Sensing the environment: lessons from fungi

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Abstract | All living organisms use numerous signal-transduction systems to sense and respond to their environments and thereby survive and proliferate in a range of biological niches. Molecular dissection of these signalling networks has increased our understanding of these communication processes and provides a platform for therapeutic intervention when these pathways malfunction in disease states, including infection. Owing to the expanding availability of sequenced genomes, a wealth of genetic and molecular tools and the conservation of signalling networks, members of the fungal kingdom serve as excellent model systems for more complex, multicellular organisms. Here, we review recent progress in our understanding of how fungal-signalling circuits operate at the molecular level to sense and respond to a plethora of environmental cues.

Mating type
A strain or clone or other isolate made up of organisms (such as certain fungi or protozoans) that are usually incapable of sexual reproduction with one another but capable of such reproduction with members of other strains of the same organism.

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Sensing the environment and ensuring appropriate cellular responses are crucial challenges confronted by all living organisms. These processes occur sequentially through reception, transduction and response pathways — failure at any step of this sequence elicits abnormal cellular states that could result in disease. Understanding this process at a molecular level has been the subject of intensive investigation to gain insights into cell growth, proliferation, differentiation and death. Fungi, which comprise one of the three main eukaryotic kingdoms, are excellent models to study environmental sensing because of their simple, but evolutionarily conserved, signal-transduction pathways that are often equivalent to those present in multicellular eukaryotic organisms. Studies of these pathways have also facilitated the development of therapies that tackle infectious diseases caused by fungal pathogens of medical and economic importance. In this review, we discuss recent advances in understanding molecular mechanisms that allow fungi to sense and adapt to their environment in response to diverse cues, including nutrients, light, gases, stress and host factors (BOX 1).

Extracellular sensing: pheromones and nutrients

Microorganisms have evolved elaborate mechanisms to sense and adapt to the environment in response to pheromone and nutrient signals. G-protein-coupled receptors (GPCRs) are the largest family of transmembrane receptors that have an important sensor function. Despite showing striking sequence and functional diversity, members of the GPCR family have a common fundamental architecture that includes a structure that incorporates seven transmembrane domains and a shared signal-transduction mechanism. GPCRs are crucial conduits for pheromone and nutrient sensing in many fungi[1–6] (FIG. 1).

Pheromones. Fungal mating is initiated by pheromone binding to a GPCR located on the cell surface. There are several types of fungal pheromone: ascomycetes express a peptide pheromone that is secreted and processed by the classic secretory pathway, whereas ascomycetes and basidiomycetes produce a second type of pheromone that undergoes N-terminal proteolysis and C-terminal farnesylation, proteolytic cleavage and carboxymethylation. This generates a mature lipophilic pheromone that is secreted through a Ste6–multi-drug resistance (MDR) related pump[7]. The involvement of GPCRs in pheromone sensing has been well studied. In some ascomycetes, such as *Saccharomyces cerevisiae*, two types of pheromone are secreted and sensed by cells of opposite mating-type through two GPCRs, Ste2 and Ste3 (REF. 8). Pheromone binding to the pheromone receptor initiates a signal-transduction pathway that begins with GDP–GTP exchange on the Gα protein Gpa1. This protein dissociates from the βγ dimer — consisting of Ste4 and Ste18 — and activates a mitogen-activated protein

Box 1 | Sensory properties of the fungi

Fungi possess almost all the senses used by humans. They can sense light, gases, chemicals and surfaces. In addition, fungi can also sense gravity and electric fields, and one fungal species (*Phycomyces blakesleeanus*) can sense adjacent objects. Light and temperature can be used to entrain and reset the fungal circadian clock. Fungal cells sense each other through secreted pheromones during mating or through small molecules produced at high cell densities for quorum sensing. As outlined in this review, the most studied sensory areas are photosensory and chemical-signalling processes. Stretch-activated receptors and Ca^{2+} channels are implicated in mechanisms for galvanotropism, gravitropism and thigmotropism. Genome-sequencing projects promise to provide valuable tools to further uncover how fungi respond to their environment and to elucidate the underlying molecular mechanisms.

kinase (MAPK) cascade (FIG. 1). This activation results in cell-cycle arrest and fusion between cells of opposite mating type. The *S. cerevisiae* pheromone receptors are well conserved in other ascomycetes, including *Schizosaccharomyces pombe*[9], *Aspergillus nidulans*[10], and *Neurospora crassa*[11].

In basidiomycetes, Ste3 pheromone-receptor homologues have also been reported, such as Pra-1 in *Ustilago maydis*[12]. In *Cryptococcus neoformans*, two pheromone receptors (Ste3α/Cprα and Ste3a/Cpra) are encoded by the mating-type locus and both are important for the sexual cycle in this organism[13].

Pheromone sensing is more complex in some homobasidiomycete mushrooms, such as *Coprinus cinereus* and *Schizophyllum commune*, which exhibit thousands of mating types. These mushrooms are unique among fungi with respect to pheromone sensing in that a given pheromone can activate several different receptors and a receptor can be activated by many different pheromones[7]. Interestingly, pheromone sensing is not required for cell–cell fusion and is only involved in the later steps of the mating process that involve nuclear migration and clamp cell fusion[14,15].

Glucose. Because glucose is the main carbon energy source, all organisms have evolved sophisticated sensing mechanisms to detect this molecule. In animals, a group of GPCRs on the tongue mediate the sensation of sweetness[16]. Fungi, especially yeasts, have developed several strategies to sense and transport glucose. In *S. cerevisiae*, several glucose-sensing systems have been reported in the literature. These include a sugar receptor (Gpr1) that senses glucose and sucrose to stimulate Gpa2, which in turn activates adenylyl cyclase (AC) resulting in increased amounts of cyclic AMP (cAMP) and activation of protein kinase A (PKA)[3,4,17]. Gpr1 homologues have been identified in other yeasts, including Gpr1 in *Candida albicans*[5] and Git3 in *S. pombe*[18]. Many filamentous fungi, such as *A. nidulans* and *N. crassa*, also have Gpr1 homologues, but possible roles for these molecules in glucose sensing remain to be explored.

Besides GPCRs, *S. cerevisiae* also expresses a family of hexose transporters (Hxts) that are involved in sugar sensing or transport. The *HXT* family comprises a subgroup of the sugar transporter-related genes, including *HXT1–17*, *SNF3* and *RGT2* (REF. 19). All contain 12 putative transmembrane domains. Characterized hexose transporters display differing affinities and expression patterns that are dependent on the sugar available. The existence of multiple high- and low-affinity hexose transporters enables cells to adjust glucose uptake in response to prevailing environmental conditions to optimize cell growth and metabolism. Two members of this family, Snf3 and Rgt2, resemble transporters but function as sensors of extracellular glucose to regulate the expression of *HXT* genes through the Hxt suppressor Rgt1. The term 'transceptor' has been coined to distinguish these sensors from other common transporters. This Snf3/Rgt2–Rgt1 pathway is conserved in other fungi: homologues of Snf3 and Rgt2 have been identified in *C. albicans*[20], *N. crassa*[21], *Kluveromyces lactis*[19] and *C. neoformans* (C.X. and J.H., unpublished observations).

