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PERSPECTIVE AND REFLECTION ARTICLE

Stress, genomes, and evolution

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Abstract Evolutionary change, whether in populations of organisms or malignant tumor cells, is contingent on the availability of inherited variation for natural selection to act upon. It is becoming clear that the Hsp90 chaperone, which normally functions to buffer client proteins against the effects of genetic variation, plays a central role in this process. Severe environmental stress can overwhelm the chaperone's buffering capacity, causing previously cryptic genetic variation to be expressed. Recent studies now indicate that in addition to exposing existing variation, Hsp90 can induce novel epigenetic and genetic changes. We discuss key findings that suggest a rich set of pathways by which Hsp90 can mediate the influences of the environment on the genome.

Keywords Hsp90 chaperone · Stress-induced mutation · Repeat instability · Evolution

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Introduction

Conrad Waddington's influential theory of genetic assimilation is a rationalization of the "inheritance of acquired characters" within the context of natural selection and Mendelian genetics. Waddington sought a non-Lamarckian explanation for how environmentally triggered traits, such as the callosities of ostriches and the hardened soles on the feet of humans, could become inherited features of an organism (Waddington 1942). He argued that morphological development must be buffered against minor environmental and genetic variations and that this canalization enhances the neutral accumulation of genetic variation. Under extreme stress, this buffering capacity is reduced, leading to the expression of latent genetic variation. Iterative rounds of selection can "assimilate" the adaptive phenotypes such that their expression gains independence from the original environmental stimulus (Waddington 1942). Waddington supported his model in *Drosophila* by applying stress, in the form of heat shock or ether vapor, to developing flies. He demonstrated that (1) morphological defects arise as a result of environmental stress, (2) the proportion of flies exhibiting these phenotypes increases under selection, and (3) continued selection for the novel traits results in their persistence, even in the absence of the original environmental stress (Waddington 1953; Waddington 1956). While these results were provocative, it remained unclear whether adaptive phenotypes could arise from such a process or if the novel phenotypes were simply degenerative outcomes of an inability to withstand stress (Williams 1966).

Over 50 years later, Rutherford and Lindquist (1998) reignited interest in genetic assimilation with the identification of a molecular basis for Waddington's observations. They proposed that the Hsp90 chaperone buffers cryptic genetic variation. Chaperones function to help fold and

stabilize client proteins that lack intrinsically robust folding properties, buffering them against small changes that might arise in the protein sequence. In this model, the surge in misfolded client proteins accompanying certain environmental stresses, such as heat shock, overwhelms the buffering capacity of chaperones, causing previously cryptic genetic variation to be expressed (Rutherford and Lindquist 1998). Although their initial experimentation in flies produced many of the same dramatic phenotypes that Waddington observed, experiments in plants revealed that the suppression of Hsp90 produces quantitative variation in leaf and stalk morphology and life-history traits such as flowering time, without impacting viability (Queitsch et al. 2002; Sangster et al. 2007; Sangster et al. 2008). Interestingly, Hsp90 appears to modulate plant resistance to insect herbivores, further supporting a role for Hsp90 in adaptive evolution (Sangster et al. 2007).

The major prerequisite for Waddington's genetic assimilation process is the presence of latent, preexisting genetic variation segregating within the population. Ruden and colleagues challenged this assumption when they demonstrated that Hsp90 could modulate the expression and assimilation of novel morphological phenotypes in isogenic populations of flies via an epigenetic mechanism (Sollars et al. 2003). Support for an epigenetic model was further strengthened by the observation that histone deacetylase inhibitors such as trichostatin A and sodium butyrate could substantially suppress the expression of the novel phenotypes and from the regulatory role Hsp90 plays in the maintenance of active chromatin (Ruden et al. 2003; Sollars et al. 2003; Tariq et al. 2009). It is therefore likely that, for a subset of assimilated phenotypes, preexisting genetic variation is not a requirement. In addition, stress-induced epigenetic changes have the potential to be adaptive, as they allow populations to rapidly and reversibly respond to changing environments (Rando and Verstrepen 2007).

If sorting through the genetic and epigenetic contributions to stress-induced phenotypes was not complex enough, the genetic assimilation model is now being further complicated by new results that suggest Hsp90 plays a role in maintaining the integrity of the genome. First, Specchia et al. (2010) reported that impairment of the chaperone leads to transposon-mediated mutagenesis in the *Drosophila* germline. The authors observed the assimilation of a *Scutoid*-like phenotype (loss of bristles and perturbed development of the compound eye) and determined that it arose from an insertional mutagenesis event in the *noc* gene. The insertion generated a truncated transcription factor and was found only in the flies exhibiting the novel phenotype. Sequencing of the *noc* gene in wild-type flies from the experiment revealed no changes to the gene sequence. These observations offer an additional explanation for the contribution of genetic background to the expression of stress-induced phenotypes, as different genetic backgrounds could lead to different transposon insertion events (Specchia et al. 2010).

