

## Protein folding sculpting evolutionary change

Susan Lindquist

### ABSTRACT

Our work suggests that the forces that govern protein folding exert a profound effect on how genotypes are translated into phenotypes, and that this in turn has strong effects on evolutionary processes. Molecular chaperones, also known as “heat shock proteins” (Hsps), promote the correct folding and maturation of many other proteins in the cell. Hsp90 is an abundant and highly specialized chaperone that works on a particularly interesting group of client proteins: metastable signal transducers that are key regulators of a broad spectrum of biological processes. Such proteins often have evolved to finish folding only when they have received a specific signal, such as the binding of a ligand or a post-translational modification. Importantly, the folding of Hsp90 clients is particularly sensitive to changes in the external and internal environment of the cell. Therefore, Hsp90 is uniquely positioned to couple environmental contingencies to the evolution of new traits. Our work has helped to define two mechanisms by which Hsp90 might influence the acquisition of new phenotypes. First, by robustly maintaining signaling pathways, Hsp90 can buffer the effects of mutations in those pathways, allowing the storage of cryptic genetic variation that is released by stress. In this case, when the Hsp90 buffer is compromised by environmental stress, new traits appear. These traits can also be assimilated, so that they become manifest even in the absence of stress, when genetic recombination and selection enrich causative variants in subsequent generations. Second, Hsp90 can potentiate the effects of genetic variation, allowing new mutations to

produce immediate phenotypes. In this case, when Hsp90 function is compromised, new traits are lost. These traits can also be assimilated, so that they are maintained under environmental stress, but this is achieved through new mutations. We have discovered these powerful evolutionary mechanisms in fruit flies, mustard plants, and fungi, but expect them to operate in all eukaryotes. Another line of work relating protein folding to the evolution of new traits involves protein-based hereditary elements known as prions. These produce changes in phenotype through heritable, self-perpetuating changes in protein conformation. Because changes in protein homeostasis occur with environmental stress, prions can be cured or induced by stress, creating heritable new phenotypes that depend upon the genetic variation present in the organism. Both prions and Hsp90 provide plausible mechanisms for allowing genetic diversity and fluctuating environments to fuel the pace of evolutionary change. The multiple mechanisms by which protein folding can influence the evolution of new traits provide both a new paradigm for understanding rapid, stepwise evolution and a framework for targeted therapeutic interventions.

Proteins are synthesized as long, linear chains of amino acids. In order to function, they must fold into intricate, stereotyped, 3-dimensional structures. Moreover, individual proteins must do so in a highly chaotic environment. Inside of a living cell the protein concentration reaches 300 mg/ml, and those many proteins possess a tremendous amount of kinetic energy. Individual proteins therefore frequently encounter folding problems due to macromolecular crowding. In fact, “off-pathway” folding events are a constant problem. Individual cells, and even entire organisms, live poised on the precipice of a protein-folding crisis, a situation that is worsened by environmental stress.

In the last 15 years, research in my laboratory in two different areas has pointed to a pivotal role for protein folding in evolutionary processes. One of these involves the action of chaperone proteins, which facilitate protein folding in the complex cellular environment, thereby helping proteins to assume their normal functions. The second, closely related line of work involves a type of hereditary element, a prion, which produces a change in phenotype through a heritable, self-perpetuating change in protein conformation. Reflecting time constraints at this symposium, I focused my presentation on the role of the chaperone protein, Hsp90, and will only touch on yeast prions very briefly.

### *Heat-shock proteins*

Nearly 5% of the protein mass of the cell is devoted to helping other proteins fold properly and to correcting problems that occur when they misfold. An important class of helper proteins (known as chaperones) is the heat-shock proteins, so-called because they

can be induced by a shift from a normal to a slightly higher growth temperature. We will concentrate here on the abundant chaperone Hsp90 and its role in facilitating the rapid evolution of novel traits. Hsp90 is essential for life in eukaryotic cells. Only small amounts of Hsp90 are required for growth and development under normal conditions, but much more is required under stressful conditions. Unlike most chaperones it is made at much higher levels than it is needed, is present in excess under normal conditions, and therefore acts as a protein-folding buffer. This enables it to facilitate protein folding even during sudden, unexpected stresses.

Also unlike most other Hsps, Hsp90 is involved in the folding of specific regulatory proteins positioned strategically in major signal transduction networks, comprising perhaps only about 2-5% of the total protein in the cell. Hsp90's client proteins are characteristically metastable proteins that do not finish folding into their final stable conformations until something else has activated them, for example the binding of a ligand, a phosphorylation event, or translocation of the protein to the membrane. Protein complexes of Hsp90 with its clients are therefore extremely dynamic and strongly affected by stress. Their metastable nature means that much higher concentrations of Hsp90 are then required to drive its client proteins into appropriate complexes. Stressful conditions also cause additional cellular proteins to become unfolded (thereby creating additional need for Hsp90 and further depleting the buffer) Together, these properties of Hsp90-client interactions have a strong influence on genetic variation is translated into phenotypic novelty and diversity.