Amino acids. Amino acids are detected by specialized sensor systems. A sophisticated general amino-acid sensor system has been identified in both *S. cerevisiae* and *C. albicans*. In *S. cerevisiae*, Ssy1–Ptr3–Ssy5 (SPS) initiates signal transduction following the sensing of extracellular, and possibly intracellular, amino acids[19,22–24]. Ssy1 is a membrane protein that resembles an amino-acid permease but functions as an amino-acid sensor. Ptr3 and Ssy5 are predicted to interact physically with Ssy1 to form a dynamic complex[19]. Binding of amino acids to Ssy1 activates the Ssy5 protease, which, in turn, proteolytically activates the latent transcription factors Stp1 and Stp2 to induce the expression of genes that encode amino-acid-metabolizing enzymes and amino-acid permeases[25]. The SPS sensor system is conserved in other fungi, and homologues of Ssy1, Ptr3 and Ssy5 that are expressed by *C. albicans* function in a pathway that senses amino acids and is required for virulence[26,27]. The general amino-acid permease Gap1 is also involved in amino-acid sensing and activates the PKA pathway in a process that is dependent on the Sch9 kinase[28].

GPCRs are also reported to sense amino acids in other fungi. In *C. albicans*, Gpr1 senses methionine to initiate a process that controls filamentation[5]. Similarly, Gpr4 senses methionine and activates cAMP signalling in *C. neoformans*[29].

Ammonium ions. Nutrient deprivation in fungi can trigger a phenotypic switch from the yeast form to filamentous growth to enable mating, search for nutrients and virulence. One aspect of this process is the perception of nitrogen availability by cell-surface ammonium permeases. All fungi studied contain at least two ammonium-ion permeases, which belong to the conserved AmtB/Mep/Rh (Rhesus) family of proteins. Within certain fungal species, one of these transporters has evolved a sensing function. Examples include Mep2 (*S. cerevisiae* and *C. albicans*) and Ump2 (*U. maydis*)[30–32]. The mechanism of ammonium-ion sensing is yet to be

Clamp cell
A bridge-like hyphal connection involved in maintaining the dikaryotic state that forms when cells in dikaryotic hyphae divide.

Protein kinase A
(PKA). A secondary messenger-dependent enzyme that has been implicated in a wide range of cellular processes, including transcription, metabolism, cell-cycle progression and apoptosis.

Rhesus proteins
Mammalian homologues of the Amt/Mep family of proteins that are expressed in many tissues and form part of the rhesus (Rh) blood-group complex.

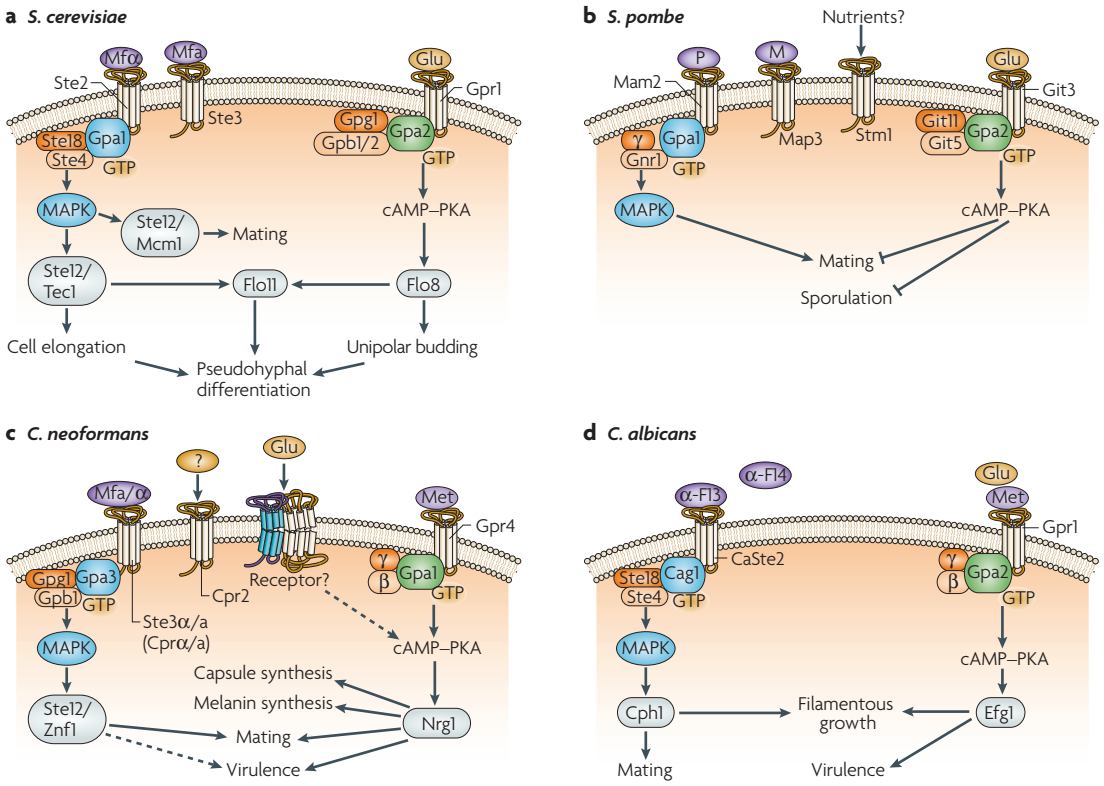


Figure 1 | **The involvement of G protein-coupled receptors in sensing extracellular signals in fungi.** **a** | In *Saccharomyces cerevisiae*, the pheromone receptors Ste2 and Ste3 sense α-pheromone (Mfα) and a-pheromone (Mfa), respectively. The binding of pheromone to a pheromone receptor stimulates the exchange of GDP for GTP on the Gα protein Gpa1, which, in turn, enables dissociation of the Ste4 and Ste18 dimer to activate the downstream mitogen-activated protein kinase (MAPK) cascade and trigger mating and pseudohyphal growth. In response to extracellular glucose (Glu), the sugar sensor Gpr1 stimulates GDP–GTP exchange on the G protein Gpa2, which activates the cyclic AMP–protein kinase A (cAMP–PKA) signal pathway and induces pseudohyphal growth. These two pathways integrate through Flo11 to regulate pseudohyphal differentiation. **b** | In *Schizosaccharomyces pombe*, the pheromone receptor Mam2 senses P-factor, whereas Map3 senses M-factor, to activate the MAPK cascade and trigger mating. The glucose sensor Git3 and the nutrient sensor Stm1 respond to extracellular glucose and nutrient signals to activate the cAMP–PKA pathway and suppress mating and sporulation. **c** | In *Cryptococcus neoformans*, the pheromone receptor Ste3α (in α cells) senses a-pheromone and Ste3a (in a cells) senses α-pheromone to activate the downstream MAPK cascade and trigger mating. A recent study also indicates that pheromone sensing could impact fungal virulence. A pheromone receptor-like GPCR protein, Cpr2, might also have a role in mating or fruiting. A GPCR protein, Gpr4, functions as an amino-acid sensor and stimulates capsule formation and mating filament production through the cAMP-PKA-signalling pathway. Glucose is important for virulence-factor production but the molecular basis of how glucose is sensed is still unclear. **d** | In *Candida albicans*, only the α pheromone factors, α-F13 and α-F14, and one pheromone receptor, CaSte2, have been identified in the pheromone-sensing system. Met, methionine.

established; however, it does not seem to relate to a change in the internal nitrogen status of the cell but rather to the presence and activity of the transporter[30]. This indicates a mechanism of sensing that is analogous to GPCRs whereby the transporter regulates a signal-transduction pathway. The cAMP–PKA and MAPK pathways are proposed as potential components of this signalling pathway; however, so far, there is no evidence that these pathways interact directly with the ammonium-ion sensors[30,32] (FIG. 2).

