Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

Self-signalling and self-fusion in filamentous fungi

Nick D Read, Alexander Lichius, Jun-ya Shoji and Andrew B Goryachev

The formation of interconnected hyphal networks is central to the organisation and functioning of the filamentous fungal colony. It is brought about by the fusion of specialised hyphae during colony initiation and mature colony development. These hyphae are normally genetically identical, and hence this process is termed hyphal self-fusion. The conidial anastomosis tube (CAT) functions in forming networks of conidial germlings during colony initiation. This hyphal type in *Neurospora crassa* is being used as a model for studies on hyphal self-signalling and self-fusion in filamentous fungi. Extraordinary new insights into the process of self-signalling that occurs during CAT self-fusion have recently been revealed by live-cell imaging of genetically engineered strains of *N. crassa*. A novel form of signalling involving the oscillatory recruitment of signal proteins to CAT tips that are communicating and growing towards each other has been observed. This 'ping-pong' mechanism operates over a very short time scale and comparisons with non-self-signalling during yeast cell mating indicate that this mechanism probably does not involve transcriptional regulation. It is proposed that this mechanism has evolved to increase the efficiency of fusion between genetically identical cells that are non-motile.

Address
Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, United Kingdom

Corresponding author: Read, Nick D (Nick.Read@ed.ac.uk)

Current Opinion in Microbiology 2009, **12**:608–615

This review comes from a themed issue on
Growth and development: eukaryotes
Edited by Judith Berman

Available online 26th October 2009

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2009.09.008

Introduction

Hyphal self-fusion is a defining feature of the lifestyle of most filamentous fungi because the majority form a colony composed of a supracellular network of genetically identical hyphae interconnected by prolific hyphal 'self-fusion' (Figures 1 and 2). This interconnected state allows the fungal colony to operate as a coordinated individual and to regulate its overall homeostasis by the interchange of nutrients, water, signalling molecules, nuclei and other organelles [1,2,3•]. The only group of 'filamentous fungi' that has not been reported to undergo vegetative cell fusion are yeasts which can undergo filamentous growth (e.g. *Candida albicans* and *Ashbya gossypii*). Interestingly, even the oomycetes, which are not true fungi but members of the Kingdom Stramenopila with a filamentous lifestyle, also undergo hyphal self-fusion. Although hyphal fusion has been studied in a wide range of different fungi, most recent work has focused on the fungal model *Neurospora crassa* [3•].

Self-fusion is brought about by specialised hyphae at two stages of colony development

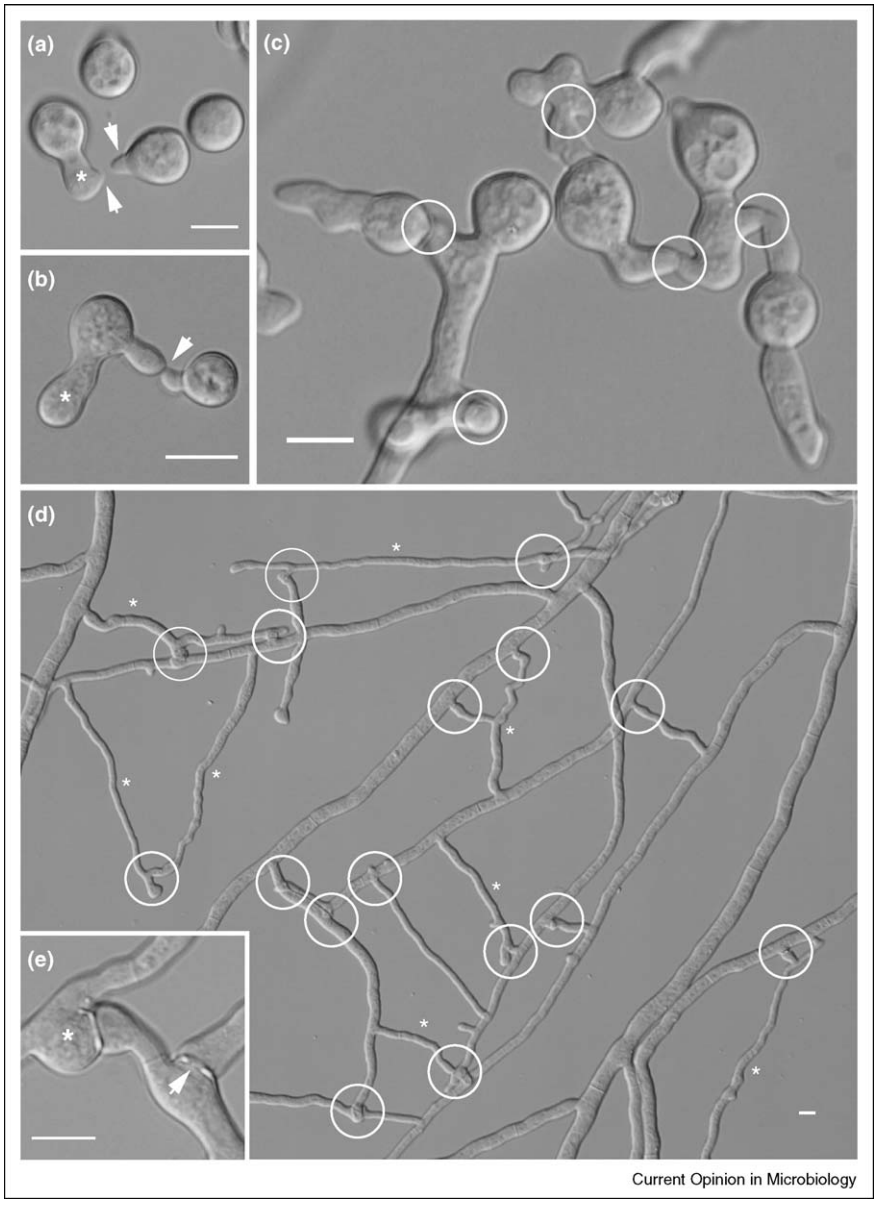
During colony establishment specialised hyphae, which are distinct from germ tubes, are involved in fusion. The most studied hyphae of this type are those formed from conidia and conidial germ tubes, and which have been termed *conidial anastomosis tubes* (CATs) [3•,4,5•,6]. CAT fusion creates an interconnected germling network during the initiation of colony development (Figure 1a–c). At later stages in the mature colony a different but related process of self-fusion occurs behind the peripheral zone of leading hyphae involved in colony extension. In this subperipheral region, specialised *fusion hyphae* arise as branches from established hyphae and these branches grow towards each other and fuse (Figure 1d,e).

