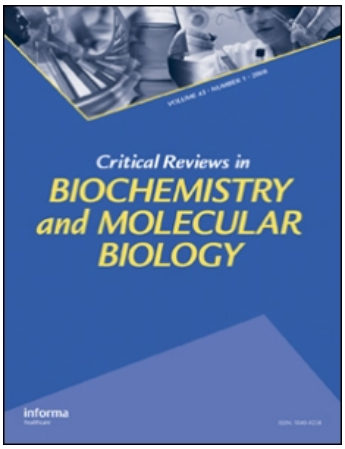

Critical Reviews in Biochemistry and Molecular Biology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713609207

The Hsp90 Capacitor, Developmental Remodeling, and Evolution: The Robustness of Gene Networks and the Curious Evolvability of Metamorphosis

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Online Publication Date: 01 September 2007

To cite this Article Rutherford, Suzannah, Hirate, Yoshikazu and Swalla, Billie J.(2007)'The Hsp90 Capacitor, Developmental Remodeling, and Evolution: The Robustness of Gene Networks and the Curious Evolvability of Metamorphosis',Critical Reviews in Biochemistry and Molecular Biology,42:5,355 — 372

To link to this Article: DOI: 10.1080/10409230701597782

URL: http://dx.doi.org/10.1080/10409230701597782

Critical Reviews in Biochemistry and Molecular Biology, 42:355–372, 2007

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230701597782

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The Hsp90 Capacitor, Developmental Remodeling, and Evolution: The Robustness of Gene Networks and the Curious Evolvability of Metamorphosis

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ABSTRACT Genetic capacitors moderate expression of heritable variation and provide a novel mechanism for rapid evolution. The prototypic genetic capacitor, Hsp90, interfaces stress responses, developmental networks, trait thresholds and expression of wide-ranging morphological changes in *Drosophila* and other organisms. The Hsp90 capacitor hypothesis, that stress-sensitive storage and release of genetic variation through Hsp90 facilitates adaptive evolution in unpredictable environments, has been challenged by the belief that Hsp90-buffered variation is unconditionally deleterious. Here we review recent results supporting the Hsp90 capacitor hypothesis, highlighting the heritability, selectability, and potential evolvability of Hsp90-buffered traits. Despite a surprising bias toward morphological novelty and typically invariable quantitative traits, Hsp90-buffered changes are remarkably modular, and can be selected to high frequency independent of the expected negative side-effects or obvious correlated changes in other, unselected traits. Recent dissection of cryptic signal transduction variation involved in one Hsp90-buffered trait reveals potentially dozens of normally silent polymorphisms embedded in cell cycle, differentiation and growth control networks. Reduced function of Hsp90 substrates during environmental stress would destabilize robust developmental processes, relieve developmental constraints and plausibly enables genetic network remodeling by abundant cryptic alleles. We speculate that morphological transitions controlled by Hsp90 may fuel the incredible evolutionary lability of metazoan life-cycles.

KEYWORDS modularity, chaperone, threshold trait, morphological evolution

I. INTRODUCTION

A surprising variety of morphological abnormalities are expressed when Hsp90 is partially disabled in heterozygous *Drosophila* mutants or when developing flies or *Arabidopsis* seedlings are treated with sublethal doses of Hsp90-inhibitory drugs–conditions expected to mimic natural reductions of Hsp90

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function by environmental stress (Rutherford & Lindquist, 1998; Queitsch *et al.*, 2002; Milton *et al.*, 2003). Flies expressing a specific abnormality produce progeny with similar defects, demonstrating that Hsp90-buffered traits are heritable. Repeated reductions of Hsp90 in particular strain backgrounds show that morphological traits are specific to strain background, and depend on the interaction between Hsp90 and cryptic polymorphisms present at unexpectedly high frequencies in phenotypically normal populations. Continued selection for abnormal flies increases trait penetrance (fraction of abnormal flies each generation) and expression of traits even when Hsp90 function is restored, suggesting that morphological phenotypes revealed and selected during stress could become fixed in nature. Indeed, control of large morphological transitions by Hsp90 is now documented for wide-ranging species, from developmental abnormalities of zebrafish (Yeyati *et al.*, 2007), laboratory and wild populations of *Drosophila* (Rutherford & Lindquist, 1998) and *Arabidopsis* (Queitsch *et al.*, 2002), to cortical patterning in *Tetrahymena* (Frankel & Nelsen, 2001; Frankel *et al.*, 2001) and developmental progression of fungi (Brunt *et al.*, 1990; Loubradou *et al.*, 1997; Brunt *et al.*, 1998; Loubradou *et al.*, 1999). Given its potential role in morphological evolution, it is intriguing that Hsp90 and its substrates are involved in life-cycle transitions of *Leishmania* parasites (Wiesgigl & Clos, 2001) and, as shown in Figure 1, metamorphosis of species spanning all major branches of metazoan phylogeny, from insects and nematodes (Birnby *et al.*, 2000), to echinoderms (Bishop & Brandhorst, 2001), ascidians (Bishop *et al.*, 2001) and molluscs (Leise *et al.*, 2004).

Hsp90 is a member of a growing group of proteins known as molecular chaperones. Protein chaperones are not enzymes, but typically act through low affinity, stoichiometric interactions with substrate proteins to promote their folding and stability by kinetic partitioning–the preferential recognition and dissociation of non-native protein conformations prevents their irretrievable aggregation and, when combined with ATP-dependent cycles of binding and release, drives accumulation of stably folded forms that are no longer recognized by chaperones (Csermely *et al.*, 1998; Frydman, 2001). Because chaperone action is stoichiometric rather than enzymatic, maintenance of the equilibrium between substrate proteins and abundant free chaperones is essential to cellular protein homeostasis

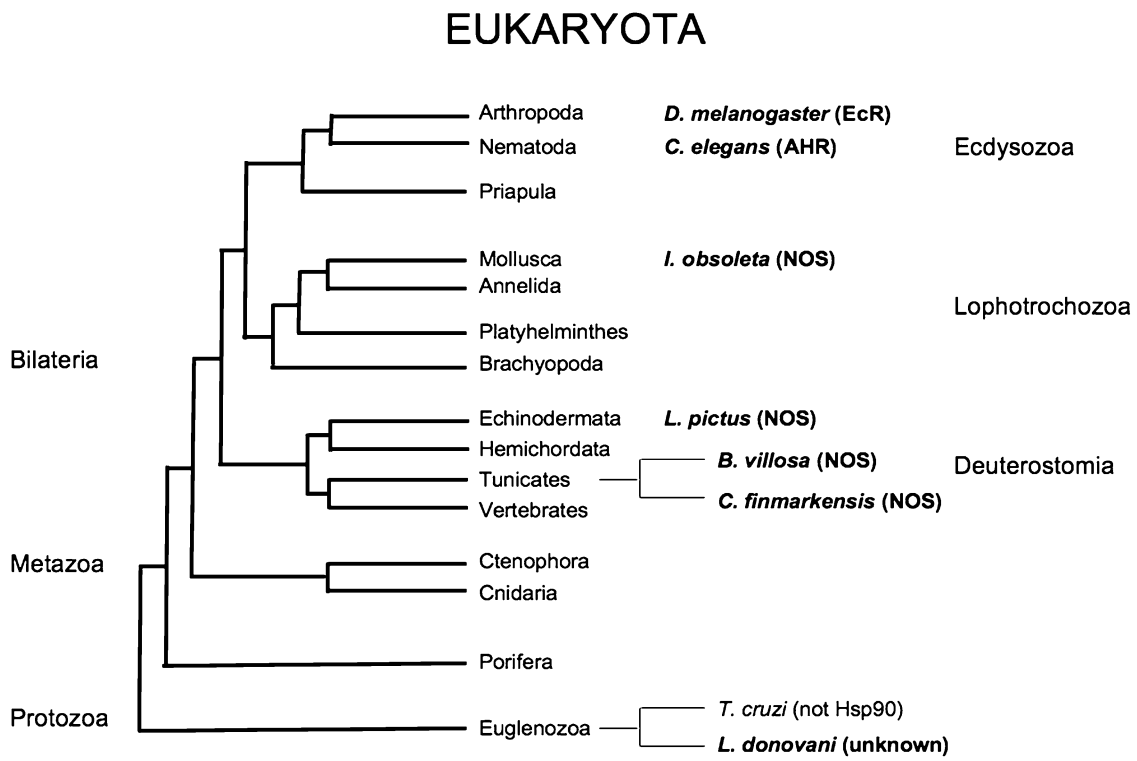

FIGURE 1 **A metazoan phylogeny highlighting at least one species from each of the three major bilaterian groups that has been shown to metamorphose via some interaction with Hsp90 (*bold*). Hsp90 substrate proteins are indicated in parenthesis. We suggest that it would be fruitful to explore the possible link of Hsp90 in regulating metamorphosis in many of the phyla that have not yet been examined, especially those that undergo a radical metamorphosis.**

