The CEK1-mediated mitogen-activated protein kinase pathway in the fungal pathogen *Candida albicans*

Elvira Román, Jesús Pla
Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Spain

Abstract

Mitogen-activated protein kinases (MAPK) mediated signal transduction pathways are essential for the adaptation of living organisms to environmental changes. In pathogenic fungi, these MAPK cascades govern the response to many types of situations, and are essential for the successful establishment of the fungus within the host. Therefore, they influence virulence and can be considered as promising therapeutic targets. In the opportunistic pathogen *Candida albicans*, the Cek1-mediated pathway was identified long time ago as an important virulence determinant in certain animal models. We will review here the recent work that reveals the role that this route plays in three important processes for the cell: osmotic adaptation, fungal morphogenesis and cell wall remodeling. We will also show the complementary (and sometimes opposite) roles that under specific circumstances the high osmolarity glycerol and CEK1 pathways play in *C. albicans* biology, especially in the context of the interaction with the mammalian host.

Introduction

Almost every living organism is constantly exposed to environmental changes that challenge its survival. Cells have, accordingly, developed different mechanisms that mediate adaptation to these changes. Mitogen-activated protein kinase (MAPK) pathways are biochemical transducers of such environmental alterations. A stimulus, normally sensed by different surface receptors, is transmitted via phosphorylation to a MAPK that becomes translocated to the nucleus of the cell where it activates different targets [normally transcription factors (TF)] that mediate either downstream or up regulation of the TF target genes, therefore allowing the correct response to the initial stimulus. To ensure the timing and intensity of the response, and due to the fact that some elements are present in more than one cascade, regulatory mechanisms must be present in the cells. These include protein phosphatases (that dephosphorylate MAPKs and other proteins),1 docking interactions (ensuring correct and tight protein-protein interactions),2 crosstalk inhibition (that ensure the inhibition of one pathway once another is active) and kinetic insulaion (that allow different cascades respond to the same stimulus differently in terms of kinetic or threshold).

MAPK-mediated signaling is attractive in the field of fungal pathogenicity. Not only MAPK routes are involved in adaptation to stress but they do also participate in processes like mating or morphogenesis that regulate important aspects of fungal virulence as occurs, for example, in *Cryptococcus neoformans*.3 4 Cells defective in certain elements of MAPK pathways are often attenuated in virulence, being unable to initiate or establish an infection in different mammalian and non-mammalian hosts.5 Selective inhibition of MAPK pathways is, therefore, a potential attractive way to control infections caused by fungi. This is especially interesting in view of the relatively limited repertoire of antibiotics available to treat fungal infections compared to bacterial ones, a fact that is largely dependent on the close similarities that exist both in the mammalian and fungal cells, both eukaryotic. Furthermore, resistance to some of the most commonly used antifungals (the azole family) is becoming frequent6 and may limit in a near future its usefulness.

Among pathogenic fungi, *Candida albicans* is probably the most extensively known model in biological research. This position is a reflection of the high incidence of the infections caused by this microbe, despite the increase in the diversity of etiological agents causing fungal infections in the last years.7 *C. albicans* is responsible for the 8-10% of bloodstream nosocomial infections in the USA8 and is a serious risk at hospital’s intensive care units. This yeast is a normal member of the human microbiota, being encountered as colonizer of the vaginal and gastrointestinal tract in about 30-70% of human population. This last location is considered the main reservoir in humans,9 upon alterations of the host immune response or mucosal barriers, translocation to the bloodstream occurs and colonization of internal organs like liver, kidneys, spleen or brain takes place, causing extremely severe diseases with mortality that can be as high as 47%.10 Immune deficiencies present in individuals with certain genetic defects (mainly neutrophils or T-helper 17 response, undergoing cancer chemotherapy and/or organ transplant and AIDS are important predisposing factors to candidiasis).11 14

