

Hypotheses

Hsp90 and Chromatin

Where is the Link?

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We biologists have become adept at deciphering the function and regulation of individual proteins and have generated vast new stores of data through genome sequencing and analyses of entire “transcriptomes” and “proteomes”. Yet we still understand little about fundamental questions such as how genotypes translate into complex phenotypes or how genomes respond to selective pressure over evolutionary time. Integrating the knowledge accumulated in disparate fields to achieve broader levels of understanding is currently our greatest challenge.

At first glance, the recent report by Sollars et al.¹ demonstrating epigenetic inheritance of a particular mutant phenotype in *Drosophila* appears to describe an odd and idiosyncratic phenomenon. Closer reading reveals it may have extensive implications: the data provide tantalizing suggestions for the integration of two biological processes—chaperone-mediated protein folding and heritable chromatin states. Such a link has been the subject of much recent speculation and scientific discussion.²

The authors utilized a *Drosophila melanogaster* stock carrying a dominant gain-of-function mutation of *Krüppel* (*Kr^{lf-1}*). This gene encodes a zinc-finger transcription factor normally required for abdominal segment patterning; the mutation causes the protein to be ectopically expressed in the eye, causing an irregular pattern of the eye facets. A genetic screen for mutations affecting the trait yielded ten different mutations that cause abnormal ectopic outgrowth of tissue near the eye, including the production of extra bristles. Mutations at all ten loci isolated affected outgrowth only when inherited from the mother. The majority of these loci were previously included in the trithorax group, members of which form regulatory complexes that help to maintain chromatin in an active state.³ Remarkably, the same ectopic outgrowth effect was observed with five alleles of Hsp90 (Hsp83 in flies), a molecular chaperone best known for maintaining metastable proteins such as signal transducers in an inactive but activatable state.⁴

The highest frequency of ectopic outgrowth was produced by an allele of the locus *verthandi*, *vtd^β*, which has been assigned to the trithorax group solely on the basis of its mutant phenotype. Strikingly, once the eye outgrowth phenotype was established in the *Kr^{lf-1}* background, *vtd^β* was not required to maintain it in future generations. Indeed, in the absence of *vtd^β*, selection could increase the penetrance of this phenotype from 25% to 74% in just four generations. As the authors note, this increase in penetrance might reflect the selection of genetic variation present in the initial stock. However, ectopic outgrowth was also generated in a highly inbred line of *Kr^{lf-1}* flies by a specific inhibitor of Hsp90. Even in this nearly isogenic line, once the trait was established, selection could increase its penetrance in the absence of further Hsp90 inhibition. Here, increased penetrance was most likely due to an epigenetic effect on chromatin structure that could be caused by changes in the function of either Hsp90 or trithorax-group genes.

Indeed, there is mounting evidence that both Hsp90 and chromatin remodeling factors may be directly involved in transcriptional regulation. Freeman and Yamamoto⁵ demonstrated that Hsp90 and its co-chaperone p23 promote disassembly of receptor-mediated transcriptional regulatory complexes. This mechanism allows fine-tuning of the transcriptional response to changes in hormone levels. However, the same phenomenon has recently been attributed to the action of the SWI/SNF complex of chromatin remodeling proteins.⁶ As both studies present convincing data, we suggest that both chaperones and chromatin remodeling complexes engage in disassembly of receptor complexes. These observations may be related to those of Sollars and colleagues. In addition to Hsp90 and chromatin remodeling factors of the SWI/SNF family, mutant alleles of transcriptional mediators and the fibroblast growth factor receptor increased the frequency of ectopic outgrowth.

Notably, the outcome of selection by Sollars et al. on this particular Hsp90-influenced trait differs from previous observations with flies in our laboratory.⁷ Rutherford and Lindquist discovered a plethora of other morphological traits which appear under Hsp90

inhibition. Some traits were clearly specific to the genetic background, suggesting that previously hidden genetic variation was responsible. Indeed, this was demonstrated for the two traits investigated in detail; both could be enriched in a population through selection on underlying genetic variation. Similar to the results of Sollars et al., selection over several generations resulted in near fixation of an eye and a wing phenotype and, after many rounds of selection, expression of the traits did not require a continued reduction in Hsp90. However, both traits were as readily inherited through males as through females, in contrast with the data of Sollars et al. Furthermore, Rutherford and Lindquist conducted the litmus test for the genetic basis of the traits: outcrossing the selected flies exhibiting high trait penetrance to unselected lines heterozygous for the Hsp90 mutation, thereby halving the enriched predisposing genetic polymorphisms. Flies receiving two wild-type Hsp90 alleles did not display the trait under physiological conditions, whereas a significant number of their heterozygous siblings with reduced Hsp90 function exhibited the phenotype. That is, continued expression of the trait when Hsp90 function was normal required the enrichment of underlying genetic variation. Should the trait have been epigenetically inherited, it would not have disappeared in outcross progeny with wild-type Hsp90 levels.

