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Hyphal Fusion

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Filamentous fungi in the Ascomycete and Basidiomycete groups grow by hyphal tip extension, branching and fusion to make an interconnected, supracellular network of hyphae that is the hallmark of these organisms (Buller, 1933; Read, 2007). Hyphal anastomosis also occurs in Zygomycetes (Griffin and Perrin, 1960), where it apparently is not a common phenomenon (Gregory, 1984), and in arbuscular mycorrhizal fungi, which are members of the Glomeromycota (Giovannetti et al., 1999, 2001). Species within the Oomycota, which undergo hyphal tip growth and branching but which are more closely related to algae and are members of the Kingdom Stramenopila, also undergo hyphal fusion (Stephenson et al., 1974).

Hyphal fusion occurs at a number of stages during the life cycle of filamentous fungi, particularly during (i) colony initiation, (ii) mature colony development, and (iii) sexual development. A wide range of important roles are performed by hyphal fusion during the life cycle. This chapter focuses on hyphal fusion in filamentous ascomycete and basidiomycete species, with emphasis on the model ascomycete fungus, *Neurospora crassa*. It reviews the different types of hyphal fusion, its mechanistic basis, and the varied functions that it serves, and it compares hyphal fusion with processes of cell fusion in fungi and other eukaryotic species. Hyphal fusion has also been recently reviewed by Glass et al. (2000, 2004), Roca et al. (2005a), Glass and Fleißner (2006), Read and Roca (2006), and Fleißner et al. (2008).

HYPHAL FUSION DURING DIFFERENT STAGES OF THE LIFE CYCLE

Hyphal Fusion During Colony Initiation

Hyphal fusion between spores and spore germlings during colony initiation is very common. It is most often observed between conidia and conidial germlings and has been demonstrated in over 73 ascomycete species (Roca et al., 2005a). Hyphal fusion has also been observed between ascospore germlings and between urediospore germlings (Read and Roca, 2006).

Tulasne and Tulasne (1863) first illustrated fusions between conidia and conidial germlings in a variety of species. An important early experimental study of fusion between germinating conidia was performed by Köhler (1930). He described fusion between germinating conidia of the same or different species via small hyphal bridges ("Fusionshyphen"), which were significantly narrower than germ tubes. Numerous other papers subsequently showed images of fused conidia and conidial germlings in culture (Hay, 1995), in asexual reproductive structures (Mesterhazy, 1973), or on host plants (Latunde-Dada et al., 1999). However, it was not fully appreciated until recently that fusion between conidia/conidial germlings involves the formation and interaction of specialized hyphae, which are different from germ tubes and which have been termed "conidial anastomosis tubes" (CATs) (Roca et al., 2003, 2005a, 2005b).

The initial characterization of CATs was made in the plant pathogen *Colletotrichum lindemuthianum* (Roca et al., 2003) and the saprotrophic species *N. crassa* (Roca et al., 2005b). They were shown to have the following combination of characteristics which distinguishes them from conidial germ tubes.

1. CATs are usually thinner and shorter than germ tubes and exhibit determinate growth. Germ tubes differentiate into vegetative hyphae of the mature colony (Fig. 1A through D) (Araujo-Palomares et al., 2007; Roca et al., 2005b). However, it is often not possible to define CATs on the basis of their width alone, and this is a problem particularly when identifying CATs that arise from germ tube tips (Fig. 1B) or as germ tube branches (Fig. 1C).
2. CATs are unbranched, while germ tubes undergo branching (Araujo-Palomares et al., 2007; Roca et al., 2005b).
3. CATs in *N. crassa* can arise directly from conidia, from germ tube tips or as subapical branches of germ tubes (Roca et al., 2005a, 2005b) (Fig. 1A through D).

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4. CAT induction is dependent on conidial density. In *N. crassa*, the optimum conidial density is ~10^6 conidia ml^{-1} (Roca et al., 2005b). Germ tube formation in *N. crassa* is not dependent on conidial density over the range of 10^2 to 10^6 conidia ml^{-1} (H.-C. Kuo and N. D. Read, unpublished data).
5. CATs home towards each other, while germ tubes tend to avoid one other. CAT homing has been most unambiguously demonstrated using optical (laser) tweezer micromanipulation (Fig. 1D). When the relative position of two CATs exhibiting chemotropic growth towards each other is changed by micromanipulation with optical traps, both CATs readjust their direction of growth back towards each other to make contact and fuse at their tips (Fleißner et al., 2005; Roca et al., 2005a; Wright et al., 2007).
6. CATs are under separate genetic control from germ tubes. A growing number of mutants have been isolated that are inhibited at specific stages of CAT fusion but undergo conidial germination and germ tube formation (Table 1).
7. CATs function in interconnecting conidial germlings and are believed to allow the young colony to act as a cooperative individual (reviewed by Riquelme et al., 1998). Germ tubes, on the other hand, function in colony establishment.

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How consistent these features of CATs are in other species will need to be carefully assessed in the future. Some species of filamentous fungi seem to lack CATs (for example, Craven et al. reported that *Alternaria alternata* does not form CATs [Craven et al., 2008]). We are not aware of any published illustrations of CAT fusion between conidial germlings in the model species *Magnaporthe oryzae* or *Aspergillus nidulans*. However, CAT formation in some species may require specific conditions (e.g., nutritional factors or conidial density) that are not commonly used experimentally.

Hyphal Fusion in a Mature Colony

A filamentous fungal colony is morphologically complex and contains a variety of different hyphal types (Bistis et al., 2003). Leading hyphae and their branches at the periphery of a colony typically grow outward, exhibit a subapical branching pattern, and tend to avoid each other (Buller, 1933). Hyphal anastomosis does not normally occur in this peripheral zone of an *N. crassa* colony (Hickey et al., 2002). Behind this peripheral zone, hyphal fusion occurs extensively to produce the characteristic interconnected state of the mature colony (Fig. 1E). Hyphal anastomosis is initiated by the formation of specialized "fusion hyphae," which arise as branches from established hyphae and branches in this part of the colony. Although fusion events are frequent within a colony, they are not uniformly distributed. Another type of hyphal fusion also occurs in mature colonies of nematophagous fungi, such as *Arthrobotrys oligospora*, where hyphal fusion is important for the formation of a range of specialized nematode-trapping structures. These traps can take the form of simple rings, constricting rings, or net-like structures (Barron, 1977; Read and Roca, 2006).

In *N. crassa*, vegetative hyphal fusion in a mature colony has been described in detail using time-lapse confocal microscopy (Hickey et al., 2002). The process can be conveniently divided into three phases: precontact, postcontact, and postfusion (Glass et al., 2000, 2004; Hickey et al., 2002; Read and Roca, 2006), as described below.

Precontact Phase

During the precontact phase in *N. crassa*, the fusion hyphae are induced and grow ("home") towards each other. The proximity of fusion hyphae to other hyphae often results in the initiation of new positively chemotropic hyphal tips that are fusion competent (Buller, 1933; Hickey et al., 2002; Köhler, 1929). The fusion hyphae in *N. crassa* can often be recognized as morphologically distinct from other hyphae. They always appear to be hyphal branches and can vary from being short, peg-like structures to reasonably long, and often dichotomously branched hyphae (Hickey et al., 2002).