CAT fusion provides a model for studying self-signalling and self-fusion

The CAT system in *N. crassa* provides a simple, experimentally amenable and genetically tractable system to study self-signalling and self-fusion in filamentous fungi [3•]. The whole process of CAT fusion can be analysed within six hours, which makes mutant screening, live-cell imaging and physiological experiments very easy to perform. CAT fusion can be divided into a continuum of events: CAT induction, CAT chemoattraction, cell–cell adhesion, cell wall remodelling/degradation, plasma membrane merger and the achievement of cytoplasmic continuity between CATs [3•] (Figure 2). Each of these processes is being analysed in detail [3•,5•,6,7,8••].

Currently the most exciting research on self-fusion is on understanding the mechanism of self-signalling. In order to orchestrate the whole process of CAT fusion, exquisite spatio-temporal coordination of signalling is necessary. However, there is an essential requirement for this mechanism of self-signalling, resulting in mutual chemotropism of CATs towards each other, to work: the two genetically identical CATs must generate physiological and functional differences between themselves. Presently we do not know what self-signalling ligands are

Figure 1



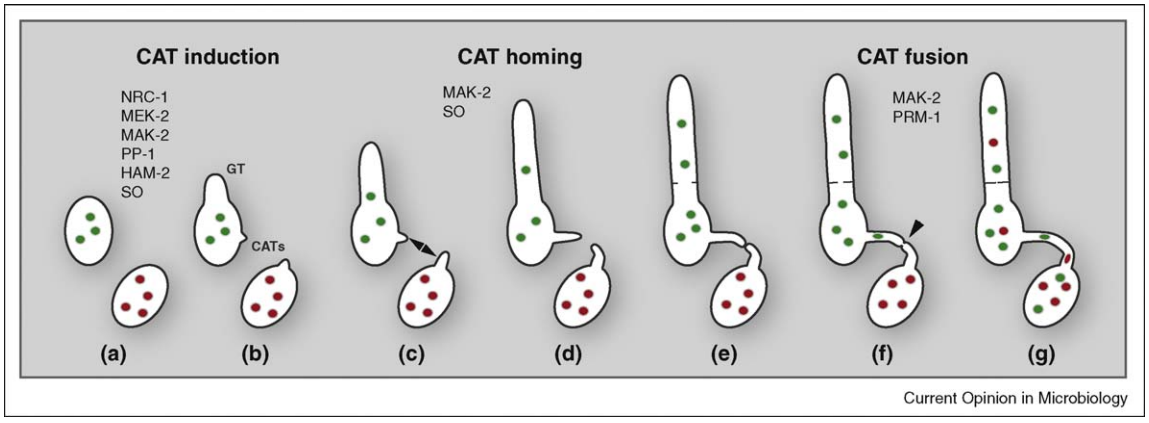
Self-fusion in *Neurospora crassa* occurs during early and late stages of colony development. **(a)** After an initial phase of isotropic expansion conidia of *N. crassa* polarise leading to the outgrowth of germ tubes (asterisk) and conidial anastomosis tubes (CATs) that can arise directly from conidia or from germ tubes (arrowheads). In this situation, the tip of the right hand CAT seems to have induced the formation of the CAT on the germ tube. **(b)** CATs chemotropically attract and become attached to each other (arrowhead indicates site of contact). Upon contact, tip growth arrests and a fusion pore is formed. **(c)** Each cell can interact with several neighbouring cells, thereby creating an interconnected germling network (CAT connections are circled). **(d)** In the subperipheral region of the mature colony (i.e. about 1–2 cm behind the leading edge of the mycelium) specialised fusion hyphae (marked with asterisks) fuse with other hyphae to establish a colony network (fusion connections circled) in a process very similar to CAT fusion. **(e)** Detail of hyphal fusion connections showing isotropic swelling upon contact (asterisk) and a fusion pore (arrowhead). Scale bars = 10 μm.

involved in CAT induction and chemoattraction, but it is likely that they are the same molecule [3•]. Furthermore, the receptor of this self-signalling molecule has not been identified and thus at this stage it remains an open question whether it is located in the plasma membrane, an internal organelle membrane or the cytoplasm.