*Hsp90 buffers polymorphisms*

The first way that Hsp90 may affect evolutionary change is by buffering genetic polymorphisms. The excess Hsp90 chaperone capacity allows a variety of different polymorphisms throughout the genome to accumulate without phenotypic consequence. Within a certain range, it keeps these polymorphisms silent because it is able to maintain the normal folding and functioning of the signal transduction pathways controlled by its client proteins. But, when stress depletes the Hsp90 buffer, phenotypes associated with these accumulated polymorphisms are revealed suddenly and in a combinatorial fashion. In this way Hsp90 acts as a “capacitor,” allowing the storage and controlled release of genetic variation. This can have enormous functional consequences for the organism.

Our first experiments suggesting this mechanism involved some Hsp90 mutants in *Drosophila* lines that we obtained from the Rubin and Hoffmann laboratories [1]. We confirmed their findings that homozygotes (completely lacking Hsp90 function) died, and heterozygotes (with reduced Hsp90 function) were normal. However, we noticed that a small number of them had astonishing, and diverse, developmental anomalies (Figure 1a). Interestingly, these rare developmental anomalies were generally specific to the genetic background into which the mutations were transferred (Figure 1b). These observations suggested that reducing Hsp90 function does not just destabilize development; instead it reveals the effects of previously hidden genetic variation that can affect virtually every trait in the fly.

Further experiments suggested that populations of these flies contained a great diversity of different genetic polymorphisms. Some rare individuals exhibited several novel and unusual traits. This itself was a big surprise, as most geneticists believed that evolution worked through small incremental mutations. When we reduced Hsp90

function by genetic mutation, or grew flies at a higher temperature, we observed novel phenotypes in those flies more frequently. When we crossed two of the rare flies together and selected for that novel phenotype, we enriched subsequent generations for that underlying genetic variation to the point where those phenotypes were maintained – that is, variation was assimilated – even when Hsp90 function was normal [1]. We hypothesized that this interface between environmental stress and protein homeostasis could have a large effect on evolution.

We then turned to the mustard plant *Arabidopsis thaliana*, an inbreeding organism with a sessile lifestyle and considerably less heterozygosity than *D. melanogaster*. We asked, again, what happened when Hsp90 function was reduced in different accession lines of *Arabidopsis*? We observed a stunning array of phenotypic abnormalities that were specific to the individual lines: e.g. leaves coming out in whorl shapes, plants growing upside-down, hairy roots, or vine-like growth (Figure 2). We also obtained the same array of phenotypes by growing the plants at elevated temperatures [2, 3]. Again, these experiments revealed hidden genetic variation when the Hsp90 buffer was reduced by stress.

Next we examined the progeny of recombinant inbred plants from different ecotypes. We studied a wide variety of normal life history traits in *Arabidopsis*, such as flowering time and number of seeds that were set. Every single trait we studied in these lines had some hidden genetic variation that was exposed when Hsp90 function was reduced, and we were able to build a crude map of quantitative trait loci (QTL) involved in these traits by linkage analysis [4, 5]. This suggested that most of the underlying variation was genetic rather than epigenetic. In addition to examining Hsp90 inhibition

with chemical compounds we used RNAi inhibition, establishing that the changes in phenotype were absolutely due to the interface between Hsp90 and genetic variation. That Hsp90 has such a massive influence on the genotype-to-phenotype map in organisms with profoundly different lifestyles, separated by at least 1 billion years of evolution, suggests this mechanism has influenced evolutionary processes very broadly.

### *Hsp90 chaperones new mutants*

Another way that Hsp90 translates genetic variation into new phenotypes is by directly chaperoning mutated proteins. Some mutations can create new activities in proteins, but often the mutated proteins are unable to fold well on their own. They therefore benefit from the Hsp90 buffer present in the cell to enable them to fold correctly into a functional state, and create new phenotypes. In this way Hsp90 acts as a potentiator of new variation rather than as a capacitor.

The first class of proteins in which we observed this phenomenon was the oncogenic kinases such as v-Src (other kinases have the same basic phenomena). Early experiments on Hsp90 were aimed at determining its basic function and that of the other heat-shock proteins. At that time we had recently developed techniques to reduce Hsp90 levels in yeast, and we introduced v-Src into those cells to model Hsp90's effects on the protein. Several different laboratories had found that oncogenic kinases were inactive when complexed with Hsp90, leading to speculation that it was a "protein repressor." We found small differences in levels of v-Src accumulation in cells with high vs. low levels of Hsp90, but the *activity* of v-Src in the two cell types was profoundly different [6] (Figure 3). Surprisingly, rather than being repressed, v-Src activity was much higher

in the cells with high levels of Hsp90. In contrast, cells with low levels of Hsp90 were able to grow normally, but unable to functionally mature the mutated, dysregulated v-Src kinase [7]. We also found that Hsp90 levels do not make nearly as much difference to the activity of c-Src, a non-mutated and much less active form of the kinase. This observation has held true for a host of oncogenic kinases and been extended to several other types of oncogenes as well. Indeed, studies are underway that suggest that reducing Hsp90 function may offer great promise as a novel therapeutic strategy for treating cancer [8-10].

In any case these studies suggested that the excess chaperone activity of Hsp90 is a latent reservoir that can be employed to fold newly mutated proteins and provide new functions. If such mutations are ever beneficial to the organism they too could enhance the rate of evolution.

