protein and the other by the complex consisting of the Far3, Far7, Far8, Far9, Far10 and Far11 proteins (Kemp and Sprague, 2003). Far1, Far3 and Far7 appear to be *S. cerevisiae* specific proteins and are missing from *C. cinerea* and *S. commune*. Other proteins induced by the pheromone response pathway and involved in cell cycle arrest are homologous to proteins of the STRIPAK complex. Far8 represents striatin (HAM-3) and Far11 STRIP1/2(HAM-2). Far9/Vps64 and Far10 are highly identical proteins and both detect the same proteins as HAM-4 in the screening of *C. cinerea* and *S. commune* genomes (Table 1). In *S. cerevisiae*, the Far protein complex prevents premature recovery from pheromone arrest, thus allowing the cells to conjugate before nuclear fusion takes place. In filamentous basidiomycetes, the nuclear fusion is not associated with the early steps of the pheromone response pathway. Old cell biological investigations on nuclear behavior in living fusing hyphae from compatible mates recorded dividing nuclei next to the fusion bridges (Lange, 1966; Bistis, 1970) as did the visualization of the spindles of dividing nuclei with tubulin antibodies at the hyphal fusions of compatible mates (Raudaskoski, 1998, Fig 2h).

Interestingly, in *S. cerevisiae* the Far complex was already suggested to localize to the nuclear membrane or membranes associated with the nuclear envelope (Kemp and Sprague, 2003) as the STRIPAK complex in *N. crassa* (Dettmann *et al.*, 2013). In the fission yeast, *S. pombe*, a SIN (septal initiation network) inhibitory phosphatase complex consisting of at

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least six proteins was identified and termed SIP. The complex contains three proteins related to the Far/HAM proteins: Far8p (Csc3, HAM-3, striatin), Far10p (Csc1p, HAM-4), and Far11p (Csc2p, Strip1/2), in addition to a new catalytic subunit of PP2A (Ppaa3p9), the scaffolding A-subunit (Paa1p) and a novel protein Ccs4p. The SIP complex binds at variable times to the nuclear SPBs (spindle pole body) and regulates the proper function of SIN (septal initiation network), the actomyosin ring assembly upon entry into mitosis and constriction, following the completion of anaphase (Singh *et al.*, 2011). The comparison of the function of complexes including Far proteins in *S. cerevisiae* and *S. pombe* has led to the suggestion that the STRIPAK complex may be functionally repurposed between different organisms (Frost *et al.*, 2012), an idea which deserves attention in dissolving the nuclear behavior as response to the pheromone signaling pathway in filamentous basidiomycetes. In this connection it is important to note that nuclear divisions in hyphae after fusion are followed by the synthesis of a new septum that is quickly dissolved (Bistis, 1970; Niederpruem, 1980).

Septal dissolution and migration hyphae

A common agreement exists regarding the necessity of septal dissolution for reciprocal nuclear exchange and migration during a compatible mating in filamentous basidiomycetes and its dependence on the *B* mating type regulated pheromone response pathway (Raper, 1966). Following the model presented for *S. cerevisiae* mating, the septal dissolution process would rely on the activation of the transcription factor Ste12 to allow the induction of mating-specific genes, which would be genes for hydrolytic enzymes necessary for breaking down the septa. Looking at the phase contrast microscopic investigations on living fusing hyphae from compatible mates it appears that septal dissolution could take place before the entry of the invading nucleus in the accepting hyphae (Lange, 1966; Bistis, 1970). Electron microscopic images of compatible or semicompatible matings with different *B* mating type genes show, in both *C. cinerea* (Giesy and Day, 1965; Casselton *et al.*, 1971) and in *S. commune* (Koltin and Flexer, 1969; Raudaskoski, 1972, 1973) broken septa in the fused hyphae (Fig 2j).

