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Cell Fusion in the Filamentous Fungus, *Neurospora crassa*

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Summary

Hyphal fusion occurs at different stages in the vegetative and sexual life cycle of filamentous fungi. Similar to cell fusion in other organisms, the process of hyphal fusion requires cell recognition, adhesion, and membrane merger. Analysis of the hyphal fusion process in the model organism *Neurospora crassa* using fluorescence and live cell imaging as well as cell and molecular biological techniques has begun to reveal its complex cellular regulation. Several genes required for hyphal fusion have been identified in recent years. While some of these genes are conserved in other eukaryotic species, other genes encode fungal-specific proteins. Analysis of fusion mutants in *N. crassa* has revealed that genes previously identified as having nonfusion-related functions in other systems have novel hyphal fusion functions in *N. crassa*. Understanding the molecular basis of cell fusion in filamentous fungi provides a paradigm for cell communication and fusion in eukaryotic organisms. Furthermore, the physiological and developmental roles of hyphal fusion are not understood in these organisms; identifying these mechanisms will provide insight into environmental adaptation.

Key Words: Cell fusion; anastomosis; filamentous fungi; signal transduction; hyphal fusion.

1. Introduction

Filamentous ascomycete fungi, such as *Neurospora crassa,* typically form mycelial colonies consisting of a network of interconnected, multinucleate hyphae. Colonies grow by hyphal tip extension, branching, and fusion *(1,2)*. In filamentous ascomycete species, hyphal cross-walls or septa are incomplete and contain a single central pore. Septal pores allow cytoplasm and organelles, including nuclei, to move between hyphal compartments, thus making the fungal colony a syncytium. The syncytial, interconnected, organization of a fungal colony enables translocation of cellular contents, such as organelles, metabolites,

nutrients, or signaling compounds, throughout the colony, presumably facilitating growth and reproduction.

Cell fusion events occur during all stages of the filamentous fungal life cycle *(3)*. These fusion events serve different purposes during the establishment and development of fungal colonies. During vegetative growth, germling fusion events between germinating, and even apparently ungerminated, asexual spores (conidia) are correlated with faster colony establishment (**Fig. 1A; refs. *4,5)***. Fusion between hyphal branches within a mature fungal colony results in the formation of a network of interlinked hyphae (*see* **Fig. 1B; refs. *1,6)***. Germling or hyphal fusion between genetically different but heterokaryon-compatible individuals leads to the formation of colonies containing genetically different nuclei (heterokaryon). Within heterokaryons, nonmeiotic or parasexual recombination can result in the formation of new genotypes *(7)*, which possibly contribute to the high adaptability of fungal species that lack sexual reproduction. In the sexual phase of the life cycle, cell fusion between male and female reproductive structures is essential for mating in out-breeding species (*see* **Fig. 1C; ref. *8)***. After mating, cell fusion is associated with ascus formation (*see*
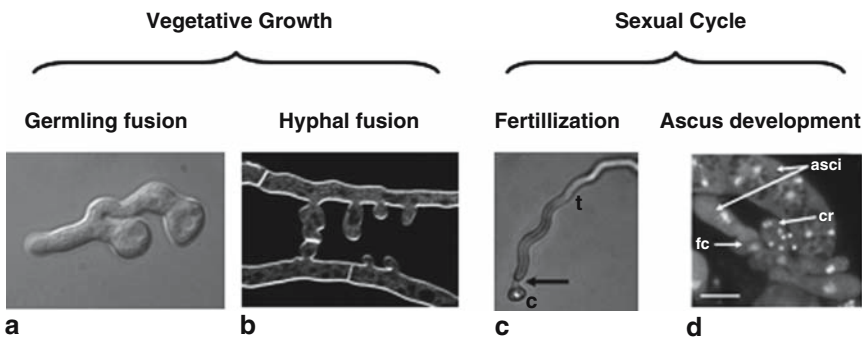

Fig. 1. Stages in the life cycle of *Neurospora crassa* in which fusion occurs. **(A)** Conidia at sufficient cell density undergo fusion between germlings. **(B)** Hyphae within the interior of the colony show chemotropism and hyphal fusion. **(C)** The sexual cycle is initiated by cell fusion between a fertile receptive hyphae (trichogyne [t]) emanating from a female reproductive structure, the protoperithecia (out of view). The trichogyne shows chemotropism toward a conidium of the opposite mating type (c). Arrow indicates fusion point. **(D)** Following fertilization, nuclei of opposite mating type (*mat A* and *mat a*) proliferate in ascogenous hyphae. Opposite mating-type nuclei pair off and migrate into the crozier (cr). In *N. crassa*, karyogamy occurs in the penultimate cell of the crozier. Hyphal and nuclear fusion occurs between the terminal cell and the subtending cell of the crozier (fc). Karyogamy, meiosis, and an additional mitotic division occurs in the ascus resulting in an eight-spored ascus. Asci, ascogenous hyphae and croziers treated with DAPI, a nuclear stain. Bar = 20 µm.