Research in *C. albicans* was long hampered by their biological peculiarities: its diploidy, absence of a sexual cycle similar to *S. cerevisiae* and lack of natural plasmids, resulted in a difficult genetic manipulation.15 16 However, its genome has been now fully sequenced,17 its almost sexual cycle (being dependent on a morphogenetic switch, the so called white-opaque transition) has been discovered18-20 and several laboratories have developed nice genetic tools such as gene deletion strategies, conditional expression systems and gene reporters.15 21-23 These tools have resulted in a quick advance in the basic knowledge of this fungal pathogen. The non-pathogenic yeast *Saccharomyces cerevisiae* has additionally served as a useful model for studying the components and functions of many genetic elements of *C. albicans*.24 It has been the case for the MAPK pathways, first identified in *S. cerevisiae* mating, and only then extended to this and other fungi. While we will emphasize for historical reasons in this review the relevance of *S. cerevisiae* as model organism, it must be stated that other fungi like *Schizosaccharomyces pombe* or even the pathogenic *Cryptococcus neoformans* may be also considered interesting comparative models for pathogenic fungi.25 27

Previous reviews from our group have highlighted the roles of MAPK pathways in *C. albicans* biology with emphasis in cell cycle regulation,28 virulence5 29 and cell wall construction.30 31 We will focus here, on the interplay between the Hog1 and the CeK1-mediated pathways as they play complementary roles in this organism. Both routes influence essential aspects of fungal pathogens biology but we will
focus here on three aspects essential for virulence: i) morphogenesis, which plays an important role in fungal virulence as a mechanism affecting the adhesion, the invasion and the dissemination of the fungus; ii) stress, which is relevant in terms of adaptation to a host-changing environment and iii) cell wall construction, which is crucial in terms of interaction of the fungi with the mammalian host and, in particular, immune cells.

**Overview of *Saccharomyces cerevisiae* mitogen-activated protein kinase pathways**

In *S. cerevisiae*, three well-characterized MAPK pathways are present: the cell integrity (CWI pathway), mediated by the Slt2 MAPK (that regulates the cell wall integrity), the high osmolarity glycerol (HOG) pathway (that responds to external increases in osmolality) and the Kss1/Fus3 cascade (involved both in filamentous and invasive growth and mating).

The CWI pathway is activated by cell wall damage through several cellular surface proteins like Mid2, Wsc1-4 and Mtl1.32-34 Once activated, the activation of the small GT-Pase Rxl1 takes place which interacts - and activates - Pck1, the protein kinase C homolog in yeast63 triggering the signal to the MAPK module (Bck1, Mkk1/2 and Slt2). Once activated, Slt2 regulates transcriptional activation through two main TF, the SBF complex and Rim13.6,37

The HOG pathway is essential for adaptation to osmotic stress regulating the accumulation of glycerol, an intracellular compatible solute.38-40 This osmotic stress also induces a transient cell cycle arrest and inhibition of protein synthesis.41 It is composed by two upstream independent branches that activate different mitogen-activated protein kinase kinase kinases (MAPKKKs). The SLN1-branch comprises the Snl1 two-component system and the phosphorylates proteins Ypd1 and Ssk1 and regulates the activation of the redundant MAPKK Ssk2/22,22,40,42-44 The SHO1-branch activates the MAPKKK Ste11 by the participation of the osmosensors Msb2 (a mucin type protein in yeast) and the Hkr1 mucin-like transmembrane protein,45,46 the transmembrane protein Oppy2,47-48 the GTPase Ste20 and the Ste11-interacting protein Ste50.49,50 Both branches converge and activate the Pbs2 MAPKK, which in turn phosphorylates the Hog1 MAPK that regulates the appropriate transcriptional response through the TF Msn1, Hot1, Sko1 and Smp1.51,54