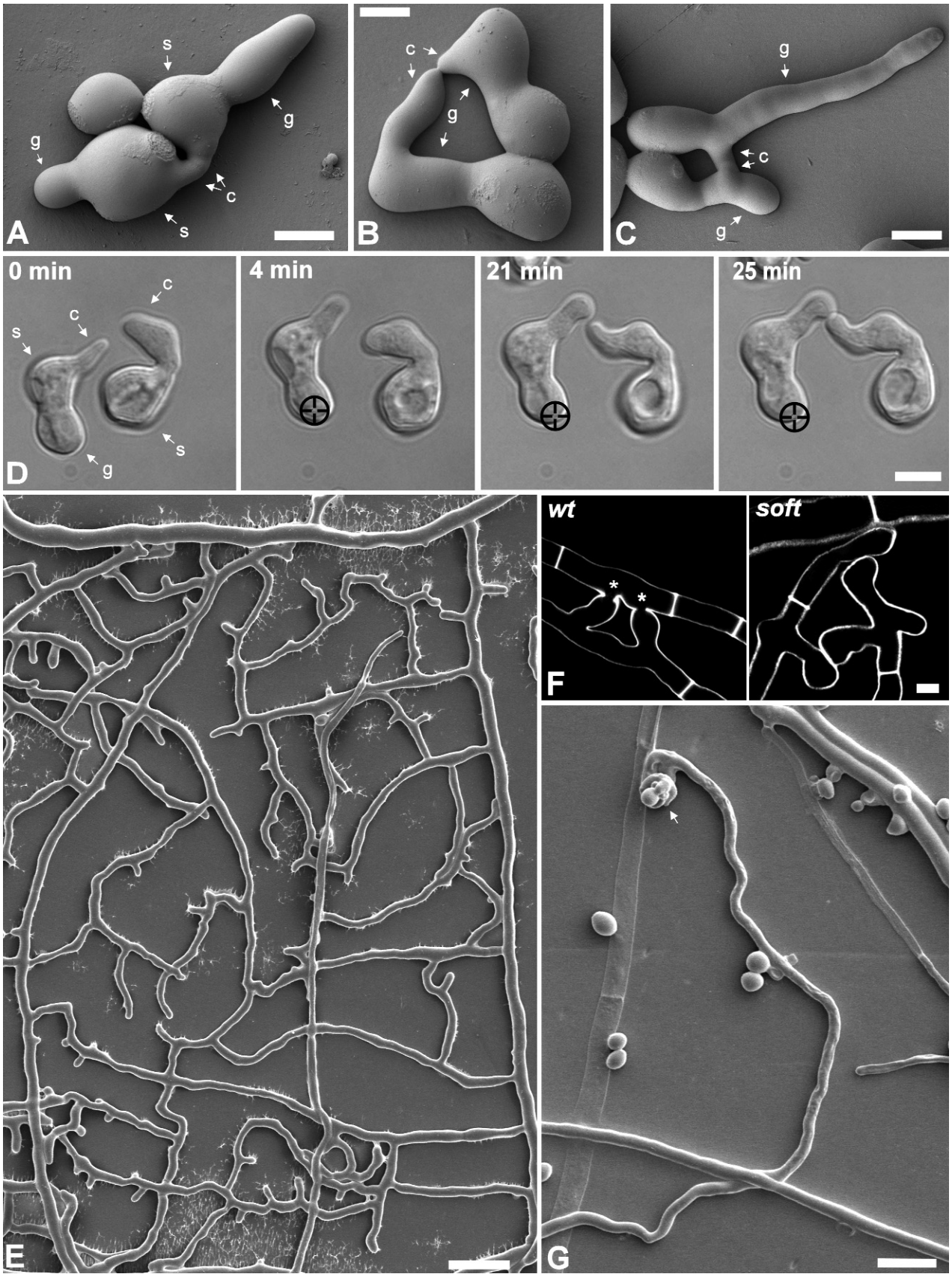
Fusion hypha formation is associated with the development of a Spitzenkörper. The Spitzenkörper is a complex organelle and protein assemblage predominated by secretory vesicles that is found in growing hyphal tips or at sites of branch initiation (Harris et al., 2005; Virag and Harris, 2006), and which is associated with polar and directional growth of hyphae (Bracker et al., 1997; Gierz and Bartnicki-Garcia, 2001; Girbardt, 1957; Grove and Bracker, 1970; Riquelme et al., 1998). During the precontact phase, the fusion hypha Spitzenkörper shares features in common with the Spitzenkörper of a growing vegetative hypha. The secretory vesicles within the Spitzenkörper can be readily stained with the membrane-selective dye FM4-64 (Fischer-Parton et al., 2000; Hickey et al., 2004). By use of this marker dye, the Spitzenkörper was stained in fusion hyphae of *N. crassa* and its behavior was monitored (Hickey et al., 2002). The formation of a new Spitzenkörper was found to precede a new fusion hypha at its site of emergence, and the positive chemotropic reorientation of fusion hyphal tips towards each other was associated with the Spitzenkörper in these tips positioning themselves towards the opposing tips (the localization of the Spitzenkörper in the growing tip was coincident with the direction of homing).

Postcontact Phase

Hyphal fusion in *N. crassa* usually results from "tip-to-tip" contact between two fusion hyphae or from "tip-to-side" contact between a fusion hypha and another hypha (Buller, 1933; Hickey et al., 2002). When the hyphal tips involved in fusion are very short branches, they are often referred to as "pegs" (Buller, 1933). During the postcontact phase, hyphal tips that will fuse cease extension growth and often undergo isotropic growth, which results in swelling of their tips (Hickey et al., 2002). This is accompanied by the adhesion of the two hyphae to each other and the digestion of their intervening cell walls. The Spitzenkörper persists during this period, suggesting that it provides secretory vesicles for wall synthesis during hyphal tip swelling and for the delivery of extracellular adhesives and cell wall-degrading enzymes during cell wall degradation and fusion pore formation (Hickey et al., 2002).

Postfusion Phase

The onset of the postfusion phase is marked by the fusion of the plasma membranes of the two anastomosing hyphae. This results in the formation of a "fusion pore" through which cytoplasm and organelles, including nuclei, flow. Fusion is often associated with dramatic alterations in the bulk flow of cytoplasm and organelles and facilitates their rapid mixing between the fused hyphae. The fusion pore increases in diameter following fusion, and one or two septa commonly form in its vicinity (Hickey et al., 2002). The Spitzenkörper persists at the site of fusion pore formation



and presumably continues to function in providing secretory vesicles containing cell wall-degrading enzymes and proteins associated with fusion pore formation and membrane merger. The Spitzenkörper disappears once the fusion pore is fully formed (Hickey et al., 2002). During the postcontact and postfusion phases, the persistence of the Spitzenkörper within a fusion hypha is a marked behavioral difference compared to that in a vegetative hypha, in which the Spitzenkörper characteristically disappears after hyphal growth has ceased (Girbardt, 1957).

Hyphal Fusion during Fertilization and in Sexual Development

In members of the Ascomycota and Basidiomycota, hyphal fusion occurs during mating-cell fusion and during the formation and maintenance of the dikaryon during the sexual phase of the life cycle. Mating-cell fusion commonly involves the fusion between a compatible hypha and a spore (microconidium or conidium in the Ascomycota and oidium in the Basidiomycota), which acts as a male fertilizing agent (or spermatium) in outbreeding species. In the Ascomycota, a fertile receptive hypha, the trichogyne, is often produced and grows out from the ascogonium of an unfertilized fruiting body. These trichogynes show positive chemotropism towards sex pheromones released by conidia (or microconidia) of opposite mating type (Fig. 1G) (Bistis, 1981; Kim and Borkovich, 2006). Mating cell fusion in some ascomycete species occurs in the absence of conidium-trichogyne fusion, but the precise mechanism(s) involved are poorly understood. In the Basidiomycota, monokaryotic hyphae of basidiomycete species show positive chemotropism towards sex pheromones released by oidia of opposite mating type (Bistis, 1970; Webster, 1980). Alternatively, compatible monokaryotic hyphae of opposite mating type can fuse in the absence of male oidia (Webster, 1980).

The dikaryotic phase is a feature of filamentous ascomycete and basidiomycete species, where nuclei of opposite mating type (in outbreeding species) or two genetically identical nuclei (in inbreeding species) are involved in the formation and proliferation of binucleate cells. In many ascomycete and basidiomycete species, dikaryotic cells are formed and maintained by specialized hyphae called crozier and clamp cells, respectively (Fig. 2A and B).