Most recently, we have demonstrated that Hsp90 is required for maintaining microsatellite repeat stability in human cells, through the regulation of double-strand break (DSB) repair (Mittelman et al. 2010). In mammalian cells, DSBs are repaired by two well-defined, homology-dependent repair pathways: strand invasion, which depends on Rad51, and single-strand annealing (SSA), which does not (Paques and Haber 1999). We showed that when Hsp90 is diverted from its normal function, the levels of active Rad51 decrease, suggesting that DSB-repair shifts towards the SSA pathway. Because the SSA pathway fixes DSBs by pairing homologous sequences on either side of the break, it would lead naturally to changes in the lengths of the repeat tract (Richard et al. 1999). Therefore, pressure from the environment might play a role in directing whether repeat tracts are repaired by the conservative Rad51-dependent pathway or the more error-prone, SSA pathway. Our results are consistent with the provocative findings from Rosenberg and colleagues, who have extensively characterized stress-induced error-prone DSB-repair in bacteria (Galhardo et al. 2007; Ponder et al. 2005).

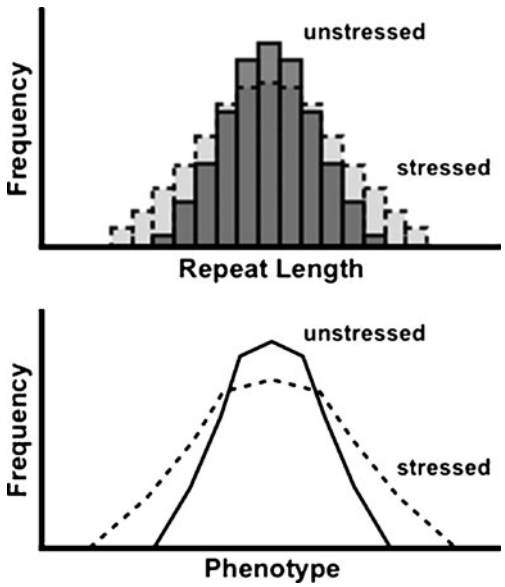

Fig. 1 Stress-induced repeat instability as a potential source of novel phenotypic variation. Under normal environmental conditions, repeat allele lengths are distributed narrowly in a population (*dark gray bars*), but when severe stress diverts Hsp90 from its normal role in maintaining genome stability, the distribution of repeat lengths broadens (*light gray bars*). Similarly, an unstressed population expresses a narrow range of phenotypes (*solid line*), which expands when stress is applied (*dashed line*). The ability of microsatellite repeats to incrementally influence gene expression suggests that stress-induced repeat instability may underlie some fraction of the increased range of phenotypes observed in response to stress

Repeat mutation has long been used as an indicator of genome stability and is of significant prognostic utility for certain types of cancer (Healy et al. 2006; Reuschenbach et al. 2009). The role of repeats as agents of disease extends beyond the two dozen or so characterized repeat expansion neurodegenerative diseases to include a wide range of neurological and morphological disorders (Albrecht and Mundlos 2005; Orr and Zoghbi 2007). This common focus on disease, however, masks the inherent utility of repeats in modulating gene function. Coding microsatellites are enriched in genes for transcription factors and other regulatory proteins, and changes in the length of these repeats exert incremental impacts on gene function (Albrecht et al. 2004; Bacolla et al. 2008; Gerber et al. 1994; Wren et al. 2000). Variations in the lengths of noncoding repeats in the promoters of genes have been shown to quantitatively affect transcription and have likely facilitated transcriptional evolution (Vinces et al. 2009). Emerging evidence implicates coding and noncoding microsatellites as important sources of common, subclinical genetic variation in morphological and behavioral traits in numerous species, including humans, dogs, and flies (Fondon and Garner 2004; Goodman et al. 1997; Kashi and King 2006; Sawyer et al. 1997). It is possible that the incremental impact of variation in intragenic repeats might provide insight into the subtle, quantitative trait variation recently described in Hsp90-supressed flies and plants (Milton et al. 2006; Queitsch et al. 2002; Sangster et al. 2007; Fig. 1).

The identification of the Hsp90 chaperone as a buffer for latent genetic variation has transformed our understanding of Waddington's theory of genetic assimilation. Although controversy exists regarding the extent to which genetic assimilation has played a role in evolution, there are clear examples of how the suppression of Hsp90, under extreme stress, can reveal potentially adaptive phenotypes. Recent studies now indicate that in addition to exposing existing cryptic variation, Hsp90 modulates the generation of novel epigenetic and genetic variation. These results imply a rich set of pathways by which the environment can influence the genome.

If Hsp90 and stress can regulate the integrity of the genome, what does this say about the relationship between the evolutionary forces of mutation and selection? Have we historically overinterpreted the conclusions of Luria and Delbruck (1943) in asserting that selection and mutation are completely independent? Could the genome be more dynamic and responsive to the environment than we previously thought? What are the implications of a malleable genome for cancer and other human diseases? Undoubtedly, these questions will be addressed as future studies continue to explore the ramifications of a genome sensitive to its environment.

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