Signalling is hypothesized to occur either in the absence of external ammonium ions or during the transport process when ammonium levels are low (as the fungal Mep proteins are only expressed under these conditions). In one model, the sensor is activated when transport occurs, thereby signalling the availability of sufficient levels of nitrogen to support a dimorphic switch. The finding that the mutation of a residue that is crucial for ammonium-ion transport in Mep2 prevents pseudohyphal growth of *S. cerevisiae*[33] supports transport-linked ammonium ion perception. Additionally, transport through Mep2 is required for the activation of the PKA pathway following addition of ammonium ions to nitrogen-starved cells[34]. Mutational analysis indicates that this pathway is distinct from the Mep2-dependent pseudohyphal pathway; however, these findings establish a precedent for transport-linked sensing of ammonium ions. One possible model is that a conformational change within the Mep2 protein during transport influences its interaction with a

Autophagy
A degradative pathway elicited by nutrient starvation by which indiscriminate portions of the cytoplasm, including organelles, are engulfed into autophagosomal vesicles for fusion with the vacuole and degradation.

signalling component. Interestingly, structural studies and molecular dynamic simulations of ammonium-ion transport mediated by bacterial Amt proteins predict the dynamic movement of residues that are involved in both ammonium-ion binding and those that gate the ammonium-ion-translocating channel[35,36]. The potential for movement of the fifth helix of the protein during ammonium-ion transport has also been noted[37].

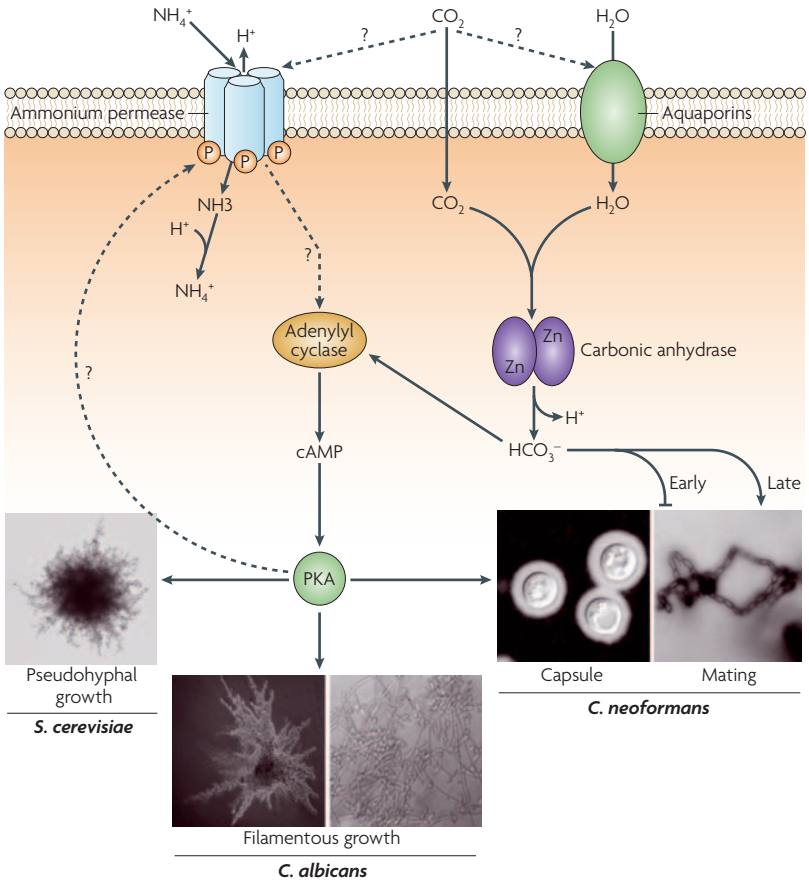


Figure 2 | **Ammonia and CO_2 sensing and metabolic pathways.** Fungal ammonium-ion permeases are homotrimeric proteins that are predicted to allow the passive diffusion of ammonia. Transport through the permease might be required to initiate signalling through the cytoplasmic domain of each monomer. The cyclic AMP (cAMP) pathway and mitogen-activated protein kinase (MAPK) pathways (not shown) are potential components of the ammonia-sensing pathway. Interestingly, a protein kinase A (PKA) consensus phosphorylation site (marked as P) is present in the ammonium-ion permeases Mep2 and Ump2 and is required for filamentous growth in *Saccharomyces cerevisiae* and *Ustilago maydis*, respectively. Extracellular CO_2 is probably transported into the cell primarily by simple diffusion owing to its non-polar characteristics. Whether CO_2 transport is facilitated by transporters, such as aquaporin or the Rhesus-like Amt/Mep proteins that function in other non-fungal systems, is not known. Once inside the cell, CO_2 is reversibly converted to bicarbonate (HCO_3^-), which is a major substrate for many carboxylating enzymes for biosynthesis of essential biomolecules. Increased amounts of HCO_3^- generated from high CO_2 concentrations activate adenylyl cyclase to produce cAMP, which, in turn, activates PKA to induce downstream targets. High amounts of CO_2/HCO_3^- enhance capsule synthesis and modulate morphological differentiation that is dependent on the mating stage (early or late) of *Cryptococcus neoformans*. These conditions also induce bud-to-hypha transitions in *Candida albicans*. Although the cAMP pathway is involved in pseudohyphal growth in *S. cerevisiae* in response to nitrogen starvation, high CO_2 alone does not promote the pathway and HCO_3^--dependent activation of adenylyl cyclase has not been reported in this yeast.

It remains to be established if conformational movements by the transporter are linked to a sensing function.

An alternative model, in which Mep2 activates signalling in the absence of ammonium-ion transport, is supported by work with *C. albicans* showing that Mep2-dependent filamentous growth occurs when cells are grown on nitrogen sources other than ammonium ions[32]. Under these conditions, the assumption is that there is little or no ammonium available to be transported and that Mep2 is in its active form. In this model, repression of signalling occurs during ammonium-ion transport by a mechanism that, again, could be linked to conformational changes in the protein. Alternatively, ammonium-ion transport could simply result in the removal of the sensor from the membrane, therefore preventing its ability to engage the relevant signalling pathway. Of interest is the extent to which other aspects of ammonium-ion metabolism influence transporter-mediated signalling transduction. *S. cerevisiae* excretes ammonium ions when grown on certain amino acids and to facilitate colony signalling[38,39]. The role of these sources of external ions in dimorphic growth has not been established.

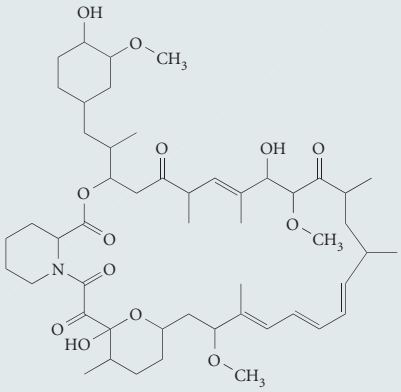
Phosphate. Membrane-transport systems that are active in cellular inorganic phosphate (P_i) acquisition have a key role in maintaining cellular P_i homeostasis. In *S. cerevisiae*, the cellular-phosphate response involves the PHO pathway, a genetic regulatory circuit that allows coordinated cellular responses and adaptation to changes in the availability of external free phosphate[40]. Recent studies indicate that phosphate sensing occurs through the phosphate-transporter protein Pho84 to initiate the PHO pathway when cells are shifted from a high P_i-containing medium to a low P_i medium. In the presence of glucose and severe phosphate limitation, the proteins Pho84 and Pho87 function as phosphate sensors to sustain rapid phosphate signalling through the activation of the PKA pathway[28,40]. It is as yet unclear how these sensing signals are integrated and coordinated by the cell.

