Progress and prospects for targeting Hsp90 to treat fungal infections

AMANDA VERI *and* LEAH E. COWEN*

Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

(*Received 27 September 2013; revised 8 November 2013; accepted 17 November 2013; first published online 20 February 2014*)

SUMMARY

Fungal pathogens pose a major threat to human health worldwide. They infect billions of people each year, leading to at least 1·5 million deaths. Treatment of fungal infections is difficult due to the limited number of clinically useful antifungal drugs, and the emergence of drug resistance. A promising new strategy to enhance the efficacy of antifungal drugs and block the evolution of drug resistance is to target the molecular chaperone Hsp90. Pharmacological inhibitors of Hsp90 function that are in development as anticancer agents have potential to be repurposed as agents for combination antifungal therapy for some applications, such as biofilm infections. For systemic infections, however, effective combination therapy regimens may require Hsp90 inhibitors that can selectively target Hsp90 in the pathogen, or alternate strategies to compromise function of the Hsp90 chaperone machine. Selectively impairing Hsp90 function in the pathogen could in principle be achieved by targeting Hsp90 co-chaperones or regulators of Hsp90 function that are more divergent between pathogen and host than Hsp90. Antifungal combination therapies could also exploit downstream effectors of Hsp90 that are critical for fungal drug resistance and virulence. Here, we discuss the progress and prospects for establishing Hsp90 as an important therapeutic target for life-threatening fungal infections.

Key words: Hsp90, fungal pathogen, *Candida*, *Aspergillus*, antifungal drug resistance, antifungal agent, azoles, echinocandins, combination therapy.

INTRODUCTION

The incidence of infectious diseases caused by fungal pathogens has reached unprecedented levels, jeopardizing food supplies, biodiversity and human health. Fungi are most renowned for their devastating impact on plants. Notorious examples include the Irish Potato famine resulting from late blight, as well as destruction of forests and urban landscapes by Dutch elm blight and chestnut blight (Money, 2007). Fungi pose a significant threat to every plant that humans cultivate, with current pressing concern for rice, wheat, coffee, cocoa and rubber crops. While largely unappreciated until recent times, fungi also cause devastating infections in animals, including some of the most striking extinctions to be observed in wildlife. Fungal outbreaks are crippling bat species and causing rapid extinction of amphibian species worldwide (Fisher *et al*. 2012). Fungal outbreaks have also directly affected humans, as with the rampant spread of *Cryptococcus gattii* infections among otherwise healthy individuals (Byrnes *et al*. 2011).

Fungal pathogens are implicated in billions of human infections worldwide each year, killing over 1·5 million people annually (Brown *et al*. 2012*a*, *b*). Fungal pathogens of humans are often opportunists, and prey upon hosts with compromised immune function. The population of immunocompromised individuals due to chemotherapy for cancer, immunosuppressive therapy for transplants, as well as infection with HIV continues to grow, resulting in an ever-increasing incidence of fungal infections (Pfaller and Diekema, 2010). The leading fungal pathogens of humans include species of *Candida, Aspergillus, Cryptococcus* and *Pneumocystis*, which together account for approximately 90% of human mortality attributable to fungal infections (Brown *et al*. 2012*a*). Mortality rates due to systemic fungal infections often surpass 50%, despite current treatment regimens (Pfaller and Diekema, 2010).

The armamentarium of antifungal drugs available for the treatment of fungal infections is limited. As with many eukaryotic pathogens, conservation with the human host provides a major challenge for identifying effective drug targets that can be exploited to selectively kill the pathogen while avoiding host toxicity. Two of the most widely utilized classes of antifungal drugs in the clinic are the azoles and the echinocandins (Fig. 1). The azoles have been a frontline antifungal agent in the clinic for decades. They inhibit a cytochrome P450, lanosterol 14α-demethylase, and thereby block the biosynthesis of a key sterol in the fungal cell membrane, ergosterol (Shapiro *et al*. 2011). The azoles exert fungistatic activity, causing growth arrest due to the depletion

* Corresponding author: Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Medical Sciences Building, Room 4368, Toronto, Ontario M5S 1A8, Canada. E-mail: leah.cowen@utoronto.ca

Parasitology (2014), **141**, 1127–1137.
doi:10.1017/S0031182013002072

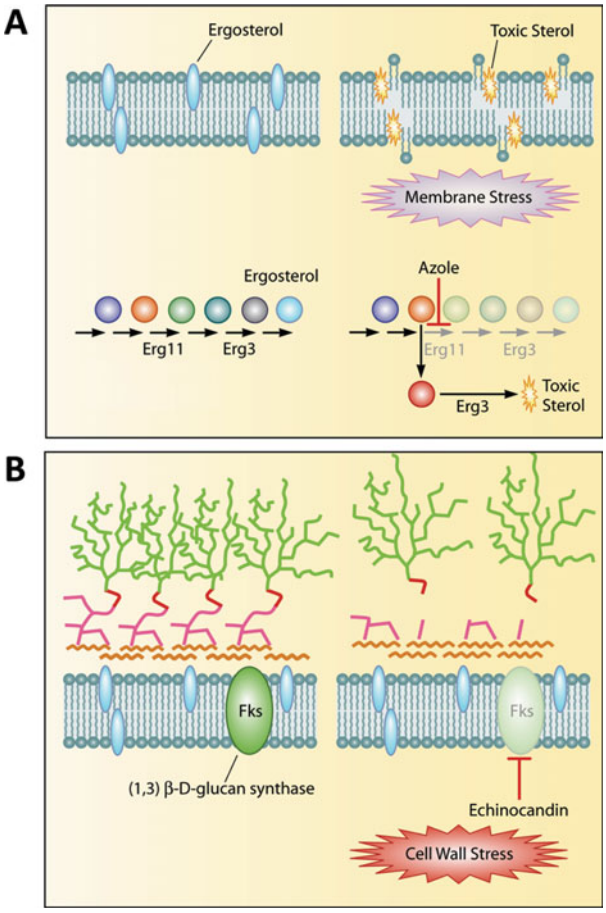

Fig. 1. Mode of action of antifungal drugs. (A) The azoles function by inhibiting the cytochrome P450 enzyme lanosterol demethylase, Erg11, blocking the production of ergosterol. Severe cell membrane stress occurs as a result of the accumulation of a toxic sterol intermediate produced by Erg3; (B) The echinocandins inhibit 1,3-β-D-glucan synthase, which synthesizes a key cell wall linker molecule. This leads to a loss of cell wall integrity and causes cell wall stress. Adapted by permission from Copyright ©American Society for Microbiology (Shapiro *et al*. 2011).

of ergosterol and the accumulation of a toxic sterol intermediate. While the azole class has expanded over the years, the echinocandins represent the only new class of antifungal drug with a distinct target to enter clinical use in decades. The echinocandins inhibit 1,3-β-D-glucan synthase, and thereby block the biosynthesis of a key linker molecule in the fungal cell wall, resulting in cell wall stress (Cowen, 2008; Cowen and Steinbach, 2008). The efficacy of all of the antifungal drugs currently in clinical use is compromised by the emergence of drug resistance in fungal pathogens. A poignant example is that the Centers for Disease Control and Prevention has ranked azole-resistant *Candida* as one of the serious threats to human health given the 46 000 infections per year in the USA alone, and that approximately 30% of patients with these bloodstream infections die during the course of hospitalization (CDC, 2013). There is a pressing need to identify novel strategies to combat fungal drug resistance and treat life-threatening fungal infectious disease.

One of the most promising new strategies to cripple fungal pathogens to emerge in recent years involves targeting key regulators of cellular stress responses. Fungi depend on stress responses to cope with the cell membrane and cell wall damage induced by antifungal drugs (Cowen and Steinbach, 2008; Shapiro *et al*. 2011). As a consequence, dismantling cellular stress response circuitry can abrogate drug resistance and dramatically enhance the efficacy of antifungal drugs. A powerful strategy to simultaneously inhibit multiple fungal stress response pathways involves targeting the molecular chaperone Hsp90 (Cowen, 2008, 2009; Cowen and Steinbach, 2008; Shapiro *et al*. 2011). As discussed earlier in this issue, Hsp90 is a highly conserved molecular chaperone in eukaryotes that modulates the stability and activation of diverse client proteins, which are most often regulators of cellular signalling (Taipale *et al*. 2010; Leach *et al*. 2012). Hsp90 functions as part of a chaperone machine, the function of which is influenced by co-chaperones and post-translational modifications. In fungi, Hsp90 exerts profound effects on cellular stress response circuitry through its interactions with a myriad of cellular regulators, which approach 10% of the proteome (Zhao *et al*. 2005; McClellan *et al*. 2007; Diezmann *et al*. 2012). Here, we discuss the progress and prospects for exploiting Hsp90 as a therapeutic target for combating fungal infections that threaten human lives.

