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The relevance of heat shock regulation in fungal pathogens of humans

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Despite being obligately associated with warm-blooded animals, *Candida albicans* expresses a *bona fide* heat shock response that is regulated by the evolutionarily conserved, essential heat shock transcription factor Hsf1. Hsf1 is thought to play a fundamental role in thermal homeostasis, adjusting the levels of essential chaperones to changes in growth temperature, for example in febrile patients. Hsf1 also regulates the expression of Hsp90, which controls the yeast-hypha transition in *C. albicans*, and we argue, might also control morphogenesis in other fungal pathogens of humans.

Key words: heat shock response, Hsf1, Hsp90, transcriptional regulation, fungal pathogenesis, fungal morphogenesis

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Numerous observations link the heat shock response with the pathogenicity of the major fungal pathogen of humans, *Candida albicans*. Firstly, yeast-hypha morphogenesis is considered to be a virulence attribute and mild heat shock is integral to most experimental conditions that are used to stimulate hyphal development in vitro.[1-3] Secondly, the induction of heat shock protein expression is associated with morphogenesis,[3,4] which is not surprising given the concomitant temperature upshift used to promote hyphal development. Thirdly, heat shock proteins are found at the *C. albicans* cell surface and these proteins are immunogenic during infections.[5-9] Fourthly, immunization with Hsp70 appears to sensitise experimental animals to systemic Candida infection, reducing their survival time apparently by hyperactivating their immune systems.[8] Fifthly, in contrast to the situation with Hsp70, autoantibodies against Hsp90 are immunoprotective, providing a degree of protection against subsequent intravenous infection with *C. albicans*,[10] and this has underpinned the development of a new antifungal therapeutic, Mycograb.[11] Therefore heat shock proteins have long been of interest to the medical mycology community.

Studies of the regulation of other stress responses in *C. albicans* have indicated that key stress regulators have been evolutionarily conserved in this pathogen compared with its benign cousin, the model yeast *Saccharomyces cerevisiae*. However, in some cases these stress regulators have been rewired such that their cellular roles have changed. For example, the Hog1 stress activated protein kinase primarily regulates osmotic stress responses in *S. cerevisiae,* whereas it also contributes to oxidative, heavy metal and cell wall stresses, and responses to antifungals and quorum sensing molecules in *C. albicans*.[12-14] Furthermore, the transcription factors Msn2 and Msn4 play key roles in the core stress response in *S. cerevisiae,* unlike their homologues in *C. albicans*.[15,16] In contrast the AP-1-like transcription factors Cap1 and Yap1 both play central roles in the oxidative stress responses in both *C. albicans* and *S. cerevisiae,* respectively.[17,18]

Given this background, we became intrigued by the heat shock response in *C. albicans*. Rather naively, we wondered whether a *bona fide* heat shock response is evolutionarily conserved in this fungal pathogen, which is apparently obligately associated with warm-blooded animals.[1] Therefore, we tested this using a combination of reductionist and genome-wide approaches, confirming that *C. albicans* does express a *bona fide* heat shock response, activating HSE-(heat shock element)-containing genes in response to an acute thermal upshift via the evolutionarily conserved heat shock transcription factor, Hsf1.[19]

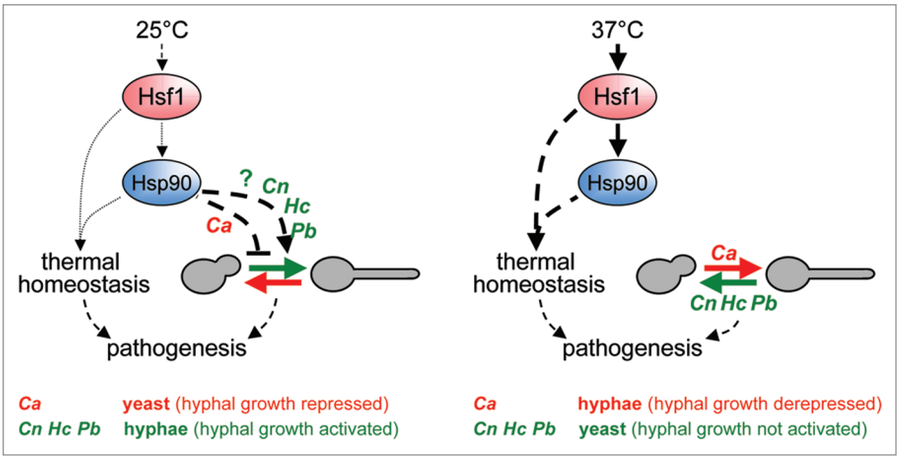

Figure 1. Speculative model linking the regulation of Hsp90 by Hsf1 to morphogenesis in fungal pathogens. Hsf1 activates Hsp90 in response to temperature upshifts and promotes thermal homeostasis in *C. albicans* via Hsp90 and other factors.[19] Hsp90 negatively regulates hyphal development in *C. albicans* (Ca).[24] Temperature upshifts also induce *HSP90* expression in *C. neoformans* (Cn), *H. capsulatum* (Hc) and *P. brasiliensis* (Pb)[31-33] and promote yeast growth rather than hyphal development in these fungi. This raises the possibility that Hsp90 might also regulate morphogenesis in these pathogenic fungi (see text). According to this model, at 25°C free Hsp90 would inhibit hyphal development in *C. albicans* (such that it grows in the yeast form), but promote hyphal growth in the other pathogens. Following a temperature shift to 37°C, Hsp90 would become sequestered for protein folding thereby mediating thermal adaptation. This sequestration would lead to the dissociation of Hsp90 from its as yet unidentified client on the Ras-PKA pathway, thereby derepressing hyphal development in *C. albicans*. In contrast, in the other pathogens this would remove hyphal activation leading to their growth in the yeast form. It should be noted that while the yeast forms of *C. neoformans*, *H. capsulatum* and *P. brasiliensis* are pathogenic, both the yeast and hyphal forms of *C. albicans* contribute to pathogenesis.[34]

