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Review

Anticipatory Stress Responses and Immune Evasion in Fungal Pathogens

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In certain niches, microbes encounter environmental challenges that are temporally linked. In such cases, microbial fitness is enhanced by the evolution of anticipatory responses where the initial challenge simultaneously activates pre-emptive protection against the second impending challenge. The accumulation of anticipatory responses in domesticated yeasts, which have been termed 'adaptive prediction', has led to the emergence of 'core stress responses' that provide stress cross-protection. Protective anticipatory responses also seem to be common in fungal pathogens of humans. These responses reflect the selective pressures that these fungi have faced relatively recently in their evolutionary history. Consequently, some pathogens have evolved 'core environmental responses' which exploit host signals to trigger immune evasion strategies that protect them against imminent immune attack.

Highlights

Fungal pathogens have evolved anticipatory behaviors that protect against imminent challenges in the host, such as immune attack.

These anticipatory behaviors are reminiscent of core stress responses in model yeasts, in that they exploit one type of signal to mount protective responses against a second type of signal that is likely to follow the first.

Anticipatory immune evasive responses differ between fungal pathogens, probably because these behaviors evolve rapidly depending on the nature of the selective pressures these fungi face and the energetic costs of the protective response.

Anticipatory immune evasion represents a potential target for antifungal therapy as blocking this response exposes the fungus to immune clearance and can attenuate fungal virulence.

Introduction

Fortunately, of the millions of fungal species that inhabit our planet [1], only several hundred fungal species cause disease in humans [2]. These have arisen within the Ascomycota, Basidiomycota, and Zygomycota [3], and this evolutionary diversity presents significant challenges for the diagnosis and treatment of potentially lethal invasive fungal infections [4]. The incidence of these infections has increased in recent decades, largely because of the rising numbers of patients undergoing procedures that render them immunocompromised and, consequently, susceptible to systemic fungal infection. Even with therapy, mortality rates for these infections remain extremely high, with some reaching over 50% [4]. More effective antifungal therapies are required to address this challenge [4], as well as to address the threat of new emerging fungal pathogens [5].

The diversity of lifestyles displayed by pathogenic fungi also represents a significant challenge for developing broad-spectrum antifungal therapies. Some pathogens normally live in the environment, and yet have evolved traits that make them potent opportunistic pathogens (e.g., *Aspergillus fumigatus*). Some are normally associated with animal reservoirs, but can cause systemic infections even in healthy individuals (e.g., *Cryptococcus*, *Histoplasma*, and *Coccidioides* species). Others exist as commensals in the microbiota of healthy individuals but can cause infection when immune defenses or the local microbiota become compromised (e.g., *Candida* species). Certain fungal pathogens have become so specialized that they are now obligately associated with a specific mammalian host (e.g., *Pneumocystis* species). Despite the diversity of their lifestyles, to colonize humans, all fungal pathogens, with the possible exception of *Pneumocystis,* require flexible nutrient adaptation, robust stress responses, and the ability to evade our immune defenses. These fitness attributes are integral to their pathogenicity [6,7].

In some cases, fungal pathogens have evolved anticipatory protective responses that are thought to enhance their fitness in the host [8]. These anticipatory responses, which have been called 'adaptive prediction' [9], evolve in microbes that occupy reasonably predictable niches where

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one type of environmental challenge is frequently followed by a second. This predictability allows the microbe to exploit the first signal to activate a pre-emptive response that protects against the second imminent challenge (Figure 1). Hence, anticipatory responses represent an evolutionary 'memory' of recent selective pressures that the fungus has faced [8]. An understanding of these selective pressures, and their corresponding adaptive responses, will highlight potential points of fragility in fungal pathogens that represent effective targets for novel antifungal therapies. Therefore, we discuss emerging information about anticipatory protective responses in fungi and their evolution. We suggest that these behaviors represent different types of core environmental response, and that they might be targeted therapeutically.

Classical Stress Responses

Anticipatory protective responses are generally based on classical responses to environmental stress. All forms of life must adapt effectively to environmental stresses, such as changes in temperature, water balance, pH, and redox status. Consequently, fungi have inherited evolutionarily ancient mechanisms for dealing with such stresses, and their dissection in model yeasts, such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, underpins our understanding of corresponding responses in pathogenic fungi. The sensitivity to the input signal and the robustness of the response differ between fungal species, but in general, the nature of the response and core regulatory circuitry are conserved (Figure 2A). However, in many pathogens, the regulation of stress responses and virulence has become entwined.