THE EMERGENCE OF HSP90 AS AN ANTIFUNGAL TARGET

The appreciation of Hsp90's role as a potentiator of antifungal drug resistance emerged following seminal studies in plants and animals, which established the profound impact of this chaperone on the translation of genotype to phenotype. Pioneering work in *Drosophila* demonstrated that Hsp90 functions as a capacitor such that it buffers the expression of genetic variation, maintaining it in a phenotypically silent state until Hsp90 function is compromised, such as by environmental stress (Rutherford and Lindquist, 1998; Rutherford, 2003). Stress can overwhelm Hsp90 function by causing an increased burden of misfolded cellular proteins, thereby providing an environmentally contingent mechanism to reveal genetic variation. In this context, inhibition of Hsp90 exposes new traits that are contingent upon cryptic genetic variation. Selection can render these traits independent of Hsp90 function, providing a mechanism by which new traits can be assimilated (Sangster *et al*. 2004). Subsequent work established that Hsp90 also buffers epigenetic variation (Sollars *et al*. 2003; Tariq *et al*. 2009), and that it functions as a capacitor in plants (Queitsch *et al*. 2002;

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

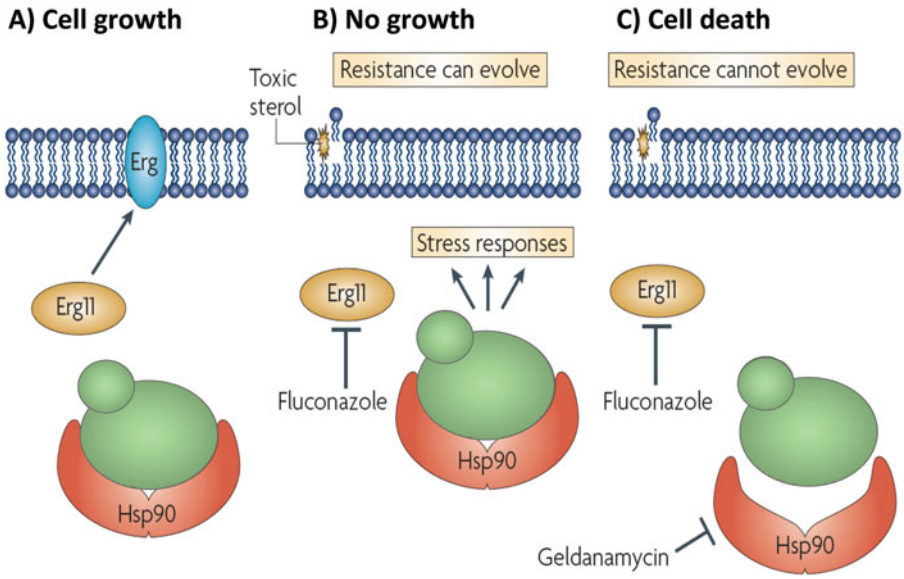

Fig. 2. Hsp90 enables the phenotypic effects of resistance mutations. (A) Under normal conditions, fungal cells contain ergosterol in their cell membranes and stress responses are not required; (B) Treatment with the azole fluconazole blocks ergosterol synthesis and leads to incorporation of a toxic sterol in the membrane, culminating in cell wall stress. Hsp90 stabilizes key regulators of cellular stress response (shown in green), enabling signal transduction pathways required for the emergence and maintenance of drug resistance; (C) Stress response pathways are blocked by Hsp90 inhibitors such as geldanamycin, leading to cell death. This prevents the evolution of drug resistance and abrogates resistance once it has evolved. Adapted by permission from Macmillan Publishers Ltd.: Nature Publishing Group (Cowen, 2008), © 2008.

Sangster *et al*. 2008*a*, *b*). Compromising Hsp90 function can also modulate RNA silencing mechanisms leading to transposon activation and the generation of novel variants (Specchia *et al*. 2010).

The scope and breadth of Hsp90's effects on phenotypic variation is stunning, as it can also have the inverse impact from capacitance in the context of acting as a potentiator. In this case, the impact of Hsp90's role in stabilizing mutant cellular regulators is to enable the emergence of new traits; compromise of Hsp90 function can mask the phenotypic effects of new mutations (Cowen, 2008). Classic precedent for Hsp90's role as a potentiator comes from mammalian cancer cells. For many cellular regulators, the acquisition of mutations that cause promiscuous activity and activate their oncogenic potential also renders them unstable and prone to misfolding. For example, oncogenic mutations in the cellular Src tyrosine kinase (c-Src) often involve truncation of the inhibitory domain, rendering the mutant v-Src protein hyperactive and contingent upon Hsp90 for stability and function (Xu and Lindquist, 1993; Xu *et al*. 1999; Falsone *et al*. 2004). In this context, inhibition of Hsp90 can reverse the oncogenic phenotypes that result from diverse genetic alterations.

Hsp90's multifaceted roles in modulating the translation of genotype to phenotype in an environmentally contingent manner motivated studies to explore the impact of Hsp90 on evolutionary process in a tractable fungal system, the model yeast *Saccharomyces cerevisiae*. This work revealed that compromise of Hsp90 function blocks the rapid evolution of resistance to azole antifungal drugs, and abrogates resistance acquired by diverse mechanisms (Cowen and Lindquist, 2005). Hsp90 enables the phenotypic effects of resistance mutations by stabilizing a key regulator of cellular stress responses (Fig. 2). Hsp90 is poised to influence diverse aspects of fungal biology and disease given that Hsp90 modulates the phenotypic effects of approximately 20% of pre-existing genetic variation in *S. cerevisiae*, where it functions as a capacitor and potentiator in almost equal frequency (Jarosz and Lindquist, 2010).

THE IMPACT OF HSP90 ON DRUG RESISTANCE OF FUNGAL PATHOGENS

Hsp90's role in enabling the emergence and maintenance of azole resistance is conserved in fungal pathogens. Hsp90's impact on drug resistance has been studied most extensively in *Candida* species, which are the fourth most frequent cause of hospital-acquired infections, imposing an economic burden on the healthcare system of over $1 billion annually in the USA alone (Miller *et al*. 2001). *Candida albicans* is the most prevalent cause of systemic candidiasis, with *Candida glabrata* emerging as a major threat due at least in part to its intrinsic resistance to azoles; together with *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*, these species account for over 90% of all cases of candidiasis (Pfaller and Diekema, 2007, 2010). Pharmacological inhibition of Hsp90 blocks the rapid emergence of azole resistance in

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

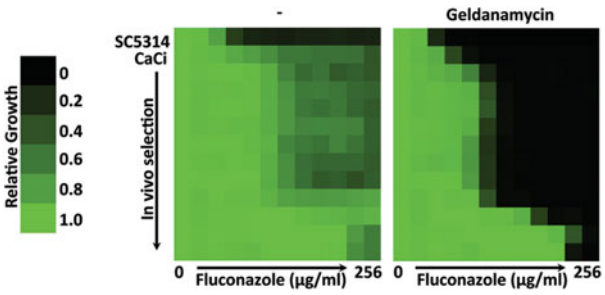

Fig. 3. Azole resistance of clinical isolates is abrogated by inhibition of Hsp90. Growth in liquid medium with increasing concentrations of the azole fluconazole was measured by absorbance at 600 nm and normalized relative to the no-drug control. Data were quantitatively displayed with colour using Treeview (see colour bar). Clinical isolates (CaCi) recovered from an HIV-infected patient undergoing fluconazole treatment are ordered with those recovered early in treatment at the top and those recovered late at the bottom. All isolates have increased growth compared with the fluconazole susceptible laboratory strain SC5314, with the isolates recovered at the latest stages showing the most robust growth at all concentrations of fluconazole tested. Inhibition of Hsp90 by geldanamycin reduces growth of all clinical isolates in the presence of fluconazole, and affects early isolates to a greater extent than isolates recovered from later stages in treatment. Figure adapted from LaFayette *et al*. (2010), © LaFayette *et al*. *PLoS Pathogens*, 2010.

C. albicans, consistent with findings from *S. cerevisiae* (Cowen and Lindquist, 2005; Cowen *et al*. 2006). Genetic or pharmacological compromise of Hsp90 function also transforms azoles from fungistatic to fungicidal (Cowen *et al*. 2009), and abrogates azole resistance that evolved in a human host by multiple mechanisms (Fig. 3) (Cowen and Lindquist, 2005). Inhibition of Hsp90 function with molecules that are in clinical development for cancer, such as 17-allylamino-17-demethoxygeldanamycin (17-AAG) transforms azoles from ineffective to highly efficacious in rescuing otherwise lethal *C. albicans* infections in the tractable invertebrate infection model, *Galleria mellonella* (Cowen *et al*. 2009). Furthermore, genetic reduction of *C. albicans* Hsp90 levels enhances the efficacy of azoles in a murine model of systemic candidiasis (Cowen *et al*. 2009). These findings provide proof-of-principle for Hsp90 as an attractive target for combination therapy to treat fungal infections.

