Hsp90 Potentiates the Rapid Evolution of New Traits: Drug Resistance in Diverse Fungi

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Hsp90 is a molecular chaperone for many signal transducers and may influence evolution by releasing previously silent genetic variation in response to environmental change. In fungi separated by ~800 million years of evolution, Hsp90 potentiated the evolution of drug resistance in a different way, by enabling new mutations to have immediate phenotypic consequences. Resistance was abrogated by Hsp90 inhibitors and by febrile temperatures, suggesting new therapeutic strategies and a clinical benefit of fever. During selection in a human host, drug resistance that was initially Hsp90-dependent evolved toward independence. Thus, Hsp90 can act in diverse ways to couple environmental contingency to the emergence and fixation of new traits.

Hsp90 is an essential molecular chaperone that regulates the folding, transport, maturation, and degradation of a diverse but select set of client proteins, many of which are key regulators of cell signaling (*1*–*3*). A common feature of many Hsp90 clients is a tendency to dwell in incompletely folded or aggregation-prone states. These proteins dynamically cycle through complexes with Hsp90 and other cofactors until their activation is engendered by the proper signal.

As a consequence of its function in chaperoning regulators of cell circuitry, Hsp90 has a capacity to buffer the expression of genetic and epigenetic variation and to release it in response to environmental stress (*4*–*6*). Hsp90 is normally expressed at much higher levels than required for basal function. It is thereby intrinsically positioned to buffer variation. Protein folding, however, is exquisitely sensitive to environmental stress. Although Hsp90 is induced to cope with stress-induced problems in protein folding, depending on the genetic variants that have accumulated in particular genomes, the demand for Hsp90 can outpace its induction. In the fly *Drosophila melanogaster*, the plant *Arabidopsis thaliana*, and likely in other organisms, compromising Hsp90's buffering capacity (by drugs, mutations, or environmental stress) produces a multitude of new phenotypes. Some of these may be stochastic, but others depend on previously silent variation acting in a combinatorial manner to produce new traits (*4*, *5*). After several generations of genetic reassortment and selection, polymorphisms that had been cryptic in progenitor organisms can become so enriched in their progeny that they produce stable phenotypes even in the absence of stress (*4*). Thus, Hsp90 may play a role in evolution by acting as a capacitor for the storage and release of genetic variation.

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In cancer cells, Hsp90 may promote the evolution of new traits in a different manner. Rather than buffering the effects of new mutations, it allows them to have immediate phenotypic consequences. For example, compared to its normal cellular counterpart c-Src, oncogenic v-Src contains several mutations that both destabilize it and derepress its kinase activity (*7*). Hsp90 chaperones the unstable protein, unleashing its promiscuous kinase activities and promoting oncogenesis (*8*, *9*).

Here, we asked whether Hsp90 could allow new mutations to have immediate phenotypic consequences and promote the emergence of new traits in free-living organisms. We examined the evolution of fungal resistance to antimicrobial agents, an ancient and ubiquitous process in nature as microorganisms evolve new strategies for competition and survival. Resistant mutations are rapidly acquired (*10*); their phenotypic consequences are large (*11*); and multiple mechanisms of resistance are known (*12*, *13*). Fungal drug resistance is also of great economic and biomedical importance; few clinically useful drugs exist and resistance has emerged for all.

We investigated resistance to two classes of drugs. Azoles are the most broadly used antifungals in the clinic. They target Erg11, which is required for the biosynthesis of ergosterol, the predominant sterol of fungal membranes (*12*, *14*). Resistance arises through multiple mechanisms, including increases in Erg11 function, increases in multidrug transporters, alterations in sterol biosynthesis, and changes in membrane composition (*10*, *12*). Echinocandins, the first new antifungal class in decades, inhibit synthesis of β-(1,3) glucan, an essential component of fungal cell walls (*14*). Our results establish an entirely new facet to the role of Hsp90 in evolutionary processes.

Hsp90 potentiates the acquisition of fluconazole resistance in *S. cerevisiae*. Using the Cre-Lox system, we constructed strains of *S. cerevisiae* in which the abundance of Hsp90 could be altered by inducible recombination (*15*). These strains had a high constitutive level of Hsp90 expression that was reducible when Cre-mediated recombination removed a cassette with an Hsp90 gene (*HSC82*) and a *URA3* marker (Re90 strains, fig. S1, A to C). Other strains had either a fixed low level of Hsp90 (Lo90 strains) or a fixed high level of Hsp90 (Hi90 strains); here, Cre-mediated recombination removed only a *URA3* marker (fig. S1, A to C).

