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Report

Axial Patterning and Diversification in the Cnidaria Predate the Hox System

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Summary

Across the animal kingdom, Hox genes are organized in clusters whose genomic organization reflects their central roles in patterning along the anterior/posterior (A/P) axis [1–7]. While a cluster of Hox genes was present in the bilaterian common ancestor, the origins of this system remain unclear (cf. [8]). With new data for two representatives of the closest extant phylum to the Bilateria, the sea anemone *Nematostella* and the hydromedusa *Eleutheria*, we argue here that the Cnidaria predate the evolution of the Hox system. Although Hox-like genes are present in a range of cnidarians, many of these are paralogs and in neither *Nematostella* nor *Eleutheria* is an equivalent of the Hox cluster present. With the exception of independently duplicated genes, the cnidarian genes are unlinked and in several cases are flanked by non-Hox genes. Furthermore, the cnidarian genes are expressed in patterns that are inconsistent with the Hox paradigm. We conclude that the Cnidaria/Bilateria split occurred before a definitive Hox system developed. The spectacular variety in morphological and developmental characteristics shown by extant cnidarians demonstrates that there is no obligate link between the Hox system and morphological diversity in the animal kingdom and that a canonical Hox system is not mandatory for axial patterning.

Results and Discussion

The Hox cluster has been the Rosetta Stone of comparative developmental biology, but its origins are unclear. Hox genes are characteristically organized in clusters whose genomic organization directly reflects domains of expression along the A/P axis [1–7]; this pattern of organization is functionally important and has been conserved across the Bilateria. The central role of Hox clusters in axial patterning in animals with very different body plans, together with functional data from arthropods and chordates, has led to the assumption that much of the morphological variation seen across the animal kingdom can be directly attributed to different numbers of Hox genes or differential use of the Hox system [9–11]. For present purposes, we define a canonical Hox system as a set of closely linked and interacting homeobox genes that are directly related to the Hox classes of *Drosophila* and mammals and that, through their combined actions, are primarily responsible for patterning most or all tissues along the anterior-posterior body axis (cf. [8, 12, 13]).

Cnidarians represent a key transition in the evolution of animal complexity and are therefore critical to understanding the origins of developmental mechanisms such as the Hox system. Although they are among the simplest of true animals at the morphological level, the Cnidaria is among the most taxon-rich phyla and cnidarians have many of the genes traditionally assumed to have arisen in the context of vertebrate complexity [14–17]. Cnidarians have genes clearly related to a number of the key homeobox gene families of bilateral animals, such as Emx, Evx, Hex, Not, and Dlx [18], and some of these are expressed in patterns strikingly like those of their putative bilaterian orthologs (reviewed in [19]). In addition, Hox-like genes have been identified in a wide variety of cnidarians (e.g., [18]) but, in contrast to a number of other key regulatory gene types, their status is often equivocal.

In an attempt to clarify the evolutionary origins of the Hox system, we characterized the Hox-like genes in two representative cnidarians, *Eleutheria dichotoma* (Hydrozoa) and *Nematostella vectensis* (Anthozoa), in terms of sequence relationships, genomic organization, and expression patterns. *Eleutheria* is a typical cnidarian in having both polyp and medusa lifecycle stages, while *Nematostella* represents the basal cnidarian class (Anthozoa). Genes related to the anterior Hox and posterior Hox/Cdx types of bilaterians are present, but most of the Hox-like genes present in cnidarians postdate the Cnidaria/Bilateria split. The organization of these genes differs between the two cnidarians, and we found no evidence for the clustered organization characteristic of true Hox genes. Patterns of expression of the corresponding genes also differ dramatically between *Nematostella* and *Eleutheria* and across a range of other cnidarians. The cnidarian genes therefore do not conform to the Hox paradigm in terms of structure, organization, or expression, and the simplest interpretation of these observations is that the Cnidaria predate the origins of the Hox system. Thus, contrary to expectations, a definitive Hox system is not essential for axial patterning in lower animals. Moreover, the spectacular range of morphological variation across the Cnidaria demonstrates that the canonical Hox system is not mandatory for the elaboration of a wide range of variations on a basic body plan.

