# The control of filamentous differentiation and virulence in fungi

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*Many members of the fungal kingdom have a distinguishing feature, dimorphism, which is the ability to switch between two morphological forms: a cellular yeast form and a multicellular invasive filamentous form. At least three pathways are involved in regulating the transition between these two forms in the budding yeast Saccharomyces cerevisiae, and evidence is now emerging that homologous signalling modules are involved in regulating filament formation and virulence in a range of human and plant fungal pathogens. Strikingly, components used to signal sexual differentiation in the response to mating pheromones are often reutilized to regulate dimorphic development, suggesting an ancient link between these processes.*

Despite their enormous diversity in size and shape, many fungi have a common morphogenetic feature: the ability to switch between a cellular yeast form and a filamentous form in response to environmental cues. The yeast-form cells divide mitotically either by budding or fission to form two independent daughters. Filaments are more complex multicellular structures. During filamentous growth, the cells do not separate after nuclear division but, rather, remain physically associated. The course of subsequent events determines the morphology of the filament. In some organisms (e.g. the budding yeast, *Saccharomyces cerevisiae*), distinct cell walls form between the daughters, but they remain attached to form chains of elongated cells called pseudohyphae (Fig. 1a). In others (e.g. the plant pathogen *Ustilago maydis*), no constrictions form at cell junctions after division, and a smooth tube called a hypha is produced (Fig. 1b). Filaments can be invasive, which means that they can penetrate the surface on which they grow. There is mounting evidence suggesting that the ability to switch between yeast and filamentous forms is a crucial determinant of fungal pathogenicity in both plants and animals.

Filamentous growth is initiated by an asymmetric cell division in which a round yeast cell divides to produce the founding elongated cell of the filament. This event is induced in response to environmental cues that differ dramatically from one organism to another: in *Saccharomyces*, it is starvation for nitrogen, in *Candida* (a human pathogen), it is serum (among other things), and, in *Ustilago*, which infects corn, it is a putative molecular signal from the host plant. Studies combining the powerful genetic and genomics tools available in *Saccharomyces* have revealed three pathways that couple afferent signals to the switch. Although many different signals can induce filamentous development, the strategies for connecting the external signal to the change in cell differentiation (the 'wiring diagram') are broadly conserved among the fungi. Moreover, there appears to be an ancient connection between filamentation, mating and virulence. Here, we review these pathways and links in *S. cerevisiae* and in four pathogens.

## Signalling filamentous growth in *S. cerevisiae*

In *S. cerevisiae*, four components of the mitogen-activated protein (MAP) kinase pathway that signals the mating pheromone response are also required for filamentous growth of diploid cells and the invasive growth of haploid cells (Fig. 2a, b)[1,2]. The shared components include three protein kinases that act in sequence, Ste20p, Ste11p and Ste7p. These enzymes are homologues of the mammalian MAP kinase signalling enzymes, PAK (p21-activated kinase), MEKK (MAPK kinase kinase), and MEK (MAPK kinase). The fourth shared component is a transcription factor, Ste12p, which acts at the terminus of both pathways. This overlap between components used to signal mating versus filamentation raises the question of how the same signalling components can be used to direct two distinct developmental events.

In haploid cells, a Ste12p homomultimer bound cooperatively to tandem Ste12p binding sites (TGAAACA; pheromone response elements or PREs) is sufficient to programme pheromone-inducible transcription[3] (see Ref. 4 for a comprehensive review of Ste12p and its interactions). During filamentous and invasive growth, Ste12p interacts with a different partner, the TEA/ATTS-family transcription factor Tec1p[5–7]. Like other family members, Tec1p binds to the sequence CATTCPy, the TEA/ATTS consensus sequence (TCS)[7]. Enhancer elements containing a PRE adjacent to a TCS are termed filamentation/invasion response elements (FREs)[7]; FREs in the promoters of the retrotransposon Ty1 and the *TEC1* gene itself are necessary and sufficient to confer gene expression that is specifically dependent on the subset of MAP kinase signalling components that are required for filamentous growth (Ste20p, Ste11p, Ste7p and Ste12p)[7].