Fig. 1D), the cell in which karyogamy and meiosis occur *(9).* Whether common cell fusion machinery is involved in both sexual and vegetative fusion events in filamentous fungi remains a question.

Fusion processes in filamentous fungi are comparable to cell fusion events in other eukaryotic organisms. Examples include fertilization events between egg and sperm or somatic cell fusion that result in syncytia (e.g., between myoblasts during muscle differentiation, between macrophages in osteoclast and giant cell formation, and during placental development; **refs. *10–14)*.** Although cell fusion events occur in a diversity of species and cell types, they require very similar cellular processes, such as cell recognition, adhesion, and membrane merger. Although in many cases cell types involved in fusion are genetically or physiologically different, such as cell fusion during mating in *N. crassa*, vegetative hyphal fusion occurs between genetically and probably physiologically identical cells. Understanding the molecular basis of hyphal fusion provides a paradigm for self-signaling in eukaryotic cells and provides a useful comparative model for somatic cell fusion events in other eukaryotes. The model organism *N. crassa* is methodically tractable *(15–17)*, thus allowing the direct comparison of the molecular basis of hyphal and cell fusion events during its life cycle.

2. Vegetative Cell Fusion
2.1. Germling Fusion

The life of a fungal individual often begins with the germination of an asexual spore, termed *conidium*. When multiple conidia are placed close to one another, numerous germling fusion events are observed *(5,18)*. As a result, numerous individual germlings become one functional unit, which subsequently develops into a mycelial colony. Germinating conidia can fuse by germ tube fusion (*see* **Fig. 1A**) or by the formation of small hyphal bridges (*fusionshyphen* or *conidial anastomosis tubes*), which are significantly narrower than germ tubes *(4,5,19)*. Fusion events among conidia show a density- and nutrient-dependent function; fusion is suppressed on nutrient-rich media. The merger of initially individual cells into functional units in response to environmental cues is found not only in fungi but also in other species such as the social amoeba of dictyostelid slime molds *(20)*.

2.2. Hyphal Fusion

After germlings create a fused hyphal network, hyphal exploration extends outward from the conidia, thus taking on the morphological aspects of a typical fungal colony *(1,2)*. In *N. crassa* and other filamentous ascomycete species, the frequency of hyphal fusion within a vegetative colony varies from the periphery to the interior of the colony *(21)*. At the periphery, hyphae grow straight

out from the colony and exhibit avoidance (negative autotropism), presumably to maximize the outward growth of the colony *(22)*. In the inner portion of a colony, hyphae show a different behavior. Instead of avoidance, certain hyphae or hyphal branches show attraction, directed growth, and hyphal fusion (**Fig. 2; refs.** *1,21,23)*. Similar to germling fusion, the frequency of hyphal fusion events depends on the availability of nutrients. Generally speaking the following rule applies: the fewer the nutrients, the more the fusion events *(4,24,25)*. For example,
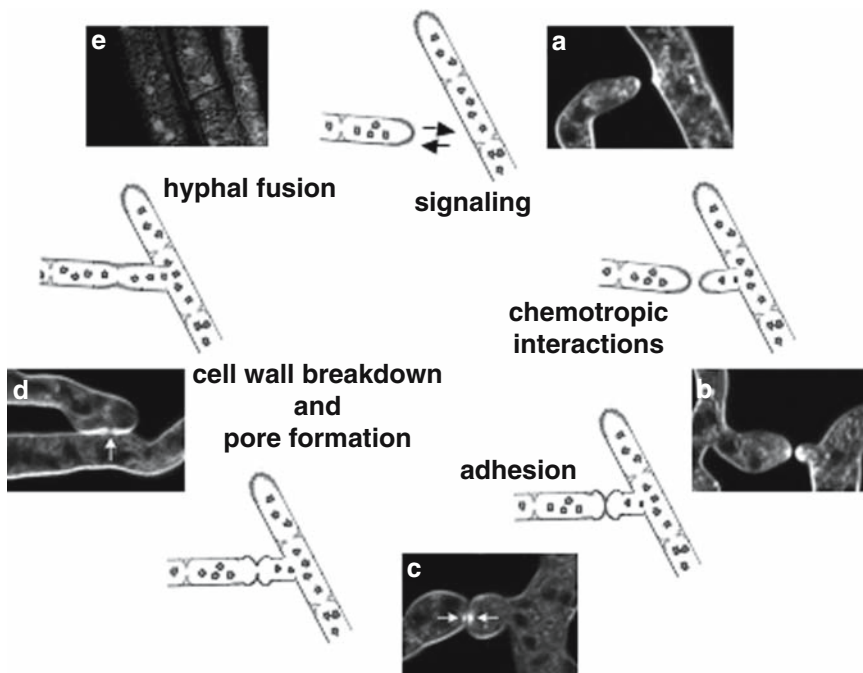

