Coupling temperature sensing and development

Hsp90 regulates morphogenetic signaling in *Candida albicans*

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Hsp90 is and environmentally contingent molecular chaperone that influences the form and function of diverse signal transducers. Here we discuss our recent findings that Hsp90 regulates the morphogenetic transition from yeast to filamentous forms required for virulence of the most prevalent fungal pathogen of humans, *Candida albicans*, and does so via cAMP-PKA signaling. This transition is normally regulated by environmental cues that are contingent upon elevated temperature to relieve Hsp90-mediated repression of the morphogenetic program. Intriguingly, Hsp90 inhibition induces filamentation independent of the canonical PKA transcription factor Efg1, in striking similarity to a select set of morphogenetic stimuli. Further investigation will determine the downstream transcription factors through which Hsp90 regulates morphogenesis and the precise mechanism of Hsp90's interaction with the cAMP-PKA pathway. *C. albicans* is one of many fungal species that undergo a morphological transition in a temperature-dependent manner, thus Hsp90's capacity to govern this key developmental program may provide insight into morphogenesis of diverse organisms.

Precise coordination of sensing and response to environmental cues is imperative for the survival of all organisms. In the leading fungal pathogen of humans, *Candida albicans*, the capacity to sense environmental signals and undergo morphological transitions is tightly linked to its virulence. In *C. albicans*, the morphological transition between yeast and filamentous growth states is regulated by diverse environmental cues, including nutrient limitation, pH, CO_2, serum and temperature (**Fig. 1A**).[1-3] Elevated temperature of 37°C is critical for *C. albicans* to undergo morphogenesis under most conditions (**Fig. 1A**), yet until recently, little was understood about the cellular signaling underpinning this temperature dependence. Numerous signal transduction pathways have been implicated in *C. albicans* morphogenesis, including the mitogen-activated protein kinase (MAPK) pathway and the cAMP-protein kinase A (PKA) pathway.[1-3] Recently, we demonstrated that the molecular chaperone Hsp90 orchestrates temperature-dependent morphogenesis in *C. albicans* in a manner that is contingent on cAMP-PKA signaling.[4]

Hsp90 is an essential molecular chaperone that responds to environmental cues and regulates the form and function of its client proteins. Many Hsp90 client proteins are regulators of cellular signaling, such as kinases and transcription factors, which dwell in incompletely folded or aggregation-prone states.[5-7] As a heat shock protein, Hsp90 is induced under conditions of stress, such as increased temperature; however, global problems in protein folding that occur at elevated temperature can overwhelm Hsp90 chaperone function. Therefore, as a thermally responsive chaperone that regulates key signal transducers, Hsp90 is uniquely poised to govern temperature-dependent traits, such morphogenesis in *C. albicans*.

We discovered that compromising Hsp90 function induces a transition from yeast to filamentous growth in *C. albicans*,
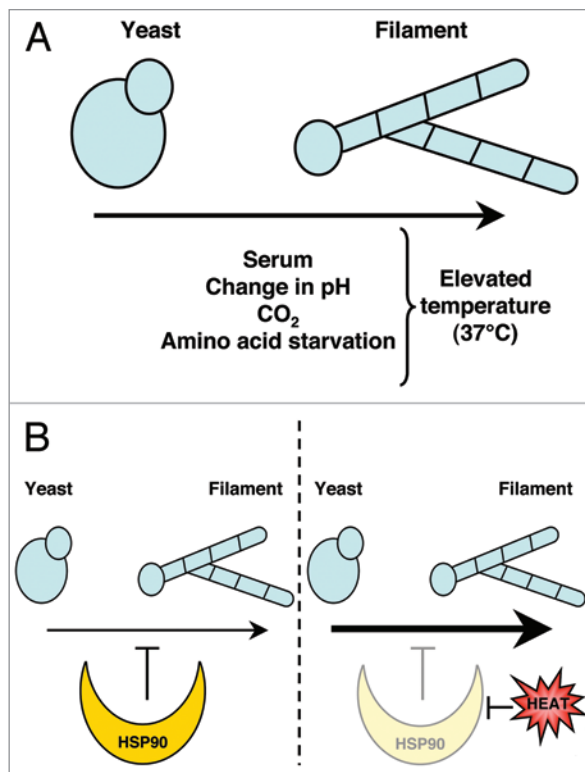
Figure 1. Temperature-dependent morphogenesis in *C. albicans*. (A) In *C. albicans*, the morphological transition between yeast and filamentous growth (hyphal and pseudohyphal) is regulated by many different environmental cues, including exposure to serum, change in pH, CO_2, and amino acid starvation. Elevated temperature of 37°C is required for *C. albicans* to undergo morphogenesis under these conditions. (B) Hsp90 exerts a repressive effect on the yeast to filament morphological transition. When Hsp90 function is compromised, for instance by elevated temperature, the yeast to filament transition is induced.

