AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology and Molecular Biology Reviews®

REVIEW

Chemotropism and Cell-Cell Fusion in Fungi

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SUMMARY Fungi exhibit an enormous variety of morphologies, including yeast colonies, hyphal mycelia, and elaborate fruiting bodies. This diversity arises through a combination of polar growth, cell division, and cell fusion. Because fungal cells are nonmotile and surrounded by a protective cell wall that is essential for cell integrity, potential fusion partners must grow toward each other until they touch and then degrade the intervening cell walls without impacting cell integrity. Here, we review recent progress on understanding how fungi overcome these challenges. Extracellular chemoattractants, including small peptide pheromones, mediate communication between potential fusion partners, promoting the local activation of core cell polarity regulators to orient polar growth and cell wall degradation. However, in crowded environments, pheromone gradients can be complex and potentially confusing, raising the question of how cells can effectively find their partners. Recent findings suggest that the cell polarity circuit exhibits searching behavior that can respond to pheromone cues through a remarkably flexible and effective strategy called exploratory polarization.

KEYWORDS Cdc42, GPCR, anastomosis, cell polarity, fungi, mating, pheromone, yeast

INTRODUCTION

Fungi inhabit a multitude of biological niches, where they proliferate as unicellular yeasts or multicellular elongated hyphae. Fungi can transition between these growth modes during the life cycle and form reproductive structures depending on the environmental conditions. Such transitions often require cell-cell fusion, of which examples are shown in Fig. 1. One prominent context in which cell fusion is critical is mating, when haploid yeast or hyphae

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The authors declare no conflict of interest.

Published 9 February 2022

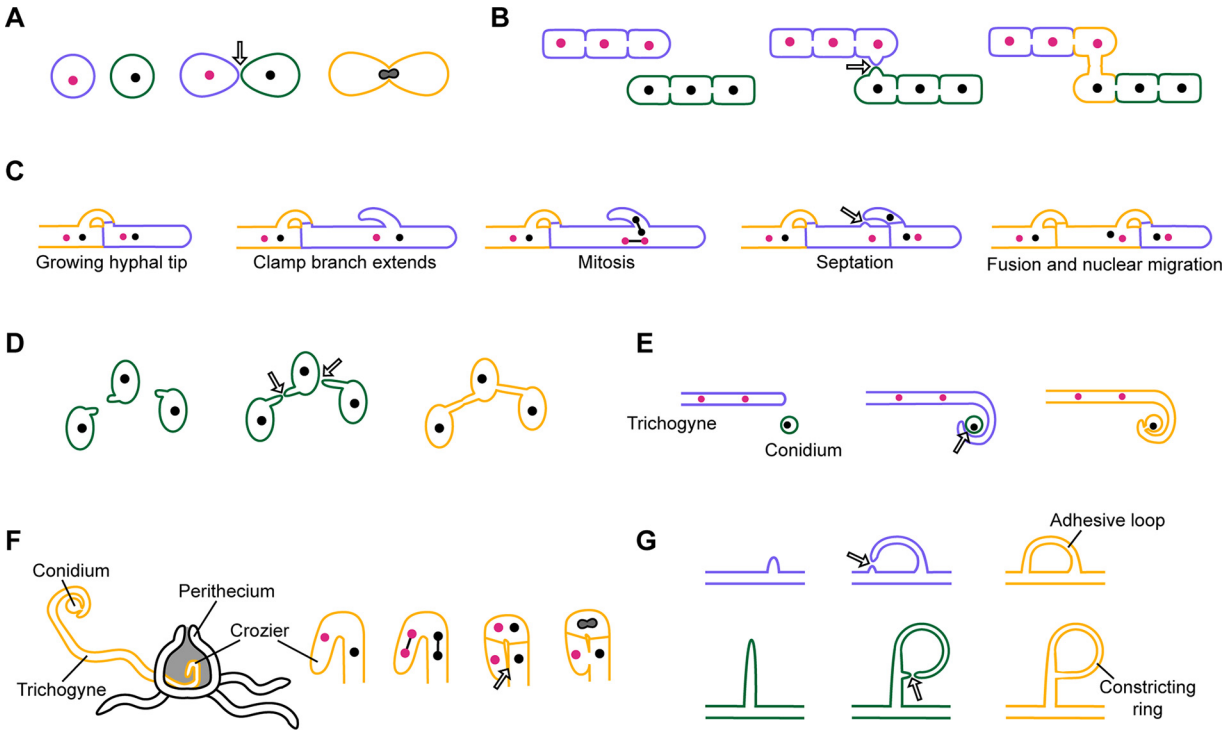

FIG 1 Examples of cell-cell fusion in fungi. Arrows indicate sites where fusion will occur. The color of the nuclei indicates mating type. A yellow outline indicates cells that have undergone fusion. (A) Mating yeast cells. (B) Mating monokaryotic hyphae (basidiomycetes). (C) Clamp connection formation in dikaryotic hyphae (basidiomycetes). To maintain a stable dikaryon, the tip (apical) cell grows a narrow protrusion that obtains a nucleus and septates to become a clamp cell, which homes toward and fuses with the neighboring subapical cell, allowing the nucleus to pass into the subapical cell. (D) Anastomosis (hyphal fusion) begins between germlings following spore germination. Later (not shown in figure), hyphae branch to form thinner fusion hyphae that similarly find each other and fuse to generate an interconnected mycelium. (E) Mating of trichogyne and conidium (filamentous ascomycetes). (F) Crozier formation (heterothallic filamentous ascomycetes). (G) Formation of adhesive loops and constricting rings in nematode-trapping fungi.

fuse with each other (1, 2) (Fig. 1A and B). Cell-cell fusion always involves the merger of both cytoplasms (plasmogamy), but it does not always result in the fusion of the parent nuclei (karyogamy). For example, hyphae that result from mating in many basidiomycete fungi are dikaryotic (2), and the maintenance of this state involves the development of clamp connections between hyphal cells through a process that also involves cell-cell fusion (Fig. 1C). In addition to sexual reproduction, cell-cell fusion plays important roles in vegetative growth of hyphal mycelia. Many fungi create specialized branches that fuse with each other to form an interconnected mycelium (1, 3–7) (Fig. 1D). Cell-cell fusion is also integral to the formation of fruiting structures (Fig. 1E and F) and other specialized structures (Fig. 1G) that are described in more detail below. Here, we discuss the general cell biological requirements for fungal cell-cell fusion and summarize recent advances from studies of model yeasts that suggest mechanisms by which fusion partners successfully find each other and fuse despite facing considerable challenges.

CHALLENGES FOR CELL-CELL FUSION IN FUNGI

Cell-cell fusion can occur only between cells whose plasma membranes are touching each other. However, the fungal plasma membrane is surrounded by a cell wall (8). Therefore, in order to fuse, two cells must (i) come into contact with each other, (ii) remove the intervening cell walls to bring their plasma membranes into contact, and (iii) fuse the membranes (Fig. 2). This process presents formidable challenges. The first challenge involves proximity; walled cells are generally immotile, so two cells cannot migrate to find each other. Instead, they must grow toward each other until they touch. In most cases, either one or both of the cells destined to fuse exhibit directional growth toward the partner (Fig. 1), suggesting that partners signal their position to each other. Such signaling at a distance is thought to be mediated by secreted factors, whose concentration gradients are decoded by receiving cells to orient their

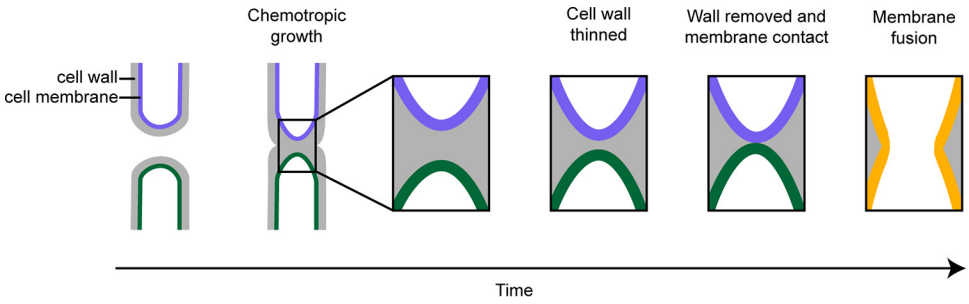

FIG 2 Challenges of cell-cell fusion. First, cells must use polar growth to come into contact with each other. Then, the cell wall between the two cells (and only that wall) must be removed to allow membrane contact and fusion.

growth. Once the cell walls are touching, the intervening wall material must be removed to allow plasma membrane contact. Here, the cells face a second challenge: fungal cells generally possess high turgor pressure created by osmotic gradients driving water flux into the cytoplasm. Turgor pushes the plasma membrane against the rigid cell wall, so any wall degradation that is not tightly targeted to the point of cell-cell contact risks the catastrophic loss of cell integrity and lysis (8). Thus, cells need to have accurate information not only about the position of the partner but also about the precise location of the contact point between the two cells once they touch. Then, they must use that information to orient polar growth and cell wall remodeling in the right direction.

