Comparative genomics of the environmental stress response in ascomycete fungi

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Abstract
Unicellular fungi thrive in diverse niches around the world, and many of these niches present unique and stressful challenges that must be contended with by their inhabitants. Numerous studies have investigated the genomic expression responses to environmental stress in ‘model’ ascomycete fungi, including Saccharomyces cerevisiae, Candida albicans and Schizosaccharomyces pombe. This review presents a comparative-genomics perspective on the environmental stress response, a common response to diverse stresses. Implications for the role of this response, based on its presence or absence in fungi from disparate ecological niches, are discussed.

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Keywords: ESR; environmental stress response; Sc_ESR; S. cerevisiae ESR; Sp_ESR; Sz. pombe ESR

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Introduction
The fungal kingdom consists of an estimated 1.5 million species whose diversity is matched by the heterogeneity of environments they inhabit [59]. The phylum Ascomycota represents the largest and most diverse group of fungi, and its members occupy diverse niches from around the globe. Free-living ascomycetes are commonly found in tree exudates, plant roots and surrounding soil, on ripe and rotting fruits, and in association with insect vectors that transport them between substrates. Many of these associations are symbiotic or saprophytic, although numerous ascomycetes (and their basidiomycete cousins) represent important plant pathogens that target a myriad of plant species, including commercial crops. Other species exist in close association with animal hosts, in either a commensal or a pathogenic framework. In nearly all of these niches, fungi comingle with other microbes in complex communities where resources are continually fluctuating. The ability to thrive in such intricate niches has been shaped by millions of years of evolution and likely driven by interactions between these species and their environment.

Many of the niches that fungi occupy are not constant, but rather their characteristics fluctuate frequently and suddenly, presenting stressful situations for their inhabitants. Free-living fungi often encounter the stress of nutrient limitation, changes in external temperature, osmolarity, humidity, pH, exposure to toxins present in the environment and competition with microbial cohorts. Pathogenic species must contend with the defence mechanisms of their host, in particular the reactive oxygen and nitrogen species generated by the immune system. Sudden changes in the external conditions can directly impact the internal environment of these
organisms and can disrupt homeostasis and normal physiology. Therefore, cells have evolved elaborate systems to monitor the features of their environment and rapidly mount defence systems against environmental stress.

Numerous studies have investigated environmental stress responses at the genomic level, using DNA microarrays to follow whole-genome expression in different organisms. By following the expression of every gene in the genome, each of these investigations has presented a global view of the physiology of stress defence. We now have the opportunity to compare and contrast fungal stress responses, thanks to the emergence of genomic studies in a variety of fungi. A comparative perspective can reveal the common features of stress defence that have been conserved over millions of years of evolution, reflecting their universal importance in surviving adversity. Conversely, responses that are specific to subsets of species highlight unique defence systems that may have been shaped by the particular habitats of those organisms.

Whole-genome expression studies have now been conducted in 'model' ascomycete fungi, including the budding yeast Saccharomyces cerevisiae, the human commensal fungus Candida albicans and the fission yeast Schizosaccharomyces pombe. Together, these studies reveal that each species responds to environmental changes with a great deal of precision in terms of the genes affected by each condition, the magnitude of their expression changes, and the kinetics of the response. However, in addition to these specialized responses to environmental stress, some species also respond with a common gene-expression response, referred to here as the environmental stress response (ESR). This response has been clearly conserved between the distant relatives S. cerevisiae and Sz. pombe, although features of the response and its regulation appear to have evolved. Less clear, however, is the conservation of such a response in C. albicans and other species. This review focuses on the comparative genomics of fungal ESR programmes, presenting commonalities and differences in the role and regulation of these responses in different fungi.

The environmental stress response

The ESR was originally described in S. cerevisiae as a set of ∼300 genes whose expression is induced and ∼600 genes whose expression is repressed in response to diverse types of stress, such as heat shock, oxidative or reductive stress, osmotic shock, nutrient starvation, DNA damage and extreme pH [20,49]. The induced and repressed genes show highly anticorrelated expression across these diverse conditions, in that the pattern of gene induction is roughly a mirror image of the pattern of gene repression. Many of the ESR genes had been previously implicated in stress defence (see below), and consistently the programme is only initiated in response to suboptimal conditions in budding yeast [49]. In fact, the magnitude and kinetics of ESR initiation appear to be graded to the severity of the stress, since cells exposed to a larger dose of stress often display larger changes in ESR gene expression. These characteristics indicate that initiation of the ESR is finely tuned to the level of stress experienced by the cell.