Hsp90's impact on drug resistance extends beyond azoles to echinocandins. Hsp90 has been found to play a key role in echinocandin resistance in *C. albicans* and *C. glabrata* (Singh *et al*. 2009; Singh-Babak *et al*. 2012), as well as in the mould *Aspergillus fumigatus* (Cowen *et al*. 2009), which causes invasive infections associated with mortality rates ranging from 40–90% (Lin *et al*. 2001). Compromising Hsp90 function reduces basal tolerance and resistance acquired in patients undergoing echinocandin treatment (Fig. 4) (Cowen and Lindquist, 2005; Cowen *et al*. 2009; Singh *et al*. 2009; Singh-Babak *et al*. 2012). The combination of Hsp90 inhibitors with echinocandins also abrogates azole resistance of *A. fumigatus* (Lamoth *et al*. 2012). Beyond the effects *in vitro*, the combination of Hsp90 inhibitors that are well tolerated in humans and echinocandins improves survival of *G. mellonella* infected with *A. fumigatus*, relative to those treated with echinocandins alone (Cowen *et al*. 2009). Further, genetic reduction of *C. albicans* Hsp90 levels improves the effectiveness of echinocandins in a mouse model of invasive infection (Singh *et al*. 2009). Together, these studies suggest that Hsp90 can be exploited as a target for combination therapy with the two leading classes of antifungal drugs, to combat the most prevalent and threatening fungal pathogens of humans.

TARGETING HSP90 TO ABROGATE DRUG RESISTANCE OF FUNGAL BIOFILMS

For many fungal pathogens, drug resistance can emerge not only as a result of the acquisition of specific resistance mutations, but also as a consequence of a change in cellular state. Biofilms represent one such cellular state that is associated with intrinsically high levels of antifungal drug resistance (Blankenship and Mitchell, 2006; d'Enfert, 2006). Fungal biofilms are complex communities that form upon adherence to specific surfaces such as medical devices and indwelling catheters. Both *C. albicans* and *A. fumigatus* cause prevalent biofilm infections, with *C. albicans* being the third most frequent cause of intravascular catheter infection and *A. fumigatus* implicated in biofilm infections on medical devices and bronchial epithelial cells (Nett and Andes, 2006; Ramage *et al*. 2009; Finkel and Mitchell, 2011). For *C. albicans*, inhibition of Hsp90 function *in vitro* reduces biofilm formation, abrogates azole resistance, and further blocks the dispersal of biofilm cells, which could otherwise serve as a reservoir for further infection (Robbins *et al*. 2011). Inhibition of Hsp90 also reduces echinocandin resistance of *A. fumigatus* biofilms *in vitro*. In a rat central venous catheter model of biofilm infection, azoles alone are ineffective while the combination of genetic or pharmacological compromise of Hsp90 function with azoles sterilizes the catheter without host toxicity (Fig. 5) (Robbins *et al*. 2011). The powerful and broad-spectrum efficacy of Hsp90 inhibitors combined with antifungals suggests that targeting Hsp90 may provide a much-needed strategy to cripple fungal pathogens.

HSP90 REGULATES KEY FUNGAL VIRULENCE TRAITS

Beyond its impact on fungal drug resistance, Hsp90 is also a key regulator of traits of central importance

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

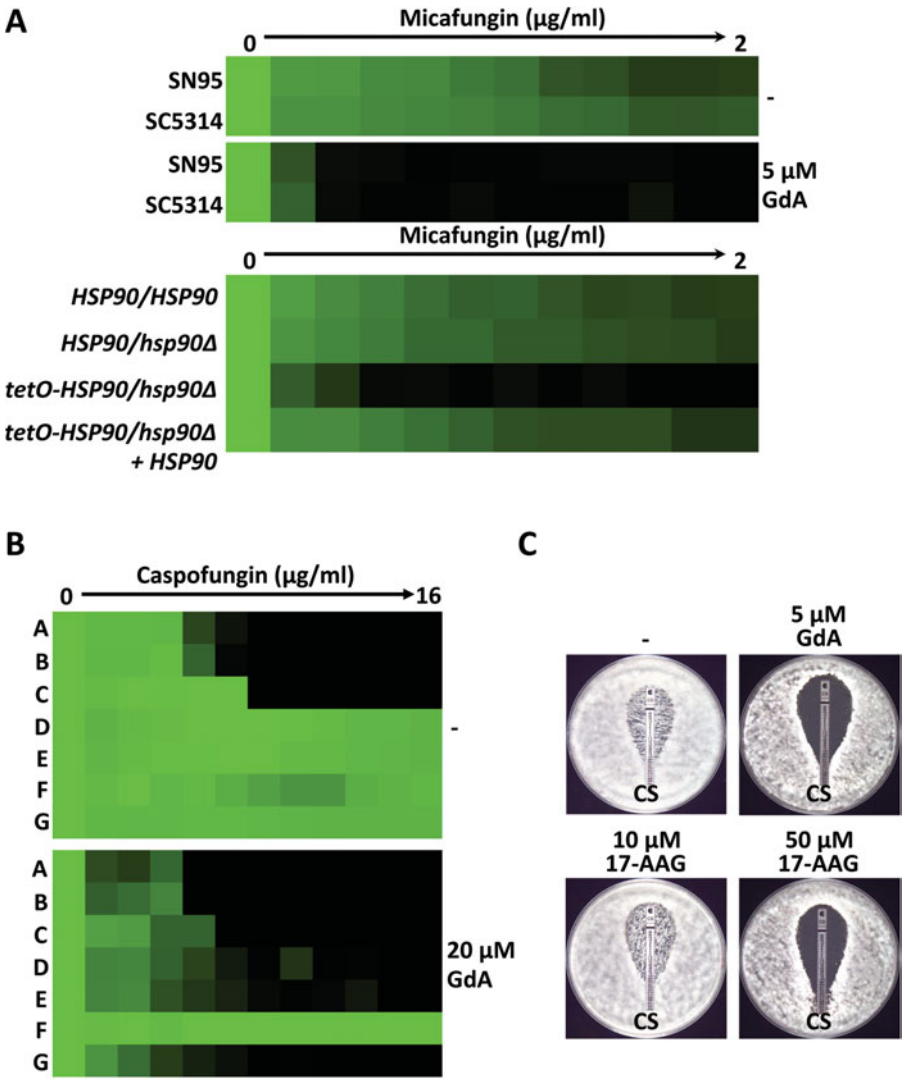

Fig. 4. Genetic or pharmacological compromise of Hsp90 function reduces basal tolerance and resistance to echinocandins. (A) Inhibition of Hsp90 by geldanamycin (GdA) reduces tolerance to the echinocandin micafungin in two laboratory strains of *C. albicans*, SC5314 and SN95; genetic compromise of *C. albicans HSP90* expression by replacing the native promoter with the *tetO* promoter abrogates tolerance to micafungin, and complementation with a wild-type *HSP90* allele restores tolerance. Data were analysed as in Fig. 3. Adapted from Singh *et al*. (2009). © Singh *et al*. *PLoS Pathogens*, 2009; (B) Hsp90 inhibition reduces resistance to the echinocandin caspofungin of *C. glabrata* clinical isolates. Isolates are arranged in the order they were recovered from a patient undergoing caspofungin treatment. Isolate A was recovered before treatment began, and isolate G was recovered after numerous rounds of caspofungin treatment. Hsp90 inhibition with GdA reduces the resistance of all of the isolates tested, with the exception of isolate F which is a petite mutant lacking mitochondrial function. Data were analysed as in Fig. 3. Adapted from reference Singh-Babak *et al*. (2012). © Singh-Babak *et al*. *PLoS Pathogens*, 2012; (C) Pharmacological inhibition of Hsp90 reduces caspofungin resistance of an *A. fumigatus* clinical isolate. Antifungal test strips produce a gradient of caspofungin with the highest concentration at the top. A reduction in tolerance is seen when Hsp90 is inhibited through addition of GdA to the plates; comparable results are observed with higher concentrations of the geldanamycin analogue 17-AAG. Adapted from Cowen *et al*. (2009). Harnessing Hsp90 function as a powerful, broadly effective therapeutic strategy for fungal infectious disease. *Proceedings of the National Academy of Sciences USA* 106/8, 2818–23; Copyright (2009), with permission from National Academy of Sciences.

for fungal virulence. For example, Hsp90 has a profound impact on the capacity of *C. albicans* to transition between yeast and filamentous growth, which is correlated with virulence (Noble *et al*. 2010). Filaments are crucial for tissue invasion, escape from macrophages, and expression of a multitude of virulence factors such as adhesins and proteases; yeasts are thought to enable early infection as well as dissemination (Gow *et al*. 2012). Filamentation is induced by numerous environmental cues, such as exposure to serum and elevated carbon dioxide, in a manner that is contingent upon elevated temperature (Shapiro *et al*. 2011). The molecular basis for the impact of temperature on morphogenesis

