Review

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The MAP kinase signal transduction network in *Candida albicans*

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MAP (mitogen-activated protein) kinase-mediated pathways are key elements in sensing and transmitting the response of cells to environmental conditions by the sequential action of phosphorylation events. In the fungal pathogen *Candida albicans*, different routes have been identified by genetic analysis, and especially by the phenotypic characterization of mutants altered in the Mkc1, Cek1/2 and Hog1 MAP kinases. The cell integrity (or MKC1-mediated) pathway is primarily involved in the biogenesis of the cell wall. The HOG pathway participates in the response to osmotic stress while the Cek1 pathway mediates mating and filamentation. Their actual functions are, however, much broader and Mkc1 senses several types of stress, while Hog1 is also responsive to other stress conditions and participates in two morphogenetic programmes: filamentation and chlamydospore formation. Furthermore, it has been recently shown that Cek1 participates in a putative pathway involved in the construction of the cell wall and which seems to be operative under basal conditions. As these stimuli are frequently encountered in the human host, they provide a reasonable explanation for the significant reduction in pathogenicity that several signal transduction mutants show in certain animal models of virulence. MAPK pathways therefore represent an attractive multienzymic system for which novel antifungal therapy could be designed.

Introduction

Living cells are exposed to a continuously changing environment. For microbial pathogens, this is especially important as the host is continuously challenging the micro-organism with different microenvironments; failure to respond to them would result in the elimination of the microbe from the host. Signal transduction pathways are major mechanisms by which all living cells sense such changes and develop adaptive responses. *Candida albicans* is an opportunistic pathogen that inhabits the human body as a commensal and is a major cause of fungal infections in humans. *C. albicans* infections normally develop as the consequence of an altered immune host response. This microbe displays different morphologies depending on the conditions of growth; these range from the unicellular (yeast-like form) to hyphae, pseudohyphae or chlamydospores. This morphological plasticity has been long considered to play a major role in virulence.

Mitogen-activated protein kinases signal transduction pathways are widespread mechanisms present in eukaryotic cells to couple environmental responses to transcriptional regulation. They comprise a conserved module of three kinases: the MAP kinase (MAPK), the MAP kinase kinase (MAPKK) and the MAP kinase kinase kinase (MAPKKK) (see Fig. 1). When upstream signals are fed into the MAPKKK (by a variety of signalling mechanisms), the MAPKKK becomes phosphorylated and in turn phosphorylates the MAPKK which, in turn, does so to the MAPK. The

MAPK normally transmits the signal to downstream transcription factors that generate a specific adaptive response. These pathways have been extensively characterized in the non-pathogenic model yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In *S. cerevisiae* they have been implicated in at least five different processes, such as adaptation to high osmolarity (HOG pathway), mating and growth (STE, invasive growth and SVG pathway) and cell integrity (also called the PKC1-mediated pathway). However, despite the evidence that functionality of the signalling through these pathways can be essential for virulence, the knowledge of their relevance in pathogenic fungi is far from complete.

We will here review the knowledge gained about signal transduction in the clinically significant fungus *C. albicans*, a model pathogenic yeast for which several genetic tools have been developed that enable precise functional analysis (De Backer *et al.*, 2000; Berman & Sudbery, 2002). Special attention will be devoted to the MAPK central core network, and its role in the biology of this fungal pathogen. We will not, however, focus on their transcriptional response and/or mediators, which have been recently the subject of excellent reviews (Whiteway, 2000; Liu, 2001; Davis, 2003).

The HOG pathway

The HOG pathway is responsible in *S. cerevisiae* for generating an adaptive response to high-osmolarity environments

Fig. 1. Main elements of the MAPK signal transduction network in *C. albicans*. Genes are represented in normal characters where deletion and/or phenotypic characterization of the corresponding strain has been carried out in *C. albicans* or, alternatively, in *italic*, where their position and role are just presumed based on the *S. cerevisiae* model and/or inspection of the genome. The existence of additional putative elements that may play a role in the activation of the corresponding MAPK is shown as question marks. The stimuli leading to the activation/repression of the pathways are shown either as stimulating (→) or inhibitory (--) arrows. It is important to note that the arrows do not establish whether the interaction/crosstalk takes place through a direct or an indirect mechanism. The role of each MAPK in the physiological response is highlighted, but it must be taken into account that the interaction between pathways extends their indicated roles and generates a proper balanced response in the fungal cell.