An orthologous response to the S. cerevisiae ESR (Sc_ESR) was subsequently identified in the fission yeast Sz. pombe. Chen et al [22] demonstrated that a common response to stress, including both induced and repressed genes, is initiated in fission yeast (originally called CESR, but referred to here as Sp_ESR for clarity) [22]. Indeed, many of the participating genes are direct orthologues of those induced and repressed in the Sc_ESR (Figure 1). Additional features of the ESR are also conserved between the two fungi, including the dynamics of ESR initiation, which is often somewhat transient. Immediately after acute stress, cells respond with large changes in gene expression, but these often subside to a new 'steady-state' level of expression over time. The transient initiation of the programme likely serves as an acclimation phase, during which time cells readjust their physiology to cope with the new conditions [49]. The similar dynamics of ESR initiation in S. cerevisiae and Sz. pombe reflect an additional level of similarity in their response to environmental stresses.

Functional classes of ESR genes

That the ESR is a response to stress is also suggested by the functions of the genes that participate in the response. Many of the genes induced in both the S. cerevisiae and Sz. pombe programmes are implicated in stress defence and include genes involved in carbohydrate metabolism (e.g.
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Figure 1. Expression of ESR genes and their orthologues in other species. The diagram shows the expression of the combined set of ESR genes defined in *S. cerevisiae* and *Sz. pombe* that have orthologues in two of the three species shown. The expression of 868 *S. cerevisiae* genes (left) along with the corresponding data for orthologues in *C. albicans* (middle) and *Sz. pombe* (right) was subjected to hierarchical clustering [33]. Data from replicate experiments were averaged. Each row in the diagram represents a gene expression profile, where red indicates induction and green represents repression in response to the denoted stress, listed above each time course experiment (indicated by coloured triangles; see original manuscripts for experimental details). Grey lines indicate missing data or lack of an orthologue in that species (data from [22,34,47,49,80]).

glycolysis, pentose-phosphate metabolism, glycerol metabolism, and trehalose synthesis), defence against reactive oxygen species (including thioredoxin, glutaredoxin, catalase, various antioxidant proteins, and genes involved in glutathione synthesis), protein metabolism (encompassing protein-folding chaperones and genes involved in ubiquitin-dependent degradation), intracellular signalling (see below), and a handful of genes implicated in DNA-damage defence and repair. Although much of the response is conserved between fungi, a small number of genes involved in fatty-acid metabolism were induced in the Sc_ESR but repressed in Sz_ESR responding to stress. The class of genes induced in the Sc_ESR is enriched for uncharacterized genes and substantially under-represented by genes essential for growth under standard conditions. This observation almost certainly reflects a bias in the study of gene function under standard laboratory conditions, as many of the ESR genes are essential under adverse conditions [50], and this underscores that much remains to be understood about the genes involved in stress defence.

An important class of genes induced in the Sc_ESR and Sp_ESR is involved in cellular signalling. Interestingly, genes encoding positive regulators and those encoding negative effectors of ESR initiation are induced as part of the programme. For example, the majority of genes induced in the Sc_ESR are regulated by the ‘general stress’ transcription factors Msn2p and Msn4p, through the stress-response element (STRE, CCCCT) found in the target genes’ upstream-regulatory regions [38,49,79,124]. Upon stress, *MSN4* expression is induced, in part by Msn2p binding of the *MSN4* upstream region, which contains STRE-like elements [56]. Both Msn2p and Msn4p, as well as broader ESR initiation, are
negatively regulated by protein kinase A (PKA) and TOR signalling [10,44,52,53,131], and genes encoding these regulators are also induced with the ESR. Positive regulators of these pathways (such as TOR1 and the PKA catalytic subunits TPK1 and TPK2) and negative effectors of the pathways (including the PKA negative regulator BCI, the phosphodiesterase PDE1 and the TOR/PKA antagonist YAK1 [45,54,58,78,131]) are also induced with the Sc_ESR. Correspondingly, in Sz. pombe the ESR includes genes encoding positive and negative effectors of PKA signalling, regulators of the ‘general-stress’ MAPK pathway that coordinates Sp_ESR activation, and the stress-regulated transcription factors Atf1 and Pcr1 [22]. Induction of these genes has been proposed to function as part of a feedback mechanism to properly modulate ESR activation in response to stress [22,49]. Strikingly, although Atf1 and Msn2/4p regulate the majority of genes induced in the Sp_ESR and Sc-ESR, respectively, the factors are not orthologous (see below). This illustrates that the feedback provided by inducing expression of these ESR regulators as part of the ESR arose through convergent evolution, underscoring the importance of this regulatory theme in stress survival.

