



# Hyphal homing, fusion and mycelial interconnectedness

N. Louise Glass<sup>1</sup>, Carolyn Rasmussen<sup>1</sup>, M. Gabriela Roca<sup>2</sup> and Nick D. Read<sup>2</sup>

<sup>1</sup>Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA

<sup>2</sup>Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, Rutherford Building, Edinburgh, UK EH9 3JH

**Hyphal fusion is a ubiquitous phenomenon in filamentous fungi. Although morphological aspects of hyphal fusion during vegetative growth are well described, molecular mechanisms associated with self-signaling and the cellular machinery required for hyphal fusion are just beginning to be revealed. Genetic analyses suggest that signal transduction pathways are conserved between mating cell fusion in *Saccharomyces cerevisiae* and vegetative hyphal fusion in filamentous fungi. However, the mechanism of self-signaling and the role of vegetative hyphal fusion in the biology of filamentous fungi require further study. Understanding hyphal fusion in model genetic systems, such as *Neurospora crassa*, provides a paradigm for self-signaling mechanisms in eukaryotic microbes and might also provide a model for somatic cell fusion events in other eukaryotic species.**

Hyphal fusion (anastomosis) occurs at crucial stages during the life cycle of filamentous fungi and serves many important functions. During the vegetative phase, fusion initially occurs between spore germlings, and later on in the interior of the mature vegetative colony. [Figure 1](#) shows the interconnected mycelial network of a fungal colony that results from multiple fusion events. It is widely assumed that vegetative hyphal fusion is important for intra-hyphal communication, translocation of water and nutrients, and general homeostasis within a colony [1,2]. However, these predicted roles still need to be analyzed experimentally.

Entry into the sexual cycle in out-crossing species involves the fusion of hyphae with other hyphae or spores of the opposite mating type. Maintenance of the dikaryotic state, which is a prelude to karyogamy, also requires the fusion of hyphae, and this commonly involves specialized structures called croziers (in the ascogenous hyphae of ascomycetes) or clamp connections (associated with the vegetative hyphae of basidiomycetes). Hyphal fusion is also involved in the formation of multihyphal aggregates from which the multicellular fruitbodies are derived.

Although morphological aspects that are associated with hyphal fusion during the life cycle of filamentous fungi have been well characterized, little is known about the signals or molecular machinery involved in hyphal

fusion, or whether common molecular mechanisms are associated with fusion events during vegetative growth and sexual development.

Hyphal fusion in filamentous fungi is comparable to cell fusion events in other eukaryotic organisms. Examples include fertilization events between egg and sperm or somatic cell fusion resulting in syncytia formation (e.g. between myoblasts during muscle differentiation), fusion between osteoclasts in bone formation, and also in placental development [3–5]. Although molecular mechanisms of non-self fusion (e.g. between *Saccharomyces cerevisiae* cells of opposite mating types) have been well characterized, molecular mechanisms associated with fusion between somatic cells in eukaryotes are not as well analyzed. Understanding the molecular basis of hyphal fusion during vegetative growth in filamentous fungi provides a paradigm for self-signaling mechanisms in eukaryotic microbial species, and might also provide a useful model for somatic cell fusion events in other eukaryotes. In this review, we focus on hyphal fusion in filamentous ascomycetes, particularly in the model system *Neurospora crassa* [6,7].