All strains exhibited the same sensitivity to the most commonly used azole, fluconazole, with growth completely arrested at 16 μg/ml (fig. S1D). To select resistant mutants, we used a rapid selection regime in which large numbers of cells are plated onto medium containing a high concentration of fluconazole (128 μg/ml). Most cells underwent ~8 doublings before growth was arrested, producing many tiny, abortive colonies (Fig. 1A). Intermediate-sized colonies were also recovered, but upon retesting, these did not have true resistance [see (*15*)]. Only colonies of the largest size (≥1.6 mm^2) had acquired robust, reproducible resistance.

Hsp90 had a profound impact on the number of large colonies recovered (Fischer's exact test, $P < 5 \times 10^{-85}$). From Hi90 and Re90 strains, 115 large colonies were obtained. All 24 that were retested grew vigorously with fluconazole (256 μg/ml) (Fig. 1B) (*16*). From Lo90 strains, only three large colonies were obtained. None showed true resistance upon retesting (*15*). Thus, the emergence of fluconazole resistance with this rapid selection regime depended on high levels of Hsp90.

Hsp90 plays a crucial role in these resistant phenotypes. Is Hsp90 required only to cope with the stress of the initial selection conditions, or is it intimately involved in enabling resistance? Cre recombinase was induced in 12 of the fluconazole-resistant mutants obtained by rapid selection (FLR strains, Fig. 1, B and C). In Hi90-FLR cells, Cre-mediated recombination had no effect on drug resistance in rich medium (Fig. 1C). In Re90-FLR strains, Cre-mediated recombination reduced Hsp90 expression and abolished resistance (Fig. 1C). All FLR strains were also resistant to voriconazole, a new azole with broader activity; this resistance was also abrogated when Hsp90 expression was reduced (*16*).

The role of Hsp90 depends on the mode of selection. *S. cerevisiae* cells exposed to two different selection regimes acquire fluconazole resistance by different mechanisms (*11*). To determine whether Hsp90 plays a role in both, we used six strains isolated in another laboratory, three by rapid selection (R1, R2, and R3) and three by gradual selection (G1, G2, and G3) (*11*). We compromised Hsp90 function pharmacologically with geldanamycin (GdA) or radicicol (RAD), structurally unrelated Hsp90 inhibitors that bind with high affinity to Hsp90's unusual adenosine triphosphate binding pocket (*17*, *18*). We used concentrations of GdA and