CAT induction involves MAP kinase signalling, HAM-2 and SO

CAT induction probably involves an extracellular CAT inducer produced by ungerminated conidia [3•,5•] or from the tips of other CATs in the vicinity (Figure 1a). CAT induction involves the NRC-1/MEK-2/MAK-2 mitogen-

Figure 2



CAT-mediated cell–cell fusion in *Neurospora crassa* and proteins involved. The three major phases of CAT-mediated cell–cell fusion are CAT induction, CAT homing and CAT fusion, and progress through a subset of consecutive stages: **(a)** Ungerminated conidia contain on average three to six nuclei (shown here in green and red to indicate their origin from different but genetically identical germlings), and initially grow exclusively by isotropic expansion. **(b)** Cell polarisation leads to the outgrowth of a germ tube (GT) and conidial anastomosis tubes (CATs). Germ tubes tend to avoid each other and are unable to fuse. **(c)** Genetically identical cells communicate by releasing an unknown chemoattractant from their tips which is perceived by opposing CAT tips (arrowheads). **(d)** Orientation along this chemoattractant gradient results in CATs growing towards each other to establish cell wall contact. **(e)** Upon contact, tip growth arrests and CATs adhere to each other, and this most likely involves adhesive secretion and the buildup of new cell wall material around the contact site in order to prevent leakage during subsequent pore formation. **(f)** Fusion pore formation (arrowhead) includes localised cell wall remodelling/degradation and plasma membrane merger. **(g)** Upon establishment of cytoplasmic continuity, organelles, including nuclei, become mixed between fused germlings. Signalling and structural molecules involved at different stages of the process are indicated (see text for details).

activated protein (MAP) kinase pathway and the transcription factor PP-1 [3•,6,9]. Orthologues of these proteins are components of the pheromone response pathway in budding yeast (Figure 3). Phosphorylation of the MAP kinase MAK-2 was found to increase during the period when CAT induction is most prolific [9]. CAT induction also involves a putative transmembrane protein called HAM-2 [10], which is an orthologue of the yeast Far11 [11], and involves a filamentous ascomycete-specific WW domain protein called SO. In contrast to *mak-2*, *nrc-1*, *pp-1* and *ham-2* mutants which all lack CATs [3•], mutants defective in *so* still form CATs but not as efficiently as the wild type [7].

CAT chemoattraction is regulated by a novel 'ping-pong' signalling mechanism involving MAK-2 and SO

CATs exhibit marked positive chemotropism towards each other. This has been most unambiguously demonstrated using optical (laser) tweezers to micromanipulate CATs which, after having their relative positions changed, readjusted their growth direction to make contact and fuse at their tips [5•,7,12]. These results provide compelling evidence for a diffusible chemoattractant released from CAT tips and for a chemoattractant receptor located at CAT tips.

If the assumption is correct that the CAT inducer and chemoattractant are the same molecule, then the findings that conidia of *mak-2*, *nrc-1*, *ham-2* and *so* mutants fail to attract wild type CATs suggest that the proteins encoded by these genes are involved in either the chemoattractant release or chemoattractant response pathways [3•]. Recent results have provided much stronger evidence for MAK-2 and SO being involved in signalling during CAT chemoattraction [8••]. Previous evidence had shown that a Δ*mak-2* mutant lacks CATs [5•] whilst a Δ*so* mutant produced CATs which were unable to undergo chemotropic growth towards other CATs [7]. It is now clear that CATs that are growing towards each other rapidly alternate between two different physiological states that may be associated with alternating signal delivery and response. This mechanism (which we have termed the '*ping-pong mechanism*') involves the rapid, anti-phase, oscillatory recruitment of MAK-2 and SO to CAT tips. It results in the simultaneous localisation of MAK-2 and SO in opposing CAT tips that are homing towards each other. This highly coordinated, oscillatory recruitment of signalling proteins is initiated when CATs are $<15\ \mu m$ apart, the period of the oscillation is 6–12 min, and each of these proteins can be repeatedly recruited to a single CAT tip four to six times during chemotropic growth. The proteins become concentrated in particulate complexes ~ 300 nm in diameter that mostly concentrate in cortical regions closest to their partner cells. Such spatio-temporal coordination of signalling allows genetically identical and developmentally equivalent cells to avoid self-stimulation and coordinate their behaviour to ultimately achieve cell fusion. A prediction resulting from this ping-pong mechanism of

signalling, in which each CAT alternates between being signal sender and signal receiver (Figure 4), is that the unidentified chemoattractant will be released in a pulsatile manner [8••].

Further insights into the functional role of MAK-2 in the ping-pong mechanism have been gained by using a variant of MAK-2 that is sensitive to inhibition by the ATP-competitor 1NM-PP1. This powerful technique [13] has allowed us to provide strong evidence that MAK-2 activity is not required for the recruitment of SO to the opposing tip but is required for its subsequent delocalisation [8••].

The final stages of CAT fusion involve MAK-2 and PRM-1

Upon making contact with each other, CATs cease tip growth and adhere to each other (Figure 2f). This is followed by fusion pore formation that involves localised cell wall remodelling and degradation, and the merging of the plasma membranes of the two CATs (Figure 2g). MAK-2 also seems to play a role in fusion pore formation [8••] and the plasma membrane protein, PRM-1 is involved in membrane merger [14]. With the development of a fusion pore, cytoplasmic continuity is achieved between the two CATs and organelles, including nuclei, interchange between the two germlings.

Other components have been revealed in studies on hyphal fusion

As indicated earlier, vegetative hyphal fusion also occurs behind the periphery of mature fungal colonies. All CAT fusion mutants so far investigated have also been found to be defective in hyphal fusion in the mature colony which supports the use of CAT fusion as a general model for studies on vegetative hyphal fusion [3•]. However, there are some morphological, developmental and physiological differences between these two processes and therefore one needs to be cautious about extrapolating from one system to the other. Nevertheless, it is tempting to speculate that proteins involved in hyphal fusion in the mature colony, including two additional MAP kinase pathways (the MIK-1/MEK-1/MAK-1 cell wall integrity pathway and the OS-4/OS-5/OS-2 osmosensing pathway), the serine–threonine protein kinase, COT-1 and GPI-anchored proteins [3•,15,16] are also involved in CAT fusion.