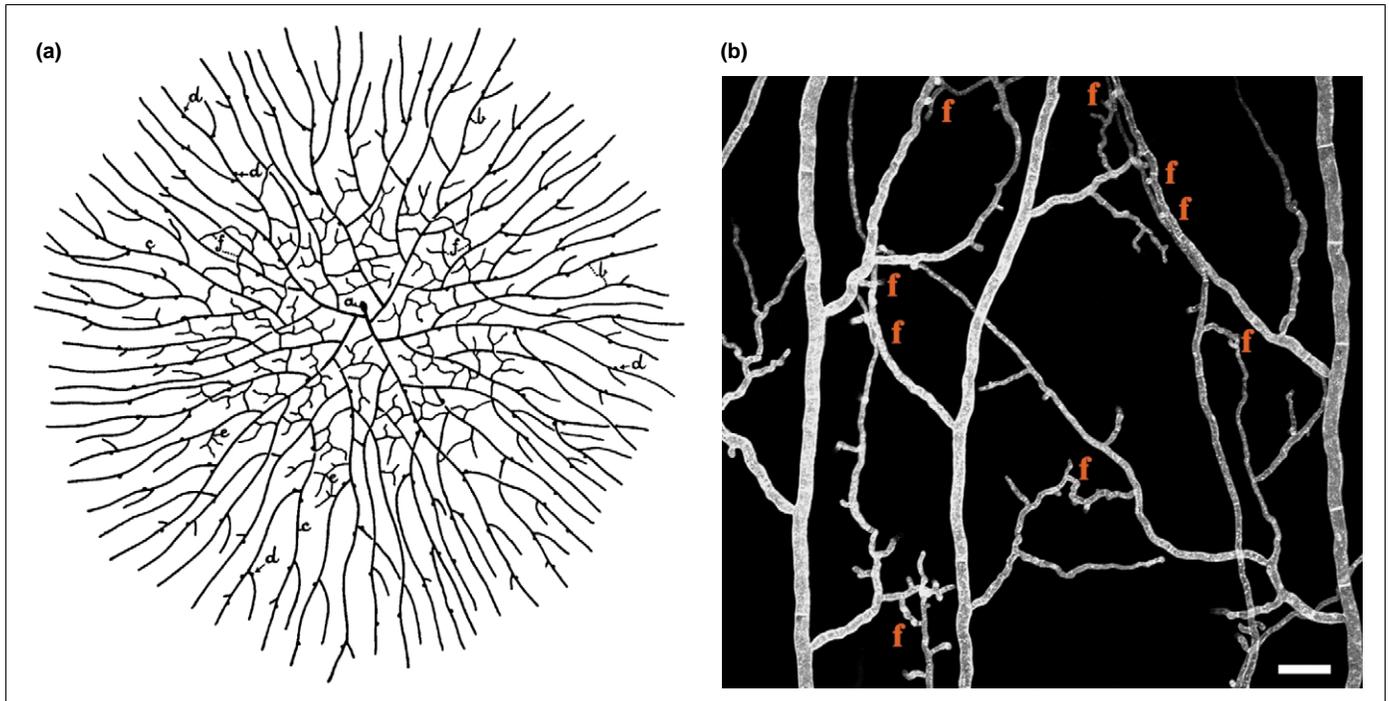
## Fusion between conidial germlings

Hyphal fusion between conidial germlings has been previously observed in numerous species [8–10]. In the plant pathogen *Colletotrichum lindemuthianum* and in *N. crassa*, it has been observed that conidia also form specialized hyphae, called ‘conidial anastomosis tubes’, which are morphologically distinct from germ tubes and function to anastomose with other conidia in close proximity [10] ([Figure 2](#)). Conidia from *C. lindemuthianum* that have undergone fusion show a higher rate of germination suggesting that fusion between conidial germlings might serve to increase or pool resources between genetically identical individuals. Even early publications provided evidence for positive autotropism being involved in fusion between conidial germlings [8]. Attraction between conidia of different species was also demonstrated, suggesting that mechanisms associated with signaling, homing and the cellular machinery of hyphal fusion are conserved among filamentous ascomycete species.

## Fusion in vegetative colonies

Hyphal fusion occurs within a single vegetative colony and also between fungal colonies to form heterokaryons,

Corresponding author: N. Louise Glass (Lglass@uclink.berkeley.edu).



**Figure 1.** (a) Drawing showing an interconnected fungal colony resulting from a single germinated spore. (b) Projection of confocal images of the interconnected colony interior of *Neurospora crassa* showing hyphal fusions (f). Scale bar represents 50  $\mu\text{m}$ . (a) and (b) reproduced, with permission, from Ref. [17].

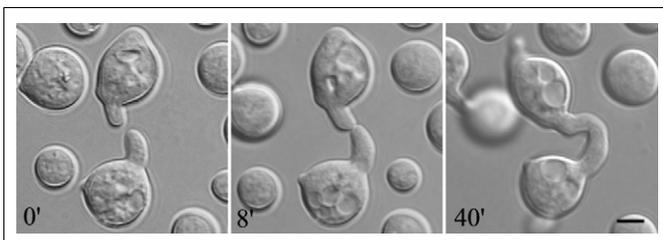
whereby genetically distinct nuclei coexist in a common cytoplasm [11]. Different stages in vegetative hyphal fusion are shown in Figure 3. In a mature filamentous fungal colony, a criterion for whether a hypha can anastomose is its presence in the colony interior rather than the periphery. The developmental switch for generating fusion-competent hyphae is unknown. It has been hypothesized that the attraction of hyphae involved in fusion is mediated by diffusible substances, which results in re-directed polarized hyphal tip growth [8,12–14] (Figure 3). We propose that these unidentified diffusible signals regulate the behavior of the Spitzenkörper, which is a complex of organelles and proteins predominated by secretory vesicles. The Spitzenkörper is found in growing hyphal tips or at sites of branch initiation, and its behavior plays an important role in hyphal morphogenesis [15,16]. Live-cell imaging of hyphal homing and fusion in *N. crassa* has shown that these processes are also intimately associated with the dynamic behavior of the Spitzenkörper (Figure 4) [17]. After making contact, hyphae involved in fusion switch from polar to isotropic growth, resulting in swelling of hyphae at the fusion point [17]. However, the Spitzenkörper persists in these hyphae and is invariably

associated with the site of the future pore (Figure 4b), suggesting that it might target vesicles involved in the secretion of extracellular adhesives and cell-wall-degrading enzymes to the future pore site. Vesicle trafficking to and from the fusion site therefore plays a central role in regulating many of the processes involved in hyphal fusion.

