

## Is Hsp90 a Regulator of Evolvability?

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**ABSTRACT** In a recent paper, Rutherford and Lindquist (1998. *Nature* 396:336–342) identified mutations in the Hsp90 protein that act to unmask hidden genetic variation with a variety of phenotypic effects. The Hsp90 protein has a number of properties that suggest a role in regulating the expression of genetic variation and therefore in adjusting the evolvability of the organism. In this paper we reflect upon the evolutionary feasibility of such mechanisms and suggest some possible ways of testing the adaptation-for-evolvability hypothesis in more detail. We conclude that Hsp90 holds promise as a molecular model system for the evolution of evolvability. *J. Exp. Zool. (Mol. Dev. Evol.)* 285:116–118, 1999. © 1999 Wiley-Liss, Inc.

It has long been known that the sensitivity of an organism's phenotype to environmental and genetic factors (phenotypic variability sensu Wagner and Altenberg, '96) is genetically regulated. Indeed, mutations with major phenotypic effects such as *Scute* in *Drosophila* and *Tabby* in mice unmask hidden genetic variation, which indicates the increased sensitivity of the mutant phenotype to genetic variation (Scharloo, '91). Similarly, extreme environmental conditions can also unmask genetic variation for traits (Hoffmann and Parsons, '97). In a recent paper, Rutherford and Lindquist ('98) have provided one of the first glimpses into the molecular mechanisms that underlie the genetic regulation of phenotypic variability. They have shown that loss of function mutations at the *Drosophila Hsp83* locus (encoding the heat-shock protein Hsp90) lead to an increase in the genetic variation of a number of phenotypic traits.

Hsp90 is a highly conserved molecular chaperone that, in addition to participating in the cell stress-response system by helping to refold denatured proteins, also has a more specific role in signal transduction. Through repeated low-affinity interactions, Hsp90 keeps inherently unstable proteins involved in signal transduction pathways poised for action. Presumably, the rampant developmental disturbances observed in *Hsp83* loss-of-function mutants, or by chemically inhibiting Hsp90 protein function, result from destabilized signal-transduction pathways that are less robust against genetic and environmental perturbations. Rutherford and Lindquist have shown that some of the exposed developmental changes are heritable and that it is possible to reinforce these variants

by selection to the point that they are expressed even when wildtype Hsp90 function is restored.

Hsp90's dual involvement in stress response and signal transduction raises the intriguing possibility that it serves to link the expression of genetic variation to environmental stress. Rutherford and Lindquist hypothesize that during stress Hsp90 becomes diverted from its function in signal transduction through its affinity for denatured proteins. The result is the expression of normally cryptic genetic variation in Hsp90-dependent transduction pathways. If this variation was purely deleterious one might expect that the regulatory proteins would have evolved to become independent of Hsp90, which seems plausible given that proteins vary in their dependence on Hsp90, or that Hsp90 through gene duplication and divergence evolved to decouple its two functions. These possibilities present Hsp90 as the first experimentally accessible model system for investigating the evolution of evolvability. Rutherford and Lindquist suggest that evolvability is increased by the unmasking of normally hidden genetic variation which might be useful in the adaptation of a species to a new environment. In this note we want to discuss some of the population genetic underpinnings and difficulties with this hypothesis, as well as make some suggestions for empirical and experimental approaches to testing it.

What then, is the relationship between genetic variability of phenotypic characters and evol-

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ability? The answer to this question depends on a number of factors. Clearly, heritable variation of adaptive characters increases evolvability, since the amount of heritable phenotypic variation limits the rate of response to natural selection. But on the other hand, there are more subtle effects by which phenotypic variability can decrease evolvability. All of them have to do with deleterious pleiotropic effects of mutations. The fitness of a mutant usually results from the balance of fitness enhancing effects on the phenotype and deleterious side effects. The chance of a phenotypic character to improve by natural selection depends on average amount of deleterious side effects of mutations ("redundant variation" sensu Riedl, '78). A phenotype that is too sensitive to genetic variation may lead to many deleterious side effects of any mutation and thus be less evolvable than one with lower variability. In fact, Gerhart and Kirschner ('97) have argued that many features of the molecular biology of a cell can be considered as means to improve evolvability by preventing deleterious effects of genetic and environmental variation. This intuition is supported by the mathematical analysis of population genetic models (Wagner, '84, '88a; Bürger, '86). The models predict that potentially adaptive genetic variation is lost to selection if it is associated with unconditionally deleterious effects on so-called "core characters." Unconditionally deleterious variation decreases evolvability, i.e., it decreases the covariance of the adaptive characters with fitness. It has been argued that this form of functional dependency between adaptive characters and core characters is general, rather than exceptional, for biological systems (Wagner, '88b). For instance, the adaptive value of variation in a locomotory organ (e.g., a bird wing) is conditional on the performance of many other characters such as the nervous, respiratory, and circulatory systems. Deleterious variation in any of these characters thus makes the variation in the locomotory organs adaptively irrelevant. Increased variation in core characters thus removes some of the potentially adaptive variation of the phenotype as a whole, and consequently decreases evolvability. That is to say that the effect of phenotypic variation on evolvability depends on the balance between the evolvability enhancing and inhibiting effects of phenotypic variation. In general, it is predicted that the more complex an organism is the more severe the negative effects of phenotypic variation (Wagner, '84, '88a,b). Phenotypic stability of core characters rather than their variabil-

ity is a prerequisite for the evolvability of complex organisms.