The heat-shock response is characterized by the induction of heat-shock proteins, which promote protein disaggregation, recycling and refolding, and the restoration of proteostasis. The response, which leads to induced thermotolerance, is regulated by an autoregulatory circuit involving the heat-shock transcription factor Hsf1, and the chaperone Hsp90 [10]. Interestingly, in *Candida albicans,* Hsp90-Hsf1 regulates genes involved in virulence as well as thermal adaptation [11]. Clearly, the heat-shock response has become integrated with the regulation of cellular morphogenesis and other key virulence factors in *C. albicans* and in other fungal pathogens [10,12].

Maintaining osmohomeostasis is also essential for fungal survival. The regulation of intracellular osmolyte concentrations and turgor pressure is dependent on the evolutionarily conserved stress-activated protein kinase (SAPK: Hog1 in *S. cerevisiae* and *C. albicans*; Sty1 in *Sz.*

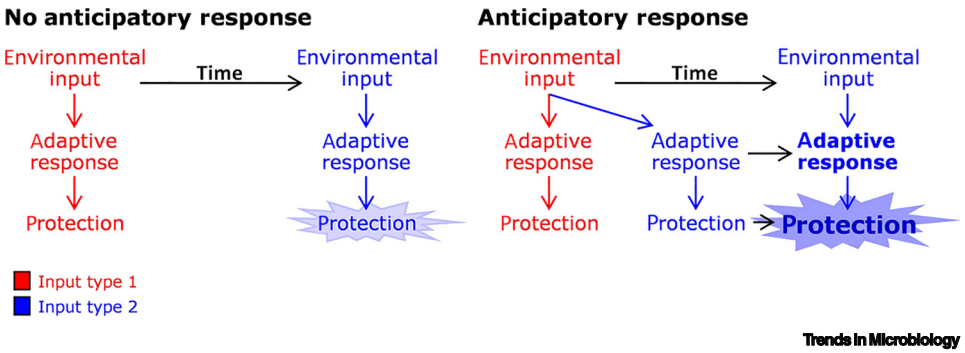


Figure 1. Anticipatory Responses Enhance Protection. In a niche where an environmental input of one type (red) is often followed by a second type (blue), in the absence of an anticipatory response (left panel) the two corresponding adaptive responses act independently to provide a degree of protection. In those microbes that have evolved an anticipatory response (right panel), the response to the second impending environmental input is activated following exposure to the first input, even before the cells are exposed to the second environmental input (e.g., adaptive prediction [9]). This can improve fitness and survival by enhancing the degree of protection against the second environmental input [9].