Intracellular nutrient signalling

In *S. cerevisiae*, the cAMP–PKA and TOR pathways respond to carbon- and nitrogen-source signals to regulate a myriad of functions including protein synthesis, ribosome biogenesis, autophagy, polarized cellular growth, cell-cycle progression and filamentation.

The Tor kinases were first identified in yeast as the targets of the antiproliferative drug rapamycin[41] and are present in all eukaryotic cells (BOX 2). The yeasts *S. cerevisiae* and *S. pombe* contain two Tor homologues, Tor1 and Tor2, whereas many other organisms studied, including mammals, contain only one Tor. The Tor proteins occur as multiprotein complexes, known as TORC1 and TORC2, in *S. cerevisiae*[42,43]. The TORC1 complex is sensitive to rapamycin and was believed to mediate all of the functions attributable to Tor except polarization of the actin cytoskeleton, which is rapamycin insensitive and was proposed to be mediated by the TORC2 complex. However, two independent

Box 2 | **Rapamycin: an antifungal, immunosuppressive and anticancer agent**



Rapamycin is a hydrophobic macrolide (see figure) produced as a secondary metabolite of the soil bacterium *Streptomyces hygroscopicus*. Rapamycin was initially discovered as an antifungal agent against *Candida albicans* and was later found to have potent immunosuppressive and antiproliferative activities. The Tor kinases were first identified in genetic screens searching for rapamycin-resistant mutants in *Saccharomyces cerevisiae*. These studies indicated a toxic mechanism of action in which rapamycin forms a stable complex with the FKBP12 prolyl isomerase and, in turn, the FKBP12–rapamycin complex binds and blocks the function of the Tor kinases. Subsequent studies in yeast using rapamycin as a molecular probe have provided insights into the structure and function of the Tor kinases, as well as the biological role of the Tor-signalling programme in transmitting nutrient signals that govern cell growth in all eukaryotic organisms ranging from yeast to mammals[166,167]. Rapamycin is an approved immunosuppressant for organ-transplant recipients and is also used to prevent restenosis following cardiac surgery. Currently, rapamycin is being tested in clinical trials for the treatment of an array of human cancers.

reports have also implicated TORC1 in controlling actin polarization[44,45].

Although it is well established in yeast that the cAMP–PKA pathway is activated by glucose, several lines of evidence support the model that the Tor-signalling cascade responds to amino-acid signals. In mammalian systems, the TOR pathway is primarily associated with the regulation of translation in response to amino-acid availability. The tumour suppressors Tsc1 and Tsc2 function through a GTP-binding protein (Rheb) to regulate Tor activity in mammals, and homologues of Tsc1 and Tsc2 control amino-acid uptake in *S. pombe*[46,47]. Furthermore, a component of the TORC1 complex, Lst8, regulates amino-acid biosynthesis[48]. In *S. cerevisiae*, the function of the Rheb homologue, Rhb1, does not seem to be linked to Tor signalling, and Tsc homologues are absent. Recently, it has been proposed that Tor responds to amino acids and, in concert with the EGO complex, regulates microautophagy to control membrane fluxes during exit from G0 phase[49]. Interestingly, the EGO complex, renamed as the Gse complex, was also shown to be required for sorting of the amino-acid permease Gap1 from the late endosome to the plasma membrane, presumably in response to amino-acid signals[50]. However, despite this compelling evidence, both the identity of the amino acid(s) sensed as well as the sensing mechanism to which Tor responds remain largely unknown. An attractive possibility is that the Ego/Gse complex is part of the intracellular amino-acid-sensing mechanism that activates Tor.

Genome-wide expression studies with rapamycin have enabled elucidation of the transcriptional programmes that are regulated by Tor[51–54]. In response to nutrients, Tor signalling allows the expression of genes that are required for ribosome biogenesis, including those encoding ribosomal proteins, ribosomal RNA (rRNA) and tRNA. Nutrient limitation or inhibition of Tor by rapamycin represses the ribosome-biogenesis genes and induces the expression of genes required for assimilation of alternative nitrogen and carbon sources as well as stress-response genes. The regulation of these genes by Tor involves the control of nuclear entry of the respective transcription factors by phosphorylation and dephosphorylation events[55–57]. In the case of nitrogen-regulated genes, this action is mediated by the protein phosphatase-2A (PP2A)-like phosphatase Sit4; for other genes, the precise regulatory mechanism is still unclear. Regulation of the ribosomal-protein genes by Tor is Sit4-independent; however, it is a more complex process as the main transcription factors, Rap1, Abf1, and Fhl1, are constitutively bound to the ribosomal gene promoters, and Tor activity modulates the ability of these factors to recruit co-activators (such as Sfp1, Ifh1 and Esa1) and co-repressors (such as Crf1)[58–64]. A recent study found that TORC1 binds directly to the 35S rRNA promoter and regulates Pol-I-dependent transcription of these genes[65]. Although the targets of TORC1 for this function are still unknown, this finding reveals a similarity between Tor signalling and other kinases that, when activated, translocate into the nucleus to phosphorylate specific targets.

Many of the non-transcriptional effects of Tor are also mediated through the phosphatase activity of Sit4, including the initiation of translation and enhanced stability of amino-acid permeases. In response to abundant nutrients Tor promotes the association of Sit4 with its regulatory subunit, Tap42. The inactivation of Tor by rapamycin or nutrient limitation dissociates the Sit4–Tap42 complex resulting in the activation of Sit4 (REF. 66). Active signalling by Tor promotes translation initiation through a dual mechanism involving phosphorylation of the Gcn2 kinase and Sit4-mediated dephosphorylation of the elongation-initiation factor eIF2α[67,68]. Regulation of Gcn2-mediated translation by cAMP–PKA is triggered by glucose stimulation or ultraviolet (UV) irradiation; however, although the mechanism involved requires Gcn2, it is independent of eIF2α phosphorylation[69].

Under conditions of limited nitrogen, *S. cerevisiae* diploid cells undergo filamentous growth. This developmental switch is complex and orchestrated by a well-defined MAPK cascade, cAMP–PKA and Tor signalling[70–72]. Sublethal concentrations of rapamycin block filamentation in *S. cerevisiae*, *C. albicans*, and *C. neoformans*. In *S. cerevisiae*, this Tor function occurs by a mechanism that requires the Sit4 phosphatase and is independent of, and parallel to, the MAP kinase and cAMP–PKA pathways.

The TORC2 complex regulates the cell-integrity pathway and polarization of the actin cytoskeleton through Rom2, the guanine nucleotide-exchange factor that activates the Rho1 GTPase; however, the mechanism for this activity is largely unknown[73]. Nutrient starvation or Tor inactivation by rapamycin triggers growth arrest and autophagy[74], and Tor activity prevents formation of the Apg13–Apg1 complex that is essential for the autophagy process. Similarly, hyperactivation of the Ras–cAMP pathway prevents formation of early membrane structures that precede and are required for autophagosome formation[75].

Restenosis
The process whereby intimal hyperplasia occurs to re-occlude a coronary artery and limit cardiac blood flow following cardiac stenting. A common complication that is markedly reduced by using stents impregnated with rapamycin.

EGO complex
A vacuolar membrane-associated multiprotein complex that consists of Ego1, Ego3, Gtr1 and Gtr2. Proposed to function in concert with TORC1 to promote microautophagy in response to amino-acid signals.

Microautophagy
The uptake of cytoplasm at the lysosomal or vacuolar surface. It is thought that this process functions to recycle the vacuolar membrane.

