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REVIEW ARTICLE

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A role for epigenetic adaption in evolution

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Abstract

The outcome of epigenetic responses to stress depends strictly on genetic background, suggesting that altered phenotypes, when induced, are created by a combination of induced epigenetic factors and pre-existing allelic ones. When individuals with altered phenotypes are selected and subjected to successive breeding, alleles that potentiate epigenetic responses could accumulate in offspring populations. It is reasonable to suppose that many, if not all, of these allelic genes could also be involved in creating new phenotypes under nonstressful conditions. In this review, I discuss the possibility that the accumulation of such alleles in selected individuals with an epigenetic phenotype could give rise to individuals that exhibit the same phenotype even in the absence of stress.

KEYWORDS

genetic assimilation, Hsp90, phenotype selection, stress response

1 | INTRODUCTION

Epigenetic responses to environmental alterations lead to altered or new phenotypes that are often adaptive, probably because the responses consist of biomolecular elements that have been naturally selected and fixed during evolution. For instance, when plants are exposed to water deficit, specific inactivation of histone deacetylase HDA6 is induced, leading to activation of genes involved in drought tolerance (Kim et al., 2017).

The outcome of epigenetic responses, such as phenocopy, depends on genetic background (Goldschmidt & Piternick, 1956), suggesting that altered phenotypes are created by a combination of induced epigenetic factors and allelic factors. Those allelic factors include cryptic mutations that might determine the characteristics of produced phenotypes when exposed (Queitsch, Sangster, & Lindquist, 2002).

In this review, I discuss the hypothesis that under the direction of selective forces, in laboratory or in the natural environment, selected individuals with epigenetically adaptive phenotypes can accumulate genes and/or alleles that contribute to adaptive genotypes.

2 | "GENETIC ASSIMILATION" HYPOTHESIZED BY C. H. WADDINGTON

In the canalization model of Waddington, embryo development is "canalized" under the influence of natural selection such that a normal body is produced even in the face of slight abnormalities of the genome or the external environment (Waddington, 1942). In addition, if an animal encounters unusual circumstances, it develops adaptive characteristics that might become newly canalized.

In the first series of experiments intended to test his hypothesis, Waddington used the *Drosophila melanogaster* Edinburgh strain S/W5, which can phenocopy a crossveinless wing in response to heat shock treatment during the pupa stage (Waddington, 1953; Figure 1). Approximately 30% of parental flies responded to heat shock and exhibited the crossveinless effect; these flies were selected and subjected to breeding. Over the course of 23 generations (F1–F23), the percentage of flies with the heat shock-induced crossveinless

phenotype increased from 30% to 97%. No crossveinless flies were produced from untreated pupae from F1 to F14. However, 1%–2% F16 flies expressed the crossveinless phenotype even in the absence of heat shock treatment. The frequency of spontaneous crossveinless flies increased thereafter, to 67%–95% in some breeding lines. Waddington interpreted these results as an indication that acquired characteristics could be "assimilated" by the genotype during selection.

Subsequently, in a second series of experiments, Waddington demonstrated genetic assimilation of the bithorax phenotype (Waddington, 1956). He found that ether treatment of *Drosophila* embryos induced bithorax phenocopy, after which repeated selection of flies with the bithorax phenotype yielded bithorax flies even in the absence of ether treatment. To explain this finding, he postulated the occurrence of new mutations that gave rise to bithorax-like flies in two independently produced fly lines. In addition, he also suggested a second possibility: that minor alleles already present might assemble together to produce the bithorax phenotype under selection pressure.

3 | CRITICISM OF GENETIC ASSIMILATION

Gerhart and Kirschner (1997) criticized the genetic assimilation hypothesis, pointing out that Waddington had mistakenly assumed that heritable changes during selection and breeding were due to stable genetic changes. Inasmuch as genetic assimilation did not occur in genetically inbred lines, they argued, the heritable changes observed by Waddington could be caused during selection by the loss of suppressor mutations pre-existing in genetically heterogeneous *Drosophila* strains.

However, as will be seen below, phenotypic variability is frequently observed in inbred *Arabidopsis* lines, particularly under stressful conditions (Queitsch et al., 2002).