MECHANISM OF POLAR GROWTH AND CELL WALL REMODELING

Fungal cells have perfected a mechanism of polar growth in which secretory vesicles carrying cell wall remodeling enzymes are directed to fuse at a specific site (the "polarity site") on the cell surface. Vesicles deliver both polysaccharide hydrolases and polysaccharide synthases to the cortex. Hydrolases are secreted enzymes that act transiently and then escape from the cell periphery by diffusion, while synthases are transmembrane enzymes that extrude carbohydrate polymers into the wall (9). The hydrolases make the local cell wall softer, allowing the turgor pressure to push the membrane outward, and thinning and deforming the cell wall at the growth site (10). The synthases add new wall polymers that become cross-linked to the existing wall, maintaining wall integrity as the cell grows. The force from turgor pressure drives cell expansion, and carefully balanced wall remodeling is required to prevent lysis. This balancing act is a result of constant monitoring by the cell wall integrity (CWI) pathway, a kinase signaling cascade that connects cell wall stress sensors to various intracellular outputs (8). Mechanical feedback via this pathway prevents excessive wall thinning and also connects to the cell polarity machinery to allow fungal cells to grow around physical obstacles (11).

For successful cell-cell fusion, at least one partner must orient its polarity site toward the other. Then, the balanced cell wall remodeling program must adjust to allow localized thinning and complete removal of the cell wall, and the two membranes must fuse. To execute this program, the partners need a sophisticated communication system to coordinate the timing and orientation of cell wall remodeling and degradation.

FUNGI COMMUNICATE USING CHEMOATTRACTANTS

In various yeasts, including the model systems *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, polarized growth toward a mating partner is guided by diffusible peptide chemoattractants called pheromones (12, 13). In these yeasts, each partner senses unique pheromones secreted by the other, allowing effective chemotropism by both cells. Many other fungi also exhibit chemotropic growth, leading to fusion in a variety of contexts. In the basidiomycete *Ustilago maydis*, time-lapse images demonstrated that individual cells in haploid suspensions deposited ~100 μm apart could home toward each other and fuse. Furthermore, after "losing" a potential fusion partner to a "rival," cells appeared to reorient on a time scale of less than 10 minutes (14). In the filamentous ascomycete *Neurospora crassa*, both mating types can form either "male" (small) or "female" (large) gametes, and the

female trichogyne (a sex-specific hypha) grows toward the male gamete (the "conidium") in a manner that depends on the pheromone released by the male cell (15–17) (Fig. 1E). Reciprocal growth by the conidium has not been reported, and pheromone receptors are not required for male fertility (15), so this process appears to be an example of unilateral pheromone guidance.

Not all guidance appears to require sex pheromones. For example, fusion of the trichogyne with the conidium is followed by migration of the male nucleus back to the female reproductive structure, where the binucleate heterokaryon generates a "crozier," a crooked finger-shaped hypha that septates to form three cells (Fig. 1F). In the middle cell, the nuclei from the two parents fuse to form a diploid, which then undergoes meiosis to form spores within an ascus. The other two cells contain only one nucleus (male or female), and these two cells orient toward each other and fuse to form a second heterokaryon (Fig. 1F). This cell goes on to form another crozier, and repeated rounds of hyphal growth, septation, and fusion yield large numbers of asci in the mature reproductive structure (perithecium). Unlike trichogyne-conidium fusion, crozier fusion does not appear to depend on sex pheromones (18).

While the best-characterized examples occur during mating, chemoattractant-guided growth also occurs during vegetative mycelium formation. Studies conducted nearly 150 years ago in *Botrytis allii* reported that one hypha appeared to trigger a second hypha to form a protrusion oriented toward the first, and these protrusions grew toward each other until the two hyphae fused (19). Careful studies of other species by Buller and colleagues (4) reinforced these observations and reported a distance dependence; "attraction" was observed only if hyphae were within 15 μm of each other. Furthermore, growth led to fusion relatively quickly; 30 to 40 minutes elapsed from the appearance of a protrusion to fusion in hyphae that were 7 to 15 μm apart, and only 20 minutes were required if they were within 5 μm of each other (4).

N. crassa also exhibits chemoattractant-guided growth during the formation of a mycelium. Germinating spores extend fusion hyphae called conidial anastomosis tubes (CATs), which can fuse with each other to form a syncytium (20) (Fig. 1D). When *N. crassa* conidia that had already extended CATs toward a fusion partner were rotated away from it using optical tweezers, cell growth reoriented toward the partner (21, 22), strongly suggesting directed growth involving a diffusible signal. Mature hyphae also branch to make fusion hyphae that can home toward each other, increasing the interconnectedness of the mycelium. Interestingly, the two *N. crassa* mating types are incompatible as fusion partners during vegetative growth. Although they can home toward one another, they are less likely to fuse than partners of the same mating type (22), and if they do fuse, the fusion product dies (23, 24). Therefore, anastomosis in *N. crassa* provides an example of chemoattraction between genetically identical cells that presumably signal via the same chemoattractant.

Other fascinating hyphal structures are formed by nematode-trapping fungi. These fungi capture and kill nematodes as nutrient sources and have potential to be used as biocontrol agents against parasitic nematodes (25). *Arthrobotrys* spp. form structures to trap nematodes by fusing a hypha either with itself (forming a ring) or with its parental hypha (forming a loop) (26–28) (Fig. 1G). When a nematode enters the lumen of a ring, the cells expand suddenly (within 1 s) to trap it. During the formation of nematode-trapping structures, fusion is preceded by the extension of a hyphal peg from the basal hypha toward the hyphal tip (similar to what is seen during clamp cell formation) (Fig. 1G). This finding suggests that as with anastomosis in *N. crassa*, chemotropism is guiding these projections. In summary, there are many examples of chemotropic growth and fusion in fungi. The best-studied instances involve bilateral communication between partners of different mating types that secrete and sense different pheromones. However, there are examples of unilateral signaling, as well as interactions that do not appear to involve the known mating pheromones. Chemotropism is also evident in vegetative contexts where the fusion partners are genetically identical, raising the question of how the cells can distinguish their potential partners from themselves.

ENCOUNTERS WITHOUT CHEMOATTRACTION?

In all of the examples discussed above, it seems clear that the polar growth of at least one partner is guided by a chemoattractant released by the other. However, that may not

always be the case. In principle, it is possible for a cell growing in an undirected manner to bump into a potential fusion partner. Although much less efficient as a way to locate the partner, such events may nevertheless explain instances of low-frequency fusion in crowded environments. A possible example of this type of fusion is the noncanonical mating between cells of the same mating type, called "fruiting," in *Cryptococcus* spp. Haploid yeast cells of the α mating type can fuse with each other to form diploid α-α hyphae (29). Fruiting is much less efficient than traditional mating, requires several weeks of incubation, and appears to occur stochastically in a population (29), perhaps suggesting that it depends on coincidental encounters between cells, rather than directed growth. Similar unisexual mating may occur in the opportunistic pathogen *Candida albicans* (30, 31).

Another scenario where chemotropism would be unable to contribute to mating encounters is when cells mate in turbulent liquid media that would scramble any long-distance chemical gradients. Nevertheless, *S. cerevisiae* cells are able to mate under these conditions, suggesting that chance encounters are able to yield fusion. Mating in liquid requires mating type-specific cell wall proteins called agglutinins that mediate adhesion between cells of the opposite mating type (32–34). Presumably, a random encounter between cells of the opposite mating type triggers agglutinin-mediated adhesion, initiating the mating process (35). A recent study using fluidic force microscopy to quantify cell-cell adhesion between single pairs of cells suggested that agglutination interactions strengthen under mechanical stress, such as that encountered by cells in a turbulent liquid environment (36). Mating in liquid requires the same pheromones that guide chemotropic growth on solid media, indicating that cell-cell communication is still important. One role for pheromones is to induce higher expression of the agglutinins, and another role is likely to be the orientation of polarity (and hence localized cell wall degradation) in the direction of the contact site with the partner. It is interesting to note that unisexual mating in both *Cryptococcus neoformans* (37) and *C. albicans* (38, 39) is greatly stimulated by the presence of pheromones from the opposite mating type. The roles of pheromone signaling in these cases remain to be elucidated.

THE PHEROMONE LANDSCAPE: WHAT DO CELLS "SEE"?

