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Review

A focus on yeast mating: From pheromone signaling to cell-cell fusion

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ABSTRACT

Cells live in a chemical environment and are able to orient towards chemical cues. Unicellular haploid fungal cells communicate by secreting pheromones to reproduce sexually. In the yeast models *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, pheromonal communication activates similar pathways composed of cognate G-protein-coupled receptors and downstream small GTPase Cdc42 and MAP kinase cascades. Local pheromone release and sensing, at a mobile surface polarity patch, underlie spatial gradient interpretation to form pairs between two cells of distinct mating types. Concentration of secretion at the point of cell-cell contact then leads to local cell wall digestion for cell fusion, forming a diploid zygote that prevents further fusion attempts. A number of asymmetries between mating types may promote efficiency of the system. In this review, we present our current knowledge of pheromone signaling in the two model yeasts, with an emphasis on how cells decode the pheromone signal spatially and ultimately fuse together. Though overall pathway architectures are similar in the two species, their large evolutionary distance allows to explore how conceptually similar solutions to a general biological problem can arise from divergent molecular components.

1. Introduction

Cells live in a chemical environment and interpret chemical cues positionally to orient growth or migration. This interpretation involves surface receptors and signaling pathways to induce local cytoskeletal and membrane reorganization for cell polarization. Unicellular yeasts are powerful models that help us understand the conceptual organization and molecular interactions underlying spatial signal detection. During sexual reproduction, yeast cells signal to partner cells by release of peptide pheromones. The pheromones activate cognate G-protein-coupled receptors which induce signaling involving a MAPK cascade and the small GTPase Cdc42, which are interpreted globally for gene expression changes and locally to drive chemotropism toward the mating partner. Ultimately, the two partner cells fuse to form the diploid zygote, which turns off mating signals.

This review provides an overview of the mating process of the two best studied yeast models, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, from signaling to cell fusion (Fig. 1). About 0.5 Gyr of evolution separates these two ascomycetes [1] which exhibit highly divergent physiologies: *S. cerevisiae* exists principally as diploid in the wild and readily mates, even immediately or shorty after spore germination [2,3]. By contrast, mating in *S. pombe* is a response to nitrogen starvation. The mating process lasts several hours [4] and leads to an unstable diploid zygote that immediately enters meiosis to form stress-resistant spores, restoring haploid progenies when nutrients are no longer scarce. It is thus not surprising that the two species exhibit notable differences in their molecular toolkit for mating. However, general pathway architectures and overall strategies for signaling, finding a partner and fusing with it are very similar, providing an interesting comparison. Our focus is on literature over the past decade. We refer the readers to our previous review [5] for some of the older literature. We first cover the mechanisms of pheromone signaling, introducing pathway components, before describing how cells interpret the pheromone signal spatially, polarize and fuse. Finally, we discuss asymmetries between cell types and how these may help ensure a faithful haploid-diploid cycle.

2. Cell communication by pheromone signaling

In both budding and fission yeasts, cell communication relies on pheromonal signaling between two partners of distinct mating types and occurs during G1 phase, the only pheromone signaling-permissive cell cycle phase. One of the mating partners secretes a lipidated pheromone through a dedicated transporter, whereas the other secreted pheromone

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is unmodified and released by secretory vesicles (Fig. 1). Binding of the pheromone to its cognate D-class G protein coupled receptor (GPCR) on the other cell type induces a conformational change that triggers the exchange of a GDP for a GTP on the coupled Gα protein, a process modulated by a regulator of G-protein signaling (RGS), a family of GTPase activating proteins (GAP) for Gα proteins. The downstream pathway is the same in both mating partners and includes activation of a MAPK cascade and Cdc42 GTPase, which both triggers cell polarization and activates a p21-activated kinase (PAK) also acting upstream of the MAPK cascade. This results in the induction of a master transcriptional regulator that promotes the expression of components of the pheromone signaling pathway.

Despite these overall similarities there are at least two major differences in pathway architecture. The first concerns the role of starvation, which is required for mating in *S. pombe*, whereas in *S. cerevisiae* limiting nitrogen triggers invasive growth in haploid cells and nitrogen withdrawal triggers sporulation in diploids. The second distinction concerns the G proteins that act downstream of the pheromone receptors. In *S. cerevisiae*, Gα-GTP binding relieves inhibition and frees Gβγ, which activates the MAPK cascade and Cdc42. In *S. pombe*, the situation is reversed with Gα-GTP acting as the main driver of pheromone signaling, while the putative Gβ Gnr1 acts as a repressor. To help readers with the sometimes confusingly identical protein names in the two species, we provide a summary table of all discussed components (Table 1).

2.1. Pheromone signaling in S. cerevisiae

Pheromone signaling in *S. cerevisiae* starts with the binding of the soluble α-factor and lipidated a-factor to their respective GPCR Ste2 (*MAT***a** cell) and Ste3 (*MAT*α cell), which leads to release of Gβγ (Ste4-Ste18) from the receptor-coupled Gα (Gpa1) (Fig. 2A). Residues driving α-factor recognition, Ste2 dimerization and heterotrimer Gαβγ binding have been identified in the recently solved dimeric Ste2 structure [6]. Freed Gβγ, anchored at the plasma membrane, constitutes the key activator of pheromone signaling [7]. It directly activates MAPK signaling by recruiting to the plasma membrane the MAPK scaffold Ste5 [8] and also binds Far1 and Cdc24, a guanine nucleotide exchange factor (GEF) that activates Cdc42 GTPase [9–11]. Beyond its role in cell polarization (see Section 3), active Cdc42 also promotes MAPK signaling via its direct effector, the p21-activated kinase (PAK) Ste20 [12], which also binds to free Gβ [13] and phosphorylates the MAPKKK Ste11 [14].

Physiologically, budding yeast cells mate when encountering a partner at close enough range in rich environments. The cells thus face a decision between engaging into sexual reproduction vs. clonal expansion. A critical step is the regulation of Ste5 membrane recruitment, which is inhibited by CDK activity in complex with G1/S cyclins [15]. Phosphorylation leads to ejection of Ste5 from the plasma membrane and thus inactivation of Ste5, which eventually undergoes proteasomal degradation in the nucleus [16]. Far1 plays a critical role in promoting the decision to reproduce sexually by acting as an inhibitor of cyclin-CDK activity in G1 phase [17], specifically preventing CDK substrate docking [18].

The MAPK scaffold Ste5 acts as the central hub of the pheromone signaling pathway by inducing at the plasma membrane the activation of the MAPK cascade, consisting of Ste11 (MAPKKK), Ste7 (MAPKK), and Fus3 (MAPK), as well as a second MAPK Kss1 that plays more minor roles in mating. Ste5 functions as a scaffold binding Ste11 and Ste7. Ste11 binds Ste5 through a Ras-binding domain-like region [19], which in many MAPKKK including in *S. pombe* binds Ras GTPase, suggesting an interesting evolutive adaptation to connect the MAPKKK to an activating membrane anchor. Ste11 also interacts with Ste50 [20], which also binds Cdc42 [21], thus reinforcing the integration of Cdc42 in the MAPK pathway. Ste5 is anchored through its N-terminus and the binding to Gβγ to the specific lipid environment of the plasma membrane [22–24]. Binding of Ste5 N-terminus to the plasma membrane relieves Ste5 autoinhibition [25], thus allowing it to direct the kinase activity of Ste7 towards Fus3 [26]. This represents a co-catalytic activity of the Ste5 'scaffold'. One interesting feature, which may explain the presence of Ste5 homologues in fungi species with MAPK duplication [27], is that Fus3 limits the Ste7-dependent activation of the MAPK Kss1 required for starvation response [28], thus redirecting pheromone signaling to Fus3 [26].