4 | HSP90 AS A CAPACITOR FOR MORPHOGENETIC MUTATIONS

Under normal conditions, most dominant mutations leading to distinct phenotypes are likely to be deleterious and are,

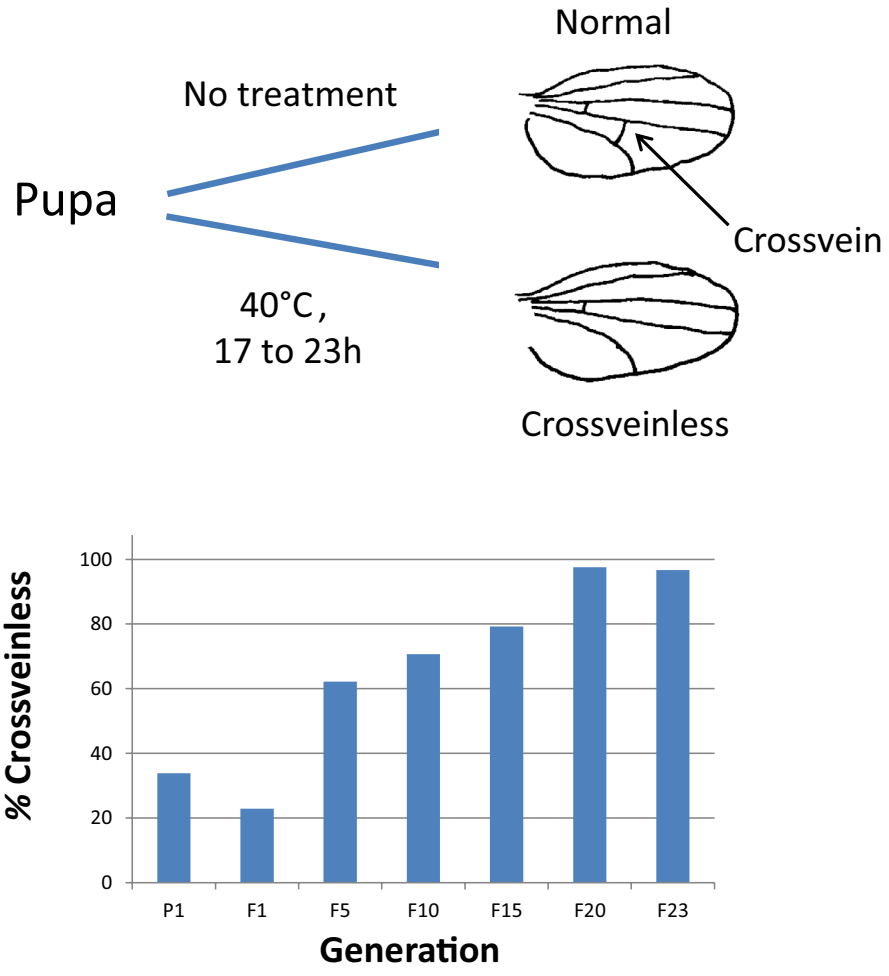

FIGURE 1 Induction of *Drosophila* crossveinless phenocopy by heat treatment (Data are quoted from Waddington, 1953)

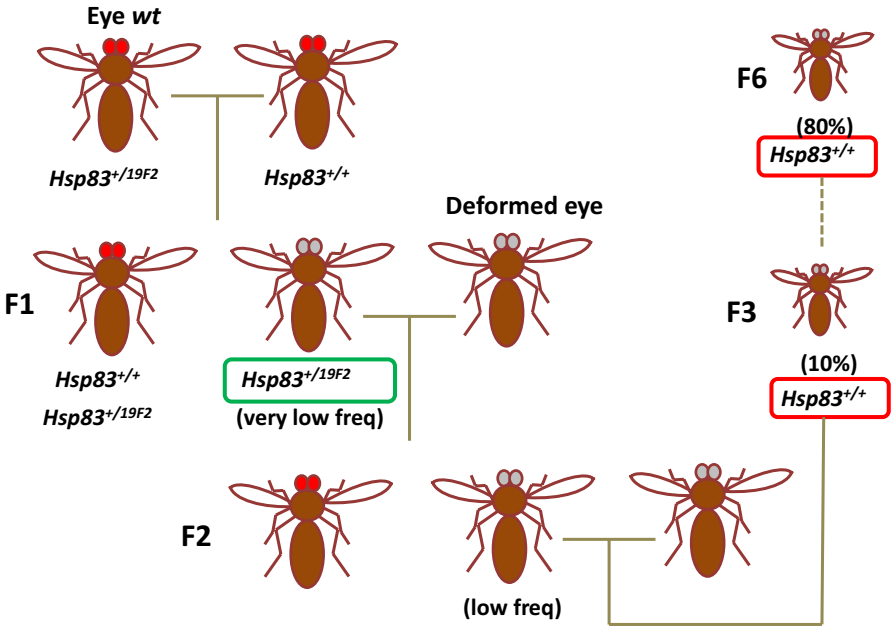

FIGURE 2 Development of abnormal eye morphology and Hsp90 mutations. Abnormal eye morphology was originally associated with Hsp90 (*Drosophila hsp83*) mutations. However, after selection and breeding, flies with deformed eyes were produced in a Hsp90 mutation-independent manner (quoted from Rutherford & Lindquist, 1998)

therefore, eliminated by natural selection. However, these mutations include some that could serve as the source for phenotypes that would be advantageous under stringent environmental conditions. Rutherford and Lindquist demonstrated that the major heat shock protein Hsp90 can function as a capacitor for morphogenetic mutations that are hidden under normal conditions, but adaptively expressed in response to environmental stresses (Rutherford & Lindquist, 1998).