Although it is possible that signalling in response to nutrients involves crosstalk between the cAMP–PKA and Tor cascades, at present the available evidence is more consistent with a model in which these pathways function in parallel and converge on downstream molecular regulators[76–78]. Future studies are needed to establish the precise molecular mechanisms by which nutrients are sensed, elucidate how these signals activate the nutrient-sensing programmes, and identify the substrates of the Tor and cAMP–PKA-signalling pathways.

Sensing gases

Fungi sense and respond to gases including CO_2 and ammonia (NH_3). In addition to its role in photosynthesis and cellular respiration, CO_2 is an important signalling molecule that is sensed by bacteria, insects and mammals. For pathogenic fungi in which the infected host is their natural habitat, adaptation to marked changes in CO_2 levels (from 0.036% to 5–6%) is required for survival and virulence. High CO_2 induces the morphogenic transition from bud to hypha in *C. albicans* and increases production of the antiphagocytic capsule in *C. neoformans*[79–81]. Cellular signalling and metabolic cascades that mediate CO_2/HCO_3^- homeostasis in pathogenic fungi have been recently elucidated (FIG. 2). Carbonic anhydrase (CA) has an essential role in CO_2/HCO_3^- homeostasis in both *C. albicans* and *C. neoformans*[82–84]. Fungal cells that lack the gene that encodes CA show severe growth defects due to a lack of bicarbonate that is essential for carboxylating enzymes catalysing fatty acids and amino-acid biosynthesis. These defects can be rescued either by air enriched with high CO_2 (5%) or by infection of the host[82–84].

AC has emerged as a central CO_2-signalling mediator[82,84]. *C. albicans* filamentation and *C. neoformans* capsule production are induced by high CO_2 in a process that is controlled by AC/cAMP signalling[85–88]. In *C. albicans*, AC mutants, but not *ras1* mutants (Ras1 is a GTP-binding protein activator of AC), cannot induce true hyphae in response to high CO_2 (REF. 82). Bicarbonate directly activates adenylyl cyclase *in vitro*[82], but it is unclear if CO_2 also directly activates the cAMP pathway. In *C. neoformans*, the AC Cac1 is directly activated by bicarbonate[84]. It therefore seems that pathogenic fungi encode a single AC that responds to both bicarbonate and G-proteins. This is in contrast to mammals in which two distinct ACs are expressed: a transmembrane AC form activated by heterotrimeric G-proteins and a soluble AC form activated by bicarbonate[89]. Interestingly, bicarbonate-dependent stimulation of soluble AC is required for human spermatozoa activation[89].

Other potential pathways influenced by high CO_2 are the mating pheromone and Hog1 MAPK cascades. In fungi, Hog1 is the most extensively studied stress-activated MAPK, and is homologous to mammalian p38 MAPK (see below). High amounts of CO_2 inhibit sexual reproduction of *C. neoformans* by repressing pheromone production[83]. This CO_2-dependent mating inhibition is due to increased amounts of intracellular bicarbonate because a *C. neoformans* CA mutant (*can2*Δ) produces normal amounts of pheromone. Interestingly, *hog1* mutations partially bypass the high CO_2-dependent mating inhibition, indicating that the Hog1 MAPK could be involved in CO_2 sensing[83]. Questions that require future studies include the mechanisms underpinning how CO_2 is sensed and transported into cells; whether CA expression or activity is modulated during host infection; and whether CA physically interacts with AC to govern the cAMP pathway.

NH_3 is known as a signalling mediator for communication between neighbouring colonies, and is found in various fungi[39]. The alkaline ammonia gas is produced in pulses by *S. cerevisiae* and influences the spatial distribution of adjacent colonies[39]. This process is distinct from that of low ammonia-induced pseudohyphal growth and requires the presence of extracellular amino acids[90]. NH_3 production is accompanied by a change in global gene expression that favours the altered metabolic needs of colonies that are deficient in nutrients and that are stressed[91]. This transcriptional profile is dependent on the transcription factor Sok2, and has been implicated in the provision of nutrients from the regulated death of cells within the centre of the colony, indicating important roles in the long-term development and differentiation of multicellular yeast colonies[92,93].

Sensing light

Light can be both a positive and a negative stimulus for fungi in that it provides a mechanism to sense the environment but also has detrimental effects, particularly at UV wavelengths. Over the past 150 years at least 100 species representing all phyla of fungi have been reported to react to light[94]. A few photosensory proteins have been identified; however, there is still much to elucidate about the downstream cellular events that are triggered by light.

Opsins are membrane-bound proteins that have seven transmembrane domains and are associated with a retinal chromophore that is attached to a conserved lysine residue within the seventh transmembrane domain. These proteins serve as the photosensors in the eyes of animals and as photosensors and light-dependent ion (H^+ or Cl^-) pumps in archaea. The opsins of archaea, bacteria and fungi are structurally related to animal opsins, but have low amino-acid similarity. The evolution of these genes has attracted attention, with support for a model of convergent evolution of the microbial and animal opsin proteins. Opsins are present throughout microbial genomes, and there is strong evidence that this distribution is due to horizontal gene-transfer events, including one before the diversification of the ascomycetes and basidiomycetes[95]. The first fungal opsin characterized was NOP-1 of *N. crassa*[96,97]. Purified NOP-1 protein shows green light-absorbance properties and no evidence of H^+ pumping. An opsin from the plant pathogen *Leptosphaeria maculans* has similar photochemistry to *N. crassa* NOP-1, but additionally is capable of light-dependent H^+ pumping[98,99]. In chytridiomycetes, there is evidence that opsins function in phototaxis; however, so far, the genes involved in this process have not been identified[100,101].

Photosensory protein
A protein with absorbance properties that overlap the wavelength spectrum to which the organism responds. Mutation of its encoding gene should disable this sensing ability.

Chromophore
The light-absorbing chemical associated with a photoreceptor protein.