Fig. 2. Stages of hyphal fusion. **(A,B)** The presence of a fusion-competent hypha often results in the formation of a peg in the receptive hypha. Peg formation is associated with the formation of a Spitzenkörper at the tip of the new peg. **(C)** Contact between fusion hyphae is associated with a switch from polar to nonpolar growth, resulting in a swelling of the fusion hyphae at the point of contact. The Spitzenkörper is associated with the site of the future pore in both fusion hyphae (arrows). **(D)** Pore formation (arrow) is associated with cytoplasmic flow. Organelles such as nuclei and mitochondria pass through the fusion pore. Septation is also often associated with hyphal fusion events. **(E)** Fusion results in cytoplasmic mixing. Fusion between one hypha labeled with cytoplasmic GFP and one carrying dsRED-labeled nuclei result in hyphae exhibiting red nuclei in green cytoplasm. Hyphae stained with FM4-64. Bar = 10 µm. (A–D, adapted from **ref.** *21.*)

addition of nitrogen to nutrient-poor media led to the largest decrease in hyphal fusion frequency in *Rhizoctonia solani (25)*.

2.3. Mechanistic Aspects of Germling and Hyphal Fusion

Mechanistically, the process of germling and hyphal fusion can be divided into three steps: (1) precontact; (2) contact, adhesion, and cell wall breakdown; and (3) pore formation and cytoplasmic flow (*see* **Fig. 2; refs. *2,21,26)*.

2.3.1. Precontact

The observed attraction between conidial germlings or fusion hyphae suggests chemotropic interactions between the fusion partners. When the relative position of two germlings showing mutual attraction is changed by micromanipulation using optical tweezers, both individuals readjust their growth toward each other to make contact and undergo fusion *(18,27)*. During hyphal fusion, the presence of a fusion-competent hypha often results in either the alteration of growth trajectory or the formation of fusion branches in a receptive hypha (*see* **Fig. 2; refs. *1,4,21)*.

The secretion of signaling molecules is a common theme in chemotactic and chemotropic cellular interactions. Instances of cell–cell communication by diffusible substances leading to cell fusion include mating in the unicellular yeast species, *Saccharomyces cerevisiae*, pollen tube growth to the ovary in plant species, or egg–sperm interaction in animals. Mating in *S. cerevisiae* requires two cells of opposite mating types. Haploid cells secrete mating-type specific pheromones, which bind to their cognate plasma membrane receptors in a partner of the opposite mating type *(28)*. Germinating pollen tubes are also thought to be guided by diffusible chemotropic substances, such as Ca^{2+} or small heat-stable molecules secreted by the style *(29,30)*. Another diffusible substance that is released by synergid cells guides the pollen tube into the ovule *(31)*. The eggs of many aquatic animal species also release chemotactic substances to attract sperm; for example, *Xenopus* egg jelly releases a cysteine-rich secretory protein, allurin, to attract sperm *(32)*. In these examples, the fusion partners are genetically and/or physiologically distinct and either secrete different signaling molecules (such as mating-type–specific pheromones) or only one partner secretes a signal that results in the attraction of the other partner. Although the involvement of secreted signals is not clear in other systems, such as in myoblast fusion during muscle development, in most cases the fusing cells are also different, such that one partner presents an extracellular or surface attractant and the other grows or migrates toward it. In *N. crassa,* there is no evidence that cells that undergo germling and hyphal fusion are genetically or physiologically different. Both cells show chemotropic interactions, indicating that both are secreting and responding to a chemotropic signal. This scenario is somewhat