The Fus3/Kss1 pathway is involved in at least 3 processes (mating, invasive and vegetative growth). Mating is induced by the presence of pheromones which is sensed by the Ste2, Ste3 receptors leading the activation of the MAPK module (Ste11-Ste7-Fus3/Kss1) through intermediate G-proteins (Ste4, Gpa1, Ste18), the Pak Ste20, the scaffold protein Ste5 and the small protein Ste50.55 Pheromone specific gene expression is controlled by the TF Ste12. Some of the mating elements are also involved in the response to nutritional starvation inducing pseudohyphal or invasive mode of growth in diploid and haploid cells respectively,56,57 such as Ste20, Ste11 or Ste7 by a mechanism that does not involved Ste5. Transcriptional response is regulated through the shared TF Ste12 but also by Tec1.58 Upstream elements of the cascade are shared with other pathways. Sho1 is required for pseudohyphal invasive growth.59 Nice epistasis analysis revealed that the mucin-like protein Msb2 acts as a sensor of the pathway upstream Sho1 and both elements mediate the activation of Ste20.60,61 This pathway shares other elements that participate in the previously mentioned HOG cascade, which are the upstream elements Sho1 and Msb2 and Opy2 that ensure Ste11 activation through its recruitment to the plasma membrane through Ste50 and Cdc42.48,62 Elements of this route do also participate in the biogenesis of the cell wall through the so-called STE vegetative growth (SVG) pathway,63 essential when a glycosylation defect is present in the cell and promotes vegetative growth in normal (that is, non-stressed) cells.60,63

**Role for the Cek1 pathway in the response to osmotic and oxidative stress in *C. albicans***

The HOG pathway was first described because of its role in the adaptation to increases in external osmolality in *S. cerevisiae*. Under this stress, Hog1 becomes activated in a Pbs2-dependent manner64 as occurs in *C. albicans*,65 and this input comes from two different upstream branches.

Initial attempts to analyze the role of the Sho1-branch in *C. albicans* involved the identification and characterization of putative elements of the route, as many of them were functional in *S. cerevisiae*. Deletion of the SSK1 regulator does not block phosphorylation to Hog1 in response to osmotic stress.66 The transmembrane adaptor molecule Sho1, that was cloned by its ability to complement the osmotic sensitivity of *S. cerevisiae* ssk1 ssk2 ssk22 mutants, is not involved in activation of Hog167 and sho1 mutants are perfectly able to grow under high osmolality (both sorbitol and sodium chloride) and transmit the stimulus to Hog1 under these conditions.68 It however, plays an important role in the activation of Cek1 under a wide range of situations. These results suggested important functional differences between both organisms. It was later confirmed that CaSte11 is not involved in transmitting the signal to Hog1 upon osmotic stress and that deletion of CaSte11 has no evident phenotype when cells are exposed to either NaCl or sorbitol. In contrast, deletion of SK2 renders cells unable to grow under high concentration of NaCl or sorbitol and unable to activate Hog1 by phosphorylation under these conditions.69 In an attempt to identify upstream elements of the branch/route, the signaling mucin Msb2 was also cloned and epistatic analysis with SHO1 was made. MSB2 encodes a transmembrane protein that interacts with Sho1 in *S. cerevisiae* and is involved in Hog1 signaling.61 Interestingly, deletion of MSB2 either alone or in combination with SHO1, had no effect on Hog1 phosphorylation but it does in Cek1 activation.60

