Genome Biology

RESEARCH HIGHLIGHT

Is canalization more than just a beautiful idea?

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Abstract

The heat-shock protein 90 (Hsp90) is currently thought to buffer eukaryotic cells against perturbations caused by pre-existing cryptic genetic variation. A new study suggests that the buffering function of Hsp90 could instead be due to its repression of *de novo* transposon-mediated mutagenesis.

In the 1940s, the developmental biologist and geneticist CH Waddington coined the concept of 'developmental stability', or the robustness of the phenotype against genetic and environmental perturbations [1,2]. It has been claimed that this robustness, termed 'canalization', has evolved under natural selection to stabilize phenotypes and decrease their variability. This is achieved by buffering the expression of traits, holding them near their optimal states despite genetic and environmental perturbations. Canalization also allows the accumulation of 'cryptic genetic variation' caused by mutations that do not affect the phenotype. Canalized traits are phenotypically expressed only in particular environments or genetic backgrounds and become available for natural selection, a mechanism that can lead to the assimilation of novel traits.

It was found some years ago that reduction in the function of the Hsp90 protein in *Drosophila* (whether by mutation or by specific inhibitors) apparently uncovered previously silent genetic variation, which led to an increase in morphological variation [3]. Hsp90 is a chaperone and heat-shock protein, which in *Drosophila* is encoded by the *Hsp83* gene. The morphological changes could become fixed and stably transmitted even if wild-type Hsp90 function were restored in subsequent generations. These findings implied that functional Hsp90 is a capacitor (that accumulates cryptic genetic variation and releases it under certain circumstances) that masks the effect of hidden or pre-existing genetic variation (Figure 1).

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The Hsp90 story in flies has become very complicated, however. Recent studies have shown that the buffering by Hsp90 is limited to specific morphological traits and does not affect others. This supports the idea that numerous mechanisms are involved in developmental buffering, and that Hsp90 is just one of many capacitors for genetic variation [1,2]. In addition, Hsp90 is a very abundant protein, in some cells accounting for up to 2% of the total protein content, and a reduction in Hsp90 activity affects the expression levels of numerous genes. A new study that implicates Hsp90 in the repression of transposon-mediated mutagenesis now further complicates the story. In work recently published in *Nature*, Specchia *et al*. [4] show that biogenesis of the small PIWI-interacting RNA (piRNA) in *Drosophila* depends on the activity of Hsp90. These results are of interest not only for the insights they provide into the molecular pathways of piRNA production, but also because they imply that Hsp90 prevents phenotypic variation by suppressing *de novo* mutation caused by the activity of transposons in the germline, one of the known roles of the piRNAs in *Drosophila*. This calls for current ideas on the buffering role of Hsp90 in flies to be revisited.

piRNAs are one class of the numerous small RNAs (around 20 to 30 nucleotides long) that are expressed by eukaryotic cells and that trigger sequence-specific gene silencing called RNA silencing [5,6]. By base pairing with target mRNAs, the small RNAs guide inhibitory complexes based on members of the Argonaute class of proteins (which includes the PIWI proteins) to the mRNAs, resulting in mRNA destruction or the inhibition of translation. RNA silencing is thought to have evolved as a form of nucleic-acid-based immunity to inactivate parasitic and pathogenic invaders such as viruses and transposable elements (transposons) [7]. In *Drosophila*, the endogenous small interfering RNA (esiRNA) pathway of RNA silencing restrains the expression of transposons in somatic cells, whereas the piRNA pathway represses transposon activity in germline cells.

Transposons are generally considered as 'selfish DNA' elements usually hidden from sight. They can move around the genome, transposing into new sites and causing insertion mutations that are frequently deleterious. Thus, host genomes have evolved multiple mechanisms for regulating transposons, including RNA silencing.

