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From fly development to evolution

A major conundrum in evolutionary biology, about as old as the discipline itself, has been the question of how major changes in body form can come about. Darwin himself insisted on a strict gradualism in all evolutionary change. Even the radical reorganization of body plans was assumed by Darwin to proceed through many small, graded steps. This view, still widely held, carries with it a number of problems, not the least of which is the glaring absence from the fossil record of the necessary continuously varying intermediate forms in evolutionary lineages. Morphological evolution appears to have proceeded fitfully, with long periods of evolutionary stasis broken by short episodes of rapid change and diversification. The rapidity of evolution during these episodes poses other problems for a strictly gradualistic interpretation: Can natural selection, acting incrementally on small, continuous variations, bring about rapid change, and do natural populations harbour enough genetic variation to support very rapid evolution?

A further problem relates to the nature of developmental processes and their underlying genetic architecture. While morphological evolution requires a certain plasticity in development, embryogenesis in most animals is remarkably stable. The homeostatic properties of developing systems, while buffering them against the vagaries of environmental fluctuations and genetic variation, would appear to impose severe constraints on the potential for evolution. Furthermore, the very complexity and sophistication of the genetic and regulatory networks associated with development in highly evolved systems (including even "simple" organisms with long evolutionary histories), greatly limit the scope for fruitful tinkering with development. How then, given the contradictory requirements for developmental stability in ontogeny and developmental flexibility in phylogeny, do large-scale morphological changes not only occur, but (apparently) come about quickly?

An exciting paper by Rutherford and Lindquist (1998) about the relation between the stress protein Hsp90 and developmental stability opens a new window on this issue, and suggests a possible cellular mechanism which may link up these problems. Most tantalisingly, this mechanism would operate under conditions of stress – destabilizing conditions such as may have called forth rapid evolutionary change, thereby linking development to environmental conditions.

Rutherford and Lindquist (1998) build on earlier results from a number of laboratories that implicate members of the heat shock protein Hsp90 family in a number of signalling pathways. Hsp90 exerts its effects by stabilizing inherently unstable components of these pathways and keeps them poised for activation in response to appropriate signals. Many of these signalling proteins are protein kinases acting in familiar signal transduction cascades. Proceeding from the hypothesis that some elements of the cellular protein folding and stabilization machinery might play a role in regulating signalling pathways (Rutherford and Zuker 1994), Rutherford and Lindquist (1998) undertook an analysis of the effects of altered levels of Hsp90 activity on development in the fruit fly *Drosophila melanogaster*. The rather dramatic results of this analysis are briefly discussed here and some of their possible implications explored.

Although Hsp90 does not appear to be ubiquitously involved in folding and assembly of nascent polypeptides or in the maintenance of protein structure, it is required for the normal functioning of a number of specific proteins. *Drosophila* Hsp90 is an essential protein, since homozygous mutants of *Hsp83* (the *Drosophila* gene coding for Hsp90) die during development. *Hsp83* heterozygotes – flies with one mutant and one wild type copy of *Hsp83* – are viable and most show no morphological abnormalities. However, some 1–5% of such individuals do show developmental aberrations affecting

a variety of morphological traits (such as deformed eyes and altered wing venation). Similar abnormalities were also obtained when *Drosophila* lines recently established from wild populations, or wild flies collected from the field, were made heterozygous for an *Hsp83* mutation. These effects were unambiguously due to lowered levels of Hsp90, and could be obtained not only in mutant stocks, but also in wild type stocks fed an inhibitor of Hsp90. The nature of the abnormalities depended on the genetic background. When *Hsp83* mutants were crossed with a particular stock, several of the progeny frequently showed similar defects, distinct from those obtained when they were crossed with other stocks. Further genetic analysis established that there were multiple genetic factors which predisposed different *Drosophila* stocks to different developmental defects when Hsp90 function was compromised. Affected flies could be bred for these defects: when lines established from flies with a particular kind of defect (thickened wing veins or deformed eyes) were subjected to selection for these traits, they showed an increase in the proportion of affected flies and the severity of the defects. Remarkably, after selection over as few as four generations, the traits became independent of the *Hsp83* mutation.