#### *Hsp90 Chaperones Responders*

A related way that Hsp90 affects evolutionary novelty is by chaperoning proteins that respond to mutations. Mutations that confer selective advantages in a new environment might not be advantageous overall because they can simultaneously create other problems. However, the abundant reservoir of Hsp90 can solve this dilemma because it chaperones a host of signal transducers that ameliorate negative effects arising from the mutated protein. We first observed this phenomenon in the evolution of anti-fungal drug resistance. The yeast *S. cerevisiae* is very adept at evolving resistance when treated with fluconazole, a widely used triazole antifungal agent. But, if Hsp90 activity is reduced pharmacologically or genetically in these cells, their ability to evolve resistance

is greatly reduced [11]. In this case Hsp90 was not folding the mutant protein. The mutation was in Erg3, which binds the azole, and the mutations simply eliminated the protein. But Erg3 is involved in sterol synthesis. When it is missing cells would normally die. They survive only because the reservoir of Hsp90 is available to direct massive maturation of calcineurin (an Hsp90 client) [11-14], which drives many protective changes.

We then tested our hypothesis in a real-time evolution experiment. We obtained a series of *Candida albicans* isolates from immunocompromised human patients over the course of 2 years of treatment (gift of Ted White, Spencer Redding and colleagues). Their genetic analysis had revealed that these patients were not being re-infected with a new strain of the pathogen. Rather, a single strain that had already infected them simply kept re-blooming. We analyzed these *in vivo* isolates and we found that even the first isolates were resistant to fluconazole, in comparison to our laboratory strain, and that the level of resistance increased over time. When we added small amounts of geldanamycin (GdA), an Hsp90 inhibitor, we found that it largely eliminated the isolates' fluconazole resistance (Figure 4) [11]. That is, in this natural evolution setting all of the mutant cells that had acquired drug resistances depended on Hsp90 for survival.

We observed that in late stages of infection some of the isolates had also evolved resistance to fluconazole that was maintained even with GdA. But these fungi had never been exposed to Hsp90 inhibitors in the patient. What drove the assimilation of the trait? New mutations that made resistance independent of Hsp90. We hypothesize that this is due to fever, because even a simple increase in growing temperature to 39°C phenocopied

the specific effects of Hsp90 inhibition [11]. Indeed fevers may generally exist to compromise an invading pathogen's ability to evolve resistance.

We have also shown that in *Aspergillus*, a major human pathogen that is separated from *C. albicans* by one billion years of evolution, resistance to a completely different class of antifungal drugs (the echinocandins) also depends on Hsp90 [11]. These experiments demonstrate that Hsp90-mediated mechanisms for acquiring drug resistance are very broadly employed by diverse human pathogens

#### *Identification of Genetic Polymorphisms Affected by Hsp90*

To get a sense of how widespread Hsp90-dependent genetic variation can be, we have now exposed 96 diverse yeast strains from many ecological niches to different growth conditions. This created a wide variety of phenotypes that were readily assayed in the lab. We compared the growth of those cells with and without subjecting them to modest Hsp90 inhibition. We found that Hsp90 inhibition can both uncover and mask traits in a manner that depends on genetic background. Every trait we studied was affected in at least some of the strains we tested (D. Jarosz, unpublished observations). These experiments provide evidence for widespread naturally occurring genetic variation, upon which Hsp90 can act. They also establish that Hsp90 acts on this variation both as a potentiator and as a capacitor, and most importantly that the effects were as often beneficial as detrimental.

To determine the nature of the genetic polymorphisms affected by Hsp90 and how they work, we took advantage of work of Kruglyak and colleagues, who mated very divergent sequenced strains of yeast to obtain meiotic segregants. They then mapped the

genomes of more than 100 of these segregants and with Schreiber and Perlstein [15, 16] identified genotypes that affected growth in the presence of many small molecules. We took advantage of this earlier work to map genetic variation associated with individual Hsp90-dependent traits. We exposed segregants from this cross to a variety of different small-molecule perturbations: several from the previous work and many new ones, along with alternative carbon sources and DNA damaging agents.

In parallel, we added an Hsp90 inhibitor (radicicol) at low levels to these meiotic segregants to reduce the Hsp90 buffer in each growth condition (Figure 5). We found that some strains gained an ability to grow and some strains lost that ability. This correlated with specific changes in genotype; radicicol on its own did not generally affect growth. If we instead stressed the cells by growing them at a higher temperature, a concomitant reduction in the Hsp90 buffer had similar effect on growth. We compiled the responses of these meiotic segregants across 100 different conditions and observed that these organisms are harboring a tremendous amount of variation that depends on Hsp90 for its phenotypic manifestation. After mapping these traits to specific places in the genome, we identified several polymorphisms that are affected by Hsp90 by performing allele replacements across the entire chromosome region implicated by the genetic analysis. Importantly, they were remarkably diverse in character, and included polymorphisms in *cis*-regulatory sequences, not only in coding regions. This again reflects Hsp90's influence on the folding of a wide variety of signal transducers.