(Posas *et al.*, 1998a; Hohmann, 2002; de Nadal *et al.*, 2002); it is composed of at least two branches. The first branch operates through the Sln1 two-component protein and the Ypd1 and Ssk1 phosphorelay proteins. Under isotonic conditions, this three-protein branch is constitutively activated so that a phosphorylated Ssk1 blocks further activation of the downstream cascade; this situation is reversed under high osmolarity, where Ssk1 becomes dephosphorylated allowing activation of the Ssk2/Ssk22 functionally redundant proteins. A second input to this cascade comes from another defined route through the Ste11 MAPKKK, the Ste11-interacting protein Ste50, the Ste20 p21-activated kinase (PAK), the small GTPase Cdc42 and the transmembrane Sho1 adaptor protein. Both signals converge at the level of the MAPKK Pbs2, which is able to activate the Hog1

MAPK by phosphorylation, thus leading to downstream effectors (Posas *et al.*, 2000; de Nadal *et al.*, 2002).

In *C. albicans* the HOG pathway has been shown to be involved in at least three separate processes: response/adaptation to stress, morphogenesis and cell wall formation. *hog1* and *pbs2* mutants are sensitive to both ionic and non-ionic osmotic stresses (San José *et al.*, 1996; Arana *et al.*, 2005). Upon addition of sodium chloride, Hog1 becomes phosphorylated in a Pbs2-dependent manner and translocates to the nucleus of the cell (Alonso-Monge *et al.*, 2003; Smith *et al.*, 2004; Arana *et al.*, 2005). Activation of Hog1 leads to glycerol accumulation, which counteracts cell dehydration, as in *S. cerevisiae* (Albertyn *et al.*, 1994; San José *et al.*, 1996). It must be stated, however, that

accumulation of other compatible solutes is also possible and that in *hog1* cells there is a basal increased glycerol level, arguing in favour of a *HOG1*-independent mechanism of glycerol accumulation. It has been also shown that the production and accumulation of D-arabitol, a metabolite exclusive to fungal cells, is regulated by the HOG pathway (Kayingo & Wong, 2005), although its relevance in the physiology of *C. albicans* cells is still not clear.

In addition to being involved in osmoadaptation, the HOG pathway plays an important role in resistance to oxidative stress. This is clearly shown by the susceptibility of *hog1* and *pbs2* mutants to oxidants like menadione, hydrogen peroxide and potassium superoxide. Furthermore, oxidative stress also activates the pathway (Alonso-Monge *et al.*, 2003), as has been recently shown in *S. cerevisiae* (Bilsland *et al.*, 2004). This mechanism is mainly dependent on the Ssk1 kinase (Chauhan *et al.*, 2003), indicating that this branch – and not the one mediated by the Sho1 adaptor protein – is specialized in sensing/transmitting oxidative stress-mediated signals. A residual basal activation of Hog1 can be observed in *ssk1* cells, but it is blocked in *ssk1 sho1* mutants (Roman *et al.*, 2005). The observed sensitivity to oxidants is consistent with the reduced virulence of *hog1* mutant cells in a mouse model of experimental infection (Alonso-Monge *et al.*, 1999) since oxidative-stress-mediated killing is essential in the control of the fungal infection by the host immune cells. Interestingly, although mutants in the HOG pathway in *S. cerevisiae* are sensitive to oxidants (Singh, 2000; Rep *et al.*, 2001), both the pattern of oxidants used and the timing of activation of this MAPK in response to these compounds clearly differ from the *S. cerevisiae* Hog1 homologue (Bilsland *et al.*, 2004), probably reflecting an adaptation to the commensal/pathogenic state in *C. albicans*. The role of the HOG pathway in oxidative stress is reinforced by recent work indicating that *C. albicans ssk1* mutants show enhanced killing by polymorphonuclear neutrophils (Du *et al.*, 2005), these cells being well-known mediators of the oxidative damage by the immune system. Interestingly, mutants altered in both branches of the pathway *ssk1*, *sho1* or *ssk1sho1* mutants, are not sensitive to osmotic stress and are able to transmit the signal to the Hog1 MAPK in response to osmotic stress, indicating the existence of additional elements in this organism to sense this type of stimulus (Roman *et al.*, 2005).