similar to cyclic adenosine monophosphate signaling in *Dictyostelium discoideum*, where a gradient of cyclic adenosine monophosphate mediates attraction of individual cells during the initiation of asexual sporulation *(20)*. However, in *D. discoideum*, all cells responds to the chemotactic signal, whereas in *N. crassa*, only cells/hyphae destined to fuse do so. The identity of the molecules that mediate chemotropic interactions during germling/hyphal fusion in any filamentous fungus, including *N. crassa*, remains enigmatic.

The chemotropic reorientation of hyphae destined to fuse is associated with alterations in the position of the Spitzenkörper or with the formation of a new Spitzenkörper associated with branch formation in the receptive hypha (*see* **Fig. 2A,B; ref. *21***). The Spitzenkörper is a vesicle-rich structure found in growing hyphal tips or at sites of branch initiation *(21,33)*. Localization of the Spitzenkörper in the hyphal apex has been associated with directionality of growth. In hyphae showing chemotropic interactions prior to hyphal fusion, the Spitzenkörper in the two partner hyphae continually reorient toward each other until the point of contact (*see* **Fig. 2B,C**). Reorientation of the Spitzenkörper and polar hyphal extension toward the fusion partner requires cellular mechanisms linking reception of the fusion signal to reorganization of the cytoskeleton. Adjustment of hyphal growth toward the fusion partner is comparable to cell polarization and shmoo formation during yeast mating *(28)*, directed pollen tube growth toward the ovary *(31)*, or the extension and/or stabilization of filopodia during myoblast fusion *(10)*.

2.3.2. Contact, Adhesion, and Cell Wall Breakdown

After making contact, hyphae involved in fusion switch from polar to isotropic growth, resulting in swelling of hyphae at the fusion point. The two Spitzenkörper of the fusion hyphae are juxtaposed at the point of contact (*see* **Fig. 2C; ref. *21***). During chemotropic interactions, vesicles targeted to the Spitzenkörper are associated with hyphal growth. However, once contact occurs, vesicles secreted to the hyphal tips via the Spitzenkörper must be involved in the cell wall degradation at the site of fusion. The localization of the two Spitzenkörper in the fusion hyphae resembles the prefusion complexes found during myoblast fusion in which vesicles line up at the sites of cell contact, forming pairs across the apposing plasma membranes *(12)*. Interpretation of Spitzenkörper behavior during hyphal fusion as a component of the prefusion complex offers an interesting working hypothesis for further analysis.

Germlings and hyphae involved in fusion events tightly adhere to one another *(18,21)*, and extracellular electron-dense material associated with fusing hyphae *(34)* may be involved in adhesion of participating hyphae. Interaction between adhesive molecules during mating in *S. cerevisiae*, termed *agglutinins*, is required to hold mating pairs together during cell wall breakdown and

plasma membrane fusion *(35)*. During prefusion complex formation in myoblast fusion, extracellular electron-dense material is also found in the area between two aligning vesicles, but not at nonpaired vesicles, suggesting a role for this extracellular material in aligning vesicles during fusion events *(36)*.

2.3.3. Pore Formation and Cytoplasmic Flow

After fusion of plasma membranes, the cytoplasms of the two participating hyphae mix. In *N. crassa,* the Spitzenkörper remains associated with the fusion pore as it enlarges (*see* **Fig. 2D,E; ref. *21***). Dramatic changes in cytoplasmic flow are often associated with hyphal fusion. Organelles, such as mitochondria, vacuoles, and nuclei, are transferred between hyphae as a result of fusion (*see* **Fig. 2E**). Septum formation near the site of hyphal fusion is also often observed. Physiological changes associated with cytoplasmic mixing upon hyphal/germling fusion are unclear but are presumed to occur; hyphae participating in fusion may be in different developmental states or be exposed to different nutritional conditions.