Remarkably, these observations provide a plausible mechanism for understanding the rapid evolution of complex traits, and suggest that that evolution can be driven in unexpected ways by exposure to certain environmental stresses. Moreover,

understanding these evolutionary processes will help us guide new therapeutic strategies for cancer [8-10] and infectious disease [17, 18].

### *Prions*

Not only have we shown that Hsp90 and environmental stress influence the interface between genotype and phenotype, but we have also found a completely different way in which protein folding affects this interface: prions [19]. Prions produce a vast array of new heritable phenotypes through a heritable, self-perpetuating change in protein conformation. I will illustrate for just one of these elements, [PSI+].

The [PSI+] prion is formed from an inactive, misfolded translation-termination factor, Sup35, which is a self-templating amyloid. The misfolded proteins are passed from mother cell to daughter and act as “seeds” to perpetuate the alternately folded prion state from generation to generation. This results in the heritable read-through of stop codons throughout the genome. We asked what the general consequences of [PSI+] might be on yeast biology, by testing seven isogenic [PSI+]/[psi-] strains for growth under ~200 conditions [20]. Remarkably, in each genetic background, [PSI+] produced a completely different constellation of phenotypes. Thus, [PSI+] rapidly uncovers previously hidden genetic variation in a stepwise fashion. Presumably, it does so by reactivating genes previously inactivated by stop-codon mutations, by ribosomal frame-shifting [21], by uncovering cryptic coding potential in the 3' untranslated regions of already active genes, and by changing the half-lives of messages through well-known mechanisms.

We recently devised high-throughput methods to scan the entire yeast genome and detect additional candidate prion-coding sequences, identifying about 200. We tested the top 100 candidates for hallmarks of prion activity: ability to form amyloids, heritability and infectivity, and ability to alter their functional state when their conformational state changes. Applying our set of assays, we found 24 prion candidates that satisfactorily met these criteria. Included in that number were all four of the known yeast prions as well as new prions involved in functions as diverse as cell wall structure and carbon metabolism [22].

Evolutionary change is fueled by genetic diversity and fluctuating environments. The [PSI<sup>+</sup>] prion allows cells to express diverse phenotypes in a heritable manner with no change in the genome. It provides a route for selection to act on genetic variants that normally have no phenotypic consequence and fix them through the reversion of stop codons. This would allow maintenance of the trait when cells return to the normal [psi<sup>-</sup>] state. When yeast cells are stressed, switching between the prion and non-prion states occurs at a higher rate [23], which may give the yeast a better chance to adapt to challenging conditions. Thus the link between prion biology, protein folding, and environmental stress seems to create a “bet-hedging” strategy. Just as with alterations in the Hsp90 buffer, the new phenotypes that arise due to prion switching are sometimes beneficial and sometimes detrimental. If a few cells (1 in 10,000 to 1 in 100,000) in the population switch to a detrimental state it wouldn’t matter very much for survival of the population. But if the traits are beneficial they could matter a great deal. Our work opens several new avenues of study, including the application of similar strategies to find

additional prions in yeast, thorough investigation of these prions *in vivo* and *in vitro*, and scanning of other organisms' genomes for prion candidates.

#### ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant R01GM025874, a SPARC grant from the Broad Institute, the G. Harold and Leila Y. Mathers Foundation, and the Howard Hughes Medical Institute. I also thank Dr. Karen Allendoerfer for preparing the transcript of the lecture on which this manuscript is based, and Drs. Daniel Jarosz and Mikko Taipale for helpful comments on the manuscript.

## Figure Legends

**Figure 1.** Examples of developmental abnormalities associated with Hsp90 deficits in *Drosophila*. (A) Thickened wing veins, (B) transformed 2<sup>nd</sup> leg with an ectopic sex comb, (C) deformed eye with an extra antenna, (D) disorganized abdominal tergites, (E) notched wings, (F) extraneous tissue growing out of tracheal pit (Reprinted from Rutherford and Lindquist, *Nature* 1998).

**Figure 2.** Maturation and activity of the mutated tyrosine kinase, v-Src, depends on hsp90. (A) V-src accumulates at the same rate when hsp90 is inhibited, but its activity is reduced. (B) C-src is much less active in general than v-src, and its activity is much less dependent on hsp90 levels.

**Figure 3.** The importance of Hsp90 in fluconazole (FL) resistance in *C. albicans*. 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, inhibition of Hsp90 by GdA (5 mM). Right, elevated temperatures reduce FL resistance of CaCi isolates. (Reprinted from Cowen and Lindquist, *Science* 2005).