In contrast to the genes induced in the ESR, which participate in a wide variety of functions, most of the genes repressed in these programmes are directly involved in protein synthesis. In both species, repressed-ESR genes encode ribosomal proteins, RNA processing and splicing factors, subunits of RNA polymerase I, II and III, and other general transcription and translation factors. Additional genes involved in growth-related processes (such as cell-cycle progression, secretion and metabolism) are repressed in both species. Consistent with these functions, the group of repressed-ESR genes in S. cerevisiae is enriched for essential genes and genes with orthologues in other species. Many of these genes are highly expressed in actively growing cells [61], and their stress-dependent repression may serve to conserve mass and energy while redirecting RNA polymerase to genes whose expression is induced by stress. One notable difference between the responses is the expression of amino acid and purine biosynthesis genes, which were identified as repressed Sp_ESR genes but show specific induction in S. cerevisiae responding to amino acid starvation. This discordance likely reflects the different environmental conditions used to define the ESR in the two species, as amino acid starvation was not included in the analysis to identify Sp_ESR genes.

Role of the environmental stress response

The diverse genes that participate in the ESR suggest that the programme protects and maintains multifarious features of the cellular system that are at risk during periods of stress. The delicate intracellular balance can be perturbed by fluctuations in the external environment, and the ESR may serve to maintain homeostasis in the face of variable conditions. Interestingly, many of the genes induced in the Sc_ESR are only required for cell survival under specific conditions, despite their common induction in response to diverse stresses [46,49,50]. This suggests that the ESR is initiated in part as a protective response to guard critical features of cellular physiology. Like many other organisms, yeast cells exposed to a mild dose of one stress can acquire resistance to a severe dose of the same or a different stress [13,41,63,72,73,83,85]; the ESR was proposed to account for this cross-stress protection in both S. cerevisiae and Sz. pombe [20,22,49]. Through a systematic investigation of this phenomenon, we have found that cross-stress protection is common in budding yeast, and when it occurs it is almost entirely dependent on the ESR transcription factors Msn2p and Msn4p (D. Berry and A. P. Gasch, unpublished data). Similarly, in Sz. pombe cells missing the transcription factor Atf1 cannot acquire the stress resistance normally afforded to starved cells or cells pretreated with peroxide [74,112]. Taken together, these results indicate that cross-stress protection is dependent on ESR regulators, strongly suggesting that the ESR accounts for acquired stress resistance in fungi.

While the ESR is often presented as a stress response, this perspective represents only one side of a two-sided coin. It is clear that initiation of the programme is triggered by suboptimal conditions and is required for stress survival. This increase in stress resistance comes at the cost of optimal cellular growth, however, as ESR activation is concurrent with decreased protein synthesis, transient cell-cycle arrest, metabolic alterations and inefficient consumption of glucose (reviewed [46]). In contrast, suppression of the ESR (i.e. high expression of protein synthesis genes and low expression of stress-defence genes) is associated with optimal
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cell growth, which occurs at the expense of stress tolerance. Thus, although initiation of the ESR is triggered by stress, suppression of the programme is required for competitive growth in the absence of stress or once cells have acclimated to the conditions. The opposing forces of stress defence and optimal growth are further emphasized by the anti-correlated expression of genes in these processes, a feature preserved in both S. cerevisiae and Sz. pombe.

Presence of an ESR in C. albicans?

The ESR is clearly conserved between S. cerevisiae and Sz. pombe, thought to have diverged >500 million years ago [11]. The presence of a common stress response is less obvious in C. albicans, however, which diverged from S. cerevisiae >200 million years ago [11]. An initial study by Enjalbert et al [34] revealed that C. albicans does not initiate a common response to mild stress under laboratory conditions; however, a subsequent study [132] identified a relatively small set of genes commonly affected by severe stress. Together, these studies indicate that C. albicans does not mediate a large common-stress response under the conditions studied to date. However, numerous observations suggest a functional link between C. albicans orthologues of ESR genes. First, many of these genes show weak but consistent similarities in stress-dependent expression compared to their Sc_ESR orthologues (Figure 1), especially the genes repressed by stress. Second, the set of C. albicans orthologues of induced-Sc_ESR genes is enriched with statistical significance for genes containing upstream elements related to the S. cerevisiae STRE [48]. Correspondingly, the group of orthologues of repressed-Sc_ESR genes is enriched for genes containing two upstream elements implicated in Sc_ESR regulation, known as PAC (GCGATGAG) and RRPE (AAAAWTTTT) [30,48,49,62]. The enrichment of these upstream elements is highly unlikely to have occurred by chance and is unlikely to have been maintained through evolution if not functional, suggesting that features of the genes’ regulation are conserved between S. cerevisiae and C. albicans. Finally, genomic studies of PKA signalling in C. albicans reveal that cells lacking cAMP show reduced expression of protein synthesis genes and elevated expression of numerous stress-defence genes [57], reflecting the same inhibitory relationship between PKA signalling and ESR initiation seen in S. cerevisiae. Together, these details suggest a functional association between C. albicans orthologues of Sc_ESR genes, one that may be related to stress defence.