Because most Hsp90 homozygous mutations are lethal, *Drosophila* Hsp90 mutations are maintained as heterozygous strain stocks. Rutherford and Lindquist found that a significant fraction (up to 5%) of these stocks, but not Hsp90 wild-type stocks, were morphologically abnormal. Hsp90 mutation-dependent malformations were detected in various tissues and organs, including wings, eyes, legs, bristles and thorax. Similar developmental abnormalities were detected in the presence of different Hsp90 mutation alleles, whereas the specific abnormalities expressed depended on the flies' genetic background. After completing extensive analyses and appropriate control experiments, they concluded that Hsp90 buffers genetic mutations that would otherwise cause morphological abnormalities (Figure 2). Curiously, introduction of two extra copies of the wild-type Hsp90 gene did not restore the abnormalities, indicating that the fixed traits ultimately became independent of the Hsp90 mutation.

Subsequently, Rutherford and Lindquist (1998) examined the heritability of the abnormal phenotypes and obtained results indicating that the traits were polygenic. Selective breeding of flies with deformed eyes showed that the frequency and severity of the abnormalities increased over the course of successive generations. Surprisingly, highly selected flies with deformed eyes had lost their original Hsp90 mutation (Figure 2).

A simple interpretation of these results is that Hsp90 is required for maintaining the cryptic state of the mutated genes, but does not silence the same genes that were already expressed. In addition, the authors proposed a threshold model in which multiple genetic determinants are required for abnormal morphologies. In this model, when the number of the determinants is increased by breeding and exceeds a hypothetical threshold, the trait no longer depends on reduced Hsp90 function. This argument is obviously dependent on the heterogeneity of fly genomes.

5 | A CASE FOR *ARABIDOPSIS THALIANA*, A SELF-FERTILIZING SPECIES

Queitsch et al. noticed that a minor portion (up to several percent) of seedlings in *Arabidopsis* accessions and inbred lines exhibited altered phenotypes specific to individual lines (Queitsch et al., 2002). Reducing Hsp90 function with Hsp90 inhibitors, such as geldanamycin (GD), yielded an array of morphological variations that were dependent on genetic backgrounds. As a result of the near homozygosity of inbred lines and accessions, this partial penetrance of altered phenotypes was unexpected. Essentially, the same phenotypes were both qualitatively and quantitatively exacerbated following treatment with GD. When plants showing abnormal morphologies without GD were selected and self-bred, their offspring exhibited higher frequencies of altered phenotypes than their parental plants in the absence of GD. Based on these results, the authors noted that genetic variants contributing to abnormal morphologies were still segregating and could be enriched by selective breeding. In addition, they

referred to stochastic processes involved in plant development that might be also buffered by Hsp90.

6 | POTENTIATION OF A MUTATION-DIRECTED PHENOTYPE BY DISTURBING CHROMATIN CONSTRUCTION

The *Drosophila* segmentation gene *Kruppel* (*Kr*) encodes Kr, a transcription factor that functions as a gap gene in the segmentation cascade of embryogenesis. The dominant gain-of-function allele $Kr^{If\text{-}1}$ causes ectopic expression of Kr in the developing eye disk and dysregulation of homeotic genes, leading to small eyes with fused ommatidia (Carrera et al., 1998). Through genetic screens, Sollars et al. (2003) identified 15 mutations in 10 maternal-effect genes that promoted ectopic outgrowth of Kr in eyes of $Kr^{If\text{-}1}$ flies. These included mutations in nine trithorax group (TrxG) genes and five mutant alleles of *hsp83,* which encodes *Drosophila* Hsp90. They observed that increased ectopic outgrowth of the Kr protein in the presence of vtd^{3}, a verthandi mutation, was maternally transmitted to progeny. Surprisingly, they also found that, although the vtd^{3} mutation was required to establish hyperectopic outgrowth in P0 flies, significant fractions of F2–F5 progeny with the vtd^{+}/vtd^{+}; $Kr^{If\text{-}1}/Kr^{+}$ genotype exhibited eye transformation, suggesting that vtd^{3} was not required for maintenance of Kr outgrowth (Figure 3; Sollars et al., 2003). The *verthandi* gene encodes Rad21, a constituent of the cohesin complex that functions in chromosome cohesion (Hallson et al., 2008). Another identified trithorax group genes as those exhibiting the similar enhancing effects, when mutated, on ectopic outgrowth included *osa* and *Trithorax-like* gene (Sollars et al., 2003). The *osa* gene encodes a transcription factor associating with the Brahma chromatin remodeling complex (Vazquez, Moore, & Kennison, 1999). The *Trithorax-like* gene encodes the GAGA factor, which has been proposed to establish a chromatin ground state for transcription (Bejarano & Busturia, 2004). Thus, these results suggested that these *trithorax* gene products could directly or indirectly suppress ectopic outgrowth of $Kr^{If\text{-}1}$. In addition, chemical inhibitors of histone deacetylases (HDACs) suppress the Kr outgrowth phenotype (Sollars et al., 2003). On the basis of these findings, they suggested that regulated remodeling and/or maintenance of chromatin structures moderately restricts ectopic outgrowth induced by $Kr^{If\text{-}1}$ and that aberrations of chromatin structures caused by mutations of some trithorax group genes promote ectopic outgrowth.