**Figure 4.** The same recombinant inbred line (RIL)-specific phenotypes are uncovered when Hsp90 buffering capacity is challenged by GDA (top) or by growth at 27 C (bottom). Line 082 seedlings, S-shaped rosettes with vertically oriented leaf blades. Line 113 seedlings, extreme hypocotyl curls and roots partially extended into air. Line 104 seedlings, abundant root hair growth. Line 134 seedlings, bent hypocotyls with rosette touching the medium surface. (Reprinted from Queitsch et al., *Nature* 2002)

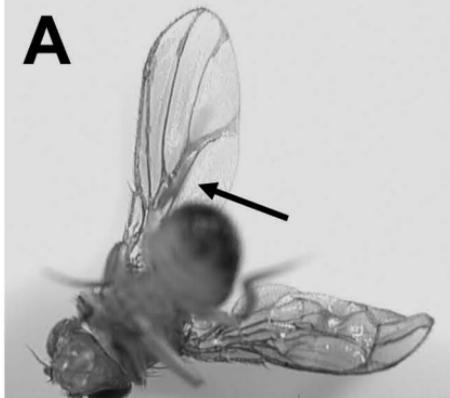
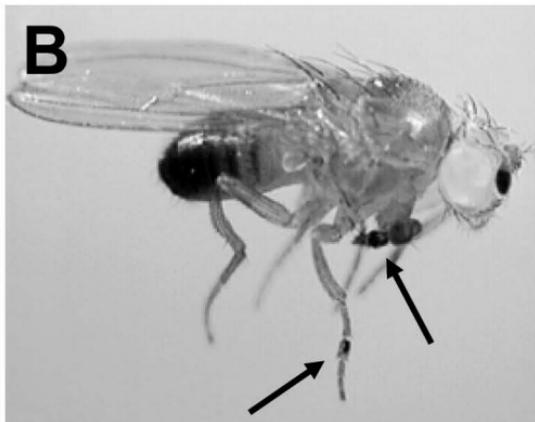
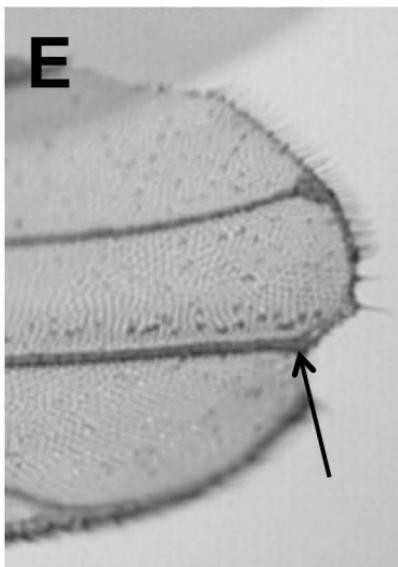
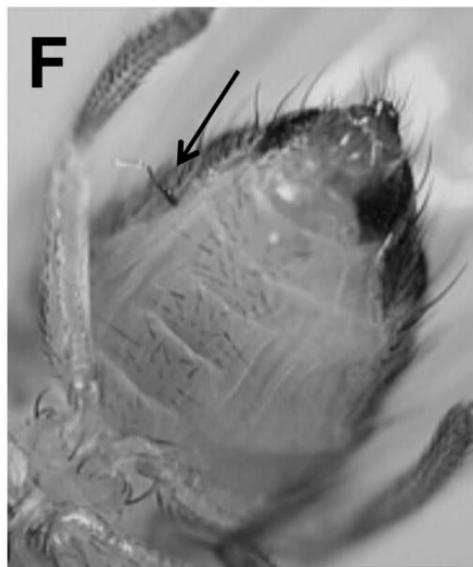
**Figure 5.** Examples of phenotypic transitions elicited in *S. cerevisiae* by reducing the Hsp90 buffer. Genotyped meiotic segregants from a cross between two sequenced genetic backgrounds are arrayed in 384-well plates and their growth was monitored across a broad array of conditions and in parallel when reducing the Hsp90 buffer. In some conditions growth is enhanced when the Hsp90 buffer is reduced, and this occurs in segregants with shared genetic polymorphisms. Growth can also be reduced when Hsp90 is inhibited. Again, the response occurs in segregants that share certain regions of their genomes.

## REFERENCES

1. Rutherford, S.L., and Lindquist, S.L. (1998). Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336-342.
2. Queitsch, C., Sangster, T.A., and Lindquist, S.L. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature* 417, 618-624.
3. Sangster, T.A., Bahrami, A., Wilczek, A., Watanabe, E., Schellenberg, K., McLellan, C., Kelley, A., Kong, S.W., Queitsch, C., and Lindquist, S.L. (2007). Phenotypic diversity and altered environmental plasticity in *Arabidopsis thaliana* with reduced Hsp90 levels. *PLoS ONE* 2, e648.
4. Sangster, T.A., Salathia, N., Lee, H.N., Watanabe, E., Schellenberg, K., Morneau, K., Wang, H., Undurraga, S., Queitsch, C., and Lindquist, S. (2008). HSP90-buffered genetic variation is common in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 105, 2969-2974.
5. Sangster, T.A., Salathia, N., Undurraga, S., Milo, R., Schellenberg, K., Lindquist, S., and Queitsch, C. (2008). HSP90 affects the expression of genetic variation and developmental stability in quantitative traits. *Proc Natl Acad Sci USA* 105, 2963-2968.
6. Xu, Y., and Lindquist, S.L. (1993). Heat-shock protein hsp90 governs the activity of pp60v-src kinase. *Proc Natl Acad Sci USA* 90, 7074-7078.
7. 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. *Proc Natl Acad Sci U S A* 96, 109-114.