The second specificity factor is the MAP kinase Kss1p, which exerts both positive and negative control over filamentation[8,9]. The function of the MAP kinase cascade (Ste20p–Ste11p–Ste7p) is to convert Kss1p from an inhibitor into an activator. The dual activities of Kss1p were illuminated by analysis of mutations that affect each of these functions[8,9] and

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identification of a physical complex between Kss1p and Ste12p[8]. The complex is disrupted in mutants of Kss1p that reduce its kinase-independent inhibitory activity[8]. Interestingly, these mutants map to the MAP kinase insertion, a peptide insert found only in MAPKs[10]. Two inhibitory proteins, Dig1p and Dig2p, each interact with Kss1p and Ste12p[11,12] – these might be important cofactors mediating the positive and negative functions of Kss1p.

The importance of the MAP kinases for developmental specificity was revealed by the knockout of *FUS3*, which encodes the MAP kinase for the mating pathway[8]. In mutants lacking Fus3p protein, Kss1p can supply MAPK function for the pheromone response pathway[8]. However, this inappropriate substitution of Kss1p for Fus3p destroys the specificity of signalling – the pheromone pathway now erroneously stimulates both mating and invasion[8]. Thus, in wild-type cells, each MAP kinase is dedicated to a specific pathway – Fus3p to the pheromone response pathway and Kss1p to the filamentation and invasion pathway.

Filamentation in *Saccharomyces* is also controlled by the cAMP pathway (Fig. 2c). As in many organisms, regulated formation of cAMP by adenylate cyclase in yeast activates cAMP-dependent protein kinase (protein kinase A, PKA), leading to diverse events. In *Saccharomyces*, perturbation of cAMP levels either through mutation of the high-affinity phosphodiesterase Pde2p or by the exogenous application of cAMP enhances filamentous growth[13–15]. Both Ras2p, a known regulator of cAMP levels, and the G protein α subunit homologue, Gpa2p, appear to act upstream of adenylate cyclase[14,15]. Ras2p has an additional distinct role in controlling FRE-dependent gene expression[16].

Another regulator implicated in invasive growth is the Rim1p zinc-finger transcription factor (Fig. 2d). This DNA-binding protein was first identified in studies of early meiotic gene expression where Rim1p was found to be necessary for the induction by starvation of *IME1*, a key regulator of meiosis and sporulation[17]. Rim1p is activated by the proteolytic removal of an inhibitory C-terminal domain, and several genes (*RIM8*, *RIM9*, *RIM13*) necessary for this cleavage-activation mechanism have been described[18]. The *RIM* genes also play a role in invasive growth: *rim1*, *rim8*, *rim9* and *rim13* mutants are defective in invasion[18]. Rim1p is homologous to the *Aspergillus nidulans* transcription factor PacC, which is also regulated by proteolysis[19].

### A MAPK pathway controls hyphal development and virulence in the asexual human pathogen *C. albicans*

*Candida albicans* is the most common fungus identified in clinical isolates. This opportunistic pathogen is capable of causing both superficial (e.g. oral thrush) as well as more serious invasive infections. Despite the lack of an identified sexual cycle in *C. albicans*, homologues of MAP kinase cascade components, *STE12* (*CPH1*), *STE7* (*HST7*), and *STE20* (*CST20*), have been isolated largely by their ability to complement the mating defects of

