

The origins of axial patterning in the metazoa: how old is bilateral symmetry?

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ABSTRACT Bilateral symmetry is a hallmark of the Bilateria. It is achieved by the intersection of two orthogonal axes of polarity: the anterior-posterior (A-P) axis and the dorsal-ventral (D-V) axis. It is widely thought that bilateral symmetry evolved in the common ancestor of the Bilateria. However, it has long been known that members of the phylum Cnidaria, an outgroup to the Bilateria, also exhibit bilateral symmetry. Recent studies have examined the developmental expression of axial patterning genes in members of the phylum Cnidaria. *Hox* genes play a conserved role in patterning the A-P axis of bilaterians. *Hox* genes are expressed in staggered axial domains along the oral-aboral axis of cnidarians, suggesting that *Hox* patterning of the primary body axis was already present in the cnidarian-bilaterian ancestor. *Dpp* plays a conserved role patterning the D-V axis of bilaterians. Asymmetric expression of *dpp* about the directive axis of cnidarians implies that this patterning system is similarly ancient. Taken together, these results imply that bilateral symmetry had already evolved before the Cnidaria diverged from the Bilateria.

KEY WORDS: *Cnidaria, Bilateria, axial patterning, Hox, Dpp*

Introduction

The ancestral bilaterian left quite a legacy. Modern-day bilaterians number some 32 phyla encompassing well over a million described species (Collins and Valentine, 2001). Bilaterians are far more abundant, far more diverse morphologically, and far more diverse ecologically than non-bilaterian metazoans (Porifera, Ctenophora, Cnidaria, and Placozoa). In addition, much greater morphological complexity is achieved within the Bilateria than within the non-bilaterian phyla. For these reasons, a great deal of recent speculation has concerned the bilaterian ancestor and the radiation of early bilaterians (*e.g.*, Peterson *et al.*, 2000; Arendt *et al.*, 2001; Collins and Valentine, 2001; Balavoine *et al.*, 2002; Erwin and Davidson, 2002; Martindale *et al.*, 2002).

Despite their morphological diversity, most bilaterians are united by a handful of fundamental body plan features including bilateral symmetry, triploblasty, a coelom, a through-gut, and a central nervous system (reviewed in Willmer, 1990). At one time or another, all of these traits have been implicated as bilaterian synapomorphies, though arguments have also been made for multiple independent origins of mesoderm, coeloms, and through-guts within Bilateria (reviewed in Willmer, 1990; Nielsen, 1995). Comparative molecular developmental data have provided support for the monophyletic origin of these traits (*e.g.*, Slack *et al.*, 1993; Arendt and Nubler-Jung, 1997; Arendt and Nubler-Jung, 1999;

Arendt *et al.*, 2001). If these traits have monophyletic origins, their widespread conservation across the Bilateria implies that they have been critical to the evolutionary success of bilaterians. If we are to understand the evolutionary significance of these traits, we must learn more about their origins and early evolution.

Conventional wisdom holds that the bilaterian condition arose from an ancestor that displayed radial symmetry instead of bilateral symmetry, diploblasty instead of triploblasty, a blind sac for a gut, no coelom, and a decentralized nerve net (reviewed in Willmer, 1990). The extant members of the phylum Cnidaria (sea anemones, corals, jellyfishes, and hydras) are widely invoked as proxies for the pre-bilaterian ancestral condition. However, careful examination of cnidarians based on morphology and the deployment of developmental regulatory genes suggests some putative bilaterian «novelties» might be traced to the cnidarian-bilaterian ancestor (CBA). For example, bilateral symmetry itself may have evolved prior to the divergence between bilaterians and cnidarians (Willmer, 1990).

The bilateral symmetry of Bilateria is a complex multigenic trait achieved by the intersection of two axes of polarity: a primary body axis (the anterior-posterior axis) and a secondary, orthogonal axis (the dorsal-ventral axis). These two axes are logically and

Abbreviations used in this paper: A-P, anterior-posterior; D-V, dorso-ventral.