3. Sexual Fusion

Fusion is also essential for fertilization during mating in filamentous ascomycete species, such as *N. crassa* (*see* **Fig. 1C,D**). Mating requires the production of a specialized female reproductive structure, termed a *protoperithecium*. Reproductive hyphae, called *trichogynes*, protrude from the protoperithecia. Trichogynes are attracted by mating-type-specific pheromones secreted by male cells (microconidia or macroconidia) of the opposite mating type *(8,37)*. After making physical contact, the tip of the female trichogyne fuses with the male cell (*see* **Fig. 1C**). Following fusion, the nucleus from the male cell migrates through the trichogyne and into the protoperithecium. Following this fertilization event, opposite mating-type nuclei proliferate in a common cytoplasm within the developing perithecium. Opposite mating-type nuclei pair off and migrate into a hook-shaped structure called a *crozier* (*see* **Fig. 1D; ref. *9***). In *N. crassa,* karyogamy occurs in the penultimate cell of the crozier, while hyphal fusion occurs between the terminal cell and the hyphal compartment nearest to the penultimate cell (*see* **Fig. 1D**). Although fusion events occur during both vegetative growth and sexual reproduction in filamentous ascomycete species, it is unclear whether signaling mechanisms and/or hyphal fusion machinery are common to both processes.

4. Identification of Fusion Mutants

Chemotropic interactions observed during hyphal and germling fusion suggest that receptors and signal transduction mechanism are involved. During mating in *S. cerevisiae*, binding of mating-type-specific pheromones to their

cognate receptors results in activation of the pheromone response mitogen-activated protein (MAP) kinase (MAPK) pathway. Activation of this signaling pathway results in G_1 growth arrest and transcriptional activation of genes associated with mating, such as *FUS1* and *PRM1 (38,39)*. Components of the MAPK pathway, such as the MAPK Fus3p, interact with proteins associated with cytoskeleton rearrangement and cell polarization, such as the formin Bni1p *(40)*. In *N. crassa,* mutations in homologs of components of the *S. cerevisiae* pheromone response pathway result in strains that cannot perform germling or hyphal fusion **(Fig. 3; ref. *19*)**. Strains containing mutations in the MAPK gene *mak-2,* the MAPK kinase (MAPKK) gene *NCU04612.3*, or the MAPKK kinase (MAPKKK) gene *nrc-1* show similar phenotypes. In addition to a failure to undergo hyphal or germling fusion, these mutants show reduced growth rates, shortened aerial hyphae, and failure to form female reproductive structures (protoperithecia; **refs. *19,41,42)***. Similarly, an *Aspergillus nidulans* mutant disrupted in a MAPKKK *STE11* homolog, *steC*, fails to form heterokaryons (indicating a
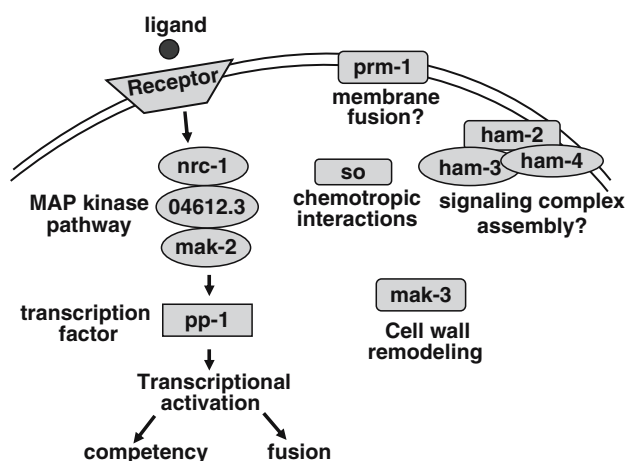

Fig. 3. In *Neurospora crassa,* mutations in the mitogen-activated protein kinase pathway components *nrc-1, NCU04612.3, mak-2,* and *pp-1* result in mutants unable to undergo germling or hyphal fusion *(19)*. In addition, mutations in *ham-2*, encoding a putative plasma membrane protein, *ham-3,* and *ham-4* result in strains unable to undergo both hyphal and germling fusion *(57,68)*. Mutations in *so* result in germling/hyphal fusion-deficient strains *(27)*. In *Fusarium graminearum,* a strain containing a mutation in the ortholog of *SLT2* fails to form a heterokaryon *(45)*; the *N. crassa* ortholog of *SLT2* is called *mak-3*. The natures of the receptor and ligand involved in anastomosis are unknown. Prm1p mediates membrane fusion in *Saccharomyces cerevisiae (38)*. Preliminary data indicate a similar role of the *N. crassa prm-1* homolog in germling/hyphal fusion. (A. Fleißner, S. Diamond, and N. L. Glass, unpublished data.)

defect in hyphal fusion) and is also affected in formation of sexual reproductive structures *(43)*. Because mutations in this MAPK pathway affect formation of sexual reproductive structures in filamentous fungi, its role in mating cell fusion has not been addressed.