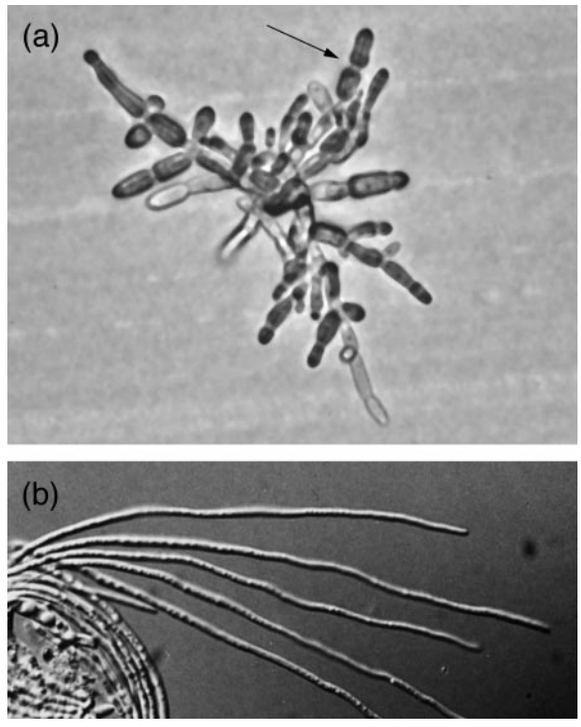


**FIGURE 1**

Filamentous forms in fungi. (a) Pseudohyphae of *Saccharomyces cerevisiae*. Shown are cells containing an activated mitogen-activated protein (MAP) kinase pathway that are growing in low-nitrogen medium. The arrow points to a constriction between two cells, which is characteristic of pseudohyphae. (b) Hyphae of *Ustilago maydis*. The dikaryons (the product of mating) are growing on charcoal medium. Note that the cells form a continuous smooth tube. (Photograph courtesy of Flora Banuett.)

*Saccharomyces* mutants[20–24]. Disruption of these genes results in defective hyphal growth on a solid medium but does not affect the characteristic induction of *Candida* hyphae by human serum[20,21,23,24]. A strain incapable of hyphal development in the presence of serum was obtained by the construction of a *cph1 efg1* double mutant. *EFG1* encodes a homologue of the *S. cerevisiae* Phd1p protein, which appears to act in a parallel non-MAPK pathway to regulate filamentous growth[25]. This *cph1 efg1* double mutant is avirulent in a mouse model of disseminated candidiasis[25]. Similarly, a mutation in the *Candida CLA4* gene, which encodes a Ste20p-related kinase, results in strains with altered yeast cell morphology that are largely deficient in hyphal development and markedly attenuated in terms of virulence in mice[26]. Thus, a MAP kinase pathway related to that of *Saccharomyces* also operates in *Candida* to regulate filamentous growth and virulence.

Negative regulators of hyphal development in *C. albicans* have also been described. A MAP kinase phosphatase mutant has been described that shows increased hyphal development, apparently owing to an effect upon Cek1, a Kss1p-related MAP kinase[27]. Likewise, a global transcriptional corepressor, Tup1, also inhibits filamentous growth[28]. In both cases, mutants that force filamentous development under conditions that do not normally induce them result in a decrease in virulence. These data suggest that