The results of Rutherford and Lindquist are strongly reminiscent of the decades-old work of Waddington and others on canalization and genetic assimilation (e.g., Waddington 1942). Waddington introduced the concept of canalization for the homeostatic properties of development. He asserted that under conditions normally encountered, homeostatic mechanisms would buffer (and so confine to a "canal" or "channel") development against deviations due to environmental fluctuations and genetic variation. However, conditions of stress, such as elevated temperatures, could disturb canalization and lead to abnormal outcomes. An abnormality could be selected for and would in time become genetically fixed; it would appear even in the absence of stress. In Waddington's language, the abnormal phenotype would become 'assimilated'. He interpreted genetic assimilation as being the result of selection for combinations of regulatory genes which were already present in the parent population.

The Hsp90 work sits very well with the older work on canalization and genetic assimilation, and provides a plausible cellular and molecular explanation for these phenomena. Laboratory as well as wild populations of *Drosophila* would appear to harbour genetic variation at a number of loci required for normal development. Under conditions in which Hsp90 function is normal, development would be buffered against the effects of variation in genes coding for members of Hsp90-dependent pathways, such as the sevenless signalling pathway (Cutforth and Rubin 1994). However, when levels of Hsp90 are decreased, development can go awry in individuals carrying combinations of variant alleles of certain genes. Because of the decrease in activity of the Hsp90-stabilized component/s of the pathway, the strength of the signal from a pathway can drop below the threshold required for normal development. The system might also generate excessively high outputs if Hsp90 is required for stabilization of some negative regulatory component of the pathway. Further selection, in which only affected individuals are used for breeding in each generation, leads to enrichment for the developmentally unstable variant alleles. If the selection is carried far enough – a mere four generations was sufficient in Rutherford and Lindquist's study – the cumulative effects of the combinations of variant alleles become strong enough to be manifested even when Hsp90 function is normal.

The results of Rutherford and Lindquist suggest a number of lines of experimentation. Immediately obvious experiments pertain to fleshing out the character of Hsp90 in this novel incarnation. The mechanisms by which Hsp90 facilitates the action of its various targets are still far from being well understood. Details of what features of its target proteins are recognized by Hsp90, the nature of contacts it makes with them, the conformational changes they undergo upon association and dissociation from Hsp90, and how these changes are related to their signalling roles, will help to clarify just what Hsp90 does.

Related to the search for Hsp90 targets and Hsp90-dependent signalling pathways is the question of just what kinds of processes might require Hsp90 function. Can Hsp90 act to regulate the effects of genetic variation only in developmental processes? There appears to be no a priori reason why this should be so. Considering the ancient origin of the machinery for the folding and maintenance of protein structure, it may well be that Hsp90 and other chaperone-like proteins have also been exploited for the regulation of metabolic processes. The known targets of Hsp90 regulation are not entirely restricted to elements of complex signalling pathways, but include members of the steroid hormone

receptor superfamily, which act in a relatively straightforward manner as transcription factors, as well as proteins not known to act in intracellular signalling pathways, such as the chloride channel CFTR and the enzyme nitric oxide synthase (Mayer and Bukau 1999). Furthermore, one of the kinases whose activity shows sensitivity to Hsp90 is the WEE1 protein, a regulator of cell division (Aligue *et al* 1994). Thus there is already evidence that signalling in processes which are not, strictly speaking, concerned with development, can be Hsp90-dependent. Hsp90 could, therefore, through its buffering action under "normal" conditions, mask genetic variation in such pathways as well. Though altered traits in features such as degradative and biosynthetic capacities may appear less dramatic than the morphological variations seen with decreased Hsp90 activity, the evolutionary significance of a "capacitor" for genetic variation affecting metabolic properties would be no less.

The central importance of the Rutherford and Lindquist finding is its implication for evolution. By masking genetic variation that would not be neutral when unmasked, and by making such variation visible to natural selection under stress conditions, Hsp90 could provide a mechanism for "storing" genetic variation. Environmental stress would make this variation available precisely under conditions in which selection has scope to act.

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Neural complexity underlying simple behaviour

A central problem in neurobiology has to do with how patterns of electrical activity convey meaningful information. That neurons and neuronal circuits of varying complexity determine behaviour is no longer in doubt. But identifying neural "networks" that control specific behaviour and the rules that these networks use to initiate and modulate appropriate responses remain difficult questions. Simple invertebrates exhibit a range of well studied behaviours and in many instances, all or most of the participating neurons have been identified. The knowledge gained from such studies can be applied to higher animals, including primates. We describe here some recent studies in invertebrate model systems that demonstrate the utility of the approach.

Spatial coding using neuronal firing rates ("Where is the neuron that fires best") has been well studied in several areas of the brain such as the visuo-motor system and the somatosensory system.