Phosphorylated Fus3 MAPK activates the master sexual transcriptional regulator Ste12 (reviewed in [29]), which promotes at least two waves of gene expression [30]. In addition, Fus3 also directly phosphorylates several important targets for mating: i) the formin Bni1 to induce actin polarization [31], ii) Far1 to trigger cell cycle arrest [32], and iii) the cell fusion regulator Fus2 (see Section 4.1) [33]. Some of these phosphorylation events may happen locally at the shmoo tip, from where Fus3 exhibits a graded distribution [34]. Activated Fus3 also binds the Gα, which, in addition to its main inhibitory role on Gβγ, has

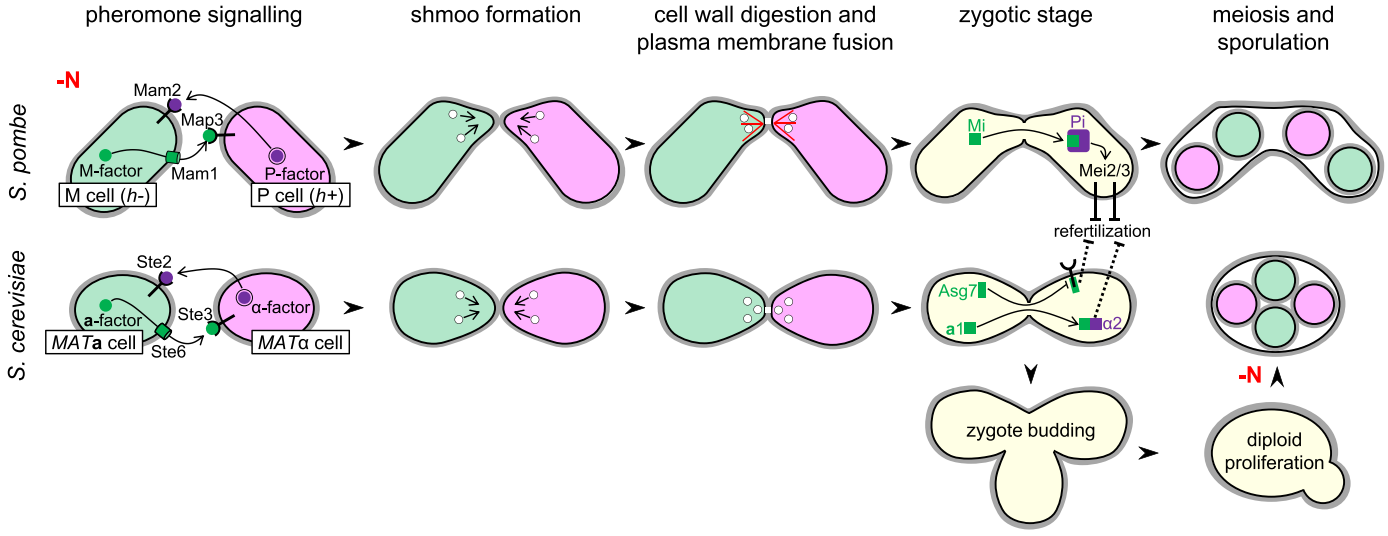

Fig. 1. Mating progression in *S. pombe* and *S. cerevisiae*. Haploid yeast cells exchange pheromone signals, stop in G1 phase and undergo polarized growth (shmooing). Upon cell-cell contact, the cell wall is digested and the plasma membranes merge, thus forming a diploid zygote. Interaction between mating-type specific proteins prevents refertilization. Top: *S. pombe* sexual differentiation is triggered by nitrogen starvation and the zygote immediately undergoes meiosis to form four haploid spores. Bottom: *S. cerevisiae* mate in rich environments and the zygote buds to form a diploid cell. Upon nitrogen starvation, diploid cells enter meiosis and sporulate.

Table 1
Functional homologues during sexual reproduction in *S. cerevisiae* and *S. pombe*.

	generic name/function	*S. cerevisiae* (MATa, MATα cell)	*S. pombe* (M/h-, P/h+ cell)
SIGNALING/POLARIZATION			
Primary role in pheromone signaling	pheromones	a-factor, α-factor	M-factor, P-factor
	pheromone transporter	Ste6	Mam1
	pheromone protease	Bar1	Sxa2
	G-protein coupled receptors	Ste2, Ste3	Mam2, Map3
	G-protein α subunit	Gpa1	Gpa1
	RGS-family regulator of Gα (GAP)	Sst2	Rgs1
	G-protein β subunit	Ste4	Gnr1 (putative)
	G-protein γ subunit	Ste18	-
	scaffold for shmoo orientation	Far1	-
	MAPK scaffold	Ste5	-
	MAPKKK adaptor	Ste50	Ste4
	MAPKKK	Ste11	Byr2
	MAPKK	Ste7	Byr1
	MAPK	Fus3	Spk1
	transcription factor	Ste12	Ste11
	Ras GTPase[a]		Ras1
	Ras GEF[a]		Ste6
Primary role in cell polarisation	Cdc42 GTPase	Cdc42	Cdc42
	Cdc42 GEF	Cdc24	Scd1
	Cdc42 GAP	? [b]	Rga3, Rga4, Rga6
	Cdc42 scaffold	Bem1	Scd2
	PAK	Ste20	Shk1/Pak1
	Formin	Bni1	? [b]
	Agglutinins and adhesins	Aga2, Sag1, Aga1, Fig2	Mam3, Map4
FUSION			
Secretory vesicle clustering	single-pass transmembrane protein	Fus1	-
	Formin	Bni1	Fus1
	Polarisome component	Spa2, Pea2	? [b]
	type V myosin for cargo transport	Myo2	Myo52
	type V myosin (structural role)	? [b]	Myo51
	tropomyosin	Tpm1	Cdc8
	tropomyosin/myosin-associated proteins	-	Rng8, Rng9
Post-vesicle clustering	Amphiphysin complex	Fus2	-
	Amphiphysin complex	Rvs161	? [b]
Membrane merging	Prm1 (multi-pass transmembrane protein)	Prm1	Prm1
	other multi-pass transmembrane proteins	Fig1	Dni1, Dni2
Post-fusion functions	transcriptional block of refertilization	a1, α2	Mi, Pi
	Other inhibitors of refertilization	Asg7	Mei2, Mei3

[a] Ras homologues Ras1 and Ras2 and their GEF Cdc25 function in the protein kinase A pathway.
[b] Question marks indicate that homologues may exist but their role during mating has not been studied.

positive contributions for chemotropism [35–37].

Pheromone signaling is tightly regulated at several levels of the pathway (Fig. 2C), starting before pheromone reception, as *MAT***a** cells secrete the diffusible α-factor protease Bar1 [38,39] that shapes the pheromone gradient to support optimal mating efficiency. Fus3 functions in negative feedback, which relies on direct binding of Fus3 to Ste5 [19,40,41], phosphorylating the same sites targeted by CDK to reduce Ste5 membrane association [19,42]. Negative feedback plays a central role in aligning the cellular response to the perceived pheromone dose [43], and in the switch-like decision to shmoo in a pheromone gradient, ensuring that cells do not engage in futile shmooing but do so only when close enough to a partner cell [44,45]. Termination of signaling occurs upon inactivation of the Gα by the RGS-family protein Sst2 [46,47], and upon phosphorylation of the pheromone receptors by the Yck1/2 kinases leading to their ubiquitination-dependent internalization (reviewed in [48]).