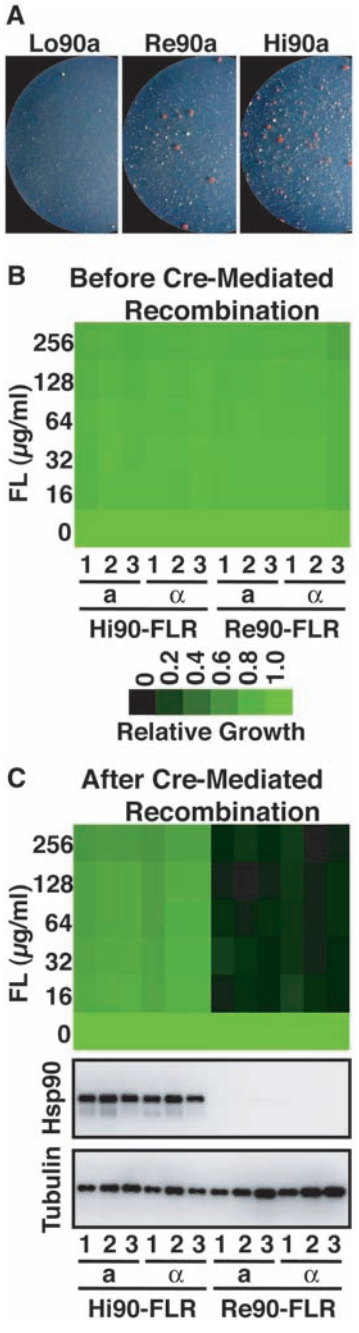

Fig. 1. Hsp90 enables rapid acquisition of fluconazole (FL) resistance and is required for its maintenance. (**A**) For both mating types (*a* and α), FL-resistant (FLR) colonies were recovered only in strains with high levels of Hsp90 (*a* shown here). Red color develops in larger colonies in this strain. (**B**) FLR strains showed strong resistance in rich medium at 23°C. Three independent Hi90-FLR and Re90-FLR strains of each mating type are shown. (**C**) Reducing Hsp90 expression by Cre-mediated recombination abrogated FL resistance. Optical densities (OD) of minimum inhibitory concentration (MIC) test plates were averaged for duplicate measurements and normalized relative to FL-free controls (see color bar). Bottom, immune blot analysis of Hsp90 levels relative to a tubulin loading control.

RAD that (i) did not impair growth on their own (Fig. 2, A to C, data points for fluconazole at 0 μg/ml); (ii) had no effect on the fluconazole sensitivities of the progenitor strains (Fig. 2A); and (iii) phenocopied the effects of genetically reducing Hsp90 in the FLR strains discussed above (*16*). GdA and RAD each abolished fluconazole resistance in the R strains (Fig. 2B), but not in the G strains (Fig. 2C). Thus, Hsp90 is required to maintain resistance acquired by rapid selection but not resistance acquired by gradual selection.

In nature, Hsp90's function can be overwhelmed by problems in protein folding that result from environmental stress, such as high temperatures (*19*). The R strains showed an enormous reduction in resistance at 39°C, whereas the G strains maintained resistance (Fig. 2D). Thus, with phenotypic consequences that are contingent on the mode of selection, environmental stress alone recapitulates the effects of impaired Hsp90 function.

Mechanism of Hsp90-independent resistance. The G strains had acquired resistance through mutations in the transcription factor Pdr1, which increase the expression of multidrug transporters such as Pdr5 (*11*). A trivial explanation for the robustness of their fluconazole resistance to GdA and RAD is that the Hsp90 inhibitors are simply being pumped out of the cell. However, G1 strains also remained resistant to fluconazole when Hsp90 was reduced genetically (fig. S2).

If this mechanism of resistance truly does not depend on Hsp90, then it should arise equally in strains with high and low levels of Hsp90 selected under a regimen that favors Pdr1-based pathways. Indeed, using such selection (*20*), triplicate populations of Hi90, Re90, and Lo90 remained static for several days and then initiated vigorous growth (fig. S3). Isolates from each population showed true resistance to fluconazole and overexpressed Pdr5 (Fig. 2E) (*16*). Thus, this mechanism of resistance can both be acquired and maintained independently of Hsp90, in contrast to the crucial role of Hsp90 in resistance acquired by rapid, acute selection.

