

The amphioxus genome in Evo-Devo: archetype or “cul de sac”?

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ABSTRACT The new discipline of “Evo-Devo” is facing the fascinating paradox of explaining morphological evolution using similar pieces or genes to build highly divergent animals. The cephalochordate amphioxus has the privilege situation of being the closest living relative to vertebrates, retaining a vertebrate-like simple body plan, and a preduplicative genome. We report two examples showing that the amphioxus genome may well be archetypal, but has been evolving since the divergence from the vertebrate lineage. Firstly, the amphioxus Hox cluster has at least 14 genes, and illustrates the phenomenon of “posterior flexibility”, or a lesser constraint of the Hox posterior genes to evolve. Secondly, an ancestral *Evx* gene was tandemly duplicated in the amphioxus genome: one of the copies (*amphiEvx-A*) has retained the chordate-specific tasks of Chordate *Evx*, while a fast evolving copy (*amphiEvx-B*) is not longer involved in archetypal tasks. Our results indicate that the amphioxus genome has particularities and oddities that remind: amphioxus is not the ancestor of the vertebrates, but its fortunate position as the closest living relative to the ancestor give amphioxus genes the privilege to serve as key landmark to understand morphological evolution.

The Evo-Devo paradox

The end of the 20th century welcomed the birth of a new discipline in Life Sciences. The initial name of “Developmental Synthesis of Evolution” shifted to “Evolution and Development” and finally to “Evo-Devo”. The rationale of Evo-Devo is: if evolution is change in morphology, as morphology depends on embryonic development, and development depends on developmental genes and gene networks, understanding how developmental genes evolve is the crux to understand evolution. The initial findings in Evo-Devo research were astonishing: similar genes performed remarkably similar roles in flies and mice. This reflects the conservation side of Evo-Devo. The current view is that most genes and developmental gene networks or gene kits are conserved among most animal phyla, and play similar roles in similar developmental processes. Most probably, all Eubilaterians had a common ancestor that already used a basic set of developmental gene kits that remained unchanged for hundreds of million years, and thus, developmental mechanisms are claiming for universality. Conservation and uniformity have lead to the tremendous, but exciting paradox of Evo-Devo: how can one evolve with conserved pieces (genes), similar kits (gene networks), and unvarying mechanisms, if evolution is basically change? Several mechanisms may explain the paradox: changes in cis-regulatory regions, changes in protein functions, changes in the connections between members of a

given gene network, or establishment of new connections between previously unconnected networks. However, another mechanism that actually is able to cope with evolution (innovation) without compromising previous roles is gene duplication, co-option and evolution of both, coding sequences and cis-regulatory regions.

Amphioxus, the origin of vertebrates, and the never-frozen genome

Amphioxus (*Branchiostoma*, Cephalochordata) is the extant sister group of vertebrates. It retains many features of the simpler vertebrate-body plan without showing major complexities, and is close to what one would expect for the ancestor of the vertebrates to be (Gee, 1996). The invertebrate/vertebrate transition was linked to events of gene duplication, most probably by two rounds of full genome polyploidization (Holland *et al.*, 1994). In particular, the finding that amphioxus had a single, archetypal vertebrate-like Hox cluster (Garcia-Fernández and Holland, 1994) compared to the four clusters of mammals, impeded the view that amphioxus has a sort of “frozen” genome and morphology, that have been totally unchanged since the divergence of Cephalochordates from the vertebrate lineage. Since then, amphioxus-one/vertebrate-more is being alleged for i) tracing the evolution of a given gene family and, ii) deducing the chordate ancestral function of such family. However, amphioxus on itself has been evolving from the common ancestor of cephalochordates and vertebrates as much as vertebrates have. Although evolutionary time seems not to have involved much morphological evolution in amphioxus (as its morphology has been basically unchanged), surely time must be detected at the genetic level, as sequence sequence divergence and oddities. We have found two of these examples where the amphioxus genome shows that it may be an important landmark in evolution, but is not frozen.

The end of the amphioxus Hox cluster: posterior flexibility and Hox 14

We undertook a chromosomal walking from the “posterior” end of the amphioxus Hox cluster, and found 4 new genes, that we called *AmphiHox-11*, *-12*, *-13* and *-14* (Ferrier *et al.*, 2000). Molecular phylogenetic analyses did not resolve whether the amphioxus posterior genes are orthologous to vertebrate posterior genes, or arose after independent duplications in the amphioxus lineage. Taking together the faster evolutionary rate of posterior genes in deuterostomes compared to the high constraint of anterior genes (groups 1 to 4), and the information available for the Hox