In *N. crassa* and related ascomycetes, ascogenous hyphae grow out from the ascogonium as a branched hyphal network that is mostly multinucleate (Beckett, 1981; Zickler et al., 1995). A hook-shaped crozier forms at the tip of an ascogenous hypha. Two nuclei within the crozier go through a simultaneous mitotic division and undergo septation across the two mitotic spindles to produce a uninucleate terminal cell and a binucleate penultimate cell, while the fourth nucleus remains in the multinucleate compartment of the ascogenous hypha (Fig. 2A). The two nuclei (the so-called "prefusion nuclei") in the penultimate cell undergo karyogamy. This cell becomes the ascus mother cell and subsequently grows out and develops into the ascus. Meanwhile, the terminal cell grows back and fuses with the subtending ascogenous hypha, allowing the nuclei in these two compartments to pair up. In *N. crassa*, the two nuclei that subtend the ascus also undergo karyogamy (Raju, 1980). In other species, these two nuclei maintain the dikaryotic state; this process is repeated as a result of further croziers forming as branches from the dikaryotic subtending cell (Berteaux-Lecellier et al., 1998; Read and Beckett, 1996). Other patterns of karyogamy have been described in other ascomycete species (e.g., in *Neotiella rutilans*) and have been reviewed by Read and Beckett (1996).

Clamp cells are found at the septa of dikaryotic hyphae of many basidiomycete species. Clamp cell formation is initiated from the apical hyphal compartment and involves the formation of a backward-growing hyphal branch at a site that is just forward of the site of the future septum. The hypha continues to grow back and forms a hook-like structure. Concomitantly, the two genetically different nuclei of the dikaryotic apical hyphal compartment divide and a septum forms at the base of the clamp cell in which one of the nuclei becomes trapped (Fig. 2B). Another septum is formed within the apical hyphal compartment, leaving the nucleus of the other mating type in the newly formed subapical hyphal compartment while keeping together two genetically different nuclei in the apical compartment. A subapical peg is typically formed in response to the presence of the backward-growing clamp cell, and the two fuse, resulting in the two solitary nuclei becoming united in the subapical hyphal compartment. This process is repeated at regular intervals to maintain binucleate hyphal compartments along the dikaryotic vegetative hypha (Badalyan et al., 2004; Buller, 1933; Todd and Aylmore, 1985). Within sexual fruiting bodies, little is known about hyphal fusion between somatic hyphae, although it has been reported in the sexual primordia of various basidiomycete species (Van der Valk and Marchant, 1978; Williams et al., 1985).

FIGURE 1 *N. crassa.* (A) Conidial anastomosis tubes (CATs) (c) that have formed directly from macroconidia (s) and fused with each other. Note that the germ tubes (s) are wider than the CATs. Bar = 5 μm. (From M. G. Roca, •. Jeffree, and N. D. Read, unpublished data.) (B) CATs (c) that have formed from germ tube (g) tips, grown towards each other, and made contact. Bar = 5 μm. (From Roca, Jeffree, and Read, unpublished.) (C) CATs (c) that have formed subapically from germ tubes (g) and have fused. Bar = 5 μm. (From Roca et al., 2005b, with permission.) (D) The CAT homing assay. The two conidia had germinated, and their CATs were homing towards each other (0 min). The left-hand germling was repositioned (here shown 4 min after repositioning). The CAT tips then changed their orientation to home back towards each other (15 and 21 min) before making contact (25 min) and subsequently fusing (not shown). The left-hand conidium remained trapped throughout the entire 25-min period without apparent inhibition of CAT growth, homing, or fusion. The position of the trap in the germ tube (g) is represented by the crosshair in the circle. Note that the germ tube is significantly wider than either CAT. Bar = 10 μm. (From Wright et al., 2007, with permission.) (E) Hyphal fusion in a mature colony that has resulted in a complex interconnected hyphal network. Bar = 100 μm. (From •. Lord and N. D. Read, cover image for 2008 issues of *Fungal Biology Reviews*, with permission.) (F) A comparison of the morphology of anastomosis between fusion hyphae in a mature wild-type colony (note fusion pores [asterisks]) and a fusion mutant (*soft*) in which fusion does not occur. Hyphae imaged by confocal microscopy after staining with calcofluor white M2R. Bar = 10 μm. (From Fleißner et al., 2005, with permission.) (G) Trichogyne that has homed towards, and wrapped around, a macroconidium (arrow) of opposite mating type. Bar = 20 μm. (From •. Kuo, •. Jeffree, and N. D. Read, unpublished data).

TABLE 1 Genes required for hyphal fusion in *N. crassa* and their roles in the fusion process

Gene[a]	Locus[a]	Function	Required for process?: Germling fusion	CAT induction	CAT homing	Hyphal fusion in mature colony	Protoperithecium formation	Mating cell fusion	Sexual development[b]	References
NCU09842	*mak-1*	MAPK	?	?	?	Yes	Yes	?	Male fertile	Maerz et al., 2008
NCU06419	*mek-1*	MAPKK	?	?	?	Yes	Yes	?	Male fertile	Maerz et al., 2008
NCU02234	*mik-1*	MAPKKK	?	?	?	Yes	Yes	?	Male fertile	Maerz et al., 2008
NCU02393	*mak-2*	MAPK	Yes	Yes	?	Yes	Yes	?	Ascospore lethal	Pandey et al., 2004; Roca et al., 2005b
NCU04612	*mek-2*	MAPKK	?		?	Yes	Yes	?	Ascospore lethal	Maerz et al., 2008
NCU06182	*nrc-1*	MAPKKK	Yes	Yes	?	Yes	Yes	?	Ascospore lethal	Pandey et al., 2004; Roca et al., 2005b
NCU00340	*pp-1*	TF[c]	Yes	Yes	?	Yes	Yes	?	Ascospore lethal	Li et al., 2005
NCU07024	*os-2*	MAPK	?	?	?	Yes	Yes	?	Male fertile	Jones et al., 2007; Maerz et al., 2008
NCU03071	*os-4*	MAPKKK	?	?	?	Yes	Yes	?	Ascospore lethal	Maerz et al., 2008
NCU00587	*os-5*	MAPKK	?	?	?	Yes	Yes	?	Ascospore lethal	Maerz et al., 2008
NCU09757	*gpig-1*	GPI-anchor	?	?	?	Yes	Yes	?	Lethal	Bowman et al., 2006
NCU06663	*gpip-1*	GPI-anchor	?	?	?	Yes	Yes	?	Lethal	Bowman et al., 2006
NCU07999	*gpip-2*	GPI-anchor	?	?	?	Yes	Yes	?	Male fertile	Bowman et al., 2006
NCU06508	*gpip-3*	GPI-anchor	?	?	?	Yes	Yes	?	Male fertile	Bowman et al., 2006
NCU05644	*gpit-1*	GPI-anchor	?	?	?	Yes	Yes	?	Male fertile	Bowman et al., 2006
NCU02794	*so*	Unknown	Yes	Yes	Yes	Yes	No	No	Perithecia not formed	Fleißner et al., 2005
NCU03727	*ham-2*	Membrane protein	Yes	Yes	?	Yes	No	No	Perithecia not formed	Roca et al., 2005b; Xiang et al., 2002
NCU09337	*prm-1*	Membrane protein	Yes	No	No	Yes	No	Yes	Ascus dominant	Fleißner et al., 2009

[a]Nomenclature and gene names according to *N. crassa*.

[b]"Male fertile" means that the mutant can be taken through a cross if used as a male, but not as a female. "Ascospore lethal" means that, even if used as a male, ascospore progeny bearing the indicated mutated gene cannot be recovered through a cross. "Perithecia not formed" indicates that mating cell fusion occurs, but subsequent sexual development from the protoperithecium is blocked; the mutant is used as a female. "Ascus dominant" indicates that mating cell fusion occurs, but subsequent sexual development from the protoperithecium is blocked when the mutant is used as a female or a male.