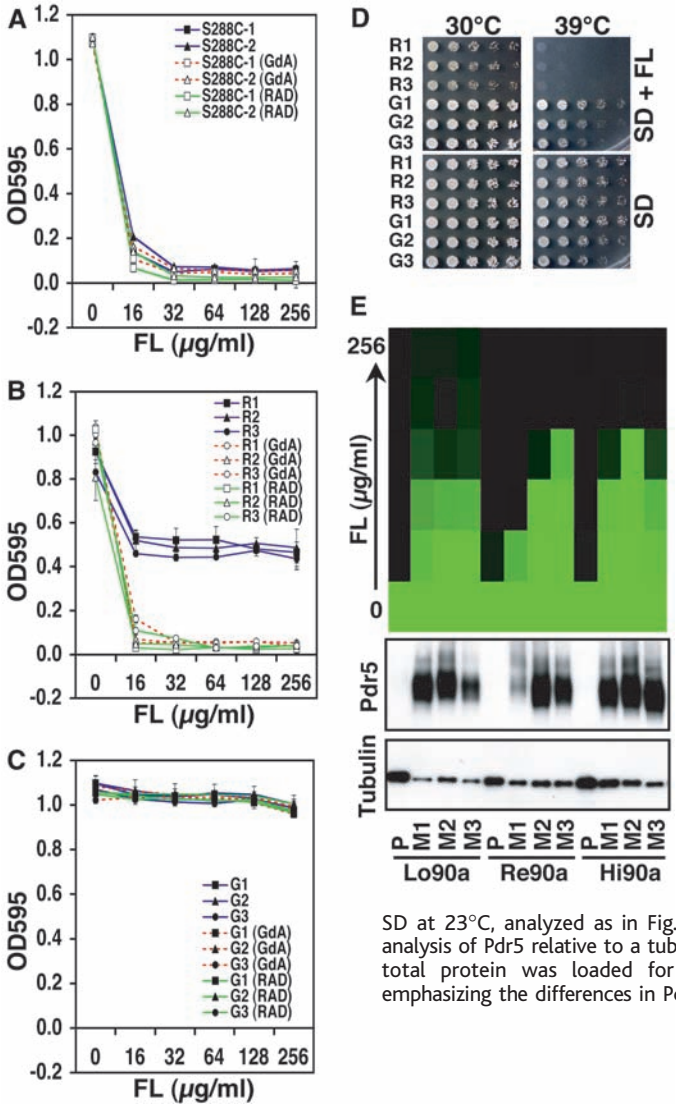

Fig. 2. Hsp90 is required for FL resistance acquired by rapid selection but not by gradual selection. Hsp90 was inhibited by geldanamycin (GdA, 5 μM) or radicicol (RAD, 5 μM) in FL MIC plates with synthetic defined (SD) medium at 30°C. (**A**) Progenitor strains; (**B**) mutants obtained by rapid selection; (**C**) mutants obtained by gradual selection. ODs of MIC test plates were averaged for three replicates. Error bars are standard deviations. (**D**) High temperature phenocopied the effects of Hsp90 inhibitors on FL resistance. Fivefold dilutions of cells (from 8×10^5/ml) were spotted on SD with FL (32 μg/ml) (top) and without FL (bottom). (**E**) Hsp90-independent FL resistance involves the increased expression of a drug pump. Top, FL resistance of the progenitor (P) and strains acquired by selection with FL (16 μg/ml) (M1 to M3) in SD at 23°C, analyzed as in Fig. 1. Bottom, immune blot analysis of Pdr5 relative to a tubulin loading control. More total protein was loaded for the progenitor strains, emphasizing the differences in Pdr5 expression.

Mechanisms of Hsp90-dependent resistance. Azoles block fungal growth by inhibiting Erg11, resulting in the accumulation of toxic intermediates in ergosterol biosynthesis (*12*). Rapid selection favors mutations in Erg3 that prevent the accumulation of toxic intermediates (*11*). Erg3 mutants have altered membrane sterol composition but can grow in the presence of azoles (*12*). Sequencing revealed that each of the 12 Hsp90-dependent strains we isolated by rapid selection contained *ERG3* mutations (table S1, Re90-FLR and Hi90-FLR).

To provide a more global view, we took advantage of a previous screen in which ~4700 viable haploid *S. cerevisiae* deletion mutants were tested for enhanced fluconazole resistance (*11*). GdA and RAD reduced resistance in all of the 11 mutants in our collection: *erg3*Δ, *erg6*Δ, *ymr102c*Δ, *ymr099c*Δ, *ypl056c*Δ, *osh1*Δ, *scs2*Δ, *cka2*Δ, *ybr147w*Δ, *ygr283c*Δ, and *ylr407w*Δ (D1 to D11, Fig. 3A) (fig. S4) (*16*). Clearly, Hsp90 can potentiate fluconazole resistance acquired through a variety of genetic lesions.

The role of calcineurin. Calcineurin is an Hsp90 client protein that regulates numerous responses to environmental stimuli, including the response to azoles (*21*–*23*). Hsp90 directly interacts with calcineurin and keeps it poised for activation (*24*, *25*). If Hsp90's effects on fluconazole resistance work through calcineurin, then inhibition of calcineurin should phenocopy Hsp90 inhibition.

Cyclosporin A (CsA) and FK506 are structurally unrelated drugs that block calcineurin function in different ways (*26*). CsA forms an inhibitory calcineurin-drug-protein complex involving Cpr1, a peptidyl-prolyl cis-trans isomerase (cyclophilin A). FK506 forms a different calcineurin-drug-protein complex involving FKBP12, a structurally unrelated peptidyl-prolyl cis-trans isomerase. Each drug strongly reduced fluconazole resistance in every one of the Hsp90-dependent mutants we tested (Fig. 3A) (fig. S4) (*16*). Thus, Hsp90 may potentiate the resistance of many different mutants through a common regulator, calcineurin.