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

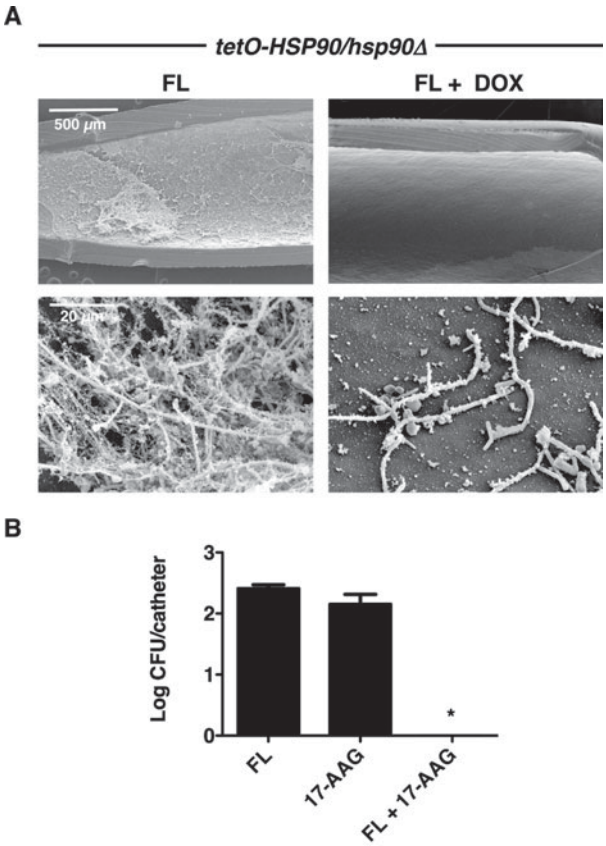

Fig. 5. Compromise of *C. albicans* Hsp90 function enhances the efficacy of fluconazole against azole-resistant infections in a rat central venous catheter model of biofilm infection. (A) Genetic reduction of *HSP90* levels was achieved using a *tetO-HSP90/hsp90Δ* strain, in which the only allele of *HSP90* is under control of a doxycycline repressible promoter. Scanning electron microscopy images of *tetO-HSP90/hsp90Δ* biofilms after 24 h of growth in rat venous catheters with or without 20 $\mu g\,mL^{-1}$ doxycycline (DOX) for transcriptional repression of *HSP90* expression, followed by treatment with the azole fluconazole (FL) for 24 h. The combination of fluconazole treatment with the genetic depletion of Hsp90 abrogates biofilm growth; (B) Pharmacological inhibition of Hsp90 with 17-allylamino-17-demethoxygeldanamycin (17-AGG) combined with azole treatment sterilizes the rat catheter. 17-AAG and FL were administered after the biofilm had formed; catheter fluid was serially diluted and plated to calculate colony forming units. Asterisk indicates $P < 0{\cdot}001$. Adapted from Robbins *et al*. (2011). © Robbins *et al*. *PLoS Pathogens*, 2011.

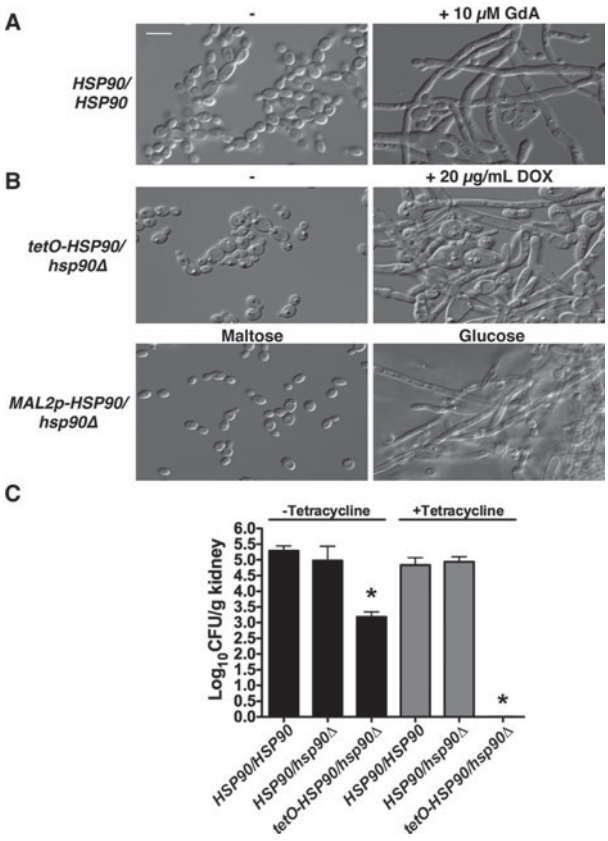

Fig. 6. Hsp90 regulates morphogenesis and virulence of *C. albicans*. (A) Pharmacological inhibition of Hsp90 with geldanamycin (GdA) induces filamentous growth under conditions that otherwise favour the yeast growth state. Scale bar represents 10 μm; (B) Genetic depletion of Hsp90 induces filamentation. Genetic repression was achieved by treating the *tetO-HSP90/hsp90Δ* strain with doxycycline or growing the *MAL2p-HSP90/hsp90Δ* strain in glucose; (C) Depletion of Hsp90 results in clearance of kidney fungal burden in a murine model of systemic infection. Genetic compromise of *C. albicans HSP90* expression simply by replacement of the native promoter with the *tetO* promoter, reduces kidney fungal burden. Further depletion of Hsp90 with tetracycline sterilizes the kidney. Asterisk indicates $P < 0{\cdot}001$. Adapted from Shapiro *et al*. (2009). Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. *Current Biology* 19/8, 621–629; Copyright (2009), with permission from Elsevier.

remained enigmatic for decades, until Hsp90 was implicated as the key temperature sensor governing this developmental programme. Compromising Hsp90 function induces filamentation under conditions that otherwise favour growth of the yeast form (Fig. 6) (Shapiro *et al*. 2009, 2012*a*). Elevated temperature compromises Hsp90, thereby relieving the repressive effect on morphogenesis. Consistent with the importance of morphological flexibility for virulence, depletion of *C. albicans* Hsp90 attenuates virulence in a murine model of systemic infection (Fig. 6) (Shapiro *et al*. 2009). Genetic studies have also implicated Hsp90 in virulence of *C. glabrata* in a murine model of disseminated disease (Singh-Babak *et al*. 2012), further validating Hsp90 as an attractive therapeutic target to treat fungal infectious disease.

CHALLENGES FOR THE DEVELOPMENT OF HSP90 INHIBITORS TO TREAT FUNGAL INFECTIONS

Despite the compelling evidence in support of targeting Hsp90 in fungal pathogens, there are

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

numerous challenges for the development of Hsp90 inhibitors to treat fungal infections. In the context of a mammalian model of fungal biofilm infections, an Hsp90 inhibitor in clinical development for cancer, 17-AAG, was highly effective in combination with an azole without host toxicity problems (Robbins *et al*. 2011). In this case, the drug delivery and fungal infection are both localized in the catheter environment as the result of the blood flow creating a fluid "lock". In contrast, when the same Hsp90 inhibitor was deployed systemically to treat a disseminated *C. albicans* infection in a murine model, there was considerable toxicity that precluded observing any therapeutic benefit (Cowen *et al*. 2009). One would expect that fungal selective Hsp90 inhibitors would ameliorate host toxicity problems (Cowen, 2013).

The development of fungal selective Hsp90 inhibitors may be quite challenging given the extensive conservation of Hsp90. The feasibility of selectively targeting Hsp90 in fungal pathogens based on structural differences compared with the human orthologues can be better assessed once structures are solved for Hsp90 from fungal pathogens. Further, selective targeting of Hsp90 in fungal pathogens may be facilitated by the divergence in ATPase activity or equilibrium of conformational states between human and fungal Hsp90 orthologues (Southworth and Agard, 2008). In support of this, conformational differences have been exploited in the development of paralogue selective inhibitors of human Hsp90 proteins (Chan *et al*. 2012; Patel *et al*. 2013). Such differences also underpin parasite selective Hsp90 inhibition with molecules that have increased affinity for *Trypanosoma brucei* Hsp90 compared with the human counterpart (Pizarro *et al*. 2013). Further analysis of Hsp90 conformational states and biochemical properties in fungal pathogens may illuminate distinct features that can be exploited for antifungal drug development. An appreciation of the upstream regulators of Hsp90 function and effectors that control drug resistance and virulence in fungi is poised to reveal alternative therapeutic strategies to inhibit the Hsp90 chaperone machine.

UP-STREAM REGULATORS OF HSP90 FUNCTION IN FUNGAL PATHOGENS

Our understanding of regulation of Hsp90 function in fungal pathogens has been largely informed by studies in mammalian systems and in the model yeast *S. cerevisiae*. Hsp90 function is modulated by ATP binding and hydrolysis, by co-chaperones, and by post-translational modifications including phosphorylation, acetylation, methylation and even nitrosylation (Kovacs *et al*. 2005; Martinez-Ruiz *et al*. 2005; Murphy *et al*. 2005; Scroggins *et al*. 2007; Mollapour *et al*. 2010, 2011; Donlin *et al*. 2012; Xu *et al*. 2012). These post-translational modifications and interactions with co-chaperones can alter Hsp90 conformational states, thereby influencing client protein recognition and chaperone function. Despite conservation of Hsp90, it is notable that the composition of the co-chaperone machinery varies considerably across the eukaryotic kingdom (Johnson and Brown, 2009). Those regulators of Hsp90 function that have been identified in fungal pathogens are far more divergent between pathogen and host than Hsp90, providing a broader window for the development of fungal selective therapeutic agents.