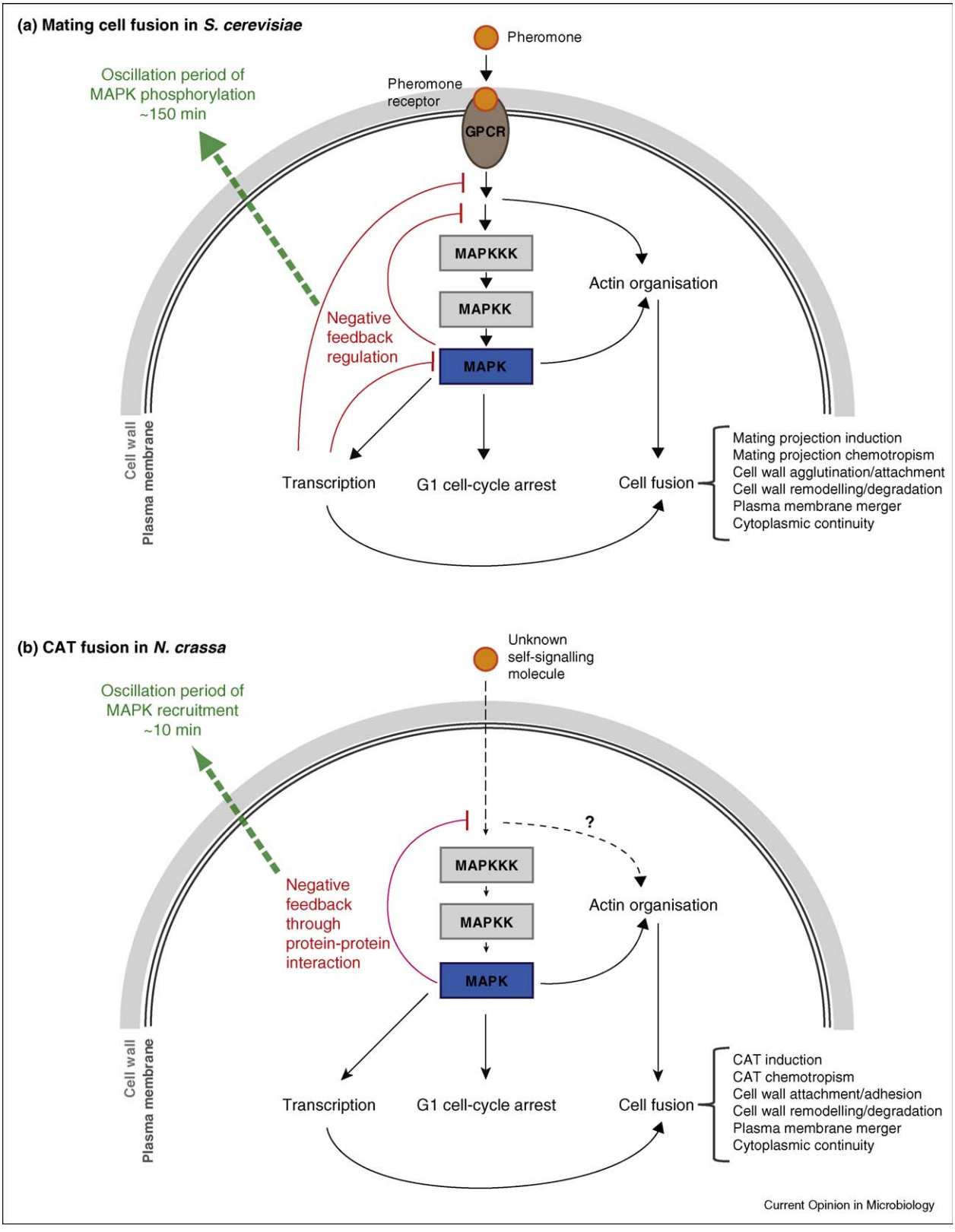
What can studies on yeast mating tell us about self-signalling in filamentous fungi?

Budding yeast mating is the most studied example of cell–cell signalling in fungi and results in non-self-fusion. During this process, pheromone binding to cognate G-protein-coupled receptors (GPCRs) leads to phosphorylation of Fus3 MAP kinase [17,18,19••] (Figure 3). Phosphorylated Fus3 arrests cells in G1 phase, promotes actin nucleation and polymerisation leading to polarised outgrowth of mating projections, and also induces expression of mating-related proteins that are involved in the formation of mating projections and cell–cell fusion. Once mating projections from two cells of opposite mating types have physically made contact, their cell walls become attached via GPI-anchored agglutinin proteins. Build up of new cell wall material around the contact site stabilises attachment and prevents leakage during subsequent fusion steps. Cell wall remodelling and local degradation of their intervening cell walls form a pore that allows the merging of plasma membranes, a process mediated by the plasma membrane protein Prm1 [19••]. As described in the previous sections, hyphal fusion in *N. crassa* also involves MAP kinase signalling and a PRM-1 protein. This indicates that in spite of their different physiological roles, molecular mechanisms underlying mating cell fusion in yeast and vegetative cell fusion in *Neurospora* are highly conserved. However, the unknown upstream signal transduction components involved in *Neurospora* self-signalling seem to be significantly different from those in budding yeast because they do not involve sex pheromone–GPCR signalling [3•] (Figure 3).