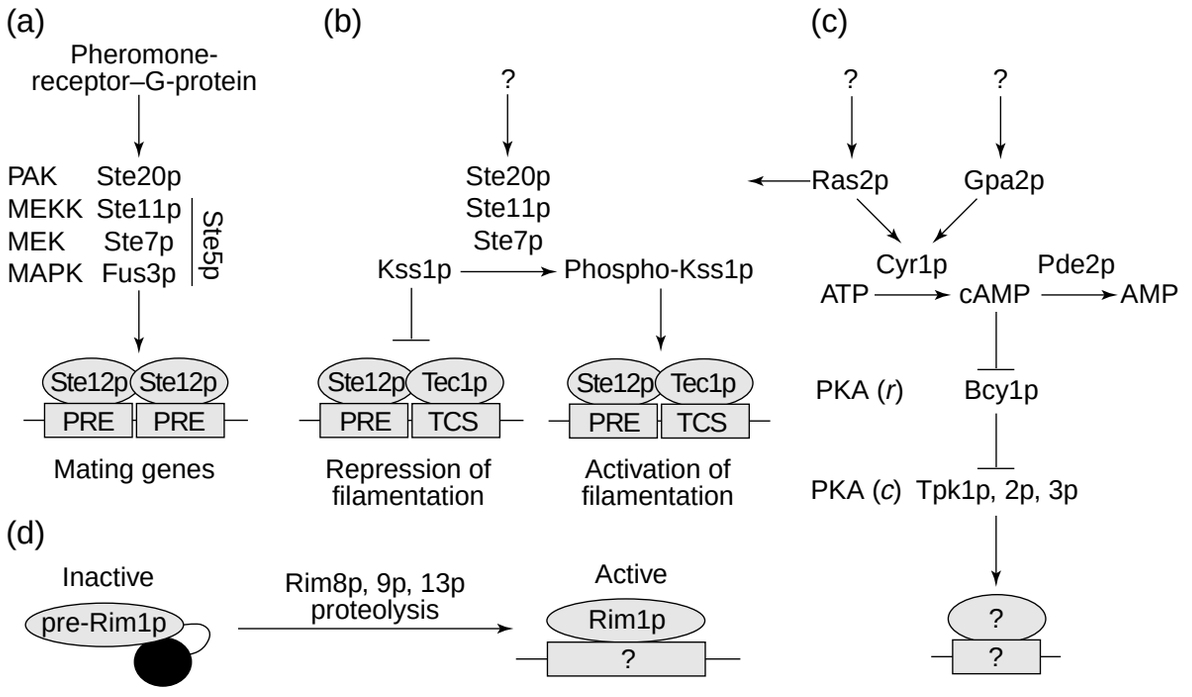


**FIGURE 2**

Mating and filamentation signalling pathways in *Saccharomyces cerevisiae*. (a) The mating mitogen-activated protein (MAP) kinase pathway. Simplified diagram of the yeast mating MAP kinase cascade. Mammalian homologues of the yeast enzymes are indicated on the left. (b) The filamentation MAP kinase pathway, depicting the two activities of Kss1p, a kinase-independent inhibitory activity and a kinase-dependent activating activity. The function of the upstream MAPK cascade is to convert Kss1p from an inhibitor of the Ste12p–Tec1p transcription factor into an activator. (c) The cAMP pathway. Ras2p and Gpa2p regulate cAMP formation, presumably via the adenylate cyclase Cyr1p. Pde2p is a high-affinity phosphodiesterase that degrades cAMP. Bcy1p is the regulatory (*r*) subunit of cAMP-dependent protein-kinase (protein kinase A, PKA). The inhibitory activity of Bcy1p is inactivated upon binding to cAMP. The three catalytic (*c*) subunit isoforms of PKA are Tpk1p, Tpk2p and Tpk3p. (d) The Rim1p proteolysis pathway. Proteolysis of the Rim1p transcription factor requires Rim8p, Rim9p and Rim13p. Cleavage converts Rim1 from an inactive to an active form that promotes invasive growth and meiosis.

the ability to switch between yeast and filamentous forms of *C. albicans* might be necessary for disease.

### Mating signalling components are required for filamentous growth and virulence in *Ustilago maydis*

Infection of corn and the induction of tumours by the smut fungus *U. maydis* offers a model system for studying plant pathogenesis. Following mating, the formation of a binucleate dikaryon (the product of haploid cell fusion in which the parental nuclei have not yet fused) results in the ability of the organism to grow in a filamentous form on solid charcoal medium and cause disease in the plants (Fig. 3)[29]. In the dikaryon, the pheromone and receptors continue to be expressed and are required for filamentous growth on charcoal medium[30,31].

Although the autocrine pheromone–receptor signalling loop is required for filamentation in the petri dish, mating pheromones and receptors are not required for filament formation and pathogenesis in the corn plant, indicating that a different signal activates filamentation in the host[30]. However, the downstream signalling components appear to be the same on the plate and in the plant. A gene encoding a G protein α subunit homologue, *gpa3*, required for pheromone signalling and filament formation on charcoal plates[32], is also required for disease development[32]. Thus, this Gα protein is likely to be coupled to the pheromone receptors encoded at the *a* mating-type locus[33] *in vitro*, but not in the plant. Similarly, a gene encoding a MAP kinase kinase homologue, *fuz7*, is required for mating and filamentous growth *in vitro* and for pathogenesis in the plant[34]. This dual requirement is also a property of *prf1*, which encodes the HMG-box transcription factor that acts downstream of the pheromone signal[35]. Taken together, these observations can be explained by proposing that the same signalling cascade is coupled to two different receptors, the pheromone receptor during growth *in vitro* and a receptor (as yet unidentified) for a putative signal present in the plant during infection.

In *Ustilago*, as in *Saccharomyces*, cAMP signalling regulates filamentation. However, the direction of regulation is reversed: a knockout of the adenylate cyclase gene activates filamentation, whereas the disruption of the gene encoding the inhibitory subunit of PKA results in decreased filamentation and a decrease in pathogenicity[36,37].

### *Magnaporthe grisea*: cAMP and a MAP kinase regulate appressorium formation and virulence

Rice blast disease is caused by *Magnaporthe grisea*. This fungus uses a specialized infection structure, the appressorium, to pierce through the rice leaf (Fig. 4). These cell projections generate sufficient pressure (80 bars) to poke holes in plastic sheets[38].