even in the absence of external cues.[4] Our results support the model that Hsp90 is a key temperature sensor that governs *C. albicans* morphogenesis such that elevated temperature is required to relieve Hsp90-mediated repression of the morphogenetic program (**Fig. 1B**). When Hsp90 function is compromised, by elevated temperature or by specific genetic or pharmacological perturbation, the yeast to filament transition is induced via the cAMP-PKA pathway.[4] Consistent with the association between morphogenetic plasticity and virulence, depletion of *C. albicans* Hsp90 attenuates virulence of the fungus in a murine model of systemic disease.[4]

Strikingly, although morphogenesis induced by Hsp90 inhibition depends on upstream inputs from the cAMP-PKA pathway, such as the GTPase Ras1, the adenylyl cyclase Cdc35 and the PKA complex itself, this morphogenetic program occurs independent of Efg1,[4] the canonical transcription factor of the PKA pathway. Efg1 is a member of the APSES family of transcriptional regulators, which is unique to the fungal kingdom. This family includes *C. albicans* proteins Efg1 and Efh1, which have roles in filamentation,[8] as well as the *Saccharomyces cerevisiae* regulators Phd1 and Sok2, which are known to regulate the pseudohyphal transition.[9,10] In *C. albicans*, Efg1 is often considered the key transcriptional regulator of morphogenesis and is required for the yeast to filament transition induced by numerous cues including serum, pH and glucose starvation.[11-14] That Hsp90-mediated morphogenesis occurs independent of Efg1, suggests either that parallel signaling pathways or alternate transcription factor(s) downstream of PKA signaling function to regulate this yeast to filament transition. Other factors thought to act downstream of PKA signaling in *C. albicans* include Flo8, Sfl1 and Tec1.[15-17]

Despite the central role of Efg1 in *C. albicans* morphogenesis, certain stimuli will induce filamentation in an Efg1-independent manner. For instance, filamentation stimulated by solid medium containing serum or by macrophage ingestion occurs independent of Efg1.[13] In this instance, the MAPK transcription factor Cph1 has been implicated as the other factor involved in filamentation, as deletion of both Efg1 and Cph1 blocks filamentation under these conditions. Further, although Efg1 has been linked to the expression of filament-specific transcripts in vitro, many of these transcripts are expressed independent of Efg1 in an in vivo intestinal tract model of *C. albicans* colonization.[18]

Notably, certain morphogenetic stimuli demonstrate a strikingly similar response pattern to inhibition of Hsp90 in that they are dependent on upstream inputs of the cAMP-PKA pathway, but are not dependent on Efg1. For instance, depletion of the cell cycle regulatory polo-like kinase Cdc5 induces filamentation that is strictly dependent on Cdc35, but not on Efg1.[19] The same holds true for filamentation induced in the presence of the DNA synthesis inhibitor hydroxyurea.[19] Additionally, depletion of the DNA-damage checkpoint regulator Rad52 triggers filamentation in a Cdc35-dependent and Efg1-independent manner.[20] In these cases, the identities of the additional pathways or transcription factors involved in regulating filamentation remain enigmatic.[19]

Regardless of the downstream transcriptional regulator involved, current data suggest that Hsp90 represses cAMP-PKA signaling.[4] Conceivably, there are three broad models that could explain this regulation (**Fig. 2**). First, Hsp90 could interact with a positive regulator of the pathway, and maintain it in an inactive conformation until Hsp90 function is compromised. Precedent for Hsp90 maintaining proteins in an inactive, but active-competent state has been well established for specific client proteins, including the heat shock factor Hsf1.[21,22] In relation to the PKA pathway, this could involve Hsp90 interacting with the positive catalytic subunits of PKA, Tpk1 or Tpk2 (**Fig. 2A**). Second, Hsp90 might stabilize a negative regulator of the cAMP-PKA pathway, such that inhibition of Hsp90 would lead to loss of function

of the negative regulator. Precedent for this regulation comes from client proteins such as the protein phosphatase calcineurin and the tyrosine kinase v-src, which both exhibit dependence on Hsp90 for function.[23,24] In the PKA pathway, this could involve Hsp90 interacting with the negative regulatory subunit of PKA, Bcy1 (**Fig. 2B**). Third, Hsp90 might interact indirectly with the cAMP-PKA pathway (**Fig. 2C**) via another regulator such as Gcn4, which is known to regulate morphogenesis and metabolic response in *C. albicans* by stimulating the cAMP-PKA pathway.[25] Of course, more complex models are possible where Hsp90 influences cAMP-PKA signaling and morphogenesis via multiple client proteins.