Phosphorylation of MAK-2 is temporally associated with germling fusion events and is dependent on functional NRC-1 *(19)*. In *S. cerevisiae*, activation of the pheromone response pathway leads to activation of the transcription factor Ste12p. In *N. crassa,* a strain containing a mutation in the *Ste12* ortholog, *pp-1,* is very similar in phenotype to a *mak-2* mutant and is defective in hyphal and germling fusion (D. J. Jacobson, A. Fleißner, and N. L. Glass, unpublished results; **ref. *42***). Live cell imaging and microscopic observations of the *N. crassa nrc-1/mak-2/pp-1* mutants indicate that they are blind to self (mutants neither attract nor are attracted to hyphae/conidia in cases where germling fusion is common in wild-type strains). Furthermore, the *nrc-1* and *mak-2* mutants do not form conidial anastomosis tubes *(18)*. These data suggest that this MAPK pathway either is involved in early communication between the fusion partners or is required for rendering conidia and hyphae competent to undergo fusion.

In *S. cerevisiae,* the *SLT2* locus encodes an MAPK that is involved in cell wall integrity. The *SLT2* MAPK pathway is downstream of the *FUS3* MAPK pathway and is required for remodeling the cell wall during shmoo formation during mating *(44)*. Initial data show that a mutant of the *SLT2* homolog in *N. crassa*, *mak-3*, is also hyphal fusion defective (A. Fleißner and N. L. Glass, unpublished results). Mutations in the *SLT2* ortholog in *Fusarium graminearum, MGV1,* resulted in a mutant that is female sterile, fails to form heterokaryons by hyphal fusion, and is substantially reduced in virulence *(45)*.

In numerous plant pathogenic filamentous fungi, homologs of components of the mating or cell wall integrity MAPK pathways are essential for pathogenic development despite their distinct infection strategies. For example, in *Magnaporthe grisea*, *Colletotrichum lagenarium,* and *Cochliobolus heterostrophus*, strains containing mutations in *FUS3* homologs are defective in appressoria formation and fail to colonize host plants *(46–48)*. Mutations in the *FUS3* homolog of the biotrophic, nonappressorium-forming grass pathogen *Claviceps purpurea* result in the inability of the fungus to colonize rye ovaries *(49)*. Possible defects in hyphal fusion have not been addressed in most of these cases. Thus, a role for germling and hyphal fusion for colony development during invasion and growth within host tissue remains unanswered.

Cells recognize extracellular signaling molecules by different types of receptors. All eukaryotes use G-protein-coupled receptors (GPCR) for cell–cell communication and sensing of environmental stimuli. Examples are the mating pheromone receptors in *S. cerevisiae* (**28,50**), cyclic adenosine monophosphate

receptors involved in cell–cell communication in *D. discoideum (20)*, or GPCRs involved in neuron guidance by extracellular chemical cues (*reviewed in* **ref. 51**). Genome sequence analysis of the *N. crassa* genome has revealed at least 10 seven-transmembrane receptors within the GPCR family *(52)*. The two mating-type-specific pheromone receptors share homology with the *S. cerevisiae* pheromone receptors Ste2p and Ste3p *(53)*. In *N. crassa*, mutations in the putative pheromone receptor gene *pre-1* result in female sterility. Female *pre-1* trichogynes are unable to detect and contact male cells of the opposite mating type, indicating a role of the PRE-1 receptor in pheromone signaling between mating partners. However, heterokaryon formation between two *pre-1* strains was comparable to wild-type strains, indicating that hyphal fusion in the *pre-1* mutant is normal *(53)*.

Binding of ligands to GPCRs results in the disassociation of an intracellular heterotrimeric G protein (Gα, Gβ, and Gγ) and subsequent activation of downstream processes *(50)*. In the *N. crassa* genome, three Gα, one Gβ, and one Gγ genes are present *(52,54)*. *gna-1* and *gnb-1* mutants do not show chemotropic interactions between a trichogyne and conidium, which is required for the initiation of the sexual cycle (*see* **Fig. 1C; ref. 53**). However, G-protein mutants show no defects in vegetative germling or hyphal fusion (A. Fleißner and N. L. Glass, unpublished results), suggesting that GPCRs are not involved in signaling vegetative fusion events. However, there is growing evidence to suggest that GPCRs could function in a G-protein-independent manner *(55)*. Together, these data indicate that signaling molecules and their receptors involved in mating cell fusion in *N. crassa* are different from those involved in vegetative germling/hyphal fusion.