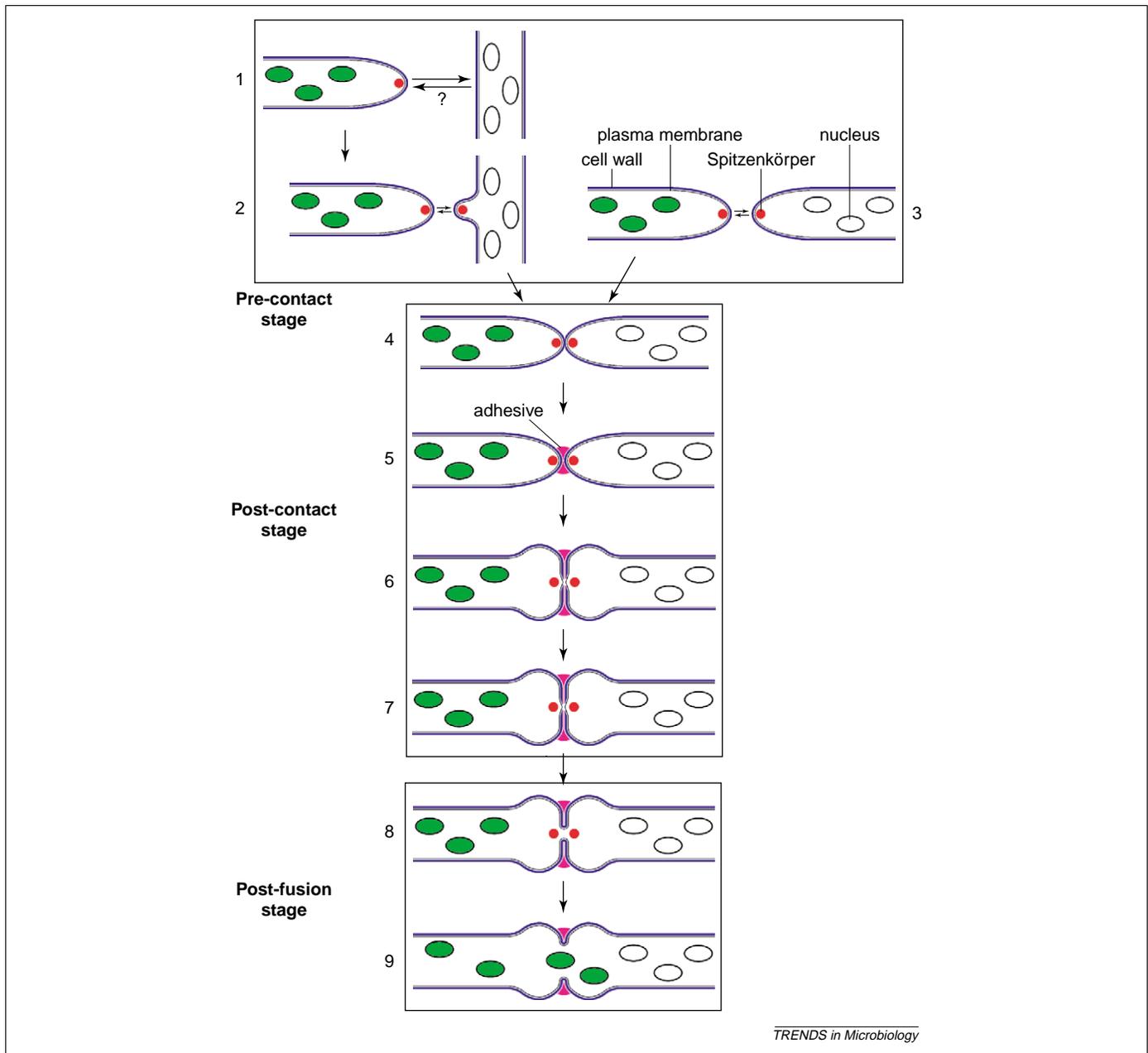
After fusion of plasma membranes occurs, the cytoplasm of the two participating hyphae mix. In *N. crassa*, the Spitzenkörper remains associated with the fusion pore as it enlarges [17]. Dramatic changes in cytoplasmic flow are often associated with hyphal fusion, and nuclei and organelles, such as vacuoles and mitochondria, also pass through the fusion pore (Figure 5). Septum formation near the hyphal fusion site is also often observed.

