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Review

Evolution of new characters after whole genome duplications: Insights from amphioxus

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ABSTRACT

Additional copies of genes resulting from two whole genome duplications at the base of the vertebrates have been suggested as enabling the evolution of vertebrate-specific structures such as neural crest, a midbrain/hindbrain organizer and neurogenic placodes. These structures, however, did not evolve entirely de novo, but arose from tissues already present in an ancestral chordate. This review discusses the evolutionary history of co-option of old genes for new roles in vertebrate development as well as the relative contributions of changes in *cis*-regulation and in protein structure. Particular examples are the FoxD, FGF8/17/18 and Pax2/5/8 genes. Comparisons with invertebrate chordates (amphioxus and tunicates) paint a complex picture with co-option of genes into new structures occurring both after and before the whole genome duplications. In addition, while *cis*-regulatory changes are likely of primary importance in evolution of vertebrate-specific structures, changes in protein structure including alternative splicing are non-trivial.

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1. Introduction

Duplicate genes arise either from whole genome duplications or by duplication of individual genes. The duplicates are generally lost unless their functions and those of the parent genes diverge. There have been three major events of whole genome duplication (WGD) in animal evolution. Two occurred near the base of the vertebrates and the third at the base of the teleost fishes about 230 mya [1]. There is some question as to whether only one or both WGDs in early vertebrates preceded the evolution of agnathans [2–4], but the current consensus is that both probably occurred before the agnathan/gnathostome split [5]. Agnathans (hagfish and lampreys), which are basal in the vertebrates, have key vertebrate characters that are lacking in the invertebrate chordates—cephalochordates (amphioxus, also called lancelets) and urochordates, (tunicates). These characters include neural crest, a telencephalon, isthmic organizer at the midbrain/hindbrain boundary, and paired eyes. The evolution of all these features subsequent to 2R WGD lends

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support to the long-standing hypothesis that the extra genes gave vertebrates the tools to elaborate upon old structures and create new ones [6]. Comparative analyses in conjunction with the amphioxus genome project provided additional support [7]. They showed that while most of the paralogs arising from WGD were lost such that humans, for example, have only about 25% more genes than amphioxus, vertebrates preferentially retained replicates of developmental genes, including those coding for transcription factors and proteins in signaling pathways [7]. It is far less likely that lineage-specific gene duplications, unless they occurred in the vertebrate ancestor, have played a role in the evolution of characters that are common to vertebrates in general.

The present review compares the invertebrate chordates to vertebrates. Amphioxus is basal in the chordates with tunicates and vertebrates as sister groups. On that basis, tunicates would seem far better than amphioxus to compare with vertebrates to understand how 2R WGD may have facilitated evolution of vertebrate characters. However, although tunicates, like amphioxus, have not duplicated their genomes, they are evolving rapidly and have very reduced genomes (70–173 Mb compared to 520 Mb in amphioxus and 3 Gb in humans) with loss of some key developmental genes (e.g. several Hox genes) and independent duplication of others (e.g. Pax2/5/8 in the appendicularian *Oikopleura dioica*). There is little if any synteny between tunicate and vertebrate genomes. Moreover, tunicates, unlike amphioxus and vertebrates, have considerably modified their adult body plans. They have largely determinate development and have, therefore, lost some features common to amphioxus and vertebrates such as the segmentation of paraxial muscles from a tailbud. Metamorphosis in the ascidian tunicate *Ciona intestinalis* is drastic with loss of the larval tail and much of the larval central nervous system (CNS) together with formation of a branchial basket with gill slits and incurrent (oral) and excurrent (atrial) siphons. This has led to lengthy discussions concerning whether or not expression domains of genes such as Pax2/5/8, engrailed and Fgf8/17/18 in the CNS and siphons represent homologies with vertebrate structures or evolved independently [8]. In contrast, although the vertebrate, tunicate and amphioxus lineages separated over 500 mya, the amphioxus (*Brahchiostoma floridae*) genome has retained a large degree of synteny with vertebrate genomes and has comparatively little loss or independent duplication of developmental genes. With highly vertebrate-like development, amphioxus is, therefore, the most appropriate organism to compare with vertebrates to understand the evolutionary origins of vertebrate-specific characters [9].