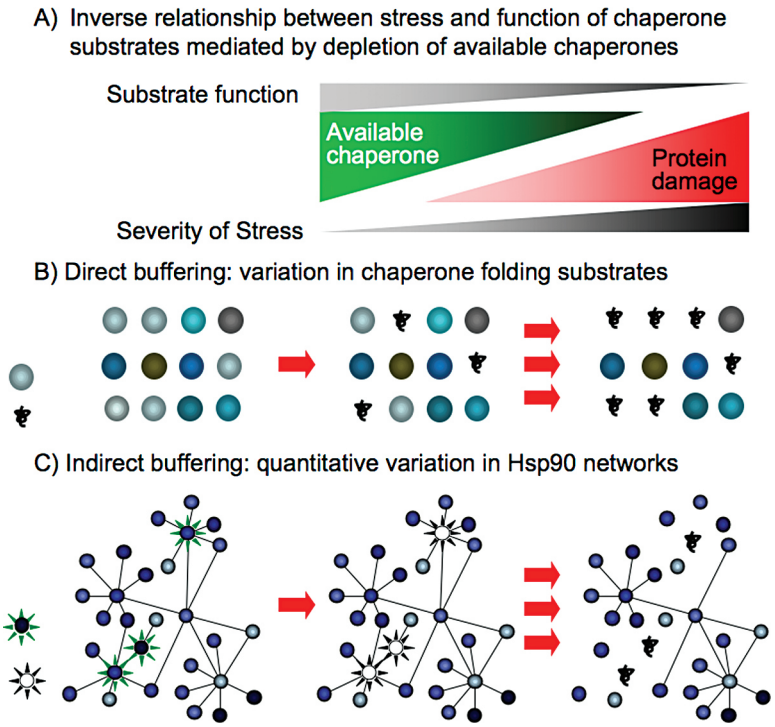

FIGURE 2 **Continuous relationship between environmental stress and genetic buffering by protein chaperones. (*A*) Stress damaged proteins deplete the pool of free chaperones, reducing their availability, first for low affinity and then for higher affinity targets. The concentration of free chaperones in the cytoplasm depends on their relative affinities for stress-damaged and normal substrates. (*B*) The activity of folding polymorphisms (*light blue*) would be directly sensitive to the level of protein folding chaperones. With increasing stress, polymorphic proteins would increasingly not be able to fold (*black squiggles*). (*C*) The activity of wild-type signal transduction clients of Hsp90 (*stars*) depends quantitatively on its chaperone function. Reduction of Hsp90 and signal transduction indirectly uncovers cryptic variants (*light blue*) in interacting genes (*blue circles*). At extreme stress, Hsp90 substrates become non-functional (*squiggles*) and signaling networks collapse.**

(Figure 2). Like most protein chaperones, Hsp90 binds to and protects nearly any newly denatured substrate. However, unlike most chaperones, Hsp90 is not required for normal protein folding but usually (*e.g.*, in non-stress conditions) is dedicated to the maturation and maintenance of a wide range of "client" proteins involved in signal transduction. Hsp90 and a large group of cochaperones keep immature and inactive clients poised for activation until availability of ligand or cofactors stabilize their conformation and transition into fully folded and active signaling complexes that, in general, no longer need Hsp90 for their stability (Rutherford & Zuker, 1994; Pratt & Toft, 1997; Richter & Buchner, 2001; Rutherford *et al.*, 2007).

In the absence of stress, cellular concentrations of Hsp90 are already in micromolar range (Hsp90 normally comprises up to 1% to 2% of soluble cellular protein; Csermely *et al.*, 1998; Picard, 2002). Given its abundance, it is remarkable that reduction of Hsp90 gene dosage by just 50% in *Drosophila* and other species is sufficient to seriously impair many Hsp90 signal transduction substrates (even though heterozygotes usually remain viable and fertile) (reviewed in Rutherford, 2000; Rutherford, 2003). As shown in Figure 2, the equilibrium between free chaperones and substrates that maintains protein homeostasis is shifted by increased concentrations of unfolded proteins during stress (Nollen & Morimoto, 2002). Despite a few-fold induction of Hsp90 and even more dramatic induction of other Hsps during cellular stress responses, diversion of chaperones to general protein damage can compromise normal protein folding and the activity of specific signal transduction substrates (Ali *et al.*, 1998; Duina *et al.*, 1998; Zou *et al.*, 1998).

Hsp90 is present in eubacteria and all eukaryotic cells. In eukaryotes, Hsp90 interfaces networks regulating fundamental processes such as the cell cycle (Helmbrecht *et al.*, 2000; Picard, 2002), growth control (Helmbrecht *et al.*, 2000), gene expression (Pratt & Toft, 1997; Cheung & Smith, 2000; Freeman & Yamamoto, 2002; Morimoto, 2002), chromatin remodeling (Rutherford & Henikoff, 2003; Ruden *et al.*, 2005;

Maloney *et al.*, 2007), proteolysis (Csermely *et al.*, 1998), and several stress responses (Nollen & Morimoto, 2002). When Hsp90 becomes limiting, substrate proteins lose activity, and developmental responses begin to fail. Our recent mapping and identification of a network of cryptic signal transduction variation controlled by Hsp90, covered in Section IX of this review, strengthens the idea that Hsp90-substrate interactions form the fundamental biochemical basis for Hsp90 genetic buffering and control of cryptic morphological variation.

Potentially two sources of genetic variation are buffered by chaperones. Genetic buffering is 'direct' when cryptic variation resides in chaperone substrates (Figure 3). Proteins with slightly different sequences can often achieve the same final fold, albeit with different stabilities. It is possible that less stable sequence variants could fold and function normally in the permissive presence of high concentrations of chaperone, but aggregate under conditions where chaperones are limiting (Fares *et al.*, 2002; Maisnier-Patin *et al.*, 2005, reviewed in Rutherford, 2003). In contrast, we think genetic buffering by Hsp90 is often 'indirect' (Rutherford, 2003). When signal transduction chaperones such as Hsp90 are limiting, client proteins are compromised even if they are not polymorphic. Our work suggests that much cryptic variation resides in proteins that are not themselves Hsp90 clients, but interact with client proteins and pathways to promote signal transduction (see Section IX). Operationally, the distinction between direct buffering by chaperone-dependent folding and indirect buffering by chaperone-dependent modulation of signaling processes is not hard and fast. However, this distinction is conceptually important. Cryptic variation controlled by Hsp90 is almost certainly enhanced by the extensive genetic interactions characteristic of developmental networks and pathways controlled by Hsp90 clients, whether or not the client proteins are polymorphic.

Disruption of the equilibrium between chaperones, normal substrates, and damaged proteins likely controls the release of variation through Hsp90 and modulates the pathology of human diseases of protein aggregation (Nollen & Morimoto, 2002). We suggest that this is true whether Hsp90 chaperone functions are reduced experimentally (by mutations or drugs) or naturally (by stress). Indeed, patterning defects in *Tetrahymena* (Frankel & Nelsen, 2001; Frankel *et al.*, 2001), parasitic transitions to mammalian hosts of *Leishmania* (Wiesgigl & Clos, 2001) and metamorphosis in several organisms (Bishop *et al.*, 2001; Bishop & Brandhorst, 2001; Leise *et al.*, 2004) can be induced by treatment with Hsp90 inhibitors and are also triggered by environmental stress.

The Hsp90 capacitor hypothesis suggests that storage and release of developmental variation by stress allows the generation of novel forms and enables diversification and rapid morphological evolution (Rutherford, 2003). Here we address two main arguments against the Hsp90 capacitor hypothesis and summarize evidence in support, suggesting that Hsp90-buffered changes could be selectable in nature. We then discuss the identification and abundance of cryptic genetic variation available to fuel Hsp90-buffered change. Cryptic variation provides the molecular basis for threshold traits, which are expressed suddenly due to nonlinearity in developmental responses, and genetic assimilation, a means by which selection on polygenic variation eventually allows thresholds to be surpassed, even in the absence of the initiating stress. These concepts are key to understanding the sudden appearance and fixation of large morphological changes controlled by Hsp90. We conclude this review by outlining the intriguing connections between Hsp90 and developmental signals associated with metamorphosis. This evidence suggests that relief of Hsp90 buffering and developmental constraint during environmental stress facilitated the evolution of large morphological transitions of metamorphosis, and may have enabled the extreme evolutionary lability of life-cycles throughout metazoan lineages.

II. CHALLENGES TO THE Hsp90 CAPACITOR HYPOTHESIS

The diversity of features controlled by Hsp90 and their ability to appear suddenly during stress make the Hsp90 capacitor an appealing mechanism to explain the rapid generation of morphological novelty observed in adaptive radiations (*e.g.*, Baker, 2006). Indeed, Hsp90-buffered morphologies in *Drosophila* seem to be endlessly variable (Rutherford & Lindquist, 1998; Milton *et al.*, 2003; and Rutherford, unpublished data), and are likely limited in their variety and form only by their dependence on Hsp90 target functions and the availability of cryptic variation in Hsp90 pathways. However, the Hsp90 capacitor hypothesis is challenged on two fronts.