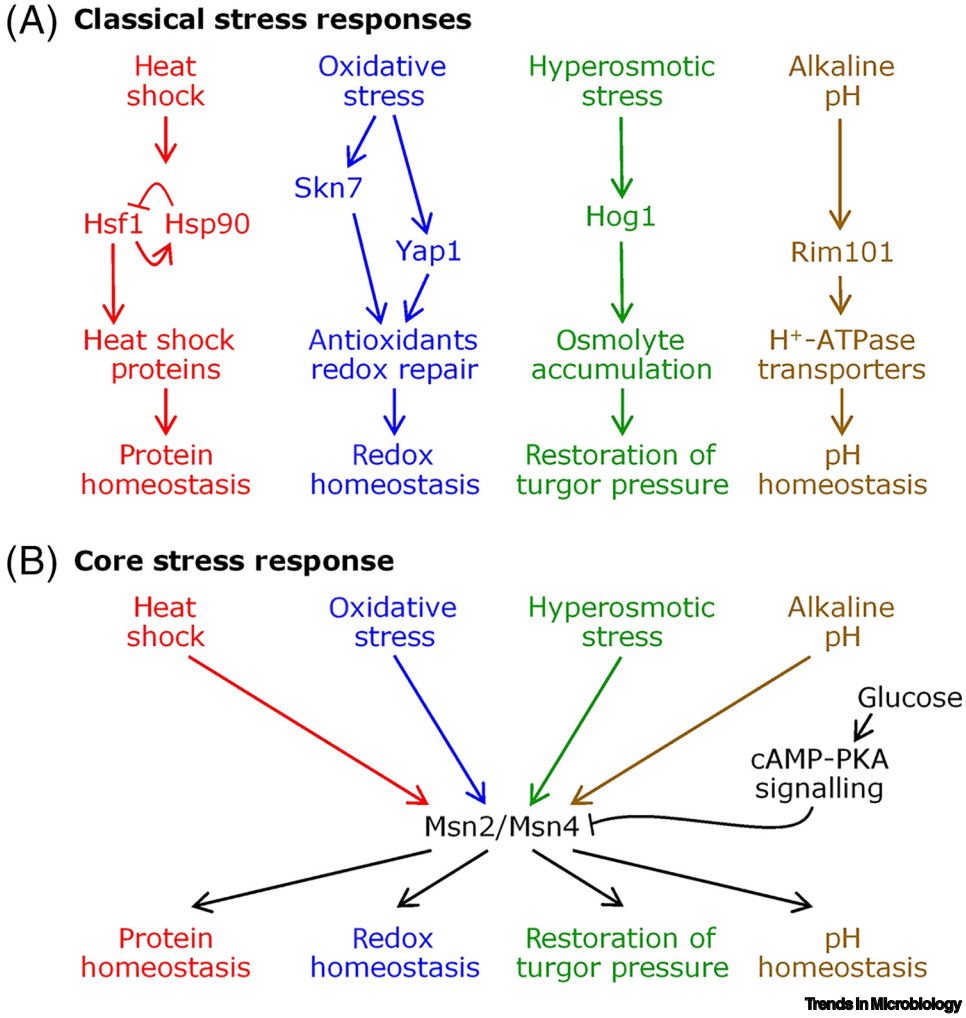


Figure 2. Classical versus Core Stress Responses. (A) Examples of evolutionarily conserved classical stress responses and regulators in *Saccharomyces cerevisiae* (see main text). The heat-shock response is regulated by an autoregulatory circuit involving Hsf1 and Hsp90 (and/or Hsp70) [10,110] and leads to the activation of heat-shock proteins, such as chaperones, that restore protein homeostasis. The oxidative stress response is regulated by Yap1 and Skn7, and results in the synthesis of reactive oxygen species (ROS)-detoxifying enzymes, antioxidants, and redox repair [111,112]. Hyperosmotic stress activates Hog1 signaling, leading to the accumulation of osmolytes and the restoration of turgor pressure [113]. Alkaline pHs trigger truncation and activation of Rim101 (PacC) and regulate the plasma membrane H^+-ATPase as well as transporter synthesis and activity. (B) In *S. cerevisiae*, these stresses also activate the core stress response (CSR), which involves the activation of stress genes containing STRE elements [19,33], leading to the upregulation of many of the functions also activated by classical stress regulators (A). The CSR is downregulated by glucose via cAMP-PKA (protein kinase A) signaling [34].

pombe; SakA in *A. fumigatus*). Osmoregulation involves crosstalk between mitogen activated protein kinase (MAPK) and cAMP-protein kinase A (PKA) signaling in fungal pathogens [13,14], and in *C. albicans* Hog1 promotes resistance to additional stresses such as oxidative, heavy metal, and cell wall stresses [15]. Significantly, Hog1/SakA signaling influences key virulence factors and enhances virulence in *C. albicans, Cryptococcus neoformans,* and *A. fumigatus* [16–18]. Therefore, this evolutionarily conserved pathway plays key roles in fungal pathogenicity as well as environmental adaptation.

Fungi respond to oxidative stress by inducing genes involved in the detoxification of reactive oxygen species (ROS) and the repair of oxidative damage [19–22]. The transcriptional response is coordinated by evolutionarily conserved AP-1-like transcription factors and response regulators. The AP-1-like factors include Yap1 in *S. cerevisiae, A. fumigatus*, and *C. neoformans*, Pap1 in *Sz. pombe*, and Cap1 in *C. albicans*; and response regulators include Skn7 in *S. cerevisiae* and *C. albicans*. In *Sz. pombe*, Pap1 is activated by the Sty1 SAPK, but in *C. albicans*, Cap1 is activated independently of the Hog1 SAPK [21,23]. Therefore, while these stress pathways have been conserved, links between them have diverged.

Responses to pH are significant for pathogenic fungi in niches such as the lung, mucosal and gastrointestinal compartments, and during survival in the phagosome/phagolysosome. Orthologous transcription factors drive responses to alkaline pH in yeasts (Rim101) and filamentous fungi (PacC) [24,25]. Significantly, Rim101/PacC signaling plays major roles in fungal pathogenicity, over and above imparting pH tolerance. In *C. albicans*, Rim101 upregulates genes involved in hyphal development, adhesion, and virulence [26,27]. In *A. fumigatus*, PacC controls tissue invasion during pulmonary aspergillosis [28]. In *C. neoformans,* Rim101 signaling modulates melanin production, capsule synthesis, and titan cell formation [24].

Clearly, key signaling pathways involved in pH, oxidative, osmotic, and thermal stress adaptation have become integral to the regulation of virulence factors in major fungal pathogens of humans.