The HOG pathway is also involved in morphogenesis. *hog1* and *pbs2* mutants display enhanced true hyphal formation as evidenced under non-inducing conditions such as low temperature, low pH on certain media or even liquid media with limited serum concentration (Alonso-Monge *et al.*, 1999; Arana *et al.*, 2005). In contrast, *ssk1* mutants also have a reduced ability to form hyphae on serum and these phenotypes are not suppressed by Hog1 overexpression (Alex *et al.*, 1998; Calera *et al.*, 2000). While enhanced filamentation in *hog1* and *pbs2* mutants could be caused by the constitutive activation of the Cek1 MAPK (see later) (Arana *et al.*, 2005; Navarro-Garcia *et al.*, 2005; Roman *et al.*,

2005), genetic analysis reveals that this is not the case, as double *hog1 hst7* mutants still show enhanced filamentation under these conditions (Eisman *et al.*, 2006), and suggests the existence of a core stress response responsible for filament formation (Smith *et al.*, 2004). The HOG pathway is involved in the formation of another morphogenetic programme: chlamydospore formation (Alonso-Monge *et al.*, 2003). Chlamydospores are thick-walled structures that have been suggested to represent resistant forms; their formation is dependent on the Efg1 morphogenic regulator among others (Sonneborn *et al.*, 1999; Nobile *et al.*, 2003) but this seems to be a process not dependent on Hog1. Recent results indicate that the defective chlamydospore formation observed in *hog1* mutants is reverted by deletion of elements of the Cek1 pathway like the *HST7* MAPKK coding gene or the *CST20* gene (Eisman *et al.*, 2006).

The HOG pathway is also involved in cell wall biosynthesis. *hog1* mutants are resistant to certain cell wall inhibitors such as nykkomycins and Congo red (Alonso-Monge *et al.*, 1999) and show an enhanced susceptibility to lysis mediated by cell wall digestion with β -1,3-glucanases, in close similarity to what is observed in *S. cerevisiae* (Alonso-Monge *et al.*, 2001). Also, in *S. cerevisiae*, low pH induces changes in the overall cell-wall architecture that are dependent on Hog1 and Pbs2 overexpression results in β -1,3-glucanase resistance (Jiang *et al.*, 1995; Kapteyn *et al.*, 2001). In *C. albicans*, deletion of *SIT4*, a type 2A-related protein phosphatase, results in reduced *HOG1* mRNA levels and impairs hyphal-specific expression of the major glucanase *XOG1* and the putative glucanase *YNR67* genes (Lee *et al.*, 2004). Susceptibility to Congo red correlates with Cek1 activation, as it is augmented in mutants of the Cek1 pathway (Cek1 and Hst7) while it is diminished in *hog1* and *ssk1* mutants, in which Cek1 phosphorylation is higher (Eisman *et al.*, 2006). These results indicate that the HOG pathway exerts a repressive role over the Cek1 pathway, similar to what has been observed in *S. cerevisiae* (O'Rourke & Herskowitz, 1998), and that this process contributes to the construction of the cell wall.

Different histidine kinases have been described in *C. albicans*: *NIK1* (Nagahashi *et al.*, 1998; Alex *et al.*, 1998), *CHK1* (Calera *et al.*, 1998) and *SLN1* (Yamada-Okabe *et al.*, 1999), which could play a role in this pathway. *CHK1* encodes a histidine kinase whose deletion alters the surface properties of the cell as revealed by a phenotype of aggregation (Calera & Calderone, 1999). This protein also influences the composition of the cell wall and *chk1* mutants have a reduced glucan content (Kruppa *et al.*, 2003, 2004). Interestingly, *chk1* mutants show an altered timing of Hog1 activation in response to oxidative or osmotic stress, and *CHK1* expression (measured as a *CHK1-lacZ* fusion) is altered in *hog1* and *ssk1* mutants (Li *et al.*, 2004). The Sln1 protein is a sensor of osmotic stress in one of the operating branches of the HOG pathway in *C. albicans* and it has been recently shown that its deletion results in constitutive activation of phosphorylated Hog1 (Roman *et al.*, 2005). Despite this last result, the role of histidine kinases in MAPK-mediated

signal transduction pathways is still uncertain, as there is no clear evidence for the control of their activity, nor does their phenotypic analysis provide a clearly assignable phenotype (Yamada-Okabe *et al.*, 1999).