Based on these findings, it can be proposed that in *C. albicans* osmotic stress activates Hog1 via exclusively the Ssk1-branch and that the Sho1 branch is really involved in activating and transmitting the stimulus to Cek1, not playing a role in osmoadaptation. From a molecular point of view, it is also not clear how this occurs. A recent work from our group has characterized Opy2. In *S. cerevisiae*, Opy2 is a membrane-anchored protein that interacts with Ste50 and is involved in Hog1 activation.47 This is mediated by the presence of a specific region in ScOpy2 that becomes phosphorylated by the Yck1/2 kinases, allowing the interaction with Ste50.62 As expected, in *C. albicans* this protein does not participate in Hog1 signaling but rather in Cek1 signaling.70 Sequence analysis of CaOpy2 reveals that this protein is devoid of the Ste50-interaction phosphorylated motif, which could explain, at least partially, the situation. This interpretation still requires experimental confirmation. However, there are still certain incongruences in this scenario. Deletion of SHO1, MSB2 and even SSK1 has not effect in Hog1 phosphorylation69 as indicated; however, and most interestingly, cells are defective (almost similar to hog1 mutants) in growth under high osmolality which in turn, points towards a role for the Cek1 pathway in growth under osmotic stress pressure by a mechanism which is, apparently, independent of Hog1 activation. These cells are able to activate Hog1 by phosphorylation, activate a HOGI-gene reporter, and increase glycerol content (a compatible solute) upon osmotic stress. In other words, activation of Hog1 is not sufficient to sustain growth when certain elements of the Cek1-pathway are missing.

The HOG pathway is also implicated in the response to other stresses, such as oxidative stress.71,72 This type of stress is, of course, more meaningful for a host-interacting microbe that is continuously exposed to the...
The Cek1-pathway and morphogenesis

One of the important characteristics of _C. albicans_ in pathogenesis is its ability to switch between different morphologies. In the yeast ( unicellular) form, cells are able to undergo transformation into hyphal cells, leading to the formation of a mycelium. Such characteristics are high temperature, the presence of serum or different chemicals (like proline or N-acetyl glucosamine) or neutral pH. _C. albicans_ can also for pseudohyphal, morphological distinct from true hyphae and chlamydomspores which are thick walled structures with a largely unknown function. Morphogenesis is highly relevant in terms of pathogenicity as illustrated by several works where mutants unable to undergo the yeast-to-hypha transition are avirulent in certain animal models. This, however, also occurs with mutants that are permanently locked in the hyphal form and recent studies indicate that more than a specific morphogenetic state, it is the ability to switch between different morphologies what really favors adaptation to the host. This situation makes sense, as within infection (or dissemination) yeast and hyphal forms may differ in terms of terms of penetration, avoiding phagocytosis, proliferation or dissemination.

Different lines of evidence indicate a positive role for the Cek1-pathway in regulating filamentation in _C. albicans_. Cells lacking the PAK Cst20, the MAPKK Hst7 or the TF Cph1 are defective in hyphal formation on solid agar medium. Analysis of the Cpp1, a putative Cek1-phosphatase, revealed that _cpp1_ mutants are hyperinvasive, and deletion of _CEK1_ in _cpp1_ mutants suppresses this invasive phenotype suggesting this as a mechanism involved in controlling the intensity of the response via dephosphorylation of the MAPK. These studies, in any case, highlight the importance of well defining the experimental conditions used to assess morphogenetic switches in _Candida_. _cek1_ mutants are apparently normal in serum-induced liquid filamentation assays. A nutritional rich medium that is commonly used as test of filamentous formation. Such conditions differ, however, from solid medium, where cells are first restrained in movement and, second, nutrients may become limiting. Like Cat20, Hst7 and Cph1, Cek1 is also required for this type of agar-invasive hypha formation on different solid media such as Lee’s, Spider (mannitol as carbon source) or Synthetic Low Ammonium Dextrose Histidine (nitrogen limiting conditions). As it happens in _S. cerevisiae_, the Sho1 adaptor protein and the Msb2 mucin are upstream elements of this cascade and also play a role in agar invasion during starvation. These results suggest that Cek1 could indeed become activated under these conditions and, in fact, it has been described that Cek1 was phosphorylated, at least partially, via a membrane protein called Ht1. It has been recently shown how cathesins inhibit _C. albicans_ morphogenesis and this results in a decrease in hyphal specific _CEK1_-mediated gene expression as well as Cek1 phosphorylation. Also, subinhibitory concentrations of hydrogen peroxide activates pseudohyphal formation in a process that seems to be also partially dependent on this MAPK pathway.

