Hsp90 and Environmental Stress Transform the Adaptive Value of Natural Genetic Variation

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Hsp90 and Environmental Stress Transform the Adaptive Value of Natural Genetic Variation

Daniel F. Jarosz[1] and **Susan Lindquist**[1,2,*]

[1]Whitehead Institute for Biomedical Research and Howard Hughes Medical Institute, 9 Cambridge Center, Cambridge, MA 02142, USA

[2]Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Abstract

How can species remain unaltered for long periods yet also undergo rapid diversification? By linking genetic variation to phenotypic variation via environmental stress, the Hsp90 protein-folding reservoir might promote both stasis and change. However, the nature and adaptive value of Hsp90-contingent traits remain uncertain. In ecologically and genetically diverse yeasts, we find such traits to be both common and frequently adaptive. Most are based on pre-existing variation, with causative polymorphisms occurring in coding and regulatory sequences alike. A common temperature stress alters phenotypes similarly. Both selective inhibition of Hsp90 and temperature stress increase correlations between genotype and phenotype. This system broadly determines the adaptive value of standing genetic variation and, in so doing, has influenced the evolution of current genomes.

Many vital proteins have difficulty reaching their final folds or are inherently unstable when they do. To contend with such problems, organisms employ protein–remodeling factors and chaperones, including a subset known as heat-shock proteins (Hsps) (1). Unlike more general chaperones, Hsp90 specializes in folding metastable signal transducers (2) and key components of multiprotein complexes. These are hubs in interaction networks (3), and Hsp90 is thereby a “hub of hubs” in regulatory circuits. Also unlike most chaperones, Hsp90 is constitutively expressed at much higher levels than required to fulfill its normal functions. The Hsp90 chaperone system, then, constitutes a large but highly specific protein-folding reservoir (4). Environmental stresses can destabilize Hsp90 clients and produce additional unfolded substrates, straining the capacity of this buffer. We have suggested that these unusual features of the Hsp90 chaperone system alter relationships between genotypes and phenotypes under conditions of environmental stress (5–8) and, in so doing, provide at least two routes to the rapid evolution of new traits: (i) Acting as a potentiator, Hsp90’s folding reservoir allows individual genetic variants to immediately create new phenotypes; when the reservoir is compromised, the traits previously created by potentiated variants disappear. (ii) Acting as a capacitor, Hsp90’s excess chaperone capacity buffers the effects of other variants, storing them in a phenotypically silent form; when the Hsp90 reservoir is compromised, the effects of these variants are released, allowing them to create new traits

*To whom correspondence should be addressed. lindquist_admin@wi.mit.edu.

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(5). To date, however, only two types of potentiated variants have been defined (2, 6), and the nature of buffered variants remains completely enigmatic. Some buffered traits map to specific chromosomal regions, suggesting a dependence on pre-existing genetic variation. But similar phenotypes can be produced by epigenetic variation (9, 10) and transposon activation (11), providing alternative explanations for their appearance. Further, the adaptive value of buffered traits remains untested.

To broadly determine the adaptive value of Hsp90's effects on the relationship between genotype and phenotype, we examined 102 genetically diverse strains of *Saccharomyces cerevisiae*, from soil, fruit, wine, sake, beer, infected human patients, etc. (table S1). We measured growth in 100 conditions, including alternative carbon sources, oxidative stressors, antifungals, DNA–damaging agents, osmotic stressors, and small molecules that perturb varied cellular processes (fig. S1 and table S2). Using two chemically unrelated Hsp90 inhibitors, radicicol (Rad) and geldanamycin A (GdA) (12, 13), we then determined the effects of reducing the Hsp90 reservoir. We used concentrations that did not induce a general stress response (fig. S2) or inhibit the growth of any strain in standard medium. However, every strain exhibited substantial changes in growth under specific conditions. These varied widely and were sometimes positive, sometimes negative (Fig. 1A). Rad and GdA produced very similar changes (table S3), confirming that they derived from Hsp90 inhibition, not off-target drug effects.