Mating cells secrete small peptide pheromones whose spatial distribution in the environment can be affected by diffusion, adsorption to surfaces, endocytosis by target cells, and degradation by proteases. In the simplest scenario in which cells are not crowded and we consider only pheromone diffusion in an unstirred medium, pheromone concentration at steady state would decay with distance from the source according to:

$$c(r) = \frac{S}{4\pi D r}$$

where $c(r)$ is the pheromone concentration at a distance r from the source, D is the pheromone diffusion constant, and S is the rate of pheromone secretion (40, 41). Although the actual concentrations depend on the specific secretion rate and diffusion constant, we can use this relation to calculate the fractional change in pheromone concentration across a receiving cell (diameter d) as a function of the distance (R) from the source to the closest edge of the receiver (Fig. 3A):

$$\textit{fractional change} = \frac{c(R) - c(R+d)}{c(R)} = \frac{d}{R+d}$$

So, for example, a detector cell or hypha of diameter 10 μm that is 20 μm away from a pheromone-secreting source would see a 33% change in pheromone concentration from one side to the other, while the same detector would experience only a 20% change if it were 40 μm away (Fig. 3A). If the detector cell were smaller (5-μm diameter), it would experience a 20% or 11% change at 20 μm or 40 μm from the source, respectively. In general, the pheromone gradient would become steeper the closer the cells were, with a maximum steepness when they are touching and about to mate. Recent analyses of mating in *S. pombe* (42) and *S. cerevisiae* (41) suggest that pheromone secretion occurs locally at sites that become oriented toward the partner, which would (by reducing R) greatly steepen the

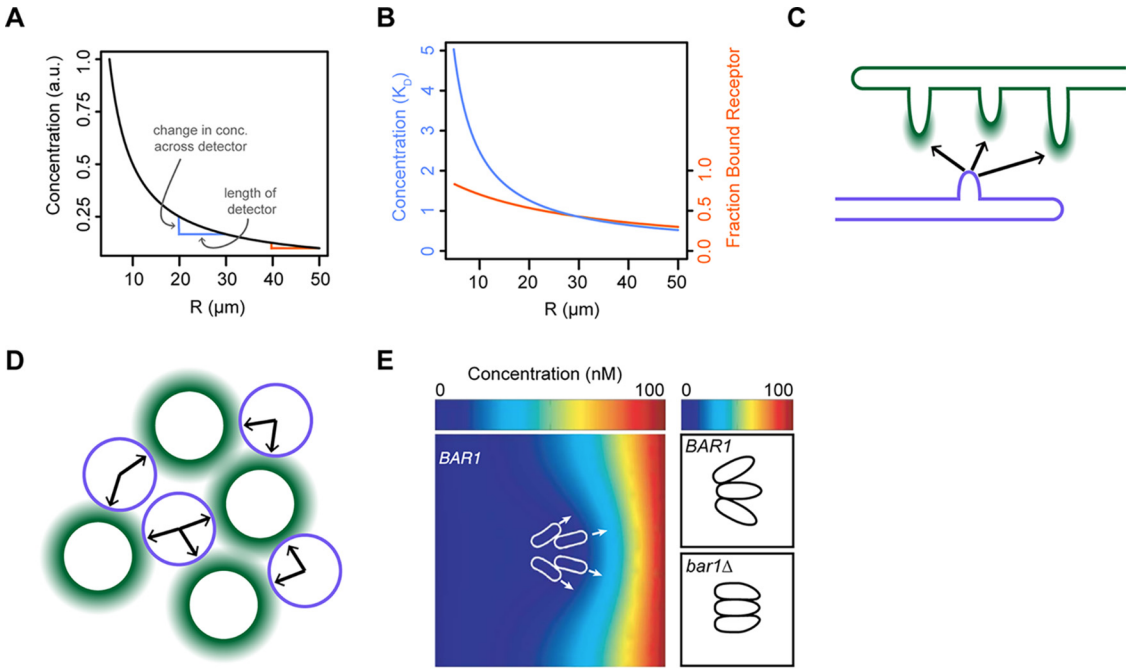

FIG 3 The pheromone landscape. (A) Theoretical steady-state gradient formed by the diffusion of a chemoattractant from a single source. Plot of pheromone concentration as a function of distance from the source (*R*). Blue and orange lines indicate the change in concentration across a 10-μm detector placed 20 μm (blue) or 40 μm (orange) from the source. (B) Comparison of steady-state pheromone concentration (blue, in units of binding K_D) and the resulting fraction of occupied receptors (orange) as a function of distance from the source (*R*). (C) Hyphal tips (green) can create multiple targets and a confusing net chemoattractant gradient for a searching hypha (purple). (D) Yeast cells often mate in environments where each cell has multiple potential fusion partners, creating a confusing net pheromone gradient. (E) Left: a computational model shows how the local secretion of a pheromone protease (Bar1) would reshape an imposed linear pheromone gradient (top color bar). Arrows indicate gradient direction sensed by each cell tip, leading to divergent growth. Right: When exposed to a linear pheromone gradient in a microfluidic device, cells secreting Bar1 exhibited self-avoidance (divergent growth), while *bar1*Δ cells did not. (Adapted from reference 58 with permission from AAAS.)

resulting pheromone gradients across mating partners. Conversely, cells at a distance perceive a lower average pheromone concentration and a shallower gradient toward the pheromone source.

To detect a pheromone gradient, cells use pheromone receptors. At steady state, the fraction of bound receptors depends on the pheromone concentration *c* and the binding dissociation constant K_D:

$$\textit{fraction of receptors bound} = \frac{c}{c + K_D}$$

Therefore, a pheromone gradient would be converted into a shallower gradient of bound pheromone (a small effect if pheromone concentrations are small compared with the K_D, but a large effect as pheromone concentrations approach receptor saturation above the K_D) (Fig. 3B). This calculation assumes steady state, but as pheromone binding/unbinding is slow in *S. cerevisiae* (43–46), it has been suggested that cells might detect gradient direction using a transient measurement of an initial wave of arriving pheromone to execute a more accurate and rapid estimation of the source direction (47).

COMPLEX PHEROMONE LANDSCAPES FROM MULTIPLE SOURCES

The discussion above has focused on the simplest case scenario in which a detector cell experiences a pheromone gradient emanating from a single source. However, this situation may be the exception rather than the rule. If multiple hyphal tips secrete pheromones, then a growing fusion hypha may be attracted to several sources (Fig. 3C). Also, yeast cells often mate in crowded environments in which each cell may have at least two and often more potential mates nearby (42, 48, 49) (Fig. 3D). In such environments, cells would

be competing with each other for mates, and experiments mixing different potential partners in the laboratory indicated that cells prefer to mate with partners producing more pheromone (50, 51). The production and secretion of high levels of pheromone may therefore be under sexual selection (like the peacock's tail) despite the significant cost to the producing cell (52).

Identifying and orienting toward a single potential mate under crowded conditions pose several difficulties. First, pheromone levels could rise to the point that they saturate the target receptors, eliminating any directional signal. Yeast cells remove the pheromone by endocytosis of pheromone-bound receptors, perhaps helping to mitigate this problem (53–56). In addition, ascomycetes produce and secrete proteases that specifically degrade the extracellular pheromones from potential partners (and not the pheromones that they produce themselves) (1). One proposed function of such proteases is to avoid receptor saturation in crowded environments (57).

Even if pheromone levels are kept below saturation, the presence of several nearby pheromone sources would yield a shallower resultant pheromone gradient that was not oriented directly toward any single source (Fig. 3D). Moreover, the locations of the pheromone sources may change, as new cells could approach or be born in the vicinity of a detector, while others cease to produce a pheromone (e.g., as a result of mating). How do cells navigate such confusing gradients to find a partner? One proposed mechanism to sharpen local pheromone gradients is based on the idea that local secretion of the pheromone protease (58) or retention of protease in the secreting cell's wall (59) would speed pheromone degradation near the detector cell. This process would allow each detector cell to create a pheromone "sink" that reshapes the surrounding pheromone landscape. Such sinks promote "self-avoidance" among detector cells, encouraging neighbors to home toward different potential partners (58) (Fig. 3E). In addition, detector cells presented with almost-equidistant sources could steepen gradients to reveal small differences, allowing homing toward just one source (58, 59). However, some conditions would seem to pose insuperable difficulties. For example, in a microcolony of yeast cells, a cell could simultaneously touch two potential partners of the opposite mating type (Fig. 3D). How could it distinguish between the partners and pick just one? As discussed further in the section Decoding the Pheromone Landscape: How Cells Find a Partner, below, recent studies have revealed an exploratory polarization strategy that can overcome such difficulties.

Interestingly, a different role for pheromone proteases was proposed for cells in turbulent liquids, where no long-range pheromone gradients exist (60). Such conditions might apply in environments like the guts of yeast-eating insects. As mentioned above, yeast cells are induced to express mating-specific agglutinins when they sense pheromones from potential partners (61). They also arrest the cell cycle in preparation for mating (62). But, the chances of mating depend on two important factors: how many potential partners there are, and how many potential competitors (cells of the same mating type) there are for mating with those partners. In a well-mixed environment, the concentration of pheromones would depend on both the prevalence of partners (which secrete pheromones and thereby elevate pheromone concentration) and competitors (which secrete protease and thereby lower pheromone concentration). Thus, protease secretion would allow cells to sense the ratio of partners to competitors and make informed choices as to whether to arrest and prepare for mating or keep proliferating.