Somewhat analogous to the oscillatory recruitment of MAK-2 during CAT fusion, Fus3 exhibits oscillatory phosphorylation, and thus activation, during yeast mating [20]. Active Fus3 also preferentially localises to mating projection tips [21,22] but oscillatory recruitment of Fus3 to these tips has not been reported. However, a key difference in the oscillatory MAP kinase behaviour of yeast compared with that in *Neurospora* is the period of MAP kinase oscillation/activity. Whereas the oscillatory recruitment of MAK-2 in *Neurospora* has a period of 6–12 min [8••], the oscillatory phosphorylation of Fus3 in yeast has a period of ~150 min. This much longer period of oscillation in yeast is achieved by transcriptional negative feedback of Fus3 mediated by Sst2 (which regulates G-protein signalling) and the Fus3 phosphatase Msg5 whose expression is upregulated by active Fus3 [20]. In addition, non-transcriptional negative feedback regulates the active Fus3 level to fine-tune the pheromone signalling during yeast mating. This feedback regulation is mediated by the inhibition of MAP kinase scaffold recruitment by active Fus3, and achieves an initial increase and a subsequent decrease to a plateau in the active Fus3 level within 5–7 min [23], a timescale similar to that of the MAK-2 oscillation period.

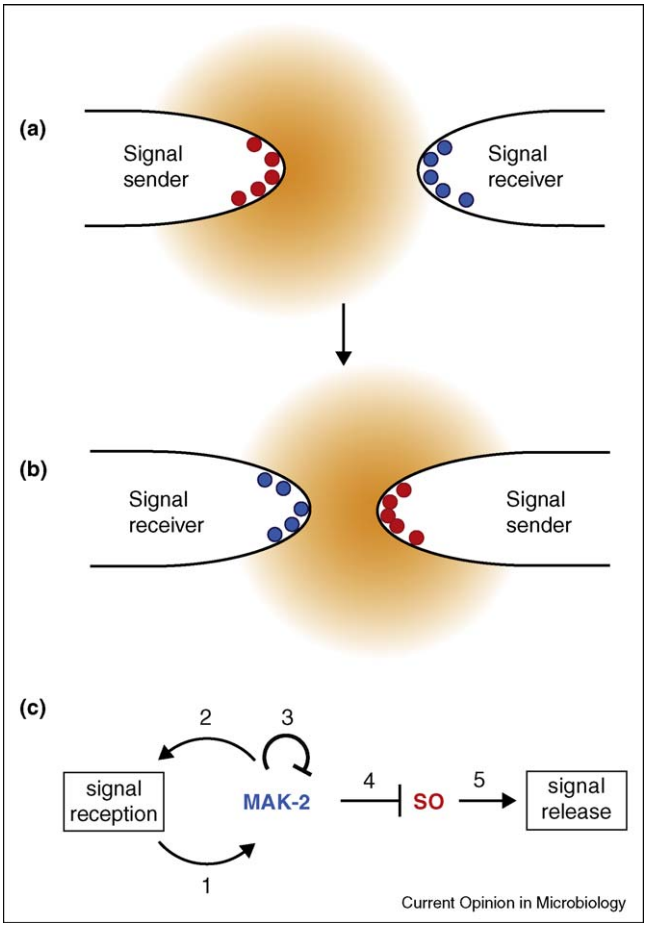
Other examples of biochemical oscillations mediated by transcriptional negative feedback have been reported to have periods greater than 90 min [24,25], whereas oscillations involving non-transcriptional negative feedback tend to have much shorter periods of ~10 min [26,23]. Thus, the oscillatory recruitment of MAK-2 is most likely achieved by negative feedback regulation that involves protein–protein interaction but not transcription (Figure 3).

Figure 3



Comparison of non-self-signalling during mating in *Saccharomyces cerevisiae* and self-signalling during CAT fusion in *Neurospora*. **(a)** Pheromone signalling in budding yeast. Pheromone binding to cognate G-protein-coupled receptors (GPCRs; Ste2 and Ste3) leads to activation of the MAP kinase

Figure 4



Ping-pong mechanism of self-signalling during CAT chemoattraction. **(a)** In the first half-period of the periodic signalling, the CAT tip on the left releases the chemoattractant signal while the one on the right responds to the signal by adjusting its growth direction along the gradient of the signalling molecule. **(b)** In the second half-period the roles reverse. **(c)** Proposed mechanism of intracellular signalling: (1) a chemoattractant–receptor complex induces local recruitment and activation of MAK-2; (2) a hypothetical positive feedback inherent in MAP kinase modules [38,39] amplifies the received signal; (3) as its local concentration increases, MAK-2-p2 (MAK-2 activated by double phosphorylation) downregulates itself by inducing disassembly of the MAP kinase protein complex through phosphorylation of its upstream components [40,39]; (4) the resulting decrease in MAK-2-p2 derepresses the accumulation of SO and leads to the formation of SO-containing protein complexes at the CAT tip; and (5) SO-stimulated chemoattractant release occurs. As the other CAT tip responds to the released chemoattractant, the sequence (1)–(5) will be repeated until the two tips physically meet [8••].

During yeast mating, negative feedback regulation of the Fus3 MAP kinase pathway is essential for the formation of multiple mating projections if the first projection fails to fuse with a mating projection from another cell [20,27••]. This suggests that downregulation of Fus3 activity is crucial for cells to respond to new pheromone stimulation. In a similar manner, downregulation of the signalling cascade involved in CAT chemoattraction may be important for MAK-2 to be dispersed from CAT tips allowing CATs to respond to the chemoattractant secreted by the opposing cell again, and thus undergo repeated ping-pong self-signalling (Figure 4).