### Fusion during sexual reproduction

Hyphal fusion occurs in the fruiting bodies of basidiomycete species [18]. However, it is unclear whether hyphal fusion is also required for the formation of female reproductive structures (protoperithecia) in filamentous ascomycetes such as *N. crassa*, although the hyphae that form the walls of these sexual organs become tightly adhered to each other [19]. Hyphal fusion is also essential for fertilization during mating. During fertilization, reproductive hyphae, known as trichogynes, protrude from protoperithecia; trichogynes are attracted to, and fuse with, male cells (microconidia or macroconidia) of the opposite mating type [20,21]. Following fertilization, nuclei of the opposite mating types proliferate in a common cytoplasm and eventually pair off and migrate into a hook-shaped structure called a crozier [22]. In *N. crassa*, karyogamy occurs in the penultimate cell of the crozier, whereas hyphal fusion occurs between the terminal cell and the hyphal compartment nearest to the penultimate cell [22,23]. Although fusion events occur during



**Figure 2.** Differential interference contrast images showing two germlings of *Neurospora crassa* undergoing homing and fusion of conidial anastomosis tubes at 8 and 40 minutes after the onset of imaging (time 0). Scale bar represents 5  $\mu\text{m}$ .



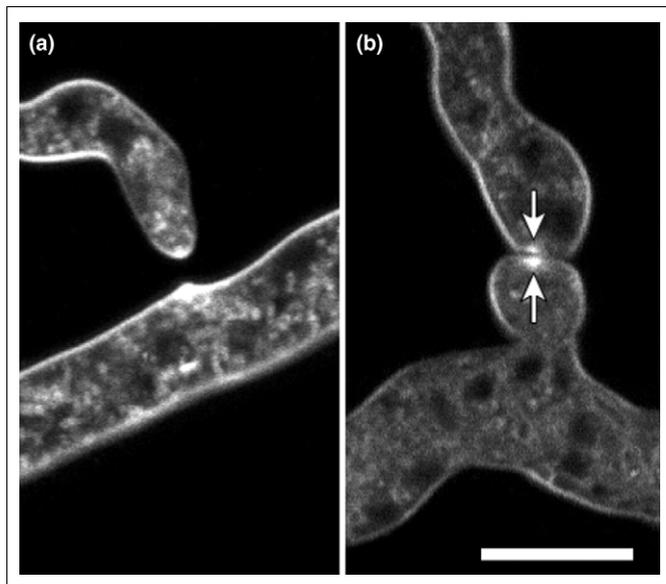
**Figure 3.** Diagram showing the pre-contact, post-contact and post-fusion stages involved in vegetative hyphal fusion. Two types of pre-contact behavior are shown: (i) a hyphal tip induces a branch and they subsequently fuse (shown in stages 1 and 2); and (ii) two hyphal tips grow towards each other and subsequently fuse (shown in stage 3). A third type of pre-contact behavior, not shown here, is tip-to-side fusion [17]. Nuclei are colored green and white to indicate that they belong to different hyphae. **Stage 1:** a fusion-competent hyphal tip secretes an unknown diffusible, extracellular signal (small arrow) which induces Spitzenkörper formation; it is not known whether the other hypha secretes a chemotropic signal at this stage. **Stage 2 and 3:** fusion-competent hyphal tips each secrete diffusible, extracellular chemotropic signals (small arrows) that regulate Spitzenkörper behavior; hyphal tips grow towards each other. **Stage 4:** cell walls of hyphal tips make contact; hyphal tip extension ceases and the Spitzenkörper persists. **Stage 5:** secretion of adhesive material at hyphal tips. **Stage 6:** switch from polar to isotropic growth, resulting in swelling of adherent hyphal tips. **Stage 7:** dissolution of cell wall and adhesive material; plasma membranes of two hyphal tips make contact. **Stage 8:** plasma membranes of hyphal tips fuse and pore formation occurs; the Spitzenkörper stays associated with the pore as it begins to widen and cytoplasm starts to flow between hyphae. **Stage 9:** pore widens, Spitzenkörper disappears; organelles (such as nuclei, vacuoles and mitochondria) often exhibit flow between fused hyphae, possibly due to differences in turgor pressure.

vegetative growth and sexual reproduction in filamentous ascomycetes, it is unclear whether common signaling mechanisms and/or hyphal fusion machinery are involved in both processes.