Next, we used an *erg3*Δ mutant to dissect the underlying molecular mechanism genetically. If CsA reduced fluconazole resistance by inhibiting calcineurin, then deletion of *CPR1* should allow the *erg3*Δ mutant to maintain resistance even when CsA is present (because the inhibitory calcineurin-drug-Cpr1 complex cannot form). This was indeed the case (Fig. 3B). As expected because Hsp90 chaperones the catalytic subunit, resistance of the *erg3*Δ*cpr1*Δ double mutant was still abrogated by Hsp90 inhibitors (Fig. 3B).

Finally, we confirmed the role of calcineurin genetically. Calcineurin is a heterodimer of a catalytic subunit (either Cna1 or Cna2) and an activating regulatory subunit (Cnb1). Double mutants *erg3*Δ*cna1*Δ or *erg3*Δ*cna2*Δ were resistant to fluconazole, consistent with catalytic subunit redundancy (Fig. 3C); *erg3*Δ mutants missing both catalytic subunits (*erg3*Δ*cna1*Δ*cna2*Δ) or missing the activating subunit (Δ*erg3*Δ*cnb1*) were sensitive. Thus, calcineurin is a critical mediator of Hsp90-dependent azole resistance.

Hsp90 potentiates the evolution of drug resistance in *Candida albicans*. *C. albicans* is an important human pathogen estimated to have diverged from *S. cerevisiae* ~800 million years ago (*27*). In *S. cerevisiae*, rapid selection favors recessive *ERG3* mutations. Because *C. albicans* is diploid, we used both a standard lab strain (CAI4) and a heterozygous *ERG3* deletion mutant (CaERG3/erg3). Both were sensitive to fluconazole, with growth completely arrested at 16 μg/ml (*16*). When large numbers of cells were plated on fluconazole (128 μg/ml), many colonies were recovered (Fig. 4A). RAD had no effect on growth in the absence of fluconazole (fig. S5). However, when cells were plated on medium with fluconazole and RAD, no resistant colonies were recovered.

Heterozygosity for *ERG3* did not produce more resistant colonies, which suggests that recessive *ERG3* mutations were not the main route to resistance. Indeed, none of the six strains we analyzed carried *ERG3* mutations (*16*). Thus, although distinct underlying mechanisms may be involved, Hsp90 plays a central role in facilitating the rapid acquisition of resistance in both *C. albicans* and *S. cerevisiae*.

Evolution of *C. albicans* drug resistance in a human host. To investigate the impact of Hsp90 on a natural evolutionary process, we used *C. albicans* clinical isolates (CaCi) collected from a single HIV-infected patient over a 2-year course of fluconazole treatment. They represent a single strain that evolved increasing levels of resistance by multiple mechanisms (*28*).

All clinical isolates were more resistant than the lab strain (CAI4) to fluconazole. Differences among clinical isolates were less apparent in rich medium (Fig. 4B) than in a defined medium that mimics the nutrient-poor environment in humans (Fig. 4C). In both media, inhibition of Hsp90 (by GdA) or of calcineurin (by CsA) reduced resistance (Fig. 4B) (*16*). Both inhibitors affected early isolates more strongly than later isolates. With respect to the effects of environmental stress, febrile temperatures reached in humans confronted by infections phenocopied the effects of Hsp90 inhibition (Fig. 4C) (*16*). Thus, under the continued selective pressures shaping pathogen evolution in this patient, resistance traits initially completely dependent on Hsp90 and calcineurin evolved toward independence, with environmental stress likely providing a driving force.

Hsp90 modulates caspofungin resistance in *Aspergillus terreus*. Finally, we turned to *Aspergillus*, filamentous ascomycetes that diverged from *Candida* and *Saccharomyces* ~1 billion years ago (*27*). They are important human pathogens and are resistant to