Relationship between self-signalling and self-fusion in fungi and other organisms

Chemoattraction between genetically identical cells, often followed by cell–cell fusion, is widely present in eukaryotes from amoebae to humans [19••,28•]. Three contrasting examples in other organisms that provide interesting comparisons with fungal self-signalling and self-fusion are, where there is, first, chemoattraction without cell fusion (e.g. *Dictyostelium*); second, chemoattraction with fusion between developmentally different cells (e.g. myoblasts); and third, chemoattraction with fusion between developmentally similar cells (e.g. macrophages).

A dramatic example of self-signalling at the cell population level, which does not lead to cell fusion, is displayed by the slime mould *Dictyostelium discoideum*. In response to starvation, *Dictyostelium* unicellular amoebae initiate the pulsatile release of cAMP and follow its gradient to form large cell aggregates that eventually differentiate into multicellular fruiting bodies [29]. In contrast to chemoattraction between two CATs in *Neurospora*, self-signalling in *Dictyostelium* amoebae is a long-range phenomenon that involves thousands of cells. Thus periodic signalling in *Dictyostelium* during chemoattraction is cell-autonomous unlike in *Neurospora* where the periodic response is induced by the presence of another CAT [8••]. Moreover, cAMP signalling in *Dictyostelium* results in an in-phase synchronisation of nearby cells while the oscillatory recruitment of MAK-2 (or SO) to homing CAT tips of *Neurospora* are always out-of-phase in the opposing CAT tips by half a period as necessitated by the ping-pong mechanism of self-signalling (see Figure 4).

(Figure 3 Legend Continued) cascade (MAPKKK; Ste11, MAPKK; Ste7, MAPK; Fus3). Activated Fus3 causes G1 cell-cycle arrest, and promotes actin nucleation and polymerisation to form mating projections. Through activation of the Ste12 transcription factor, Fus3 also induces expression of mating-related proteins which are required for mating projection formation, attachment between two cells of opposite mating types and ultimately cell–cell fusion. The signalling pathway also involves negative feedback loops (red lines) consisting of transcriptional and non-transcriptional feedback regulation, which are crucial for cells to optimally respond to the pheromone stimulus. The transcriptional negative feedback loop leads to oscillatory phosphorylation of Fus3 with a period of ~150 min. **(b)** Signalling during CAT fusion in *Neurospora*. An unidentified chemoattractant is recognised by an unknown receptor(s), and activates the MAP kinase cascade (MAPKKK = NRC-1; MAPKK = MEK-2; MAPK = MAK-2). Downstream events in CAT fusion, which are probably regulated by MAK-2, seem to be similar to those of yeast mating, and involve G1 cell-cycle arrest, actin organisation, cell wall attachment and cell fusion. Somewhat analogous to the oscillatory phosphorylation of Fus3, MAK-2 shows oscillatory recruitment to CAT tips. A key difference to signalling during yeast mating is the period of the MAK-2 oscillation which is markedly shorter (~10 min) than that of Fus3, suggesting a distinct regulatory mechanism for the signalling pathway which does not involve transcriptional feedback loops (see text for details).

One of the most intriguing phenomena involving chemoattraction between developmentally different cells is the fusion of stem cells with differentiated tissue cells which may potentially result in the reversal of cellular senescence and tissue regeneration [30]. Fusion of muscle progenitor cells (myoblasts) during muscle development and regeneration is perhaps the best-characterised example in this class. In *Drosophila*, fusion is initiated by founder cells that do not fuse with each other but instead attract and fuse with undifferentiated fusion-competent myoblasts [31]. This initial fusion event results in a nascent myotube that subsequently grows by 2–25 successive fusion events, the number of which is strictly developmentally regulated. Nascent mammalian myotubes, formed by the initial fusion of several differentiated myoblasts, subsequently grow by releasing cytokine interleukin-4 (IL-4) which chemoattracts more undifferentiated myoblasts with which they fuse [32]. Thus, in contrast to *Neurospora*, both signalling and fusion during muscle development are asymmetric and unilateral. However, all fusion events during myotube elongation are strictly pair-wise and require polarised 'tip-to-tip' attachment of fusing cells, which is a feature in common with hyphal self-fusion.

Perhaps the best-characterised example of self-fusion among developmentally similar cells is the fusion of mammalian macrophages. Uninucleate, macrophages fuse occasionally to form osteoclasts and giant cells. Interestingly, prostaglandins and IL-4, which mediate activation and chemoattraction of myoblasts, are also involved in macrophage fusion that results in the differentiation of osteoclasts and giant cells. Other cytokines and growth factors, such as RANKL and M-CSF, have also been reported to regulate the complex process of macrophage fusion and differentiation [33]. RANKL induces the expression of the dendritic cell-specific transmembrane protein DC-STAMP [34] that is crucial for macrophage fusion during multinucleate osteoclast formation [35]. Interestingly, expression of DC-STAMP in one of the interacting macrophages is sufficient for fusion to occur [35], suggesting that two fusing macrophages are in different physiological states. One cell may take the role of fusion-competent 'founder' cell and expresses DC-STAMP, whilst the other may act as a 'follower' cell that expresses a so far unknown DC-STAMP ligand [36,37]. Thus it seems that macrophages 'differentiate' into founder and follower cells (equivalent to signal sender and receiver cells) to avoid self-stimulation, whereas in CAT chemoattraction two interacting cells rapidly alternate between two physiological states to achieve this. These different strategies to achieve different physiological states in a population of genetically identical cells may be attributed to macrophages being motile and *Neurospora* conidia being non-motile. Thus macrophages are capable of exhibiting migratory movement to a fusion partner [37] whilst sessile conidia depend on being close enough to a potential fusion partner in order to bridge the distance by CAT growth and fusion. The ability to alternate between being a signal sender and receiver allows any fusion-competent cell to fuse with any other fusion-competent cell of similar genetic background in its vicinity. Therefore, the ping-pong mechanism of signalling might function to increase the efficiency of fusion between immobile cells.