Anticipatory Stress Responses

Studies of 'classical' stress responses are defining fungal adaptation mechanisms for specific stresses under defined laboratory conditions. However, host niches are complex and dynamic: complex in that fungal pathogens are exposed to multiple stimuli, and dynamic in that these stimuli change over time. It is important to understand how adaptation to one environmental input affects subsequent responses to other inputs, as these effects can be dramatic [25,29].

Adaptation to a mild stress provides transient protection against a subsequent acute dose of the same stress by activating a so-called 'molecular memory' [30], one example of which is induced thermotolerance. In some cases, one type of stress can provide protection against subsequent exposure to a different stress, yielding stress cross-protection. For example, in *S. cerevisiae,* exposure to thermal, salt, or nutrient stress protects against subsequent freezing [31], and salt protects against subsequent oxidative stress [32]. This cross-protection is mediated by the core stress response (CSR).

The CSR was first revealed by genome-wide transcriptional profiling of *S. cerevisiae* cells exposed to diverse environmental insults. This revealed a core set of genes that is commonly induced by different stresses, which include heat-, oxidative-, osmotic-, and pH-stress genes, as well as carbohydrate metabolism and energy-generating genes [19,33]. This response is activated by the transcription factors Msn2 and Msn4 [19,33], and is downregulated by cAMP-PKA signaling [34] (Figure 2B). The evolutionarily related pathogenic yeast, *Candida glabrata*, exhibits an analogous CSR that is also regulated by Msn2 and Msn4 [35]. *Sz. pombe* is evolutionarily distant from these yeasts, but also displays a CSR. However, this CSR is dependent on the Sty1 SAPK (Hog1 orthologue) and Atf1, rather than Msn2/4 orthologues [20]. *A. fumigatus* also displays a core response to heat, oxidative, and osmotic stresses [36] which is partially dependent on SakA (Hog1 orthologue) signaling [37]. Therefore, evolutionarily diverse fungi display CSRs, but there are differences in the regulatory circuitry involved.

Intriguingly, some pathogenic fungi display large CSRs (e.g., *C. glabrata* and *A. fumigatus*), but others do not (e.g., *C. albicans*). *C. albicans* displays minimal overlap between the gene sets induced by oxidative, osmotic, thermal, and heavy-metal stresses, and minimal cross-protection between these stresses [21,38]. Furthermore, in *C. albicans,* Msn2/4 homologues play no obvious roles in heat, osmotic, ethanol, or nutrient responses [39]. This raises interesting questions. How did CSRs evolve? If the fitness advantages offered by stress cross-protection drove the evolution of CSRs in many fungi, why not in *C. albicans*?