Flies with the double mutation, $Kr^{If\text{-}1}hsp83^{e3A}$, also exhibited increased ectopic outgrowth of Kr. Treatment of $Kr^{If\text{-}1}$ larvae with GD also promoted outgrowth. These results provide a clue regarding the molecular mechanism by which Hsp90 buffers some mutations, as previously demonstrated

(a)

	Genotype	Phenotype
	$Kr^{+/+}$	Normal eye
	$Kr^{If-1/+}$	Ectopic expression of Kr
P0	$Kr^{If-1/+}$, $vtd^{-/-}$	Eye transformation
F2	$Kr^{If-1/+}$, $vtd^{+/+}$	Eye transformation

(b)

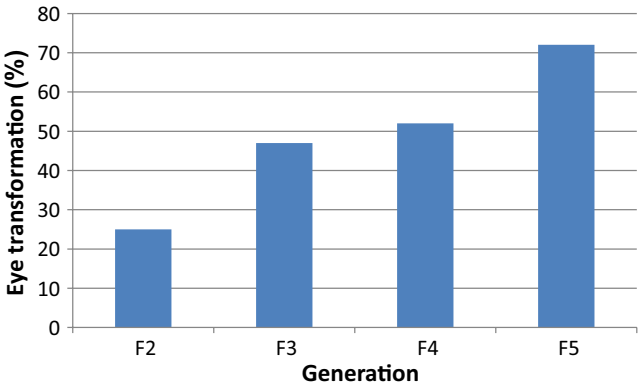

FIGURE 3 Deformed eye development due to a combination of a *Kruppel* mutation and a trithorax mutation. (a) A relationship between genotype and phenotype. (b) Selection followed by breeding increased the percentage of flies with transformed eyes (Data are quoted from Sollars et al., 2003)

by Rutherford and Lindquist (1998). A more recent study showed that Trithorax requires Hsp90 for maintenance of active chromatin at sites of gene expression (Tariq, Nussbaumer, Chen, Beisel, & Paro, 2009). On the other hand, Gangaraju et al. (2011) suggested that the *Drosophila* Piwi-interacting RNA (pi-RNA) pathway mediates Hsp90-dependent suppression of phenotypic variation. These results indicate that epigenetically altered phenotypes induced by stimuli could be inherited by offspring.

7 | THE FIRST STEP TOWARD APPEARANCE OF NEW PHENOTYPES TO BE SELECTED

As described above, in Waddington's experiments, ether treatment or heat shock gave rise to altered morphologies (Waddington, 1953, 1956). Lindquist and her associates used treatment with GD to induce unusual phenotypes (Rutherford & Lindquist, 1998, Queitsch et al., 2002). Finally, Sollars et al. (2003) found that GD treatment promoted ectopic outgrowth of Kr, which was associated with abnormal eye morphology. These experimental results suggest that stressful stimuli induce alteration of epigenetic states that lead to unmasking of hypothetical cryptic mutations (Queitsch et al., 2002) and promote expression of $Kr^{If\text{-}1}$ (Sollars et al., 2003). Waddington noted that

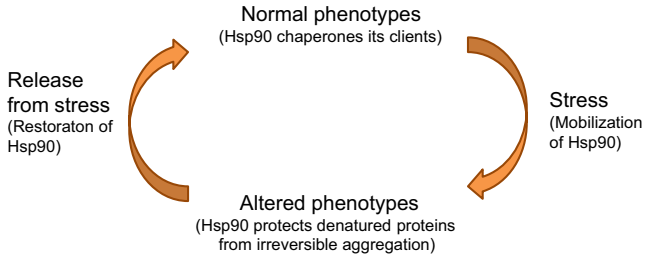

FIGURE 4 Modulation of Hsp90 function by stress

phenocopy characteristics were segregated into progeny in a polygenic manner, implying that many new mutations would be involved (Waddington, 1953). Together, these results suggest that epigenetic control of cryptic or less influential alleles, including mutations, is a critical determinant for the appearance of new phenotypes.