4.1. Proteins Mediating Membrane Fusion

Although cell fusion events are essential for the development of most eukaryotic organisms, the molecular basis of the final step of this process, the fusion of plasma membranes, is only poorly understood. In *S. cerevisiae,* one of the few proteins predicted to be involved in this process is Prm1p. *PRM1* encodes a plasma membrane protein and is found only in fungal species. *prm1* mutants show a significant fusion defect during mating, resulting in the accumulation of prezygotes. Preliminary data indicate that mutations in the *N. crassa prm-1* ortholog also results a fusion defect during germling and hyphal fusion (A. Fleißner, S. Diamond, and N. L. Glass, unpublished results). Further studies will evaluate if *prm-1* is required for fusion of the female trichogyne with the male cell during sexual development. These experiments will reveal whether the different cell fusion events during the *N. crassa* life cycle, which are initiated by different cell–cell communication mechanisms, share the same membrane fusion machinery.

4.2. Genes of Unknown Function: so and ham-2, ham-3, and ham-4

The *N. crassa so* mutant (allelic to *ham-1*) is deficient in both germling and hyphal fusion *(27)* and exhibits an altered conidiation pattern and shortened aerial hyphae. The *so* locus encodes a protein of unknown function, which contains a WW domain predicted to be involved in protein–protein interactions. Homologs of *so* are present in the genomes of filamentous ascomycete fungi but are absent in other eukaryotic species. These data indicate that some aspects of tip growth, polarization, and germling/hyphal fusion require functions that are specific to filamentous fungi. Interestingly, the SO protein accumulates at septal plugs of injured hyphae *(56)*; SO is not essential for wound sealing but contributes to the speed of septal plugging. A possible connection between its function in germling/hyphal fusion and wound sealing is unclear.

The *so* mutant forms female reproductive structures (protoperithecia), and mating cell fusion between the *so* trichogynes and male cells is unimpaired. However, fertilization by a male cell does not result in entry into sexual reproduction *(27)*. Thus, the block in sexual reproduction in the *so* mutant occurs postfertilization. It is possible that *so* may be required for development of the ascogenous hyphae and for the second sexual fusion event during ascus formation (*see* **Fig. 1D**).

In *N. crassa,* the *ham-2* (hyphal anastomosis) locus encodes a putative transmembrane protein *(57)*. *ham-2* mutants show a pleiotropic phenotype, including slow growth, female sterility, and homozygous lethality in sexual crosses. In addition, *ham-2* mutants fail to undergo both hyphal and germling fusion. Laser tweezer experiments showed that *ham-2* mutants are blind to self (fail to attract or be attracted to a wild type during germling fusion events; **ref. *18***), similar to the *mak-2* mutants described earlier. Subsequently, a function for a homolog of *ham-2* in *S. cerevisiae,* termed *FAR11,* was reported. Mutations in *FAR11* result in a mutant that prematurely recovers from G_1 growth arrest following exposure to pheromone *(58)*. Far11p was shown to interact with five other proteins (Far3p, Far7p, Far8p, Far9p, and Far10p). Mutations in any of these other genes give an identical phenotype as *far11* mutants. It was proposed that the Far11 complex is part of a checkpoint that monitors mating cell fusion in coordination with G_1 cell-cycle arrest. Apparent homologs of genes encoding several of the proteins that form a complex with Far11p in *S. cerevisiae* are lacking in *N. crassa*, including *FAR3* and *FAR7* (**2**). Preliminary data show that mutations in the homologs that are present in *N. crassa, FAR8* and *FAR9/10* (*ham-3* and *ham-4*, respectively), result in phenotypes similar to the *ham-2* mutant, including defects during germling/hyphal fusion (C. Rasmussen, A. Fleißner, A. Simonin, M. Yang, and N. L. Glass, unpublished results). These data indicate that homologs of proteins of the *S. cerevisiae* Far11 complex might also physically interact in *N. crassa* and that this interaction is essential