8. Cullinan, S.B., and Whitesell, L. (2006). Heat shock protein 90: a unique chemotherapeutic target. *Semin Oncol* *33*, 457-465.
9. Dai, C., and Whitesell, L. (2005). HSP90: a rising star on the horizon of anticancer targets. *Future Oncol* *1*, 529-540.
10. Whitesell, L., and Lindquist, S.L. (2005). HSP90 and the chaperoning of cancer. *Nat Rev Cancer* *5*, 761-772.
11. Cowen, L.E., and Lindquist, S.L. (2005). Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science* *309*, 2185-2189.
12. 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 J* *21*, 546-559.
13. Fox, D.S., and Heitman, J. (2002). Good fungi gone bad: the corruption of calcineurin. *Bioessays* *24*, 894-903.
14. Imai, J., and Yahara, I. (2000). Role of HSP90 in salt stress tolerance via stabilization and regulation of calcineurin. *Mol Cell Biol* *20*, 9262-9270.
15. Perlstein, E.O., Ruderfer, D.M., Ramachandran, G., Haggarty, S.J., Kruglyak, L., and Schreiber, S.L. (2006). Revealing complex traits with small molecules and naturally recombinant yeast strains. *Chem Biol* *13*, 319-327.
16. Perlstein, E.O., Ruderfer, D.M., Roberts, D.C., Schreiber, S.L., and Kruglyak, L. (2007). Genetic basis of individual differences in the response to small-molecule drugs in yeast. *Nat Genet* *39*, 496-502.

17. Cowen, L.E. (2008). The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. *Nat Rev Microbiol* 6, 187-198.
18. 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., et al. (2009). Harnessing Hsp90 function as a powerful, broadly effective therapeutic strategy for fungal infectious disease. *Proc Natl Acad Sci U S A* 106, 2818-2823.
19. Shorter, J., and Lindquist, S.L. (2005). Prions as adaptive conduits of memory and inheritance. *Nat Rev Genet* 6, 435-450.
20. True, H.L., and Lindquist, S.L. (2000). A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 407, 477-483.
21. Namy, O., Galopier, A., Martini, C., Matsufuji, S., Fabret, C., and Rousset, J.P. (2008). Epigenetic control of polyamines by the prion [PSI+]. *Nat Cell Biol* 10, 1069-1075.
22. Alberti, S., Halfmann, R., King, O., Kapila, A., and Lindquist, S. (2009). A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell* 137, 146-158.
23. Tyedmers, J., Madariaga, M.L., and Lindquist, S. (2008). Prion switching in response to environmental stress. *PLoS Biol* 6, e294.

**A****B****C****D****E****F**

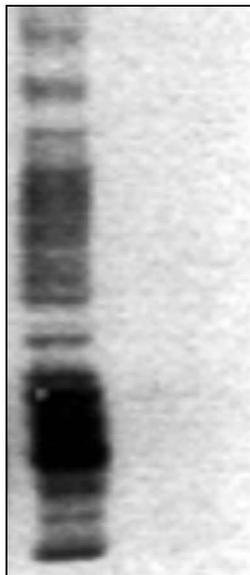


**src accumulation**



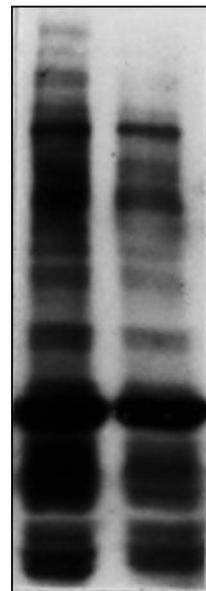
Wt ↓ *hsp90*

**src activity**



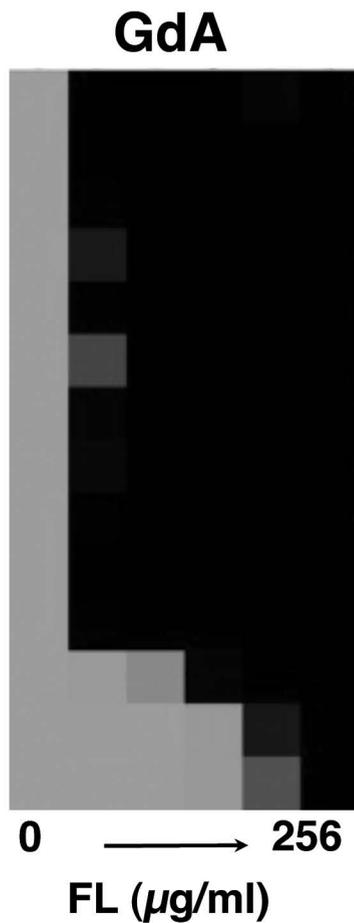
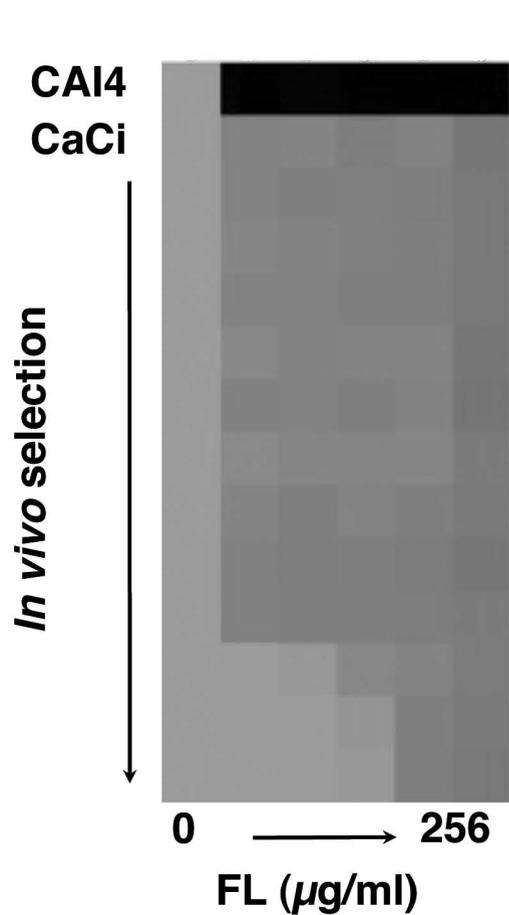
Wt ↓ *hsp90*