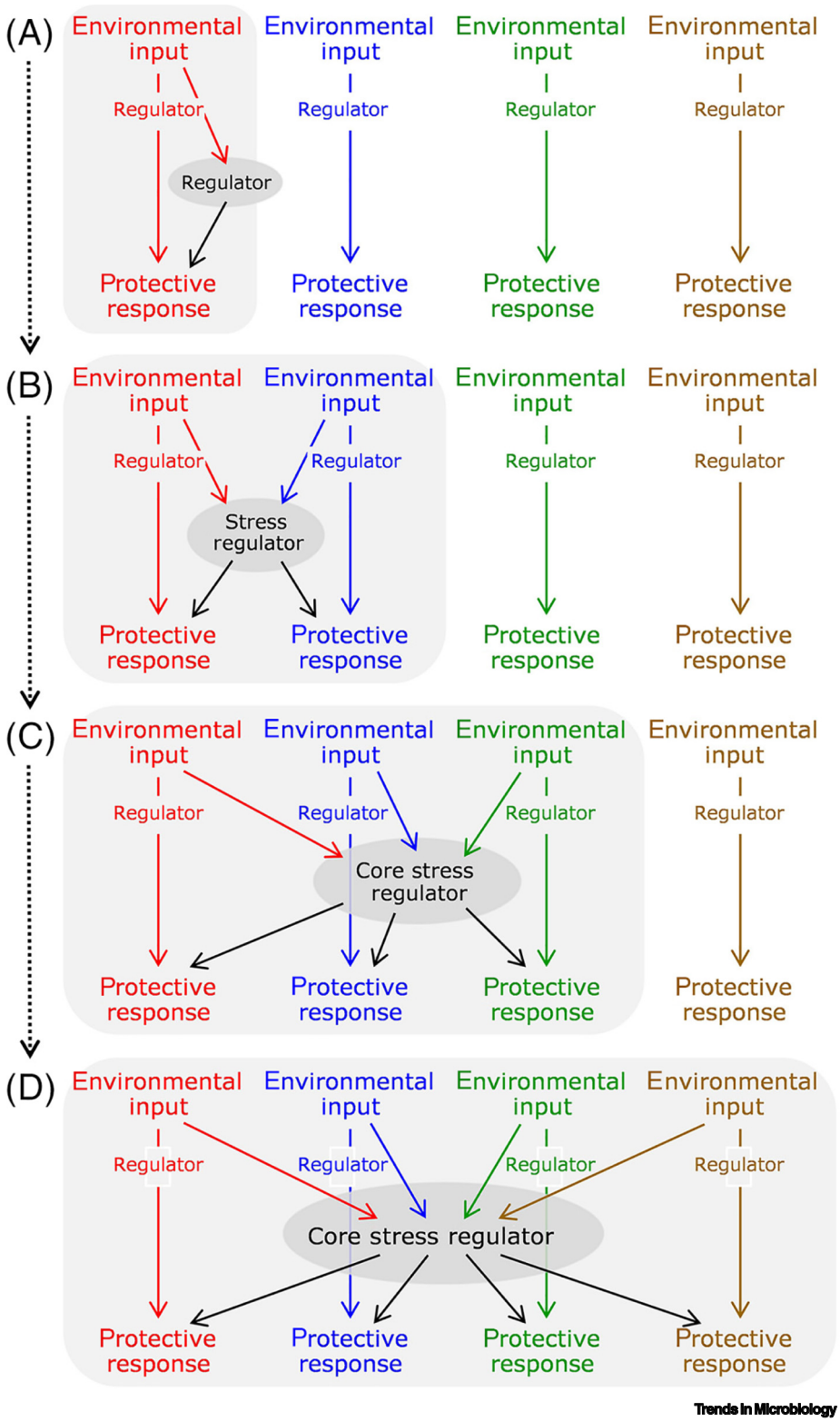
CSRs are energetically demanding [40,41]. Therefore, CSRs are not likely to have arisen in one step, in their entirety. Rather, CSRs probably emerged in a stepwise fashion, through the accumulation of individual protective responses [41] (Figure 3). During their domestication, *S. cerevisiae* cells probably evolved regulatory circuitry that permitted anticipation of impending environmental challenges, which has been termed 'adaptive prediction'. For example, fermentation leads to a rise in temperature, which is followed, predictably, by a switch from fermentative to respiratory growth and, consequently, an increase in intracellular ROS. Therefore, *S. cerevisiae* 'learned' to exploit thermal stress to activate oxidative stress resistance even before ROS levels increase [41]. *S. cerevisiae* can rapidly evolve anticipatory behaviors, within 50–150 generations [42]. Therefore, anticipatory protective responses can arise rapidly in niches that change in a reasonably predictable manner.

Therefore, the development of anticipatory protective responses probably underlies the development of CSRs in domesticated yeasts, such as *S. cerevisiae* and *Sz. pombe* [19,20,33], and in environmental fungi, such as *Aspergillus* species [36]. Why then does one pathogenic *Candida* species display a large CSR (*C. glabrata* [35]) whereas another does not (*C. albicans* [21,38])? The answer probably lies in the nature of the selective pressures that these species have faced in their recent evolutionary histories.

Classical Immune Evasion

Host immunity provides a major barrier to colonization by opportunistic fungal pathogens [43,44]. Innate immune cells detect fungal cells by recognizing pathogen-associated molecular patterns (PAMPs) on the fungus via host pattern-recognition receptors (PRRs) [45,46]. Host cells express a variety of Toll-like and C-type lectin receptors that recognize mannan, β-glucan, melanin, and chitin in fungal cell walls. The recognition of β-glucan by dectin-1 plays a major role in the activation of proinflammatory responses [47,48]. Mannan and melanin also simulate inflammation, whereas chitin exerts anti-inflammatory effects [49–51]. However, antifungal immune responses are influenced by the spatial organization of these components in the fungal cell wall [52] and cooperativity between the host receptors that recognize them [49,53]. Proinflammatory PAMP–PRR interactions lead to the phagocytic uptake of the fungus, phagolysosomal maturation and acidification, and the bombardment of the fungus with reactive chemical species and other toxic molecules [43]. Cytokine signaling promotes the recruitment of additional innate immune cells to infection sites and, in the longer term, activation of adaptive immunity [54].