Why C. albicans does not initiate an ESR under conditions that activate the response in other species is not clear, but may be linked to the specific niche of this species [34]. The dimorphic fungus exists as part of the mucosal flora of its human host, in both yeast and hyphal forms. Immunocompromised individuals are susceptible to pathogenic infection, which requires fungi to switch between the yeast and hyphal states [81,84]. In fact, numerous environmental features of the host can trigger this morphological transition, including changes in temperature, pH, nutrient levels and serum exposure, among others [36]. Thus, C. albicans may have evolved to respond to such conditions by triggering a change in cellular states, whereas S. cerevisiae and Sz. pombe initiate the ESR to maintain cellular homeostasis. That the C. albicans orthologues of ESR genes share the same upstream sequence motifs raises an alternative possibility, that C. albicans does mount an ESR-like response, but only under limited conditions or in a specific morphological state. Most laboratory studies are conducted on the amenable yeast form grown under standard conditions; the response to stress in hyphal cells or mixed cultures has not been investigated on a global scale. Finally, it is formally possible that the ESR was largely lost in C. albicans some time after the species split from the S. cerevisiae lineage. Distinguishing between these possibilities will require genomic investigations of stress defence throughout the natural life cycle of C. albicans.

Regulation of the environmental stress response

ESR regulation in Sz. pombe

The ESR represents a common response to stress, and therefore a logical mode of ESR regulation would be to control the response with the same signalling components under many different conditions. This regulatory logic represents the model of ESR regulation in Sz. pombe. The stress-responsive
Figure 2. The Hog1–Sty1 pathways in budding and fission yeast. Proteins involved in Hog1 signalling in \textit{S. cerevisiae} (left) and Sty1 signalling in \textit{Sz. pombe} (right) are shown. Some intermediate proteins and additional Hog1-dependent transcription factors have been omitted for simplicity. Dashed arrows represent conditions that lead to Hog1p phosphorylation in the absence of detectable nuclear Hog1p (see text for references).

MAP kinase Sty1p (also known as Spc1 and orthologous to the human stress-activated kinase p38) is activated by diverse stresses, including heat shock, oxidative and osmotic stress, radiation, DNA-damaging drugs, heavy metals, starvation and other conditions (Figure 2) \cite{28,29,65,82,127,134,136}. Consequently, cells lacking \textit{sty1} are sensitive to a wide variety of stressful conditions. Sty1 is activated by dual threonine/tyrosine phosphorylation mediated by the upstream MAPKK, Wis1 (Figure 2) \cite{82,127,139}. Upon phosphorylation by Wis1, Sty1 translocates to the nucleus where it phosphorylates Atf1 and other transcription factors, which then regulate changes in gene expression \cite{42,43,71,128,141}. Chen \textit{et al} \cite{22} showed that nearly all of the genes induced and repressed in the \textit{Sp}_ESR require Atf1 for proper expression; however, genes repressed in the \textit{Sp}_ESR are largely independent of Atf1, revealing the presence of additional Sty1-dependent factors.

The mechanism of Wis1–Sty1 activation is not entirely clear; however, it seems to involve multiple upstream branches that play different roles under different conditions. One upstream branch utilizes a two-component regulatory system involving the histidine kinases Mak1/2/3, the relay protein Mpr1, and the response regulator Mcs4 \cite{17,88,92}. Activation of this branch of the pathway leads to phosphorylation of two MAPKKKs, Wis4 and Win1, which in turn phosphorylate Wis1 and consequently activate Sty1 \cite{118,122,126,129}. Interestingly, the two-component regulators are important for Wis4 and/or Win1 activation in response to low doses of oxidative stress, but apparently not other conditions, implicating additional inputs into the pathway \cite{17,112,118}. Wis4 and Win1 are partially
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Figure 3. Dependence of ESR initiation on the Sty1/Hog1 pathways. (A) Average expression of Sp_ESR genes is shown in wild-type and sty1- cells 15 min after 0.5 mM hydrogen peroxide treatment, 15 min after 30 °C to 39 °C heat shock, 15 min following 0.5 mM cadmium chloride exposure, and 60 min after treatment with 0.02% MMS. The average expression is shown for genes induced in the Sp_ESR (iESR), ribosomal protein genes (RP) repressed as part of the ESR, and remaining protein synthesis genes (PS) repressed as part of the ESR (data from [22]). (B) The average expression of iESR, RP and PS genes in the Sc_ESR is shown in wild-type and hog1 ∆ cells 20 min after exposure to 0.5 M potassium chloride (data from [99]).