[c]TF, transcription factor.

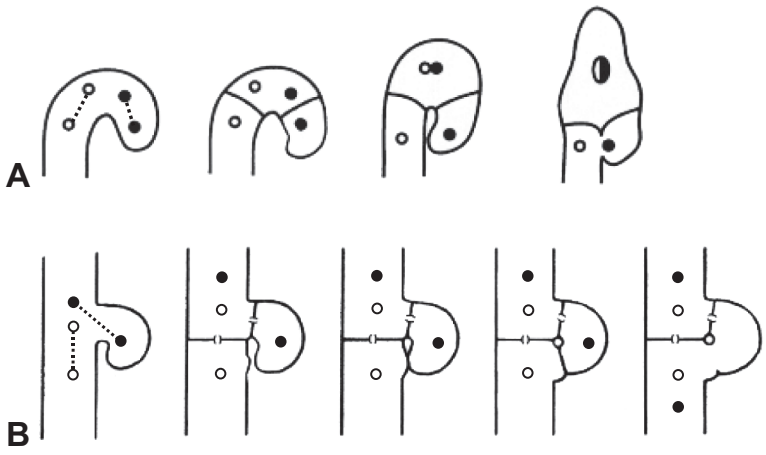


FIGURE 2 (A) Diagram of crozier cell fusion in a typical ascomycete species. (B) Diagram of clamp cell fusion in a basidiomycete species. See "Hyphal Fusion in a Mature Colony" for details.

MECHANISTIC BASIS OF VEGETATIVE HYPHAL FUSION

Induction

CAT induction probably involves an extracellular CAT inducer produced by ungerminated conidia. Evidence for this comes from observations that CAT formation is dependent on macroconidial concentration (Roca et al., 2005b), CATs form when conidia are close together, and culture filtrates can induce CAT fusion (Leu, 1967). CAT induction may involve a form of quorum sensing (i.e., a mechanism whereby cells sense their density by detecting extracellular molecules secreted by the population) (Miller and Bassler, 2001). In *Venturia inaequalis*, germling fusion via CATs was found to be frequent when conidia were in close proximity. Fusion was not observed between two isolated conidia mounted close to one another (Leu, 1967). However, when multiple conidia were placed close to one another under the same conditions, numerous germling fusion events were observed. Fusion between isolated conidial pairs in *V. inaequalis* was induced by the addition of culture filtrates from the same or different isolates. The observed fusion induction was apparently species specific, because culture filtrates of *Venturia pirina* did not induce fusions between *V. inaequalis* conidia. The identity of the self-signaling inducer is unknown.

The composition of the growth medium can affect fusion frequency among conidial germlings, and this may relate to the frequency of CAT induction. In *Leptosphaeria coniothyrium*, *Sclerotinia fructigena*, *Botrytis* sp., *Fusarium* sp., and *N. crassa*, the frequency of germling fusion was reduced on rich organic media (such as 1% malt or potato dextrose agar), but fusion was frequent when these media were diluted (Köhler, 1930; Laibach, 1928). In contrast, the frequency of germling fusion in *V. inaequalis* was found to be significantly greater on complete medium than on basal medium or water agar (Leu, 1967). It is not clear whether a nutrient or another factor in the medium is causing these inhibitory or stimulatory effects.

CAT induction in *N. crassa* requires a putative transmembrane protein (HAM-2) (Xiang et al., 2002) and the NRC-1/MEK-2/MAK-2 mitogen-activated protein (MAP) kinase pathway (see below); strains containing mutations in the genes encoding these proteins do not form CATs (Roca et al., 2005b). Furthermore, phosphorylation of MAK-2 increased during the period when CAT formation/hyphal fusion is most prolific (Pandey et al., 2004). In *Saccharomyces cerevisiae*, stimulation of the pheromone response pathway required for mating cell fusion results in activation of a transcription factor, Ste12 (Errede and Ammerer, 1989), which regulates the expression of genes involved in polarized growth, cell adhesion, cell wall breakdown, and membrane merger (Cross, 1988; Gammie et al., 1998; Heiman and Walter, 2000) (see below). In *N. crassa*, a strain containing a deletion of the *ste12* ortholog, *pp-1*, is very similar in phenotype to the *nrc-1*, *mek-2*, and *mak-2* mutants, fails to form CATs, and is defective in germling fusion and hyphal fusion in mature colonies (Li et al., 2005; D. Li, A. Fleißner, and N. L. Glass, unpublished observations) (Table 1). CAT induction may also involve a filamentous-ascomycete-specific WW domain protein called SOFT (SO) because *so* mutants produce fewer CATs than the wild type (Fleißner et al., 2005). AQ4

Much less is known about what makes hyphae in the subperipheral region of the mature colony competent to undergo fusion, but the process involves the induction of specialized fusion hyphae (see "Hyphal Fusion in a Mature Colony" above). It is not known whether this is primarily regulated by a program of gene expression that is specific to this region of the colony or whether environmental factors are important in initiating the expression of hyphal fusion genes. However, an unknown self-signaling inducer is involved. This inducing compound seems to be produced by the tips of fusion hyphae because the close proximity of a fusion hyphal tip to a trunk hypha in *N. crassa* can induce the formation of a new fusion hypha in its vicinity (Buller, 1933; Hickey et al., 2002). It is not unreasonable to postulate that the same molecule may function as both the CAT and fusion hypha inducer.

As indicated in "Hyphal Fusion in a Mature Colony" above, nematode traps produced by nematophagous fungi result from hyphal fusion. They can be induced by the presence of nematodes, and Pramer and Stoll (1959) coined the term "nemin" to describe the inducer. Nordbring-Hertz et al. (1989) later showed that di- and tripeptides containing valine were very effective in inducing traps under nutrient-poor conditions.

Chemoattraction

A key feature of CATs or fusion hyphae is that they are attracted towards one other. In *N. crassa*, when the relative

position of two conidial germlings showing mutual attraction is changed by micromanipulation using optical tweezers, the CAT tips of both germlings adjust their growth towards each other to make contact and undergo fusion (Fleißner et al., 2005; Roca et al., 2005b; Wright et al., 2007) (Fig. 1D). These observations provide compelling evidence for a diffusible chemoattractant released from CAT tips and for a chemoattractant receptor located at the CAT tips. However, as with the CAT/fusion hypha inducer(s) (see "Induction" above), the identity of the chemoattractant is unknown. It is possible that the inducer and chemoattractant are the same self-signaling ligand. In *S. cerevisiae*, both the induction of the formation of mating cell protuberances (shmoos) and chemotropic interactions associated with mating are regulated by the secretion of peptide sex pheromones (**a**-pheromone or α-pheromone) that bind to their cognate G-protein-coupled receptors (Ste3p or Ste2p, respectively) in a cell of the opposite mating type (Cross, 1988; Elion, 2000).

The process of vegetative hyphal fusion between compatible cells in filamentous fungi involves the fusion of genetically identical cells (i.e., self fusion), in contrast to mating-cell fusion in *S. cerevisiae*, which involves the fusion of two genetically nonidentical cells (i.e., nonself fusion). The best-studied example of chemoattraction between genetically identical cells in eukaryotes is found in the slime mold *Dictyostelium discoideum*, in which extracellular cyclic AMP (cAMP) acts as a chemoattractant that results in aggregation of amoebas (Manahan et al., 2004). The discovery that the *N. crassa* genome encodes three G-protein-coupled receptor-like proteins that resemble *Dictyostelium* cAMP receptors (Borkovich et al., 2004; Galagan et al., 2003) suggested the possibility that cAMP may serve a similar chemoattractant role in filamentous fungi. However, cAMP plays no apparent role as a CAT chemoattractant in *N. crassa*, because a *cr-1* mutant lacking cAMP was shown to undergo CAT homing after optical tweezer-based micromanipulation (Roca et al., 2005b).