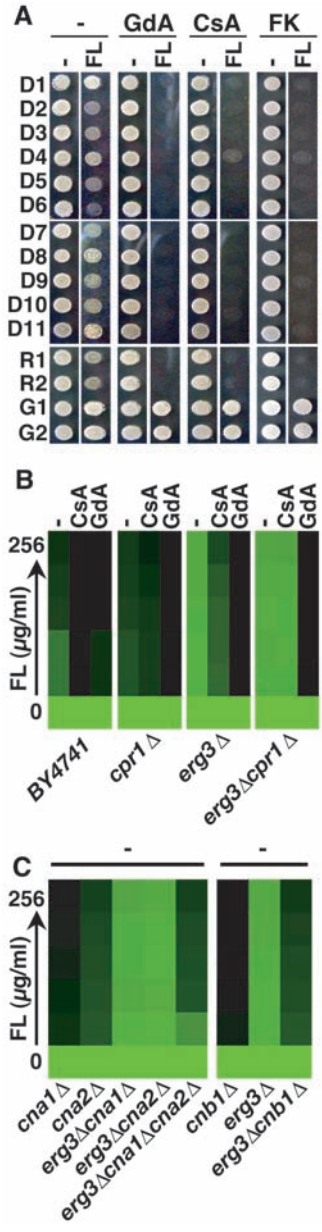

Fig. 3. Hsp90 and calcineurin are required for FL resistance acquired through diverse mutations. (**A**) Cells (4×10^6/ml) were spotted on rich medium with or without FL (64 μg/ml) at 30°C. Top two panels, deletion mutants; bottom panel, strains obtained by rapid (R1, R2) and gradual (G1, G2) selection. Hsp90 was inhibited by GdA (5 μM); calcineurin was inhibited by cyclosporin A (CsA, 20 μM) and FK506 (FK, 10 μM). Concordant results were obtained by MIC testing, but the magnitude of the inhibitors' effects on resistance differed among mutants (fig. S4). (**B**) FL resistance of the parental strain (BY4741) and *erg3*Δ and *cpr1*Δ single and double mutants in rich medium at 30°C, with CsA (20 μM), or with GdA (5 μM), analyzed as in Fig. 1. (**C**) FL resistance of *erg3*Δ and calcineurin mutants in rich medium at 30°C.

many antifungal drugs (*29*). We used antifungal test strips to create a gradient of drug concentration in solid medium. Hsp90 and calcineurin inhibitors strongly reduced the resistance of a clinical isolate of *C. albicans* to fluconazole and voriconazole, but did not affect the basal resistance of *A. terreus* (Fig. 5). Calcineurin inhibitors increase the sensitivity of *Aspergillus* to the echinocandin caspofungin (*30*, *31*). An Hsp90 inhibitor had an equally strong effect (Fig. 5). We found similar effects on clinical isolates of *A. fumigatus* (*32*). In contrast, inhibitors of calcineurin or Hsp90 did not alter the sensitivity of *C. albicans* to caspofungin (Fig. 5). Thus, Hsp90 has profound but distinct effects on drug resistance in evolutionarily distant fungal pathogens.

Discussion. Our results establish a distinct role for Hsp90 in the evolution of adaptive traits. In *S. cerevisiae* and *C. albicans*, fungal species separated by ~800 million years of evolution, Hsp90 potentiates the emergence of azole resistance by enabling diverse new mutations to have immediate phenotypic consequences. In *A. terreus*, Hsp90 is required for the high basal resistance to an echinocandin. Hsp90's role in drug resistance is to enable crucial responses to specific stresses, namely changes in the composition of cell membranes and cell walls. In this respect Hsp90 functions in concert with calcineurin, itself a key sensor of environmental stress and regulator of cell signaling.

Previous work suggests that Hsp90 might affect the evolution of new traits in two different ways. First, Hsp90 can promote the storage of cryptic genetic variation; when Hsp90 buffering capacity is compromised, new traits appear (*4*, *5*). Second, Hsp90 can chaperone mutated cell regulators that are prone to misfolding but have activated oncogenic potential (*9*, *17*); when Hsp90 function is compromised, new traits are lost. In the evolution of fungal drug resistance, new traits are also lost when Hsp90 function is compromised. Here, however, Hsp90 does not directly chaperone and activate mutated proteins. Rather, mutated proteins lose function, and it is their loss of function that confers resistance. Hsp90 chaperones a normal (unmutated) regulator of cell signaling (calcineurin), potentiating the circuitries that sculpt adaptive phenotypes. Because many mutations are expected to exert stress on cellular processes and because Hsp90 chaperones so many signal transducers, this Hsp90-mediated mechanism for the evolution of new traits is likely to play a much broader role than in the evolution of drug resistance alone. Further, although these three ways by which Hsp90 affects the acquisition of new traits were uncovered in different model organisms, they are likely to operate in the same organisms in concert.