2.2. Pheromone signaling in S. pombe

In *S. pombe*, sexual differentiation is triggered upon nitrogen

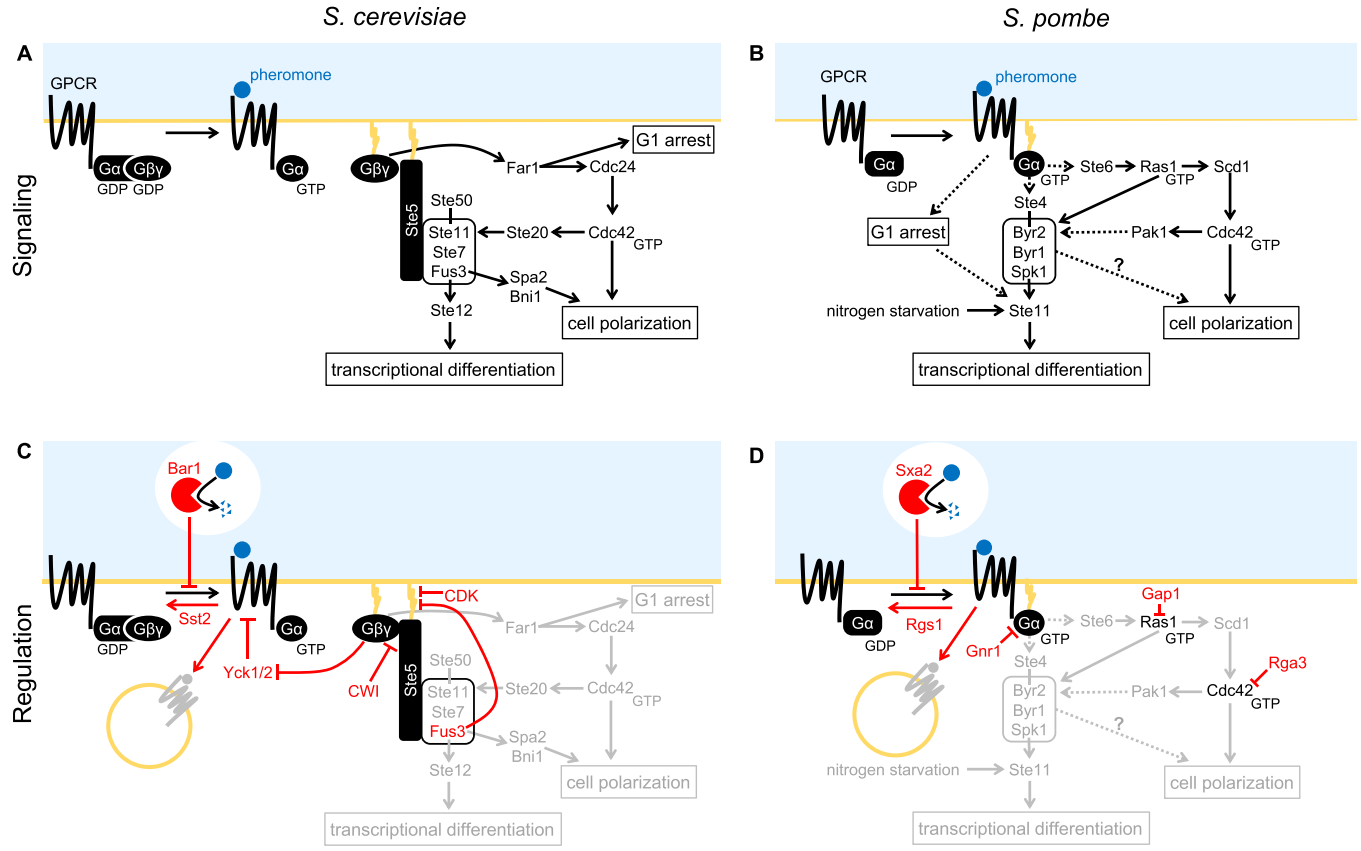

Fig. 2. Pheromone signalling in *S. cerevisiae* and *S. pombe*. Upon pheromone binding, the receptor catalyzes the formation of Gα-GTP. A) In budding yeast, this induces the release of Gβγ, which recruits the MAPK adaptor Ste5 as well as Far1 and Cdc24, the GEF of Cdc42. Active Cdc42 induces cell polarization and activates Ste20, which phosphorylates Ste11 (bound to its scaffold Ste50). MAPK activation, supported by the co-catalytic role of Ste5, results in induction of polarization and in Ste12-driven transcriptional differentiation. B) Upon nitrogen starvation, Gα-GTP of fission yeast acts as the main inducer of the pheromone signaling pathway. Activation of Ras1, through its GEF Ste6, induces both the MAPKKK Byr2 (and its scaffold Ste4) and Cdc42 activity through its GEF Scd1. In addition to its key role in cell polarization, Cdc42 also activates Pak1 and thus the MAPK cascade, which triggers Ste11-dependent transcriptional differentiation. C) Scheme of pheromone signaling in *S. cerevisiae* as in (A) on which negative controls that prevent hyperactivation of pheromone signaling are indicated. See text for details. D) Scheme of pheromone signaling in *S. pombe* as in (B) on which negative controls that prevent hyperactivation of pheromone signaling are indicated. See text for details.

starvation, which leads to G1 arrest and expression of the main transcription factor Ste11 (not to be confused with its homonym in *S. cerevisiae*), whose expression is repressed by the cAMP and TORC1 pathways, as well as CDK activity, during vegetative growth (reviewed in [49]). Amongst the numerous targets of Ste11 are the pheromones, the receptors and several components of the pheromone signaling pathway, including Ste11 itself [50,51]. Fission yeast pheromones, soluble P-factor and lipidated M-factor, produced by P (*h*+) and M (*h*-) cells respectively, activate the GPCR on the opposite cell type: Mam2 (in *h*-) and Map3 (in *h*+) (Fig. 2B). Pheromone binding triggers the activation of the associated Gα protein (Gpa1), which leads to signaling transduced by the MAPK cascade – Byr2 (MAPKKK), Byr1 (MAPKK), and Spk1 (MAPK) – and the Cdc42 GTPase, which also promotes MAPK signaling through its target Shk1/Pak1 [52]. Spk1 MAPK interacts with the central transcription factor Ste11 and induces its activation [53], thus ensuring a transcriptional positive feedback loop that locks cells in the sexual differentiation program.

As noted above, in fission yeast active Gα represents the central inducer of pheromone signaling and triggers local and global responses. Consequently, the two major Gβ binding partners in *S. cerevisiae*, Ste5 and Far1 do not exist in *S. pombe*. The presence and role of these scaffolds has indeed been substantially reshaped through fungal evolution [27]. Instead, activation of the MAPKKK Byr2 requires the Ste50-like protein Ste4 (not to be confused with its *S. cerevisiae* homonym), with which it interacts [52,54,55]. Similarly, the *Cryptococcus neoformans* Ste50 homologue, which lacks a Ste5 homologue [56], is required for pheromone signaling and binds to the MAPKKK [57]. The *Kluyveromyces lactis* Ste50 homologue also binds the MAPKKK, as well as the Gα [58], suggesting that in *S. pombe* Ste4 may translate Gpa1 activation to the MAPKKK Byr2. Byr2 activation also requires the Ras1 GTPase, which is activated by a mating-specific GEF Ste6, itself under control of Ste11 transcription. This is an architecture similar to the mammalian ERK pathway, components of which can functionally partly replace the *S. pombe* MAPK cascade [59,60]. Ras1 is also thought to promote activation of the Cdc42 GEF Scd1 for cell polarization.

As in *S. cerevisiae*, the pheromone signaling pathway is finely regulated at several levels (Fig. 2D). Its hyperactivation is deleterious both for individual cells where untimely activation can lead to unregulated fusion attempts and cell lysis [61–63], and at the population level as it leads to preferential mating between sister cells, likely reducing genetic exchanges [64]. First, M-cells release the P-factor protease Sxa2 to degrade extracellular P-factor and promote mating [65–68]. Second, the receptor cytoplasmic tail is targeted to induce its internalization resulting in interruption of signaling [69,70]. Third, the cytoplasmic tail of Mam2 recruits the RGS-family protein Rgs1 [70], which inhibits the Gα [71,72]. Finally, Gnr1, a putative Gβ protein, interacts with Gpa1 to inhibit pheromone signaling [73].

3. Finding a mating partner

3.1. Overview of the yeast cell pairing strategy

Polarization of mating partners towards each other requires the spatial decoding of pheromone gradients. This represents an important challenge, especially as yeast cells measure only a few micrometers. Thus, global pheromone gradients may lead to only small differences in concentrations around cells' peripheries. Part of the solution to this problem lies in the use of mobile polarity patches, which serve both as sites of pheromone secretion and pheromone perception (reviewed in [74]).

Local pheromone secretion from the polarity patch provides the advantage to concentrate release from a narrow source, leading to steeper pheromone landscapes than would be the case if the whole cell served as source. Pheromone gradients are further sculpted by proteolytic cleavage of the non-lipidated pheromone by a secreted protease (Bar1 and Sxa2), which prevents pheromone accumulation in the environment and allows discrimination of even close sources. These elements significantly sharpen pheromone gradients, allowing concentration discrimination potentially within the width of a polarity patch.

Although details differ, the overall patch organization and its behavior are similar in budding and fission yeasts. The polarity patch is organized around the GTP-bound form of Cdc42 GTPase, which activates formins to assemble actin cables and recruits the exocytic machinery for local secretion. In mitotic cells, Cdc42 activity is promoted by positive feedback recruitment of its own GEF (reviewed in [75,76]). It is assumed that similar regulation takes place during mating but that negative regulation initially destabilizes the patch. Indeed, in cells exposed to low pheromone levels, patches are unstable, displaying either assembly-disassembly behavior or random walk at the plasma membrane. In both species, patch mobility likely serves to escape a pre-polarized position at the former division site and promote outbreeding [64,77]. To what extent and how the patch directionally moves up-gradient is still under debate, but it is clear that upon perception of high pheromone concentration, the patch is stabilized. Because pheromone secretion occurs locally at the mobile patch, the encounter of two patches in partner cells leads to their stabilization by stimulation, which forms the basis of cell pairing.