In close contrast with the situation with _CEK1_, the HOG pathway plays an opposite role in _C. albicans_ morphogenesis. Under non-

### Table 1. Roles and stimuli related to the high osmolarity glycerol and Cek1 pathways in _C. albicans_.

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<thead>
<tr>
<th>Type of stress</th>
<th>Role of HOG and SVG pathways</th>
<th>Activation of the MAPKs</th>
<th>Differences with <em>S. cerevisiae</em></th>
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<tr>
<td>Osmotic</td>
<td>HOG pathway is essential for survival</td>
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<td>SVG is dispensable for survival</td>
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<td>Hog1 becomes phosphorylated</td>
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<td>Cek1 becomes desphosphorylated</td>
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<td>Only one MAPKK Ssk2</td>
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<td>No apparent role for the SHO1 branch</td>
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<tr>
<td>Oxidative</td>
<td>HOG pathway is essential for survival</td>
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<td>SVG is dispensable for survival</td>
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<td>Hog1 becomes activated</td>
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<td>Only one MAPKK Ssk2</td>
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<td>Ssk1 is essential for signaling</td>
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<td>No apparent role for the SHO1 branch</td>
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<td>Cell wall damage</td>
<td>Opposite but dependent roles:</td>
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<td></td>
<td>SVG is essential for survival</td>
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<td></td>
<td>SVG compensates defects in the HOG pathway</td>
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<td>Cek1 becomes activated</td>
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<td>Cek1 is constitutively phosphorylated in the absence of a functional HOG pathway</td>
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<td>Absence of a functional HOG pathway</td>
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<td></td>
<td>induces activation of the SVG pathway even in the absence of stimuli</td>
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HOG, high osmolarity glycerol; SVG, STE vegetative growth; MAPKs, mitogen-activated protein kinases; MAPKK, mitogen-activated protein kinase kinase kinase.
inducing conditions, the absence of Hog1 (MAPK) leads to enhance hyphal formation, a phenotype also observed with pbs2 mutants (MAPKK). As occurs with the hog1 mutant, cells lacking the TF SKO1 (a target of the HOG pathway in S. cerevisiae) also show an increased ability to form hyphae under non-inducing conditions. This correlates with transcriptional analysis that indicates repression of certain hyphal related genes (such as ECE1 or HWP1); interestingly, this repression was observed at both 30 and 37°C, while the repression of filamentation mediated by Sko1 was only observed at 37°C. An important difference with the situation in S. cerevisiae is that in C. albicans there is only one MAPKKK involved in this negative regulation (CaSSK2) and only Cask2 mutants, but not Castle11 mutants, are hyfamitileous. In agreement with this, the transcription profile of different filamentous growth-regulated targets is similar in ssk2 and hog1 mutants. Interestingly, however, the repression mediated by the HOG pathway is independent on the Cek1 MAPK. This was evidenced when the hyperfiamenation phenotypes of the hog1 mutants were still present when CLA4, CST20, HST7 or CPP1 were deleted.

It must be mentioned here that certain elements of the Cek1 pathway (CST20, CLA4, HST7, CEK1, CPH1 or CPP1) do not affect the production of chlamydospores, thick walled structures with unknown function in Candida. However, deletion of certain upstream elements of the Cek1 route restores the inability of hog1 mutants to form these structures. These data demonstrate that both routes (Cek1 and HOG) regulate in an independent and opposite manner two different morphogenetic processes in C. albicans (Figure 1).

### The Cek1 route as a sensor of cell wall damage and glycosylation

The fungal cell wall is an essential structure for fungal cells that changes continuously in response to the environmental and growth conditions. Not only regulates the shape and morphology of the cell but its integrity is essential for the survival in the host. Since mammalian cells lack this structure, it is a preferred target for new antifungals such as echinocandins. We provide here some recent findings that indicate a role for this route in modeling the cell wall.