The Cek1-mediated pathway

In *S. cerevisiae*, a conserved MAPK pathway mediated by the Fus3/Kss1 MAPKs participates in at least three different processes: mating, invasive growth and vegetative growth. Mating leads to the formation of diploid cells from haploid sexually compatible cells (α and α type) and was the first process in which the MAPK core was discovered to participate (reviewed by Elion, 2000). The participating elements in this cascade are the Ste20 MAPKKK, the Ste7 MAPKK, the functionally partially redundant Fus3/Kss1 MAPKs and the scaffold protein Ste5. Under nitrogen starvation diploid cells enter the pseudohyphal mode of growth (Gimeno *et al.*, 1992), while haploid cells undergo a related morphological transition in response to glucose depletion that results in invasive growth (Palecek *et al.*, 2002). The two processes are functionally different and, as an example, neither Fus3 nor Ste5 participates in the invasive pathway (Elion, 2001). Additionally, the two pathways differ in their upstream elements: mating is triggered through Ste2 or Ste3, the pheromone receptors and the coupled trimeric G protein encoded by the *GPA1* (α subunit), *STE4* (β subunit) and *STE18* (γ subunit) genes (reviewed by Bardwell, 2004). Other upstream elements that regulate this cascade are the GTP-binding protein Ras1, which acts as an activator of the cascade required for the transition from yeast to pseudohyphal growth in *S. cerevisiae* (Mösch *et al.*, 1996), and Cdc42. This last protein is a Rho family GTPase that controls multiple signal transduction pathways. In fact, Cdc42 and its GTP-GDP exchange factor, Cdc24, are essential for polarized growth during budding, mating and pseudohyphal growth and also mediate signalling towards the high-osmolarity response pathway (Raitt *et al.*, 2000).

Elements of this MAPK route also participate in the biogenesis of the cell wall through the so-called SVG (sterile vegetative growth) pathway that promotes vegetative growth under basal (non-stressed) conditions and is essential in mutants deficient in protein mannosylation (Lee & Elion, 1999; Cullen *et al.*, 2000). The SVG pathway also shares elements with the HOG route and the Sho1-Ste20-Ste11 branch drives the specific response either to Ste7-Kss1 or to Pbs2-Hog1 (Posas & Saito, 1997; Posas *et al.*, 1998b). At the end of this multifunctional pathway, the Ste12 transcriptional activator mediates the expression of mating, invasive-specific genes or cell wall biosynthesis in concerted action with other regulators such as the repressors Dig1/Dig2 or the transcriptional factors Tec1 and Mcm1. These last elements bind to specific sequences in the promoters called FRE (filamentation response elements) or PRE (pheromone response elements) respectively (reviewed by Schwartz & Madhani, 2004).

The existence of orthologues of this pathway in *C. albicans* was discovered a long time ago. The Cek1 MAPK (*Candida*

ERK-like kinase, a *C. albicans* KSS1 homologue) was isolated by its ability to confer a cell-cycle-specific arrest when overexpressed in *S. cerevisiae* (Whiteway *et al.*, 1992). Later, the Hst7 MAPKK (Leberer *et al.*, 1996) (a Ste7 homologue) and the Cst20 PAK (a Ste20 homologue) were identified as well as other upstream and downstream elements (Csank *et al.*, 1997; Schroppel *et al.*, 2000; Ushinsky *et al.*, 2002). The Cph1 transcription factor (a Ste12 homologue) is supposed to lie at the end of this route (Liu *et al.*, 1994) while the VIH-type Cpp1 phosphatase is supposed to be involved in Cek1 dephosphorylation, since some phenotypes of *cpp1* mutants are reversed in *cpp1 cek1* cells (Csank *et al.*, 1997).

Several reports indicate that this pathway is involved in morphogenesis and filamentation. Deletion of *CEK1* – as well as *HST7* – results in hyphal formation defects on certain solid media such as SLAD, in which the nitrogen source is limiting (Csank *et al.*, 1998). It has also recently been shown that nitrogen starvation induces the Mep2 ammonium permease that in turn activates filamentous growth on these media and it has, therefore, been proposed to trigger the Cph1/Cek1-mediated pathway in a Ras1-dependent manner (Biswas & Morschhauser, 2005). The Cdc24/Cdc42 GTPase module is required for hyphal morphogenesis and elongation, a process independent of the cell cycle, in contrast to what happens in *S. cerevisiae* pseudohyphal growth. In the presence of serum, however, all the mutants in the pathway are able to filament, indicating that this route is neither the only nor the main mechanism leading to hyphal formation (Whiteway, 2000; Liu, 2001). The relevance of the Cek1-mediated pathway is reinforced by the reduced virulence of mutants altered in some of their components (Marcil *et al.*, 2002). In contrast to what occurs in *S. cerevisiae*, Tec1 (Guhad *et al.*, 1998; Csank *et al.*, 1997; Lo *et al.*, 1997) is not regulated by Cph1 (Ste12 orthologue) in *C. albicans*. Tec1 may be regulated by Efg1 and/or Cph2, both bHLH (basic helix-loop-like) proteins implicated in the yeast-to-hypha transition in *C. albicans*. Efg1 is the transcriptional factor underlying the AMPc-dependent protein kinase pathway, while Cph2 has not been clearly assigned to any signalling route. The existence of different signalling cascades regulating the yeast-to-hypha transition evidences the relevance of this process in *C. albicans* physiology.