First, the evolution of an evolutionary mechanism such as evolvability (heritable control over the *capacity*

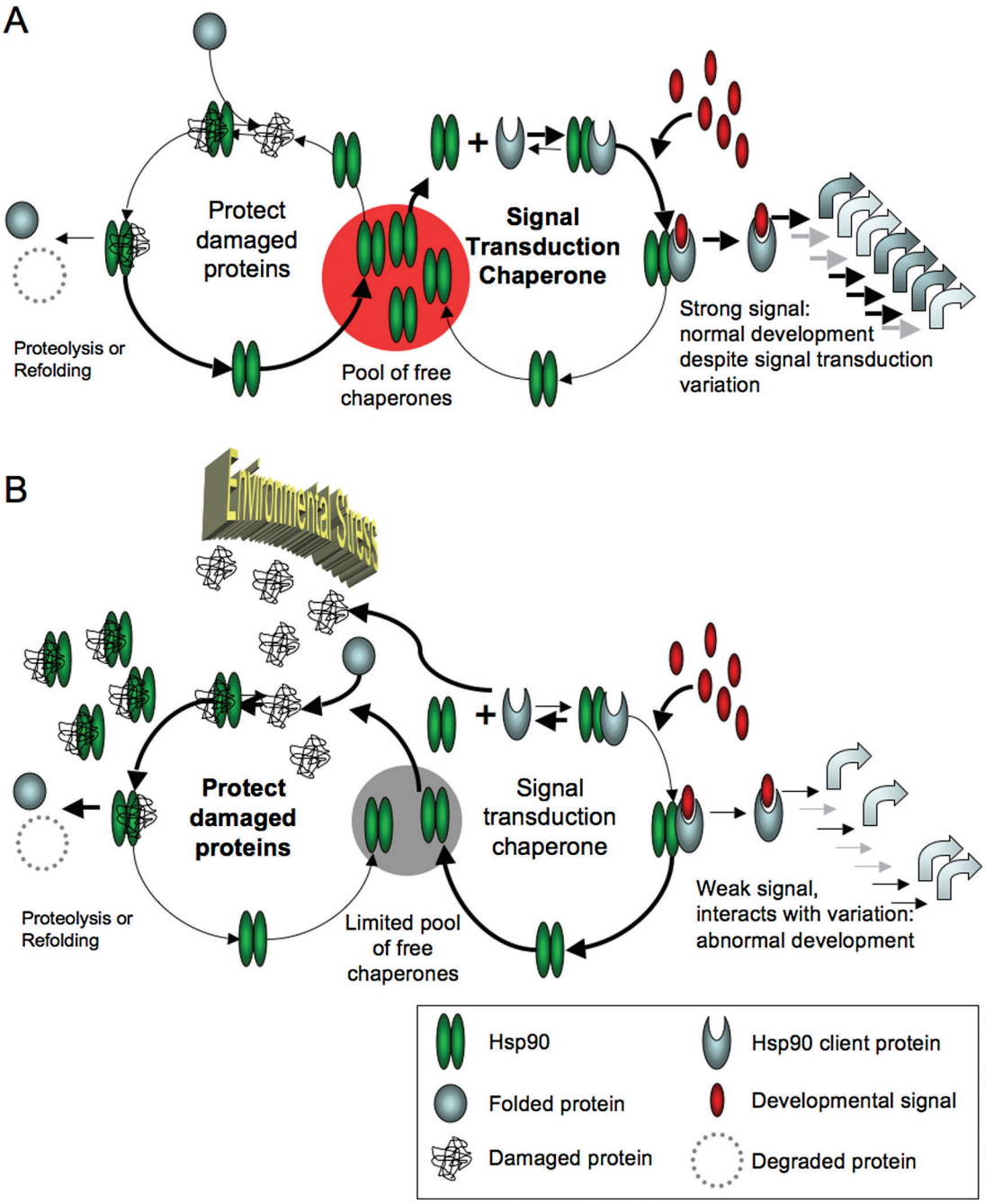

FIGURE 3 **The dual functions of Hsp90 as a chaperone for signal transduction substrates and as a general heat shock protein for stress-damaged substrates provides a stress-sensitive control of the pool of Hsp90 and cellular protein homeostasis. Even though Hsp90 is one of the most abundant cellular proteins even in normal conditions, during severe stress free Hsp90 is diverted for emergency maintenance or degradation of damaged proteins. (*A*) Many signal transduction proteins have a quantitative requirement for Hsp90 maintenance of late-stage folding intermediates and remain poised until developmental signals (*red*, see key) trigger their activation and maturation. (*B*) During protein stress Hsp90 is diverted to damaged protein, the pool of free Hsp90 is reduced and signaling is quantitatively impaired. Coupled with strain-specific variation that weakens signaling (*gray arrows*), this could result in abnormal development.**

to evolve–for example, control of mutation rate as is highlighted by other articles in this series) is considered highly unlikely in traditional population genetic models (Wagner & Altenberg, 1996; Dickinson & Seger, 1999; Gibson & Wagner, 2000). Although more recent models have begun to define conditions under which evolvability might evolve (Agrawal *et al.*, 2005; Masel, 2005), the study of evolvability is problematic because traditionally only the most clearly adaptive traits have been considered proper focus for evolutionary studies. Even considering competition between groups of related individuals, the strength of selection

for population-level traits, or during episodic selection in rare periods of stress is weak compared to the strength of selection for individual traits. This is especially true for traits like evolvability, where the potential benefit to the group (survival because a rare individual has a beneficial change) comes at the expense of most individuals (which will almost never benefit by expression of random morphological variation). The adaptive value of robustness (*e.g.*, failure to express genetic variation) due to genetic buffering (canalization) may be easier to imagine than the evolution of evolvability. However the evolution of genetic canalization is also problematic, for reasons too complicated to cover here. However, detailed arguments for and against the possible evolution of canalization and evolvability have been recently reviewed (Radman *et al.*, 1999; Gibson & Wagner, 2000; Partridge & Barton, 2000; Meiklejohn & Hartl, 2002).

Protein folding and refolding after stress is almost certainly the most ancient, and logically the primary adaptive role of Hsp90 and other chaperones, which likely drove early chaperone-substrate co-evolution (Csermely *et al.*, 1998; Frydman, 2001). Chaperones are not known to be specifically involved in bacterial signal transduction, suggesting it may have been during the evolution of multicellular eukaryotes and expansion of signaling networks that chaperones became coopted as a regulatory mechanism for signal transduction (Csermely *et al.*, 1998). And only after Hsp90 was already integral to developmental networks could the set of Hsp90 client proteins have been conceivably selected for properties of evolvability (Rutherford, 2003). However, whether control of variability by Hsp90 evolved as an adaptation, or simply emerged as an unselected byproduct of other more critical functions, is peripheral to the Hsp90 capacitor hypothesis and irrelevant to the question of whether and how Hsp90 buffering impacts evolvability and morphological evolution.

A second and more serious challenge to the Hsp90 capacitor hypothesis is whether Hsp90-buffered changes can be adaptive. To contribute to adaptive evolution, Hsp90-buffered changes must be selectable in nature. The existence of many bizarre morphological adaptations suggest that it is impossible to predict *a priori* what sort of changes could be adaptive in any unpredictably changed environment. In microevolutionary time-frames, however, developmental mutations that cause large changes in morphology often suffer from a lack of specificity (pleiotropy) and are associated with deleterious side effects and correlated fitness costs. To be advantageous in a stressful and novel environment, Hsp90-buffered morphologies must be modular, able to respond to selection independent of correlated fitness costs and unselected changes in other traits. The most serious and oft cited challenge to the Hsp90 capacitor hypothesis has been that the morphological phenotypes controlled by Hsp90, although selectable in the lab, are necessarily "monstrous" or "unconditionally deleterious" in nature (Dickinson and Seger, 1999; Wagner *et al.*, 1999; Gibson & Wagner, 2000; Meiklejohn & Hartl, 2002; Queitsch *et al.*, 2002). If so, Hsp90-buffered changes could not be refined by natural selection, and would not contribute to evolutionary adaptation under any circumstance. In the following section, we discuss experiments designed to critically test whether Hsp90-buffered changes could be advantageous, and thus promote adaptive evolution.