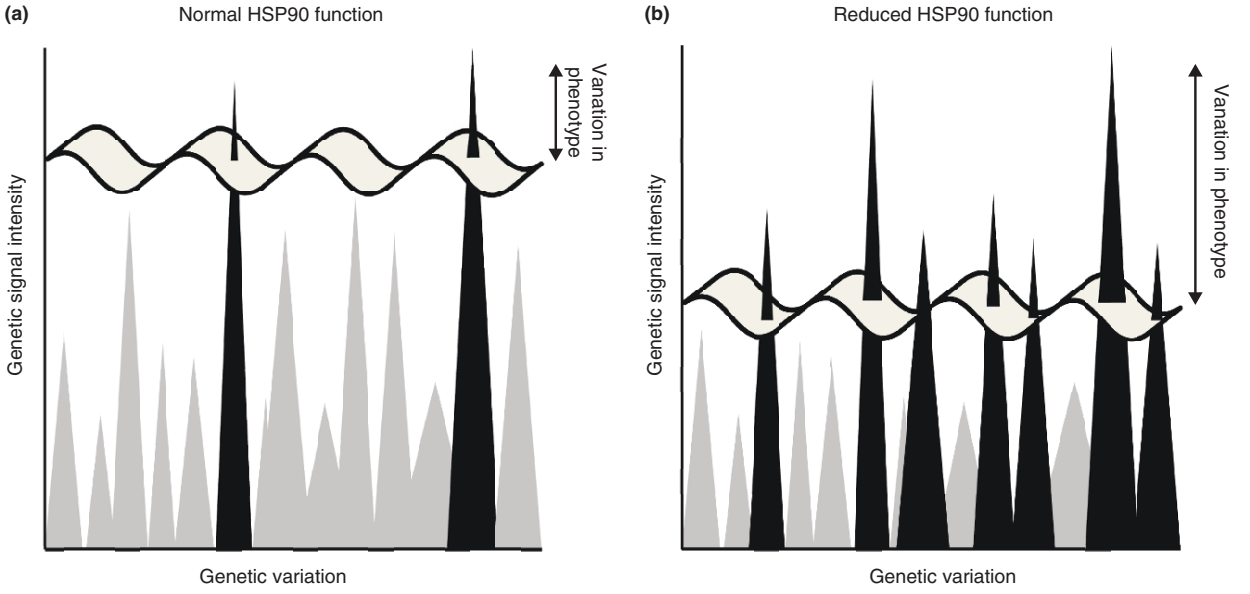

Figure 1. A model for the buffering role of Hsp90 in canalization. Hsp90 conceals cryptic genetic mutations. **(a)** When Hsp90 is normal, underlying genetic variation (gray peaks) is hidden and genetic signal inputs (black peaks) are outputted normally, resulting in a phenotype that varies within normal limits. **(b)** When Hsp90 is impaired, hidden genetic variation is revealed, resulting in altered genetic signal inputs (black peaks) that are abnormally outputted. These altered outputs may lead to an abnormal phenotypic variation.

Transposition is also potentially adaptive by occasionally providing a source of genetic diversity [8]. Thus, a transposable element is often defined as a natural, endogenous, genetic toolbox for mutagenesis. In addition, transposon defense mechanisms have recently been shown to be co-opted or borrowed to provide additional regulatory complexity for host genes [7-9].

The production of esiRNAs from their longer precursor transcripts requires the processing activity of the ribonuclease Dicer. By contrast, the production of piRNAs is independent of Dicer. *Drosophila* has three distinct PIWI proteins, AGO3, Aubergine, and Piwi, all of which exhibit the small RNA-guided ribonuclease ('Slicer') activity. Deep sequencing and bioinformatic analyses of *Drosophila* piRNAs suggest a model for piRNA biogenesis in which PIWI subfamily proteins guide the 5' end formation of piRNAs by reciprocally cleaving or slicing long sense and antisense transcripts of transposons. Thus, in this amplification loop, which is called the ping-pong cycle, transposons are both a source of piRNAs and a target of piRNA-mediated silencing. However, classification of piRNAs according to their origins indicated that piRNAs derived from a particular piRNA cluster locus are exclusively loaded onto one of the PIWI proteins, Piwi, indicating that those piRNAs are produced by a pathway independent of the ping-pong cycle. This pathway is called the primary processing pathway [5,6]. The mechanism of their production, however, has been largely unclear.

During spermatogenesis in *Drosophila* males, antisense piRNAs derived from the repetitive *Suppressor of Stellate* [*Su(Ste)*] locus on the Y chromosome silence the X-linked *Stellate* locus. In *Su(Ste)* and piRNA pathway mutants, piRNAs targeting *Stellate* are lost, causing crystals of Stellate protein to form in primary spermatocytes [6]. Specchia *et al*. [4] found that mutations in the *Hsp83* gene encoding Hsp90, or treatment with the specific Hsp90 inhibitor geldanamycin also caused the accumulation of crystalline aggregates in primary spermatocytes, suggesting that Hsp90 is involved in a piRNA-mediated mechanism that silences the expression of repetitive sequences and transposons. Consistent with this, the authors found that Hsp90 mutations result in a marked reduction in the accumulation of piRNAs corresponding to *Su(Ste)* and various transposon sequences. Conversely, the expression of various types of transposons was upregulated in both the ovaries and the testes of Hsp90 mutants. These results showed that Hsp90 represses the expression of transposons through piRNA-mediated mechanisms (Figure 2a).