In *S. cerevisiae*, the interaction of **a** or α-pheromone with Ste3p or Ste2p, respectively, results in the disassociation of a heterotrimeric G-protein (Gα=Gpa1p, Gβ=Ste4p, and Gγ=Ste18p). Interaction between Gβγ and Ste20p activates a MAP kinase cascade (Ste11p-Ste7p-Fus3p) (Cross, 1988; Elion, 2000). Although mating-cell fusion in *N. crassa* involves a similar interaction of cognate pheromones and G-protein-coupled receptors (Li et al., 2007), mutations in *N. crassa* genes encoding the sex pheromones (*mfa-1* and *ccg-4*), the pheromone receptors (*pre-1* and *pre-2*), and heterotrimeric G-proteins (*gna-1*, *gnb-1*, and *gng-1*) do not affect vegetative hyphal fusion, as assessed by heterokaryon tests and germling fusion assays (Kim and Borkovich, 2004, 2006; Li et al., 2007). However, strains containing deletion mutations in *mak-2* (the *N. crassa* ortholog of *FUS3*) and *nrc-1* (the *STE11* ortholog) are unable to undergo germling or hyphal fusion in mature colonies (Pandey et al., 2004; Roca et al., 2005b) (Table 1). Furthermore, conidia of *mak-2* and *nrc-1* mutants do not attract wild-type CATs, indicating that neither of these mutants produces chemoattractant (Table 1) (Roca et al., 2005b). We have recently found that MAK-2-green fluorescent protein (GFP) localizes to the tips of CATs undergoing chemotropic homing towards each other (A. Fleißner, M. G. Roca, N. D. Read, and N. L. Glass, unpublished observations). These studies indicate that the MAK-2 MAPK pathway is required both for the initiation of fusion and for chemotropic interactions. Recent data indicate that a strain containing a deletion mutation of the predicted MAPKK in the MAK-2 MAP kinase pathway, termed *mek-1*, is also vegetative-fusion defective (Maerz et al., 2008) (Table 1). Interestingly, the vegetative-fusion defects of *mak-2* and *nrc-1* mutants are suppressed by mutations in *cot-1* (Maerz et al., 2008); *cot-1* encodes a protein kinase of the NDR Ser/Thr protein kinase family, which is required for hyphal tip elongation in *N. crassa* (Yarden et al., 1992). The requirement of the MAK-2 MAP kinase pathway for vegetative hyphal fusion is also conserved in other filamentous ascomycete fungi. For example, in *A. nidulans*, a mutant disrupted in the MAPKKK *nrc-1* homolog, *steC*, fails to form heterokaryons (Wei et al., 2003).

In addition to genes encoding components of the MAK-2 MAP kinase pathway, an *N. crassa* locus called *soft*, which encodes a filamentous ascomycete-specific WW domain protein, is required for vegetative hyphal fusion (Fig. 1F) (Fleißner et al., 2005; Wilson and Dempsey, 1999). *so* mutants form CATs, but these fail to show chemotropic interactions (Table 1; Fig. 3), suggesting that *so* mutants are impaired both in the production/secretion and reception/transduction of a chemoattractant signal (Fleißner et al., 2005). Functional SO-GFP is found in the cytoplasm in hyphae, but upon injury, SO-GFP localizes rapidly to septal pore plugs. SO-GFP localization was shown to be Woronin body (*hex-1*) independent. Importantly, *so* mutants show a decrease in efficiency of septal plugging upon injury (Fleißner and Glass, 2007). In the homothallic filamentous ascomycete species *Sordaria macrospora*, strains containing mutations in the *so* ortholog (*pro40*) are also defective in vegetative hyphal fusion (Rech et al., 2007); Pro40, like SO, localizes to septal pore plugs (Engh et al., 2007). Similarly, mutations in *so* orthologs in plant pathogens such as *Fusarium oxysporum* and *Alternaria brassicicola* also resulted in mutants that fail to undergo vegetative hyphal fusion (Craven et al., 2008; Prados Rosales and Di Pietro, 2008) (see "Hyphal Fusion and Plant Pathogenesis" below).

In *N. crassa,* the *ham-2* (acronym for hyphal anastomosis) locus encodes a putative transmembrane protein (Xiang et al., 2002); *ham-2* mutants fail to undergo both germling and hyphal fusion in mature colonies (Table 1; Fig. 3). Similar to the *mak-2* and *nrc-1* mutants described above, *ham-2* mutants fail to attract wild-type CATs, suggesting that HAM-2 is required for chemoattractant production/secretion and reception/transduction (Roca et al., 2005b). In *S. cerevisiae*, mutations in the *ham-2* ortholog, *FAR11*, result in mutants that prematurely recover from G_1 growth arrest following exposure to pheromone (Kemp and Sprague, 2003). Far11p was shown to interact with five other proteins (Far3p, Far7p, Far8p, Far9p, and Far10p). Homologs of genes encoding *FAR3* and *FAR7* are lacking in *N. crassa* (Glass et al., 2004). Preliminary data show that mutations in *N. crassa* homologs of *Far8* and *Far9/10* (*ham-3* and *ham-4*, respectively) result in mutants displaying phenotypes similar to the *ham-2* mutant, including a block in vegetative hyphal fusion (A. Simonin, C. G. Rasmussen, M. Yang, and N. L. Glass, unpublished results). In *S. macrospora*, strains containing mutations in the *ham-2* ortholog (*pro22*) results in mutants that show a dramatic reduction in fusion frequency and fail to develop mature fruiting bodies (Rech et al., 2007). The predicted *N. crassa* HAM-3 protein shows significant similarity to proteins of the striatin family. The striatin family of proteins act as scaffolding proteins that organize signaling complexes; for example, formation of a complex between striatin and the