III. PHENOTYPIC MODULARITY AND INTRINSIC EVOLVABILITY OF HSP90-BUFFERED TRAITS

Biological evolvability refers to a species, lineage or population's capacity for adaptive evolution through the processes of random mutation and selection (Wagner & Altenberg, 1996; Rutherford, 2003). At the population level, 'extrinsic evolvability' depends on gene frequencies, selective pressures and environmental and ecological contexts that are all outside of individual (and genetic) control. Extrinsic evolvability is examined retrospectively in the adaptive radiations of certain lineages (*e.g.*, Yang, 2001), which is perhaps most intuitive, and it is operationally defined as response to selection (Houle, 1992), measured in increasing mean trait values in offspring relative to parent populations (Falconer & Mackay, 1996).

The concept of evolvability becomes complicated at the boundary between group effects and individual effects. 'Intrinsic evolvability' depends on features of individual development that determine how heritable variation will be expressed (Rutherford, 2003). In addition to the mutation rate (as covered in other articles of this series), heritability, variability and the expression of mutation (genetic buffering) control the response to selection and are all potentially under genetic control. However, the translation of genetic variation into phenotypic variation is not a property of a single gene, but is mediated by groups of genes and interconnections between components of developmental networks.

A key component of intrinsic evolvability is modularity, which is present at all levels of biological organization, from populations to molecules (Davidson, 2004; Hansen, 2003; Rutherford, 2003; Schlosser & Wagner, 2004). Here we consider intrinsic evolvability and modularity as they relate to the ability of traits expressed by individuals (or individual genotypes) to change independently of one-another (phenotypic modularity) depending on Hsp90 and its interactions with different genetic backgrounds.

If the cryptic alleles responsible for large morphological changes controlled by Hsp90 caused the expected deleterious side effects, resulting in un-modular or correlated response to selection, Hsp90-buffered variation could not be selected in nature. To test whether Hsp90-buffered changes are necessarily correlated with deleterious side-effects, we measured fitness costs associated with artificial selection of deformed eye *dfe*, a large change in eye morphology that is controlled by Hsp90 in both laboratory and wild flies. A *dfe* abnormality initially appeared and reappeared in the progeny of a single male fly crossed to several related, but unaffected females. As shown Figure 4, during artificial selection deformed flies were crossed together, producing a higher frequency of abnormal progeny in each generation. In the fourth generation, affected and normal flies were split into 3 high lines (HE1-3) selected for *dfe* flies and 3 low lines (LE1-3) selected against flies with eye deformities. By generation 15, the penetrance of *dfe* was about 80% to 90% in the high penetrance lines and near zero in the low lines.

Interestingly, during selection the Hsp90 mutation that had originally revealed the *dfe* phenotype was quickly lost in both high and low lines by simultaneous natural selection against the mutant Hsp90 allele (Rutherford & Lindquist, 1998). (Not surprisingly, even in phenotypically normal heterozygotes there are apparently deleterious fitness effects of Hsp90 reduction and consequent reductions in development signaling.) The variety and abundance of cryptic alleles affecting *dfe* and a threshold trait mechanism called genetic assimilation allowed the *dfe* trait to become expressed to

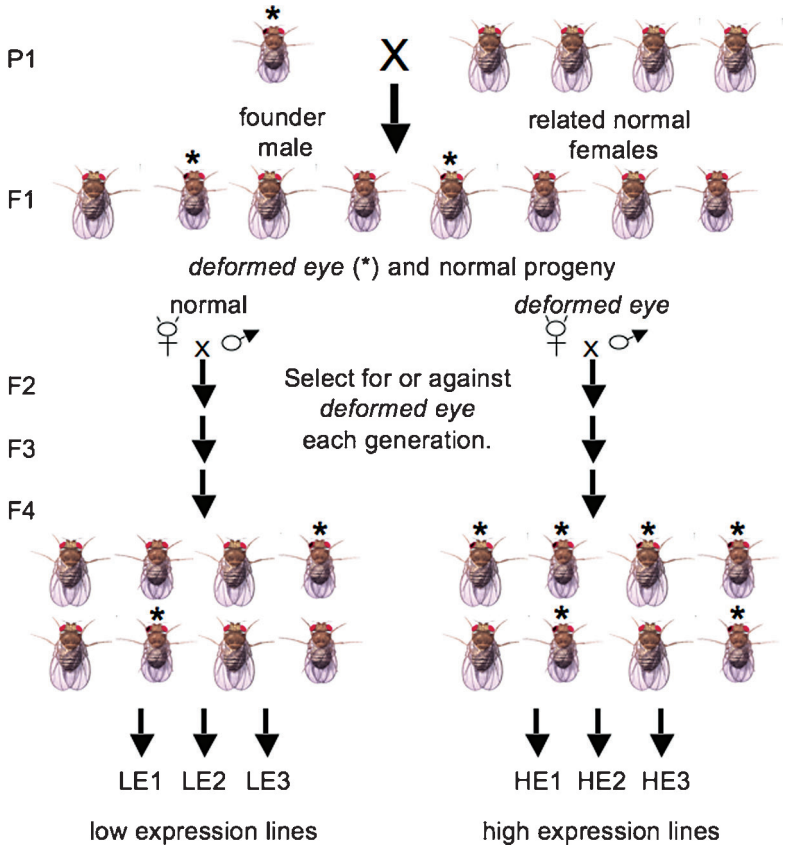

FIGURE 4 **Artificial selection of the deformed eye trait in *Drosophila*. The selection started from just a single male (*P*1) with a deformed eye (*asterisk*). Crossing of the founder male with genetically related normal females resulted in deformed and normal progeny (*F*1). Crossing within deformed or normal flies and selection for or against *deformed eye* each generation produced low (LE1-3) and high (HE1-3) expression lines, respectively (Rutherford & Lindquist, 1998; Carey *et al.*, 2006).**

high penetrance independent of deleterious alleles. Genetic assimilation will be discussed in detail in Section VII of this review; we note here that genetic assimilation of traits revealed under low Hsp90 conditions would be necessary for the continued expression of adaptations in populations adjusting to a new environment and is an important feature of the Hsp90 capacitor hypothesis.

After ~40 generations of selection and loss of the original Hsp90 mutation we were unable to detect fitness effects of the *dfe* polymorphisms. Even 120-fold differences in penetrance between the genetically related high- and low-penetrance *dfe* selection lines did not translate into detectable differences in absolute fitness between high and low lines or wild-type flies in hatch rates, egg-to-adult viability and lifetime female fecundity (Carey *et al.*, 2006). By contrast, scoring tens of thousands of flies raised in competition experiments allowed us to measure the fitness costs of the *white* eye-color mutation in the selection line backgrounds relative to wild-type alleles. However, in the same experiment there was no measurable difference between the high and low lines in relative fitness. We do not suggest that there could not be subtle reductions in fitness or that the described morphological changes associated with *dfe* are adaptive in this or any context. However, the high relative fitness of *dfe* flies is consistent with previous observations that even the most extreme Hsp90-buffered phenotypes were generally restricted to particular morphological features and could be readily transmitted to the progeny of affected flies and enriched to high frequency by selection (Rutherford and Lindquist, 1998; Milton *et al.*, 2003). This suggests that there could be conditions in nature where the adaptive value of an Hsp90-buffered change could outweigh the initial fitness costs, allowing a morphological adaptation, initially revealed under stress, to be further sculpted and refined by selection.

Our recent work, discussed in Section IX of this review, shows that cryptic variation in signaling networks controlled by Hsp90 is surprisingly abundant. The abundance and highly polygenic nature of Hsp90-buffered variation and the relative independence of the morphological changes controlled by Hsp90 enables expression of adaptive traits without strongly deleterious and correlated effects on reproduction or viability, *e.g.*, modularity as opposed to pleiotropy. In the next section we consider threshold traits, which support the mechanism of genetic assimilation and provide the opportunity for natural selection of alternative alleles that produce large changes without compromising fitness. Near trait thresholds, deleterious alleles (such as Hsp90 mutations) may be readily replaced by less deleterious alleles with similar phenotypic effects.

IV. THRESHOLD TRAIT MECHANISM OF Hsp90 BUFFERING

It was initially puzzling that Hsp90 had little effect on the normally variable quantitative traits and did not control every trait in every genetic background (Milton *et al.*, 2003). The most significant advance in our understanding of Hsp90 buffering and the biological control of variation was prompted by the surprising realization that Hsp90 is specific for highly discrete and previously invariable threshold traits, both qualitative and quantitative. Strong and consistent effects of Hsp90 are specific to variation in certain highly invariant quantitative traits, most of the Hsp90-buffered traits were previously studied models for threshold trait selection and canalization. The threshold trait specificity of Hsp90 unifies current knowledge of development with Hsp90 buffering, genetic networks, signal transduction and classical population genetic models of threshold traits (Milton *et al.*, 2006). Threshold models show how genetic and nongenetic sources of variation are simultaneously buffered by Hsp90 and how, once variation in previously invariable traits is expressed during stress, the predicted (and observed) response to selection (extrinsic evolvability) is dramatically increased (Milton *et al.*, 2006).