The results cited above raise the question of whether the kind of phenotypic variation, caused by changes in the level of Hsp90 protein, in balance increases or decreases evolvability. The variations in *Drosophila* wing vein, eye and head morphologies (Rutherford and Lindquist, '98) resemble unconditionally deleterious variations that are predicted to decrease evolvability. Whether, or under what conditions, Hsp90 is an enhancer of evolvability is a question that can be investigated experimentally. We propose the following experiments to address this problem. First, the question of whether evolvability is enhanced can be investigated by comparing the response of Hsp90 geldanamycin-inhibited and Hsp90-wild-type control fly strains to artificial selection on life-history traits. Second, the question of whether enhanced evolvability can convey a competitive advantage could be investigated by allowing a Hsp90-inhibited fly strain and a control strain (labeled with some neutral marker) to each adapt to a novel environment and subsequently compete. The outcome of this experiment can be directly compared to the result of first subjecting the same strains to competition before they adapt to the new environment. It may even be possible to directly estimate the heterozygote fitness of *Hsp83* loss-of-function mutants relative to wild-type alleles when they compete in a novel environment.

Hsp90 may also influence evolvability by structuring the co-variability of traits. The evolvability of a complex organism depends on how its variability is structured into semi-independent modules (Bonner, '88; Raff, '96; Wagner, '96; Wagner and Altenberg, '96). If functionally related traits must change in unison to preserve functionality then co-variation of the characters is advantageous to evolvability. A striking aspect of Hsp90 is that it affects many seemingly unrelated proteins simultaneously. Do these proteins have anything in common? The involvement of Hsp90 in the cellular response to temperature may provide a clue to this question. Adaptation to changes in temperature is a recurrent challenge on both ecological and evolutionary time scales, and clearly, adjusting the temperature optima of a large number of enzymes simultaneously is a daunting task. Could it be that Hsp90 serves to correlate the temperature adaptation of many proteins simultaneously? And, in particular, does it serve to correlate the temperature adaptation of regulatory proteins that need to be adjusted in concert

to preserve functionality? Hsp90 may achieve this role by facilitating the accumulation and subsequent release of genetic variation in target interacting proteins, thereby increasing the chances of simultaneously exposing mutations that work together in the new temperature regime. Alternatively, evolutionary changes in Hsp90 regulation or activity may directly and simultaneously adjust the temperature optima of its targets. These hypotheses could be tested by comparing Hsp90 function in closely related species living under different temperature regimes. Indeed, there is evidence that the regulation of another heat-shock protein, Hsp68, differs among lizard species with different temperature requirements (Ulmazov et al., '92). Similar studies of the Hsp90 system are urgently needed.

Even if it can be shown that the Hsp90 system is able to enhance evolvability, the question remains, "What selective forces shaped it?" While Rutherford and Lindquist do not explicitly propose a scenario for the evolutionary origin of the Hsp90 system, one could certainly think of a scenario in which the *Hsp83* function evolved *because* of its possible role as a mechanism for evolvability. This suggestion, however, shares a problem with similar ideas for biological mechanisms with a potential influence on evolvability, like recombination or mutation rates (Maynard Smith, '78). The informal consensus among population geneticists is that evolvability is difficult to select for even if in principle possible (Dawkins, '89; Kauffman, '93; Wagner and Altenberg, '96). The problem is that the advantage only comes into effect during the potentially rare episodes of adaptive evolution. In fact, in phases of adaptive equilibrium many of these traits are selected against. The question, then, is whether the benefits in terms of evolvability outweigh the disadvantages these traits have in equilibrium, and the answer to this depends on how often the population experiences directional selection. To answer this question requires theoretical work to assess what pattern of environmental changes is necessary to give evolvability enhancers a (geometric) fitness advantage. These studies need to be supplemented by careful field observations like those gathered on Darwin's finches on the Galapagos (Grant, '86) to compare the predicted with the actual patterns of environmental changes.

Is Hsp90 a regulator of evolvability? Hsp90 clearly holds great promise as a model system for understanding the genetic basis for evolvability, and indeed shows some indications of (broad-sense) adaptation for this purpose. However, it is too early to conclude that the Hsp90 system is a clear-cut example of a mechanism directly favoring evolvability. Fortunately, the theoretical developments and experimental evidence needed to firmly test this idea are within our reach.

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