Hsp90's role in the evolution of fungal drug resistance has broad therapeutic implications. Calcineurin inhibitors have antifungal potential, but their immunosuppressant effects are problematic (*33*). Hsp90 inhibitors might provide a better strategy. Drugs structurally related to GdA are currently in phase I/II clinical trials as anticancer agents (*34*, *35*). Hsp90 inhibitors are effective in overcoming fungal drug resistance at concentrations that are clinically well tolerated. Inhibiting Hsp90 may render resistant fungal pathogens more responsive to treatment and, when given early in therapy, may impede the de novo evolution of resistance. Hsp90 inhibitors may provide an even broader therapeutic paradigm; calcineurin and Hsp90 inhibitors also have potent antimalarial activity (*25*).

Hsp90 is a central player in the ancient and highly conserved heat-shock response. Although Hsp90 is induced in response to heat stress, its capacity to maintain client proteins can be compromised by severe stress. One form of heat shock, fever, is a conserved response to infection. The potential risks and benefits of fever to the infected host remain difficult to decipher (*36*). Increased temperatures can abolish fungal drug resistance, which provides an explicit mechanism by which fever, in the modern era, might be beneficial to the host. Historically, fever may have benefited the host by sensitizing the cellular circuitry of fungal pathogens and impeding the deployment of other virulence mechanisms.

Strikingly, traits acquired by different Hsp90-mediated effects can evolve to have little dependence on Hsp90. This can occur through the enrichment of preexisting polymorphisms during continued rounds of mating and selection (*4*). Here, in fungi, this occurred via additional mutations. Repeated episodes of fever may provide

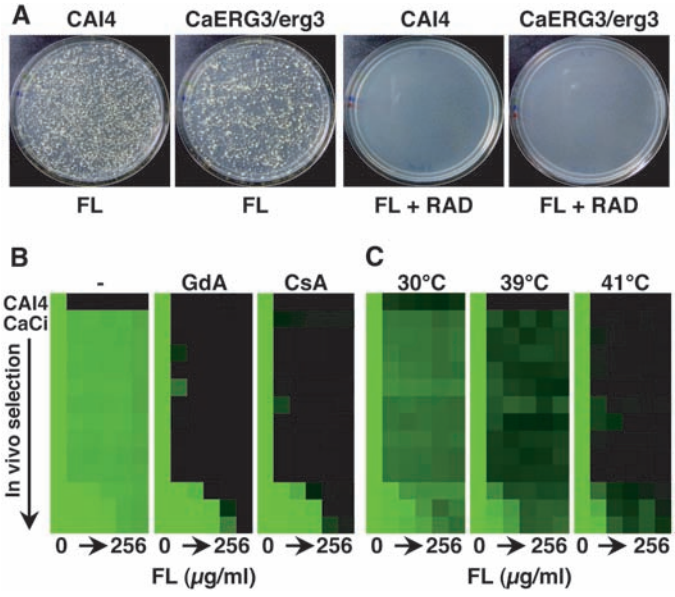

Fig. 4. The importance of Hsp90 in FL resistance in *C. albicans*. (**A**) Hsp90 inhibition blocked the emergence of FL resistance in a strain wild-type for *ERG3* (CAI4) and a heterozygous *ERG3* deletion mutant. Left, selection with FL (128 μg/ml) alone; right, selection with FL plus 1 μM RAD. (**B**) FL resistance of clinical isolates was initially Hsp90-dependent but evolved toward independence. Left, FL sensitivity of CAI4 and resistance of serial clinical specimens (CaCi) isolated from an HIV patient receiving FL; isolates are ordered sequentially, with those recovered early at the top. Middle and right, inhibition of Hsp90 by GdA (5 μM) or calcineurin by CsA (20 μM) in rich medium with FL at 30°C, analyzed as in Fig. 1. (**C**) Elevated temperatures reduce FL resistance of CaCi isolates in synthetic medium (RPMI).