8 | THE MAJOR STRESS PROTEIN HSP90 AS A KEY PLAYER IN STRESS RESPONSES

As shown above, Hsp90 is likely to be a key player in stress responses whose outcomes are transgenerationally inherited from parents to progeny (Rutherford & Lindquist, 1998; Sollars et al., 2003). Both ether treatment and heat shock are stressors that might induce stress responses including mobilization of Hsp90. It is plausible that Hsp90 also played a key role in Waddington's experiments (Waddington, 1953, 1956). Thus, I will briefly summarize the involvement of Hsp90 in stress responses.

Hsp90 is indispensable for life in all eukaryotes even under nonstressful conditions (Lindquist & Craig, 1988). Although Hsp90 elicits its chaperone functions under both normal and stressful conditions, it has distinct sets of target proteins in the presence and absence of stress. Among numerous client and substrate proteins of Hsp90, those involved in signal transduction are especially functionally significant (Schopf, Biebl, & Buchner, 2017). Furthermore, Lindquist and her associates systematically analyzed Hsp90-client interactions under normal conditions and found that kinases rather than transcription factors and E3 ligases specifically interact with Hsp90 (Taipale et al., 2012).

Hsp90 clients are more or less structurally unstable proteins that are stabilized in complex with Hsp90. For instance, we previously showed that purified protein kinase II (CKII) tends to aggregate under physiological conditions, but is stable and kinase active in complex with Hsp90 (Miyata & Yahara, 1992). Hsp90 also interacts with AKT, a serine/threonine kinase, and protects it from destabilization by protein phosphatase 2A (Sato, Fijita, & Tsuruo, 2000). In human, Taipale et al. (2012) showed that in interactions with wild-type client proteins, Hsp90 does not bind particular sequence motifs in most cases, but instead associates with intrinsically unstable kinases.

It should be noted that Hsp90 usually interacts with its clients with the aid of cochaperones, such as Cdc37 (Schopf et al., 2017). Vaughan et al. (2006) co-expressed human Cdc37 and Cdk4 kinase in cultured insect cells and isolated complex containing the two human proteins and insect Hsp90. They analyzed the structure of the purified complex by single-particle electron microscopy at a resolution of ~19 Å. Comparison of their results with the crystal structure of Hsp90 obtained previously (Ali et al., 2006) indicated that the conformation of the kinase in the complex changed as the Hsp90 ATPase cycle proceeded. Recently, Verba et al. (2016) performed a 3.9 Å cryo-electron microscopic analysis of the isolated Hsp90-Cdc37-Cdk4 complex and showed how Cdc37 and Hsp90 chaperone the folding of Cdk4, enabling it to interact with cofactors such as cyclins.

As reviewed by Buchner and his associates (Schopf et al., 2017), Hsp90 interacts with the tumor suppressor p53, the tau protein and the glucocorticoid receptor in distinct modes.

9 | HSP90 AS A STRESS SENSORY MOLECULE

As described above, treatment with GD causes aberration in gene expression leading to defects in developmental processes. Heat shock is well known to mobilize pre-existing intracellular heat shock proteins (Hsps) including Hsp90 and induce synthesis of Hsps to protect cells against fatal damage (Lindquist & Craig, 1988). We found that, when incubated at high temperatures, Hsp90 acquires a novel chaperone activity toward unfolded proteins that is associated with its self-oligomerization (Yonehara, Minami, Kawata, Nagai, & Yahara, 1996). These results suggest that, at high temperatures, Hsp90 discontinues its chaperone functions involving its usual clients, including various protein kinases. Thus, morphological abnormalities induced by heat treatments during development may be the consequence of Hsp90 changing its substrate preference from its normal clients to unfolded proteins (Figure 4).

10 | HSP90 AS A KEY MODULATOR OF EPIGENETIC STATE

Csermely, Kajtar, Hollosi, Oikarinen, and Somogyi (1994) reported that Hsp90 induces condensation of chromatin. We screened random short peptides for binding to Hsp90 using

surface plasmon resonance measurements and found that peptides with histone sequences and histones themselves bound to Hsp90 with high affinity (Schnaider, Oikarinen, Ishiwarari-Hayasaka, Yahara, & Csermely, 1999). These results raised the possibility that Hsp90 might be involved in chromatin reorganization during steroid action, mitosis and cellular stresses.