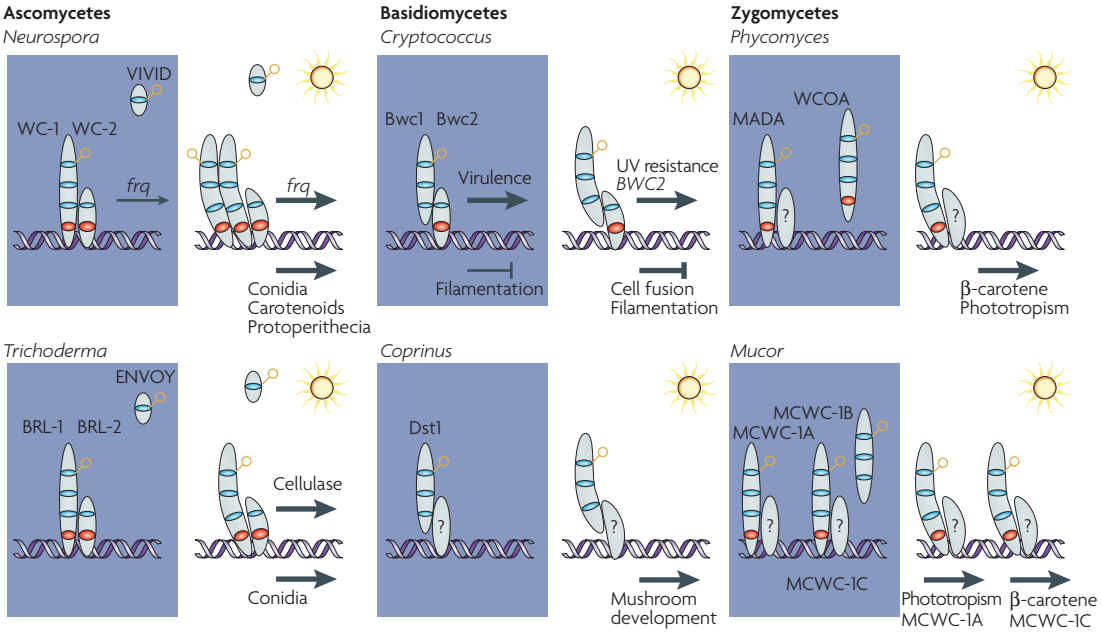


Figure 3 | **Blue-UV light sensing through the white collar-1 protein family.** Ascomycetes (*Neurospora crassa*, *Trichoderma atroviride* and *Hypocrea jecorina* (previously known as *Trichoderma reesei*)), basidiomycetes (*Cryptococcus neoformans* and *Coprinus cinereus*), and zygomycetes (*Phycomyces blakesleeanus* and *Mucor circinelloides*) all sense blue-UV light through the activity of white collar-1 (WC-1) homologues. The interacting WC-2 proteins have been identified in some species and are predicted to function in others. The zygomycetes have several copies of WC-1, including WCOA and MCWC-1B that have an unknown function. The downstream effects of light exposure mediated by these proteins are divergent in different organisms. The blue oval indicates a Per–Arnt–Sim (PAS) or a LOV domain; the red oval indicates a zinc-finger DNA binding domain; the yellow loop indicates a flavin molecule. Line thickness denotes the magnitude of the transcriptional response.

Phytochromes are red/far-red light photoreceptors with bilin (tetrapyrrole) chromophores that are present in prokaryotes, plants and slime molds, and have been recently identified in fungi. In *A. nidulans*, light of red and far-red wavelengths regulates the sexual–asexual differentiation transition by a mechanism that is dependent on the FphA phytochrome[102]. The FphA protein binds bilin and has red/far-red light-absorbance properties, and its loss blocks *A. nidulans* responses to these wavelengths. An area for future investigation is the interaction between blue and red/far-red light responses in *A. nidulans*, analogous to that observed in some plant light responses.

The first fungal photosensor identified was white collar-1 (WC-1) of *N. crassa*[103], and this system has been extensively studied with emphasis on the circadian clock of this fungus and how it is regulated by the WC-1 and WC-2 proteins, the clock protein FRQ and interacting factors[104,105]. More recently, *wc-1* homologues have been identified in basidiomycetes[106–108] and zygomycetes[109,110], as well as other ascomycetes[111,112]. This information extends the function of *wc-1* homologues in photosensing across the fungal kingdom.

All WC-1 proteins feature a domain (known as LOV) that interacts with a photoreactive flavin molecule, such as flavin-adenine dinucleotide (FAD) for *N. crassa* WC-1 (REF. 113), and two additional Per–Arnt–Sim (PAS) domains (FIG. 3). A zinc finger, DNA-binding domain is found in WC-1 homologues from *N. crassa* and other ascomycetes, and the zygomycetes *Phycomyces blakesleeanus*, *Rhizopus oryzae* and *Mucor circinelloides*. By contrast, the basidiomycete WC-1 homologues currently identified lack this domain[106]. In *N. crassa*, WC-1 interacts with a second protein (WC-2) that contains PAS and zinc-finger domains. Similarly, in *C. neoformans* Bwc1 interacts with a protein (Bwc2) that is structurally similar to WC-2 to form a dimeric transcription-factor complex. In *N. crassa*, the WC-1–WC-2 complex binds DNA in the dark and is hypothesized to bind another molecule of WC-1 in the presence of light. The detailed mechanism of action of WC-1 proteins after light exposure remains to be elucidated. The LOV domain is conserved in other environment-sensing proteins: in *Arabidopsis thaliana* the Phot1 protein, which mediates phototropism, is comprised of two adjacent LOV domains and a serine/threonine-kinase domain, indicating conservation in function across kingdoms at the light-sensing level but not at the level of signal transduction.

The ability to adapt to the intensity of light has been reported for some fungi — in *N. crassa* this process is regulated by VIVID, a blue-light sensor[114,115]. VIVID is a small protein that includes a LOV domain that mediates flavin interactions. *Hypocrea jecorina* (previously known as *Trichoderma reesei*) expresses a similar, small LOV-domain-containing protein called ENVOY, although it functions in a divergent manner from VIVID and cannot complement an *N. crassa vivid* mutant[116]. The gene that encodes ENVOY is a cellulose-inducible gene that can also be induced by light. The light responses of a second

LOV
A domain found in proteins that sense light, oxygen or voltage that physically interacts with a flavin molecule.

Trichoderma species have been investigated at the molecular level. In *Trichoderma atroviride* many light responses are mediated by BLR-1 and BLR-2, homologues of the WC-1 and WC-2 proteins of *N. crassa*. Mutation of the *brl-1* or *brl-2* genes results in reduced growth rate and defects in the expression of a subset of photo-regulated genes[111,117].

In summary, recent discoveries reveal that fungi share similar photosensor capabilities with each other and with distantly related organisms. However, ongoing fungal genome projects continue to reveal additional candidate photosensors, and there is still much to learn about how light regulates fungal physiology in conjunction with other signalling pathways.

Sensing stress

Sensing and responding to stress is required for fungal survival. Mammals have two MAPK pathways — p38 and Jun N-terminal kinase (JNK) — to relay stress-related signals that control cellular survival, differentiation and apoptosis. Similarly, fungi have sophisticated signalling cascades to sense and respond to different types of stress including osmotic shock, temperature, high salt, UV irradiation, oxidative or nitrosative damage, and exposure to antifungal drugs. In fungi, Hog1 is the most extensively studied stress-activated MAPK, homologous to mammalian p38 MAPK. Hog1 is activated by a unique two-component phosphorelay system that has been best studied in *S. cerevisiae*[118]. Under normal conditions, a membrane sensor histidine kinase, Sln1, is constitutively activated by autophosphorylation and subsequently phosphorylates the phosphotransfer protein Ypd1, which, in turn, transfers phosphate to the Ssk1 response regulator. The phosphorylated version of Ssk1 inhibits both the Ssk2 and Ssk22 MAPKKKs, which then block activation of the Pbs2 MAPKK–Hog1 MAPK system. In response to stress, the two-component phosphorelay system is rapidly repressed, resulting in the activation of Ssk2 and Ssk22, which activates Pbs2 and Hog1. Hog1 also responds to oxidative and metal-ion stress[119,120]. Pbs2 and Hog1 are also activated by a pathway that consists of the transmembrane protein Sho1, Cdc42, Ste20 p21-activated kinase, Ste11 MAPKKK and its interacting protein Ste50, and Pbs2 (which has a scaffolding role). In other fungi, however, Sho1 does not seem to be a major regulator of Hog1. For example, *C. neoformans* does not contain a Sho1-like membrane osmosensor, and various Sho1 functions are largely Hog1-independent in *C. albicans*[121]. A third branch for Hog1 activation that involves the membrane protein Msb2 has also been reported[122]. Activated, phosphorylated Hog1 is translocated into the nucleus, where it activates transcription factors (such as Msn2, Msn4, Hot1 and Skn7) or repressors (such as Sko1) thereby regulating expression of downstream effector proteins that counteract stresses. For example, hyperosmolarity triggers Hog1 to activate expression of genes that are involved in glycerol synthesis, which counteracts external osmotic shock.