Conclusions

Much of the molecular machinery involved in chemotropic growth, cell adhesion and cell fusion during hyphal self-fusion seems to be shared with that involved in non-self-fusion between mating yeast cells. Key differences between these processes appear to lie in the mechanisms of signal perception and regulation in order to ensure cell–cell recognition ultimately leading to cell–cell fusion. It may also have evolved to increase the efficacy of fusion between genetically identical, non-motile cells. The intriguing ping-pong signalling so far seems to be a unique and sophisticated mechanism of self-signalling which allows two genetically identical cells to be in different physiological states. Important challenges for achieving a better understanding of fungal self-signalling in the future will be to identify: firstly, the CAT inducer/chemoattractant and its receptor, secondly, components which act upstream of MAP kinase signalling, thirdly, additional components of the ping-pong mechanism and lastly, the regulatory networks involved in the different stages of cell fusion.

Acknowledgement

This work was supported by funding from the Biotechnological and Biological Sciences Research Council (grant # BB/E010741/1) to NDR.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Fricker MD, Boddy L, Bebber D: **Network organisation of filamentous fungi**. In *Biology of the Fungal Cell.* Edited by Howard RJ, Gow NAR. Berlin: Springer-Verlag; 2007:309-330.

2. Read ND: **Environmental sensing and the filamentous fungal lifestyle**. In *Fungi and their Environment.* Edited by Gadd GM, Watkinson SC, Dyer PS. Cambridge, UK: Cambridge University Press; 2007:38-57.

3. • Read ND, Fleißner A, Roca MG, Glass NL: **Hyphal fusion**. In *Cellular and Molecular Biology of Filamentous Fungi.* Edited by Borkovich KA, Ebolle D. Washington DC: American Society of Microbiology; 2010:260-273.
This chapter provides the most comprehensive and up-to-date review of hyphal fusion during vegetative and sexual development in filamentous fungi.

4. Roca MG, Davide LC, Mendes-Costa MC, Wheals A: **Conidial anastomosis tubes in *Colletotrichum***. *Fung Genet Biol* 2003, **40**:138-145.

5. • Roca MG, Arlt J, Jeffree CE, Read ND: **Cell biology of conidial anastomosis tubes in *Neurospora crassa***. *Eukaryot Cell* 2005, **4**:911-919.
This paper was the first study to demonstrate in *N. crassa* that conidial anastomosis tubes are morphologically and physiologically distinct from germ tubes and under separate genetic control.

6. Roca MG, Read ND, Wheals AE: **Conidial anastomosis tubes in filamentous fungi**. *FEMS Microbiol Lett* 2005, **249**:191-198.

7. Fleißner A, Sarkar S, Jacobson DJ, Roca MG, Read ND, Glass NL: **The *so* locus is required for vegetative cell fusion and postfertilization events in *Neurospora crassa***. *Eukaryot Cell* 2005, **4**:920-930.

8. •• Fleißner A, Leeder AC, Roca M.G, Read ND, Glass NL: **Oscillatory recruitment of signaling proteins to cell tips promotes coordinated behavior during cell fusion in *Neurospora crassa***. *Proc Natl Acad Sci USA* (in press).
This paper shows the oscillatory recruitment of the MAK-2 and SO proteins during CAT chemoattraction for the first time.

9. Pandey A, Roca MG, Read ND, Glass NL: **Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa***. *Eukaryot Cell* 2004, **3**:348-358.

10. Xiang Q, Rasmussen CG, Glass NL: **The *ham-2* Locus, encoding a putative transmembrane protein, is required for hyphal fusion in *Neurospora crassa***. *Genetics* 2002, **160**:169-180.

11. Kemp HA, Sprague GF: **Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast *Saccharomyces cerevisiae***. *Mol Cell Biol* 2003, **23**:1750-1763.

12. Wright GD, Arlt J, Poon WCK, Read ND: **Optical tweezer micromanipulation of filamentous fungi**. *Fung Genet Biol* 2007, **44**:1-13.

13. Bishop AC, Ubersax JA, Petsch DT, Matheos DP, Gray NS, Blethrow J, Shimizu E, Tsien JZ, Schultz PG, Rose MD *et al.*: **A chemical switch for inhibitor-sensitive alleles of any protein kinase**. *Nature* 2000, **407**:395-401.

14. Fleißner A, Diamond S, Glass Nl: **The *Saccharomyces cerevisiae PRM1* homolog in *Neurospora crassa* is involved in vegetative and sexual cell fusion events but also has postfertilization functions**. *Genetics* 2009, **181**:497-510.

15. Bowman SM, Piwowar A, Al Dabbous M, Vierula J, Free SJ: **Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa***. *Eukaryot Cell* 2006, **5**:587-600.

16. Maerz S, Ziv C, Vogt N, Helmstaedt K, Cohen N, Gorovits R, Yarden O, Seiler S: **The nuclear Dbf2-related kinase COT1 and the mitogen-activated protein kinases MAK1 and MAK2 genetically interact to regulate filamentous growth, hyphal fusion and sexual development in *Neurospora crassa***. *Genetics* 2008, **179**:1313-1325.