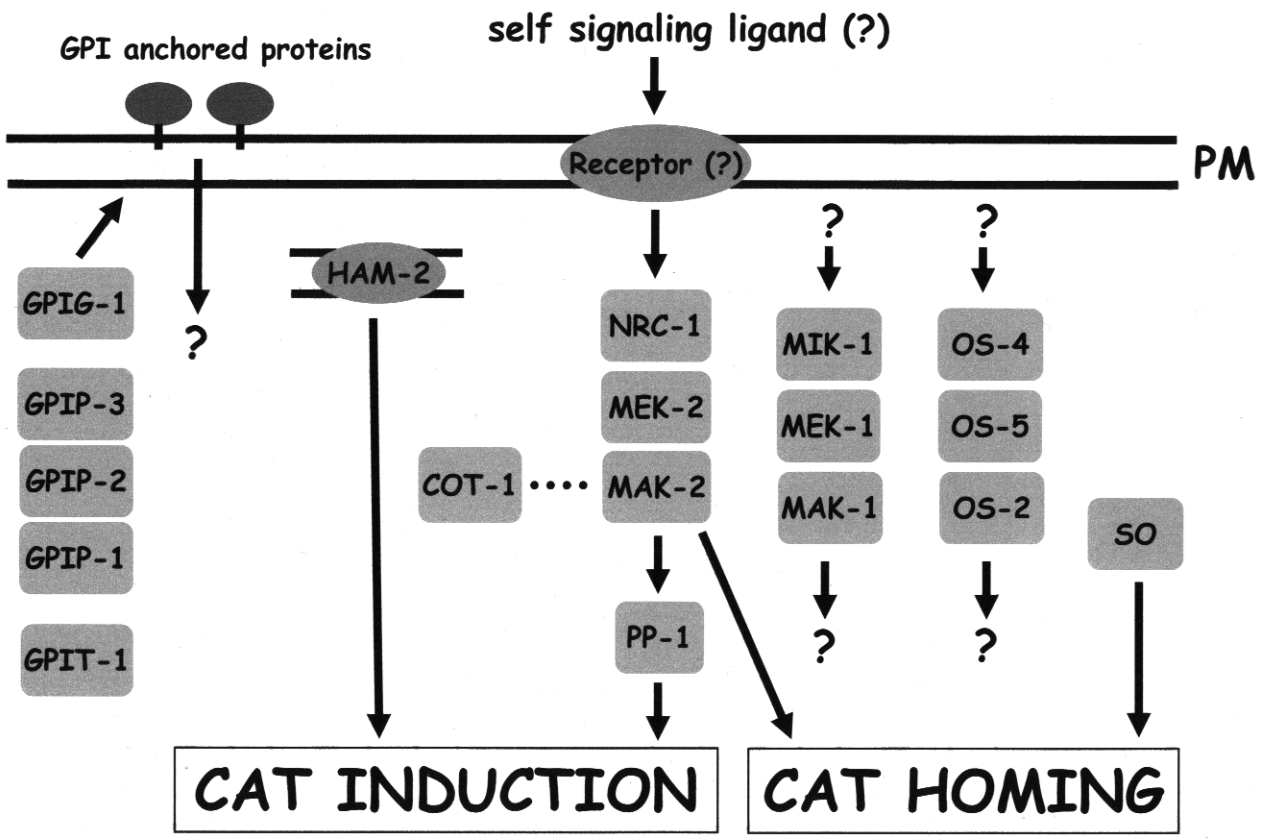


FIGURE 3 Working model of the signaling involved during vegetative hyphal fusion in *N. crassa* (see the text for details). The self-signaling ligand and receptor responsible for the process of self-fusion are unknown. GPIG1, GPIT1, GPIP1, GPIP2, and GPIP3 are involved in the GPI protein anchoring pathway (Bowman et al., 2006). It is not known which stage(s) of vegetative hyphal fusion these proteins regulate. HAM-2 is a predicted transmembrane protein (Xiang et al., 2002), although it is not known which cellular membrane it is associated with. NRC1-MEK2-MAK2 (Li et al., 2005; Maerz et al., 2008; Pandey et al., 2004; Roca et al., 2005b), MIK1-MEK1-MAK1 (Maerz et al., 2008), and the OS4-0S5-OS2 (Maerz et al., 2008) are three MAP kinase pathways, and PP1 (Li et al., 2005) is the transcription factor predicted to be at the base of the MAK2 pathway. Upstream elements of the MAK1 and OS2 MAP kinase pathways and the stage(s) of vegetative hyphal fusion that they regulate are unknown. Mutations in *cot-1* suppress the vegetative hyphal fusion defect of *mak-2* (Maerz et al., 2008). SO is an ascomycete-specific WW domain protein (Fleißner and Glass, 2007; Fleißner et al., 2005).

estrogen receptor is required for estrogen-induced activation of a MAP kinase signal transduction pathway (Lu et al., 2004). In *S. macrospora*, mutations in the *ham-3* ortholog (*pro11*) result in a mutant unable to complete sexual development; full fertility was restored by expression of a mouse striatin cDNA (Poggeler and Kuck, 2004). These data suggest that HAM-2/Pro22, HAM-3/Pro11, and HAM-4 might physically interact in filamentous ascomycete fungi and that this interaction might be involved in vegetative hyphal fusion.

In the tips of hyphae within the mature colony, including chemotropic fusion hyphae, the position of the Spitzenkörper determines directional growth (Harris et al., 2005; Hickey et al., 2002; Virag and Harris, 2006). However, an obvious Spitzenkörper is not observed in germ tubes (Araujo-Palomares et al., 2007) or CATs (Roca et al., 2005b). The absence of a recognizable Spitzenkörper may be due to the reduced concentration of vesicles in the slow-growing germ tube and CAT tips, and this may relate to their slow extension rates. Nevertheless, germ tube and CAT tips probably possess key components of the hyphal tip growth machinery such as the polarisome and exocyst protein complexes that have been characterized in *S. cerevisiae*. The polarisome is responsible for directing cytoskeletal and other cell components towards sites of localized cell expansion at sites of growth, while the exocyst is involved in secretory vesicle docking and fusion with the plasma membrane (Harris et al., 2005; Virag and Harris, 2006). Chemotropic growth must involve the rapid transduction of chemoattractant signals perceived at the plasma membrane to the tip growth machinery (Spitzenkörper/polarisome/exocyst). This machinery responds rapidly and sensitively to direct secretory vesicles involved in wall synthesis to appropriate locations on the apical plasma membrane of CATs or fusion hyphae (Read, 2007). This is thought to involve the directed polymerization of actin microfilaments, along which secretory vesicles are transported towards sites of vesicle fusion within the tips of CATs and fusion hyphae.

Of possible significance here is that cytochalasin-A and latrunculin B, drugs that disrupt F-actin polymerization, perturb germling fusion (Rasmussen, 2007; M. G. Roca and N. D. Read, unpublished data).

Cell-Cell Adhesion, Cell Wall Breakdown, and Membrane Merger

The molecular basis of cell-cell adhesion, a shift from polar to isotropic growth, cell wall degradation, and plasma membrane merger during the final stages of hyphal fusion are little understood in filamentous fungi. Upon physical contact, the two Spitzenkörper of two fusion hyphae are juxtaposed at the point of contact (Hickey et al., 2002). The localization of the two Spitzenkörper in fusion hyphae resembles the prefusion complexes found during muscle myoblast fusion, in which vesicles line up at the sites of cell-cell contact, forming pairs across the apposing plasma membranes (Dworak and Sink, 2002).

In *S. cerevisiae*, mating-type-specific adhesins are expressed in response to pheromone, enabling the fusion partners to tightly adhere at their shmoo tips (reviewed by Lipke and Kurjan, 1992). However, *S. cerevisiae* genes encoding mating-type-specific agglutinins are not conserved in the genome of *N. crassa* (Glass et al., 2004), indicating that different proteins may be involved in cell-cell adhesion. Recent data from *N. crassa* demonstrate that glycosylphosphatidylinositol (GPI)-anchored proteins are required for hyphal fusion. Mutations in *gpig-1*, *gpip-1*, *gpip-2*, *gpip-3*, and *gpit-1* genes, which encode components of the *N. crassa* GPI anchor biosynthetic pathway, resulted in mutants with pleiotropic phenotypes and that were unable to undergo vegetative hyphal fusion, as assessed by heterokaryon formation (Bowman et al., 2006) (Table 1; Fig. 3). Chitinases, such as the GPI-anchored chitinase ChiA in *A. nidulans*, which localizes to the tips of germ tubes, at hyphal branching sites, and at hyphal tips (Yamazaki et al., 2008), might play a role in cell wall remodeling and degradation at sites of hyphal fusion during fusion pore formation.

In *S. cerevisiae*, one of the few proteins implicated in plasma membrane merger during mating is Prm1p; *prm1Δ* mutants show a ~50% reduction in mating cell fusion (Heiman and Walter, 2000). Deletions of the *PRM1* homolog in *N. crassa* (*Δprm1;* mutations in *prm1* are ascus dominant) showed a ~50% reduction in germling fusion (Fleißner et al., 2009). In addition, *N. crassa Δprm1* mutants have a similar reduction in the frequency of trichogyne-conidium fusion during sexual reproduction, as well as defects in crozier cell fusion (see "Relationship between Vegetative Hyphal Fusion and Sexual Development" below). These data suggest that in *N. crassa*, PRM1 is involved in cell fusion events during both the vegetative and sexual stages of the *N. crassa* life cycle and may thus be part of the general cell fusion machinery.

