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Evolutionary loss of melanogenesis in the tunicate *Molgula occulta*

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Abstract

Background: Analyzing close species with diverse developmental modes is instrumental for investigating the evolutionary significance of physiological, anatomical and behavioral features at a molecular level. Many examples of trait loss are known in metazoan populations living in dark environments. Tunicates are the closest living relatives of vertebrates and typically present a lifecycle with distinct motile larval and sessile adult stages. The nervous system of the motile larva contains melanized cells associated with geotactic and light-sensing organs. It has been suggested that these are homologous to vertebrate neural crest-derived melanocytes. Probably due to ecological adaptation to distinct habitats, several species of tunicates in the Molgulidae family have tailless (anural) larvae that fail to develop sensory organ-associated melanocytes. Here we studied the evolution of *Tyrosinase* family genes, indispensible for melanogenesis, in the anural, unpigmented *Molgula occulta* and in the tailed, pigmented *Molgula oculata* by using phylogenetic, developmental and molecular approaches.

Results: We performed an evolutionary reconstruction of the tunicate *Tyrosinase* gene family: in particular, we found that *M. oculata* possesses genes predicted to encode one Tyrosinase (Tyr) and three Tyrosinase-related proteins (Tyrps) while *M. occulta* has only *Tyr* and *Tyrp.a* pseudogenes that are not likely to encode functional proteins. Analysis of *Tyr* sequences from various *M. occulta* individuals indicates that different alleles independently acquired frameshifting short indels and/or larger mobile genetic element insertions, resulting in pseudogenization of the *Tyr* locus. In *M. oculata, Tyr* is expressed in presumptive pigment cell precursors as in the model tunicate *Ciona robusta*. Furthermore, a *M. oculata Tyr* reporter gene construct was active in the pigment cell precursors of *C. robusta* embryos, hinting at conservation of the regulatory network underlying *Tyr* expression in tunicates. In contrast, we did not observe any expression of the *Tyr* pseudogene in *M. occulta* embryos. Similarly, *M. occulta Tyr* allele expression was not rescued in pigmented interspecific *M. occulta* × *M. oculata* hybrid embryos, suggesting deleterious mutations also to its *cis*-regulatory sequences. However, in situ hybridization for transcripts from the *M. occulta Tyrp.a* pseudogene revealed its expression in vestigial pigment cell precursors in this species.

Conclusions: We reveal a complex evolutionary history of the melanogenesis pathway in tunicates, characterized by distinct gene duplication and loss events. Our expression and molecular data support a tight correlation between pseudogenization of *Tyrosinase* family members and the absence of pigmentation in the immotile larvae of *M. occulta*. These results suggest that relaxation of purifying selection has resulted in the loss of sensory organ-associated melanocytes and core genes in the melanogenesis biosynthetic pathway in *M. occulta*.

Keywords: Pigmentation, Tyrosinase evolution, Transposable elements, Pseudogenes, Phylogeny

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