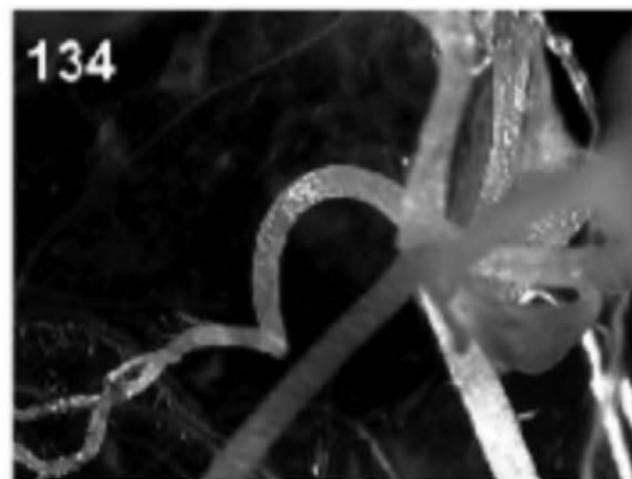
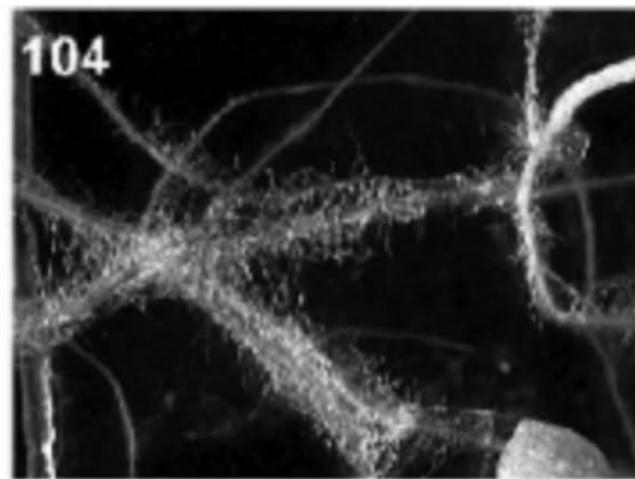
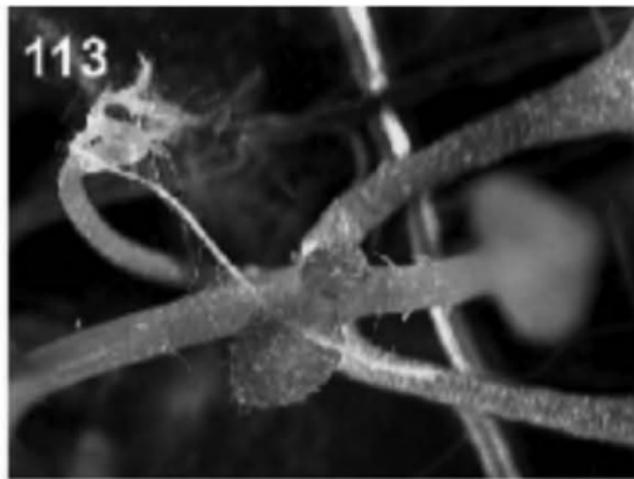
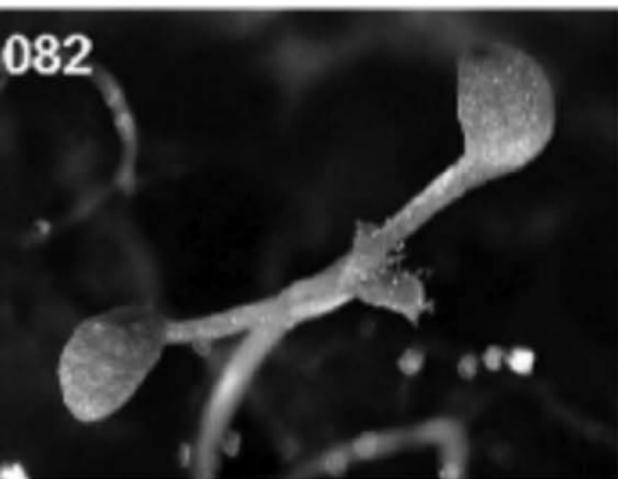
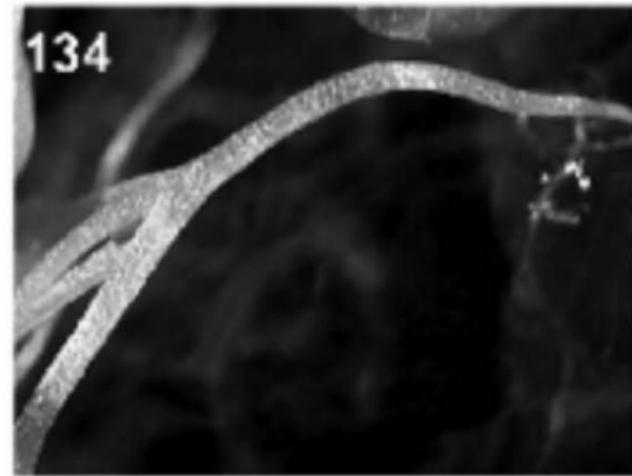
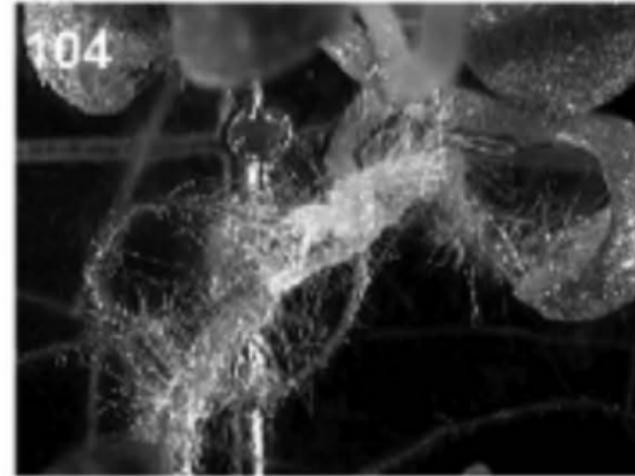
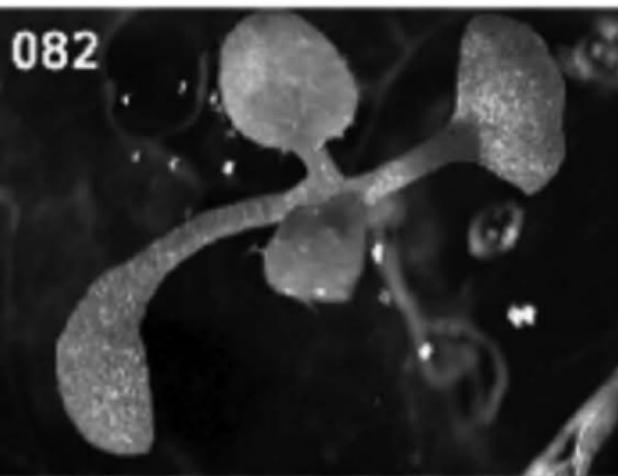
**activity**