Specchia *et al*. [4] examined the effect of Hsp90 mutations on transposon mobility in individual flies and found that in homozygous Hsp90 null mutants, several transposons had jumped into new sites within the genome. They further showed that approximately 1% of Hsp90 mutants screened (30 out of 3,220 flies) exhibited morphological abnormalities. Together, these findings suggested that the phenotypic variation observed among

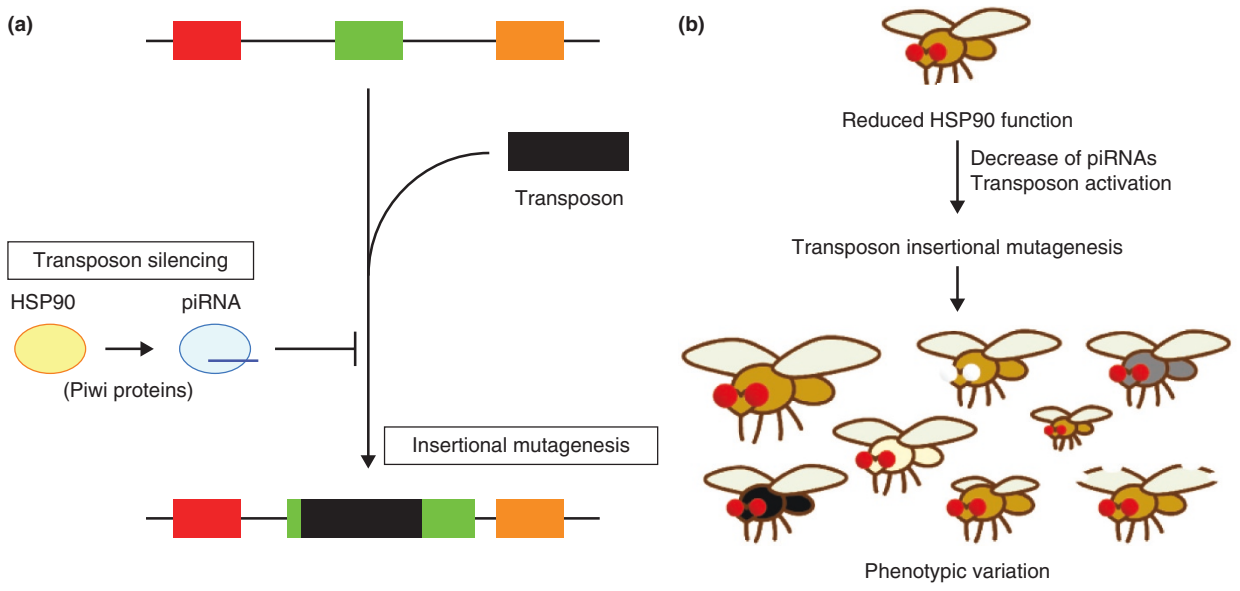

Figure 2. Hsp90 functions in the suppression of transposon-mediated 'canonical' mutagenesis via the piRNA pathway. (a) Activated transposons can move from one site in a genome to another by the process of transposition and so have the potential to induce ***de novo*** gene mutations by insertion. Hsp90 could be involved in the control of transposition by affecting piRNA biogenesis. **(b)** Reduced Hsp90 function leads to transposon-mediated insertional mutagenesis and the induction of morphological variability, such as variation in eye and body color.

Hsp90 mutants could be due to *de novo* mutations produced by activated transposable elements rather than to the buffering of pre-existing cryptic genetic variation. For example, among the abnormalities observed by Specchia *et al.* [4] among their Hsp90 mutants was a fly resembling the Scutoid phenotype (in which there is a loss of bristles from the head and thorax of the adult), which is caused by a mutation in the *noc* gene. The authors demonstrated that the coding sequence of the *noc* gene in this fly was indeed interrupted by an *I*-element-like transposon sequence. This indicates that the Scutoid phenotype found in the screen was caused by a *de novo* mutation and not by the expression of a pre-existing genetic variation (Figure 2b).

As well as suggesting that a reinterpretation of the buffering role of Hsp90 [3] might be needed, these new findings also provide evidence supporting a model in which Hsp90 is involved in the control of transposon activity in germ cells by affecting piRNA biogenesis. piRNAs in *Drosophila* are produced almost exclusively in germ cells from intergenic repetitive genes, transposable elements and piRNA clusters by two pathways: the primary processing pathway, and the amplification 'ping-pong' loop [5,6]. Mature piRNAs are loaded onto the PIWI subfamily of Argonaute proteins, and the amplification loop is known to be independent of Dicer but dependent on the Slicer activity of PIWI proteins. However, the mechanisms of primary piRNA processing remain elusive. How does Hsp90 function in piRNA biogenesis and which of the two piRNA production pathways is it involved in? Hsp90 can, for example, be co-purified with the Slicer activity of Ago2, one of the mammalian Argonaute proteins [10].

Hsp90 could play a role in the biogenesis of small silencing RNAs either as a chaperone for the correct folding of the Argonaute proteins or by providing an assembly platform for components of the small RNA biogenetic machinery to promote the loading of small RNAs onto the Argonaute proteins. It will be important to ascertain whether Hsp90 interacts with the PIWI proteins in flies and has a role in their function, such as ensuring their correct cellular localization, and also whether mutations in Hsp90 affect either or both of the two piRNA biogenesis pathways. It will also be interesting to examine whether Hsp90 is required for the esiRNA pathway that silences transposable elements in somatic cells. Further investigation should reveal the role of Hsp90 in RNA silencing and help expand our understanding of transposon regulation by RNA-silencing pathways.

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