The reproducible character of the traits suggests that they arose from pre-existing variation rather than de novo mutation. To investigate quantitative trait loci (QTLs) that might underlie such traits, we analyzed 104 densely mapped haploid progeny from a cross between BY4716 (BY), a common laboratory strain originally obtained from a rotten fig, and RM11-1a (RM), a vineyard isolate (14). The coding sequences of these strains diverge by approximately 0.5%. Most genes were polymorphic in this cross but, importantly, not Hsp90. Our results agreed well with earlier studies under 23 overlapping conditions (15), demonstrating the robust consequences of the variation segregating in this cross.

Again, we reduced the Hsp90 reservoir, and again, this strongly affected growth in specific strains in different conditions ([Fig. 1B, fig. S3, and supporting online material (SOM)]. With the genetic diversity present in just these two parents, the patterns of growth changes in their progeny were highly diverse (Fig. 1C–F), establishing the independent segregation of many Hsp90-contingent alleles. No QTLs influenced growth in 5 μM Rad or GdA alone: Variations in drug pumps or detoxification pathways did not confound the analysis.

In three conditions (trichostatin A, iodoacetate, and chlorhexidine), we could not map Hsp90-contingent QTLs. This might reflect epigenetic effects or the segregation of too many underlying variants (SOM). The vast majority of Hsp90-contingent phenotypes, however, could be mapped (table S5 and fig. S4). The QTLs responsible derived equally from BY and RM: Relaxed pressures of laboratory cultivation did not skew the nature of the accumulated variation.

Hsp90 acted as a potentiator of variation almost as frequently as a capacitor: 44 previously apparent QTLs disappeared when the reservoir was reduced, and 63 previously silent QTLs became apparent (tables S4 and S5). With a false discovery rate of 0.05, at most three would have been expected by chance. As previously suggested (7, 8), reducing the Hsp90 reservoir produced genetically complex traits in a single step: Fully one–third of the traits involved two QTLs, and 15% involved three or more.

As expected in such analyses, the QTLs encompassed many polymorphic alleles. To identify causative variants, we dissected four. In each case, we used three otherwise diverse progeny that carried BY sequence in the relevant region and three that carried RM sequence. In these

four sets of six strains, we substituted every candidate gene, one by one, with the allele of the opposite parent.

For the first QTL, Hsp90 acted as a capacitor for rapamycin resistance latent in the RM genome. Segregants with both RM and BY sequence were sensitive to the compound. When the Hsp90 reservoir was reduced, those with RM sequence gained the ability to grow. The QTLs spanned eight genes, but all Hsp90-dependent growth effects were conferred by the open reading frame (ORF) of *NFS1* (Fig. 2A).

Nfs1 is a cysteine desulfurase that acts as a sulfur donor in tRNA thiolation (16). Rapamycin targets the highly conserved TOR proteins, which regulate growth in all eukaryotes. It does so primarily via the protein synthesis machinery (17). Other mutations in this same tRNA modification pathway confer rapamycin sensitivity. Furthermore, Nfs1 function is known to depend on Hsp90 (18). Thus, changes in the Hsp90 reservoir are logically linked to polymorphisms in this region.

For the second QTL, Hsp90 acted as a potentiator for deoxycholate (DOC) resistance conferred by standing variation in the RM genome. Segregants carrying RM sequence were DOC-resistant, whereas those carrying BY sequence were sensitive. When the Hsp90 reservoir was reduced, strains carrying RM sequence lost resistance. Allele replacements demonstrated that this resistance arose entirely from the RM *PDR8* ORF (Fig. 2B).

DOC facilitates fat emulsification in the intestine and acts as an anti-microbial agent (19). *PDR8* encodes a transcription factor not known to depend on Hsp90. To determine whether RM polymorphisms caused Pdr8 to become an Hsp90 client, we examined other Pdr8-dependent phenotypes: growth in NaCl, hygromycin B, and LiCl (20). Reducing Hsp90 didn't affect any of these (fig. S6), suggesting that RM Prd8 does not require Hsp90 for function. More likely, RM polymorphisms exert their effects via Hsp90's interaction with another, DOC-specific element of Pdr8's circuitry.