PHEROMONES WITH DIFFERENT PHYSICAL PROPERTIES

Further complexity in the pheromone landscape can arise from differences in the intrinsic properties of the pheromones themselves. In ascomycetes, the two mating types secrete pheromones with different physical properties, which has led to speculation that different pheromones may convey distinct types of information. One mating type makes unmodified peptide pheromones, and the other type makes prenylated peptide pheromones containing unsaturated fatty acid modifications (usually a farnesyl group) (63, 64). Both types of pheromones are generated from larger primary translation products via a complex series of maturation events, and they are secreted by different mechanisms: exocytic release from

secretory vesicles in the case of unprenylated pheromones and dedicated membrane transporters in the case of prenylated pheromones. In basidiomycetes, which can have many more mating types, all pheromones appear to be of the prenylated variety (65). The pheromone-directed proteases discussed above target the unprenylated pheromones; although activities capable of degrading prenylated pheromones have been reported (66), none have so far been identified molecularly.

Prenylation adds a hydrophobic tail that is often used to promote membrane association of intracellular proteins (67). Thus, one might expect that the solubility of a peptide pheromone in aqueous media would be reduced by prenylation, although it is unclear whether the physiological concentrations of such pheromones exceed the solubility threshold. It is also unclear whether there are extracellular factors (perhaps secreted by signaling cells) that might act as effective carriers for prenylated pheromones; early studies on prenylated **a**-factor pheromones in *S. cerevisiae* indicated that it was associated with high-molecular-weight species of an undetermined nature (68, 69).

The different physical properties of prenylated and unprenylated pheromones have led to the interesting speculation that they may be optimized for signaling in different environments (63). Prenylated pheromones may preferentially partition into the outside leaflet of cell membranes, solid/liquid surfaces, and/or air-water interfaces. One recent study suggested that different pheromones may provide long-range global (unprenylated pheromone) or shorter-range directional (prenylated pheromone) information (70). However, for the moment, it remains unclear whether and how prenylation affects pheromone distribution in any system.

DECODING THE PHEROMONE LANDSCAPE: HOW CELLS FIND A PARTNER

The best-understood examples of pheromone guidance occur in the budding yeast *S. cerevisiae* and the fission yeast *S. pombe* (13, 71). In each case, there are two haploid mating types: one secretes a prenylated pheromone and expresses receptors that recognize an unprenylated pheromone, while the other secretes the unprenylated pheromone and expresses receptors that recognize the prenylated pheromone (Fig. 4A). Receptors are members of the 7-transmembrane G protein-coupled receptor (GPCR) family and bind only the pheromone made by the opposite mating type. Pheromone receptor binding activates a G protein that triggers two signaling pathways. The first activates a mitogen-activated protein kinase (MAPK) cascade leading to cell cycle arrest in G_1 phase, transcriptional induction of mating genes (including agglutinins, signaling proteins, and fusion proteins), and establishment of a polarity site (Fig. 4B). The second influences the location of the polarity site, as discussed below (Fig. 4C). These two pathways can account for almost all experimental findings, but there are hints that GPCRs may have additional G protein-independent effects, whose molecular nature remains unclear (72).

Polarity establishment involves the accumulation of the conserved Rho-family GTPase Cdc42 and associated proteins at a site on the cell cortex (73). Cdc42 switches between active GTP-bound and inactive GDP-bound states; guanine nucleotide exchange factors (GEFs) activate Cdc42 by promoting the exchange of bound GDP for GTP, while GTPase activating proteins (GAPs) inactivate Cdc42 by promoting GTP hydrolysis (Fig. 4D). GTP-Cdc42 is recognized by effectors, which are proteins that selectively bind the active form. Some effectors bind to the Cdc42 GEF, leading to a positive feedback loop in which increasing the GTP-Cdc42 concentration leads to the recruitment of GEF from the cytoplasm, which locally activates more Cdc42 (74–76). Other effectors promote actin orientation toward the polarity site, leading to vesicle traffic and hence polarized growth.

Exposure to pheromones triggers polarization even if there is no pheromone gradient (77, 78). Similarly, artificial activation of the mating MAPK pathway leads to polarization even in the absence of pheromones (79). Under such conditions, when information about the location of a potential partner is unavailable, it is thought that initial stochastic Cdc42 activation suffices to initiate positive feedback, leading to polarization (80, 81). Although stochastic fluctuations can initiate polarity at more than one site, positive feedback at the polarity sites makes them compete for the limited pool of cytoplasmic polarity factors available, and this

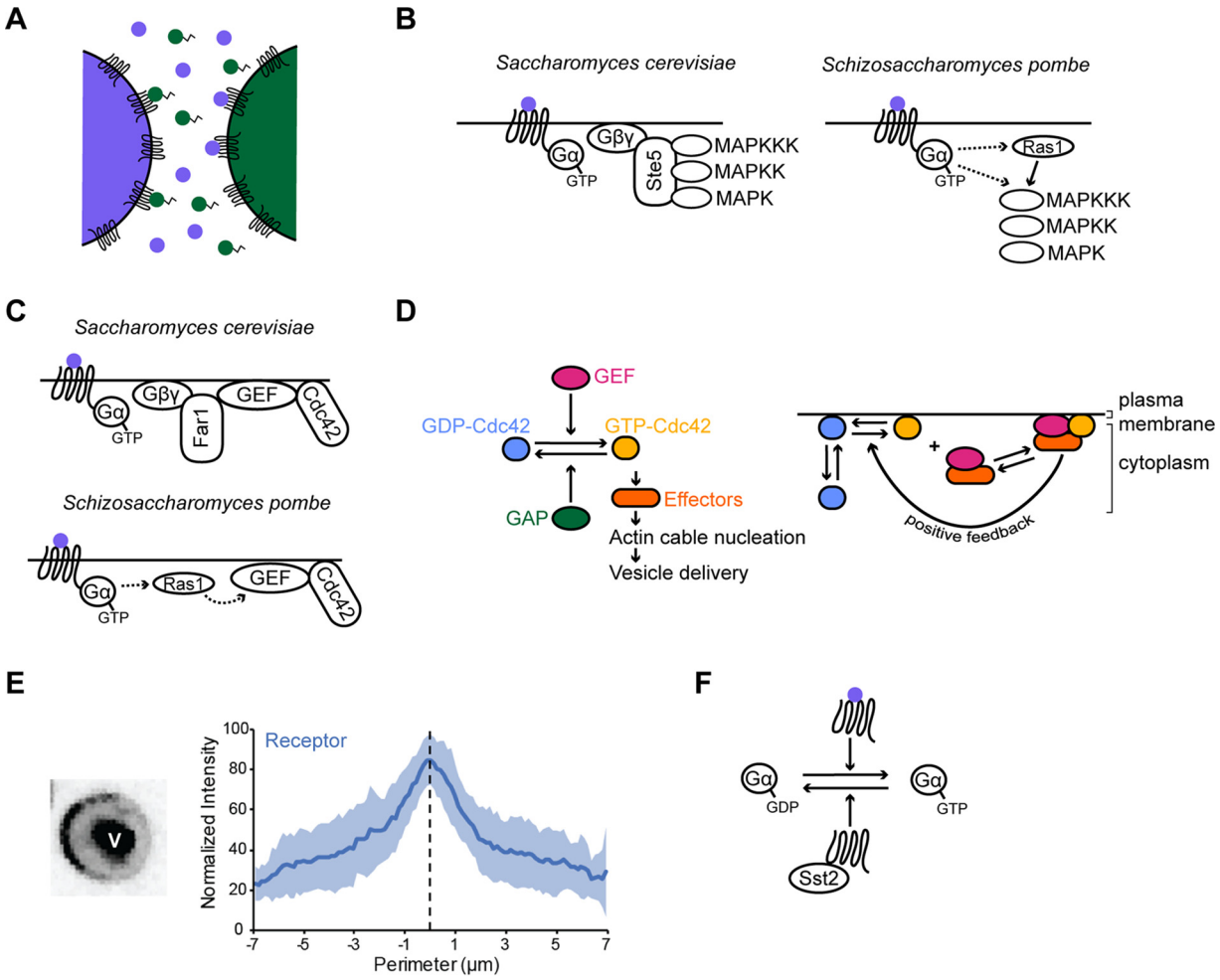

FIG 4 Pheromone response in yeasts. (A) Cells of opposite mating type secrete unique prenylated (green) or unprenylated (purple) pheromones that are detected by G-protein-coupled receptors on cells of the opposite mating type. (B) Signaling from GPCR to MAPK for *S. cerevisiae* and *S. pombe* (dashed lines indicate where molecular links are not fully known). (C) Signaling from GPCR to Cdc42 for *S. cerevisiae* and *S. pombe* (dashed lines indicate where molecular links are not fully known). (D) Left: Conversion of Cdc42 between inactive and active forms, catalyzed by GEFs and GAPs. Active Cdc42 binds effectors that lead to local secretion. Right: Positive feedback loop leads to Cdc42 clustering; active Cdc42 can recruit effector-GEF complexes from the cytoplasm, thereby activating neighboring Cdc42. Locally depleted inactive Cdc42 is replenished by binding from the cytoplasm. (E) Pheromone receptor distribution is not uniform on the cell cortex. Left: Ste2-sfGFP in a cell in G_1 (cortical signal is receptor; V, vacuolar signal from undegraded sfGFP). Right: Quantification of Ste2-sfGFP membrane distribution. Dark line is average from 71 cells, and shading represents standard deviation. (Adapted from reference 93.) (F) Mechanism for ratiometric sensing; pheromone-bound/active receptor promotes conversion of Gα-GDP to Gα-GTP. Inactive receptor binds Sst2, a GAP that converts Gα-GTP to Gα-GDP. Thus, Gα-GTP reports the ratio of active to inactive receptors.