Although some aspects of ESR regulation are similar between Sz. pombe and S. cerevisiae, regulation of the ESR seems to have diverged significantly. First, although S. cerevisiae has an orthologue of the transcription factor Atf1, called Sko1p, this regulator is only activated in response to osmotic shock in budding yeast [90,110,116,138]. Both Atf1 and Sko1p bind CRE elements (TGACGTCA) upstream of their target genes and can repress a set of genes in the absence of stress [22,25,28,64,90,101,138]. Both proteins are also converted to activators under inducing conditions, which also trigger phosphorylation by Sty1 or its orthologue in S. cerevisiae, Hog1p [28,87,110,111,141]. However, whereas Atf1 regulates many of the genes induced in the Sp_ESR under diverse conditions [22], Sko1p regulates a smaller set of genes in response to osmotic shock, and most are not part of the Sc_ESR [56,108]. These results show that, although Atf1 and Sko1p are conserved in these fungi, as are details of their activation, their roles in stress defence and the set of genes they regulate have significantly diverged.

Divergent regulation of the ESR in budding and fission yeast

Although some aspects of ESR regulation are similar between Sz. pombe and S. cerevisiae, regulation of the ESR seems to have diverged significantly. First, although S. cerevisiae has an orthologue of the transcription factor Atf1, called Sko1p, this regulator is only activated in response to osmotic shock in budding yeast [90,110,116,138]. Both Atf1 and Sko1p bind CRE elements (TGACGTCA) upstream of their target genes and can repress a set of genes in the absence of stress [22,25,28,64,90,101,138]. Both proteins are also converted to activators under inducing conditions, which also trigger phosphorylation by Sty1 or its orthologue in S. cerevisiae, Hog1p [28,87,110,111,141]. However, whereas Atf1 regulates many of the genes induced in the Sp_ESR under diverse conditions [22], Sko1p regulates a smaller set of genes in response to osmotic shock, and most are not part of the Sc_ESR [56,108]. These results show that, although Atf1 and Sko1p are conserved in these fungi, as are details of their activation, their roles in stress defence and the set of genes they regulate have significantly diverged.

In S. cerevisiae, the function of Atf1 seems to be supplanted by the transcription factors Msn2p and/or Msn4p (Msn2/4p) [38,79,124]. These Cys2
Hisz zinc finger proteins reside in the cytosol in the absence of stress but rapidly relocate to the nucleus in response to suboptimal conditions, triggered by changes in their phosphorylation patterns [10,52,53]. Once in the nucleus, the factors induce gene expression through upstream STRE elements (CCCCT) in the promoters of their targets [79,124]. Interestingly, the set of Msn2/4p targets is enriched for genes containing STREs, as well as other C-rich upstream sequences distinct from the published STRE that may represent alternative binding sites for the transcription factors [48]. Sz. pombe apparently lacks orthologues of Msn2/4p, consistent with the idea that the transcriptional induction of Sp_ESR and Sc_ESR genes has diverged. It should be noted that additional, condition-specific regulators are also required for full induction of Sc_ESR genes (reviewed [46]); however, their relationship to Msn2/4p activity is not well understood.

Regulation of the Sc_ESR may also have evolved at the level of the upstream signalling pathways that control the response. Like all characterized fungi, S. cerevisiae has an orthologue of Sty1 known as Hog1p (Figure 2) [15,68]. Hog1p is also activated through dual phosphorylation by an upstream MAPKK (Pbs2p) that triggers Hog1p nuclear localization and interaction with transcription factors at target-gene promoters and with the elongation complex within open reading frames [2,3,14,27,40,109,111,115]. The activity of numerous transcription factors is dependent on Hog1 activity (including Sko1p, Msn2/4p, Smp1p, Hot1p and Msn1p) [2,3,26,115–117]. In addition to regulating gene expression, both Sty1 and Hog1p also control cell-cycle progression under stressful conditions [4,23,37,82,102,127,146], and both have been linked to sexual development (Sty1 is required for mating and meiosis in Sz. pombe [65,128,141], whereas Hog1p suppresses the mating pathway under osmotic shock conditions in S. cerevisiae [55,97,126,147]).