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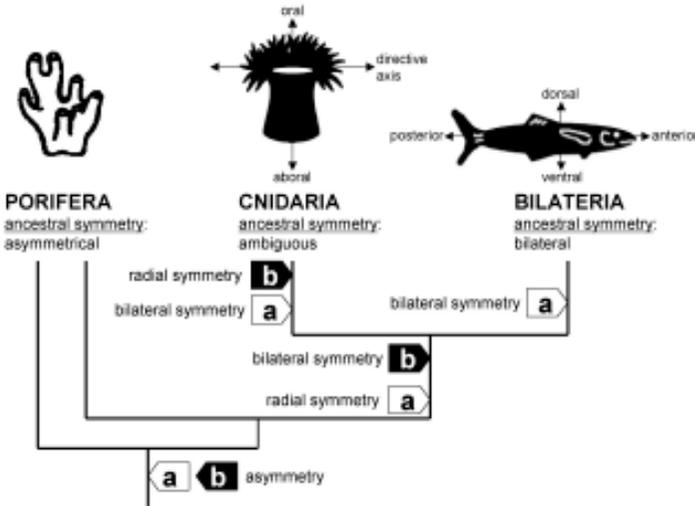


Fig. 1. Hypotheses on the origins of bilateral symmetry in metazoans. The sponges (phylum Porifera) are likely a polyphyletic group (Medina *et al.*, 2001). Sponges lack a fixed primary body axis and they lack symmetry (Nielsen, 1995). This lack of symmetry in sponges may represent the ancestral metazoan condition. In hypothesis A, the absence of symmetry in the ancestral metazoan was followed by the evolution of radial symmetry sometime before the divergence of cnidarians and bilaterians. Bilateral symmetry then would have evolved independently in bilaterians and cnidarians. In hypothesis B, the cnidarian-bilaterian ancestor was a bilaterally-symmetrical animal. Radial symmetry would have evolved subsequently within the Cnidaria.

evolutionarily separable. Indeed, different gene networks are responsible for patterning each axis. For example, Hox genes play an evolutionary conserved role in patterning the A-P axis of all bilaterians studied to date (Slack *et al.*, 1993). Patterning of the D-V axis in both vertebrates and insects depends on the activities of *decapentaplegic (dpp)* and *short gastrulation (sog)* homologs (Holley *et al.*, 1995; Ferguson, 1996).

Bilateral symmetry was already firmly entrenched in the most recent common ancestor of the Bilateria. Therefore, comparisons among extant bilaterians cannot shed light on the origin of bilateral symmetry. It is necessary to examine outgroup taxa. The

sponges, phylum Porifera, are an early off-shoot from the metazoan phylogeny and an outgroup to the Eumetazoa, a clade comprising all other animals (Fig. 1). Sponges are generally asymmetrical in form, and they lack any kind of fixed primary body axis (Kaandorp, 1991; Kaandorp, 1994; Nielsen, 1995). The sponges probably diverged from the Eumetazoa prior to the invention of axial patterning.

The Cnidaria are a basal eumetazoan lineage and an outgroup to the bilaterians. Some cnidarians exhibit bilateral symmetry (Stephenson, 1926). All cnidarians possess a primary body axis, the oral-aboral axis. In those species exhibiting bilateral symmetry, there is a secondary axis of polarity, orthogonal to the oral-aboral axis. This secondary axis has been called the directive axis (Stephenson, 1926). Did bilateral symmetry evolve independently in the Cnidaria and the Bilateria from an ancestral condition of radial symmetry (hypothesis A in Fig. 1), or did bilateral symmetry evolve in the common ancestor of the Cnidaria and the Bilateria (hypothesis B in Fig. 1)?

Efforts to reconstruct the symmetry of the cnidarian-bilaterian ancestor (CBA) using phylogenetic inference are frustrated by the lack of homologous structural landmarks in cnidarians and bilaterians and ambiguity over the ancestral condition for cnidarians. While it is clear that the most recent common ancestor of bilaterians exhibited bilateral symmetry, and it is widely accepted that the most recent common ancestor of the sponges lacked symmetry, the ancestral cnidarian may have exhibited radial symmetry, biradial symmetry, tetradial symmetry or bilateral symmetry (Fig. 1; Willmer, 1990). Regardless of the character state assignment for the CBA, it is clear that derived forms of symmetry have evolved both within the Bilateria (where the echinoderms evolved pentaradial symmetry) and within the Cnidaria.