Mutants of the SVG pathway (cek1, hst7, cst20, clau, msb2 and shol) are susceptible to compounds that alter biogenesis of the cell wall such as Congo red, calcofluor white, echinocandins or the glucanase-enriched zymolase. Defects in the cell wall, either by the addition of drugs or by specific mutations in cell wall genes (such as Och1), result in the activation of Cek1. Cek1 phosphorylation has been described to be mainly Sho1-dependent, since shol mutants blocked completely this activation under all stimuli described except for tunicamycin phosphorylation that is mainly driven through Msb2. In close contrast, the absence of SSK1, SSK2, PBS2 or HOG1, render cells with increased resistance to the presence in the media of these cell wall inhibitors. Mutants in the HOG pathway constitutively activate Cek1 and the absence of the Cpp1 phosphatase confers a resistant phenotype. This phosphorylation is completely abolished when we delete any of the elements of the SVG pathway (as it occurs in shol hog1, hst7 hog1 or cek1 hog1 double mutants) becoming susceptible to the presence of these compounds. This indicates that the Congo red resistance observed in mutants lacking a functional HOG pathway is probably due to a hyperactivation of the Cek1 mediated pathway.

The precise role of the Cek1 pathway in cell wall biogenesis is difficult to determine given the involvement of the two remaining cascades (Hog1 and Mkc1-dependent) and other signaling routes in this process. For example, the calcineurin pathway participates in regulating chitin synthesis upon a cell wall stress in coordination with the HOG and PKC MAPK pathways. The PKC1-pathway, regulated by the MAPK Mkc1 (homologous to ScSIT2), is essential for the integrity of the cell wall and becomes activated upon the addition of different cell wall inhibitors such as zymolase, calcofluor white, tunicamycin, etc., or due to gene deletions as it occurs in N-mannan mutants such as mns1. It is not surprising, also, that different morphological states differ in their cell wall and multiple hyphal specific genes have been described (as ALS3, ALS8, HWP1, HYR1, etc...), some of them being regulated by Efg1 at its transcriptional level. Multiple growth and stress conditions affect the cell wall composition and structure. The medium and growth conditions (low pH and temperature) and cell wall stress, due to the addition of external compounds or due to an internal mutation (as mnn9, pm1, bg1, phr1, etc...), renders in cell wall differences, C. albicans, a salvage mechanism similar to the one described in S. cerevisiae that becomes activated under cell wall stress, the so called compensatory mechanism, is expected to take place, resulting, among others, in an increased chitin level on the lateral cell wall that can be monitored by the integrity of this structure. In addition, the role of Cek1 has been recently shown to be influenced by the Hsp90 chaperone or the small Hsp21 which in turn indicates a tight temperature-dependent role for Cek1 in the response to cell wall damage and stress.

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![Figure 1](image-url) "The high osmolarity glycerol (HOG) and Cek1 pathways play opposite and independent roles in morphogenesis. Positive (induction, in red) and negative (repressive, in black) roles of the HOG and Cek1 routes in filamentous growth and chlamydospore formation. The corresponding elements are shown in hierarchical order, indicating also the triggering events and the corresponding responses."
dependent regulation of the pathway.

An important role for this pathway is emerging as sensor of glycosylation alterations. As occurs in S. cerevisiae, changes in glycosylation activate Cek1 and hyperactivation of the MAPK is observed when N or O-glycosylation is impaired. Either by mutants defective in this process or by the addition of certain chemicals. This activation is dependent, at least in the case of tunicamycin, on Msb2 and results in the induction of some PMT genes through the transcription factor Ace2. In S. cerevisiae, the cleavage and the secretion of the extra-cellular Msb2 N-domain by the aspartyl protease Yps1 is required for the activation of Msb2 and therefore for transmitting the signal to Kss1, with the secreted domain showing an inhibitory role. In C. albicans, mutational analysis reveal that the transmembrane domain of Msb2 is essential for signaling to Cek1, while a specific N-proximal domain also seems to play an inhibitory role as deletion of this region results in permanent hyperactivation of the route. It has been recently reported that in C. albicans, the secreted aspartyl protease Sap5 may be the processing protease responsible for Msb2 shedding. Therefore, Msb2 fulfills a clear role as a sensor of wall damage that connects this stimulus via Sho1 with a cell wall biogenesis cascade.