for vegetative germling/hyphal fusion. Interestingly, HAM-3 shows significant similarity to proteins of the striatin family. In mammals, genes belonging to the striatin family are principally expressed in neurons *(59)*. Striatin proteins accumulate in dendritic spines in neurons at the point of cell–cell contact or synapses. Striatin family proteins act as scaffolding proteins that organize signaling complexes; for example, formation of a complex between striatin and the estrogen receptor is required for estrogen-induced activation of a MAPK signal transduction pathway *(60)*. In *Sordaria macrospora,* a species related to *N. crassa,* mutations in the *ham-3* homolog (*pro11*) result in a mutant unable to complete sexual development, but full fertility was restored by expression of a striatin cDNA from mouse *(61)*. These data indicate that the homologous proteins carry out similar cellular functions in fungi and animals. Further characterization of the function of HAM-3 in *N. crassa* will allow interesting comparisons between neuronal and hyphal signaling and might reveal conserved cellular mechanisms.

5. Physiological and Morphogenetic Consequences of Fusion

There are many advantages associated with hyphal fusion within a colony and between colonies, including increased resource sharing and translocation, increased colony cooperation, hyphal healing, and exchange of genetic material. For example, in *Colletotrichum lindemuthianum*, conidia that undergo fusion exhibit a higher rate of germination compared with single unfused conidia *(5)*. Also, conidia grown in low-nutrient environments show an increased rate of fusion *(4)*. These observations suggest that fusion between conidial germlings may serve to increase or pool resources that are important for colony establishment.

It is widely assumed that vegetative hyphal fusion within an established colony is important for intrahyphal communication, cooperation, translocation of water and nutrients, and general homeostasis within a colony. Fusing to create a hyphal network could be important in influencing hyphal patterns of growth and morphogenesis in filamentous fungi. Formation of a connected network may also facilitate signaling within a colony (by molecules, proteins, or perhaps electric fields; *reviewed in* **ref. *62***), which may also affect behavior and development of a filamentous fungal colony. In nature, fungal colonies exploit diverse environments with unequal distributions and types of nutrient sources. The ability to form a hyphal network may be needed for coordinated behavior between the different parts of a fungal colony and nutrient transport from sources to sinks *(6)*.

As well as facilitation of long-distance nutrient transport, vegetative hyphal fusion can function as a healing mechanism to repair hyphal connections when the fungal network has been damaged. Hyphal tips growing out from either side of damaged compartments will eventually find each other, fuse, and reestablish

the hyphal network *(1)*. In fungi that are asexual, fusion between different individuals can be a means of exchanging genetic material through the formation of a heterokaryon *(7,63)*.

Fusion between different colonies has potential disadvantages. Hyphal fusion between individuals increases the risk of transfer of deleterious infectious elements, parasitism, or resource plundering, such as the competitive acquisition of resources by one colony from another (*reviewed in* **ref. *64***). Many fungi have developed mechanisms for nonself-recognition that result in programmed cell death, which is assumed to minimize the amount of exchange between individual colonies *(64–66)*. Many of the nonself-recognition mechanisms occur following hyphal fusion between genetically different colonies, raising the intriguing possibility that a link is present between the hyphal fusion and programmed cell death machinery in filamentous fungi.

6. Conclusion

Cell fusion events are essential for the vegetative and sexual development of filamentous ascomycete fungi. Live cell imaging has revealed that the processes of cell–cell communication and cell fusion are complex and highly regulated. Characterization of the components required for sexual fertilization versus vegetative fusion indicates that upstream components of the signaling pathways differ. Future analyses will reveal if the machinery associated with fusion processes are similar between sexual and vegetative fusion events. *Neurospora crassa* is an attractive model system with which to study the molecular basis of cell–cell communication and cell fusion in eukaryotes and to dissect similarities and differences in the processes of sexual and vegetative fusion: the genome has been sequenced and annotated *(54)*, well-established molecular and cell biology techniques are available (**ref. *52; see also* http://www.nih.gov/science/models/neurospora/); and whole-genome microarrays *(67)* and knockout mutants for every single gene are in progress *(17)*. It is currently unclear what the adaptive role of germling and hyphal fusion is in filamentous fungi and what selective advantages it provides. Further analysis of these issues will provide significant insight into environmental adaptation and the evolution of form and function in multicellular microorganisms.

Acknowledgment

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