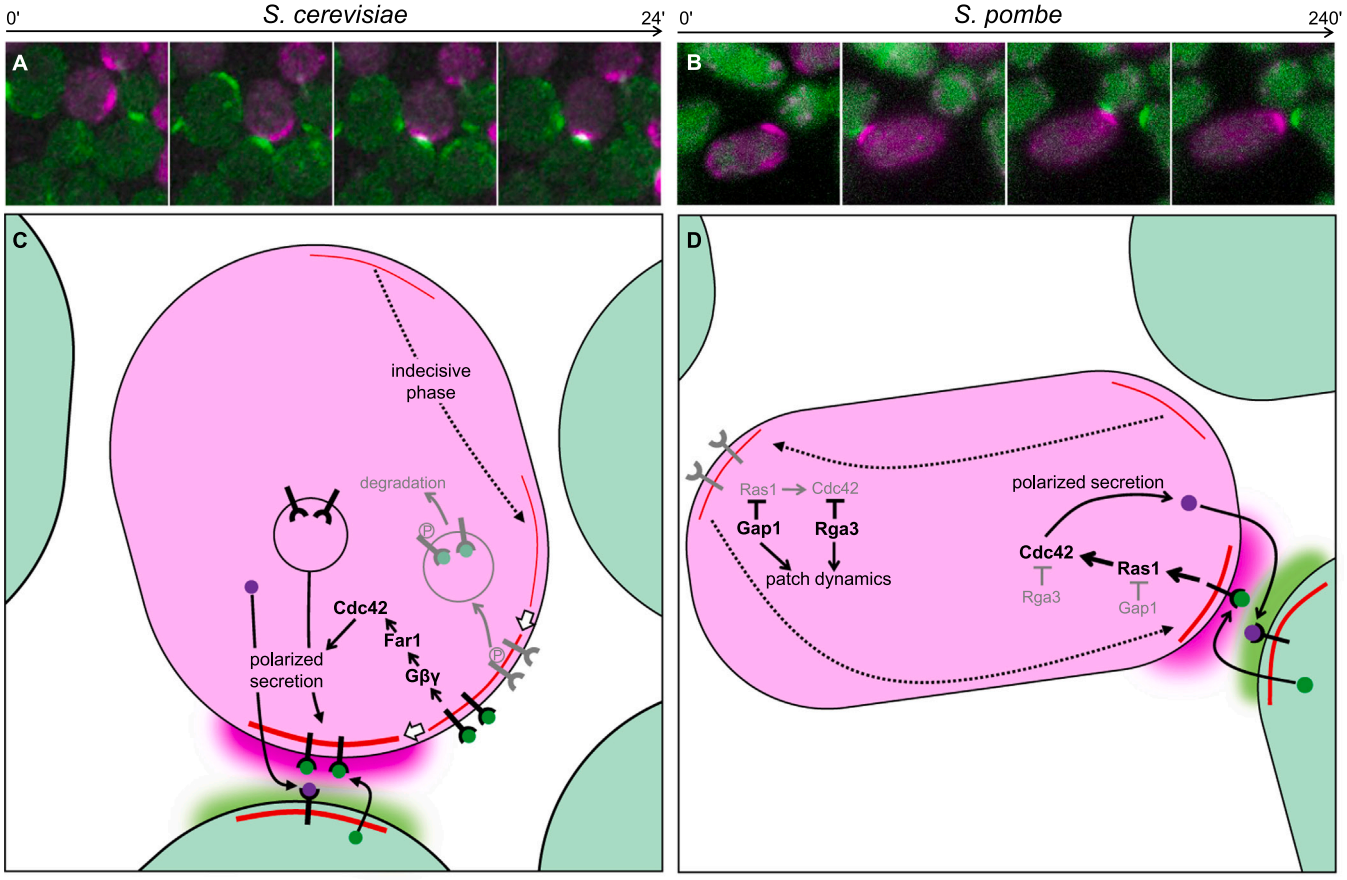

Fig. 3. Dynamics of the polarity patch in *S. cerevisiae* and *S. pombe*. A) Lateral displacement of the Cdc42-GTP polarity patch (labelled with Bem1-tdTomato, magenta) in a *MAT*α budding yeast cell towards the Bem1-GFP-labelled patch (green) of a *MAT***a** mating partner. The time lapse shows selected time points over 24 min of imaging and is a kind gift from Manuella Clark-Cotton and Daniel Lew (Duke University). B) Assembly-disassembly of the Cdc42-GTP polarity patch (labelled with Scd2-GFP, magenta) in *h-* fission yeast cell surrounded by *h+* mating partners with Scd2-mCherry-labelled polarity patches (green). The time lapse shows selected time points over 240 min of imaging. C) Following an indecisive phase (dotted arrow), the polarity patch of budding yeast moves laterally towards the gradient until it stabilizes at the high pheromone concentration, opposite to the patch of a mating partner. This lateral displacement is supported by the destabilization of the patch downgradient, where previously activated receptors are phosphorylated and internalized to be eventually degraded. Upgradient, recently activated receptors induce the activation of the Gβγ-Far1-Cdc42 axis, supporting polarized secretion of the pheromones and the receptors, thus supplying local pheromone signaling and polarized growth. D) In fission yeast, the polarity patch moves by assembly-disassembly around the cell, regulated by a competition between positive and negative signaling. When pheromone concentration is low, inhibition by the GAPs Gap1 and Rga3 lead to disassembly of the patch. In proximity of the patch of a partner, high pheromone concentration overcomes GAP inhibition and triggers signaling via Ras1 and Cdc42, thus supporting local secretion of the components of the pathway, including pheromones. This positive feedback loop between the mating partners ensures patch stabilization and polarized growth.

3.2. Pheromone-dependent polarization in S. cerevisiae

The polarity patch in *S. cerevisiae* is characterized by Cdc42 activity (Cdc42-GTP, its GEF Cdc24, and scaffold Bem1), as well as by the machineries to secrete pheromones (including the exocytic vesicular Rab GTPase Sec4 and the exocyst tethering complex) and to perceive them (Ste2, Ste4-Ste18, Far1) [78–81]. The initial position of the polarity patch is influenced by the position of the previous division site and the bud site selection machinery, in particular the GTPase Rsr1/Bud1 [10,82]. Competition between the bud site selection pathway and the pheromone pathway, both converging on Cdc24, is particularly evident when the gradient points in opposite direction from the bud site signal [77]. The patch is thus often initially poorly positioned and has to re-align to the pheromone gradient through the local sensing correction mechanism described below. However, even with this competition, the initial position of the patch is biased towards the pheromone source, suggesting that the cells also have the ability to sense the pheromone gradient globally [83].

The movement of the patch is first characterized by an indecisive phase of large random steps that eventually bring it closer to the gradient source [83,84]. During the second phase, patch movements are more constrained and chemotactic to reach the patch of the mating partner [81,84] (Fig. 3A). Patch motility is primarily driven by the local actin-mediated delivery of exocytic vesicles that dilute polarity factors [78,85] and may also deliver a Cdc42 GAP [79]. The patch chemotactic movement and its stabilization at shmoo site depend on the Gβγ-Far1-Cdc24 axis [78,80,82,84,86]. Graded pheromone distribution is thought to be perceived across the width of the patch itself, leading to higher Cdc42 activity on the upgradient side [81,84], and thus displacement of the patch upgradient (Fig. 3C). Perception of high pheromone levels, in close proximity to the patch of a mating partner, induces high MAPK activity [83,87] and stabilizes the polarity patch [78,80,82]. Several observations illustrate the importance of the communication feedback between mating partners: i) patch stabilization at a default position in one partner due to saturating levels of pheromone strongly compromises the chemotropic behavior in the partner cell [86,88], ii) in presence of a mating partner with a constitutively mobile patch, the cell cannot stabilize its own polarity patch [86], iii) cells often present two polarity patches, each transiently stabilized by a potential partner until patch competition through high protein dynamics favors the stabilization of only one patch, thus avoiding double mating events [89].

Mechanisms of gradient sensing (discussed in greater details in [74]) revolve around regulation of the pheromone receptor, of which only the α-factor receptor Ste2 has been well characterized. Ste2 localizes to the polarity patch [83,86,90], where, naïve and unphosphorylated, it is delivered by exocytosis and recruits Sst2 [91]. Signaling through Ste4-Far1-Cdc24 to promote local Cdc42 activation is operated by pheromone-bound unphosphorylated Ste2, which is rapidly turned off by phosphorylation (and internalization). This negative regulation contributes to polarity patch movement by depleting 'used' receptor and allowing rapid refresh of receptor activation at the site of new naïve receptor delivery [81,92] (Fig. 3C). Of note, receptor phosphorylation by the kinases Yck1/2 is itself regulated: upon recruitment by activated Gα, Fus3 phosphorylates Gβ [35], which increases its affinity to Yck1/2, titrating the kinases away from the receptors and prolonging the active state [92] thus preserving pheromone signaling and ensuring efficient polarization and mating [93]. Regulation by RGS Sst2 is required for chemotropism [94], and is more complex than sole inhibition of Gα, as Sst2 also acts positively by promoting receptor retention at the plasma membrane [95]. Furthermore, because Sst2 binds unoccupied Ste2, the level of active Gα was proposed to depend on the ratio of pheromone-bound to free Ste2 molecules, rather than the absolute number of bound ligands, providing a mechanism to compensate for inhomogeneous receptor distribution for global sensing [83,96].