Upon tunicamycin addition, Cek1 is not only activated, but there is also a boost in the amount of Cek1. This is probably related to the fact that Cek1 function is regulated both post-translationally and by proteolysis degradation through the ubiquitin proteasome degradation pathway. Moreover, Cek1 is also regulated by proteasome and it has been suggested to be a short lived protein. This was demonstrated by using a conditional proteasome mutant (pre1) where Cek1, but not other MAPKs such as Hog1, was found to be faster degraded.

While a role for the Cek1 pathway in cell wall construction seems to be clear, the precise mechanism by which this takes place is not. At the molecular level, the surface of cek1 mutants is more leaky as revealed by electron microscopy studies (Romin E., unpublished data) and there are important differences in the mannan, similar to those showed by mutants in Chk1 or Sho1. Importantly, this results in an increased exposure of β-glucan, a major component of the cell wall. In yeast cells, glucan in normally hindered under the mannan layer and is not accessible to outer cells, being only accessible in bud scars. In fact, a genetic network has been recently described in S. cerevisiae where several signaling elements like Clu1 or Slt2 (the Mkc1 homologue) have been positioned. As bud scars are not present in true filaments, the switch from unicellular mode of growth to mycelium has been proposed as a morphological mechanism of escape from immune cells. Therefore, dimorphism may play a role in regulating the immune response. In fact, cekl and hst7 mutants show an altered β-glucan exposure; this, has functional consequences as the Dectin-1 mediated immune response is different in hDCs and transiently transfected Dectin 1 cells. This altered response could partially explain the defects in virulence associated to cek1 mutants. This statement may be a simplification of the real situation during infection, where several changes do take place, in particular as the result of treatment with the anti-fungals echinocandins, inhibitors of β-glucan synthesis.

**Concluding remarks**

It is now clear that while similar cascades exist both in S. cerevisiae and C. albicans and most elements are found in both organisms, important differences do exist. In particular, we provide in this review evidences from different research groups that reveal the role of the Cek1 cascade in morphogenesis and the regulation of cell wall remodeling rather than participating in the adaptation to osmotic stress. Why is this occurring? One can obviously invoke evolutionary reshaping of signaling pathways. It is conceivable that in its natural environment, S. cerevisiae may be more prone to experience osmolarity changes; this could occur in wine/grapes and the surface of other biological material where it can be exposed to subsequent cycles of dehydration. In contrast, C. albicans seems to have evolved closely linked to humans, and in this host, changes in osmolarity (up to 1M where the phenotype of hog1 is evident) are not normally encountered. It has been hypothesized that in the kidneys, increases in osmolarity are high, but this is not a common evolutionary niche for C. albicans, which is normally found in the gastrointestinal tract and vagina. Maybe C. albicans has uncoupled this branch from feeding into the HOG pathway and accommodated it in the Cek1 pathway, which is a more necessary event to accomplish cell wall biogenesis during the commensal status (see below). In this context, it must be also remembered that at the top of the Cek1 pathway lies a mucin that connects glycosylation defects with signaling events. However, the secreted portion of this protein is also an important defense mechanism against antimicrobial peptides such as LL-37 or histatins in vitro, which may play an important role in fungal infections. Such antimicrobial peptides are frequently encountered in habitats like the vaginal and oral/gastrointestinal mucosal surfaces where C. albicans normally inhabits. Evolutionary pressure may have connected both functions for adaptation to the host.

Conditions encountered by pathogenic fungi are therefore different from environmental organisms. MAPK routes have adapted by evolution in the way they sense, trigger and respond to these stimuli. Knowledge of them will allow exploiting the difference that exist between fungal and mammalian routes, an aspect that can be productive in terms of antifungal research in the future.

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