17. Dohlman HG, Thorner JW: **Regulation of G protein-initiated signal transduction in yeast: paradigms and principles**. *Annu Rev Biochem* 2001, **70**:703-754.

18. Qi M, Elion EA: **MAP kinase pathways**. *J Cell Sci* 2005, **118**:3569-3572.

19. •• Chen EH, Grote E, Mohler W, Vignery A: **Cell–cell fusion**. *FEBS Lett* 2007, **581**:2181-2193.
This minireview provides the most recent overview on cell–cell fusion in a variety of eukaryotic model systems, including yeast cell mating, somatic cell and gamete fusion in nematodes, myoblast fusion in mammals and flies, as well as macrophage fusion in mammals. Major players of molecular mechanisms involved in cell–cell signalling and plasma membrane fusion are put into context.

20. Hilioti Z, Sabbagh W, Paliwal S, Bergmann A, Goncalves MD, Bardwell L, Levchenko A: **Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis**. *Curr Biol* 2008, **18**:1700-1706.

21. Maeder CI, Hink MA, Kinkhabwala A, Mayr R, Bastiaens PI, Knop M: **Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signalling**. *Nat Cell Biol* 2007, **9**:1319-1326.

22. Slaughter BD, Schwartz JW, Li R: **Mapping dynamic protein interactions in MAP kinase signaling using live-cell fluorescence fluctuation spectroscopy and imaging**. *Proc Natl Acad Sci U S A* 2007, **104**:20320-20325.

23. Yu RC, Pesce CG, Colman-Lerner A, Lok L, Pincus D, Serra E, Holl M, Benjamin K, Gordon A, Brent R: **Negative feedback that improves information transmission in yeast signalling**. *Nature* 2008, **456**:755-761.

24. Lev Bar-Or R, Maya R, Segel LA, Alon U, Levine AJ, Oren M: **Generation of oscillations by the p53–Mdm2 feedback loop: a theoretical and experimental study**. *Proc Natl Acad Sci U S A* 2000, **97**:11250-11255.

25. Hirata H, Yoshiura S, Ohtsuka T, Bessho Y, Harada T, Yoshikawa K, Kageyama R: **Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop**. *Science* 2002, **298**:840-843.

26. Jacquet M, Renault G, Lallet S, De Mey J, Goldbeter A: **Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in *Saccharomyces cerevisiae***. *J Cell Biol* 2003, **161**:497-505.

27. •• Moore TI, Chou CS, Nie Q, Jeon NL, Yi TM: **Robust spatial sensing of mating pheromone gradients by yeast cells**. *PLoS One* 2008, **3**:e3865.
This paper showed detailed observation of *S. cerevisiae* chemotropic growth in response to the pheromone gradient with different steepness and concentration. This paper, along with [21], also raised insight into how negative feedback regulation on the signalling pathway contributes for better sensing the chemoattractant gradient.

28. • Oren-Suissa M, Podbilewicz B: **Cell fusion during development**. *Trends Cell Biol* 2007, **17**:537-546.
This review focuses on ways to identify fusogens that are proteins or protein complexes that directly mediate fusion of lipid bilayers. In this context, proposed models for viral, intracellular and developmental membrane fusion mechanisms are discussed.

29. Rappel WJ, Loomis WF: **Eukaryotic chemotaxis**. *Wiley Interdiscip Rev: Syst Biol Med* 2009, **1**:141-149.

30. Stolzing A, Hescheler J, Sethe S: **Fusion and regenerative therapies: is immortality really recessive?** *Rejuvenation Res* 2007, **10**:571-586.

31. Richardson BE, Nowak SJ, Baylies MK: **Myoblast fusion in fly and vertebrates: new genes, new processes and new perspectives**. *Traffic* 2008, **9**:1050-1059.

32. Jansen KM, Pavlath GK: **Molecular control of mammalian myoblast fusion**. *Methods Mol Biol* 2008, **475**:115-133.

33. Vignery A: **Macrophage fusion: molecular mechanisms**. *Methods Mol Biol* 2008, **475**:149-161.

34. Yagi M, Ninomiya K, Fujita N, Suzuki T, Iwasaki R, Morita K, Hosogane N, Matsuo K, Toyama Y, Suda T, Miyamoto T: **Induction of DC-STAMP by alternative activation and downstream signaling mechanisms**. *J Bone Miner Res* 2007, **22**:992-1001.

35. Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N, Morita K, Ninomiya K, Suzuki T, Miyamoto K *et al.*: **DC-STAMP is essential for cell–cell fusion in osteoclasts and foreign body giant cells**. *J Exp Med* 2005, **202**:345-351.

36. Vignery A: **Macrophage fusion: the making of osteoclasts and giant cells**. *J Exp Med* 2005, **202**:337-340.

37. Ishii M, Saeki Y: **Osteoclast cell fusion: mechanisms and molecules**. *Mod Rheumatol* 2008, **18**:220-227.

38. Kholodenko BN, Birtwistle MR: **Four-dimensional dynamics of MAPK information-processing systems**. *Wiley Interdiscip Rev: Syst Biol Med* 2009, **1**:28-44.

39. Shin SY, Rath O, Choo SM, Fee F, McFerran B, Kolch W, Cho KH: **Positive- and negative-feedback regulations coordinate the dynamic behavior of the Ras–Raf–MEK–ERK signal transduction pathway**. *J Cell Sci* 2009, **122**:425-435.

40. McKay MM, Ritt DA, Morrison DK: **Signaling dynamics of the KSR1 scaffold complex**. *Proc Natl Acad Sci U S A* 2009, **106**:11022-11027.