#### Genetic control of vegetative hyphal fusion

Our hypothesis is that some of the machinery involved in mating cell fusion in *S. cerevisiae* might be used for vegetative hyphal fusion in filamentous ascomycetes. Many of the components involved in signal transduction

and the machinery of mating cell fusion in *S. cerevisiae* are conserved in *N. crassa* (<http://www-genome.wi.mit.edu/annotation/fungi/neurospora/>) [7] (Table 1). Mutations in potential orthologs of some of these genes in filamentous fungi result in mutants that are unable to undergo hyphal fusion or that fail to form heterokaryons, a process that requires hyphal fusion. In *S. cerevisiae*, components that are required for late events associated with mating cell fusion do not appear to be as conserved in the *N. crassa* genome (Table 2), including *FUS1*, *FUS2*, *FIG1* and *FIG2*.



**Figure 4.** Confocal images of hyphal homing and fusion in *Neurospora crassa* stained with FM4–64. (a) One hyphal tip growing towards a hyphal peg (pre-contact stage). Brightly fluorescent Spitzenkörper are present in both the hyphal tip and emerging peg. (b) Post-contact stage. The adherent hyphal tips have swollen but their fluorescent Spitzenkörper have persisted even though hyphal extension has ceased. Scale bar represents 10  $\mu\text{m}$ . Reproduced, with permission, from Ref. [17].

Mutations in *FUS1* or *FUS2* result in *S. cerevisiae* mutants that re-orient their growth toward a mating partner (schmoos), but fail to undergo plasma membrane fusion [24]. Other genes that are important for cell fusion in *S. cerevisiae* are conserved in *N. crassa*. One example is *PRM1*, which encodes a protein that is potentially involved in plasma membrane fusion in *S. cerevisiae* (Table 2) [25].

A homolog of *PEA2*, which is required for correct polarization of the cytoskeleton during schmoos formation in *S. cerevisiae* [26], is also missing from the *N. crassa* genome. It is the only component of the polarisome (which might have functional similarities to the Spitzenkörper) that does not appear to be conserved in the *N. crassa* genome. It is possible that other proteins might perform analogous functions to Pea2p for cytoskeletal polarization involved in hyphal fusion in *N. crassa*.

**Table 1.** Conservation of pheromone response genes

<i>S. cerevisiae</i> <sup>a</sup>	<i>N. crassa</i> <sup>b</sup>	Function <sup>c</sup>	Refs
<i>STE20</i>	<i>NCU03894.1</i> (e-109)	P21 activated kinase	[47]
<i>STE11</i> <sup>d</sup>	<i>nrc-1</i> <i>NCU06182.1</i> (9e-81)	MAPKKK	[47]
<i>STE7</i>	<i>NCU04612.1</i> (e-66)	MAPKK	[47]
<i>FUS3</i> <sup>d</sup>	<i>mak-2</i> <i>NCU02393.1</i> (e-125)	MAPK	[47]
<i>STE5</i>	ND <sup>e</sup>	Scaffold protein	[47]
<i>STE12</i> <sup>d</sup>	<i>pp-1</i> <i>NCU00340.1</i> (4e-61)	Transcription factor	[48]

<sup>a</sup>ORF (open reading frame) translations were retrieved from the *Saccharomyces* Genome Database (<http://www.yeastgenome.org/>).

<sup>b</sup>Blastp was used to query the *Neurospora* Genome (<http://www-genome.wi.mit.edu/annotation/fungi/neurospora/>) with e value cutoff of 1e-9, BLOSUM 62 matrix and seg filtering. The protein with the lowest e value was chosen as the potential ortholog.

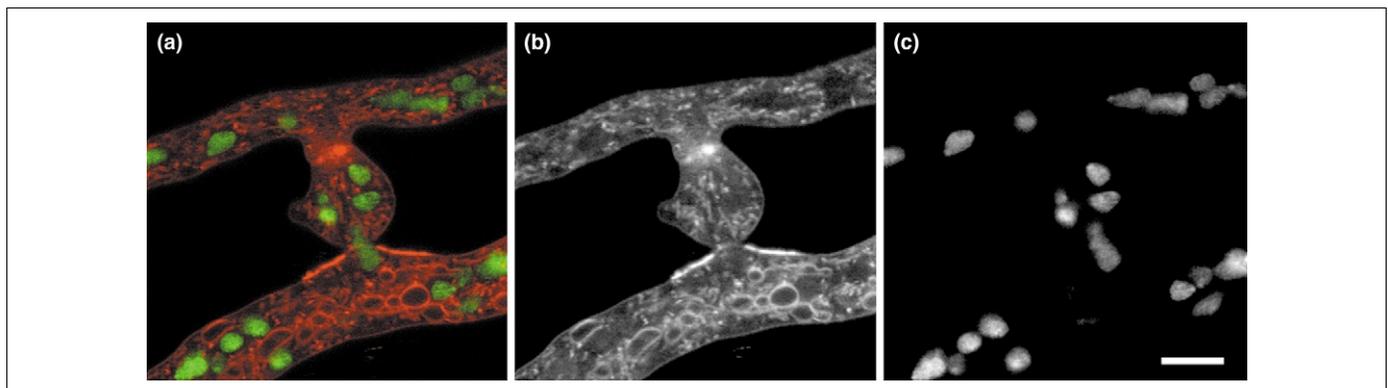
<sup>c</sup>Function defined either biochemically or by phenotype of mutant in *S. cerevisiae*.  
<sup>d</sup>Mutation in these orthologous genes in filamentous fungi results in mutants defective for hyphal fusion.