Accumulating evidence indicates that Hsp90 elicits its functions in response to stresses in various ways related to transcriptional control and remodeling of chromatin states. For instance, Hsp90 and its cochaperone p23 participate in recycling of the RSC chromatin remodelers and promote reorganization of chromatin states (Echtenkamp et al., 2016). This molecular function of the Hsp90-p23 complex is similar to that of the chaperone-induced dissociation of hormone receptor complexes bound to DNA, allowing recruitment of the receptors to their hormone-binding competent states (Freeman & Yamamoto, 2002). In addition, Hsp90 and Hsp70 are involved in the removal of promoter-bound nucleosomes, making the trans-activation site of the GAL promoter available for binding of transcription factors (Floer, Bryant, & Ptashne, 2008). These results showed that Hsp90 dissociates pre-existing transcriptional regulatory complexes and reconstitutes their elements into complexes with new functions when organisms are exposed to new environments.

For some coding and noncoding *Drosophila* RNAs, RNA polymerase II pauses 25–50 bases downstream of the promoter in order to quickly respond to transcription activating stimuli (Rougvie & Lis, 1988). This RNA polymerase II stalling is mediated by the negative elongation factor complex (NELF; Yamaguchi et al., 1999). Because Hsp90 stabilizes NELF, mobilization of Hsp90 by stresses effectively activates target genes involved in responses to stresses and developmental signals (Greer et al., 2015; Sawarkar, Sievers, & Paro, 2012).

Furthermore, evidence has been obtained in *Drosophila* systems that Hsp90 and Trx, a TrxG protein, function cooperatively in maintaining the active state of chromatin (Tariq et al., 2009).

Tah1 and Pih1, Hsp90 cochaperones, are required for Hsp90-dependent tyrosine phosphorylation of various cellular and viral proteins targeted by Hsp90, including v-Src (Zhao et al., 2005). These two cochaperones, likely together with Hsp90, are stoichiometrically and functionally associated with Rvb1 and Rvb2, essential components of some chromatin remodeling complexes.

Taken together, these observations indicate that, on exposures of living organisms to new environmental conditions, Hsp90 functions as the sensory machinery for stress and serves as an effector molecule that dissociates and reconstitutes transcription-regulatory complexes, leading to new epigenetic states.

11 | CREATION OF INDIVIDUAL PHENOTYPES BY COMBINATORIAL ACTIONS OF ALLELIC AND EPIGENETIC FACTORS

I attach importance to the fact that the most epigenetic responses depend on genetic background. For instance, as mentioned by Goldschmidt and Piternick (1956), the type and frequency of heat-induced phenocopies in *Drosophila* are dependent on the genetic background. Moreover, this principle appears to hold true for other stimuli-induced phenotypes. This implies that induced phenotypes are created by combinations of epigenetic and allelic factors.

To consider the mechanism involved in creation of new phenotypes using both induced and pre-existing factors, I refer to the model discussed by Rutherford and Lindquist (1998). First, I postulate an allele or alleles, that when appropriately expressed, specify the characteristics of a new phenotype. Such alleles are considered to be the determinants for a variety of traits in *Arabidopsis* (Queitsch et al., 2002; see red circles in Figure 5). Next, I postulate ten additive determinants, either allelic or epigenetic, that affect a trait if six of them are simultaneously positive, the new phenotype develops (Figure 5a). Notably in this regard, Rutherford and Lindquist (1998) considered only allelic determinants and dealt with epigenetic determinants such as treatment with Hsp90 inhibitors as factors that lowered the threshold for new phenotypes. By contrast, in my model, I tentatively postulate three epigenetic factors that become potentially positive in response to stress and seven genetic alleles that collaboratively support the expression of the first type of determinant (red circles in Figure 5). If the total number of these positive determinants exceeds six, a new phenotype is created. When individuals are exposed to stress, two epigenetic factors become positive (Figure 5, orange circles). Although an individual harboring only two positive allelic determinants (green circles in Figure 5a) still shows a normal phenotype under stressful conditions, another individual harboring four positive determinants expresses the new phenotype (Figure 5a). It should be noted that individuals harboring more positive allelic determinants could respond to stress more sensitively and manifest the new phenotype (Figure 5b). The inequality shown in Figure 5b holds as long as stressful conditions continue. Thus, successive breeding followed by repeated selection of the new phenotype could cause accumulation of positive allelic determinants in selected individuals.

When a sufficient number of positive allelic determinants accumulated, the new phenotype might be maintained even in individuals missing positive epigenetic determinants (Figure 5c). In fact, it has been clearly shown that Hsp90 mutations and treatment with Hsp90 inhibitors are functionally equivalent in terms of the development of