This differentiation event is induced by the growth of *Magnaporthe* on a hydrophobic surface. Although the appressorium is a projection of a single cell rather than a multicellular filament, its formation requires two familiar signalling pathways: a cAMP pathway and a MAP kinase pathway. Disruption of the gene for PKA results in strains unable to induce appressoria in response to cAMP and unable to infect rice plants[39] (although the precise phenotype of the PKA knockout has recently become controversial; see Ref. 40 for a review). Likewise, disruption of a gene encoding a Fus3p/Kss1p-related MAP kinase, *PMK1*, also produces a defect in appressorium development[41]. Because *pmk1*-knockout strains still respond to exogenous cAMP to effect early appressorium development, it appears that the cAMP/PKA pathway and MAPK pathways play distinct roles in the development of this key virulence determinant[41]. In addition to the structural similarity of Pmk1 to Fus3p (indeed Pmk1 can function in mating in *Saccharomyces*)[41], another tantalizing observation suggests a link between mating and appressorium formation in this organism: activation of the *M. grisea* pheromone pathway prevents appressorium formation and inhibits pathogenicity[42].

### Signalling filamentous growth in the human pathogen *Cryptococcus neoformans*: *Saccharomyces* in disguise?

The human fungal pathogen *C. neoformans* preys primarily on immunocompromised individuals[43]. Although capable of infecting virtually any tissue, the organism has a propensity to invade the central nervous system and cause meningitis, a frequently fatal condition. When haploid cells of the opposite mating type (*MAT***a** and *MAT*α) are mixed under conditions of starvation, they fuse to form a filamentous dikaryon[44]. Mating signalling is probably initiated by mating pheromones; a candidate gene encoding α-pheromone has been identified in the *MAT*α locus, and its introduction into a *MAT***a** strain results in filamentous growth[45]. At the termini of the dikaryotic hyphae, nuclear fusion occurs followed by immediate initiation of meiosis and spore formation. Recent work has implicated a gene encoding a heterotrimeric G protein α subunit, *GPA1*, in mating[46]. A *gpa1*-disruption mutant is defective in mating and filament formation as well as in several activities required for virulence, including melanin and capsule production[46]. These defects are rescued by the exogenous application of cAMP, consistent with the possibility that this G protein subunit functions by regulating adenylate cyclase activity (as has been proposed for Gpa2p in *S. cerevisiae*)[46].

There is an intriguing link between mating type, virulence and filament formation in *Cryptococcus*. Most of the strains isolated from patients with cryptoccal infections are *MAT*α. Surprisingly, only this α mating type forms filamentous structures in response to nitrogen starvation (haploid fruiting; Fig. 5)[47]. Haploid hyphal development requires a gene(s) tightly linked to or within the *MAT*α locus. An α-specific Ste12p homologue (*STE12*α) has been identified by the ability of its gene to complement the filamentation defect of a *MAT****a*** strain[48]. Remarkably, the DNA segment that encodes *STE12*α is completely missing in *MAT****a*** strains[48]. Gene-knockout analysis demonstrates that *STE12*α is indeed required for haploid fruiting (J. Kwon-Chung, pers. commun.).

Although it is the nonfilamentous yeast cells that are found in individuals infected with *Cryptococcus*, these data suggest an intimate connection between the filamentation signalling pathway and virulence in this species. Moreover, the common requirement for Ste12p family members between such distantly related fungi (*Saccharomyces* is an ascomycete whereas *Cryptococcus* a basidiomycete) suggests a striking degree of conservation in the regulatory pathways that control filamentous growth.

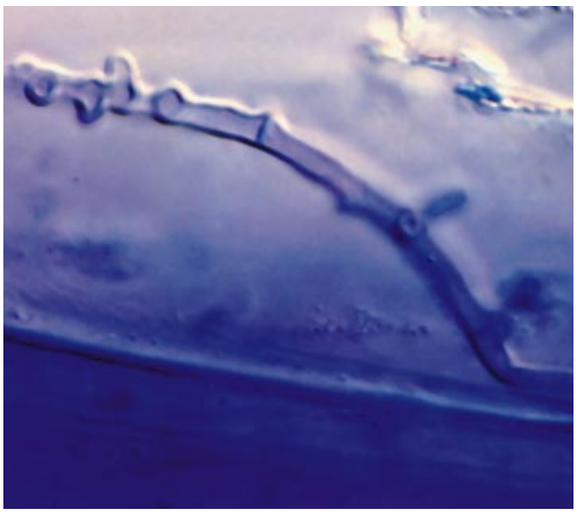

**FIGURE 3**

Infection by *Ustilago maydis*. Invasive hypha growing in a cell of the corn plant. (Photo courtesy of Flora Banuett.)