For the third QTL (Fig. 2C), Hsp90 acted as a capacitor for hydroxyurea (HU) resistance latent in the BY genome. Segregants carrying BY sequence were initially more sensitive than those carrying RM sequence. Reducing the Hsp90 reservoir increased the resistance of segregants carrying BY sequence but not RM sequence. This trait proved to be conferred by *MEC1*.

HU reduces intracellular deoxynucleotide triphosphate concentrations, eliciting replication stress (21). Mec1 coordinates multicomponent repair and checkpoint pathways that differ for different damage responses (22). A major QTL that conferred resistance to ultraviolet (UV) radiation also proved to map to *MEC1*. In this case, however, Hsp90 acted as a potentiator. The UV resistance of strains carrying the BY allele was lost when the Hsp90 reservoir was reduced. Because Hsp90 inhibition affected two Mec1 functions in different ways, these results suggest that Mec1 is an Hsp90 client, whose partitioning between diverse complexes is affected by Hsp90-contingent polymorphisms.

For the fourth QTL, Hsp90 acted as a capacitor for CDNB (1-chloro-2,4-nitrobenzene) resistance latent in the RM genome. Segregants with either RM or BY sequence were sensitive to this oxidative stressor. When the Hsp90 reservoir was compromised, those with RM sequence gained the ability to grow. Allele replacements established RM *NDI1* as the causative variant, but here, the polymorphisms resided in the 3′ untranslated region (Fig. 2D), rather than in the ORF.

NDI1 encodes an oxidoreductase that defends against oxidative stresses (23). We found that CDNB normally had little effect on *NDI1* mRNA levels. But when the Hsp90 reservoir was

reduced, transcripts produced by *NDI1* in response to CDNB stress increased by ~100-fold in segregants with RM relative to those with BY sequence. Increased *NDI1* transcripts fully explained the phenotype: Forced overexpression of the normally ineffective BY allele (using a *Gal1* promoter) was sufficient to confer CDNB resistance (Fig. 2D).

How might changes in Hsp90 affect the expression of genetic variation in nature? Hsp90 is induced by environmental stress (4). We've postulated that this increase is sometimes insufficient to maintain the folding reservoir, changing the manifestation of genetic variation (24). Indeed, all four alleles analyzed above were affected by a simple temperature stress (growth at 39°C) in the same manner as by Hsp90 inhibition (Fig. 3A–D). Moreover, the same phenotypes were elicited by genetic deletion of one of two Hsp90 alleles, confirming that they are due to changes in Hsp90 function. Far more broadly, we find that even with the abundant genetic diversity present in our wild strains, the effects of temperature on phenotypic transitions were similar to those of Rad and GdA (Pearson correlation ~0.61 and ~0.56, respectively, and see SOM).

We took advantage of the fact that 48 of these strains have been sequenced to ask whether their genomes carry an impress of Hsp90's selective forces. As previously reported in other strains and circumstances (25), across the ~100,000 polymorphisms present here with the 100 growth conditions we used, the correlation between genoptye and phenotype was relatively weak (Spearman correlation ~0.35) in the absence of Hsp90 inhibition. Similar strains often had divergent phenotypes, and divergent genotypes often produced similar phenotypes.

The correlation between genotype and phenotype became much stronger when the Hsp90 reservoir was reduced (Spearman correlation ~0.54; Fig. 4). Ten million random data permutations did not produce a single increase of such magnitude ($P < 10^{-7}$). This transition was evident across diverse ecological niches. A simple increase in growth temperature had a similar effect (Spearman correlation ~0.48). It is difficult to imagine how environmental stress in general, and Hsp90 in particular, could have such a strong impact on genotype-phenotype correlations unless it had acted though the evolutionary history of these strains to influence the retention of a broad swath of genetic variation.