Primary fungal pathogens, such as *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, and *Cryptococcus gattii,* must either evade or subvert immune clearance mechanisms. Immune evasion strategies are also relevant to opportunistic fungal pathogens, such as *C. albicans, C. glabrata*, *A. fumigatus*, and *C. neoformans* [4]. Of these, *C. albicans* is intriguing because it is generally thought to be associated with warm-blooded animals [55] and is carried as a commensal in the gastrointestinal and urogenital tracts of many healthy individuals [56,57]. Therefore, this opportunistic pathogen must have evolved effective immune evasion strategies. We divide these into 'classical' and 'anticipatory' immune evasion



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strategies, the latter being triggered before immune cells are encountered. However, this division may be less clear than inferred.

Fungal pathogens exhibit three types of classical immune evasion strategy: 'attack', 'control', and 'stealth' [58,59]. Fungal attack involves the secretion of molecules that actively counteract immune components. These include the induction of superoxide dismutases (SODs) that detoxify host-generated ROS, expression of secreted aspartate proteases that degrade host proteins [60–62], and the secretion of the toxin, candidalysin, by *C. albicans* [63]. Hypha formation helps *C. albicans* to escape from, and kill, phagocytes through the induction of the NRLP3 inflammasome via candidalysin or pyroptosis [64–66]. *C. albicans* can also kill macrophages through metabolic competition for glucose [66]. Meanwhile, *A. fumigatus* attenuates immune cell function by synthesizing gliotoxin [67] and by shedding galactosaminogalactan, which induces neutrophil killing through the activation of natural killer (NK) cells [68].

Fungal control involves the suppression or manipulation of immune defenses [58,59]. For example, *C. neoformans*, *C. albicans*, *C. glabrata*, *Candida krusei*, and *H. capsulatum* have developed mechanisms that inhibit phagosomal maturation, allowing them to thrive within phagocytes [69–72], and some fungi can escape phagocytic cells via nonlytic expulsion [73,74]. *C. albicans* synthesizes farnesol, which attenuates cytokine production by macrophages [75], and has also evolved mechanisms that inhibit fungal killing by the complement system [76].

Stealth is where the fungus evades immune recognition, thereby avoiding phagocytic attack [58,59]. *C. albicans* activates yeast–hypha morphogenesis, which reduces the ease with which immune cells can phagocytose the fungus, partly through reduced exposure of proinflammatory PAMPs [77] and partly through difficulties in engulfing long fungal filaments [78]. However, macrophages that attempt to engulf lengthy hyphae can form 'frustrated phagosomes' which, while not fully engulfing the hypha, can inhibit its growth [79].

Similarly, *C. neoformans* evades phagocytosis by forming large titan cells [80,81], and by synthesizing a thick polysaccharide capsule that masks immunostimulatory cell wall components [82]. Similarly, other pathogens mask immunostimulatory components in their cell walls: *C. albicans*, *H. capsulatum*, and *A. fumigatus* all mask β-glucan and melanin with mannan, α-glucan, and the rodA hydrophobin, respectively [83–85].

Clearly, evolutionarily divergent fungal pathogens have evolved an array of strategies to evade immune clearance.

Anticipatory Immune Evasion

As described earlier, some fungi have evolved anticipatory responses that protect them against impending stresses [9]. We argue that certain immune evasion strategies also represent anticipatory behaviors because, in these cases, the fungus activates the response before encountering immune cells.

Figure 3. Core Stress Responses Probably Evolved via the Accumulation of Anticipatory Protective Responses. (A) Four hypothetical environmental inputs trigger evolutionarily conserved stress pathways, each with its own specific regulator (red, blue, green, brown), to activate input-specific responses. The red pathway has an additional regulator (black). Over evolutionary time (broken lines from A to B to C to D), additional stress pathways come under the control of the black regulator: (B) blue pathway; (C) green pathway; and (D) brown pathway. In the process, the black regulator evolves from a stress-specific regulator into a core stress regulator capable of providing stress cross-protection. At each stage (from A to B to C to D), the shaded boxes illustrate the sequential addition of pathways that come under the regulation of this core stress regulator during the evolution of this hypothetical core stress response.

Some forms of anticipatory immune evasion represent fungal attack. For example, *C. albicans* upregulates oxidative stress genes in response to glucose [25]. Plasma levels of glucose are sufficient to enhance resistance to acute oxidative stress, and to protect the fungus against subsequent exposure to neutrophils. Therefore, glucose triggers an anticipatory response that actively counteracts immune components (ROS) to protect *C. albicans* against impending phagocytic attack [6]. The early activation of efficient micronutrient-scavenging mechanisms by *C. albicans* during hyphal development [86,87] also appears to represent an anticipatory response, as the fungus prepares for iron- and zinc-limiting environments before the hypha penetrates tissue to enter these environments [88]. Similarly, the induction of *SOD* genes during hyphal growth [62] protects the invading hypha in anticipation of impending immune attack and ROS exposure [89].