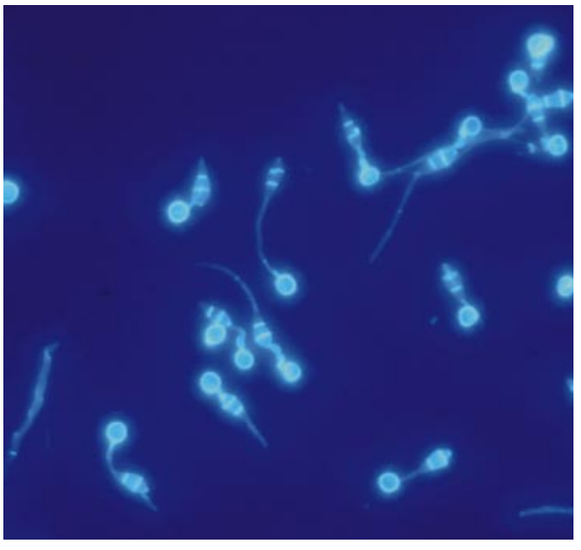

**FIGURE 4**

Appressoria of *Magnaporthe grisea*. Fluorescence micrograph of a field of ellipsoidal *Magnaporthe* cells that have extended appressoria (the thin projections that terminate in a circular foot-like structure). The cells are stained with the fluorescent chitin-binding dye calcofluor white. (Photo courtesy of John Hamer.)

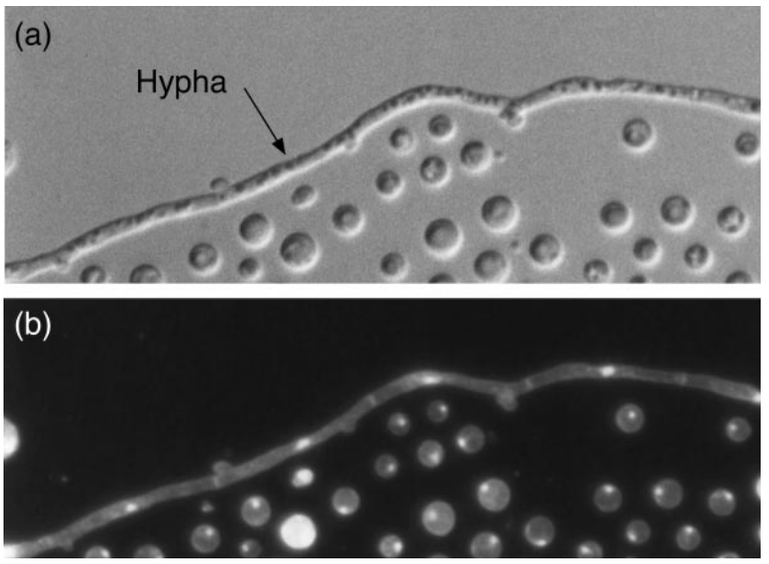


**FIGURE 5**

Hypha of haploid *Cryptococcus neoformans* strain growing across a field of round yeast-form cells. (a) Bright field view. (b) Fluorescence micrograph of same field in which DNA is stained with DAPI and the cell walls are stained with calcofluor white. (Photo courtesy of Brian Wickes.)

## Signalling dimorphic development: the big picture

This comparison of fungal developmental signalling mechanisms illustrates some common themes that underly the change in cell type.