The biochemical investigation of cell wall structures in *S. commune* has shown that the major components of the cell wall are β-glucan (R-glucan) and chitin. In addition, the cell walls contain α-glucan (S-glucan) and proteins (Wessels, 1965). Enzymes hydrolyzing these compounds play an important role in breaking down the septa during mating interactions. In extracts from several homokaryotic mycelia, the β-glucanase activity was low, it was also low in extracts of heterokaryons with similar *B* mating type genes and in young dikaryotic mycelia. In an established mycelium with different *B* but similar *A* mating type genes, a 30-fold increase in the specific activity of intracellular β-glucanase was noted. The analyzed *Bon* mutant strain also had a high intracellular level of β-glucanase activity. These results (Wessels, 1966, 1969, 1971; Wessels and Niederpruem, 1967) led to the suggestion that β-glucanase and possibly some other enzymes function in the removal of the septal barrier prior to nuclear migration and that the synthesis of these enzymes is regulated directly by the mating type genes, especially by the *B* genes. It was further demonstrated through treatment of hyphal-wall preparations of *S. commune* with β-glucanase and chitinase that the septa of the hyphae disappeared, while the hyphal walls remained intact (Janzen and Wessels, 1970). This indicated that the septa are formed of chitin and β-glucan but α-glucan is missing. In the hyphal walls, α-glucan appears to be present and preserves the wall structure during septal dissolution.

It is interesting to ponder, whether the high enzymatic activity causing the septal break-down continues in the hypha as a consequence of continuous pheromone receptor interaction. In a *S. commune* model, with different *B* specificities in a hypha, the pheromone receptors are placed in the cell membrane and the secreted pheromones into the cell wall. The interaction between receptors and pheromones would take place leading to the activation of genes needed for septal dissolution when the nuclei with the different *B* specificities are closely located (Schuurs *et al.*, 1998). When the nuclei with different *B* specificities are apart, the pheromone response pathway is not activated. A *C. cinerea* diploid strain with alike *A* mating type but different *B* genes lacked septal dissolution as well as clamp fusions (Casselton *et al.*, 1971). This difference between the diploid mycelium and mycelium with different *B* genes (*B* heterokaryon) was thought to indicate that septal dissolution would not occur when the same cell has two different *B* alleles, but the nuclei with different *B* specificities should be localized on different sides of the septum for its dissolution. This model seeks its counterpart from the distribution of *B* genes during clamp cell fusion.

Already in the early stages of cell biological work, the hyphae with dissolved septa and an irregular number of nuclei were named migration hyphae (Raudaskoski, 1972, 1973). This phenotype is also typical for a *Bon* mutant (Raudaskoski,1984,1998). Counting the nuclear number during the development of the *Bon* mutant germlings indicated that at stage I, in germlings grown from 0 to 11 h, most cells were binucleate; at stage II from 17 to 55 h, uninucleate and at stage III from 58 to 69 h, the cells contained varied numbers of nuclei. The ratio of nuclei to cells remained constant during the transition from stage II to stage III. Changes in nuclear distribution results from the movement of the nuclei from cell to cell, and the movement is associated with the disruption of the dolipore septa (Koltin and Flexer, 1969). The experiments designed to observe the presence of migration nuclei in compatible matings indicated that in *S. commune*, the fertilizing nucleus moved in the accepting compatible mycelium with a speed of 0.5–3.0 mm h^{-1} in some cases 5.5 mm h^{-1} (Snider and Raper, 1958) and in *C. cinerea* with roughly the same speed (Kües, 2000). Much faster movements, 40 mm h^{-1}, were recorded in the young mycelium of *Coprinellus congregatus* but in the old mycelium the nuclear movement dropped to 1.25 mm h^{-1} (Ross, 1976). Unfortunately, *C. congregatus* is a bipolar species with no specific *B* mating type gene activity (Bastouill-Descollonges and Manachere, 1990).