Patch stabilization leads to persistent local growth, forming a growth projection (or shmoo). Long shmoos can be observed in cells treated with pheromone, but they are usually short in mating mixtures, where cell pairing occurs at close range. All mobile patch molecular components also localize at the shmoo tip [10,80,91,97–99], whose shape reflects the stability of the polarity patch and the focusing of the secretion site [78,92]. Focusing of secretion relies on the polarisome, formed by the core proteins Pea2 and Spa2 and by the formin Bni1, in absence of which cells form broad shmoo structures and exhibit mating defects [100,101]. The complex nucleates actin for transport of myosin V-driven cargoes. Bni1 and Spa2 are also direct targets of Gα-directed Fus3 MAPK phosphorylation, indicating mating-specific regulation of actin assembly [31,102].

3.3. Polarization and polarized growth in S. pombe

Upon reception of pheromone from the mating partner, fission yeast cells assemble a polarity patch that is characterized by similar composition as in *S. cerevisiae*: Cdc42 activity (Cdc42-GTP, its GEF Scd1 and scaffold Scd2), as well as the secretory machinery (myosin Myo52, Rab GTPase Ypt3 and exocyst complex), the M-factor transporter in M-cells, and at least some of the pheromone reception and transduction machineries (Gpa1 and Ras1-GTP) [63,64,68]. Different from the lateral displacement observed in *S. cerevisiae*, these patches undergo rapid assembly and disassembly around the cell cortex, until high local pheromone concentration stabilizes them, in close proximity to the patch of a mating partner [63,64,68] (Fig. 3B). Lateral movements have occasionally been observed and more frequently in cells lacking the Cdc42 GAP Rga3 [103], suggesting that correction mechanisms similar to those described in *S. cerevisiae* also exist.

Regulation of Cdc42 and Ras1 GTPases plays a central role in the dynamics of the patch and the regulation of polarized growth (Fig. 3D). Activation of Ras1 by its mating specific GEF Ste6 (not to be confused with the homonym pheromone transporter in *S. cerevisiae*) [104] allows to temporally and locally overcome the strong inhibition by its GAP Gap1 [63]. If pheromone signaling, and thus likely Ste6 activity, is not sufficient, the dynamic patch disassembles and reassembles elsewhere in the cell. Thus, Ras1 inhibition by Gap1 ensures patch dynamics and prevents premature patch stabilization, which would lead to untimely fusion attempts and cell lysis [63,68]. However, Gap1 is not the sole negative input, as patch dynamics still happens in *gap1*Δ cells exposed to very low pheromone signal. Although deletion of all Cdc42 GAPs (Rga3, Rga4, and Rga6) does not abolish patch dynamics, the GAP Rga3 plays a role, and removal of Rga3 results in cells that mate preferentially with their sister [103]. These observations, and similar "default" polarization from cell poles in mutants with pheromone signaling hyperactivation (such as mutants in *rgs1* or preventing receptor internalization), leading to preferential pairing with sister cells, suggest that patch dynamics may be an adaptive mechanism to promote outbreeding [64].

Upon patch stabilization by high local pheromone concentration, the cell undergoes polarized growth (shmoo) towards the mating partner. Though shmoos can occasionally extend over several micrometers, the vast majority of cell pairing occurs at close range. Polarized shmoo growth is thought to occur essentially like polar growth during the mitotic cycle with local delivery by type V myosin Myo52 of cell wall remodeling enzymes, which allow local cell wall expansion driven by turgor pressure [64,105]. Myo52 forms a crescent at the shmoo tip and remains highly dynamic until the recruitment of the mating-specific formin Fus1, whose function is dispensable for polarized growth but essential for cell fusion. A positive feedback loop between Myo52 and Fus1 then ensures their focalization and the formation of the fusion focus (see Section 4.3) [105].

4. Fusion of the mating partners

The process of cell fusion is morphogenetically complex, as yeast cells are encased in a cell wall that protects them from both external

insults and strong internal turgor pressure. As cells pair, partner cells first establish a prezygotic stage, in which cell walls come into contact and become irreversibly attached. The transition to the zygotic stage then requires both local cell wall digestion and plasma membrane fusion. Deciphering the fusion process has been challenging because many of the proteins present at the contact site between partner cells are already present and may have functions in polarity patches and/or sexual differentiation. In addition, mutants often exhibit only partial fusion impairment, suggesting redundancy and robustness in cell fusion pathways. However, genetic dissection has revealed important fusion-specific factors and functions that illuminate the process.

Studies in budding and fission yeast have largely focused on different aspects of the process, and revealed distinct molecular players, which we detail below. However, there are strong similarities that suggest a common overall strategy (Fig. 4). In both species, cell wall piercing involves the clustering and release at the zone of cell contact of secretory vesicles, thought to carry cell wall hydrolytic enzymes [105,106]. Secretory vesicle clustering depends on type V myosins following specialized actin structures, whose formation relies on mating-specific factors [105,107]. As yeasts live in a hypoosmotic environment, the time and position of cell wall removal are carefully controlled to avoid cell lysis. The subsequent process of plasma membrane merging remains poorly understood, but requires the transmembrane protein Prm1, conserved throughout fungi [108,109]. Expansion of the fusion pore and further cell wall remodeling yields a continuous cytosol in a seamless envelope, where the two nuclei fuse to generate the diploid zygote. We refer readers to [110] for a review on karyogamy.

4.1. *Vesicle clustering and wall digestion in S. cerevisiae*

In *S. cerevisiae*, the mechanisms of secretory vesicle clustering at the cell contact site are known to involve two gene products, the pheromone-induced Fus1 protein and the polarisome component Spa2 [106]. Fus1 is a one-pass O-glycosylated transmembrane protein [111], which acts as a scaffold for the cell fusion machinery and localizes to the shmoo tip following a specific secretory pathway requiring Cdc42, Cdc24, and the exomer subunit Chs5 [112–115]. Though cell fusion-specific roles have been difficult to distinguish from general cell polarization functions, formin-dependent actin assembly is likely critical for cell fusion [106,116,117]. The polarisome may play a dual function in directing the localization of fusion factors to the zone of cell-cell contact and promoting vesicle clustering. Together with Fus1, actin-based transport promotes the localization of a second pheromone-induced protein, Fus2, which is a key factor acting after