Interestingly, left-right wing asymmetry, almost certainly unconditionally deleterious in any environment imaginable, was the one highly invariable trait that was independent of Hsp90 control, and would therefore remain buffered during environmental stress (Milton *et al.*, 2006). The functional distinction by Hsp90 between plausibly adaptive and maladaptive traits and between variable and threshold traits (which are by definition invariable) transcends the historical dichotomy in evolutionary biology between quantitative traits, thought to provide most of the material for evolutionary change, and qualitative traits, best known as major developmental mutations that are generally viable only in the artificial environment of the laboratory. As illustrated in Figure 5, increased sensitivity to genetic background and simultaneously to multiple types of nongenetic variation occurs as a natural consequence of steep phenotypic response (thresholds)

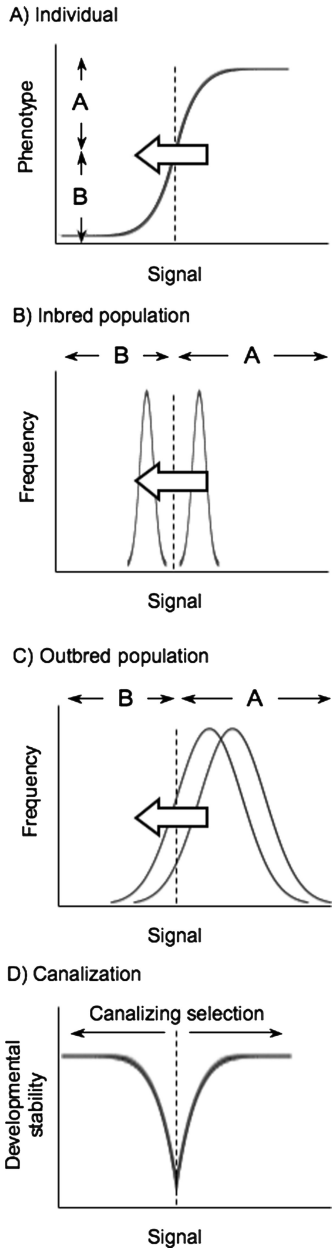

FIGURE 5 **Schematic representation of the threshold trait model. (*A*) Relationship between developmental signal (X-axis) and phenotype (Y-axis) at the level of individual. Quantitative difference in signal level is converted into discrete (two or several sets) of phenotypes according to the threshold (*dotted line*). Here the threshold defines phenotypes A and B. If an individual has cryptic variation sensitive to Hsp90-buffering level, reduction of Hsp90 buffering (*arrow*) would lower the signal below the threshold, converting phenotype A to phenotype B. The probability of change increases near the threshold. (*B* & *C*) Histograms showing narrow and wide distribution of the signal level in inbred (*B*) and outbred (*C*) populations, respectively. The inbred population has little variation, resulting in narrow distribution of signal levels, while the outbred population has high variation, resulting in a wide distribution of the signal levels. (*D*) Developmental stability is minimal at the threshold. Canalization of development results from the process of canalizing (or stabilizing) selection. Traits canalized through evolution of threshold (*e.g.* switch-like) responses would be robust to genetic, environmental, or stochastic variation in individuals or populations far from the threshold, but sensitive to all types of variation at the threshold.**

to continuous differences in Hsp90-dependent signaling. Because of abundant natural variation in signal transduction pathways and nonlinear developmental responses (*e.g.*, thresholds, or biological switches–see below), when Hsp90 function and the strength of signaling are decreased in individuals or across populations, developmental thresholds are crossed and the probability of abnormal phenotypes is increased.

V. CRYPTIC GENETIC VARIATION

The powerful, but highly deterministic view of the cell embodied by "one gene-one enzyme" and more recent metaphors of the genome as a "parts list" or "blueprint" for development need to be re-examined with the realization that developmental genes (parts) are largely the same across divergent metazoans (Nichols *et al.*, 2006). Overlapping networks of combinatorial gene interactions control most developmental decisions (Levine & Davidson, 2005). Redundancy, quality-control features and capacity for recovery allow developmental networks to achieve phenotypes that are generally robust to perturbations and small quantitative differences in signal transduction. For example, stereotypical adult phenotypes like digit and vertebrae number are nearly invariant in normal populations despite environmental variation and abundant genetic polymorphisms. A rash of models and empirical results of the past 5 years has documented many examples of biological robustness and mechanisms by which developmental stability is achieved (Barkai & Leibler, 1997; Alon *et al.*, 1999; von Dassow *et al.*, 2000; de Visse *et al.*, 2003; Volfson *et al.*, 2006; Lander, 2007). On the other hand, the extreme robustness of developmental networks and near identitiy of the "parts lists" among metazoans, raise the even more pressing question of where variation resides to drive macroevolutionary change.

Understanding how large gene networks are remodeled during the evolution of development or by diseases such as cancer is one of the most formidable problems in biology today. We suggest that conditionally neutral or cryptic variation is a critical piece of this puzzle. In classical quantitative genetic models, pervasive cryptic variation is hidden by genotype-by-environment interactions (GXE), epistasis (GXG) and dominance, which are a ubiquitous feature of the genetic architecture of all organisms (Falconer & Mackay, 1996; Bergman & Siegal, 2003). Estimates of DNA polymorphism in *Drosophila* or human populations suggest that there are

hundreds of thousands to millions of nucleotide differences between the genomes of unrelated individuals–so much variation that each individual is essentially genetically unique, despite the fact that individuals of a species share the same characteristic phenotypes, especially for the most discrete (threshold) traits (Moriyama & Powell, 1996). A renewed interest in the importance of cryptic variation in evolution and human health highlights the importance of understanding canalization at the molecular level (Rutherford, 2000; Gibson & Dworkin, 2004). Indeed, our imprecise knowledge of this hidden quantitative trait variation–how much and which of the extensive nucleotide variation is functional and under what conditions it is expressed–confounds our ability to understand basic principles of evolutionary change and remains one of the largest obstacles to realization of the medical promise of the genome era.

Several features make Hsp90 buffering distinct from typical GXE or epistasis. First, Hsp90 buffering is specific to strong environmental stress (rather than a simple environmental change) and relief from Hsp90 buffering would happen simultaneously to all individuals in a population, regardless of genotype. Highly polygenic Hsp90-buffered variation affects particular developmental pathways dictated by the expressed set of Hsp90 substrate proteins. Membership in the set of Hsp90-dependent clients and signaling pathways could easily evolve through the evolution of Hsp90-dependence, determined by destabilizing mutations of individual signal transduction proteins (Rutherford, 2003). Second, Hsp90 is specific for morphological novelties and typically invariant quantitative traits. These traits would normally have little opportunity to evolve, but may be most likely to contribute to novel adaptations in a radically changed environment. Third, restriction of Hsp90 effects to threshold traits means only individuals with very high or very low signaling levels would be seen (and generally removed) by stabilizing selection. This would have little impact on individuals at intermediate (optimal) signaling levels and liability. Fourth, our work suggests that robustness and evolvability are manifest on a trait-by-trait basis. The specific set of traits controlled by Hsp90 almost certainly results from the set of Hsp90 clients and their sensitivities to stress. Critically invariant traits (such as left-right wing asymmetry) can be buffered independent of Hsp90 and potentially adaptive threshold traits (Milton *et al.*, 2003, 2006). Finally, as a "highly connected node" in the topology of developmental networks Hsp90 is expected to be critical to the integrity and robustness of developmental processes (Rutherford *et al.*, 2007). Because of Hsp90's position in the network architecture of development, it has the potential to coordinate variation affecting several processes simultaneously.

VI. GENETIC ASSIMILATION

Beginning in the early 1940s, C.H. Waddington promoted the idea that cryptic variation, initially revealed by environmental stress, can provide a driving force for evolution through a mechanism he called genetic assimilation. In the most famous example (Waddington, 1942), Waddington introduced genetic assimilation to account for how callosities on the "knees" of ostriches, which appear during embryogenesis, could have evolved. Waddington suggested that initially the callosities were a physiological response to environmental insult. However, if this thickening and toughening of the skin made the ostriches more fit, those that were more *physiologically* responsive (*e.g.*, made better, faster callosities in response to chaffing) would have left more offspring. The selected enrichment for genes that enhance the physiological response, could then result in an ability to respond to lower and lower levels of environmental stimulation, eventually resulting in the developmental production of the callosities in the absence of environmental input.