Electron microscopic investigations in *S. commune* revealed microtubules around the interphase nuclei and astral microtubules radiating from the SPB attached to the nuclear envelope (Raudaskoski, 1998, Fig 2h). A part of the nuclear movement in migration hyphae is probably a consequence of separation of daughter nuclei at telophase (Raudaskoski,

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1998), today known to be dependent on the motor molecule dynein, which is a major microtubule (MT)-minus-end -directed motor in eukaryotic cells. Dynein is known to associate with the cell cortex and MTs (Roth *et al.*, 2014). The interaction of the cortical dynein with astral MTs could help to drive the processes of the fast telophase nuclear separation (Girbardt, 1971). In the basidiomycete *U. maydis* 10 kinesin genes and two genes encoding the N-terminal and C-terminal parts of one dynein heavy chain are known (Steinberg, 2007). A similar situation with a bipartite dynein gene (Kothe, personal communication) and with at least 9 kinesin genes was found in *C. cinerea* and *S. commune* (Raudaskoski, unpublished). The function of these motor molecules in nuclear and other cell organelle movement is still unresolved as is the role of the actin cytoskeleton.

Clamp cell fusion

The clamp cell formation, the outgrowth for the clamp cell, the cessation of the nuclear pair below the site of clamp cell formation and the movement of one of the nuclei into the clamp cell, the synchronous division of the nuclei in the hypha and in the clamp cell, the formation of the septa in the hypha and at the base of the clamp cell as well as the migration of the nucleus enclosed in the clamp cell to the subapical cell are all processes involving cytoskeletal elements (Runeberg *et al.*, 1986; Salo *et al.*, 1989; Raudaskoski *et al.*, 1991, Kamada *et al.*, 1993; Tanabe and Kamada,1994,1996; Figs 3 and 4). The last step in the fusion of the clamp cell with the subapical cell is dependent on the *B* mating type genes, and on the pheromone response pathway, while the previous steps are controlled by the *A* mating type genes (Casselton and Olesnicky, 1998). Recently, cell biological evidence has been obtained regarding the location of the pheromone receptor at the wall surrounding the fusing clamp connection both *in vivo* and using immune staining (Erdmann *et al.*, 2012).

After septum formation, the backward-looped, unfused clamp cell containing a nucleus (pseudoclamp, hook cell) is attracted by a small peg induced at the subapical cell at the site of the clamp cell tip indicating signaling exchange between the tip of the hook and the subapical cell before the fusion (Kües, 2000; Kües *et al.*, 2002; Badalyan *et al.*, 2004). This phase is not usually recorded perhaps due to its short duration but is clearly shown in *C. cinerea* and in a few other filamentous basidiomycetes (Badalyan *et al.*, 2004). When both the nuclei of *S. commune* dikaryon carried a deletion mutation of the RasGTPase-activating protein Gap1, the unfused stage of the clamp cell was elongated and the presence of the peg became more evident, although the deformed clamp cell tip growth in the mutant often seemed to miss the peg and, instead, fused with the tip of a nearby growing branch (Schubert *et al.*, 2006). These observations indicate that a Ras protein is involved in the regulation of the *B*-signaling pathway, at least in directing clamp cell fusion. Unfortunately, these observations have not yet been possible to confirm by a constitutively active Ras1 protein, which represents an equivalent stage of the Ras in the dikaryon with deleted Gap1 proteins (Knabe *et al.*, 2013). The negative result could perhaps be caused by the involvement in the process of the Ras2 protein present in the *S. commune* genome. Instead, the ectotopic expression of a constitutively active Cdc42 protein deformed all those processes in the clamp cell formation that are known from cell biological studies to require actin (Weber *et al.*, 2005, Fig 3). The development of septa in dikaryotic hyphae at clamp connections or in the haploid hyphae takes place exactly at the site where the nuclear division has occurred. This reminds the situation in the fission yeast *S. pombe*, in which the SIP complex (Singh *et al.*, 2011) comparable to STRIPAK complex (Frost *et al.*, 2012) regulates the transition from mitosis to septal synthesis at the site of nuclear division.

When discussing the clamp cell fusion, it is interesting to return to the ideas presented by Casselton *et al.* (1971) suggesting that when a cell contains two different *B* specificities, the *B* function is not expressed; the dolipore septa is not broken down and clamp cell fusion fails. During the development of the clamp cell connection, the nuclei with different *B* mating type genes are separated one in the clamp cell and the other in the subapical cell by formation of the septum at the base of the clamp cell and in the hypha. Both nuclei are probably in the G1 phase of the cell cycle, due to recovering from telophase. The situation could lead to a local expression of the pheromone response pathway at the clamp cell tip and the peg of the subapical cell resembling the mating of yeast cells and perhaps including proteins from the STRIPAK complex. In this situation the model of proximity of the nuclei in controlling the pheromone response pathway is not valid (Schuurs *et al.*, 1998), since the nucleus in the subapical cell and the one in the unfused clamp cell are not in close proximity. It could, however, be possible that the pheromone receptor interaction inducing septal dissolution in migration hyphae works under different rules than in the clamp cell fusion.