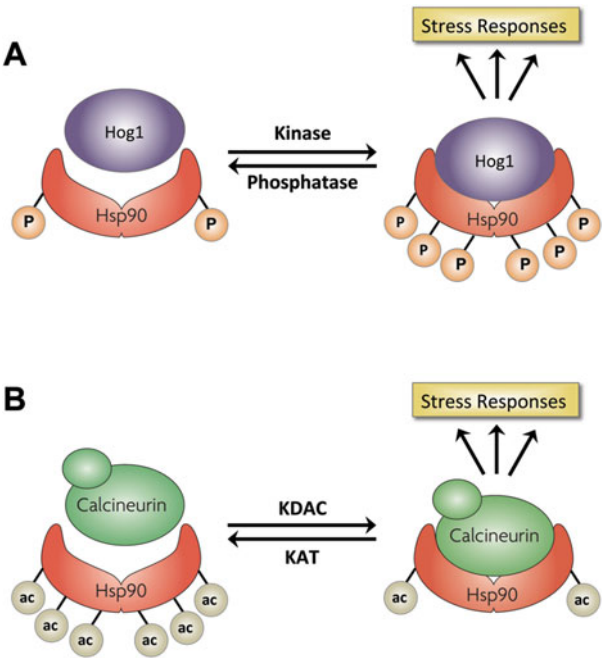

Fig. 7. Upstream regulators of fungal Hsp90 function. (A) Hsp90 function is regulated by phosphorylation. The stability and activation of kinase clients such as Hog1 are dependent on the phosphorylation of Hsp90 by kinases such as CK2. This is important for key cellular stress responses; (B) Hsp90 function is regulated by acetylation. The acetylation level of Hsp90, balanced by lysine deacetylases (KDACs) and lysine acetyl transferases (KATs), influences its interactions and ability to stabilize client proteins. Inhibition of lysine deacetylases (KDACs) blocks the interaction between Hsp90 and its client protein calcineurin, and alters the stability and function of numerous other clients, thereby blocking key responses to drug-induced cellular stress. Adapted from Cowen (2013). The fungal Achilles' heel: targeting Hsp90 to cripple fungal pathogens. *Current Opinion in Microbiology* 16/4, 377–384; Copyright (2013), with permission from Elsevier.

Regulators of Hsp90 function in fungal pathogens have been explored in most detail in *C. albicans*. The first analysis of a co-chaperone demonstrated that Sgt1 physically interacts with Hsp90, and that depletion of Sgt1 phenocopies depletion of Hsp90, abrogating drug resistance and inducing filamentation (Shapiro *et al*. 2012*b*). Sgt1 is far more

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

divergent between fungi and humans than Hsp90, providing an alternative therapeutic target and motivating the study of additional co-chaperones.

Beyond co-chaperones, post-translational modifications have recently been implicated as key for modulating Hsp90 function in *C. albicans*. As with *S. cerevisiae* and mammalian cells (Mollapour *et al*. 2011), Hsp90 function is regulated by phosphorylation mediated by protein kinase CK2 in *C. albicans* (Fig. 7) (Diezmann *et al*. 2012). Deletion of CK2 regulatory subunits reduces phosphorylation of Hsp90, as well as the co-chaperone Cdc37, thereby compromising Hsp90 client protein stability and function (Diezmann *et al*. 2012). Consistent with an impact on Hsp90 function, CK2 has been implicated in regulation of azole resistance (Bruno and Mitchell, 2005). Beyond phosphorylation, acetylation provides an additional post-translation modification with a profound impact on Hsp90 function. Inhibition of lysine deacetylases (KDACs) with the broad-spectrum agent trichostatin A impairs the stability and function of multiple Hsp90 client proteins, and abrogates azole resistance (Fig. 7) (Robbins *et al*. 2012). The KDACs of functional importance for drug resistance in *S. cerevisiae* are Hda1 and Rpd3 (Robbins *et al*. 2012), while the KDACs in *C. albicans* remain to be identified. Notably, the regulatory subunits of the Hda1 complex are not conserved in metazoans providing opportunities for development of fungal specific inhibitors of the Hsp90 chaperone machinery.

DOWN-STREAM EFFECTORS OF HSP90 IMPORTANT FOR DRUG RESISTANCE AND VIRULENCE

Given Hsp90's pleiotropic effects on cellular signalling, it is not surprising that Hsp90 modulates drug resistance and virulence though multiple down-stream effectors. In *C. albicans*, the first Hsp90 client protein identified was calcineurin (Cowen and Lindquist, 2005; Cowen *et al*. 2006; Singh *et al*. 2009), a serine threonine protein phosphatase and key regulator of resistance to azoles and echinocandins (Cruz *et al*. 2002; Sanglard *et al*. 2003; Singh *et al*. 2009). Hsp90 physically interacts with the catalytic subunit of calcineurin in both *S. cerevisiae* and *C. albicans*, and depletion of Hsp90 leads to reduction of calcineurin levels (Imai and Yahara, 2000; Singh *et al*. 2009). Inhibiting calcineurin function with the structurally unrelated immunosuppressants cyclosporin A and FK506 provides a powerful strategy to abrogate drug resistance of diverse fungal pathogens (Steinbach *et al*. 2007; Lamoth *et al*. 2012). Calcineurin is also required for virulence in species of *Candida, Cryptococcus* and *Aspergillus* (Bader *et al*. 2003; Sanglard *et al*. 2003; Steinbach *et al*. 2006; Miyazaki *et al*. 2010*b*; Reedy *et al*. 2010; Chen *et al*. 2011, 2012, 2013; Singh-Babak *et al*. 2012; Zhang *et al*. 2012; Juvvadi *et al*. 2013). A central challenge for the development of calcineurin inhibitors for the treatment of fungal infections is the development of analogues that retain antifungal activity in the absence of immunosuppressive effects due to inhibition of calcineurin in the host (Blankenship *et al*. 2003).

Another Hsp90 effector that modulates resistance to both azoles and echinocandins in *C. albicans* is the terminal mitogen activated protein kinase of the Pkc1 cell wall integrity pathway, Mkc1. Hsp90 stabilizes Mkc1, enabling crucial responses to drug-induced stress (LaFayette *et al*. 2010). While Hsp90 stabilizes both the phosphorylated and un-phosphorylated form of Mkc1 in *C. albicans* (LaFayette *et al*. 2010), Hsp90 interacts exclusively with the phosphorylated form of the orthologue Slt2 in *S. cerevisiae* (Millson *et al*. 2005). Compromising signalling through this cell wall integrity pathway reduces drug resistance, and attenuates virulence of *Candida* species (LaFayette *et al*. 2010; Miyazaki *et al*. 2010*a*). Signalling through Pkc1 also regulates virulence in *Cryptococcus neoformans* (Gerik *et al*. 2005). Chemical genomic approaches have recently been successful in the identification of Hsp90 interactors on a global scale in *C. albicans*, revealing a multitude of additional interactors that include novel regulators of drug resistance and virulence, as well as new therapeutic targets (Diezmann *et al*. 2012).

CONCLUSIONS AND OUTLOOK

Hsp90 has emerged as a fungal Achilles' heel given that it is a hub of cellular circuitry required for fungal drug resistance, stress response, morphogenesis and virulence (Cowen, 2013). The development of combination therapies with an Hsp90 inhibitor deployed with an antifungal drug may be facilitated by the multitude of Hsp90 inhibitors, including 17 agents currently in clinical development for cancer (Trepel *et al*. 2010; Neckers and Workman, 2012). Hsp90 inhibitors in clinical development show promise for combination therapy with antifungals in the context of mammalian models where the fungal infection and drug delivery is localized (Robbins *et al*. 2011). To overcome host toxicity issues in the context of disseminated fungal infections and systemic drug delivery, it is likely that fungal selective Hsp90 inhibitors will be required (Cowen *et al*. 2009). Alternatively, targeting regulators of Hsp90 function that are more divergent between pathogen and host, such as co-chaperones or KDACs, or down-stream effectors required for drug resistance and virulence, such as calcineurin, could provide the foundation of effective combination therapies (Cowen, 2013). Further development of inhibitors of the Hsp90 chaperone network as combination therapeutic agents to treat fungal infections will be facilitated through collaborative efforts

https://doi.org/10.1017/S0031182013002072 Published online by Cambridge University Press

between industry and academia. This may provide a broader paradigm for treatment of infectious disease caused by other eukaryotic pathogens, such as protozoan parasites that are the causal agents of malaria and trypanosomiasis (Pallavi *et al*. 2010; Shahinas *et al*. 2010, 2012, 2013). An appreciation of the importance of combination therapeutic strategies, which are the foundation for the treatment of HIV (Bock and Lengauer, 2012), tuberculosis (Zumla *et al*. 2012) and malaria (Eastman and Fidock, 2009), promises to dramatically accelerate the discovery of novel treatment strategies to minimize the global health burden of fungal infectious disease.