Phenocopies are phenotypes that resemble known developmental mutations, but are environmentally induced by particular stresses during specific windows of development (Mitchell & Petersen, 1982). Selection on cryptic alleles in *Drosophila* that alter their sensitivity to phenocopy traits provided the original experimental demonstrations of genetic assimilation. In one example, Waddington showed that heat treatment of pupae of a specific age induced a crossveinless phenotype on the wings of a small fraction of flies. By selectively breeding the crossveinless flies, the frequency of flies producing the crossveinless wing phenocopy was increased in the next generation (Waddington, 1953, 1957). The selected lines eventually produced a number of flies with "phenocopies" in the absence of heat treatment. Similar genetic assimilation has been demonstrated for Hsp90-buffered traits, which eventually became independent of Hsp90 during selection (Rutherford & Lindquist, 1998). Genetic assimilation was key to Waddington's ideas about the interplay between evolution and developmental responses, and remains a critical feature of

the Hsp90 capacitor hypothesis, as it allows for continued presence of selected phenotypes independent of Hsp90, particularly after evolutionary and/or physiological adaptation has reduced the environmental stress response.

VII. CLASSICAL THRESHOLD TRAIT MODELS

Genetic assimilation has not been embraced by mainstream evolutionary theory, perhaps because it is often associated with large and sudden morphological changes and often severe environmental stress. Despite its distinctly Lamarckian flavor, genetic assimilation is predicted by standard quantitative genetic theory in threshold trait models (Wright, 1934; Falconer & Mackay, 1996). Continuously variable traits (*e.g.*, height) approximate Gaussian distributions in outbred populations because of selection against extreme phenotypes. It is assumed that continuous trait values of individuals (*e.g.*, height), or approximately continuous (meristic) trait values (*e.g.*, *Drosophila* bristle numbers), directly reflect continuous differences in the strength of underlying developmental variation, and that these differences result from numerous polymorphisms with additive contributions to the trait. Studies of continuous *Drosophila* bristle number traits largely confirm the classical models, and show that continuous variation for bristle number probably resides in many of the same developmental signaling genes shown by developmental studies to be responsible for bristle cell fate determinations (Mackay, 1996).

In contrast with the relatively well behaved continuous traits, threshold traits are much less understood and characterized by sharply discontinuous phenotypes that fall into two or a few highly discrete categories. They are accessible by standard quantitative models if one assumes that the developmental processes underlying threshold traits are also continuous and normally distributed (*e.g.*, have a similar genetic architecture to the better studied continuous traits). Sewall Wright (1934) developed the first threshold trait models to describe and predict the non-mendelian inheritance of genetic polymorphisms controlling the appearance of extra digits in lines of guinea pigs. The fraction of guinea pigs with extra toes in any particular line or cross could be measured and was used to infer, using inverse probability density functions, the mean and variance of hypothetical liability distributions and the positions of trait thresholds on a standard deviation scale.

In the case of Hsp90 buffering and threshold traits, liability is directly represented by the strength of Hsp90-dependent signaling and known developmental pathways, allowing the powerful application of threshold models with explicit molecular basis. For example, based on empirically determined parameters, we estimate that ~50% reduction of Hsp90 reduces the strength of signaling liability for abnormal bristle numbers by more than 2 standard deviations (SD) (Milton *et al.*, 2006). Interestingly, such a shift explains both the appearance of bristle number abnormalities and increased variation at lower thresholds, and the disappearance of abnormal bristle numbers and reduced variation at upper thresholds. The presence of both upper and lower thresholds for expression of abnormalities is an expected consequence of stabilizing selection for optimal levels of developmental signaling.

Molecular enhancer and suppressor screens in inbred *Drosophila* lab strains demonstrate the direct interaction of Hsp90 at both upper and lower developmental thresholds, where discrete phenotypic transitions are produced by abrupt (switch-like) phenotypic responses at thresholds in the level of the underlying developmental signals (Rutherford, 2000, 2003). The highly specific genetic interactions of Hsp90 with heterozygous signaling mutants, which would normally have no phenotype on their own, are a model for Hsp90 interactions with polygenic variation in outbred strains. When Hsp90 is reduced, signal transduction substrates begin to fail, signaling is reduced and variations in other pathway genes are expressed in either inbred lab strains or, as our work has shown, in outbred populations.

VIII. BIOLOGICAL SWITCHES

As discussed above, thresholds represent nonlinear response of phenotype to continuous differences in the underlying signal, and may be best exemplified by discrete cell fate decisions during development. Current models of nonlinearity and cooperativity in developmental pathways provide a concrete molecular and mechanistic foundation for understanding developmental thresholds in the context of normal signal transduction pathways (Ferrell, 1996, 1998; Gardner *et al.*, 2000). In one of the best studied examples, the mitogen activated protein kinase (MAPK) cascades, many of the upstream kinases are known Hsp90 substrate proteins.

In general, MAPK pathways are characterized by "ultrasensitivity" (Goldbeter & Koshland, 1984), steep sigmoidal and "switch-like" responses (Ferrell, 1996, 1998). Close to the threshold even a small reduction in MAPK signaling could have a large effect on the response (Figure 5). Significantly, nonlinearity also provides developmental buffering away from the threshold, where large changes in the strength of developmental signals would have little phenotypic effect.

Our results suggest that the pathways and traits protected by Hsp90, such as MAPK pathways in *Drosophila*, are generally characterized by nonlinear threshold responses to continuous variation in signaling (Cutforth & Rubin, 1994; Dickson *et al.*, 1996; van der Straten *et al.*, 1997). Thresholds account for both robustness to variation in the strength of signaling and the sensitivity of Hsp90 dependent responses. Dramatic morphological shifts could respond to selection, depending on the chance segregation of cryptic polymorphisms specific to different genetic backgrounds. Complex phenotypes controlled by Hsp90 also show a threshold response to environment (Rutherford & Lindquist, 1998) and genetic factors (Rutherford, unpublished data).

In multicellular organisms, the development of adult phenotypes results from multiple cell fate decisions and thresholds that are elaborated through the interaction of cell-cell signaling pathways and downstream transcription factors (Barolo and Posakony, 2002). At each step, the production of a discrete decision eliminates the continuous variation preceding the decision. This sequential digitization may be an important aspect of developmental noise reduction. It remains to be seen whether the switch paradigm describing small mathematically modeled circuits in single cells (O'Farrell, 2001) or engineered circuits in bacteria (Gardner *et al.*, 2000) will translate into the threshold trait expression of adult phenotypes.

IX. CRYPTIC POLYMORPHISMS IN Hsp90-BUFFERED GENE NETWORKS

To identify the molecular basis of cryptic variation and the relevant Hsp90 substrates and pathways in the *dfe* selection lines, we used complementary and unbiased genome-wide (autosomal) screens for polygenic suppressors and single gene enhancers of *dfe* penetrance. Conservative estimates based on recombination and quantitative trait loci (QTL) mapping now suggest that the small founding population (5 flies) from which the three *dfe* high lines were derived carried at least 15 QTL, or cryptic alleles, that influence eye development in the context of the *dfe* background. However, surprisingly few of the 15 QTL were shared between the 3 high lines despite extensive noncomplementation when the high lines were crossed with each other and with the low lines. A less conservative estimate suggests that there may be as many as 28 QTL segregating in the 3 high lines, with evidence for extensive overlap between the lines.

In a complementary and independent mapping approach, we used the *Drosophila* deletion set to identify regions that when deleted (or mutant) enhanced *dfe* penetrance (Rutherford, unpublished data). This identified more than 40 narrowly defined regions corresponding to over 75 genes that enhance *dfe* penetrance, with several QTL regions containing clusters of enhancing genes. The genetic positions of QTL and enhancers correspond more closely than would be expected by chance, supporting the idea that these very different approaches identified the same sets of genes. This work suggests that an extensive network of cryptic variation controls the *dfe* trait, and identifies dozens of candidate polymorphisms and affected pathways in Hsp90-dependent signaling networks.

Many enhancers of *dfe* fall into well defined networks (modules) of cell cycle, growth control, apoptosis, and differentiation containing classical Hsp90 clients. Reduction of Hsp90 and client functions in turn exposed variation hidden in genes interacting with the same networks controlled by its clients (Rutherford, unpublished data). For example, a regulatory circuit involving eight genes, including the E2f transcription factor, controls the length of the cell cycle in *Drosophila* (Reis and Edgar, 2004). This circuit acts through negative feedback onto E2f from the major cell division kinases (CDKs) regulating G1/S and G2/M transitions, and compensates during G1 and G2 growth phases to maintain the overall rate of the cell cycle. All eight of the genes in this circuit are enhancers of *dfe* and our data suggest five of these may be polymorphic in normal populations. Three genes in the network (*wee*, *Cdk1*, and *Cdk2*) are also known Hsp90 targets in flies and other organisms. Through these client proteins, Hsp90 capacitor could destabilize robust circuit design by reducing either negative (*wee*) or positive regulators (CDK). Natural variation in other members of the circuit could then express effects that would either enhance or reduce the overall cell-cycle rate. Our work suggests that many cell cycle

polymorphisms segregate in normal flies without disrupting development, but could contribute to cancer or morphogenesis in the appropriate context.