Despite these shared features, Sty1 and Hog1p seem to play significantly different roles in general-stress defence. The lore had been that Hog1p is only activated by osmotic shock (and related conditions that similarly affect membrane fluidity and/or turgor pressure [60,100,114]), since hog1Δ cells were not reported to be particularly sensitive to unrelated stresses and little Hog1 phosphorylation was originally reported under those conditions [16,125]. However, recent evidence indicates that Hog1p can be phosphorylated (albeit to lower levels than after osmotic stress) by diverse stresses, including heat shock, oxidative stress, weak-acid stress, heavy metal toxicity and starvation [6,12,26,60,70,100,130,133,142]. This is reminiscent of the multi-stress activation of Sty1 in Sz. pombe. Surprisingly, however, many of these stresses fail to activate detectable nuclear accumulation of Hog1p, despite increased phosphorylation of the kinase [12,133,142], raising questions as to Hog1p’s role in gene activation under these conditions. Even during osmotic shock, when Hog1p is required for cell survival, expression of Sc_ESR genes is only partially dependent on the kinase (Figure 3B). This is in contrast to the case in Sz. pombe, where expression of Sp_ESR genes is heavily dependent on Sty1. Together, these results reflect significantly different dependence on Sty1/Hog1p for ESR initiation in the two species.

One possible explanation for this difference is that the route to Hog1p activation has diverged in the two species. As with the Sty1 pathway, Hog1p can be activated by a two-component regulatory system involving the single histidine kinase in budding yeast, Sln1p, its response regulator Ypd1p, and the two-component regulator Ssk1p, although considerable differences exist in the functions of these systems in the two fungi [75,76,107]. Under non-stress conditions in S. cerevisiae, constitutive Sln1p activity represses Ssk1p, and this essential function is required to prevent the lethality of constitutive Hog1 activation [76,107]. Specific stresses inhibit Sln1p activity, thereby relieving Ssk1p inhibition and allowing it to phosphorylate the MAPKKK proteins, Ssk2p and Ssk22p, that lie upstream of Pbs2p [75,105,135]. The majority of conditions that increase Hog1p phosphorylation do so through the Sln1-dependent branch of the pathway (Figure 2), whereas in Sz. pombe this branch is largely specific to oxidative stress [60,70,99,100,114,130,133]. In addition, an alternative, well-characterized mode of Hog1 activation occurs via the Ste20–Ste11 phosphorylation cascade in S. cerevisiae [75,98,104,106]. These kinases are activated by at least two upstream membrane sensors, Sho1p and Msb2p [98,99,106,113]. Sho1p is activated by high osmolarity and heat.
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shock, perhaps in response to regional cell-surface defects, given Sho1’s localization to sites of polarized growth [99,106,113,114,142]. Msb2p is another membrane-bound sensor that interacts with Sho1p, although a role for Msb2p in the response to high osmolarity has only been observed in the context of ssk1∆sho1∆ cells [24,98]. No known homologues of Sho1p or Msb2p exist in Sz. pombe, [68] and the Sz. pombe orthologues of Ste20p and Ste11p are not reported to be sensitive to stress. However, the Sz. pombe orthologues of both the Ste20p and Ste11p kinases are required for mating and meiosis, as are Wis1 and Sty1, providing a functional link between these kinases [51,65,77,91,128,141,143]. Components of the Ste20–Ste11 branch perhaps serve as the alternative, as-yet unidentified route to Sty1 activation in fission yeast.

Numerous lines of evidence suggest that the Sc_ESR is not generally regulated by the Hog1 pathway in S. cerevisiae, which raises questions concerning how the response is coordinated in budding yeast. Current indications suggest that S. cerevisiae regulates the ESR with different signalling pathways under different conditions. The clearest example is seen for the Mec1p pathway in budding yeast. This signalling cascade coordinates gene-expression changes and cell-cycle arrest in response to DNA damage [120]. Microarray studies have shown that Mec1p and the downstream kinase Dun1p are required for proper initiation of the Sc_ESR in response to DNA-damaging conditions, but not in response to heat shock [47]. Other signalling pathways have been implicated in the condition-specific regulation of sets of ESR genes, including PKC, Snf1, HOG, PKA, TOR and others (reviewed [46]). Many of the conditions that activate these pathways also activate Msn2/4p, suggesting that these condition-specific pathways may converge by regulating the transcription factors. We have also recently found a common role for the histone deacetylase Rpd3p in coordinating genes induced and repressed in the Sc_ESR (Alejandro-Osorio et al., submitted). Rpd3p was previously shown to induce the expression of number of genes in response to salt and heat stress [27]. Consistent with the details presented above, the function of Rpd3p at these promoters is dependent on Hog1p in response to osmotic shock, but not in response to heat shock [27]. This further underscores the condition-specific role of Hog1p in Sc_ESR regulation and the importance of additional upstream regulatory pathways in initiating the programme.