Comparative molecular studies of development may provide evidence relevant to the symmetry of the CBA. If the mechanisms used to pattern the primary and secondary axes are homologous in cnidarians and bilaterians, then bilateral symmetry should be regarded as a synapomorphy of Bilateria plus Cnidaria, and not a synapomorphy of bilaterians. Two questions must then be answered: (1) are homologs of the axial patterning genes utilized by bilaterians found in the genomes of cnidarians, and (2) are

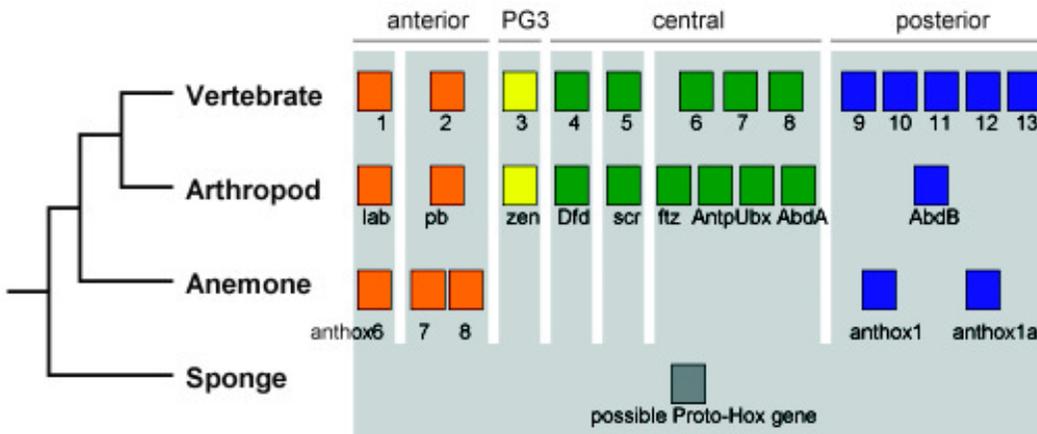


Fig. 2. Distribution of Hox genes among major metazoan lineages. The likely ancestral Hox complements for vertebrates and arthropods are shown (Finnerty and Martindale, 1998). The sea anemone *Nematostella* is used to represent the cnidarian condition (Finnerty, 2001). A single Hox-like gene has been identified in sponges (Degnan *et al.*, 1995) and this may represent a direct descendant of the original «Proto-Hox gene» (Finnerty, 2001). Each of the Hox subclasses (anterior, group 3, central and posterior) are represented by a different color. Vertical gray bars indicate likely orthology. The term *anthox* is short for *anthozoan homeobox* gene. The numbering of cnidarian hox genes is related to their order of discovery and bears no relationship to the numbering of vertebrate paralogy groups.

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these genes deployed along the body axes in a manner consistent with their deployment in bilaterian metazoans. Recent studies suggest that the answer to both questions is yes. In other words, the current data favor the interpretation that bilateral symmetry evolved once in the common ancestor of cnidarians and bilaterians.

Hox genes and the primary body axis

The adult body plan of sponges (phylum Porifera) reveals no regionalization and therefore, no organism-wide axis of polarity (Kaandorp, 1991; Kaandorp, 1994; Nielsen, 1995). In contrast, the adult body plan of cnidarians (sea anemones, corals, jellyfishes, and hydras) reveals obvious regionalization along the primary body axis, the oral-aboral (OA) axis. The OA axis passes through the center of the cylindrical polyp forms or the hemispherical medusa forms. The polyp can be divided into three major body regions: head, column, and foot or physa.

Embryological evidence implies homology between the OA axis of cnidarians and the AP axis of bilaterians. Metazoan blastulae and gastrulae commonly display monaxial symmetry about their animal-vegetal axis (Beklemishev, 1969). This primary body axis of the embryo becomes the primary axis of the adult in many cases. Later in development, one can recognize an «antero-posterior» axis passing through the apical sense organ (anterior) and the blastopore (posterior) of diverse metazoan embryos including cnidarians, protostomes, and deuterostomes (Nielsen, 1995). However, the relationship between embryological and adult axes is not consistent across the Metazoa (reviewed in Holland, 2000). Furthermore, considering the widespread utilization of «set-aside» cells in indirectly developing metazoans (Davidson *et al.*, 1995), the existence of structural homology between embryos does not necessarily imply structural homology between adult body plans.

In all bilaterians that have been examined, the Hox genes play a conserved role in patterning the anterior-posterior axis (Slack *et al.*, 1993). Hox genes represent a subset of the larger family of homeobox containing transcription factors (Duboule, 1994). A cluster of seven or eight Hox genes was present in the bilaterian ancestor (Finnerty and Martindale, 1998). Typically, each individual Hox gene is expressed in a band of cells representing a subset of the animal's anterior-posterior axis. Different Hox genes have distinct domains of spatial expression along the primary body axis. Collectively, the staggered Hox expression domains specify regional identity over the majority of the body axis, as each region of the body

comes to express a different Hox gene or combination of Hox genes. The «Hox code» effectively subdivides the bilaterian body plan into distinct regions. Does the Hox code operate during cnidarian development? If the oral-aboral axis of a cnidarian is homologous to the anterior-posterior axis of bilaterians, then we may expect staggered Hox expression domains along the oral-aboral axis of cnidarians.

