The Echinoid *hbox12* Multigene Family: Copy Number Variation and Spatiotemporal Expression

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Evolution of individual members of multigene families can result from changes in their *cis*-regulatory landscapes, leading to differences in spatiotemporal expression and function. The archetypal member of the echinoid *hbox12* family is an homeobox-containing gene expressed exclusively by dorsal blastomeres, where it governs the dorsal-ventral gene regulatory network during embryogenesis of the sea urchin *Paracentrotus lividus*. The coding sequence of *hbox12* is highly similar to that of the *pmar1/micro1* genes, which in other sea urchin species are expressed solely by micromeres, where they drive their specification. The apparently different functions across species, despite the broad sequence similarity, suggest that *hbox12* and *pmar1/micro1* are paralogs whose *cis*-regulatory sequences diverged following multiple rounds of duplication from a common ancestor.

We describe the inventory of the *hbox12* genes in *P. lividus*, highlighting that gene copy number variation occurs across individual sea urchins. We also show that the distinct members of the *hbox12* family exhibit substantial differences of spatiotemporal expression. While some genes were broadly expressed throughout development, others were transiently expressed in either animal blastomeres or micromeres of the early embryo. Similar to *pmar1/micro1*, in the latter case transcription relied on the Wnt/β-catenin signaling, despite the absence of conservation in their *cis*-regulatory regions.