Our hypothesis that Hsp90 plays a role in evolutionary processes remains controversial because of a paucity of hard evidence (26). Here, we establish that Hsp90 operates on roughly 20% of the pre-existing genetic variation in *S. cerevisiae* to both preserve phenotypic robustness and provide a broad conduit to diversification. Further, environmental stress creates a dynamic interface for transitioning between these effects in a manner that has left an impress on current genomes. Half of the traits buffered by Hsp90 and half potentiated by it had beneficial effects on growth; the other half were detrimental. What might maintain such contrasting adaptive effects? Many proteins in regulatory hubs are metastable, essential for life, and constitutively dependent on Hsp90. The need to preserve these functions during environmental stress might provide all the selective pressure needed to maintain this protein-folding reservoir. The accumulation of new Hsp90-contingent alleles might simply be an inevitable consequence of its existence. Once established, however, the capacity of the reservoir to facilitate the appearance of new traits——evolvability——might have provided an additional selective advantage. Theory holds that natural selection is unable to sustain mechanisms for "evolvability" because genetic recombination would inevitably separate evolvability genes from the alleles on which they act (5, 24). Negating this objection, Hsp90-contingent polymorphisms are dispersed throughout the genome; loss of some through genetic re-assortment would be balanced by the gain of others.

A particular advantage of the Hsp90 system is that it provides a route to genetically complex traits in a single step, via combinatorial gain and loss of phenotypic variation in response to environmental stress. Under selective pressure, multiple mechanisms could lead to the fixation of such traits (5, 24). In *Drosophila*, at least, Hsp90 can also create new traits by affecting epigenetic variation (10) and transposon-mediated mutagenesis (11), and it probably affects genome stability by other mechanisms as well (5). The strength of the Hsp90 buffer and the wealth of mechanisms by which it creates heritable new traits in response to environmental change may help to explain two long-puzzling features of evolution, the stability of phenotypes over long periods and their rapid diversification in response to changing selective pressures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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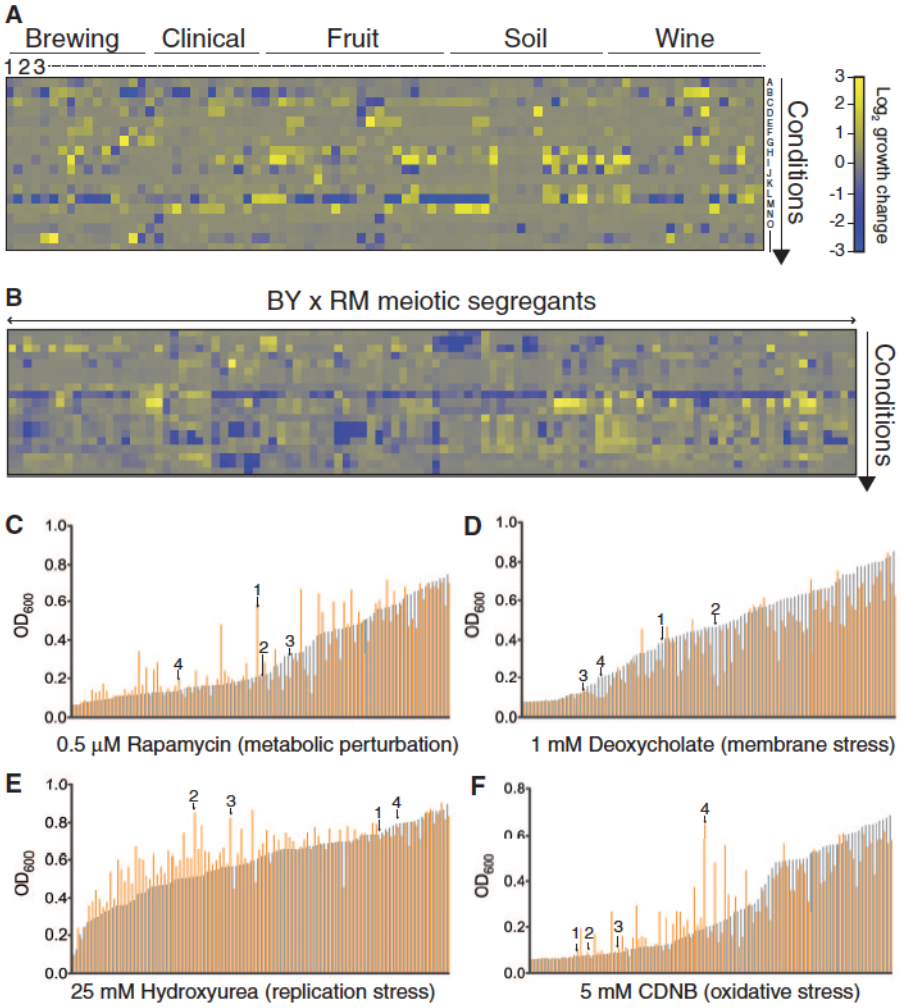