process yields a single winning polarity site after a few minutes (82–85). This competitive aspect of the polarity system can explain why mating cells polarize to one and only one site, yielding monogamous mating to a single partner; any cell orienting toward more than one partner has several polarity sites, which compete to yield only one successful site that leads to fusion (86).

ORIENTING POLARITY ESTABLISHMENT TOWARD A PARTNER

To orient polarization toward a partner, a cell must sense and decode a pheromone gradient. This process is best understood in *S. cerevisiae*, where gradient decoding relies on a receptor-initiated pathway involving the scaffold protein Far1 (Fig. 4C). Pheromone binding to the receptor triggers activation of a heterotrimeric G protein, generating separate Gα-GTP and G$\beta\gamma$ subunits at the membrane. Gα-GTP is inactivated rapidly back to Gα-GDP by a Regulator of G-protein Signaling (RGS) protein called Sst2, and Gα-GDP rebinds G$\beta\gamma$ (87). The rapid inactivation means that concentrations of free Gα-GTP and G$\beta\gamma$ rise only locally near sites with pheromone-bound receptors. Free G$\beta\gamma$ binds Far1, which also binds the

Cdc42 GEF (88, 89). This process leads to local activation of Cdc42 at sites with more pheromones. In this way, the location of the polarity site is influenced by the spatial distribution of the pheromone-bound receptors (88–92).

In principle, the Far1 pathway would allow a cell to translate an external pheromone gradient into an internal gradient of Cdc42 activation, biasing polarity establishment to occur in the right direction. However, faithful decoding of a pheromone gradient in this manner would occur only if the receptors were distributed uniformly across the cell surface. Otherwise, pheromone binding and polarity establishment would occur preferentially where receptors were most concentrated. Surprisingly, however, pheromone receptors are not distributed uniformly in yeast and instead exhibit a steeply varying distribution with highest concentration at sites of recent secretory activity (Fig. 4E) (93). This finding raised the question of how cells could decode a pheromone gradient without being confused by the steeper receptor gradient.

An attractive solution to this conundrum involves ratiometric sensing by pheromone receptors (93–95). The core idea is that instead of simply responding to the local concentration of pheromone-bound receptors, yeast cells instead respond to the local ratio of bound to unbound receptors (Fig. 4F). If pheromone concentrations were uniform, a region of the cell membrane that is enriched for receptors would have more bound receptors than a region with lower receptor density, providing a false local signal. However, the enriched region would also have more unbound receptors, so the local ratio of bound to unbound receptors would accurately reflect the pheromone concentration.

A mechanism for ratiometric sensing came from the observation that Sst2, the yeast RGS that inactivates the G protein, is recruited to the membrane by binding to inactive pheromone receptors (96). This means that where unbound receptor density is high, Sst2 concentration is also high, and G protein activation is short-lived. Experiments replacing Sst2 with an RGS that was distributed uniformly regardless of where the receptors are concentrated demonstrated that yeast cells do indeed perform ratiometric sensing through Sst2 (93, 94). Ratiometric sensing not only protected cells from being misled by uneven receptor distribution but also improved gradient decoding by amplifying shallow pheromone gradients (93).

MOBILE POLARITY SITES ENABLE ERROR CORRECTION

Despite employing ratiometric sensing, live-cell imaging of mating pairs with fine time resolution revealed that initial polarity sites were often misoriented with respect to potential mating partners (93, 97). In such cases, error correction required relocation of the polarity site. In both budding and fission yeast, early polarity sites displayed a remarkable degree of mobility, with two predominant motile behaviors. One behavior involves the disappearance of one polarity site and appearance of a new site at another location. This behavior was particularly prevalent in fission yeast but also occurred in budding yeast (93, 98). The second form of mobility involves the gradual displacement of a polarity site along the cell cortex and occurred primarily in budding yeast (78, 93, 97, 99, 100).

Because polarity sites are assembled using positive feedback, one would expect them to be difficult to disassemble or move; positive feedback would tend to reinforce what is already there. So why are polarity sites mobile? One possibility is that early polarity sites have only weak positive feedback and few active polarity proteins, so that molecular noise can result in relocation of the site (99, 101). In budding yeast, the mating MAPK promotes polarization, so weak early MAPK activity may explain why early sites are so unstable (93, 99). However, polarity sites remain mobile (albeit due to more gradual lateral displacement) in cells with high MAPK activity and strong polarity (78, 81). An analysis of such mobility has highlighted the role of actin-directed vesicle traffic.

Movement of the polarity site occurs on a timescale of minutes, whereas individual proteins in the polarity site exchange between membrane and cytoplasm on a timescale of just a few seconds (78). This finding indicates that the site does not move as a solid physical entity. Instead, the centroid of a cluster of polarity factors is displaced when more factors are gained on one side (and lost from the other side) of the cluster. Polarity site movement is reduced greatly upon actin depolymerization (78, 81, 99), and imaging of moving polarity sites

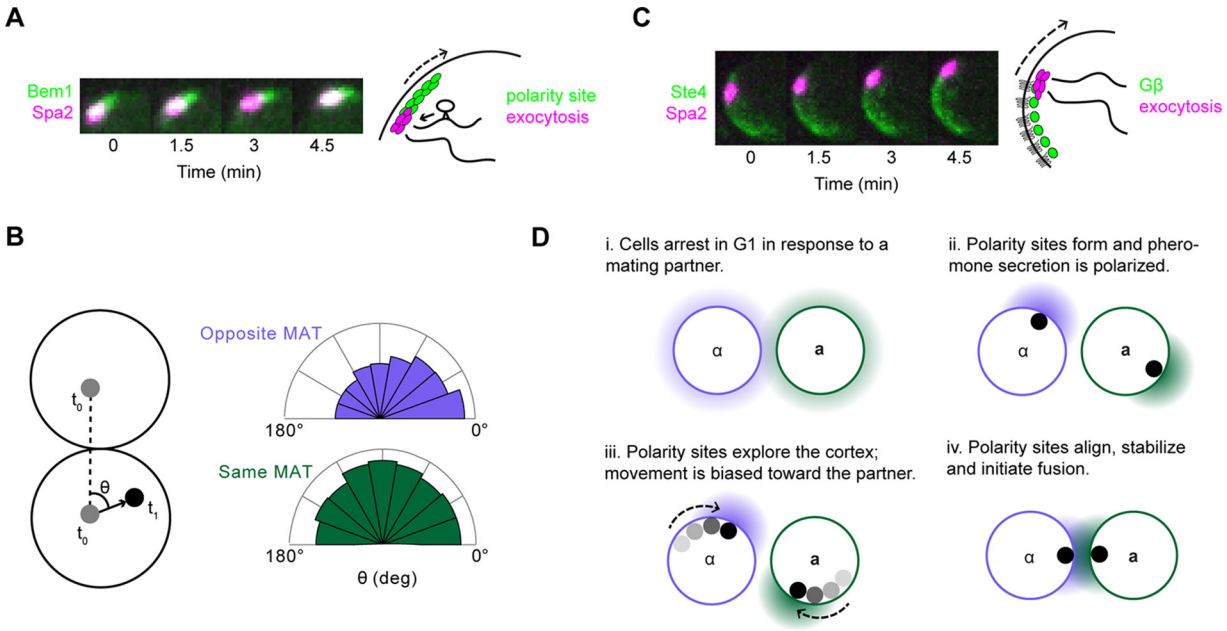

FIG 5 Polarity site movement during yeast mating. (A) Vesicle delivery (marked by Spa2) trails the polarity site (marked by the Cdc42 effector Bem1) in *S. cerevisiae*. (Adapted from reference 81.) (B) Left: Polarity site movement illustrated for two touching cells of the opposite mating type. In the bottom cell, the polarity site movement in a 2-minute interval is illustrated by the arrow (t_0 to t_1). The optimal direction of movement (toward the partner cell's polarity site) is indicated by the dashed line. The angle of polarity site movement (θ) relative to the optimal direction is shown. Top right: θ from cells with opposite mating type partners is biased toward small angles (i.e., toward the partner). Bottom right: θ from cells with same mating type partners shows no bias. (Adapted from reference 105.) (C) Pheromone receptor and G$\beta\gamma$ (marked by Ste4) surround and trail the site of vesicle delivery (Spa2). (Adapted from reference 81.) (D) Exploratory polarization model.

showed that actin cables deliver vesicles to the "back" side of the polarity factors (Fig. 5A). The fusion of vesicles at the polarity site would dilute the local polarity factors (by adding membrane locally), and computational modeling showed that physiological rates of vesicle traffic could promote the displacement of the polarity site (78, 81, 102, 103). However, quantitatively recapitulating the movement of the polarity site required the incorporation of other factors, including the presence of a Cdc42-directed GAP on the vesicles (104).