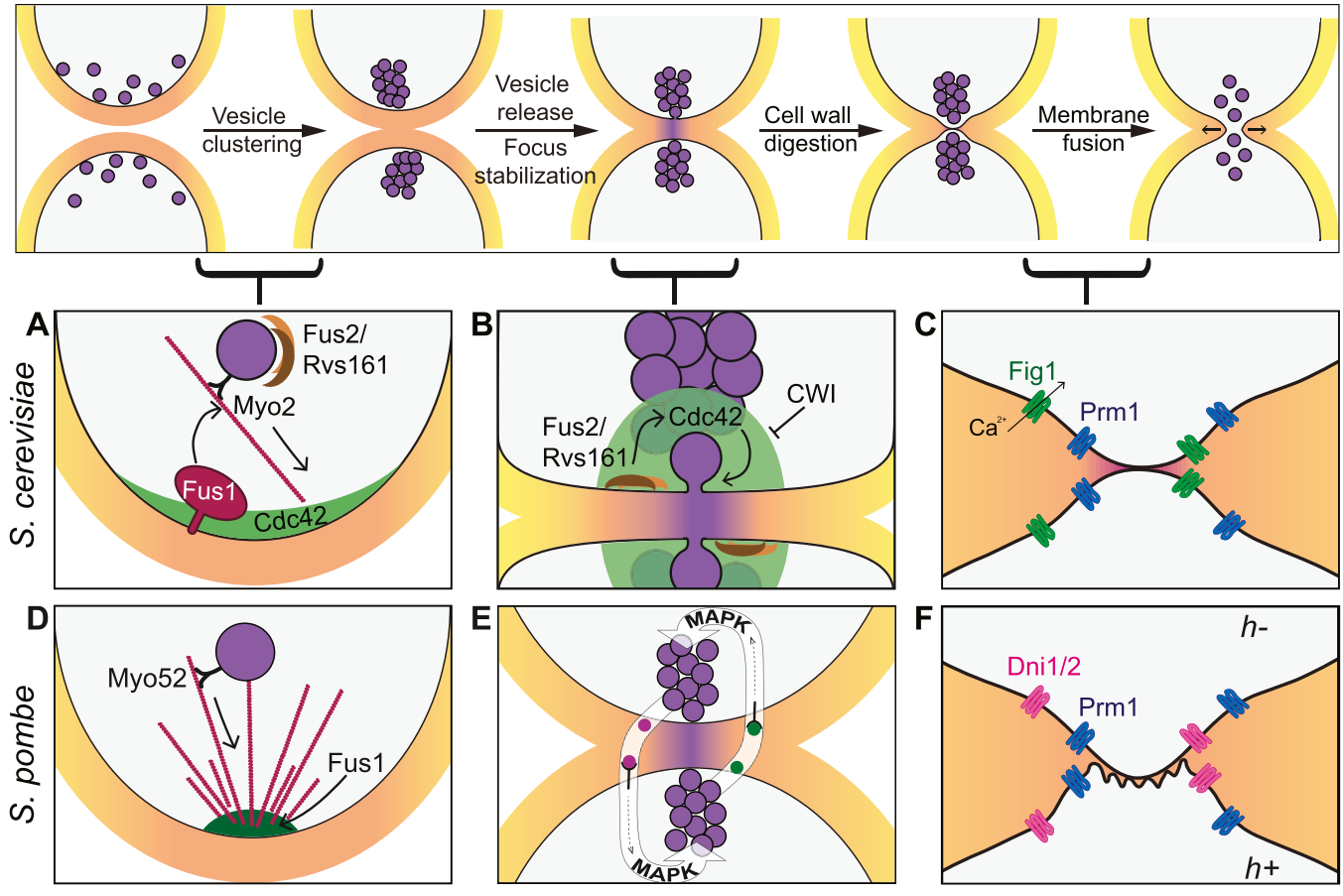

Fig. 4. Cell-cell fusion in *S. cerevisiae* and *S. pombe*. The top panel provides a general scheme of cell fusion in both species. After polarization of partner cells towards each other, clustering of secretory vesicles at facing positions and release of their content (purple) leads to cell wall (orange) digestion. Plasma membranes fuse and the fusion pore expands. The two rows below highlight specific steps and molecular components in *S. cerevisiae* (A-C) and *S. pombe* (D-F). In *S. cerevisiae*, A) the amphiphysin-like complex Fus2-Rvs161 is transported along actin cables (red) on Myo2-driven vesicles and localizes to the shmoo tip in a manner dependent on Cdc42 activity (green) and the transmembrane scaffold Fus1. B) Membrane flattening in pre-zygotes is proposed to be sensed by Fus2-Rvs161 to recruit a Cdc42 cluster, which may promote vesicle content release and thus cell wall digestion. C) The tetraspan proteins Prm1 and Fig1 are important for membrane fusion. In *S. pombe*, D) the mating-specific formin Fus1 (dark green) nucleates the fusion focus, an actin aster underlying the clustering of the vesicles brought by Myo52. E) Fusion focus stabilization relies on a positive feedback loop, driven by local pheromone secretion and MAPK signaling. This leads to local cell wall digestion. F) As the cell wall is digested, fission yeast pairs exhibit a morphological asymmetry, where the *h*- cell membrane protrudes into a wavy, less tense *h*+ cell membrane. Prm1 and the other tetraspan proteins Dni1/2 are then important for membrane fusion.

vesicle clustering [106,107,118] (Fig. 4A). Within the polarisome, the direct interaction of Pea2 and Myo2 [119] and condensate formation by Spa2 [120] suggest possible mechanisms for focusing the actin structure underlying vesicle clustering.

Despite their localization dependency, Fus2 and Fus1 play additive functions, as only double mutants fully block fusion prior to cell wall digestion [121]. Fus2 forms an amphiphysin-like complex with the BAR protein Rvs161, predicted to preferentially bind positively curved membranes [122,123]. As partner cells contact each other, flattening of the previously negatively curved plasma membrane is proposed to induce a conformational change in the Fus2-Rvs161 complex directing the recruitment of a cluster of Cdc42 GTPase at the fusion site, just prior to fusion [99] (Fig. 4B). The kelch protein Kel1, which binds Fus2, also plays a role in this pathway [124]. Cdc42 cluster recruitment is essential for cell fusion, as a *cdc42* mutant allele that carries a point mutation preventing this recruitment partially blocks late fusion steps after vesicle clustering. Nevertheless, it exhibits normal cell polarization and pheromone signaling [123]. The Cdc42 cluster then likely promotes cell fusion by signaling local secretory vesicle release. Indeed, exocytosis itself is required late in the cell wall digestion process as acute secretion block immediately arrests fusion [125]. Post-fusion, Fus2 may also play roles during pore expansion as it localizes to the edge of the expanding pore [118].

4.2. Coordination of cell wall digestion with cell-cell contact in *S. cerevisiae*

To avoid cell lysis, the decision of where and when to degrade the cell wall and open the fusion pore(s) must be precisely coordinated. Several mechanisms have been proposed. One model suggests that the zone of cell fusion restricts diffusion of cell wall hydrolases, such that cell wall removal will only occur in the narrow region in between the two partners [126]. However, the cell fusion defects of mutants that fail to cluster vesicles indicate that restricted diffusion in the cell wall is not sufficient. A second model, explained above, proposes that membrane flattening, as growing shmoos push against each other when forming the pre-zygote, is sensed by the Fus2-Rvs161 complex, allowing Cdc42 recruitment leading to vesicle release [99]. Finally, fusion is likely to be signaled by high-level pheromone signaling, as is also the case in *S. pombe*, as cell fusion requires high level pheromones [127] and sustained Fus3 MAPK activity [87].

4.3. Vesicle clustering and cell wall digestion in *S. pombe*

As noted for other aspects of the mating process, despite overall conceptual conservation, there are important differences in the molecular components regulating cell fusion between *S. cerevisiae and S. pombe*. Most notably, neither Fus1 and Fus2 are conserved beyond *Saccharomycetales* species and so neither exists in *S. pombe*. Instead, *S. pombe* expresses a dedicated, pheromone-induced formin, which has allowed a detailed study of the specialized actin fusion focus that orchestrates vesicle clustering at the pre-zygotic stage [105,128]. Confusingly, this formin is also named Fus1, but bears no sequence similarity to the Fus1 transmembrane scaffold in budding yeast.

In the fusion focus, linear actin filaments are thought to form an aster with their barbed ends concentrated near the plasma membrane, allowing the directional concentration of vesicular cargoes (Fig. 4D). Ultrastructural information is consistent with this view, showing a dense assembly of secretory vesicles, though the precise location of actin filament barbed ends has not been mapped [129]. The type V myosin Myo52 is the principal motor protein responsible for the transport of cargo vesicles, which contain cell wall degrading enzymes to promote cell wall degradation [105]. Live imaging of the process shows a progressive reduction of the distance between the two foci, likely representing progressive cell wall erosion. The fusion foci in the two partners join as cytosols merge and disappear post-fusion.

The focusing of actin filaments is strictly dependent on Fus1 formin, which itself localizes at the shmoo tip [130] and forms a tight cluster through unknown mechanisms. Several additional actin-binding proteins that localize to the fusion focus also reinforce this local concentration. These include profilin Cdc3 [131], tropomyosin Cdc8 [132,133] and its associated coiled-coil complex Rng8-Rng9 [133], calmodulin Cam22 [105], and type V myosin Myo51 in addition to Myo52 [105,134]. As mentioned earlier, these two motor proteins function in a positive feedback with Fus1 and contribute to focusing the actin structure, as cells lacking these motors show a broad Fus1 signal along the zone of cell fusion [105]. Type V myosins have overlapping functionalities, as only double mutants cells are fully fusion-defective. However, in contrast to the transport function of Myo52, Myo51 acts as a structural component of the fusion focus together with the Rng8-Rng9 complex, which is recruited by tropomyosin and promotes the coalescence of the focus likely by cross-linking tropomyosin-decorated actin filaments [133]. Myo51 also plays roles in pore expansion after cell fusion [105]. Finally, concentration of Fus1 formin at a single location is helped by capping proteins, which cap the ends of Arp2/3-nucleated filaments and prevent the diversion of Fus1 formin to other actin structures [135].