ACKNOWLEDGEMENTS

We thank members of the Cowen lab for helpful discussions.

FINANCIAL SUPPORT

A. V. was supported by an Ontario Graduate Scholarship and a University of Toronto Open Fellowship. L. E. C. was supported by a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund, by a Canada Research Chair in Microbial Genomics and Infectious Disease, by a Ministry of Research and Innovation Early Researcher Award, by Natural Sciences and Engineering Research Council Discovery Grant # 355965 and by Canadian Institutes of Health Research Grants MOP-86452 and MOP-119520.

REFERENCES

Bader, T., Bodendorfer, B., Schroppel, K. and Morschhauser, J. (2003). Calcineurin is essential for virulence in *Candida albicans*. *Infection and Immunity* **71**, 5344–5354.

Blankenship, J. R. and Mitchell, A. P. (2006). How to build a biofilm: a fungal perspective. *Current Opinion in Microbiology* **9**, 588–594.

Blankenship, J. R., Steinbach, W. J., Perfect, J. R. and Heitman, J. (2003). Teaching old drugs new tricks: reincarnating immunosuppressants as antifungal drugs. *Current Opinion in Investigational Drugs* **4**, 192–199.

Bock, C. and Lengauer, T. (2012). Managing drug resistance in cancer: lessons from HIV therapy. *Nature Reviews Cancer* **12**, 494–501. doi: 10.1038/nrc3297.

Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G. and White, T. C. (2012*a*). Hidden killers: human fungal infections. *Science Translational Medicine* **4**, 165rv113. doi: 10.1126/scitranslmed.3004404.

Brown, G. D., Denning, D. W. and Levitz, S. M. (2012*b*). Tackling human fungal infections. *Science* **336**, 647. doi: 10.1126/science.1222236.

Bruno, V. M. and Mitchell, A. P. (2005). Regulation of azole drug susceptibility by *Candida albicans* protein kinase CK2. *Moecular Microbiology* **56**, 559–573.

Byrnes, E. J., III, Bartlett, K. H., Perfect, J. R. and Heitman, J. (2011). *Cryptococcus gattii*: an emerging fungal pathogen infecting humans and animals. *Microbes and Infection* **13**, 895–907. doi: 10.1016/j.micinf.2011.05.009.

Centers for Disease Control and Prevention (2013). *Antibiotic Resistance Threats in the United States, 2013*. U.S. Deparment of Health and Human Services. Centers for Disease Control and Prevention, Atlanta, USA.

Chan, C. T., Reeves, R. E., Geller, R., Yaghoubi, S. S., Hoehne, A., Solow-Cordero, D. E., Chiosis, G., Massoud, T. F., Paulmurugan, R. and Gambhir, S. S. (2012). Discovery and validation of small-molecule heat-shock protein 90 inhibitors through multimodality molecular imaging in living subjects. *Proceedings of the National Academy of Sciences USA* **109**, E2476–E2485. doi: 10.1073/pnas.1205459109.

Chen, Y. L., Brand, A., Morrison, E. L., Silao, F. G., Bigol, U. G., Malbas, F. F., Jr., Nett, J. E., Andes, D. R., Solis, N. V., Filler, S. G., Averette, A. and Heitman, J. (2011). Calcineurin controls drug tolerance, hyphal growth, and virulence in *Candida dubliniensis*. *Eukaryotic Cell* **10**, 803–819. doi: 10.1128/EC.00310-10.

Chen, Y. L., Konieczka, J. H., Springer, D. J., Bowen, S. E., Zhang, J., Silao, F. G., Bungay, A. A., Bigol, U. G., Nicolas, M. G., Abraham, S. N., Thompson, D. A., Regev, A. and Heitman, J. (2012). Convergent evolution of calcineurin pathway roles in thermotolerance and virulence in *Candida glabrata*. *G3:Genes, Genomes, Genetics* **2**, 675–691. doi: 10.1534/g3.112.002279.

Chen, Y. L., Lehman, V. N., Lewit, Y., Averette, A. F. and Heitman, J. (2013). Calcineurin governs thermotolerance and virulence of *Cryptococcus gattii*. *G3:Genes, Genomes, Genetics* **3**, 527–539. doi: 10.1534/g3.112.004242.

Cowen, L. E. (2008). The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. *Nature Reviews Microbiology* **6**, 187–198.

Cowen, L. E. (2009). Hsp90 orchestrates stress response signaling governing fungal drug resistance. *PLoS Pathogens* **5**, e1000471. doi: 10.1371/journal.ppat.1000471.

Cowen, L. E. (2013). The fungal Achilles' heel: targeting Hsp90 to cripple fungal pathogens. *Current Opinion in Microbiology* **16**, 377–384. doi: 10.1016/j.mib.2013.03.005.

Cowen, L. E. and Lindquist, S. (2005). Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science* **309**, 2185–2189.

Cowen, L. E. and Steinbach, W. J. (2008). Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. *Eukaryotic Cell* **7**, 747–764.

Cowen, L. E., Carpenter, A. E., Matangkasombut, O., Fink, G. R. and Lindquist, S. (2006). Genetic architecture of Hsp90-dependent drug resistance. *Eukaryotic Cell* **5**, 2184–2188.

Cowen, L. E., Singh, S. D., Kohler, J. R., Collins, C., Zaas, A. K., Schell, W. A., Aziz, H., Mylonakis, E., Perfect, J. R., Whitesell, L. and Lindquist, S. (2009). Harnessing Hsp90 function as a powerful, broadly effective therapeutic strategy for fungal infectious disease. *Proceedings of the National Academy of Sciences USA* **106**, 2828–2823. doi: 10.1073/pnas.0813394106.

Cruz, M. C., Goldstein, A. L., Blankenship, J. R., Del Poeta, M., Davis, D., Cardenas, M. E., Perfect, J. R., McCusker, J. H. and Heitman, J. (2002). Calcineurin is essential for survival during membrane stress in *Candida albicans*. *EMBO Journal* **21**, 546–559.

d'Enfert, C. (2006). Biofilms and their role in the resistance of pathogenic *Candida* to antifungal agents. *Current Drug Targets* **7**, 465–470.

Diezmann, S., Michaut, M., Shapiro, R. S., Bader, G. D. and Cowen, L. E. (2012). Mapping the Hsp90 genetic interaction network in *Candida albicans* reveals environmental contingency and rewired circuitry. *PLoS Genetics* **8**, e1002562. doi: 10.1371/journal.pgen.1002562.

Donlin, L. T., Andresen, C., Just, S., Rudensky, E., Pappas, C. T., Kruger, M., Jacobs, E. Y., Unger, A., Zieseniss, A., Dobenecker, M. W., Voelkel, T., Chait, B. T., Gregorio, C. C., Rottbauer, W., Tarakhovsky, A. and Linke, W. A. (2012). Smyd2 controls cytoplasmic lysine methylation of Hsp90 and myofilament organization. *Genes and Development* **26**, 114–119. doi: 10.1101/gad.177758.111.

Eastman, R. T. and Fidock, D. A. (2009). Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. *Nature Reviews Microbiology* **7**, 864–874. doi: 10.1038/nrmicro2239.

Falsone, S. F., Leptihn, S., Osterauer, A., Haslbeck, M. and Buchner, J. (2004). Oncogenic mutations reduce the stability of SRC kinase. *Journal of Molecular Biology* **344**, 281–291.

Finkel, J. S. and Mitchell, A. P. (2011). Genetic control of *Candida albicans* biofilm development. *Nature Reviews Microbiology* **9**, 109–118. doi: 10.1038/nrmicro2475.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L. and Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**, 186–194. doi: 10.1038/nature10947.

Gerik, K. J., Donlin, M. J., Soto, C. E., Banks, A. M., Banks, I. R., Maligie, M. A., Selitrennikoff, C. P. and Lodge, J. K. (2005). Cell wall integrity is dependent on the PKC1 signal transduction pathway in *Cryptococcus neoformans*. *Molecular Microbiology* **58**, 393–408. doi: 10.1111/j.1365-2958.2005.04843.x.

Gow, N. A., van de Veerdonk, F. L., Brown, A. J. and Netea, M. G. (2012). *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nature Reviews Microbiology* **10**, 112–122. doi: 10.1038/nrmicro2711.

Imai, J. and Yahara, I. (2000). Role of HSP90 in salt stress tolerance via stabilization and regulation of calcineurin. *Molecular and Cellular Biology* **20**, 9262–9270.

Jarosz, D. F. and Lindquist, S. (2010). Hsp90 and environmental stress transform the adaptive value of natural genetic variation. *Science* **330**, 1820–1824. doi: 10.1126/science.1195487.