<sup>e</sup>ND, not detected.

#### Pheromone response mitogen-activated protein (MAP) kinase pathway

Several mutants in filamentous fungi that contain mutations in the putative orthologs of the *S. cerevisiae* pheromone response mitogen-activated protein (MAP) kinase pathway are defective in hyphal fusion [27,28]. These observations suggest that the pheromone response MAP kinase pathway is either involved directly in the hyphal fusion process or in rendering hyphae competent to undergo fusion. An *Aspergillus nidulans* strain disrupted in the *STE11* ortholog *steC* (a MAP kinase kinase kinase, MAPKKK), resulted in a mutant that fails to form heterokaryons [27]. In addition, the *steC* mutant has a slow growth rate and an altered conidiophore morphology. A *N. crassa* *STE11* mutant, *nrc-1*, also exhibits a pleiotropic phenotype [29]. A deletion mutant of *nrc-1* (the gene for non-repressible conidiation) is de-repressed for conidiation, is female sterile and shows an ascospore autonomous lethal phenotype. The *nrc-1* mutant is also defective in both self and non-self hyphal fusion [28].

In many plant pathogens, conidial germination induces a complex morphogenetic program that results in the formation of an infection structure called an appressorium. Plant pathogens, such as *Magnaporthe grisea*, *Colletotrichum lagenarium* or *Cochliobolus heterostrophus*, that have mutations in orthologs of *FUS3*, the MAP kinase component of the pheromone response



**Figure 5.** Confocal images showing fused hyphal branches of *Neurospora crassa* through which nuclei are flowing. Membranes stained with FM4–64; nuclei labeled with H1 histone-GFP. (a) Merged image. (b) FM4–64 staining alone. (c) GFP labeling alone. Scale bar represents 10  $\mu\text{m}$ .

**Table 2. Conservation of polarisome and cell fusion genes**

<i>S. cerevisiae</i> <sup>a</sup>	<i>N. crassa</i> <sup>b</sup>	Function <sup>c</sup>	Refs
<i>PRM1</i>	<i>NCU09337.1</i> (e-49)	Plasma membrane fusion	[25]
<i>FUS1</i>	ND <sup>d</sup>	Vesicle localization	[24]
<i>FUS2</i>	ND	Unknown	[24]
<i>RVS161</i>	<i>NCU01069.1</i> (2e-78)	Endocytosis/actin	[24]
<i>FIG1</i>	ND	Calcium transporter	[49,50]
<i>FIG2</i>	ND	GPI anchored protein	[50]
<i>FIG4</i>	<i>NCU08689.1</i> (0.0)	Polyphosphoinositide phosphatase	[50]
<i>KEL1</i>	<i>NCU00622.1</i> (3e-60)	Kelch domain	[51]
<i>CHS5</i>	<i>NCU07435.1</i> (4e-60)	Polarize Fus1p	[52]
<i>SPA2</i>	<i>NCU03115.1</i> (e-19)	Scaffold protein	[53]
<i>BNI1</i>	<i>NCU01431.1</i> (2e-83)	Formin	[54]
<i>BUD6</i>	<i>NCU08468.1</i> (2e-73)	Actin cytoskeleton	[55]
<i>PEA2</i>	ND	Actin regulator	[26]

<sup>a</sup>ORF translations were retrieved from the *Saccharomyces* Genome Database (<http://www.yeastgenome.org/>).

<sup>b</sup>Blastp was used to query the *Neurospora* Genome (<http://www-genome.wi.mit.edu/annotation/fungi/neurospora/>) with e value cutoff of 1e-9, BLOSUM 62 matrix and seg filtering. The protein with the lowest e value was chosen as the potential ortholog.