4.4. Coordination of cell wall digestion with cell-cell contact in *S. pombe*

The timing of cell wall digestion in fission yeast has been linked to the stabilization of the fusion focus, for which pheromone signaling plays an important role. Components of the pheromone signaling machinery (including receptors, active Ras GTPase and the MAPK cascade) all concentrate at the fusion focus late in the process, promoting local pheromone signaling [61,63]. Two pre-zygotic stages can be defined after fusion focus formation: (1) uncommitted, in which engagement requires active pheromone signaling and cell fusion can be blocked by washing out external pheromones; and (2) committed, where the cell pair is irreversibly engaged in fusion. Reaching the committed stage is not a matter of global pheromone concentration, but of where it is sensed, which is controlled by positive feedback between fusion focus assembly and the pheromone signaling cascade on the focus [61] (Fig. 4E). Active receptors lead to local Ras1 activation and MAPK cascade recruitment. In turn, local signaling promotes fusion focus stabilization, through an unknown mechanism [61,63]. Stabilization of the focus, which normally occurs as cells near each other, is proposed to promote cell wall digestion by locally increasing cell wall hydrolytic activity [136]. However, cell-cell contact is not strictly required, as cells will attempt fusion without a partner (and therefore lyse) if engineered to prematurely engage the feedback loop either by co-expression of a pheromone-receptor pair [61] or upon constitutive activation of Ras1 GTPase [63].

4.5. The fusion of the plasma membranes

The plasma membrane merging that happens upon cell wall digestion is likely helped by both protein and lipid membrane components to overcome the repulsive forces that normally keep membranes apart in an aqueous environment [137]. In other membrane fusion systems, including vesicular, viral or other cell-cell fusions, the process is driven by fusogenic proteins or complexes. These usually include a single-pass transmembrane protein containing an additional hydrophobic domain, and other facilitator transmembrane proteins. Insertion of the hydrophobic domain into the partner membrane upon a conformational change promotes the bilayer merging [137]. To qualify as a fusogen, a gold standard is that the protein should be sufficient to promote membrane fusion in a reconstituted system. To date, no fusogen has been identified in fungal cell-cell fusion.

Nevertheless, the pheromone-induced protein Prm1, a 4-pass transmembrane protein localizing to the shmoo tip promotes membrane fusion in all fungi where it has been investigated, including in budding

and fission yeasts [108,109,138,139]. In budding yeast and *Neurospora crassa, prm1* deletion blocks the fusion process in about half of all cell pairs after cell wall removal but prior to membrane merging [108,139]. In *S. pombe*, *prm1* deletion blocks fusion in almost all cells, but aberrant cell wall is detected between the partners, probably resulting from a rapid repair response [109]. How Prm1 acts at the molecular level is not yet well described. One proposed mechanism in budding yeast involves homodimer formation and reduction of a disulfide bridge to release a hydrophobic extracellular loop that could interact with the neighboring membrane [140,141]. Alternatively, in fission yeast, Prm1 is proposed to promote lipid (especially phosphatidylserine and sterol) microdomain formation to promote cell fusion [109].

Additional transmembrane proteins, such as the claudin-family 4-pass proteins Fig1 and Dni1/2 in budding and fission yeast respectively, help membrane merging [142–144]. *S. cerevisiae* Fig1 helps regulate Ca^{2+} influx, which occurs in bursts throughout the mating process [145], and alleviates cell lysis during cell fusion [143] (Fig. 4C). In fission yeast, membrane fusion is Ca^{2+}-independent [142], and Dni2 serves to restrict Dni1 localization at the fusion site [144] (Fig. 4F). The Hex2 Golgi protease is also proposed to modify another still unknown factor that acts together with Prm1 in budding yeast [146]. Pheromone receptors have also been proposed to promote membrane fusion through heterotypic interaction, although point mutants block cell fusion before cell wall removal [147].

Finally, the lipidic composition of the fusing membranes also determines the fusion process. The shmoo tip is enriched in ergosterol and phosphatidylinositol 4,5-*bis*phosphate [148,149]. The absence of the ergosterol biosynthetic enzymes Erg4 and Erg6 leads to fusion defects in *S. cerevisiae*, likely due to the accumulation of precursors rather than absence of ergosterol [148,150]. Indeed, a recent study in *N. crassa* links the structure of ergosterol precursors with membrane fusion defects [151].

4.6. Cross regulation with cell wall integrity and osmolarity pathways

Yeast cells can simultaneously perceive and integrate several signals. As non-motile cells, yeasts have well-developed ways to cope with various external stresses. In particular, maintenance of cell wall integrity is critical to their survival to avoid lysis due to their strong internal turgor pressure. Because mating is the one situation where the cell wall has to be breached to allow cell fusion, the pheromone-MAPK signaling is strongly interconnected with stress-signaling MAPK cascades that monitor the cell wall and osmolarity: the cell wall integrity (CWI) and high osmolarity glycerol (HOG) pathways, as recently reviewed for *S. cerevisiae* [152].

In *S. cerevisiae*, the phosphorylation of Ste5, the central hub of pheromone signaling, has emerged as a key regulatory point for the crosstalk between pheromone signaling and the CWI pathway [153,154]. Pkc1, an upstream signaling kinase of the CWI pathway, phosphorylates Far1 and Ste5, decreasing its binding to Gβγ and preventing its cortical recruitment, to reduce hyperactivation of the pathway (Fig. 2C). This regulation protects against cell lysis upon mechanical stress and at the fusion site [153]. A similar cross-talk was identified in response to oxidative stress [154]. CWI signaling through the mechano-sensor Mid2 (which means mating-induced cell death) and Pkc1 also impairs the formation of the Cdc42 cluster at the fusion site, further protecting the cell from untimely fusion attempts [155] (Fig. 4B). In *S. pombe*, cross-talk with the CWI pathway has not been studied, but the accumulation of the mechano-sensor Wsc1 at the site of cell-cell contact [156] suggests an important contribution.

Turgor pressure regulation is a key factor during mating and cell fusion. In *S. cerevisiae*, osmotic unbalance between partners blocks cell fusion [157]. By contrast, in *S. pombe*, turgor pressure difference between cell types is proposed to underlie their asymmetric ultrastructure (see Section 5.2) and promote the fusion process [129]. In *S. cerevisiae*, the HOG and the pheromone pathways share several components, including the MAPKKK Ste11, the scaffold protein Ste50 and the Cdc42 effector PAK Ste20 kinase [158,159]. Different interfaces of Ste50 [159] and different kinetics of the Cdc42 activatory input [160] are used to insulate the two pathways. Pheromone signaling is proposed to downregulate the HOG pathway to slightly reduce cellular osmolarity and thus the risk of cell lysis. Indeed, upon pheromone stimulation, Fus1 interferes with the HOG MAPK signaling through competition with the osmosensor Sho1 [112], leading to downregulation of HOG targets [161]. In cells pre-adapted to a high osmolarity environment, pheromone signaling and the CWI pathway also indirectly reactivate HOG to induce glycerol efflux and thus a drop in turgor pressure [158], leading to fast glycerol turnover in mating cells. It will be interesting to further investigate the role of turgor pressure and cell wall mechanical properties in cell fusion.

5. System's design for the haploid-diploid life cycle

5.1. Role of adhesins

The vast majority of studies on cell polarization and cell fusion are performed on solid (agar) surface. However, yeast cells may find themselves in (semi-)liquid environments, for instance in wood sap, decaying fruits or insect guts, in which pheromone gradients are easily perturbed by flows. Cell agglutination likely reduces this perturbating factor to allow the formation of stable pheromone gradients even in liquid environments [162].

Early work defined the role of mating-specific **a**- and α-agglutinins in *S. cerevisiae* to promote cell attachment [163]. The **a**-cell expresses the **a**-agglutinin, consisting of the glycosylphosphatidylinositol (GPI)-linked glycoprotein Aga1 bound to Aga2 by disulfide bridges. Aga2 C-terminal peptide exhibits high affinity (in the low nM range) to the GPI-linked, glycosylated α-agglutinin Sag1/Aga1 expressed on the α-cell. Additional adhesive interactions take place between Aga1 and Fig2, expressed in both cell types. The strength of individual agglutinin binding was recently estimated at about 100 pN, with cell-cell adhesion force increasing with time and upon physical stress [164]. This strength is likely critical for successful mating, as reprogramming of yeast adhesion through artificial protein binding pairs showed a linear dependence on mating success with binding affinity and thus likely strength of adhesion [165]. In *S. pombe*, the only studied mating-specific adhesin, Map4, is only expressed in P-cells and also promotes agglutination [166]. The M-cell-specific adhesin Mam3, only identified in genome-wide approaches so far, also promotes efficient mating [167–170]. Interestingly, agglutinin-dependent cell adhesion also promotes, in poorly understood ways, mating on solid substrates [166,171].