Analogous stress-activated MAPK pathways have been studied in diverse fungi (FIG. 4). Although similar to mammalian p38 or JNK MAPK cascades, the range of stresses that other fungal Hog1 MAPK networks respond to is broader than that seen in *S. cerevisiae*. *S. pombe* and *C. albicans* contain the Sty1/Spc1 and Hog1 MAPK cascades, respectively, and both respond to a myriad of environmental stimuli (FIG. 4), as shown by the pleiotropic defects seen in the stress responses of *sty1* and *hog1* mutants[123–125]. In addition to stress control, the fungal HOG pathway regulates cell-cycle progression, sexual development and morphological differentiation. In *S. pombe*, *spc1* mutants are defective in G2 arrest and the conjugation/mating process[126]. In *C. albicans*, *hog1* mutants are hyperfilamentous in serum and show cytokinesis defects in high osmolarity conditions[127]. Similarly, in the filamentous fungus *A. nidulans*, SakA is involved in the repression of sexual development (cleistothecia formation) and also has a role in the observed stress resistance of asexual conidiospores[128]. In the rice blast fungus *Magnaporthe grisea*, the MAPK Osm1 negatively regulates appressorium morphogenesis[129]. Interestingly, SakA is not a major regulator of osmotic response in *A. nidulans*[128] and Osm1 is dispensable for the generation of appressorium turgor pressure[129], indicating that other signalling pathways contribute to control intracellular osmotic pressure.

Unlike ascomycete fungi, the basidiomycete *C. neoformans* has evolved a specialized HOG pathway. Hog1 of *C. neoformans* is constitutively phosphorylated under normal conditions and represses the synthesis of two major virulence factors, capsule and melanin, and sexual reproduction[130]. Exposure to stress conditions results in the rapid dephosphorylation of Hog1, which then induces the appropriate cellular responses against the offending environmental stimuli, including hyperosmosis, heat and oxidative stress, and UV irradiation[130]. Whether Hog1 is similarly regulated in other basidiomycete fungi remains to be investigated.

Although stress-activation of MAPKs by two-component phosphorelay systems is a common process in fungi, it is not widespread in mammals therefore making these pathways an attractive antifungal target. For example, the phenylpyrrole drug fludioxonil shows antifungal activity against plant and human pathogenic fungi by hyper-activating the HOG pathway through the two-component system[131–133]. For different fungal species, however, the structure and mechanism of activation by two-component phosphorelay systems differ. Although *S. cerevisiae* contains a single sensor kinase (Sln1), most fungi express multiple sensor kinases[134], which could explain why the *S. cerevisiae* HOG pathway responds to a narrower range of stresses. *C. albicans* expresses three sensor histidine kinases, Sln1, Chk1 and Nik1/Cos1, which are distinctly and redundantly involved in osmosensing, cell-wall biogenesis, morphogenesis, quorum sensing and virulence[135]. *N. crassa* contains 11 sensor histidine kinases, including OS-1, which controls osmotic-stress response and antifungal resistance to fungicides[134,136]. Recently, a *C. neoformans* two-component phosphorelay system was identified and investigated[133]. The response regulator Ssk1 is responsible for the constitutive phosphorylation of *C. neoformans* Hog1 and most of the resulting Hog1 phenotypes[133]. Among seven sensor kinases (Tco1–7) that are expressed by *C. neoformans*, Tco1 and Tco2 have both distinct and redundant roles in transmitting a subset of stress signals that trigger the Hog1 MAPK[133]. In contrast to

Two-component phosphorelay system
First identified in various bacterial systems and subsequently found in lower eukaryotes including fungi. The signalling is achieved by phosphotransfer from a histidine residue in the sensor histidine kinase to an aspartate residue in the response regulator.

Serum
The liquid component of blood that consists of proteins, lipids and many low molecular-weight molecules.

Appressorium
An enlarged fungal filament that is used for penetration through the surface of the host plant.

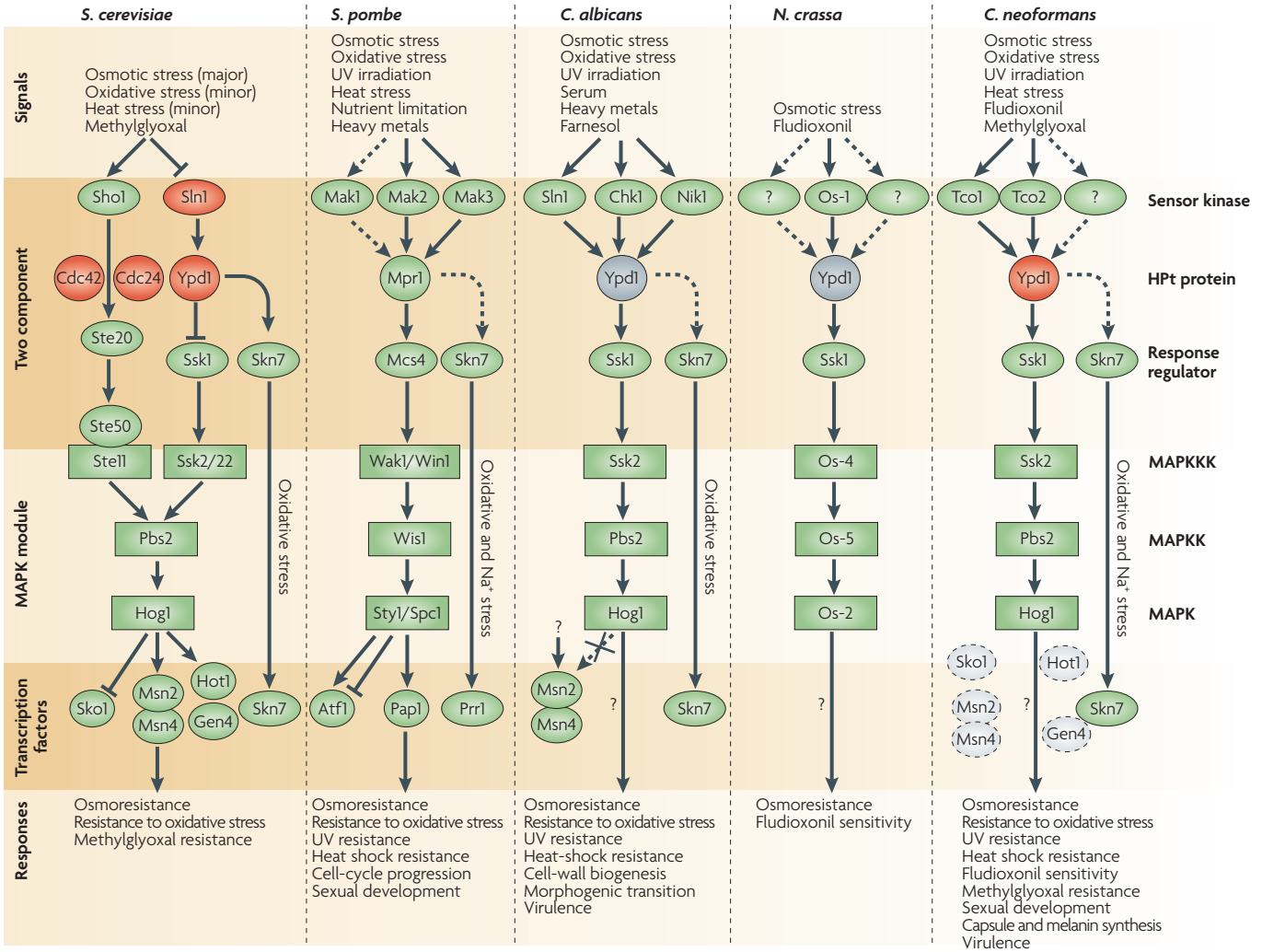


Figure 4 | **Fungal stress-response mechanisms.** The diagram illustrates the two-component phosphorelay and stress-activated mitogen-activated protein kinase (MAPK) pathways found in ascomycete fungi (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans* and *Neurospora crassa*) and basidiomycete fungi (*Cryptococcus neoformans*). Solid lines or arrows indicate known signalling routes and dotted arrows indicate hypothesized signalling events. Component colours indicate the following: essential for cell viability (red), dispensable for normal growth (green), identified but not characterized (grey). Note that *C. albicans*, the normal biological niche of which is warm-blooded animals, does not respond to heat stress (unlike *S. cerevisiae*, *S. pombe*, and *C. neoformans*). However, *C. albicans* does sense serum and the quorum-sensing molecule, farnesol, to induce morphological changes[125], reflecting the unique evolutionary adaptations of this fungus. Interestingly, the basidiomycete *C. neoformans* does not contain many of the known Hog1 transcription factors that are expressed by the ascomycete fungi. In *S. cerevisiae*, the Sho1–Ste20–Ste50-signalling pathway is not a two-component phosphorelay system. Hpt protein, histidine-containing phosphotransfer protein.