The cell cycle receives input from diverse developmental signals. Altogether, *dfe* trait architecture may involve at least six of eight canonical signaling pathways believed to have been present throughout the evolution of metazoans from sponges to humans (Pires-daSilva & Sommer, 2003; Nichols *et al.*, 2006). In addition to the cell-cycle network, disruption of at least four other developmental processes, including morphogen gradient stability, protein synthesis and degradation, and regulation of cell and tissue growth seem to be required to produce the morphological changes. Hsp90 has substrate proteins in a number of *dfe* pathways, with which it interacts simultaneously and at multiple levels. Thus, it seems that only through collaboration of several misregulated signaling networks can robust phenotypes be perturbed to produce morphological variation.

The ability to maintain intrinsic flexibility and evolvability of genetic networks despite their normally constant developmental outcomes may be unique to Hsp90 and perhaps just a handful of other highly connected proteins, suggesting that the most important role of Hsp90 in evolution could be its capacitor function. Hsp90 has several properties critical to its role as an "evolvability gene." First, Hsp90 can both hide and release pre-existing but cryptic genetic variation. Second, Hsp90 is a hub in developmental regulatory networks with a position high in the hierarchy of network and subnetwork architecture. This allows Hsp90 to control multiple processes at once. Third, Hsp90 provides physiological control of phenotypic variability, altering the genotype-to-phenotype map in response to changes in selective environments. These features are key to the ability of the Hsp90 capacitor to coordinate the interplay of developmental networks and pathways in sculpting morphological change.

X. Hsp90 AND LIFE HISTORY EVOLUTION

Now that we have reviewed the unique attributes of Hsp90 and how it can act as a genetic capacitor to control the accumulation and release of cryptic variation, in this section we speculate on how genetic assimilation of threshold traits controlled by Hsp90 may have contributed to morphological transitions associated with the evolution of life histories. The development of widely diverse body plans and morphologies is controlled by a cascade of highly conserved transcription factors and inductive signaling molecules that govern the read out of the individual genomes in time and space to produce remarkably distinct morphologies (Levine & Davidson, 2005). One key to producing such unique morphologies using the same sets of parts is the modular nature of gene promoter regions, which direct gene expression to different tissues at different times, often with exquisite timing and precision (Levine & Davidson, 2005; Swalla, 2006). Promoters often contain several separate clusters of enhancers that control the timing and tissue specificity of gene expression. While multiple binding sites can be redundant, there can also be remarkable interaction between the sites to produce evolutionarily conserved and robust outcomes. We first consider how Hsp90 might be involved in the environmental cues triggering metamorphosis, we then consider how alternate destabilization and support of combinatorial transcription factors through Hsp90 may have enabled promoter evolution and the morphological remodeling of metamorphosis.

Marine organisms often exploit alternative life history strategies, with benthic (bottom-dwelling) animals frequently free-spawning eggs that develop into larvae that live in the plankton and may or may not feed (Strathmann & Eernisse, 1994). This separation in time and space of adult sexual organisms from developmental stages and larval growth is thought to be selectively advantageous for slow moving animals who would otherwise not disperse far on the ocean floor. Predation pressure and selection is much higher on eggs and larvae on the sea floor than in the plankton (Allen & McAlister, 2007). However, for planktonic larvae, premature metamorphosis would mean settling in the open ocean and certain death for the adults who thrive on the bottom in the productive intertidal zones near shores. The transition from larval to adult stages is therefore almost always tightly regulated by an environmentally sensitive competent period that delays metamorphosis until the proper cues are detected by the larvae (Bishop *et al.*, 2006; Roberts *et al.*, 2007). Interestingly, in both sea urchins and ascidians, Hsp90 inhibitors trigger metamorphosis when applied during the competent period (Bishop & Brandhorst, 2003).

The molecular basis of competency is not well understood (Bishop *et al.*, 2006; Roberts *et al.*, 2007). However, nitric oxide synthase (NOS), an Hsp90 substrate in vertebrates (Garcia-Cardenã *et al.*, 1998), is critical for delaying metamorphosis of competent larvae in snails,

ascidians and sea urchins (Bishop *et al.*, 2001; Bishop & Brandhorst, 2001; Leise *et al.*, 2004). When competent ascidian or sea urchin larvae are treated with inhibitors of either Hsp90 or NOS, they spontaneously trigger metamorphosis, suggesting NOS inhibits the metamorphic cues (Bishop & Brandhorst, 2003). We suggest that Hsp90, through its action on NOS, may interpret environmental cues that trigger the morphological transitions associated with metamorphosis, and could have been a key component in their evolution.

Several reports over the past few years show an intimate connection between Hsp90 and morphological remodeling of metamorphosis in animals from marine snails to the dauer larvae of *C. elegans* (Figure 1; Bishop *et al.*, 2001; Baker, 2006). Morphological transitions of metamorphosis are often dramatic, and can involve fundamental changes in highly robust or canalized traits like symmetry and basic body plan (Strathmann & Eernisse, 1994; Swalla, 2006). Within the deuterostomes, our invertebrate relatives, organisms can express completely different body plans and symmetry in larval *versus* adult stages. In many echinoderms, a bilateral planktonic larva undergoes a radical metamorphosis into a spiny, radially symmetric adult whose body axes are completely different (Swalla, 2006). Another example is the swimming tadpole larva of tunicates, which have a chordate body plan that metamorphoses into a radically different filter-feeding and totally sessile adult that is not bilaterally symmetrical, completely lacks a dorsal nerve chord and is hardly recognizable as an animal (Swalla, 2006). Given the often drastic morphological changes associated with the process, it is remarkable that metamorphosis has evolved several times independently within the metazoans (Degnan & Degnan, 2006; Swalla, 2006).

In several metazoan groups, the global timing and coordination of embryonic and larval developmental events is controlled by systemic lipophilic signaling molecules, which include steroids, thyroid hormone (TH), retinoids and 20-hydroxyecdysone (Ec) (Riddiford *et al.*, 2000; Escriva *et al.*, 2004). These signaling molecules activate members of the large nuclear receptor superfamily, which contains over 150 members that can combine as homodimers and heterodimers to transcriptionally regulate large sets of genes (Escriva *et al.*, 2004). Interestingly, many nuclear hormone receptors are also classical Hsp90 substrate proteins (Pratt & Toft, 1997; Cheung & Smith, 2000; Freeman & Yamamoto, 2002; Morimoto, 2002). Transcriptional activation of different groups of genes by spatially and temporally controlled expression of various steroid hormone ligands, combined with tissue specific expression of different combinations of ligand binding receptors and binding partners is thought to orchestrate many of the morphological changes associated with metamorphosis (Furlow & Neff, 2006).

For example, in the Ecdysozoa (Figure 1), or molting insects, Ec plays a critical role in the proper timing and sequence of development and metamorphosis (Riddiford *et al.*, 2000). The steroids Ec and juvenile hormone (JH) are expressed in temporally and spatially controlled pulses to coordinate embryonic and larval development. Ec binds to and activates nuclear receptors (EcR), which are homologous to vertebrate THR, to coordinate the timing of metamorphosis (Riddiford *et al.*, 2000). While vertebrate THR is not a classical Hsp90 substrate protein, *Drosophila* EcR has demonstrated genetic and biochemical dependence on Hsp90 and its co-chaperones, both *in vitro* and *in vivo* (Arbeitman & Hogness, 2000). In echinoderms, TH triggers metamorphosis in competent larvae (Chino *et al.*, 1994) and is hypothesized to play a role in the evolution of direct development in some echinoderm groups (Heyland *et al.*, 2005). A unique feature of ascidian tadpole larvae, however, is that they do not metamorphose in response to TH, despite TH blockers' ability to block metamorphosis, suggesting that TH is necessary but not sufficient for metamorphosis in some species (Davidson *et al.*, 2004). Regardless, the 20-hydroxyecdysone receptor (EcR) of insects and binding partners for TH receptors (THR) of deuterostomes are similar to vertebrate retinoid X receptor (RXR) heterodimeric classes of steroid receptors (Escriva *et al.*, 2004). Vertebrate RXR requires Hsp90 to attain full transcriptional activity (although this functional association is less widely recognized because the THR and RXR classes of nuclear receptors do not co-purify with the Hsp90 chaperone complex, as do many of the steroid receptors; Holley & Yamamoto, 1995).