Common themes in ESR regulation

Despite the significant differences in ESR regulation between S. cerevisiae and Sz. pombe, a number of common themes emerge. First, both species utilize some combination of condition-specific regulators (e.g. different upstream activators of Sty1/Hog1p or distinct signalling pathways in S. cerevisiae) and ‘general-stress’ factors that function under diverse conditions (such as Sty1-Atf1 in Sz. pombe or Msn2/4p and Rpd3p in S. cerevisiae). This logic supports precision in how cells monitor environmental features, by allowing multiple, overlapping sensory systems to activate common regulators that guarantee ESR initiation under diverse circumstances. This network organization also imparts robustness to the system, making ESR initiation less sensitive to loss of any one of the multiple pathway inputs [67]. A second theme in ESR regulation is positive and negative feedback, as presented above. Inducing the expression of genes encoding ESR regulators probably promotes the precise modulation of ESR activation in a manner insensitive to subtle environmental fluctuations. At the same time, combined positive and negative feedback would provide a mechanism for rapid ESR suppression once cells have acclimated to the conditions or when the stress has been removed. The latter is especially important for successful competition for limited resources in complex, microbial niches.

A third commonality in ESR regulation is that subsets of ESR genes can be controlled by condition-specific transcription factors in addition to the general factors discussed here. One clear example exists for ESR genes involved in oxidative stress defence in budding and fission yeast. In response to reactive oxygen species, these genes are induced by transcription factors specific to oxidation (including Pap1 in Sz. pombe and Yap1p in S. cerevisiae). However, in response to diverse other conditions, the same genes are controlled by the ESR regulators Atf1 or Msn2/4p in Sz. pombe and S. cerevisiae, respectively [49,92,112,136]. Similar conditional regulation exists for other subsets of ESR genes [7,117,137]. This situation provides condition-specific precision in the fine details of...
how the ESR is initiated. Finally, both responses appear to be negatively regulated by PKA signalling [22,25,44,78,89]. As PKA signalling is associated with optimal growth in both species, ESR suppression may be similarly governed by this pathway in the absence of stress.

**Orthologous regulators in C. albicans**

Many of the regulators discussed above have also been implicated in stress defence in *C. albicans*, most notably Ca_Hog1. Cells lacking this kinase are sensitive to a panel of stresses (including osmotic shock, oxidative stress, heavy metal treatment, antifungal drugs, and caffeine) that all lead to increased Ca_Hog1 phosphorylation [6,86,123,132]. Cells lacking *Ca_HOG1* also fail to alter the expression of the few genes implicated in the Ca_ESR in response to multiple conditions, and mutant cells cannot acquire resistance to severe stress after mild-stress treatment [35,132]. These phenotypes suggest that Ca_Hog1p plays a role in general-stress resistance, similar to Sty1 in *S. pombe*. The mutant also shows impaired virulence, likely affected by its inability to survive the reactive oxygen burst of the host immune system [5,6]. The defect in virulence may be exacerbated by aberrant morphological switching in the *Ca_hog1* mutant: these cells show derepression of hyphal-specific genes and more readily switch to the hyphal state [5]. Sc_Hog1 is known to suppress activation of the mating pathway during osmotic stress [97] and perhaps Ca_Hog1 plays a similar role in suppressing signalling that activates hyphal expression.

As in both *S. cerevisiae* and *S. pombe*, Ca_Hog1 can be activated by multiple upstream branches (reviewed [69]). One branch consists of at least one of the three histidine kinases, Ca_Sln1, and its downstream effector Ca_Ssk1 [18,19,21,86,145]. As in *S. cerevisiae*, constitutive Ca_Sln1 activity largely represses Ca_Ssk1 and therefore Ca_Hog1, although unlike in budding yeast, deletion of *Ca_SLN1* is not lethal and a small amount of phosphorylated Hog1 exists even in unstressed cells [86,132,145]. A second upstream branch of the Hog1 pathway utilizes the *C. albicans* orthologue of Sho1p [119]. This result suggests that the stress-responsive role of Sho1 is common to *C. albicans* and *S. cerevisiae*, even though no such role exists in more distantly related species [68].

Although Ca_Hog1 plays a clear role in stress defence, the regulators that function downstream of the pathway are not known. *C. albicans* has an orthologue of Sc_Msn4p and a second protein with homology to Sc_Msn2p, called Mnl1; however, these proteins are not required for general-stress tolerance under standard laboratory conditions. Nicholls *et al* [94] demonstrated that a strain lacking both factors shows no increase in stress sensitivity and no defect in stress-dependent gene expression. Although Ca_Msn4 could weakly mediate STRE-dependent induction in an *msn2Δmsn4Δ S. cerevisiae* strain, Ca_Msn4 did not induce expression from the STRE-containing reporter in *C. albicans* [94]. Recently, however, Mark Ramsdale and Alistair Brown (personal communication) have demonstrated an important role for Mnl1 in the response to weak acid; cells lacking Mnl1 are sensitive to this stress and fail to properly induce expression of genes containing a modified STRE element in their upstream regions. These results imply a stress-defensive role for the transcription factor Mnl1p, although features of its activity appear to have diverged from its counterpart in *S. cerevisiae*.