Johnson, J. L. and Brown, C. (2009). Plasticity of the Hsp90 chaperone machine in divergent eukaryotic organisms. *Cell Stress and Chaperones* **14**, 83–94. doi: 10.1007/s12192-008-0058-9.

Juvvadi, P. R., Gehrke, C., Fortwendel, J. R., Lamoth, F., Soderblom, E. J., Cook, E. C., Hast, M. A., Asfaw, Y. G., Moseley, M. A., Creamer, T. P. and Steinbach, W. J. (2013). Phosphorylation of calcineurin at a novel serine-proline rich region orchestrates hyphal growth and virulence in *Aspergillus fumigatus*. *PLoS Pathogens* **9**, e1003564. doi: 10.1371/journal.ppat.1003564.

Kovacs, J. J., Murphy, P. J., Gaillard, S., Zhao, X., Wu, J. T., Nicchitta, C. V., Yoshida, M., Toft, D. O., Pratt, W. B. and Yao, T. P. (2005). HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Molecular and Cellular Biology* **18**, 601–607.

LaFayette, S. L., Collins, C., Zaas, A. K., Schell, W. A., Betancourt-Quiroz, M., Gunatilaka, A. A., Perfect, J. R. and Cowen, L. E. (2010). PKC signaling regulates drug resistance of the fungal pathogen *Candida albicans* via circuitry comprised of Mkc1, calcineurin, and Hsp90. *PLoS Pathogens* **6**, e1001090. doi: 10.1371/journal.ppat.1001069.

Lamoth, F., Juvvadi, P. R., Gehrke, C. and Steinbach, W. J. (2012). *In vitro* activity of calcineurin and heat-shock protein 90 (Hsp90) inhibitors against *Aspergillus fumigatus* azole- and echinocandin-resistant strains. *Antimicrobial Agents and Chemotherapy* **57**, 1035–1039. doi: 10.1128/AAC.01857-12.

Leach, M. D., Klipp, E., Cowen, L. E. and Brown, A. J. (2012). Fungal Hsp90: a biological transistor that tunes cellular outputs to thermal inputs. *Nature Reviews Microbiology* **10**, 693–704. doi: 10.1038/nrmicro2875.

Lin, S. J., Schranz, J. and Teutsch, S. M. (2001). Aspergillosis case-fatality rate: systematic review of the literature. *Clinical Infectious Diseases* **32**, 358–366. doi: 10.1086/318483.

Martinez-Ruiz, A., Villanueva, L., Gonzalez de Orduna, C., Lopez-Ferrer, D., Higueras, M. A., Tarin, C., Rodriguez-Crespo, I., Vazquez, J. and Lamas, S. (2005). S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. *Proceedings of the National Academy of Sciences USA* **102**, 8525–8530. doi: 10.1073/pnas.0407294102.

McClellan, A. J., Xia, Y., Deutschbauer, A. M., Davis, R. W., Gerstein, M. and Frydman, J. (2007). Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell* **131**, 121–135. doi: 10.1016/j.cell.2007.07.036.

Miller, L. G., Hajjeh, R. A. and Edwards, J. E., Jr. (2001). Estimating the cost of nosocomial candidemia in the United States. *Clinical Infectious Diseases* **32**, 1110. doi: 10.1086/319613.

Millson, S. H., Truman, A. W., King, V., Prodromou, C., Pearl, L. H. and Piper, P. W. (2005). A two-hybrid screen of the yeast proteome for Hsp90 interactors uncovers a novel Hsp90 chaperone requirement in the activity of a stress-activated mitogen-activated protein kinase, Slt2p (Mpk1p). *Eukaryotic Cell* **4**, 849–860. doi: 10.1128/EC.4.5.849-860.2005.

Miyazaki, T., Inamine, T., Yamauchi, S., Nagayoshi, Y., Saijo, T., Izumikawa, K., Seki, M., Kakeya, H., Yamamoto, Y., Yanagihara, K., Miyazaki, Y. and Kohno, S. (2010*a*). Role of the Slt2 mitogen-activated protein kinase pathway in cell wall integrity and virulence in *Candida glabrata*. *FEMS Yeast Research* **10**, 343–352. doi: 10.1111/j.1567-1364.2010.00611.x.

Miyazaki, T., Yamauchi, S., Inamine, T., Nagayoshi, Y., Saijo, T., Izumikawa, K., Seki, M., Kakeya, H., Yamamoto, Y., Yanagihara, K., Miyazaki, Y. and Kohno, S. (2010*b*). Roles of calcineurin and Crz1 in antifungal susceptibility and virulence of *Candida glabrata*. *Antimicrobial Agents and Chemotherapy* **54**, 1639–1643. doi: 10.1128/AAC.01364-09.

Mollapour, M., Tsutsumi, S., Donnelly, A. C., Beebe, K., Tokita, M. J., Lee, M. J., Lee, S., Morra, G., Bourboulia, D., Scroggins, B. T., Colombo, G., Blagg, B. S., Panaretou, B., Stetler-Stevenson, W. G., Trepel, J. B., Piper, P. W., Prodromou, C., Pearl, L. H. and Neckers, L. (2010). Swe1Wee1-dependent tyrosine phosphorylation of Hsp90 regulates distinct facets of chaperone function. *Molecular Cell* **37**, 333–343.

Mollapour, M., Tsutsumi, S., Truman, A. W., Xu, W., Vaughan, C. K., Beebe, K., Konstantinova, A., Vourganti, S., Panaretou, B., Piper, P. W., Trepel, J. B., Prodromou, C., Pearl, L. H. and Neckers, L. (2011). Threonine 22 phosphorylation attenuates Hsp90 interaction with cochaperones and affects its chaperone activity. *Molecular Cell* **41**, 672–681. doi: 10.1016/j.molcel.2011.02.011.

Money, N. P. (2007). *The Triumph of the Fungi: A Rotten History*. Oxford University Press, New York, NY, USA.

Murphy, P. J., Morishima, Y., Kovacs, J. J., Yao, T. P. and Pratt, W. B. (2005). Regulation of the dynamics of Hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. *Journal of Biological Chemistry* **280**, 33792–33799.

Neckers, L. and Workman, P. (2012). Hsp90 molecular chaperone inhibitors: are we there yet? *Clinical Cancer Research* **18**, 64–76. doi: 10.1158/1078-0432.CCR-11-1000.

Nett, J. and Andes, D. (2006). *Candida albicans* biofilm development, modeling a host-pathogen interaction. *Current Opinion in Microbiology* **9**, 340–345.

Noble, S. M., French, S., Kohn, L. A., Chen, V. and Johnson, A. D. (2010). Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. *Nature Genetics* **42**, 590–598. doi: 10.1038/ng.605.

Pallavi, R., Roy, N., Nageshan, R. K., Talukdar, P., Pavithra, S. R., Reddy, R., Venketesh, S., Kumar, R., Gupta, A. K., Singh, R. K., Yadav, S. C. and Tatu, U. (2010). Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of HSP90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *Journal of Biological Chemistry* **285**, 37964–37975. doi: 10.1074/jbc.M110.155317.

Patel, P. D., Yan, P., Seidler, P. M., Patel, H. J., Sun, W., Yang, C., Que, N. S., Taldone, T., Finotti, P., Stephani, R. A., Gewirth, D. T. and Chiosis, G. (2013). Paralog-selective Hsp90 inhibitors define tumor-specific regulation of HER2. *Nature Chemical Biology* **9**, 677–684. doi: 10.1038/nchembio.1335.

Pfaller, M. A. and Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews* **20**, 133–163.

Pfaller, M. A. and Diekema, D. J. (2010). Epidemiology of invasive mycoses in North America. *Critical Reviews in Microbiology* **36**, 1–53.

Pizarro, J. C., Hills, T., Senisterra, G., Wernimont, A. K., Mackenzie, C., Norcross, N. R., Ferguson, M. A., Wyatt, P. G., Gilbert, I. H. and Hui, R. (2013). Exploring the *Trypanosoma brucei* Hsp83 potential as a target for structure guided drug design. *PLoS Neglected Tropical Diseases* **7**, e2492. doi: 10.1371/journal.pntd.0002492.

Queitsch, C., Sangster, T. A. and Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624.

Ramage, G., Mowat, E., Jones, B., Williams, C. and Lopez-Ribot, J. (2009). Our current understanding of fungal biofilms. *Critical Reviews in Microbiology* **35**, 340–355. doi: 10.3109/10408410903241436.

Reedy, J. L., Filler, S. G. and Heitman, J. (2010). Elucidating the *Candida albicans* calcineurin signaling cascade controlling stress response and virulence. *Fungal Genetics and Biology* **47**, 107–116. doi: 10.1016/j.fgb.2009.09.002.

Robbins, N., Uppuluri, P., Nett, J., Rajendran, R., Ramage, G., Lopez-Ribot, J. L., Andes, D. and Cowen, L. E. (2011). Hsp90 governs dispersion and drug resistance of fungal biofilms. *PLoS Pathogens* **7**, e1002257. doi: 10.1371/journal.ppat.1002257.