Other Proteins Involved in Vegetative Hyphal Fusion

A number of mutants have been found to be defective in vegetative hyphal fusion, but the precise stages at which they are blocked have yet to be determined (Table 1). These include strains containing mutations in two additional MAP kinase pathways predicted in filamentous ascomycete genomes (Lengeler et al., 2000; Xu, 2000). The first of these MAP kinase pathways is orthologous to the *S. cerevisiae* cell wall integrity MAP kinase pathway and includes a MAPKKK (Bck1p), two redundant MAPKKs (Mkk1p/2p), and a MAPK (Slt2p/Mpk1p). In *S. cerevisiae*, the polarized growth of mating projections involves new cell wall synthesis, which requires activation of a cell wall integrity MAP kinase, Mpk1p (Slt2p) (Buehrer and Errede, 1997); during pheromone-induced morphogenesis, Mpk1-GFP is localized at the shmoo tip (Changwei et al., 2007). In *F. graminearum*, mutations in the *MPK1* ortholog, *MGV1*, resulted in a mutant that fails to form heterokaryons via hyphal fusion (Hou et al., 2002). Similarly, in *N. crassa*, mutations in the *MPK1* ortholog, *mak-1*, and predicted upstream kinases, *mek-1* and *mik-1*, also result in strains that fail to undergo hyphal fusion (Maerz et al., 2008) (Table 1; Fig. 3). In *M. grisea* and *Colletotrichum lagenarium*, mutations in the MAP kinase gene orthologous to *MPK1* affect conidial germination, sporulation, ability to form appressoria, and plant infection (Kojima et al., 2002; Xu, 2000; Xu et al., 1998). Defects in vegetative fusion were not assessed in these mutants.

A third MAP kinase pathway predicted in filamentous ascomycete genomes is orthologous to the osmosensing MAP kinase pathway in *S. cerevisiae* and has been extensively studied in a number of filamentous ascomycete fungi (Furukawa et al., 2005; Vitalini et al., 2007; Zhang et al., 2002). In *N. crassa*, strains containing deletion mutations in the MAPKKK (*os-4*), the MAPKK (*os-5*), or the MAPK (*os-2*) genes show hyphal fusion defects (Maerz et al., 2008) (Table 1). Future studies will reveal the relationship, interaction, and function of these MAPK pathways in vegetative hyphal fusion (Fig. 3).

RELATIONSHIP BETWEEN FUSION DURING VEGETATIVE GROWTH AND SEXUAL DEVELOPMENT

Relationship between CAT Fusion and Hyphal Fusion in a Mature Colony

CAT fusion is being used as a model to study fundamental aspects of vegetative hyphal fusion because it is a much simpler and more experimentally amenable system than fusion within a mature colony (Read and Roca, 2006). Data obtained so far suggest that many features of CAT fusion are common to the fusion of hyphae in a mature colony. All germling fusion mutants identified so far are also defective in hyphal fusion in mature colonies (Fleißner et al., 2005; Roca et al., 2005b). However, there are differences between the two processes of hyphal fusion. First, there are morphological and developmental differences. CATs are short (Fleißner et al., 2005; Roca et al., 2005b), while fusion hyphae vary from being short peg-like structures to much longer, and often dichotomously branched hyphae (Hickey et al., 2002). Second, there are physiological differences. Cytoplasmic and organelle mixing between hyphae is usually very rapid between hyphae in the mature colony following fusion but is very slow between fused CATs (Roca and Read, unpublished). We speculate that there might be slight differences in the turgor pressures of fusion hyphae which result in the rapid bulk flow of cytoplasm and organelles (Hickey et al., 2002). It may be that the turgor pressure differential between fusing germlings is much less or nonexistent (Read and Roca, 2006). Further identification and characterization of hyphal fusion mutants should more clearly define similarities and differences between fusion of CATs and fusion hyphae and should determine how far CAT fusion can be used as a model for vegetative hyphal fusion in general.

Relationship between Vegetative Hyphal Fusion and Sexual Development

All of the fusion mutants so far identified in *N. crassa* have a pleiotropic phenotype and are affected in aspects of sexual development (Table 1). For example, the *N. crassa mak-2*, *mek-2*, and *nrc-1* mutants fail to form female reproductive structures (protoperithecia) or develop defective ones, suggesting that hyphal fusion may be important during the development of fruiting bodies (e.g., in the development of the protoperithecial or perithecial wall). Fusion mutants also show reduced growth rates and conidiation defects (Kothe and Free, 1998; Li et al., 2005; Maerz et al., 2008; Pandey et al., 2004; •. Lichius, •. Lord, and N. D. Read, unpublished results). Similar results have been observed with hyphal fusion mutants identified in other species (Craven et al., 2008; Hou et al., 2002; Rech et al., 2007; Vallim et al., 2000; Wei et al., 2003).

Two stages of sexual development in *N. crassa* and related species that involve hyphal fusion are mating-cell fusion and crozier cell fusion (see "Hyphal Fusion in a Mature Colony" above). Mechanistically mating cell fusion appears reminiscent of germling and hyphal fusion in mature colonies: cells communicate over a spatial distance, grow towards each other, and fuse. However, the molecular basis of vegetative and sexual cell communication may differ. In particular, pheromone, pheromone receptor, and G-protein mutants are defective in chemotropic interactions during mating but are apparently not defective in vegetative fusion (see "Chemoattraction" above). In contrast, mutations in *soft* (*so*) result in strains that lack chemotropic interactions between CATs and are defective in germling fusion and fusion between hyphae in the mature colony (Fleißner et al., 2005). Both the *N. crassa so* mutant and the *S. macrospora so* mutant (*pro40*) form protoperithecia (Engh et al., 2007), but further sexual development is blocked. However, in *N. crassa*, the *so* mutant shows normal trichogyne-conidium chemotropic interactions and undergoes normal mating-cell fusion (Table 1). In addition, fusion of crozier cells in fruiting bodies (perithecia) of both the *so* and the *ham-2* mutants are not affected (Fleißner et al., 2005; Xiang et al., 2002). These data support the hypothesis that cell-cell communication during vegetative hyphal fusion and fusion during sexual development employ different signaling molecules and respective receptors for chemotropic interactions. In contrast, the *N. crassa Δprm1* mutants have normal chemotropic interactions during germling and mating cell fusion but show ~50% reduction in both germling and trichogyne-conidium fusion (Fleißner et al., 2009). These observations indicate that vegetative and sexual fusion may require common components of the machinery involved in plasma membrane merger. Future comparison of different types of hyphal fusion at different stages during the fungal life cycle will be important to distinguish molecular components universally involved in cell fusion from those that are specific to individual cell fusion pathways.

ROLES OF HYPHAL FUSION

Cooperation between Conidial Germlings

Cooperation among relatives is a ubiquitous phenomenon in ecological systems and involves the activities of one individual benefiting one or more other individuals (for a review, see Sachs et al., 2004). Germling fusion during colony establishment is an excellent example of cooperative behavior; cooperation within and between fungal individuals can affect fitness attributes of filamentous fungi (Pringle and Taylor, 2002). Most germling and hyphal fusion mutants show pleiotropic growth defects, indicating a role for many of these genes in processes in addition to vegetative hyphal fusion. Thus, effects of germling fusion on cooperation and colony development are difficult to assess. However, one fusion mutant, *so*, shows a wild-type maximal linear colony extension rate (Fleißner et al., 2005), although *so* mutants show a significant delay in achieving wild-type rates. These observations suggest that cooperation via germling fusion during colony establishment may result in the attainment of a maximal extension rate by the mature colony in a shorter period of time (Roca and Read, unpublished).

Maintenance of Physiological Homeostasis in a Mature Colony

Hyphal fusion results in a supracellular, syncytial state in a filamentous fungal colony. This interconnected organization enables translocation of cellular contents, such as organelles, metabolites, nutrients, or signaling compounds throughout the colony, presumably facilitating growth and reproduction (Buller, 1933; Rayner, 1996). In *N. crassa*, fusion is often associated with dramatic alterations in cytoplasmic flow; organelles, including nuclei, pass through fusion pores (Hickey et al., 2002). Such dramatic changes in cytoplasmic flow and movement of organelles suggest that filamentous fungi must adapt to the physiological consequences of hyphal fusion within a fungal colony. Anastomosis between hyphae within a single colony allows the cooperation of hyphae and enables fungi to establish complex functional units that show coordinated growth and exploration of their environment (Buller, 1933; Rayner, 1996). Cytoplasmic continuity can be restored by growth of hyphae through dead hyphal compartments, followed by hyphal fusion with living sectors (Buller, 1933). Self fusion between multiple colonies can allow them to act cooperatively in supporting one or more large fruiting bodies, such as toadstools (Buller, 1933). Thus, in general terms, vegetative hyphal fusion contributes significantly to the general homeostasis within a colony.