Fig. 1.

Reducing the Hsp90 reservoir creates diverse phenotypes. Representative growth changes elicited by Hsp90 inhibition. The scale bar indicates $\log_2$ of the ratio of growth in each condition with and without 5 mM Rad for (**A**) wild strains and (**B**) BY × RM progeny. (**C** to **F**) Examples of rank-ordered growth distributions after 64 hours of growth of BY × RM progeny with (orange bars) and without (gray bars) 5 mM Rad.

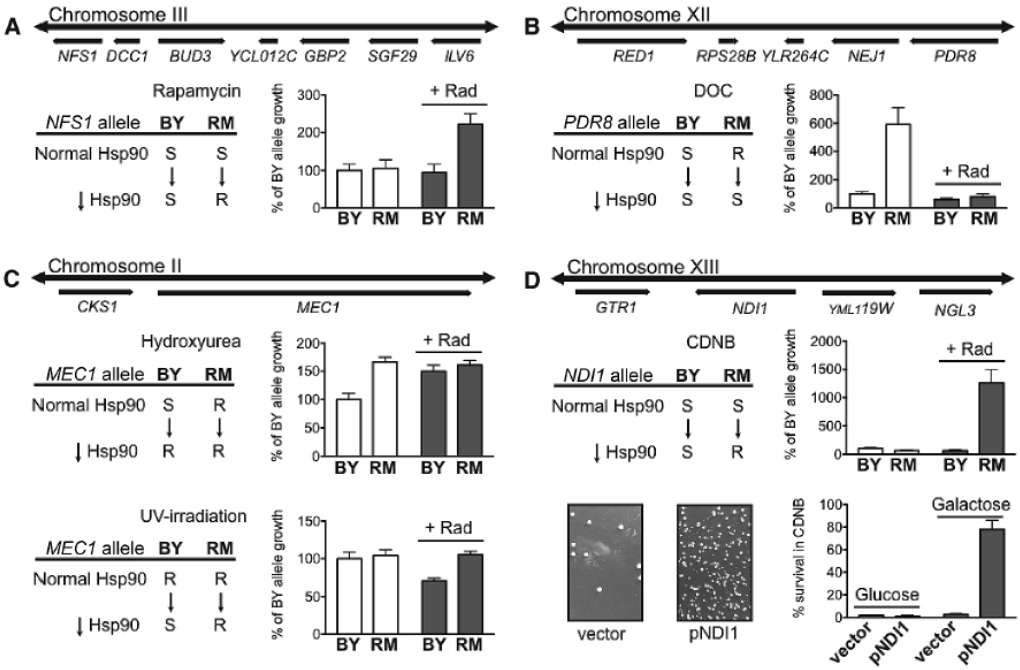

Fig. 2.

Genetic dissection of Hsp90-contingent alleles. The growth of allele-replacement strains with (solid bars) and without (open bars) 5 mM Rad is normalized to that of the BY allele–replacement strain in each condition without Rad. (**A**) QTLs conferring Hsp90-buffered rapamycin resistance, due to the RM *NFS1* allele (44 hours). (**B**) QTLs conferring Hsp90-potentiated DOC resistance, due to the RM *PDR8* allele. (**C**). QTLs conferring Hsp90-buffered HU resistance, due to the BY *MEC1* allele (25 hours). Hsp90-potentiated resistance to UV-irradiation was due to the same allele (20 J/m^2; 25 hours after irradiation). (**D**) QTLs conferring CDNB resistance, due to polymorphisms in the 3′-untranslated region (UTR) of RM *NDI1* (44 hours). Overexpression of BY *NDI1* rescues CDNB toxicity. Error bars in the entire figure represent the standard deviation of three biological replicates.