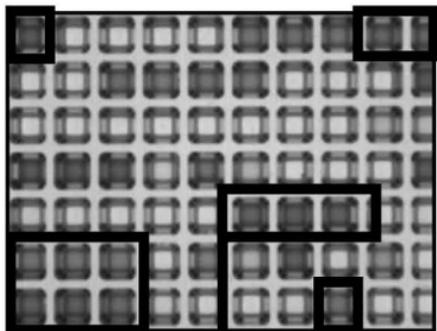


Wt ↓ *hsp90*

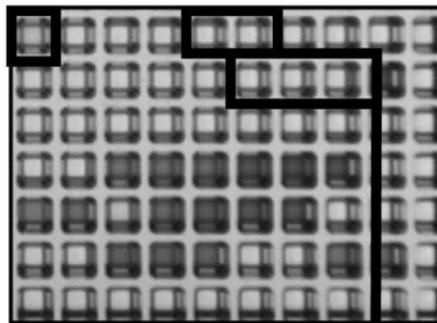
Long exposure





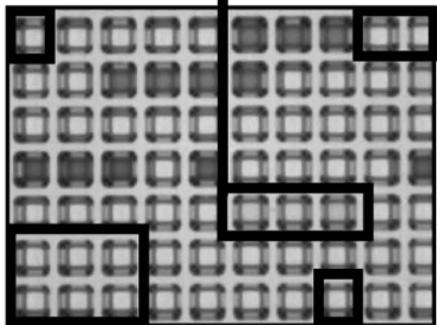


SMP#1 Alone



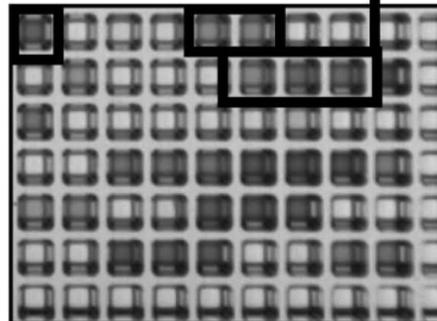
SMP#2 Alone

Trait appears



SMP#1 + Radicicol

Trait disappears



SMP#2 + Radicicol