2. How genes acquire new functions

New functions for genes can be acquired in two ways—by the evolution of new regulatory elements, allowing gene expression in new domains or suppressing expression in old ones or by changes in proteins such as point mutations, exonization of intronic sequences, acquisition of new protein domains from elsewhere in the genome (for example, the TGFβ-receptor domain in amphioxus Dkk3 [10]), or changes in alternative splicing. To date, studies relating gene duplication to the acquisition of vertebrate-specific characters have largely focused on changes in *cis*-regulatory DNA, leading to gene expression in new domains and their integration into existing gene networks. There is less information on how changes in protein structure affect the acquisition of new characters. In all likelihood, a clear picture will not emerge until gene networks and the functions in vivo of co-expressed isoforms in development are better characterized.

The original hypothesis for the fate of genes after duplication is the duplication–degeneration–complementation model [11]. This initially concerned the partitioning of the several expression domains of the original gene amongst the duplicates by degeneration of different regulatory elements from each replicate, thus preventing loss of the replicates. Subsequent models have added the possibility of acquisition of new regulatory elements or neofunctionalization, which, for the teleost-specific WGD, has been estimated to affect about 25% of the retained duplicates [12]. New regulatory elements (enhancers) can arise by duplication of existing ones or from transposable elements or from coding regions of genes that have decayed after gene duplication as well as from point mutations [13,14]. There are many examples where the several replicates remaining after 2R WGD have partitioned the ancestral expression domains and/or gained new ones in new structures. However, there are also examples where several replicates are expressed in overlapping patterns in vertebrate-specific structures. Presumably, in such instances the parent gene was co-opted to an ancestral structure before WGD and subsequently function diverged somewhat.

3. New structures are built upon old foundations

Many, if not most, vertebrate-specific structures have been built upon those existing in an invertebrate ancestor. Examples are migratory neural crest, the midbrain/hindbrain organizer and neurogenic placodes (e.g. the trigeminal, lateral line, otic placodes). These structures and others have evidently evolved by co-option of additional genes into existing gene networks. For example, the acquisition of new expression domains in the central nervous system (CNS) has been correlated with co-option of gene duplicates after 2R WGD. A comprehensive study involving in situ hybridization of early larvae for genes from 33 families with singletons in amphioxus and two or more copies in vertebrates [15] found that expression of 13/33 gene families was conserved between amphioxus and *Xenopus*, but 15/33 of the genes were not expressed in the amphioxus CNS while at least one of the corresponding vertebrate duplicates was. An additional 7 gene families were expressed in the amphioxus CNS but had expanded domains in the CNS in vertebrates. Thus, about half of these genes had probably acquired new enhancers (yet to be characterized) subsequent to gene duplication [15].

3.1. Evolution of neural crest involved co-option of genes for neural crest specification

Homologs of the genes specifying the neural plate proper and its edges are similarly expressed in amphioxus and vertebrates (Fig. 1). However, homologs of genes specifying vertebrate neural crest are not expressed at the edges of the neural plate in amphioxus. Thus, neural crest clearly evolved by the incorporation of additional genes into the gene network already in place at the neural plate border in the ancestral chordate. This is due chiefly to the cooption of gene duplicates subsequent to 2R WGD. Very few neural crest genes appear to be newly evolved in vertebrates, and they are involved in terminal differentiation [16]. Amphioxus has homologs of most of the genes directing specification and migration of neural crest in vertebrates [i.e. Snail/Slug, AP-2, FoxD3, Twist, id, cMyc and sox 9/10 (SoxE)], but only Snail is expressed in the edges of the neural plate in amphioxus (Fig. 1) [17,18]. While it has been assumed that the acquisition of new gene expression domains must be accompanied by the acquisition of new enhancers, as yet, there are only a few studies clearly demonstrating the evolution of new enhancers in neural crest. One concerns the transcriptional repressor FoxD3. FoxD genes are one of 23 subclasses of the Fox family of transcription factors [19,20]. The single FoxD gene in amphioxus is expressed in the notochord, somites and in the forebrain [21,22]. In vertebrates, expression has been partitioned amongst the duplicates (5