Heterokaryon Formation and Parasexuality

Anastomosis between hyphae of genetically different, but heterokaryon-compatible genotypes, can lead to genetic diversity via parasexual recombination and formation of novel genotypes (Pontecorvo, 1956; Swart et al., 2001). This process has been postulated to contribute to the high adaptability of fungi in species that lack sexual reproduction and genetic diversity generated via meiotic recombination. However, in nature, the formation of heterokaryons is restricted by heterokaryon incompatibility (also termed vegetative or somatic incompatibility) (see chapter 21; see also Glass and Dementhon, 2006; Leslie, 1993; Saupe, 2000; and Worrall, 1997). Thus, at least for filamentous ascomycete species, it is unclear how much gene flow and recombination occur within natural fungal populations as a consequence of hyphal fusion and heterokaryon formation.

Hyphal Fusion and Plant Pathogenesis

Hyphal fusion can also be important in the pathogenicity of some plant pathogens. Studies involving a number of plant pathogenic fungi have shown that genes encoding components of MAP kinases are essential for infection of host plants (Kojima et al., 2002; Lev et al., 1999; Takano et al.,

2000; Xu, 2000; Xu et al., 1998). These observations led to the hypothesis that cell fusion and plant infection might share common signaling pathways and that cell fusion might be a prerequisite for successful host colonization (Craven et al., 2008; Prados Rosales and Di Pietro, 2008). For example, mutation of *mak-2* MAPK orthologs in plant pathogenic fungi such as *M. grisea*, *C. lagenarium*, or *Cochliobolus heterostrophus* results in strains that are defective in appressorium formation and also fail to colonize host plants when inoculated through wound sites (Lev et al., 1999; Takano et al., 2000; Xu, 2000; Xu et al., 1998). In the non-appressorium-forming "ergot fungus" *Claviceps purpurea*, mutations in the *mak-2* ortholog similarly result in nonpathogenic strains that show an inability to infect through preformed lesions (Mey et al., 2002). For wild-type *C. purpurea*, strictly oriented growth along the pollen tube path to reach the base of the ovary is a prerequisite of successful host infection (Tudzynski and Scheffer, 2004). Mutations in the MAP kinase gene orthologous to *N. crassa mak-1* in *M. grisea* and *C. lagenarium* result in strains affected in conidial germination, sporulation, ability to form appressoria, and plant infection (Kojima et al., 2002; Xu, 2000; Xu et al., 1998); defects in vegetative fusion have not been determined in these mutants. It is unclear whether defects associated with pathogenesis in these mutants are a consequence of a lack of vegetative hyphal fusion or whether they relate to alternative roles of MAP kinase pathways in these fungal pathogens.

Recent studies of the interaction between *F. oxysporum* and tomato revealed that conidia germinating on the root surface readily fused with each other, forming a mycelial network that adhered to the plant surface. Strains containing mutations in genes encoding orthologs of *mak-2* or *so* (*fmk1* or *fso1*, respectively) were germling fusion defective. Interestingly, mycelia of the *fmk1* and *fso1* mutants were easily detached from the root surface, indicating a function for network formation in plant colonization. However, while the *fmk1* mutant was unable to infect the plant, *fso1* strains showed only slightly reduced virulence (Prados Rosales and Di Pietro, 2008), suggesting that germling fusion might contribute to infection but is not essential for pathogenicity. In contrast, inactivation of the *so* ortholog in *A. brassicicola* (*Aso1*) led to a mutant exhibiting hyphal fusion defects as well as a lack of pathogenicity on the host plant (cabbage). While the mutant was able to penetrate and initially colonize the host, it failed to invade plant tissue. It was suggested that hyphal anastomosis in *A. brassicicola* may play a role in facilitating transport of nutrients from the host plant throughout the invading colony (Craven et al., 2008). The extent to which anastomosis contributes to virulence and pathogenicity might very well depend on the individual infection strategies of different phytopathogenic species. Future analyses employing a variety of different host pathogen systems will be essential to fully reveal the roles of hyphal fusion during fungal pathogenesis.

COMPARISON OF HYPHAL FUSION WITH OTHER SYSTEMS INVOLVING CELL FUSION

Many of the processes required for hyphal fusion in filamentous fungi during vegetative growth are also required during cell fusion processes in general, including signaling by diffusible substances, directed cell growth or movement towards each other, attachment of the two cell types to one another, production and targeting of enzymes to the attachment site, and fusion of the plasma membranes of the interacting cells. Hyphal fusion in filamentous fungi is comparable to somatic cell aggregation and fusion events in other eukaryotic organisms. In cellular slime molds such as *D. discoideum*, amoebae exhibit a form of self signaling and show chemoattractive movement towards each other (Manahan et al., 2004). The adjustment of hyphal growth towards the fusion partner is comparable to cell polarization and shmoo formation during yeast mating (Kurjan, 1993), directed pollen tube growth towards the ovary (Higashiyama et al., 2003), or the extension and/or stabilization of filopodia during myoblast fusion (Chen and Olson, 2004). Cell fusion is involved in the fusion of multinucleate vegetative plasmodia of the acellular slime mold *Physarum polycephalum* resulting in the formation of larger plasmodia (Collins and Haskins, 1972). In colonial marine invertebrates, such as *Hydractinia* and *Botryllus*, fusion can occur between compatible colonies (Buss and Grosberg, 1990; Litman, 2006), while in mammals, examples of somatic cell fusion events that result in syncytia include myoblast fusion during muscle differentiation, fusion between osteoclasts in bone formation, and trophoblast fusion during placental development (Chen et al., 2007; Cross et al., 1994; Dworak and Sink, 2002; Jee and Nolan, 1963; Paululat et al., 1999; Shemer and Podbilewicz, 2003; Vignery, 2000). Understanding the molecular basis of hyphal fusion during vegetative growth in filamentous fungi may provide a paradigm for self-signaling and self-fusion mechanisms in eukaryotic microbial species, as well as provide a useful model for somatic cell fusion events in complex, multicellular species.

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AUTHOR QUERIES

AQ1: General note to authors: ASM style capitalizes specific taxon names, but the vernacular form is lowercase: the Basidiomycetes, a basidiomycete.

AQ2: In sentence "CATs function in interconnecting conidial germlings and are believed to allow the young colony to act as a cooperative individual (reviewed in 93),"correct that in "reviewed in 93" 93 refers to Riquelme et al., 1998, as edited? If not, please correct.

AQ3: "fruitbody" changed to "fruiting body" throughout per preferred usage in literature/Websters' 11th edition.

AQ4: Okay to replace "See section 3" with "see below" (since this is section 3)? If you want to be more specific, please write in the complete heading.

AQ5: Please give initials for Lichius and Lord (unpublished results).

AQ6: Sachs, 2004, is not in References. Correct that you mean Sachs et al., 2004? If not, please make appropriate entry in References and make sure Sachs et al., 2004, is cited.

AQ7: Some chapter numbers have changed. Chapter 21 is now Katz and Kelly, Glucose. If this is not the chapter you mean, please consult Dr. Borkovich for correct chapter number.

AQ8: For Leu, 1967, if Madison is not okay, please correct.

AQ9: Please give missing initials for researchers cited for unpublished data in legend to Fig. 1.

AQ10: In Table 1, references were given by number; these have been changed to the Author/Date format. Please verify that all reference citations are correct. Also, in footnote *b*, okay to insert semicolon before "the mutant is used as a female"?