Interestingly, Cek1 is also activated by different environmental signals. When stationary-phase cells are diluted in fresh rich medium, Cek1 phosphorylation peaks in the following 1–2 h and slows down as the cells again enter stationary phase, indicating that the activity of this MAPK is regulated by growth signals. This process is dependent on the Sho1 adaptor protein (Roman *et al.*, 2005). Cek1 activity and/or expression could, however, also be regulated by quorum sensing. Tyrosol and farnesol have been recently identified in *C. albicans* and shown to be involved in morphogenesis (Hornby *et al.*, 2001; Chen *et al.*, 2004) and a recent study claimed that farnesol reduces the levels of *HST7* and *CPH1* mRNAs (Sato *et al.*, 2004); therefore, relief of quorum-sensing-mediated repression in restart of growth

could lead to transient activation of the Cek1 kinase. The analysis of *sho1* mutants, which fail to activate the Cek1 kinase and display sensitivity to Congo red – as well as other cell-wall-interfering compounds – indicates that this route is involved in the construction of the cell wall in addition to promoting invasion under certain conditions (Roman *et al.*, 2005).

In *C. albicans*, elements of this pathway are also relevant for mating (Magee *et al.*, 2002). *C. albicans* cells are able to undergo a parasexual cycle that results in the formation of tetraploid cells either *in vitro* (Magee & Magee, 2000) or *in vivo* within the mouse (Hull *et al.*, 2000). This process, whose clinical and biological relevance outside the laboratory remains to be precisely assessed (Johnson, 2003), depends on the *CPH1* and *HST7* genes, as *cph1* and *hst7* mutants are defective in mating. Interestingly, *cst20* or *cek1* are only partially defective (Chen *et al.*, 2000) but deletion of another MAPK, Cek2 (a *FUS3* homologue) in *cek1* cells renders them unable to mate (Chen *et al.*, 2000). These results indicate that the two proteins play complementary roles and are functional in mating, similar to what occurs in the sexual cycle of *S. cerevisiae*. However, their function in other differentiation programmes remains to be determined. *C. albicans* produced specific mating-type pheromones which bind to conserved pheromone receptors (Ste2/3) (Bennett *et al.*, 2003); this interaction triggers the trimeric G protein encoded by orthologues of *CAG1*, *STE4* and *STE18*, activating the MAPK cascade. No scaffold protein has been identified in *C. albicans* in order to prevent crosstalk between signalling routes. Transcriptional analyses in response to pheromone revealed the upregulation of a set of cell-surface and secreted protein genes previously implicated in virulence in a mouse model of systemic candidiasis, apart from the expression of specific mating genes (Bennett *et al.*, 2003). This observation suggests that certain aspects of cell–cell communication in mating may have been evolutionarily adapted to account for host–pathogen interactions in *C. albicans*. In addition, the mating efficiency depends largely on nutrient signals, and optimal mating has been reported in Lee's and Spider media, which induce filamentation/invasive growth in *C. albicans*, providing further evidence of the relationship between the mating and filamentation pathways (Chen *et al.*, 2002; Lockhart *et al.*, 2003).

The cell integrity pathway

In *S. cerevisiae*, initial indications of the cell integrity pathway were obtained through the analysis of conditional mutants that lysed upon a shift to the non-permissive temperature of 37 °C (Torres *et al.*, 1991) without osmotic protection (normally carried out using sorbitol or sodium chloride) (for reviews see Heinisch *et al.*, 1999; Cid *et al.*, 1995). The basic kinase module consists of the Bck1 protein (bypass of C protein kinase) MAPKKK, the redundant *MKK1/MKK2* MAPKKs and the Slt2 MAPK (also called Mpk1). Recent studies have revealed that it integrates signals derived from several other pathways such as the

nutrient-regulated TOR pathway and that it could respond to cell wall damage by a set of membrane-located sensors such as Wsc1 and Mid2. Although originally shown to be involved in cell integrity, the Slt2 pathway has now been shown to play a role in morphogenesis, cell cycle and oxidative stress response among others.