An analysis of polarity site movement in yeast cells exposed to mating partners showed that the direction of movement was biased by the pheromone gradient (105) (Fig. 5B). The ability of a pheromone gradient to bias the direction of movement was dependent on an intact Far1 pathway, suggesting that the external gradient must be translated into an internal GEF gradient that biases Cdc42 activation to the up-gradient side of the polarity site. During polarity site movement, new pheromone receptors are delivered mainly to the polarity site, while older receptors are endocytosed and degraded. This results in a zone of high receptor concentration surrounding and trailing the moving polarity site (Fig. 5C). It is truly remarkable that such a small zone (only 2 μm across) can detect local pheromone gradients; gradients must be very steep in order to produce a detectable signal over such a short distance. As discussed further below, recent work has suggested that cells can indeed create suitably steep local gradients.

Do mobile polarity sites also mediate chemotropism in hyphal systems? Certainly, the hyphal tip can turn to follow chemoattractant gradients (see above). In hyphae, a vesicle-supply center (the Spitzenkörper) near the tip is thought to dictate growth orientation (106). If this hypothesis is correct, then gradients sensed by cell surface receptors would have to somehow influence the Spitzenkörper location. We speculate that receptors influence Cdc42 localization, as they do in yeasts, and that the actin cytoskeleton transmits this influence to the Spitzenkörper. But while yeast cells deliver vesicles to the plasma membrane at a leisurely 50 vesicles/min (102), hyphae can grow much more rapidly, delivering up to 2,000 vesicles/min in *Ashbya gossypii* and an astonishing 60,000 vesicles/min in *N. crassa*, replacing the entire plasma membrane at the polarity site every few seconds (107, 108). Such rapid traffic would

overwhelm a yeast-style polarity circuit (103); hyphal cells must have more robust circuits that can withstand bombardment by vesicles.

Biased mobility of a polarity site allows the cell to improve the orientation of a polarity site that was misoriented initially. However, for this strategy to be effective, the cell must somehow recognize when proper orientation has been achieved and stop moving the polarity site once that happens.

KNOWING WHEN TO STOP: POLARITY SITE MOVEMENT CEASES WHEN PARTNER CELLS ALIGN

How do cells know when to stop moving their polarity sites? The observation that mobile polarity sites are accompanied by a zone that is enriched in pheromone receptors suggested that, after polarity is established, cells detect and respond to the pheromone level predominantly at the polarity site (41, 42, 81, 97). Such "local sensing" is in contrast to the "global sensing" that occurs before cells establish polarity (13, 109). Local sensing means that a cell would sense higher levels of pheromone when a polarity site is oriented toward a pheromone source than when it is not. In experiments where cells are exposed to different levels of uniform pheromone, polarity site movement slows and eventually ceases as pheromone levels are raised (78, 81, 98). Thus, it is attractive to speculate that polarity site movement stops when the local pheromone concentration near the site rises above some threshold (the "threshold" model).

How would a high pheromone concentration stabilize the polarity site? Here, again, an intact Far1 pathway is required; cells with a defective Far1 pathway continue moving the polarity site even when partner sites become aligned (41, 105). In polarized cells exposed to high levels of pheromone, the Far1 pathway acts like a positive feedback loop; Cdc42 activity leads to local insertion of new pheromone receptors that bind pheromones rapidly, recruiting Far1 and associated GEF that activates more local Cdc42. Computational simulations suggest that this feedback loop can stop movement at high pheromone concentrations (81).

For the threshold model to work, the pheromone concentration must be above the threshold on one side of the cell and below the threshold on the other. Are the pheromone levels starkly different on different sides of the small (4-μm diameter) yeast cell? Computational simulations indicate that if pheromones were secreted globally around the surface of a partner cell, then pheromone levels across a receiving cell could differ by as much as 4-fold. However, if pheromones were secreted locally at a polarity site that was directed toward the receiving cell, pheromone levels across that cell could differ by as much as 20-fold, providing a robust basis for a threshold model to operate (41). Supporting this possibility, pheromone secretion factors are somewhat enriched at mobile polarity sites (41, 42).

Evidence supporting the need for cells to secrete pheromones at mobile polarity sites came from experiments where a wild-type cell was mixed with a mutant partner with aberrant polarity site behavior (41). When the mutant failed to polarize and secreted pheromones globally, the wild-type partner did not stabilize its polarity site even when oriented correctly toward the partner. Similarly, wild-type cells did not stop polarity site movement when paired with partners that kept moving their polarity sites or partners that misoriented their polarity sites. These findings imply that in order for a cell to stabilize its polarity site toward a partner, the partner must reciprocally stabilize its own polarity site in the correct orientation. This idea is consistent with the hypothesis that only local pheromone secretion directed toward a partner yields a high enough local concentration to rise above the threshold needed for stabilization.

EXPLORATORY POLARIZATION: A STRATEGY TO FIND A MATE IN COMPLEX ENVIRONMENTS

In aggregate, the findings discussed above led to the exploratory polarization model (Fig. 5D). In this model, each cell establishes mobile polarity sites that explore the cortex, eventually becoming stably oriented toward the partner (42, 93). In a context where many

cells or hyphae are present and secreting pheromones, the pheromone landscape may be quite confusing, with net gradients that do not necessarily indicate the location of a single partner. Moreover, the net gradient may itself be quite unstable, as growth, division, and fusion of nearby cells change the distribution of pheromone sources. Navigating such a landscape by attempting to decode the gradient direction is difficult and (at least in budding yeast) quite error-prone. Exploratory polarization avoids this confusion by changing the basic question that cells need to answer; instead of "where is the (net) pheromone gradient pointing?," cells can succeed by answering the simpler question "is my polarity site sensing high enough pheromone levels?" Local sensing of subthreshold pheromone levels leads to continued exploration, while local sensing of suprathreshold pheromone levels leads to stabilization and successful polarity site alignment. This coincidence-detection feature allows successful pairing with a single partner even if a cell is surrounded by eligible mates (Fig. 3D).

Exploration can occur via a mixture of appearance/disappearance and lateral movement of the polarity site. When appearance/disappearance predominates, the location of each new appearance may be biased by the pheromone gradient, as interpreted through ratiometric sensing by cell surface receptors and transmission to Cdc42 via the Far1 pathway (93). When lateral movement predominates, the direction of movement can be biased by the pheromone gradient in a similar manner (105). Appearance/disappearance and movement may arise from similar molecular mechanisms, as deletion of a Cdc42-directed GAP in fission yeast can switch behavior from one to the other (110).

Exploratory polarization arises from the cell biology of polarized secretion in fungi, which enables local pheromone emission and local pheromone sensing. Where a polarity site exists, secretory vesicles are delivered to that site, with at least three relevant consequences: (i) local pheromone secretion (either directly from the vesicles, for unprenylated pheromones, or indirectly by delivering transporters, for prenylated pheromones); (ii) local pheromone sensing, due to the delivery of new receptors to the polarity site; and (iii) mobility of the polarity site, enabled by dilution of polarity factors and delivery of GAPs. To convert these features into successful partner selection further requires a pathway that faithfully translates the spatial distribution of pheromones into localized Cdc42 activation. In addition to biasing the location of polarity site appearance and the direction of polarity site movement, this pathway stabilizes the polarity site once suprathreshold levels of pheromone are detected. In *S. cerevisiae*, this pathway involves Far1, which links G$\beta\gamma$ to the Cdc42-activating GEF. Far1-related proteins can be found in many fungal clades, although not in *Archiascomycota* (111). Other pathways may substitute the same functionality; in *S. pombe*, the pathway involves the Ras GTPase, which is activated downstream of Gα-GTP and recruits a Cdc42-activating GEF (13).