Other forms of anticipatory immune evasion are more representative of fungal stealth. For example, *C. neoformans* exploits elevated carbon dioxide or bicarbonate concentrations to activate melanin and capsule production, which provides protection against imminent immune attack [90]. *C. albicans* exploits other host signals, such as lactate, hypoxia, iron limitation, and neutral pH, to activate masking of the proinflammatory PAMP, β-glucan [91–95]. This β-glucan masking reduces phagocytic recognition and attenuates immune responses against the fungus [91,93,94,96]. β-Glucan 'masking' is mediated by the exoglucanase, Xog1, which 'shaves' exposed β-glucan from the cell surface [97]. Some other, but not all, pathogenic *Candida* species display hypoxia-induced β-glucan masking, but there is no obvious relationship between phylogeny and phenotype [93]. Meanwhile, an analogous anticipatory response has arisen in the evolutionarily distant species, *H. capsulatum*. This fungus activates the Eng1 endoglucanase in its pathogenic yeast form, but not in the saprobic hyphal form, leading to reduced β-glucan exposure and attenuated immune detection [98].

Clearly, certain pathogenic fungi have evolved mechanisms by which they can exploit specific host signals to trigger anticipatory responses that protect against impending immune attack. The lack of phylogenetic clustering for β-glucan masking phenotypes, for example, is consistent with the idea that these anticipatory responses have evolved recently in evolutionary terms, in response to temporally related, niche-specific, selective pressures.

Parallels between Anticipatory Responses

There are ineluctable parallels between CSRs and anticipatory immune evasion. Both provide protection against an imminent challenge, albeit an environmental stress in one case and phagocytic attack in the other. Both must provide fitness advantages that outweigh the energetic cost of activating the pre-emptive response [40,41]. In both cases, this energetic cost must impose a selective pressure to disarm the anticipatory response should the fitness benefits no longer outweigh the cost, that is, should the fungus evolve a new niche. This could explain the lack of a CSR in *C. albicans*.

In which case, why does another opportunistic pathogen, *C. glabrata,* retain a CSR [35]? *C. glabrata* is more closely related to *S. cerevisiae* than to *C. albicans* [99]. However, given the speed with which anticipatory responses appear to evolve [42] this is unlikely to account for their CSR differences. Instead, unlike *C. albicans, C. glabrata* might have retained an environmental reservoir [100] in which a CSR provides a fitness advantage. Alternatively, *C. glabrata* might have evolved (or retained) a CSR because its infection strategy differs from that of *C. albicans* [88,101]. *C. albicans* generally persists in the host by evading phagocytotic attack. By contrast, *C. glabrata* is able to survive phagocytosis and replicate within macrophages [88,101], a strategy presumably enhanced by the CSR.

Concluding Remarks and Future Perspectives

To summarize, anticipatory protective responses are widespread in fungi. Examples have been reported in domesticated yeasts, environmental fungi, and fungal pathogens. In some cases, the accumulation of anticipatory protective responses appears to have led to the emergence of CSRs, and in others to the accumulation of pre-emptive immune evasion mechanisms. In essence, anticipatory stress responses (CSRs) and anticipatory immune evasion represent different types of core environmental response (Figure 4). *C. albicans*, *C. glabrata*, and many other contemporary fungal species simply display core environmental responses that have been evolutionarily tuned to the corresponding niche.

The elaboration of core environmental responses is of interest both academically and translationally (see Outstanding Questions). Anticipatory protective responses appear to evolve with alacrity, emerging rapidly within specific niches, or fading once their energetic costs outweigh their fitness benefits. Therefore, anticipatory protective responses probably reflect, with reasonable accuracy, the selective pressures that the organism faces within the niche where it has recently evolved. For some fungi, this niche might be obvious, but for others, this is not the case. For example, *C. albicans* has been isolated from the environment [55,102–104], but these isolations might represent contamination by a warm-blooded animal. Does *C. glabrata* occupy an environmental niche, as suggested by bioinformatic studies [100]? Unbiased examination of their anticipatory protective responses would provide valuable clues about the niches in which these human commensals have actually evolved. Similarly, the definition of anticipatory protective responses in newly discovered fungi would provide valuable insights into their niches and lifestyles. Such responses could be probed by phenotypic screening for cross-protection, for example.