**Prospect of ESR-like responses in other fungi**

Hints of a common stress response exist in other fungal species, by nature of shared phenotypes of mutant strains. For example, strains of the euascomycetes *Neurospora crassa*, *Magnaporthe grisea* and *Aspergillus* species that lack the respective orthologue of Hog1 were each shown to be sensitive to a panel of diverse stresses, suggesting a role for Hog1 orthologues in general-stress defence, similar to that played by Sty1 in *S. pombe* [31,32,66,96,148]. To date, there has been no whole-genome analysis of general-stress responses in these species. However, a recent study characterized genes induced by reactive nitrogen species in the euascomycete *Histoplasma capsulatum* [95]. Few of the identified genes were also induced by other stresses; however, one notable and intriguing exception was the orthologue of the Sp_ESR transcription factor Atf1, raising the possibility of a conserved role in general-stress defence.

The orthologue of Hog1 in the human pathogen *Cryptococcus neoformans* also plays a role in stress
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resistance, mating and virulence. The general-stress-responsive role of the kinase is evident from the sensitivity of \( Cn \_hog1 \) cells to osmotic stress, heat shock, UV exposure and oxidative stress [8]. Strains lacking the kinase also show reduced virulence in a mouse model [8], similar to the reduced virulence seen in hog1 mutants of \( C. \) albicans. Interestingly, \( C. \) neoformans serotypes of varying virulence show corresponding differences in Hog1 activity [9]. Serotype A strains show high basal levels of Hog1 phosphorylation, which probably accounts for the increased resistance of this strain to stress (and perhaps also its elevated virulence). In response to stress, Hog1 is rapidly dephosphorylated in this serotype, although the kinase still transits to the nucleus under these conditions. In contrast, strains of the less virulent serotype D show low levels of basal Hog1 phosphorylation and a rapid increase in phosphorylation upon stress, coupled with its nuclear localization. These results highlight the importance of this signalling pathway, and the importance of stress defence, in pathogenesis.

Concluding remarks

The ESR represents a common gene expression response to stress, one that has been conserved in multiple fungi in the billion years of independent evolution since \( S. \) cerevisiae and \( Sz. \) pombe diverged. The conservation of this programme implicates its importance to the lifestyles of these fungi. Both budding and fission yeast exist in variable environments in the wild, and stressful environmental changes may occur simultaneously or in tandem. The acquired stress resistance provided by ESR initiation has likely provided a significant competitive advantage to these fungi. Evidence suggests than ESR-like responses may exist in other ascomycete fungi, with clear differences in when and how the responses are initiated. It will be particularly interesting to uncover how these differences correspond to the natural environments of the fungi, which may implicate the selective pressures that have contributed to ESR maintenance or loss through evolution.

Despite conservation of the ESR in \( S. \) cerevisiae and \( Sz. \) pombe, however, regulation of the ESR has clearly diverged. The two species utilize different transcription factors to coordinate the ESR, and the Sty1/Hog1 pathways play different roles in general-stress vs. condition-specific regulation. How this regulatory network was free to evolve in the face of strong selective pressure to maintain the ESR is not clear. Considering the phylogeny of these species and the roles of the p38/Sty1/Hog1 kinases in each organism strongly suggests that the ancestral role of the pathway was one related to general-stress resistance. The Hog1 pathway may have specialized along the \( S. \) cerevisiae lineage into one activated only by limited stressful conditions. Elucidating the evolutionary history of this pathway will serve as a useful model for the evolution of signal transduction in general.

The comparative analysis presented here also emphasizes the importance of Hog1 and stress resistance in the virulence of pathogenic fungi, including \( C. \) albicans, \( Aspergillus \) fumigatus and \( C. \) neoformans [69,144]. The involvement of Hog1 may be through initiation of the ESR, or could reflect an additional function of Hog1, unrelated to regulating gene expression [140]. Interestingly, the role of the general-stress response in virulence has been clearly demonstrated in a variety of pathogenic bacteria [1,39,103]. Thus, the importance of such general-stress responses in pathogenicity is a feature present across kingdoms. A deeper understanding of these responses, including features that are conserved or have evolved across species, will contribute to our knowledge of and ability to control pathogenicity, as well as a broader understanding of how organisms sense and respond to environmental stresses.

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