HSP90 inhibition causes heterochronies in the skull ossification sequences of Pleurodeles waltl (Urodela). Evolutionary implications.

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Heat-shock protein 90 (HSP90) is an ubiquitous chaperone in eukaryotes that facilitates folding of proteins both in normal conditions and in response to environmental stress. In addition, HSP90 is able to buffer genetic variation resulting from random mutations by regulating intracellular protein degradation mechanisms.
It has been shown that experimental HSP90 inhibition produces anatomical variability during embryonic development in *Arabidopsis*, *Drosophila* and zebrafish. It is not clear whether these anatomical changes are caused by the expression of genomic cryptic mutations or by modifications of HSP90 dependent molecular pathways. However, several results suggest that HSP90 modulation is a relevant mechanism underlying the *de novo* appearance of character states.
In order to test whether HSP90 is involved in the generation of apomorphic phenotypes in tetrapods, we pharmacologically inhibited HSP90 in *Pleurodeles waltl* (Urodela) embryos (n=141), by exposition to 10 µM Radicicol during the first 24 hours of development. Control specimens (n=20) received no treatment. The 54 surviving larvae were euthanatized at stages 46 (n=25) and 50 (n=29), and subjected to whole-mount double staining with alcian blue and alizarin red.
All the control and 7 (13%) of the treated specimens were phenotypically normal. The remaining 47 (87%) larvae showed 20 abnormal phenotypes best described as heterochronic skull ossification sequences. Interestingly, these heterochronies fit the ossification sequences described in other urodele families. We propose that modulation of HSP90 function during the embryonic development, caused naturally by environmental stress, may underlay the evolutive modifications of skull ossification sequences in urodeles. New studies on the inheritance of the phenotypes obtained in untreated embryos from treated parents may confirm the plausibility of this hypothesis.

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