Robbins, N., Leach, M. D. and Cowen, L. E. (2012). Lysine deacetylases Hda1 and Rpd3 regulate Hsp90 function thereby governing fungal drug resistance. *Cell Reports* **2**, 878–888. doi: 10.1016/j.celrep.2012.08.035.

Rutherford, S. L. (2003). Between genotype and phenotype: protein chaperones and evolvability. *Nature Reviews Genetics* **4**, 263–274.

Rutherford, S. L. and Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342.

Sanglard, D., Ischer, F., Marchetti, O., Entenza, J. and Bille, J. (2003). Calcineurin A of *Candida albicans:* involvement in antifungal tolerance, cell morphogenesis and virulence. *Molecular Microbiology* **48**, 959–976.

Sangster, T. A., Lindquist, S. and Queitsch, C. (2004). Under cover: causes, effects and implications of Hsp90-mediated genetic capacitance. *BioEssays* **26**, 348–362.

Sangster, T. A., Salathia, N., Lee, H. N., Watanabe, E., Schellenberg, K., Morneau, K., Wang, H., Undurraga, S., Queitsch, C. and Lindquist, S. (2008*a*). HSP90-buffered genetic variation is common in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **105**, 2969–2974. doi: 10.1073/pnas.0712210105.

Sangster, T. A., Salathia, N., Undurraga, S., Milo, R., Schellenberg, K., Lindquist, S. and Queitsch, C. (2008*b*). *HSP90* affects the expression of genetic variation and developmental stability in quantitative traits. *Proceedings of the National Academy of Sciences USA* **105**, 2963–2968. doi: 10.1073/pnas.0712200105.

Scroggins, B. T., Robzyk, K., Wang, D., Marcu, M. G., Tsutsumi, S., Beebe, K., Cotter, R. J., Felts, S., Toft, D., Karnitz, L., Rosen, N. and Neckers, L. (2007). An acetylation site in the middle domain of Hsp90 regulates chaperone function. *Molecular Cell* **25**, 151–159.

Shahinas, D., Liang, M., Datti, A. and Pillai, D. R. (2010). A repurposing strategy identifies novel synergistic inhibitors of *Plasmodium*

falciparum heat shock protein 90. *Journal of Medicinal Chemistry* **53**, 3552–3557. doi: 10.1021/jm901796s.

Shahinas, D., Macmullin, G., Benedict, C., Crandall, I. and Pillai, D. R. (2012). Harmine is a potent antimalarial targeting Hsp90 and synergizes with chloroquine and artemisinin. *Antimicrobial Agents and Chemotherapy* **56**, 4207–4213. doi: 10.1128/AAC.00328-12.

Shahinas, D., Folefoc, A., Taldone, T., Chiosis, G., Crandall, I. and Pillai, D. R. (2013). A purine analog synergizes with chloroquine (CQ) by targeting *Plasmodium falciparum* Hsp90 (PfHsp90). *PLoS ONE* **8**, e75446. doi: 10.1371/journal.pone.0075446.

Shapiro, R. S., Uppuluri, P., Zaas, A. K., Collins, C., Senn, H., Perfect, J. R., Heitman, J. and Cowen, L. E. (2009). Hsp90 orchestrates temperature-dependent *Candida albicans* morphogenesis via Ras1-PKA signaling. *Current Biology* **19**, 621–629.

Shapiro, R. S., Robbins, N. and Cowen, L. E. (2011). Regulatory circuitry governing fungal development, drug resistance, and disease. *Microbiology and Molecular Biology Reviews* **75**, 213–267. doi: 10.1128/MMBR.00045-10.

Shapiro, R. S., Sellam, A., Tebbji, F., Whiteway, M., Nantel, A. and Cowen, L. E. (2012*a*). Pho85, Pcl1, and Hms1 signaling governs *Candida albicans* morphogenesis induced by high temperature or Hsp90 compromise. *Current Biology* **22**, 461–470. doi: 10.1016/j.cub.2012.01.062.

Shapiro, R. S., Zaas, A. K., Betancourt-Quiroz, M., Perfect, J. R. and Cowen, L. E. (2012*b*). The Hsp90 co-chaperone Sgt1 governs *Candida albicans* morphogenesis and drug resistance. *PLoS ONE* **7**, e44734. doi: 10.1371/journal.pone.0044734.

Singh, S. D., Robbins, N., Zaas, A. K., Schell, W. A., Perfect, J. R. and Cowen, L. E. (2009). Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. *PLoS Pathogens* **5**, e1000532. doi: 10.1371/journal.ppat.1000532.

Singh-Babak, S. D., Babak, T., Diezmann, S., Hill, J. A., Xie, J. L., Chen, Y. L., Poutanen, S. M., Rennie, R. P., Heitman, J. and Cowen, L. E. (2012). Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. *PLoS Pathogens* **8**, e1002718. doi: 10.1371/journal.ppat.1002718.

Sollars, V., Lu, X., Xiao, L., Wang, X., Garfinkel, M. D. and Ruden, D. M. (2003). Evidence for an epigenetic mechanism by which Hsp90 acts as a capacitor for morphological evolution. *Nature Genetics* **33**, 70–74.

Southworth, D. R. and Agard, D. A. (2008). Species-dependent ensembles of conserved conformational states define the Hsp90 chaperone ATPase cycle. *Molecular Cell* **32**, 631–640. doi: 10.1016/j.molcel.2008.10.024.

Specchia, V., Piacentini, L., Tritto, P., Fanti, L., D'Alessandro, R., Palumbo, G., Pimpinelli, S. and Bozzetti, M. P. (2010). Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons. *Nature* **463**, 662–665. doi: 10.1038/nature08739.

Steinbach, W. J., Cramer, R. A., Jr., Perfect, B. Z., Asfaw, Y. G., Sauer, T. C., Najvar, L. K., Kirkpatrick, W. R., Patterson, T. F., Benjamin, D. K., Jr., Heitman, J. and Perfect, J. R. (2006). Calcineurin controls growth, morphology, and pathogenicity in *Aspergillus fumigatus*. *Eukaryotic Cell* **5**, 1091–1103.

Steinbach, W. J., Reedy, J. L., Cramer, R. A., Jr., Perfect, J. R. and Heitman, J. (2007). Harnessing calcineurin as a novel anti-infective agent against invasive fungal infections. *Nature Reviews Microbiology* **5**, 418–430.

Taipale, M., Jarosz, D. F. and Lindquist, S. (2010). *HSP90* at the hub of protein homeostasis: emerging mechanistic insights. *Nature Reviews Molecular Cell Biology* **11**, 515–528. doi: 10.1038/nrm2918.

Tariq, M., Nussbaumer, U., Chen, Y., Beisel, C. and Paro, R. (2009). Trithorax requires Hsp90 for maintenance of active chromatin at sites of gene expression. *Proceedings of the National Academy of Sciences USA* **106**, 1157–1162. doi: 10.1073/pnas.0809669106.

Trepel, J., Mollapour, M., Giaccone, G. and Neckers, L. (2010). Targeting the dynamic *HSP90* complex in cancer. *Nature Reviews Cancer* **10**, 537–549. doi: 10.1038/nrc2887.

Xu, W., Mollapour, M., Prodromou, C., Wang, S., Scroggins, B. T., Palchick, Z., Beebe, K., Siderius, M., Lee, M. J., Couvillon, A., Trepel, J. B., Miyata, Y., Matts, R. and Neckers, L. (2012). Dynamic tyrosine phosphorylation modulates cycling of the *HSP90-P50(CDC37)-AHA1* chaperone machine. *Molecular Cell* **47**, 434–443. doi: 10.1016/j.molcel.2012.05.015.

Xu, Y. and Lindquist, S. (1993). Heat-shock protein Hsp90 governs the activity of pp60v-src kinase. *Proceedings of the National Academy of Sciences USA* **90**, 7074–7078.

Xu, Y., Singer, M. A. and Lindquist, S. (1999). Maturation of the tyrosine kinase c-src as a kinase and as a substrate depends on the molecular chaperone Hsp90. *Proceedings of the National Academy of Sciences USA* **96**, 109–114.

Zhang, J., Silao, F. G., Bigol, U. G., Bungay, A. A., Nicolas, M. G., Heitman, J. and Chen, Y. L. (2012). Calcineurin is required for pseudohyphal growth, virulence, and drug resistance in *Candida lusitaniae*. *PLoS ONE* **7**, e44192. doi: 10.1371/journal.pone.0044192.

Zhao, R., Davey, M., Hsu, Y. C., Kaplanek, P., Tong, A., Parsons, A. B., Krogan, N., Cagney, G., Mai, D., Greenblatt, J., Boone, C., Emili, A. and Houry, W. A. (2005). Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the Hsp90 chaperone. *Cell* **120**, 715–727.

Zumla, A., Hafner, R., Lienhardt, C., Hoelscher, M. and Nunn, A. (2012). Advancing the development of tuberculosis therapy. *Nature Reviews Drug Discovery* **11**, 171–172. doi: 10.1038/nrd3694.