5.2. Asymmetries between mating partners

Yeasts are generally considered isogamous species, where both partners undergo symmetric processes during mating. However, a number of differences between partners have been noted, most notably linked to a fundamental asymmetry in the properties of the pheromones from each mating types. Indeed, while *MAT*α/*h*+ cells secrete a soluble α/P-factor by exocytosis, the **a**/M-factors from *MAT***a**/*h*- cells are lipidated and have to be exported through dedicated transporters: Ste6/Mam1 [172,173]. While this asymmetric setup may not be absolutely essential, as basidiomycete fungi only use lipidated pheromones, it is universal across ascomycetes, and *S. cerevisiae* engineered to communicate through only **a**-type or only α-type pheromones can mate [174], but with low efficiency. This low efficiency may be due in part to the function of other cell type-specific factors [175], but there is also a proposed inherent advantage of asymmetry linked to the difference in pheromone diffusion in the extracellular medium: α/P-factor freely diffuses and thus reaches remote cells, whereas hydrophobic **a**/M-factor acts at closer range. Thus, *MAT***a**/*h*- cells that receive soluble pheromones may be considered long-range sensors.

In *S. cerevisiae*, sensing by *MAT***a** cells allows them to receive low pheromone concentration from a remote partner to induce exploration of the environment [176]. Although this low concentration may not be sufficient for sustained MAPK activation [177] and cell cycle arrest, it drives a shift from classical axial budding to bipolar budding, which acts as a search mechanism until a daughter cell is close enough to a gradient source to arrest and polarize toward the mating partner [176–178]. Although filamentous growth is not observed in *S. pombe*, it would be interesting to investigate whether related species that undergo filamentous growth, such as *Schizosaccharomyces japonicus* [179,180], use a similar strategy to improve mating efficiency. In liquid environments, both pheromones may have long-distance effects to induce agglutination, as shown for M-factor in *S. pombe* [181].

The relative role and importance of lipidated and unlipidated pheromones is not yet settled. Using autocrine cells (expressing the receptor of the pheromone they secrete), it was possible to create *S. pombe* mating pairs that either only rely on M- or P-factor detection, and to demonstrate that perception of M-factor, but not P-factor, is sufficient for mating [62]. The key role of M-factor is also supported by the invariance of the M-factor and its cognate Map3 receptor in *S. pombe* natural variants, whereas the P-factor/Mam2 pair shows more sequence diversity [65]. Interestingly, extensive mutagenesis identified an M-factor mutant that only allows efficient mating through a mutated cognate receptor, thus leading to reproductive isolation [182,183]. Although similar systematic analysis has, to our knowledge, not yet been performed on **a**-factor/Ste3 in *S. cerevisiae*, combinations of mutations in Ste2 lead to preferential recognition of *K. lactis* α-factor over conspecific pheromone [184], suggesting that evolution of either pheromone/receptor pair could contribute to speciation.

The solubility of α/P-factor also presents a significant challenge both to properly interpret the directionality of the gradient and upon reaching saturating levels. Thus, among the few genes that are expressed in a mating-type specific manner are Bar1 and Sxa2 [50,51], proteases responsible for the degradation of α/P-factor by the long-range sensors *MAT***a**/*h*- respectively. Upon release in the extracellular medium, the proteases induce the degradation of pheromones, thus supporting high mating efficiency, especially in high pheromone concentration environment [65,68,185,186]. In *S. cerevisiae*, where pheromone reception induces cell cycle arrest before mating, Bar1 confers a measure of sex-ratio in the population, preventing differentiation of excess *MAT***a** cells relative to the number of *MAT*α, thus avoiding high sexual competition between *MAT***a** cells and minimizing the growth arrest cost [187,188]. Locally, by shaping the pheromone gradient, Bar1 drives self-avoidance between *MAT***a** cells, thus increasing exploration of their environment [186,188].

Beyond the differences due to the chemical properties of the pheromones, additional cell type-specificities have been noted especially during the fission yeast fusion process. The myosin Myo52 concentrates and stabilizes first at the fusion focus of the *h*- cell [105], probably helping the alignment of the two opposing fusion foci. The fusion process in fission yeast also exhibits asymmetries at the ultrastructural level [129]. One cell, frequently the *h*-, protrudes into its partner, displaying a smooth and tense plasma membrane, whereas its partner exhibits a floppy membrane (Fig. 4F). This asymmetry is proposed to be linked to differences in turgor pressure and ratios between exocytic and endocytic activities in the two cell types. Finally, a third asymmetric event has been described after cell fusion, but before karyogamy: transcriptional induction of meiosis happens first in the *h*+ nucleus and then in the *h*- nucleus [189]. This asymmetry is conferred by a bipartite transcription factor, composed of the nuclear-resident Pi homeobox, already present in the *h*+ cell nucleus, and the activating cytosolic peptide Mi, expressed in the *h*- cell, which diffuses fast to the *h*+ nucleus upon cell fusion. Thus, although morphologically indistinguishable, yeast cells exhibit multiple asymmetries during their sexual reproduction, which may inform on the origin and relevance of anisogamy.

5.3. Blocks to multiple matings

The sexual life cycle requires a faithful alternance between the haploid and the diploid state. The mating process thus needs to result in the fusion of exactly two partners to form the diploid state, which can be reduced to the haploid state through meiosis. Indeed, the fusion of three cells would result in triploids from which meiosis would generate aneuploid, often inviable progenies. Recent studies have started revealing some of the mechanisms preventing mating with more than one partner.

Haploid cells rarely engage with more than one partner at a time, a property likely linked to the dynamics of and competition between polarity patches than ensure singularity [64,81,89]. However, some mutant situations disturbing patch competition can lead to (near-) simultaneous dual fusion events [81,89]. The merging of partner cell cytosols upon fusion then imposes a block to mating with further partners. In fission yeast, the Mi-Pi bipartite transcription factor mentioned above is an important element, as its design promotes the fast induction of the *mei3* gene in the newly-formed zygote [189]. In turn, Mei3 protein prevents mating in zygotes in two ways: by promoting CDK activity for entry in the meiotic cycle, thus leaving the pheromone signaling-permissive G1 phase, and by promoting the activation of the Mei2 RNA-binding protein, which imposes the zygotic fate independently of meiotic progression [190]. Transcriptional regulation by homeobox transcription factors may be conserved across ascomycetes, as the **a**1/α2 homeobox complex in budding yeast also contributes in blocking zygote mating. However, its function is only apparent in absence of a second, likely faster block, that involves the *MAT***a**-expressed Asg7 inhibiting the *MAT*α-expressed **a**-factor receptor Ste3 upon cell fusion [89]. Inhibition of the mating pathway by other signaling cascades, as discussed in Section 4.6, may also serve to suppress further zygotic fusion attempts.

6. Conclusions and open questions

Despite important progress in our understanding of the mechanisms of mating and fusion in yeast, there remain many open questions. The concept of local sensing at the polarity patch needs further study to better dissect the contribution of stochastic vs chemotropic patch mobility and the mechanisms thereof, which may be different in the two species. It is also unclear to what extent initial patch position is biased by a form a global sensing. For cell fusion, how partner cell walls merge in pre-zygotes, whether through covalent polymer linkages or perhaps with help of agglutinins, is completely unexplored. This would help understand whether and how cell walls provide mechanical resistance against turgor-driven forces as cells push against each other. Whether mechanical forces contribute to membrane merging and the nature of the fusogenic machinery are still unknown. There is finally an almost blank slate on figuring out how the freshly formed zygote completes its morphogenetic process to expand the fusion pore, yet restricts any further fusion event.

The evolutionary divergence of *S. cerevisiae* and *S. pombe* yeasts, yet the strong conceptual similarities in how they orient in a pheromone gradient and locally digest their cell wall to fuse, raise interesting questions about how the general organization of a cellular function is preserved through evolution. Indeed, the two species have very different physiologies, where starvation induces filamentation for food foraging in the first but sex for production of resistant spores in the second. Divergences in their molecular toolkit are apparent throughout, but are perhaps most striking in the shift from Gα to Gβ-based signaling downstream of receptor activation. How did a reversal of signaling logic occur during evolution? Better understanding of the molecular links between receptor activation and downstream signaling in *S. pombe*, but also in other fungi, would help address this question. Finally, an intriguing question is whether the asymmetries observed between mating types in isogamous yeast mating represent a first evolutionary step towards anisogamy.

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Conflict of interest

The authors declare no known conflict of interest.

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