In *C. albicans*, the *pkc1* mutant (protein kinase C, Pkc1, lies upstream of the MAPK core) shows a striking osmotically dependent phenotype that has complicated its study (Paravicini *et al.*, 1996). Mkc1, the homologue of the *S. cerevisiae* Slt2/Mpk1 MAPK, plays a role in maintaining cellular integrity and cell wall formation as deduced from the osmotically remediable sensitivity of mutant cells to certain cell-wall-interfering compounds (Navarro-García *et al.*, 1995). *mkc1* mutants do not display a drastically altered cellular composition but differ from wild-type strains in the deposition of surface mannan (Navarro-García *et al.*, 1998). In addition, overexpression of Mkc1 results in enhanced pseudohyphal formation when *S. cerevisiae* cells are subjected to nitrogen starvation (Navarro-García *et al.*, 1998), indicating its relevance in polarized growth. Recent studies indicate that this MAPK is essential for invasive growth under embedded conditions and in the process of biofilm formation (Kumamoto, 2005). Mkc1 signalling is therefore relevant in those processes dependent on physical interaction with external surfaces. Recent studies also indicate that the pattern of Mkc1 activation (despite the fact of the existence of the TEY motif, characteristic of cell growth MAPKs, instead of the TGY motif, characteristic of stress MAPKs: Kultz & Burg, 1998) closely resembles that of the general stress-activated kinases. In fact, Mkc1 is phosphorylated in response to oxidative stress, osmotic stress, antifungal drugs, calcium ions and low-temperature shocks among others (Navarro-García *et al.* 2005) and its oxidative-stress-mediated phosphorylation is partially dependent on an intact HOG pathway (Arana *et al.*, 2005; Navarro-García *et al.*, 2005). Mkc1 therefore behaves similarly to Mkp1, an Slt2 homologue in *Pneumocystis carinii* (Fox & Smulian, 1999) and to *S. cerevisiae* Slt2 (Vilella *et al.*, 2005), in both cases being also activated by oxidative stress. Evidence for the role of Mkc1 in cell integrity is also reinforced by the recent observation that *pmr1* mutants, defective in a P-type ATPase that drastically changes the composition of the cellular surface, constitutively activate Mkc1 (Bates *et al.*, 2005). Not surprisingly, *mkc1* mutants display a reduced virulence in the mouse model of systemic infection (Diez-Orejas *et al.*, 1997).

Conclusions

The network of MAPK signalling pathways represents an array of cascades that are essential for morphogenesis, cell growth, biogenesis of the cell wall and stress response, among others, in *C. albicans*. The homology of these pathways with those of other fungal models has helped in their identification but their precise role in *C. albicans* reveals particular features in this dimorphic fungus that could reflect its adaptation to specific forms of life. For example, all

of them play a role in morphogenesis but they seem to be required under different conditions and to be activated by different stimuli; the cell integrity Mkc1 MAPK is important for growth under embedded conditions, while Cek1 and Hog1 kinases are critical under nitrogen starvation. In addition, the stimuli leading to their activation can be shared in some cases; for example Mkc1 and Hog1 are both activated by oxidative stress, but most probably generate a rather different response. A level of crosstalk among the pathways is already hinted at (see Fig. 1) but needs further analysis. MAPK-mediated signal transduction pathways are related to virulence. This is clearly demonstrated by the loss or reduction of virulence in strains deleted for essential elements of these cascades, in models of experimental infection. However, their actual relevance *in vivo* (that is, activation of the corresponding pathways during infection) represents one of the main issues to be addressed in the future. This should contribute not only to precisely defining the role of these signalling pathways for an essential capacity of *C. albicans*, but also to establishing better criteria regarding the definition of virulence factors (Odds *et al.*, 2003). The use of these *in vitro* models that mimic the main steps relevant to pathogenesis (adhesion, invasion, colonization, dissemination, etc.) will surely enable us to determine which of them are relevant. The identification of the main targets of the pathways through combined genomic, proteomic and biochemical approaches will be essential and may contribute to their validation as novel antifungal targets.

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