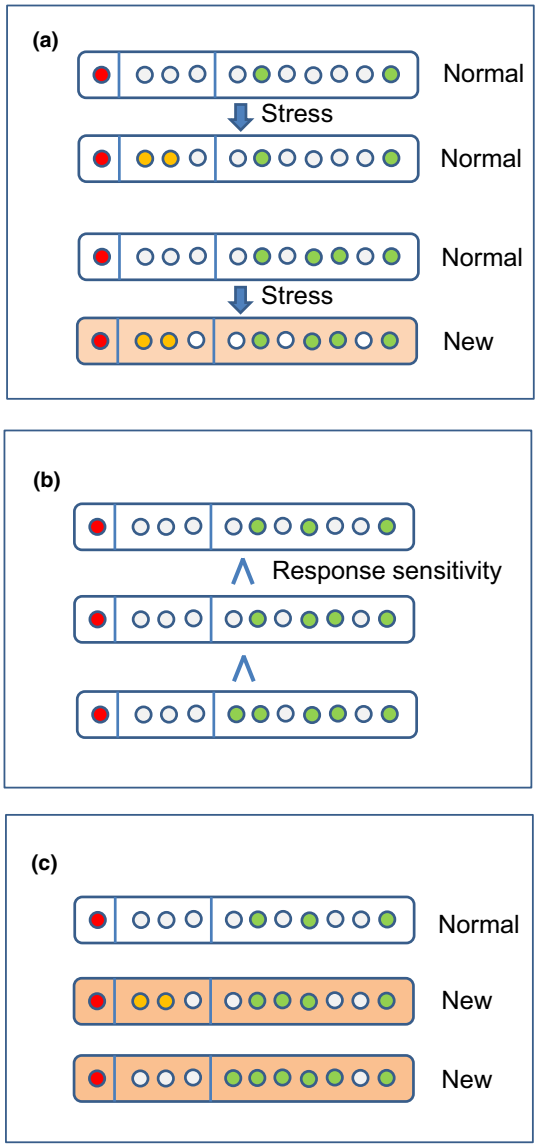

FIGURE 5 Schematic model of the combinations of alleles and epialleles that lead to phenotypes. The red circle indicates a genetic determinant that specifies the characteristics of a phenotype. In some cases, multiple such determinants may be considered. Three epigenetic and seven genetic determinants are postulated. White circles are negative determinants. Orange circles are positive epigenetic determinants. Green circles are positive genetic determinants. (a) The first row represents an individual with a normal phenotype and two positive genetic determinants. When an individual in the first row is stressed, and two orange circles are induced (second row), the phenotype is not altered. By contrast, an individual with four positive genetic determinants (third row) acquires a new phenotype when stressed (fourth row). (b) Individuals with more positive determinants tend to acquire a new phenotype more easily when stressed. For this reason, when individuals expressing a new phenotype are experimentally selected, they accumulate positive genetic determinants. (c) An individual with six positive determinants acquires a new phenotype even when unstressed (third row)

new phenotypes in *Drosophila* (Rutherford & Lindquist, 1998) and *Arabidopsis* (Queitsch et al., 2002; Samakovli, Thanou, Valmas, & Hatzopoulos, 2007). Analogously, the *Drosophila* transcription factor ATF-2 (dATF-2) mutant dATF-2PB is equivalent to inactivation of dATF-2 by stress (Seong, Li, Shimizu, Nakamura, & Ishii, 2011).

It is worth mentioning, however, that this replacement of epigenetic determinants by allelic ones is distinct from irreversible alterations of epigenetic states induced by functional defects in Hsp90 (Sollars et al., 2003), although both could positively affect transgenerational epigenetic inheritance of new phenotypic characteristics.

12 | TRANSGENERATIONAL EPIGENETIC INHERITANCE AS A MEANS OF STRENGTHENING EPIGENETIC RESPONSES

Recently, multiple studies have demonstrated that epigenetic phenotypes are transgenerationally inherited by offspring (Crossniklaus, Kelly, Ferguson-Smith, Pembrey, & Lindquist, 2013). In addition, several of these studies have shown some of the molecular mechanisms directing transgenerational inheritance. For example, Seong et al. (2011) showed that stress-induced heterochromatin disruption is passed on to the successive generations both maternally and paternally in a non-Mendelian fashion.

Another study showed that elevated resistance to harmful stresses in *Caenorhabditis elegans* induced by hormesis can be transmitted to offspring (Kishimoto, Uno, Okabe, Mono, & Nishida, 2017). In that study, epigenetic memories generated in parental somatic cells were transferred to germ cells and then to subsequent generations.

However, adaptive epigenotypes established in response to stimuli are less stable than genotypes, particularly on discontinuation of the stimuli. Thus, the significance of epigenetic transgenerational inheritance in terms of adaptive evolution remains elusive (Crossniklaus et al., 2013).

I suggested above that higher sensitivity to stress by individuals that have more positive allelic determinants gives rise to accumulation of positive determinants in individuals expressing an adaptive phenotype. For the same reason, transgenerational epigenetic inheritance of acquired characteristics could also contribute to strengthening of epigenetic responses and, thus, to accumulation of positive determinants. Seong et al. (2011) demonstrated that both stress sensitivity and transgenerational inheritance are heightened when fly embryos are exposed to stress during every generation.