<sup>c</sup>Function defined either biochemically or by phenotype of mutant in *S. cerevisiae*.

<sup>d</sup>ND, not detected.

pathway, are defective in appressorial formation [30–32]. These mutants also fail to colonize host plants when inoculated through wound sites, which bypasses the requirement for appressorium development. In addition, these MAP kinase mutants were reported to be impaired in conidiogenesis, aerial hyphae formation and female fertility. In *N. crassa*, a strain containing a disruption of the ortholog of *FUS3* (*mak-2* in *N. crassa*) results in a mutant with a phenotype very similar to a *nrc-1* mutant (see above). In particular, the *mak-2* mutant fails to undergo hyphal fusion [28]. Although unreported in the MAP kinase mutants constructed in plant pathogens, our prediction is that these mutants are also hyphal fusion defective and fail to make an interconnected mycelial network, an aspect that might be important for pathogenesis.

One of the downstream components of the pheromone response pathway in *S. cerevisiae* is *STE12*, which encodes a transcription factor [33]. Mutations in *STE12* homologs in *A. nidulans* result in mutants that are affected in sexual development [34]. In *M. grisea*, strains containing a deletion of the *STE12* ortholog (*MST12*) formed defective appressoria and were unable to form invasive hyphae within the plant when inoculated into wound sites [35]. In contrast to the *A. nidulans steC* (MAPKKK) and *M. grisea PMK1* (MAPK) mutants described previously, obvious defects in vegetative growth, conidiation or conidial germination were not observed in *mst12* or *steA* mutants. In *N. crassa*, a strain containing a mutation in the *STE12* ortholog (*pp-1*) has recently been identified (P. Bobrowicz and D.J. Ebbole, unpublished). The *pp-1* mutant is very similar phenotypically to the *mak-2* mutants (de-repressed for conidiation, female sterile and an ascospore autonomous lethal phenotype). The *pp-1* mutant is also hyphal fusion defective (D.J. Jacobson and N.L. Glass, unpublished). These data suggest that the MAP kinase pathway related to the pheromone response pathway in *S. cerevisiae* is required for vegetative hyphal fusion in filamentous fungi. Components of this pathway might also be involved in other aspects of growth and reproduction, such as ascospore germination and conidiation.

Microscopic analysis and live-cell imaging of the hyphal fusion process suggests that a diffusible factor mediates

attraction of participating hyphae [8,12–14,17], possibly by activating MAP kinase pathways [28]. Mating in *S. cerevisiae* is induced by the production of mating cell-specific peptide pheromones,  $\alpha$ -pheromone and  $\alpha$ -pheromone, which bind to their cognate membrane-bound receptors (Ste3p or Ste2p), and ultimately result in the activation of the *FUS3* MAP kinase pathway [36]. It is unclear, however, how self-signaling is accomplished during vegetative hyphal fusion in filamentous fungi or what the nature of the self-signaling molecule is. In *N. crassa*, expression of the pheromone precursor genes was shown to be mating type-specific and regulated by the mating type (*mat*) locus [37]. The mating-specific pheromones of *N. crassa* are apparently not involved in vegetative hyphal fusion because *mat* deletion mutants easily form heterokaryons [38,39].

#### Cell wall integrity MAP kinase pathway

In *A. nidulans* and *M. grisea*, mutations in the MAP kinase gene orthologous to the *SLT2* MAP kinase gene in *S. cerevisiae* affect conidial germination, sporulation and sensitivity to cell-wall-digesting enzymes [40,41]. The *SLT2* locus in *S. cerevisiae* encodes a MAP kinase that is involved in cell wall integrity [36]. Mutations in the *SLT2* ortholog in *Fusarium graminearum*, *MGV1*, resulted in a mutant that was female sterile and failed to form heterokaryons by hyphal fusion [42]. In *S. cerevisiae*, the *SLT2* MAP kinase pathway is downstream of the *FUS3* MAP kinase pathway and is required for remodeling the cell wall during schmoos formation [43]. These data suggest that the cell wall remodeling MAP kinase pathway might also be required for hyphal fusion in filamentous fungi. The *SLT2* MAP kinase and cell integrity pathway is highly conserved in *N. crassa*, although mutants in this pathway are currently lacking.