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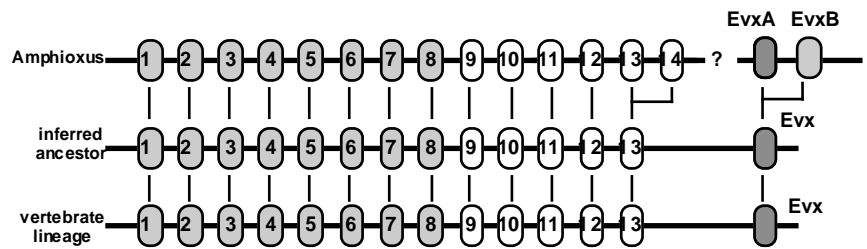


Fig. 1. Presumed organization of the extended Hox cluster of the last common ancestor of chepalocordates and vertebrates. The cluster depicted below represents the Hox/Evx complement just prior to cluster duplication in the vertebrate lineage.

cluster of other deuterostomes (summarised in Ferrier *et al.*, 2000), we favour the scenario for an ancestral cluster of at least 13 genes, where the relationships of posterior genes are obscured by a fast evolutionary rate of the “tail” part of the cluster (Fig. 1). We suggest that the higher rate of evolution of posterior genes is due to the phenomenon of “posterior flexibility”, which reflects a higher constraint of anterior genes to evolve, while posterior genes are less constrained. Nonetheless, vertebrate posterior genes clearly group in groups 9 to 13. This implies that in the lineage of vertebrates some type of constraint was acting on posterior genes, after the divergence of amphioxus and before cluster duplication: the biological reason for such constraint is unclear, and may be related to the invention or elaboration of pan-vertebrate structures in which posterior Hox genes are involved. A survey of such structures may include tail fins and paired appendages, trunk neural crest, posterior mesodermal tissues, hindgut, and urogenital system.

Amphioxus Evx: the archetypal and the fast-evolving genes

Evx are homeobox genes present from cnidarian to vertebrates and seem to have a basic function in patterning the posterior part of the embryo in all bilaterians. In addition, they are expressed in the central nervous system. In particular lineages, Evx genes have acquired specific functions: they behave as pair-rule genes in higher insects; in vertebrates they are essential for tailbud development, and are expressed in the midbrain/hindbrain boundary and during appendage development. We discovered that amphioxus has two Evx genes that are closely linked (Ferrier *et al.*, 2001), and probably arose after a tandem gene duplication event (Fig. 1). Phylogenetic analyses clearly show that AmphiEvx-A is a sister member of both vertebrate Evx-1 and Evx-2 genes, while Evx-B is a highly divergent Evx gene. AmphiEvx-A may be the member that has retained the archetypal Chordate Evx function, whereas Evx-B may have acquired a particular role in the amphioxus lineage. AmphiEvx-A is expressed during gastrulation in a ventro-posterior domain, in all three germ layers in the most posterior part of the neurula, and in pairs of neural cells in the nerve cord. In 36–48 h larvae is expressed in the post-anal tailbud. Evx-B is not expressed in early embryos, and after hatching, generalised ectodermal expression is detected, in a manner unrelated to any other Evx gene. Both the molecular comparison and the expression patterns strongly suggest that AmphiEvx-A is a prototypical chordate Evx gene, retaining the ancestral features of the family in chordates. Thus, the basal chordate involvement of Evx genes may well be the pan-bilaterian role in gastrulation and neurogenesis, plus a pan-chordate role in tailbud development, whereas the

relation of Evx to the midbrain/hindbrain boundary may be a vertebrate innovation, together with a latter involvement in appendage development.

Lessons from an archetypal but not the archetype

Amphioxus morphology may have remained astonishingly invariant since the origin of vertebrates, several hundred of millions years ago, but the amphioxus genome has not escaped evolution. Posterior flexibility, a putatively duplicated Hox 14 gene, and a fast evolving copy of Evx are examples that reflect “Evo” in the amphioxus genome, that have not been able to modify amphioxus “Devo”. Thus, amphioxus duplicated genes which do not involve major morphological changes may unveil the genome changes which are conservative. In contrast, comparisons of the molecular changes that have eventually produced evolutionary innovations (e.g., genome duplication at the origin of vertebrates) may provide insights in the mechanistic of evolutionary potentialities and constraints. Yet, the amphioxus genome is the closest representative of the ancestral genome of vertebrates, and may be invaluable to infer such ancestral genome and the ancestral role for a particular gene in chordates. The inferences that the ancestral Hox cluster predating amphioxus divergence had at least 13 genes, and that Evx has a pan-chordate role in tailbud development whereas it acquired a vertebrate-specific role in midbrain/hindbrain boundary, are examples of the lessons that the closest-to-the-ancestor-but-not-the-ancestor amphioxus can give.

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