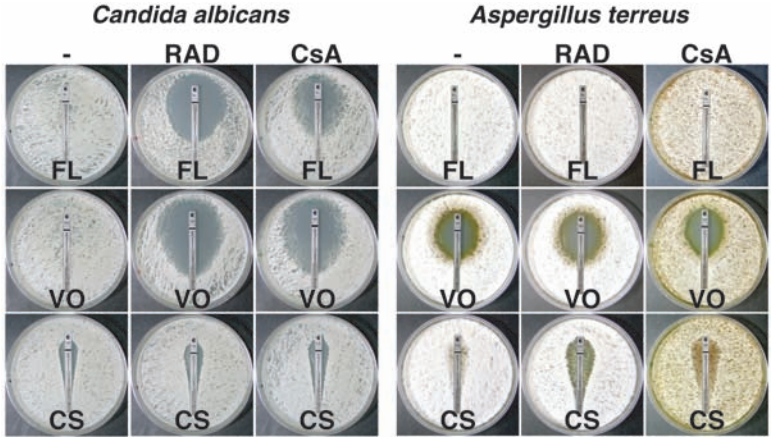

Fig. 5. Hsp90 potentiates resistance to different drugs in *C. albicans* and *A. terreus*. Resistance of *C. albicans* clinical isolate T118 and *A. terreus* soil isolate ATCC 10020 to two azoles [fluconazole (FL) and voriconazole (VO)] and to an echinocandin [caspofungin (CS)] is shown on rich medium. Antifungal test strips (Etest, AB Biodisk) produce a gradient of drug concentration, highest at the top (*15*). Plates contained RAD (5 μM) or CsA (20 μM). For MIC values, see table S2.

ideal selective conditions for the emergence of Hsp90 independence. A very different way in which changes in protein folding can potentiate the acquisition of new traits and provide a route to their genetic assimilation has been described for a yeast prion (*37*). There will likely be many other mechanisms by which spontaneous and environmentally induced changes in protein folding (*19*) and cell signaling (*38*, *39*) promote the emergence of new traits.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/309/5744/2185/DC1
Materials and Methods
Figs. S1 to S5
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REPORTS

Influence of Gravity Waves on the Internal Rotation and Li Abundance of Solar-Type Stars

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The Sun's rotation profile and lithium content have been difficult to understand in the context of conventional models of stellar evolution. Classical hydrodynamic models predict that the solar interior must rotate highly differentially, in disagreement with observations. It has recently been shown that internal waves produced by convection in solar-type stars produce an asymmetric, shear layer oscillation, similar to Earth's quasi-biennial oscillation, that leads to efficient angular momentum redistribution from the core to the envelope. We present results of a model that successfully reproduces both the rotation profile and the surface abundance of lithium in solar-type stars of various ages.

Rotation plays a crucial role in stellar evolution. Low-mass stars, including the Sun, are known to start their life with a large surface rotational velocity and then spin down with time because of a magnetically dominated stellar wind linked to their external convection zones. The interplay between the loss of angular momentum through a wind and its redistribution inside the star creates velocity gradients that induce mixing of elements. In the case of a fragile element such as lithium, which is destroyed by proton capture at a relatively low temperature (~2.5 million degrees) not too far below the convection envelope, surface depletion is thus expected. The lithium atmospheric abundance has been determined in many stars for which a fair estimate of the mass and age is feasible. These data allow an estimate of the extent, magnitude, and temporal evolution of chemical transport in the outermost stellar radiative regions, which may be linked directly to the instantaneous distribution of angular momentum in these objects. Furthermore, the quasi-flat seismic solar rotation profile (*1*, *2*) tells us that the characteristic time scale for the evolution of angular momentum has to be shorter than the age of the Sun.

Sophisticated stellar models that take into account hydrodynamic processes induced by rotation (i.e., meridional circulation and shear mixing) fail to reproduce the major observational constraints described above (*3*, *4*), although they are successful in reproducing the abundance anomalies and evolution characteristics of more massive stars (*5*). For solar-type stars with relatively extended convective envelopes that are strongly spun down by magnetic braking in their infancy, these models predict large rotation gradients within the interior, which are not consistent with helioseismology (Fig. 1). This is due to the too-low efficiency of the invoked hydrodynamic instabilities in redistributing angular momentum.

Two mechanisms have been proposed to explain the near uniformity of the solar rotation profile. The first rests on the possible existence of a magnetic field in the radiation zone (*6*, *7*). The second invokes traveling internal gravity waves (IGWs) generated at the base of the convection envelope (*8*, *9*). For either of these solutions to be convincing, they must be tested with numerical models coupling these processes with rotational instabilities and should explain all the aspects of the problem, including the lithium evolution with time.

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