#### Membrane proteins and unknown components

A gene required for hyphal fusion in *N. crassa*, *ham-2* (hyphal anastomosis), encodes a putative transmembrane protein [44]. The *ham-2* mutants show a pleiotropic phenotype, including slow growth, female sterility and homozygous lethality in sexual crosses. Recently, a function for a homolog of *ham-2* in *S. cerevisiae* was

reported. Mutations in *FAR11* result in a mutant that prematurely recovers from G1 growth arrest following exposure to pheromone [45]. Far11p was shown to interact with five other proteins [Far3p, Far7p, Far8p, Far9p (also called Vps64p) and Far10p] [45]. Mutations in any of these genes give an identical phenotype to the *far11* mutants (i.e. premature recovery from growth arrest following exposure to pheromone). In *S. cerevisiae*, it is possible that the Far11p complex could be part of a checkpoint that monitors mating cell fusion in coordination with G1 cell cycle arrest. However, genes encoding several of the proteins that form a complex with Far11p in *S. cerevisiae* are apparently not conserved in *N. crassa*, including *FAR3* and *FAR7* (Table 3). Far9p and Far10p share regions of predicted protein identity and recover the same predicted protein when analyzed in *N. crassa* database searches.

Another *N. crassa* mutant, *ham-1*, shows substantial reduction in its ability to undergo both self and non-self hyphal fusions [46]. The *ham-1* mutant is similar morphologically to wild-type, with the exception of slightly slower growth rate, shortened aerial hyphae and female infertility. The *N. crassa* gene that complements the *ham-1* mutation shows similarity to a *S. cerevisiae* gene of unknown function (A. Fleissner *et al.*, unpublished). It will be of interest to determine whether the *S. cerevisiae* homolog has a role in mating.

### Concluding remarks

Understanding the mechanism and role of hyphal fusion in the biology of filamentous fungi is still in its early stages. Many outstanding questions remain. What are the diffusible chemotropic molecules responsible for causing fusion-competent hyphae to grow towards each other and how do they regulate Spitzenkörper behavior? Is the signal transduction machinery involved in regulating hyphal homing and fusion between conidial germlings the same or different to that involved in homing and fusion between hyphae in the colony interior? Are other signaling pathways (e.g. calcium signaling) also involved? How similar are the signaling and fusion machineries involved in vegetative stages to those involved in sexual stages of the life cycle?

**Table 3. Conservation of mating-mediated G1 arrest genes**

<i>S. cerevisiae</i> <sup>a</sup>	<i>N. crassa</i> <sup>b</sup>	Function <sup>c</sup>	Refs
<i>FAR1</i>	ND <sup>e</sup>	Cell cycle inhibitor	[56]
<i>FAR3</i>	ND	G1 arrest	[45]
<i>FAR7</i>	ND	G1 arrest	[45]
<i>FAR8</i>	<i>NCU08741.1</i> (4e-10)	G1 arrest	[45]
<i>VPS64/FAR9</i>	<i>NCU00528.1</i> (7e-19)	G1 arrest	[45,57]
<i>FAR10</i>	<i>NCU00528.1</i> (2e-17)	G1 arrest	[45]
<i>FAR11</i>	<i>ham-2</i> <sup>d</sup>	G1 arrest	[45]
	<i>NCU03727.1</i> (6e-14)		

<sup>a</sup>ORF translations were retrieved from the *Saccharomyces* Genome Database (<http://www.yeastgenome.org/>).

<sup>b</sup>Blastp was used to query the *Neurospora* Genome (<http://www-genome.wi.mit.edu/annotation/fungi/neurospora/>) with e value cutoff of 1e-9, BLOSUM 62 matrix and seg filtering. The protein with the lowest e value was chosen as the potential ortholog.

<sup>c</sup>Function defined either biochemically or by phenotype of mutant in *S. cerevisiae*.

<sup>d</sup>Mutation in these orthologous genes in filamentous fungi results in mutants defective for hyphal fusion.

<sup>e</sup>ND, not detected.

Hyphal fusion during vegetative growth in filamentous fungi is a complex and highly regulated process. It is unclear what physiological or developmental roles hyphal fusion serves in these organisms and what selective advantage it provides. All of the hyphal fusion mutants so far described in filamentous fungi have pleiotropic phenotypes. This observation suggests that failure to undergo fusion might be important for several developmental or physiological processes. However, it is also possible that all of the fusion mutants so far identified contain mutations in genes that are also required for other aspects of the biology of these organisms. Comparative analysis of genome sequences of filamentous fungi will be illuminating for aspects on conservation of signaling and fusion machineries, particularly in comparisons between ascomycete and basidiomycete species, such as *Ustilago maydis* ([http://www-genome.wi.mit.edu/annotation/fungi/ustilago\\_maydis/](http://www-genome.wi.mit.edu/annotation/fungi/ustilago_maydis/)) and *Coprinus cinereus* ([http://www-genome.wi.mit.edu/annotation/fungi/coprinus\\_cinereus/](http://www-genome.wi.mit.edu/annotation/fungi/coprinus_cinereus/)).

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