6. Summary

Most of the filamentous basidiomycetes possess a tetrapolar mating system (Raper, 1966) in which the *A* and *B* mating-type loci encode homeodomain transcription factors and pheromones and pheromone receptors, respectively. The hyphal fusions in filamentous basidiomycetes are a prerequisite for sexual reproduction initiated by interaction of pheromones with receptors, encoded by the *B* mating type genes. However, the occurrence of hyphal fusions in homokaryotic and heterokaryotic mycelia irrespective of composition of mating type genes (Ahmad and Miles, 1970; Smythe, 1973; Raudaskoski, 1998) have suggested that the pheromone receptor interaction occurs after hyphal fusions (Vaillancourt *et al.*, 1997; Casselton and Olesnicky, 1998; Olesnicky *et al.*, 1999). The inability to induce the *B* pathway with a synthetic farnesylated peptide functioning in a yeast assay with *C. cinerea* receptors, or to isolate native pheromones from culture filtrates (Olesnicky *et al.*, 1999) have further supported this view. In filamentous ascomycetes the STRIPAK (Striatin-Interacting Phosphatase and Kinase) protein complex and several other proteins have been shown by genetic tools and biochemical analyses to be indispensable for fusions of conidial anastomoses tubes (CATs) and vegetative hyphae (Read *et al.*, 2012; Leeder *et al.*, 2013). Instead the signaling molecules and their receptor(s) for the initial phase of hyphal fusions are not yet

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known (Leeder *et al.*, 2011). The genomes of *C. cinerea* and *S. commune* possess genes homologous to the genes encoding the proteins of STRIPAK complex in ascomycetes (Table 1), which suggests that the STRIPAK complex proteins could also be involved in the hyphal fusions of filamentous basidiomycetes.

The proteins of the pheromone response pathway in filamentous basidiomycetes are not yet well characterized and the counterparts are sought from the pheromone response pathway in the yeast *S. cerevisiae*. The yeast pheromone response pathway also includes Far proteins homologous to the STRIPAK complex proteins (Bloemendal *et al.*, 2012), which further emphases the need to clarify the relationship between the STRIPAK complex and pheromone response pathway proteins. The sexual reproduction of tetrapolar basidiomycetes requires also the homeodomain proteins encoded by the *A* mating type genes regulating the clamp cell development. The *A* mating type regulated pathway may also have connections with STRIPAK complex proteins in the regulation of the septal development after synchronous nuclear division at the clamp cell development. The SIP complex in the fission yeast *S. pombe* (Singh *et al.*, 2011) contains three proteins related to the STRIPAK complex proteins. The SIP complex regulates the proper location of SIN (septal initiation network) at the sites where the diving nuclei have been located. New observations on the last step in the clamp cell development, on the *B*-pathway regulated clamp cell fusion with the subapical cell, have raised interest in the possibility of extracellular pheromone-receptor communication in this process (Badalyan *et al.*, 2004; Schubert *et al.*, 2006; Nakazawa *et al.*, 2011a).

The investigation of proteins homologous to the STRIPAK complex proteins could bring in new information about the regulation of sexual reproduction in filamentous basidiomycetes. This can only be achieved by cell and molecular biological research applying efficient gene deletion procedures (de Jong *et al.*, 2010; Ohm *et al.*, 2010b; Nakazawa *et al.*, 2011b), localization of nuclei (Fig 4) and the proteins from different regulation pathways in living hyphae as well as applying different biochemical methods to reveal the protein interactions at complexes associated with hyphal fusions and with signaling pathways regulated by *A* and *B* mating type genes in filamentous basidiomycetes.

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