Why has a heat shock response been conserved in *C. albicans*? The answer lies partly in the observation that Hsf1 is essential for viability in *C. albicans,* as in other yeasts.[19] This essentiality appears to be due to the fact that Hsf1 is required for the basal expression of key chaperones that are required for protein folding, even in the absence of heat shock.[19] However, this explanation seemed unsatisfactory at the time because, given the extent of transcriptional rewiring in *C. albicans*,[15,16,20-22] couldn't alternative regulators have evolved to drive the basal expression of such vital chaperones? Therefore, we reasoned that the role of Hsf1 might have diverged in *C. albicans* such that it contributes to other stress responses in this pathogen. We tested this only to find that the Hsf-dependent HSE-reporter is not activated in *C. albicans* by the other stresses we tested.[19]

At this point we reconsidered the possible roles of the heat shock response in the wild. Molecular biologists regularly apply acute insults to provoke dramatic molecular responses that are easy to assay experimentally. However, in reality the cellular systems we study may have evolved to maintain homeostasis in the face of more modest environmental changes. A good example of this is the osmotic stress response in *S. cerevisiae*, which appears perfectly adapted to the maintenance of homeostasis when faced with repeated osmotic challenges.[23] By analogy, the heat shock response may have evolved in *C. albicans* to maintain homeostasis in response to less acute thermal challenges than experimentalists tend to apply in vitro. This pathogen, which probably evolved as a commensal organism, is likely to be exposed to modest thermal challenges such as, for example, those imposed by a febrile patient, or by the impact of ambient temperature upon the skin. We tested this idea by measuring the degree of activation of the Hsf1-HSE regulon at different growth temperatures, finding that the activity of this regulon increased as the growth temperature increased above 30°C.[19] In light of these observations, we suggest that the primary role of Hsf1 in the wild is to act as a thermostat that tunes the levels of essential chaperones to growth temperature. In this case "heat shock" is probably a misnomer for this transcription factor, and the term "thermosensor" might be more appropriate.

Hsp90 is an essential chaperone that is regulated by Hsf1 in *C. albicans* (**Fig. 1**). Recently, Leah Cowen's group elegantly demonstrated that Hsp90 plays a central role in the thermoregulation of the yeast-hypha transition. They showed that the heat shock protein Hsp90 negatively regulates hyphal development by repressing Ras-protein kinase A (PKA) signalling.[24] The downregulation of Hsp90 activity, either by genetic or pharmacological means, derepressed filamentation in a manner that was dependent upon Ras1, adenylyl cyclase and PKA. Hsp90 is known to interact physically with regulators (its clients) to modulate their activity,[25] and this presumably underpins the ability of this chaperone to act as an "evolutionary capacitor" that affects the emergence of drug resistance in *C. albicans*.[26,27] (Indeed this fundamental property of Hsp90 appears to have influenced the evolution of organisms throughout the eukaryotic kingdom[28]). Therefore, the impact of Hsp90 upon morphogenesis is thought to be mediated via an interaction with a client on the Ras-PKA pathway,[29] and this mechanism is likely to be regulated in turn by Hsf1 (**Fig. 1**). Indeed, chaperonins such as the CCT complex have previously been shown to modulate Ras-PKA signalling in *C. albicans*,[30] suggesting that several classes of chaperone regulate this pathway. The conundrum is how Hsp90 can inhibit hyphal development when Hsp90 expression levels increase during hyphal development.[4] The answer probably lies in the sequestering of Hsp90 for protein refolding during the thermal upshift. This sequestration might release the morphogenetic regulator from Hsp90, thereby allowing this client to activate hyphal development.

Temperature also regulates morphogenetic transitions in other pathogenic fungi. Unlike *C. albicans, Histoplasma capsulatum, Cryptococcus neoformans* and *Paracoccidiodes brasiliensis* are saprobes, occupying environmental niches outside their mammalian host. These fungi all exist in filamentous forms in the

environment, but grow in the yeast form in the host, and these morphogenetic transitions can be induced by thermal shifts in vitro. In *H. capsulatum, C. neoformans* and *P. brasiliensis,* Hsp90 expression levels have been shown to increase in response to the elevated temperatures that induce their yeast growth form.[31-33] Hence the Hsf1-Hsp90 module might play a role in the regulation of morphogenesis in these fungi (**Fig. 1**). However, at least in *P. brasiliensis,* the increase in Hsp90 expression is transient, inferring that additional factors must play a role in the maintenance of the yeast growth form. Ras-PKA signalling would be an obvious candidate. Also, while elevated temperatures induce hyphal development in *C. albicans,* they promote yeast growth in these other pathogens. Therefore, Hsp90 presumably exerts opposite effects upon hyphal development in these species (**Fig. 1**). For example, the interaction of Hsp90 with a key regulator might inhibit this client in one species, but activate this client in another.

To summarise, while heat shock proteins have been of interest to the medical mycology community for several decades, recent work has now begun to shed light on the regulation of this response in *C. albicans*. It is now becoming clear that heat shock regulation not only impacts upon thermal homeostasis in *C. albicans*, but also upon morphogenesis (and hence fungal pathogenesis) and drug resistance (and hence antifungal therapy). We argue that these observations may be of relevance to other clinically relevant fungal pathogens.

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