S. cerevisiae, in which Sln1 negatively regulates Hog1, Tco1 and Tco2 positively regulate Hog1 of *C. neoformans*. The sensor kinases expressed by *S. pombe*, *N. crassa* and other fungi also positively regulate the HOG pathway[137,138].

Similar to the upstream components of the HOG pathway, the downstream regulators seem to have significantly diverged among fungi. For example, regulation of Hog1-dependent downstream factors in *C. albicans* in response to general and oxidative stresses differs from the mechanism of regulation in non-pathogenic model yeasts[139,140]. Furthermore, some yeast transcription factors controlled by Hog1 are missing in *C. neoformans* (Msn2, Msn4, Hot1 and Sko1) or have different or no obvious function in *C. albicans*[141].

The nutrient sensing Tor and the cAMP–PKA cascades also have significant roles in controlling stress responses in conjunction with the HOG pathway. In *S. cerevisiae*, either Tor inhibition or reduced PKA activity is strongly correlated with the induction of transcription of genes that contain stress regulated elements (STREs)[52,55,142]. Furthermore, the Msn2 and Msn4 transcription factors that control the general stress response are activated by the Hog1 pathway and are negatively regulated by Tor and PKA-dependent nuclear export[143,144]. The Sko1 transcriptional repressor that is controlled by Hog1 is also controlled by PKA by a process that modulates the nuclear localization of Sko1 (REF. 145). Similarly, the oxidative-stress-response regulators Yap1 and Skn7/Pos9

Stress regulated element (STRE). A region in the promoter of genes (consensus sequence CCCCT) to which transcription factors bind to mediate stress-induced transcription.

Calcineurin
A serine/threonine-specific protein phosphatase that is activated by calcium–calmodulin.

Indole-3-acetic acid
(IAA). An auxin plant hormone.

Auxin
A class of plant growth substance (often called phytohormones or plant hormones).

are also under the control of the cAMP–PKA pathway[146]. These findings support the contention that nutrient deprivation is processed by *S. cerevisiae* as an environmental stress.

Sensing the host

Animal–fungi interactions. During host infection, pathogens encounter various factors that are unique to the host. Serum has both positive and negative influences on the proliferation and virulence of pathogenic fungi. In *C. albicans*, serum induces morphological differentiation of cells from the yeast form to the virulent hyphal form[86,87]. In *C. neoformans*, serum enhances the biosynthesis of the capsule and its antiphagocytic ability greatly contributes to the pathogenesis of cryptococcosis[80]. The cAMP-signalling pathway seems to be largely responsible for mediating the serum-elicited pathogenic phenotypes as mutant *C. albicans* that lack cAMP-signalling factors do not respond to serum[86,87]. The identity of serum factors that activate the cAMP pathway in fungi is not known. On the other hand, fungal pathogens must adapt to, and survive, in serum. Recent findings in *C. neoformans* and *A. fumigatus* indicate a role for calcineurin in serum survival through its role in mediating Ca^{2+} homeostasis in *C. neoformans* and phosphate homeostasis in *A. fumigatus*[147,148].

Host protein receptors that recognize fungal molecules have been identified, including the mannose-receptor protein dectin-1 and Toll-like receptors in animal cells that bind fungal cell-wall carbohydrates, and the leucine-rich resistance proteins in plants that recognize fungal avirulence proteins. Whether fungi sense their host during the infection process through the use of equivalent receptors is an intriguing possibility.

Plant–fungi interactions. Many fungi, including some soil-borne yeasts, are found associated with plants or plant debris and can sense plant-derived signals. Plant hormones can potentially alter yeast morphology. Indole acetic acid (IAA) triggers pseudohyphal growth of *S. cerevisiae* and auxin transporters are important for IAA sensing[149]. The human fungal pathogen *Cryptococcus gattii* has an ecological association with trees especially *Eucalyptus* species. This fungus might sense plant-derived signals and develop a beneficial relationship with plants to complete its life cycle. Indeed, recent studies reveal that IAA stimulates mating of *C. gattii* (C.X. and J.H., unpublished data).

Fatty acids, including some plant-derived lipids, mediate signals involved in plant–fungal interactions or alter fungal morphology. In the corn smut fungus *U. maydis*, a mixture of triacylglycerides containing corn oil stimulates filamentation[150]. Plant surface lipids (waxes) induce the development of infection structures (appressoria) in plant pathogenic fungi, including the *Colletotrichum* species[151] and *M. grisea*[152]. In *A. nidulans*, the Psi factors, derived from linoleic acid, function as sexual-sporulation hormones[153]. Recently, asexual and sexual sporulation have been linked to fatty-acid metabolism in *A. nidulans*[154] and virulence of *A. fumigatus*[155]. Animal fatty acids such as prostaglandin E_2 and thromboxane B_2 enhance serum-induced germination by *C. albicans* and *C. neoformans*[156].

Mechanosensitive responses in fungi. Fungal cells can sense surfaces and modify their behaviour accordingly. Recent studies reveal that touch, hearing and other mechanical senses function through the activity of an ion channel, the mechanosensation channel, homologues of which are present in fungi, plants and animal species[157]. Thigmotropism is common in fungi — *Uromyces appendiculatus* senses the dimension of host topographical signals through Ca^{2+} sensing and a mechanosensitive channel and subsequently penetrates plant stomata guard cells to cause disease[158]. Mechanosensitive channels, including the Ca^{2+} channels Yvc1 and TrpY1, have also been identified in *S. cerevisiae*[159,160], *S. pombe*[161] and *C. albicans*[162].

Many plant pathogenic fungi, including *M. grisea* and *Colletotrichum gloeosporioides*, produce pathogenesis-related appressoria when in contact with certain hydrophobic and hard surfaces[163]. Indeed, studies have revealed that the hydrophobicity and hardness of the host surface is an essential signal to trigger appressorium formation in *M. grisea*[164].

From fungi to humans

This article has focused on the molecular mechanisms used by the fungal kingdom to sense and adapt to a plethora of environmental cues. We have strived to cover both the model yeasts as well as fungi that are pathogenic to animals and plants. As underscored by the drugs FK506 and cyclosporin, which have both immunosuppressive and antifungal activities by targeting the calcineurin pathway in humans and fungi, respectively[165], signal-transduction systems used by fungi have emerged as amenable and informative models for the study of widely conserved mechanisms of signal transduction. Recent advances in genome sequencing projects combined with functional and comparative genomics should further facilitate an understanding of the interconnections between these two major eukaryotic kingdoms.

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Competing interests statement
The authors declare no competing financial interests.

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