13 | LIKE EPIGENETIC RESPONSES, PRIONS ALSO CREATE NEW PHENOTYPES

The *Saccharomyces cerevisiae* Sup35 is a protein normally involved in the termination of translation. When Sup35 is converted into the prion form [PSI^+], its function termination of translation is perturbed, leading to production of read-through proteins (True & Lindquist, 2000). Anomalous read-through products and Sup35 aggregates might give rise to novel characteristics in host cells (True, Berlin, & Lindquist, 2004; True & Lindquist, 2000). Moreover, conversion from [PSI^-] cells to [PSI^+] is associated with the emergence of new phenotypes, such as caffeine-resistance (True et al., 2004). Intriguingly, when caffeine-resistant [PSI^+] cells were outcrossed to another caffeine-sensitive strain, many progeny maintained caffeine-resistance even after the cells were cured of [PSI^+] by treatment with guanidine hydrochloride which dissociates protein aggregates.

Yeast cell transformation by [PSI^+] depends on the genetic background of cells (True & Lindquist, 2000). The conversion of a [PSI^+]-dependent caffeine-resistant phenotype to a [PSI^+]-independent phenotype is reminiscent of the loss of Hsp90 mutation-dependence of *Drosophila* eye abnormalities (Figure 2). On the basis of these findings, Halfmann and Lindquist (2010) referred to "the conjecture of West-Eberhard that in some cases genes may be followers rather than leaders in evolution" (West-Eberhard, 2005), that is, selectable phenotypes may be leaders in evolution. However, as I described at the beginning of this review, adaptive phenotypes, such as drought-tolerance, are based on biomolecular genetic elements that could have been naturally selected and fixed during evolution.

(Suzuki, Shimazu, and Tanaka (2012) revealed that another yeast protein, Mod5, has RNA isopentenyltransferase activity and is converted into the prion form [MOD^+] under stressful conditions. [MOD^+] cells exhibit high ergosterol levels, leading to resistance to antifungal agents. The authors suggested that prion conversion could provide a mechanism for fast on-demand adaptations in stressful environments to complement relatively slow genetic adaptations (Suzuki et al., 2012).

Like prion conversion, viral infection also induces phenotypic alterations of host cells by modulating their epigenetic states. However, the host genome is frequently disordered by interactions with the viral genome. Accordingly, I will not address viral infection in this review.

14 | COUNTER-ARGUMENTS

In this review, I described a scenario in which adaptive responses are induced on environmental alterations before acquisition of adaptive genotypes. Now, let me briefly discuss a case that seemingly conflicts with this scenario. Trinidadian guppies, *Poecilia reticulata*, have adapted to living with cichlid predators in the mainstream of the Guanapo River. However, in headwater streams, some populations derived from those in the mainstream have been separated from the predators. Ghalambor et al. (2015) transplanted Trinidadian guppies captured in the mainstream into two cichlid-free streams and monitored alterations in brain gene expression 1 year later (three or four guppy generations). Based on comparison with native cichlid-free populations, the authors concluded that the transplants had adapted to cichlid-free environments. On the other hand, when fishes from the same source were reared in tanks in the absence of the predator cue, changes in gene expression were in the opposite direction relative to those in the adapted transplants. The authors suggested that the direction of plastic response of gene expression to environmental alterations is opposite to that of evolution. An important question remains as to whether or not genome diversification was induced by the transplantation, probably followed by natural selection, within three or four generations. However, because the authors did not mention the expression of new genes or alleles, the answer to the above question is likely to be "no."

15 | CONCLUSION

In this review, I described a scenario for adaptive evolution of laboratory animals, plants and yeast that can be summarized as follows. First, environmental stresses induce new phenotypes by utilizing epigenetic adaptive systems consisting of epigenetically inducible elements and allelic ones. Second, among individuals expressing new phenotypes, those with adaptive phenotypes are experimentally selected and subjected to successive breeding. Inasmuch as individuals harboring more genetic elements that contribute to the expression of adaptive phenotypes could respond more sensitively to stresses, and those elements would consequently accumulate in adaptive offspring. It should be noted that the degree of adaptation increases as selection and breeding proceed. Third, when a sufficient number of those genetic elements have accumulated in adapted individuals, the epigenetic elements may no longer be required to maintain the adaptive phenotypes, giving rise to genetically adapted individuals.

To adequately adapt to environmental alterations, organisms must search widely for useful alleles and accumulate them in individuals, who are then subject to selection. Thus, it is critical that, in the initial steps of adaptation, selected groups should be appropriately large and genetically diverse. The scenario described above would meet these requirements. In addition to stress responses, prion activation and probably also viral infection may also contribute to evolution in nature.

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