Although the precise model of Hsp90 regulation of *C. albicans* morphogenesis remains elusive, our results suggest that Hsp90 likely influences activation of the cAMP-PKA cascade at the level of the PKA complex itself.[4] We found that while the quorum-sending molecules farnesol and dodecanol block the morphogenetic response to serum,[26] they do not block the response to Hsp90 inhibition.[4] Serum has been shown to directly stimulate Cdc35,[27] suggesting that Hsp90 is likely to regulate morphogenesis via an effector downstream of Cdc35. Notably, deletion of upstream regulators of PKA block the morphogenetic response to Hsp90 inhibition, suggesting that PKA activation remains dependent on upstream input. This is consistent with *ras1* null mutants being defective in response to serum-induced filamentation, despite the fact that serum stimulates a factor downstream of Ras1.[27] Further, our findings that deleting Ira2, Pde1 or Pde2 does not phenocopy Hsp90 inhibition suggest that Hsp90 does not stabilize these negative regulators upstream of PKA.[4] As the *efg1* mutant still filaments in response to Hsp90 inhibition, we propose a model in which Hsp90 inhibition leads to activation of the PKA complex itself and induction of filamentous growth.

Our study provides the foundation from which to dissect the cellular circuitry through which *C. albicans* employs an environmentally responsive chaperone to couple temperature change with a key morphog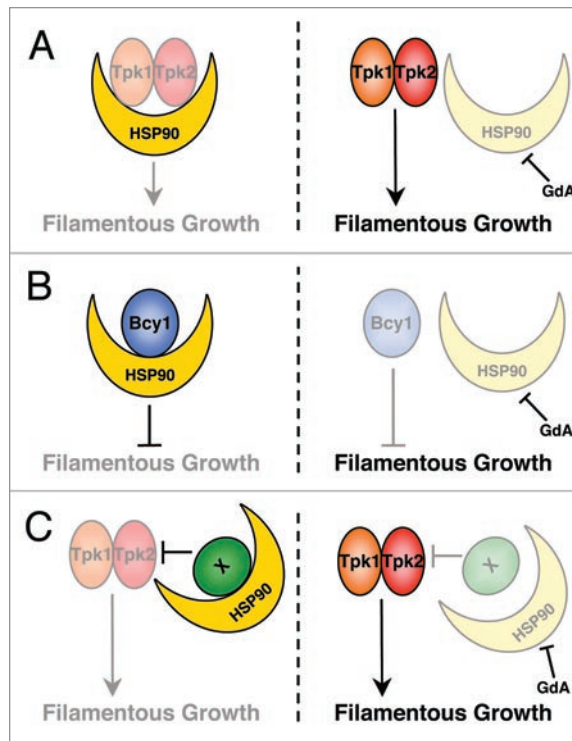enetic program. *C. albicans* represents one of many fungal species that undergo a morphological transition in a temperature-dependent manner. Intriguingly, the systemic dimorphic fungi, including *Histoplasma capsulatum* and *Paracoccidioides brasiliensis*, exhibit the reverse transition. These fungi grow as yeast at elevated temperature within their human hosts, but in filamentous forms at ambient temperature in the environment.[28] This raises the tantalizing possibility that Hsp90 may regulate temperature-dependent morphogenesis in other pathogenic fungi. Both *H. capsulatum* and *P. brasiliensis* have known Hsp90 homologs[29] and it has been demonstrated that *P. brasiliensis* differentially expresses many heat shock proteins, including Hsp90, during its morphological transition.[30] Dissecting the mechanisms by which Hsp90 regulates temperature-dependent developmental transitions in diverse species is poised to reveal how environmental response programs have been rewired throughout evolution.

Figure 2. Models for Hsp90's interaction with the PKA pathway. (A) Hsp90 could interact with a positive regulator of the cAMP-PKA pathway and maintain it in an inactive, but active competent state. This could involve Hsp90 interacting the positive catalytic subunits of PKA, Tpk1 or Tpk2. (B) Hsp90 could stabilize a negative regulator of the cAMP-PKA pathway, such that inhibiting Hsp90 would destabilize the negative regulator. This could involve Hsp90 interacting the negative regulatory subunit of PKA, Bcy1. (C) Hsp90 might interact indirectly with the cAMP-PKA pathway. This could occur via a positive regulator such as Tpk1 or Tpk2, or by a negative regulator. More complex models are possible, where Hsp90 could regulate cAMP-PKA signaling via multiple client proteins.

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