An understanding of anticipatory protective responses also offers translational opportunities. For example, dissecting the mechanisms that underlie fungal immune evasion will reveal strategies by which this immune evasion might be blocked. This has been demonstrated for lactate- and hypoxia-induced β-glucan masking in *C. albicans,* where pharmacological inhibition of secreted exoglucanase has been shown to increase the immune visibility of *C. albicans* and reduce its

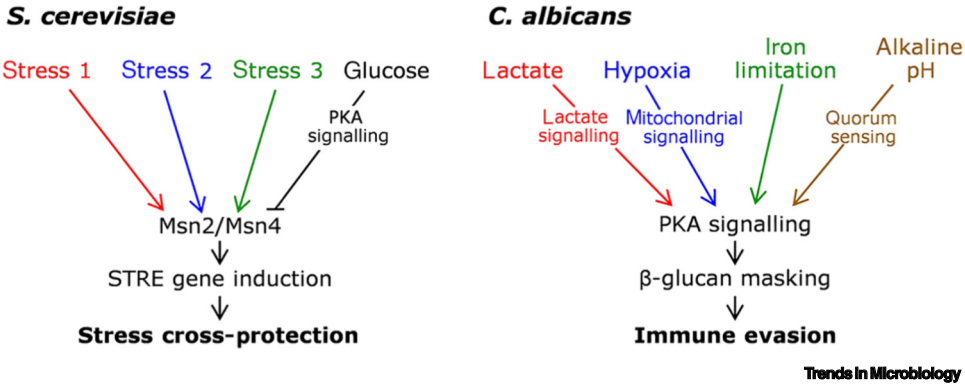

Figure 4. Comparison of Core Environmental Responses in *Saccharomyces cerevisiae* and *Candida albicans*. The core stress response in *S. cerevisiae* is essentially a core environmental response that, through Msn2/Msn4- and protein kinase A (PKA)-mediated signaling, controls the expression of core stress genes containing the STRE element to drive anticipatory responses that promote stress cross-protection (see main text). *C. albicans* displays a different type of core environmental response that exploits specific host signals (lactate, hypoxia, iron limitation, and alkaline pH) to trigger β-glucan masking and, consequently, immune evasion. These anticipatory responses are triggered by signal-specific upstream pathways, which converge on PKA signaling, to protect against impending immune attack [91,93–95]. These core environmental responses reflect the niches in which these domesticated and pathogenic yeasts have recently evolved (see main text).

Outstanding Questions

- What are the net fitness benefits of anticipatory immune evasion in particular niches? For example, to what extent does β-glucan masking enhance the colonization of *C. albicans* in mucosal niches, in tissues, and in the bloodstream. Does β-glucan masking provide protection after phagocytosis by innate immune cells?
- Does the core stress response enhance the fitness of *C. glabrata* after phagocytosis by innate immune cells?
- Does anticipatory immune evasion protect against amoebic predation, and hence offer fitness benefits in environmental niches?

The answers to these questions will help to reveal the cost-benefits of certain types of anticipatory response in pathogens with different lifestyles.

- How rapidly can anticipatory responses emerge in a diploid fungus like *C. albicans*?
- How quickly are anticipatory responses lost once the temporal links between the driving selective pressures are lost?

Addressing these questions will help us to understand the rate at which anticipatory protective responses can emerge and be lost.

- What types of protective anticipatory behavior are present in newly discovered fungal species, or in fungal species whose niche evolutionary has not been well defined?

Given the speed with which protective anticipatory behaviors emerge, an understanding of these behaviors would shed light on the selective pressures that have been faced by a fungal species in their recent evolutionary history, and hence provide valuable clues about their niche.

- Do small-molecule inhibitors of anticipatory immune evasion enhance immune recognition and clearance in animal models of fungal infection?
- Do such inhibitors enhance therapy when used in combination with antifungal drugs in clinical use?

These questions address the potential therapeutic benefits of targeting anticipatory immune evasion.

virulence [97]. Therefore, drugs that block fungal immune evasion [97], or that promote exposure of proinflammatory PAMPs by modulating cell wall remodeling pathways [105], might prove useful for antifungal therapy. Such drugs could conceivably be useful for the treatment of systemic infections, but would not assuage immunopathological conditions such as vulvovaginal candidiasis or psoriasis, for example [106,107]. By analogy, agrochemicals that block anticipatory protective responses in crop pathogens, or enhance their exposure of molecular signatures that trigger plant immunity, could potentially help to address threats to food security. The lifestyles of plant and human pathogens differ significantly, and therefore the nature of their anticipatory protective responses is likely to differ too. Hence, the development of an agrochemical that blocks these responses is unlikely to compromise antifungal therapy in the clinic. This would be infinitely preferable to the agricultural usage of azoles, which has led to the emergence of drug-resistant strains of *A. fumigatus* in the clinic [108,109].

Acknowledgments

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