ATTRACTION TO SELF: FINDING A GENETICALLY IDENTICAL PARTNER

Chemoattraction can clearly succeed in bringing together partners of two different mating types. Indeed, it has been speculated that mating types evolved to exploit the advantages of chemoattraction between dissimilar cells (112); although such mating systems reduce the number of potential partners, different mating types can protect cells from "the apparent inevitability of self-stimulation during sexual signaling" (113). Nevertheless, there appear to be many instances in fungal biology in which chemoattraction occurs between cells of the same mating type. How do the cells in these instances prevent self-stimulation by the chemoattractants that they themselves secrete?

Self-attraction is thought to occur during sexual reproduction of true homothallic (self-fertile) fungi like *Sordaria macrospora*, a relative of *N. crassa*. *N. crassa* reproduction involves female and male gametes of different mating types (Fig. 1E), whereas the single mating type of *S. macrospora* generates only female sexual structures. Within these fruiting bodies, two genetically identical cells are thought to fuse as a prelude to generating crozier structures (Fig. 1F) with diploid ascus mother cells that undergo meiosis to produce spores. Interestingly, *S. macrospora* express pheromones and receptors homologous to the cognate pheromone-receptor pairs that guide mating in *N. crassa*, and *S. macrospora* mutants lacking both pheromone receptors are infertile (114). Mutants lacking only one pheromone or

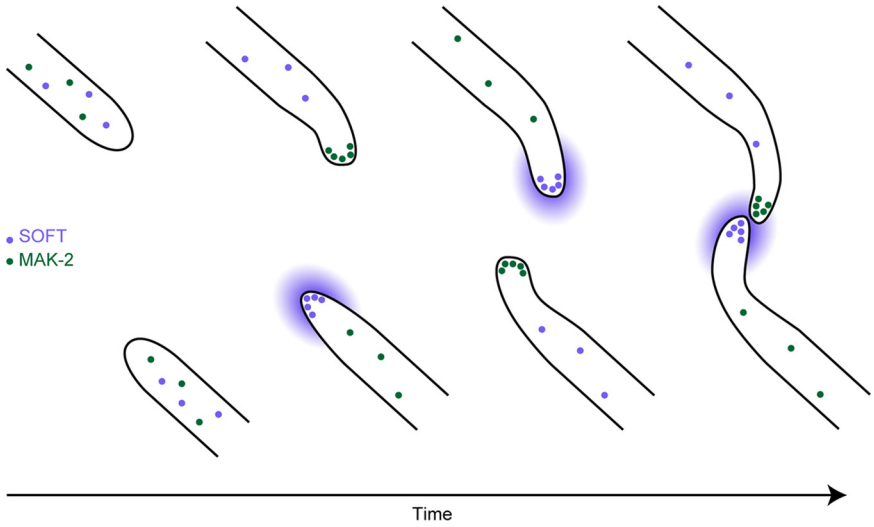

FIG 6 Self-signaling during hyphal anastomosis. Genetically identical conidial anastomosis tubes (CATs) in *N. crassa* grow toward each other alternating between states with either the scaffold SOFT (purple) or the MAPK MAK-2 (green) concentrating at the tip. According to the "ping-pong model," an unknown chemoattractant (purple) is secreted from SOFT-enriched tips and received by MAPK-enriched tips, allowing chemotropic growth by alternating self-signaling between hyphae. Switches between the signaling and receiving states may involve feedback loops that render the system excitable.

pheromone receptor are fertile, suggesting that the two cognate pheromone-receptor pairs may operate redundantly in parallel. These observations led to the suggestion that *S. macrospora* sexual structures produce cells with distinct "functional mating types" that express different combinations of pheromones and receptors (115). This setup would enable pheromone-driven cell homing and fusion, as occurs during mating between genetically different mating types.

The idea that distinct functional mating types can develop due to environmental rather than genetic influences is reminiscent of well-known instances of environmental sex determination in animals (e.g., turtles, crocodiles) and plants (e.g., ferns, mosses) that are responsive to temperature, nutrients, or other cues (116). However, in *S. macrospora*, the cells posited to develop different functional characters are both present within the same small fruiting structure, and it is unclear what environmental difference may be significant enough to cause the differentiation of adjacent cells into distinct functional mating types. One possibility is that very small initial differences may become amplified by communication between the developing cells. A striking example of such a system comes from studies of vegetative hyphal fusion.

During the early stages of vegetative mycelial development in *N. crassa*, germinating spores extend CATs (specialized fusion hyphae) that use chemotropism to grow toward other genetically identical CATs (20, 22, 117). While this process does not require the pheromones and pheromone receptors that mediate mating, it does require the same downstream MAPK signaling pathway (22, 118, 119). Remarkably, the MAPK demonstrates oscillatory, out-of-phase localization to the tips of CATs as they approach each other (Fig. 6) (118). Another protein required for fusion (SOFT) shows reciprocal localization, concentrating at the tips of CATs lacking MAPK (Fig. 6) (118). These findings suggested that as CATs approach each other, they switch back and forth between two modes: in one mode (tip-localized SOFT), the CAT tip emits a chemoattractant but does not respond to it; and in the other mode (tip-localized MAPK), the CAT tip detects the chemoattractant and uses it to guide polarized growth toward the partner. According to this "ping-pong" model, a given cell acts as either an emitter or a receiver at any given time but not both. This ingenious hypothesis explains how cells can escape the self-stimulation trap.

Intriguingly, homologues of SOFT also appear to be important for the construction of adhesive nets (Fig. 1G) by the nematode-trapping fungus *Duddingtonia flagrans*. Mutants lacking

SOFT displayed loop closure defects and spiraling hyphae, suggesting that cell-cell communication by a ping-pong mechanism may be important for normal trap formation (120).

The molecular mechanisms that underlie ping-pong switching remain to be determined, as does the identity of the hypothesized chemoattractant and receptor system. However, a plausible theoretical model has been proposed in which the MAPK pathway is wired to exhibit "excitable" behavior (121). Excitable systems contain both a rapid positive feedback loop (so that an initial rise in signaling is amplified quickly) and a delayed negative feedback loop (so that signaling is terminated after a characteristic time, ushering in a refractory period until the negative factors dissipate). In the case of CATs, initiation of a membrane-associated MAPK burst would not occur until two CAT tips grew close enough (about 15 μm) to increase local chemoattractant levels. The first CAT tip to "fire" a MAPK burst would then turn off the MAPK and (during its MAPK refractory period) switch to emitting the chemoattractant. This process, in turn, would trigger a MAPK burst in the partner CAT, starting a ping-pong series of switches between emitting and sensing until the CAT tips meet and fuse. Consistent with this model, a membrane-tethered MAPK disrupts signaling (122). The ability of cells to switch between functionally distinct states, analogous to different mating types, in a coordinated manner provides a very elegant mechanism to extend chemoattraction paradigms to genetically identical partners.

REMOVAL OF THE INTERVENING CELL WALL

Once partner cells come into contact, they must degrade the cell walls at the contact site in order to fuse. During polar growth, all cells secrete cell wall hydrolases to soften the cell wall at the growth site (123), and it seems likely that secreted hydrolases are also responsible for cell wall degradation between fusing cells. To explain how the balance between degradation and synthesis is tilted toward degradation in fusing cells, one hypothesis proposed that the difference is purely geometric (124). After cell-cell contact, hydrolases escaping by diffusion from one partner can act on the cell wall of the other partner and continue degradation until they can escape the contact site (Fig. 7A). Because the diffusional escape of the hydrolases takes longer in this geometry than it does during polar growth, hydrolytic activity increases and outpaces synthesis.

Although the idea that polar growth and fusion differ only in terms of geometry is elegantly simple, it seems insufficient to explain wall degradation because there are mutants in *S. cerevisiae* that impair cell wall degradation between partners without affecting polar growth (125). These mutants revealed that wall degradation requires, in addition, extreme focusing of vesicle traffic to the contact site (126). Genetic analyses suggested that focusing by just one of the partners suffices for efficient wall degradation. A detailed examination of the process in *S. pombe* showed that upon cell-cell contact, the actin cytoskeleton was remodeled to form an aster-like "fusion focus" that delivered secretory vesicles to a very narrow zone between the partners (127). This narrowing of secretion delivered a more concentrated bolus of hydrolases to the contact site (Fig. 7B). Unlike the secreted hydrolases, cell wall synthases (also present on secretory vesicles) are transmembrane proteins whose local concentration was not affected by narrowing of the secretion zone. Thus, focusing of secretion allows hydrolysis to outpace synthesis and remove intervening cell wall at the contact site.