The involvement of Hsp90 in steroid receptor signaling and other processes associated with metamorphosis in multiple metazoan lineages suggests that the buffering capacity of Hsp90 controls hidden variation affecting morphological changes important for evolution of life history transitions. As an evolutionary capacitor, Hsp90 buffering would retain the evolvability of life history traits and during stress release morphological variation, allowing effective adaptive radiation

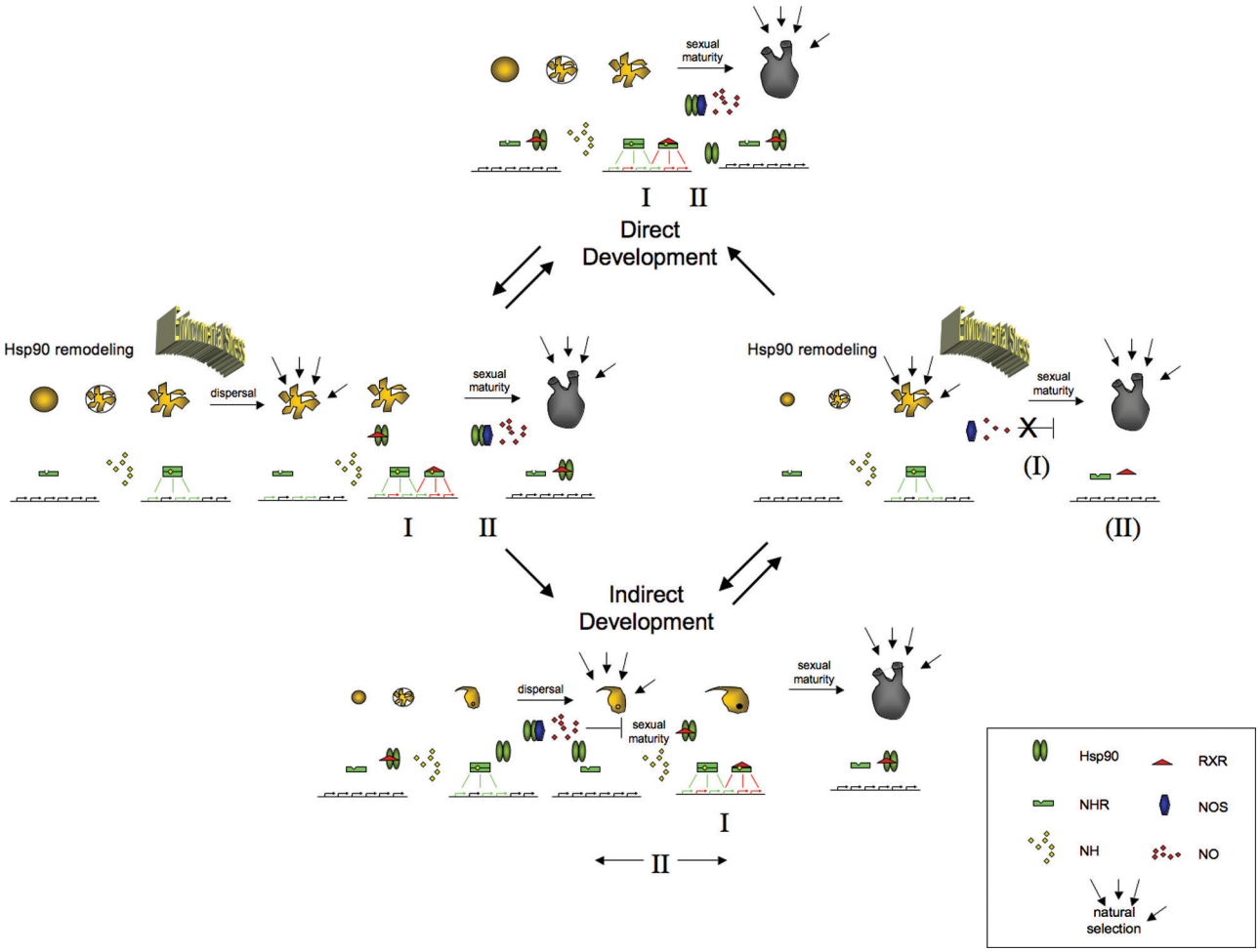

FIGURE 6 **Hypothetical scenario for Hsp90 capacitor activity in evolvability of life-history transformations of metazoans. Whether a direct or an indirect developer was the basal ancestor of the bilateria, all major groups within the bilateria are polyphyletic for metamorphosis. Metamorphosis generally involves steps mediated by Hsp90-controlled signaling, including nuclear hormone receptor (NHR; I) and nitric oxide signaling (NO; II). (*I*) NHRs involved in metamorphic transitions across the metazoans are both Hsp90-independent (*e.g.*, thyroid hormone receptor) and Hsp90-dependent (*e.g.*, Retinoid X receptor [RXR]). (*II*) Nitric oxide synthase (NOS) is also an Hsp90-dependent target in mammals (Garcia-Cardenã *et al.*, 1998). Interestingly, NHRs in mammals control gene transcription as homodimers (*green arrows*) or as heterodimers with RXR (*red arrows*). We suggest that morphological transition by NHR signaling and sexual maturation by NO signaling might be coincidentally altered via Hsp90-buffering. Reduction of Hsp90 capacitor function, which may occur under severe environmental changes, could expose variation in the timing (*e.g.*, off-rate) (Freeman & Yamamoto, 2002), strength and specificity of transcriptional control of NHR during morphogenesis and could alter the timing and strength of NO signaling and shift the timing of sexual maturation.**

into a range of environments. It has been previously suggested that Hsp90 capacitor functions could have contributed to the evolvability of life histories during dramatic climactic changes in the early evolutionary history of metazoans (Baker, 2006). Here we extend this idea to recent data supporting a role for Hsp90 in the evolution of developmental networks. The hypothetical model shown in Figure 6 depicts a composite of signaling events important for metamorphosis across metazoans. When Hsp90 function is reduced, both competency (through NOS) and transcriptional control through hormone response elements specific to the heterodimer form (shown in red) would be affected, as would the timing, turnover, and dissociation of several active steroid receptor transcription complexes. We suggest that this deregulation of the timing and targets of steroid signaling is a destabilizing event providing variation allowing developmental network remodeling and the evolution of heterochrony (changes in the timing of specific developmental phases or events), both critical features of the evolution of metamorphosis. Not only do many nuclear receptors require Hsp90 for activity, Hsp90 and a co-chaperone p23 are necessary for transcriptional timing, receptor recycling, chromatin remodeling and assembly of activated transcriptional complexes (Freeman and Yamamoto, 2001). Indeed, a

characteristic feature of steroid hormone action is the ability to respond rapidly, on the order of minutes, to fluctuations in hormone levels. Such fine tuned temporal control of transcription may be crucial for their biological function as global regulators of morphogenesis during development.

It is intriguing that Hsp90 is implicated in the evolvability of new life history stages in several groups of animal phyla (Baker, 2006). Whether this shuffling of genetic pathways also contributes to the evolution of body plans will need further investigation (Swalla, 2006). The transition from monophasic to biphasic life histories alters the adaptive landscape an organism faces, and it has been suggested that separation of feeding and reproduction can relieve selective pressures on the adult. A survey of metamorphosis among the major animal groups provides tantalizing clues suggesting a genetic capacitor function of Hsp90 linking the evolution of life histories and associated morphological changes with environmental stresses, adaptation and evolutionary radiations.

XI. CONCLUDING REMARKS

Despite the central importance of adaptive evolution for understanding the emergence of biological diversity, the shape and texture of adaptive landscapes remain unknown. The time and average number of mutations required for a novel adaptation to appear, and the expected size of allelic effects remain largely unresolved. Under the assumptions of gradualist models of evolution, phenotypes map onto a smooth and monotonic fitness landscape, with a single peak of maximum fitness. On such landscapes, genetic capacitors are unlikely, because the changes expected to be favorable are slow, small and quantitative rather than rapid, large and qualitative. However, under alternative assumptions pioneered by Wright, adaptations can be large on a rugged fitness landscape with many local optima. The sudden production of novel adaptations independent of correlated fitness costs would facilitate peak shifts from previously adapted optimal phenotypes to distant phenotypic optima for the new environment, effectively bypassing low fitness intermediates. Some evolutionary transitions, such as those that occur between direct and indirect development, are likely to demand simultaneous adaptations of multiple features such as delayed sexual maturation, developmental heterochrony and morphogenesis. During stressful events triggering the need for evolutionary adaptations to a novel life-cycle, developmental remodeling by Hsp90 could allow the simultaneous release of variation affecting several processes, increasing the chance for productive and coordinated changes in multiple systems. We suggest that by allowing simultaneous selection of multiple previously neutral alleles contributing to large phenotypic transitions at trait thresholds, evolutionary capacitors such as Hsp90 may fulfill the predictions of both of the historically dichotomous models of evolutionary change, bridging the gap between microevolutionary process and macroevolutionary reality.

ACKNOWLEDGMENTS

We are grateful to R. Howsmon and K. F. Gorman for Figure 4, and to K. F. Gorman, R. Howsmon, C. Carey, J. R. Knapp, and B. M. Huynh for providing unpublished data. We are grateful to Yuichiro Suzuki, Mariko Bushcamp, HeeSun Kim, and Amanda Rychel for critical comments on the manuscript. Support by NIH R01 GM068873 (SR). YH is supported by Lady Tata Memorial Trust.

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Editor: Susan M. Rosenberg