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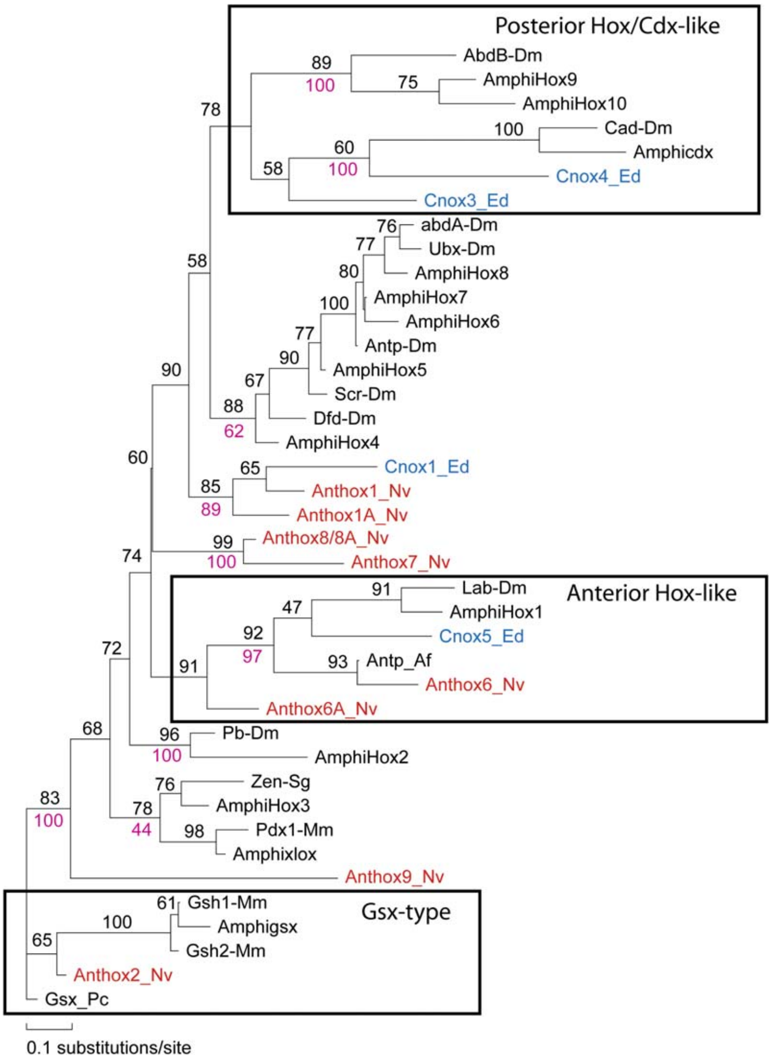

Figure 1. Phylogenetic Analyses Identify Some Cnidarian Hox-like Genes as Relatives of Anterior Hox or Posterior Hox/Cdx Types of Bilateria, but "Intermediate" Genes Are Missing

Numbers on branches reflect the percentages of 1000 ML bootstrap replicates supporting the topology shown. Bayesian posterior probability values are shown below some of the critical nodes. *Nematostella* sequences are coded in red, those from *Eleutheria* in blue. As it is not clear what might be the most appropriate outgroup, and the nature of the outgroup to some extent determines internal topology (since it affects the position of the root), analyses were unrooted. However, for clarity, the tree is shown as if rooted via the *Podocoryne carnea* Gsx sequence (Gsx_Pc; encoded by GenBank #AAG09805), which is the ortholog to *Nematostella* anthox2. Note that although some cnidarian sequences are related to the "anterior" (group 1) Hox or posterior Hox/Cdx classes of bilateral animals, true intermediate (groups 2–8) Hox genes are clearly resolved from the cnidarian sequences. Conversely, independent duplications have increased Hox-like gene complexity within the Cnidaria.

Phylogenetic Analyses Reveal No Clear Orthologies to True Hox Classes

When the Gsx-type genes (which are clearly distinct) are excluded, a total of four Hox-like genes are present in *Eleutheria* [20, 21], and five genes of this type have previously been identified in *Nematostella* [22]. In the present study, we identified three novel Hox-like genes by analysis of the unassembled genomic sequence data now available for *Nematostella*. One of these, designated as anthox8A, encodes an identical homeodomain to anthox8 but differs significantly outside this. In addition, a gene related to anthox6 (anthox6A) and a highly derived gene (anthox9) were identified. The derived position of anthox9 is reflected in its position in the phylogenetic trees; note that although its predicted sequence has the Hox-like characteristic Glu residue at homeodomain position 15, the Ile residue at position 16 appears to be without precedent in the Antp superclass (only LIM and atypical homeodomains have anything other than a Leu residue at position 16 [23]).

To investigate relationships between the cnidarian Hox-like genes and the true Hox classes of higher animals, phylogenetic analyses were conducted with all of available sequences from *Nematostella* and *Eleutheria* (Figure 1 and Supplemental Data available with this article online). The results confirm several key aspects of previously published studies [7, 13, 18, 24] but do not support others. *Nematostella* anthox1/1A and anthox7/8/8A have been duplicated independently, as have several other homeobox gene loci in *Acropora* [25]. Clear support was obtained for common origin for anterior Hox genes and a class of cnidarian genes related to *Nematostella* anthox6 that also includes anthox6A and *Eleutheria* Cnox-5ed. Our analyses also confirm the high similarity between a group of cnidarian genes that includes *Eleutheria* Cnox-4ed and posterior Hox genes, although interestingly *Nematostella* appears not to have a gene of this type. Here we refer to the classes of cnidarian genes that are related to the anterior and posterior Hox/Cdx groups as the "anterior Hox-like" and "posterior Hox/Cdx-like" types, respectively. However, whereas the affinities of these gene types are well supported, other cnidarian Hox-like genes have no clear relationship to true Hox classes. Whereas orthology relationships have sometimes been suggested, a simpler interpretation is that the common ancestor had genes that later gave rise to the anterior and posterior Hox genes, but that intermediate Hox genes