If wall degradation is caused by a focusing of secretion, what is it that triggers such focusing? An important factor is the local concentration of pheromones. In *S. cerevisiae*, mutants with decreased pheromone secretion, as well as specific N-terminal pheromone receptor mutants, stall the mating process after the cells have oriented growth and touched but before degradation of the intervening cell wall (128, 129). In *S. pombe*, cells engineered to secrete the pheromone that would be secreted by the opposite mating partner (which therefore undergo autocrine receptor activation) often lyse, suggesting that elevated local pheromone self-signaling suffices to induce a fusion focus and degrade the wall even in the absence of a partner (130). Pheromone levels are expected to rise as partner cells approach each other, and contact would make them rise further due to the lengthened diffusional escape paths for secreted molecules like pheromones or hydrolases (Fig. 7A). Thus, the geometry of the contact site may enable a rise in local

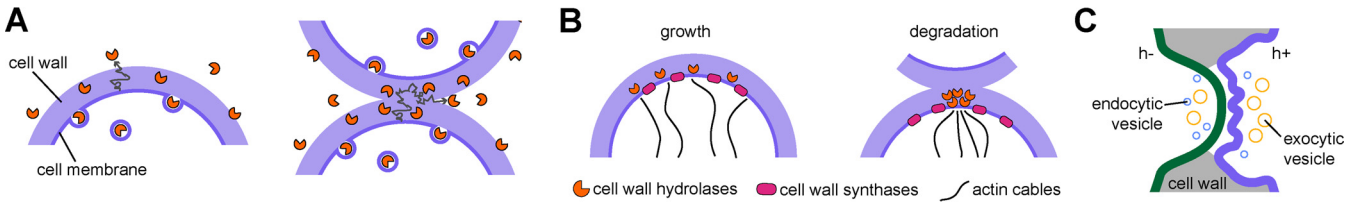

FIG 7 Cell wall removal and membrane fusion. (A) The juxtaposition of cell walls between cells engaged in polar growth could enrich secreted hydrolases to promote enhanced degradation of the intervening wall. In the absence of cell-cell contact, secreted cell wall hydrolases escape rapidly via diffusion, acting only transiently. Upon cell-cell contact, diffusional escape paths are longer, allowing hydrolases more time to promote wall degradation. Gray lines indicate possible diffusional paths. (B) Focusing of secretion would alter the local ratio of hydrolases to synthases, enabling wall degradation. During vegetative growth, dispersed delivery of cell wall hydrolases and synthases to the growing tip maintains a balance between hydrolase and synthase activity. If actin cables become more tightly focused, it would concentrate the cell wall hydrolases, allowing them to outpace synthase activity to promote wall degradation. (C) Plasma membrane asymmetry during mating in *S. pombe*. One mating partner (green) has a taut, smooth plasma membrane that pushes into the slack, wavy membrane of the opposite partner (purple). The difference is correlated with a difference in the local abundance of exocytic and endocytic vesicles. An osmotic imbalance yielding higher hydrostatic pressure in the green cell may drive fusion.

pheromone levels that activates the local MAPK (131, 132) which acts to focus secretion and degrade the wall (130).

Pheromone levels may not be the only factor inducing more focused secretion. In *S. cerevisiae*, the polarity regulator Cdc42 becomes more focused following cell-cell contact, and this focusing is proposed to depend on the local membrane curvature at the contact site (133, 134). Focusing is mediated by a BAR-domain curvature-sensitive protein called Fus2, which localizes to a tight focus. Fus2 can bind and focus active Cdc42, but this does not appear to happen during polar growth—it occurs only when cell-cell contact flattens the local curvature (133).

AVOIDING LYSIS

Focused secretion can mediate wall degradation, but in order to avoid catastrophic lysis, such degradation must be targeted precisely to the contact site. As discussed earlier, fungal cells growing under turgor pressure protect themselves from lysis using constant surveillance of cell wall integrity (CWI) by a stress response pathway. The CWI pathway includes cell wall stress sensors, a Rho-activated protein kinase C, and a MAPK cascade (8). This pathway acts to promote the repair of cell wall wounds (135), prevent lysis of growing cells subjected to compressive stress (136, 137), and prevent excessive cell wall thinning during polar growth (10). In hyphae of *N. crassa*, the CWI pathway also participates in self-signaling and components accumulate at sites of cell-cell contact (20). The CWI pathway is activated during pheromone-induced polar growth in yeast (138), and pathway hyperactivation can block the degradation of the cell wall between partners (139). One of the ways that the CWI pathway acts to protect cells from lysis is by dismantling cell polarity, thereby defocusing secretion (136, 140). In mating cells subjected to stress, the pathway can act by inhibiting the localization of the scaffold proteins Ste5 and Far1 (Fig. 4B and C), thereby antagonizing the mating MAPK, causing depolarization (141).

A remaining mystery concerns how CWI-mediated protection is overridden to allow cells to degrade intervening cell walls during mating. It is attractive to speculate that cell-cell contact somehow signals to the CWI pathway that at this site it is now safe to allow wall degradation. In addition to influencing pheromone levels and membrane curvature, cell-cell contact engages adhesive cell wall proteins (Aga1p and Fig2p) that are required for mating (142). The role of these adhesins on solid media (where there is no need to protect from turbulence) is unclear, but they are in the right place at the right time to participate in CWI regulation.

FUSION

Once the intervening cell walls are removed, the plasma membranes of the partner cells fuse. The best current candidate fusogen is Prm1 (143–146). Prm1 is a multipass transmembrane glycoprotein that forms disulfide-linked dimers that must be targeted to the contact site to promote fusion (147, 148). Cells lacking Prm1 accumulate mating

pairs that have closely apposed plasma membranes after removing the intervening cell walls. Some of them do go on to fuse, suggesting that other fusion mechanisms are possible. Cells lacking Prm1 are prone to lysis and very reliant on Ca^{2+}-dependent wound healing (20, 149, 150), so one possibility is that repair of near-death membrane ruptures provides a backup pathway that can lead to fusion.

A recent study used correlative light and electron microscopy to reveal fascinating new details of the ultrastructure of cell-cell fusion in the fission yeast *S. pombe* (151). Although mating yeast are considered isogamous (with gametes of both mating types playing similar roles in mating), this study revealed differences between the mating types at the late stages of the process. One partner exhibited a taut, smooth plasma membrane, while the other had a slack, wavy membrane (Fig. 7C). The wavy partner had markers of increased exocytosis and decreased endocytosis compared with the smooth partner, suggesting that excess plasma membrane accumulates specifically at the contact site in the wavy partner. Conversely, manipulations of turgor suggested that high turgor was particularly important in the smooth partner and that the turgor-driven protrusion of its plasma membrane into the slack partner might drive fusion. The differences between partners were correlated with (although not absolutely determined by) mating type. This study draws intriguing parallels between yeast mating and fusion of some animal cells, in which a protrusive cell drives fusion with a nonprotrusive partner (151, 152).

CONCLUSIONS

Cell-cell fusion is an integral part of fungal life cycles but poses particularly difficult challenges for cells that rely on an intact and unperforated cell wall to maintain their integrity. First, polar growth must be oriented to find suitable partners. This step is achieved by having one partner secrete a chemoattractant (often a pheromone) that is detected by the other. Pheromone receptors translate pheromone binding into local activation of the polarity regulator Cdc42, which helps to guide the direction of polarization. This strategy requires distinct partner cells, to avoid the confusion that would arise if one cell could detect its own chemoattractant. In many cases, the fusion partners are genetically distinct and wired to express different pheromones and receptors. However, even genetically identical partners appear to be able to differentiate or switch between different functional states that either secrete or sense specific attractants. A second challenge arises when cells need to find partners in crowded environments, where it may not be possible to detect an informative net gradient. To solve this issue, mating yeast cells use an exploratory polarization strategy in which both partners assemble mobile polarity sites that both secrete a pheromone and detect the partner's pheromone. When partner cells' polarity sites are oriented toward each other, their position is stabilized, and polar growth occurs until there is contact. At that point, a third challenge arises, namely, the cells must degrade the intervening cell walls at the contact site while avoiding any wall removal at other sites, which would cause lysis. Wall degradation is achieved by focusing secretion precisely to the contact site, allowing secreted hydrolases to locally outpace cell wall synthesis and remove the intervening wall. Juxtaposition of the partner plasma membranes then leads to fusion, with recent advances indicating an asymmetric process where one partner protrudes into the other to trigger fusion.

Our knowledge about fungal cell-cell fusion mechanisms remains very incomplete. Events that occur hidden away in specialized structures remain difficult to access, and even for fusion events occurring out in the open, there are many unanswered questions. How are switches between functional identities orchestrated so as to present genetically identical cells with distinct partners? How is stabilization of a mobile polarity site regulated so as to occur only upon precise co-orientation? How is the focusing of secretion that drives cell wall degradation constrained to occur only at contact sites? Fungal cell biology has yet to reveal many of its deepest mysteries.

ACKNOWLEDGMENTS

We thank Tim Elston, Amy Gladfelter, Joe Heitman, Masa Onishi, and Corrina Robertson for thoughtful feedback on drafts of this work.

M.R.C.-C. was a Howard Hughes Medical Institute Gilliam Fellow and received a Graduate Diversity Enrichment Program Award from the Burroughs Wellcome Fund. This work was funded by NIH/NIGMS grant R35GM122488 to D.J.L.

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