- First, the signalling systems that control dimorphism are conserved between distantly related fungi. This conservation of pathways among diverse species raises a central question of modern biology: how is diversity generated if species contain orthologous genes specifying the same pathways? Presumably, these pathways are expressed in the context of underlying differences that modulate the outcomes. The different morphologies and behaviours characteristic of each species could be generated by subtle, quantitative differences in the inputs and outputs of each pathway. Alternatively, developmental diversity could be produced through the coupling of the signalling pathways to qualitatively different cell-autonomous and environmental inputs and their linkage to distinct target effectors. Fungal dimorphism offers a unique opportunity to test these possibilities.
- Second, dimorphism in fungi is controlled by multiple signalling pathways. At least three parallel pathways control the switch to filamentous growth in *Saccharomyces*. Multiplex signalling also occurs in other fungi. How cells integrate the information from different pathways to effect a change in cell type is not known. The answer will require a complete definition of the downstream effectors – transcription factors and their target genes.
- Third, signalling pathways that regulate mating and filamentous development are intimately connected because they utilize common components. This connection is complete in the *Ustilago* dikaryon where the entire pheromone signalling pathway from ligand to receptor to signalling cascade drives filamentous growth in dikaryons and mating in haploids. It is partial in *Saccharomyces*, where a subset of the mating MAP kinase signalling components is used to drive filamentation. Hints of this link are present in *Cryptococcus* where haploid fruiting is controlled by the mating-type locus-associated *STE12α* gene. Finally, in *Magnaporthe*, mating signalling antagonizes formation of the infection structure. Given that mating and filamentous growth both involve the production of cell projections, these associations might reflect the evolutionary origins of the signalling pathways in a common morphogenetic process.
- Finally, signalling pathways that control filamentous differentiation are required for pathogenesis. The simplest interpretation of this observation is that dimorphic development is required for virulence. In *M. grisea*, MAPK and cAMP signalling promote the formation of a highly specialized infection structure that is essential for invasion into the host. However, in other systems, the connection is less certain because it is possible that the signalling pathways control more than filamentous growth. The molecular definition of the targets of these signalling pathways should bring this issue into sharper focus.

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# Vertebrate photoreceptor cell development and disease

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*Photoreceptors provide an excellent model for studies of vertebrate neuronal differentiation, and many human diseases resulting in blindness primarily affect photoreceptors. There is therefore great interest in studying the cellular and molecular mechanisms of photoreceptor development. This article discusses our current understanding of this process, including the recent discovery of the homeodomain transcription factor Crx and its potential role in diseases affecting human vision.*

The neural retina is an exquisitely sensitive light detector, utilizing photoreceptor cells to carry out phototransduction – a series of signal amplifications that enable detection of a single photon of light. In vertebrates, there are two classes of photoreceptors: rods and cones. Cones function in bright-light (daylight) amplitudes and are responsible for colour vision. Rods are sensors of dim light and do not discern colour. Human vision relies heavily upon cones, of which there are three types – blue, green and red – and uses only one variety of rod photoreceptor. The human retina also contains a centrally localized, cone-rich region called the fovea, found in the centre of a yellowish spot within the retina named the macula. The fovea is the area of highest visual acuity in the retina. By contrast, mice and rats rely almost entirely on rod-mediated vision. Approximately 75% of all cells in the mouse retina are photoreceptors, and 97% of these are rods. The mouse has two varieties of cones – with sensitivities to middle (M) and short (S) wavelengths – but does not have a fovea or macula (see Refs 1 and 2 for review).

The phototransduction cascade is initiated by the capture of light by 11-*cis*-retinal, a chromophore bound by the opsin proteins – rhodopsin in rod photoreceptors and cone opsins in cone photoreceptors. The proteins that carry out phototransduction are located in an elaborate and highly specialized membranous structure, the outer segment. The biochemistry, ultrastructure and cellular physiology of photoreceptors have been studied in detail[1]. The photoreceptor outer segment appears to be relatively fragile, degenerating in response to many environmental and/or genetic perturbations, resulting in blindness[3,4].

## Human photoreceptor disease

The genetic diseases of photoreceptors can be broadly classified into the inherited macular dystrophies, cone–rod dystrophies (CRDs) and rod–cone dystrophies (commonly termed retinitis pigmentosa, RP). The CRDs are characterized by loss of cone-mediated vision in the first decade of life or later, with subsequent loss of rod-mediated vision[5]. Conversely, RP is notable for initial loss of rod function, followed by loss of cone-mediated vision[4]. The majority of known RP genes, as well as the genes associated with CRD, encode proteins of the photoreceptor outer segment (see the RetNet Web site: `http://utsph.sph.uth.tmc.edu/www/utsph/RetNet/home.htm`). Many of these proteins are required for phototransduction or outer segment structure. The processes whereby mutations in rod-specific genes eventually cause cone disease in RP remain obscure.

Understanding of the causes of eye disease with polygenic or multifactorial bases, such as age-related macular degeneration (AMD), might result from studies of monogenic ailments. For example, a digenic form of RP occurs in patients who are doubly heterozygous for mutations in the unlinked photoreceptor-specific genes encoding ROM1 and

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