Rutherford and Lindquist did not propose that all Hsp90-dependent phenotypes require hidden genetic variation, only that many likely do and that the two studied in detail surely did. It must also be noted that the phenotype examined by Sollars et al. does not have a purely epigenetic origin, as it depends on the presence of the predisposing mutant *krüppel* allele. Studies with *Arabidopsis* in the Lindquist lab also revealed a wide variety of traits when Hsp90 was reduced. Some of these were due to genetic variation, but others were non-propagatable in isogenic lines, indicating that Hsp90 buffers normal development from the destabilizing effects of stochastic processes.⁸ Sollars et al. now add epigenetic inheritance to the known panoply of Hsp90-dependent phenomena, previously including altered developmental stability and buffered genetic variation. Given the remarkable variety of traits produced upon the reduction of Hsp90 in both *Drosophila* and *Arabidopsis*, it is of great interest to determine the relative contribution of epigenetic versus genetic mechanisms, and, indeed, the capacity of the two to work in concert.

Here we hypothesize a mechanism to explain the epigenetic inheritance of the ectopic outgrowth phenotype reported by Sollars et al. and the as yet undefined link between Hsp90 and trithorax-group proteins, drawing together several different lines of investigation from a variety of sources. First, the absence of the locus *verthandi* results in 55% ectopic outgrowth in the F1 generation of flies containing the *K^zJ^{F-1}* mutation, while the Hsp90 alleles and trithorax-group proteins produce a significantly smaller penetrance (5–15%). So how might mutations at *vtid* affect phenotype so dramatically and in a heritable manner? One clue might be the unusual nature of this locus. As deletion alleles of *vtid* dominantly suppress *polycomb* mutations, *vtid* was originally defined as a trithorax gene.⁹ Trithorax and polycomb genes maintain patterns of Hox developmental gene expression, with trithorax-group proteins generally functioning as activators of transcription and polycomb-group proteins generally functioning as repressors.³ The *vtid* locus has been cytogenetically mapped near to the centromeric heterochromatin of the left arm of chromosome.³ Our examination of the *Drosophila* genome^{10,11} failed to yield any annotated genes in the minimal *vtid* region. Furthermore, translated BLAST¹² and gene prediction¹³ searches did not reveal any putative genes; we found only one complete

non-LTR retrotransposon and fragments of several others, indicating that *vtid* is likely not a protein-coding gene.

Immediately adjacent to the *vtid* locus is *α-catenin* gene, which is involved in cell adhesion during eye development.¹⁴ *α-catenin* interacts with *β-catenin*,^{14,15} a central component of the *Wingless* developmental signaling pathway. Further, the various functions of *β-catenin* are in a tightly regulated equilibrium; binding of *β-catenin* to adhesion complexes has been shown to antagonize its signaling ability.^{16–18}

It is tempting to speculate that deletion of the *vtid* locus results in the spread of the nearby heterochromatin and subsequent heritable silencing of *α-catenin* and possibly other loci. The ectopic outgrowth phenotype then results from disruption of the *β-catenin* equilibrium. The *krüppel* mutation may contribute by destabilizing *wingless* signaling domain regulation, which is demonstrated to be altered in abnormal flies by Sollars et al. Importantly, as predicted by this hypothesis, excess *wingless* signaling in the eye results in strikingly similar phenotypes to the ectopic outgrowth.¹⁹

Other non-coding elements which stably and heritably influence chromatin structure, such as *Fab7*, *Mcp*, and *bxd*, have been identified in *Drosophila*.^{20,21} These elements have been shown to recruit trithorax and polycomb group proteins, presumably supporting both transcriptionally silenced and activated states of regulated homeotic genes.^{3,20} Our speculation that *vtid* may include such a cellular memory module²² accounts for all of the data presented by Sollars et al. if one invokes that heterochromatic spread due to loss of one *vtid* element may be transmitted to an intact homolog by a trans-silencing mechanism, potentially aided by the proximity of this locus to the centromere.²³ Such a phenomenon would be similar to the transvective, dominant *trans*-silencing of both copies of the brown gene when one is rearranged to be near a heterochromatic region.²⁴

If ectopic outgrowth is caused by expansion of the heterochromatin, it is not surprising that the phenotype is produced by deficiencies in trithorax proteins, including SWI/SNF homologs that promote an activated chromatin state at their target genes. Likewise, pharmacological means of generally activating chromatin would be expected to suppress the aberrant phenotype, as observed by Sollars and colleagues when they fed histone deacetylase inhibitors to fly lines expressing ectopic outgrowth.

In this scenario, what is the function of Hsp90? SWI/SNF proteins function in large multi-protein complexes which may require the assistance of a chaperone such as Hsp90 for assembly or regulation. In this case, reduction in activity of either chaperone or chromatin remodeling complexes would result in the phenomenon observed by Sollars et al. Alternatively, as both SWI/SNF proteins and Hsp90 are likely involved in regulating transcription through the disassembly of hormone-dependent transcriptional complexes, a reduction in activity of either may show aberrant epigenetic effects at specific loci.

The data of Sollars and colleagues suggest an interplay between both genetic and epigenetic factors in the genesis of chromatin-factor and Hsp90-dependent phenotypes. The possible evolutionary impact of such epigenetic phenomena will depend primarily on their stability and secondarily on factors such as frequency of incidence, genetic factors promoting stabilization, selection strength, and gene flow within the population. As epigenetic phenomena are often unstable, they are more likely to contribute directly to the genesis of phenotypic plasticity rather than to complete fixation of a novel trait. Should the trait prove advantageous, fixation may eventually occur due to selection for genetic stabilization of the trait.

Thus, the report by Sollars et al. adds yet another unexpected facet to our understanding of the multitude of ways in which phenotypic variation is created. The results of this study, combined with those of others,^{7,8,25} greatly expand the newly emerging concept that protein folding may dramatically influence the translation of genotype into phenotype.

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