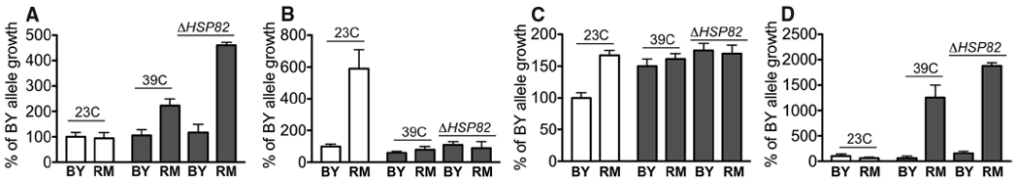

Fig. 3.

Environmental stress recapitulates phenotypic effects of Hsp90 inhibition. Calculations and symbols are as in Fig. 2. Growth of allele-replacement strains at 23°C, 39°C, or after a deletion of one of the Hsp90 genes, *Hsp82*, at 23°C, is shown. (**A**) *NFS1* (0.5 μM rapamycin; 44 hours). (**B**) *PDR8* (1 mM DOC; 80 hours). (**C**) *MEC1* (25 mM HU; 25 hours) (**D**) RM intergenic region between *NDI1* and *GTR1* (5 mM CDNB; 44 hours). Because the *HSP82* deletion reduces Hsp90 function more than does 5 μM Rad, it often creates stronger phenotypes.

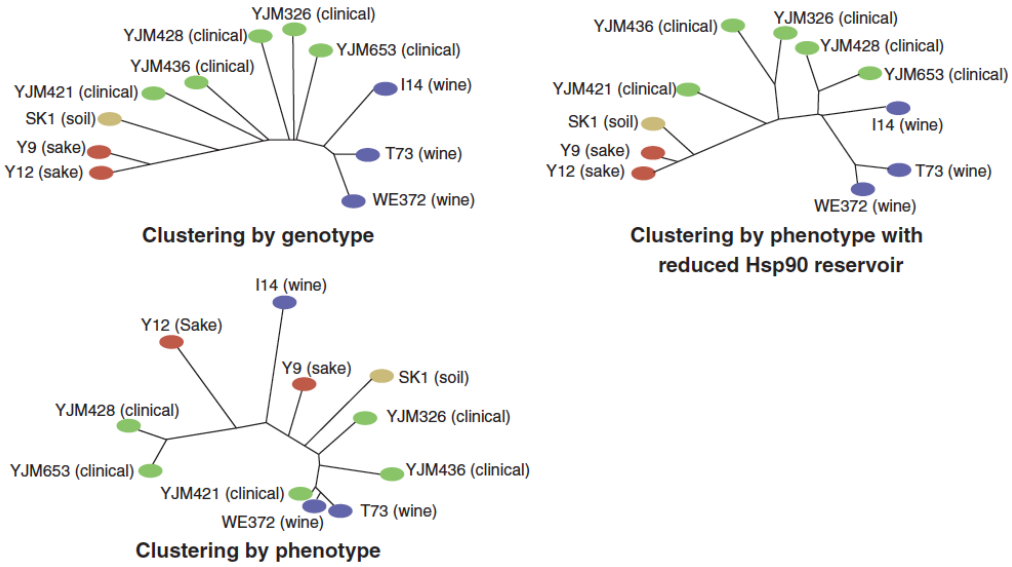

Fig. 4.

Hsp90 inhibition and environmental stress improve the corrrelations between genotype and phenotype. Phylogenetic clustering is derived from (27) and (25). Phenotypic clustering is described in the SOM.