Several Hox genes have been recovered from cnidarians (Murtha *et al.*, 1991; Schummer *et al.*, 1992; Miller and Miles, 1993; Naito *et al.*, 1993; Aerne *et al.*, 1995; Gauchat *et al.*, 2000), with as many as five Hox genes identified from a single species, the sea anemone *Nematostella* (Finnerty, 2001). The precise homology of cnidarian Hox genes has been a matter of debate, but a recent consensus seems to have emerged: cnidarians appear to possess Hox genes from the anterior class and the posterior class (Fig. 2; Finnerty and Martindale, 1997; Finnerty, 1998; Martinez *et al.*, 1998; Gauchat *et al.*, 2000). Cnidarian genes with obvious homology to the *central Hox* class or the group 3 genes (*e.g.*, *zen*) have not been recovered (Finnerty, 2001).

Recently, the spatial expression of Hox genes has been studied in cnidarians by *in situ* hybridization (Gauchat *et al.*, 2000; Masuda-Nakagawa *et al.*, 2000; Finnerty *et al.*, 2003). The expression of two Hox genes was studied in *Hydra*, in adult animals and over the course of regeneration and asexual budding (Gauchat *et al.*, 2000). The *anterior-Hox* gene *cnox1* was expressed at high levels in the future head region of developing buds. The

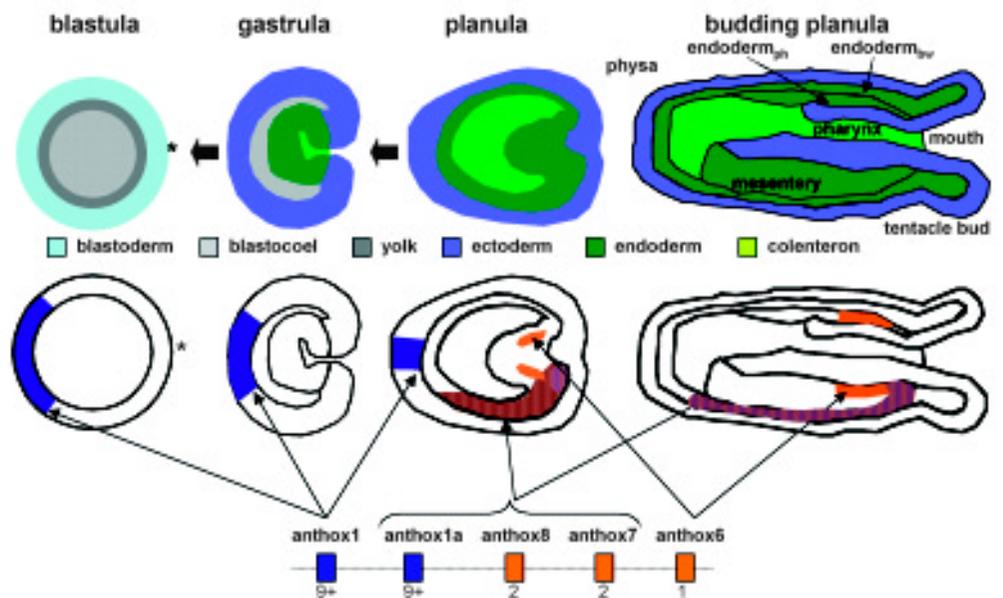


Fig. 3. Hox gene expression in the sea anemone *Nematostella*. The diagrams are based on recent *in situ* hybridization experiments (Finnerty *et al.*, 2003). The same gene coloration scheme is maintained from Fig. 2. The temporal developmental progression proceeds from left to right. The upper panel of diagrams reveals the germ layer composition of animals sectioned longitudinally through the primary body axis. The lower panel of diagrams depicts the expression of Hox genes. The temporal and spatial expression of *anthox1a*, *anthox7* and *anthox8* are practically indistinguishable. All embryos and larvae are shown with the blastopore (*) or mouth facing right and the future aboral end facing left. The preferred swimming direction is shown with an arrow. This is known as the apical pole of the larva. The endoderm lining the body wall (*endoderm_{bw}*) can be distinguished from the endoderm lining the outer wall of the pharynx (*endoderm_{ph}*). The lumen of the pharynx is lined with ectoderm.

posterior-Hox gene *cnox3* was expressed in scattered cells in the head region of adults. The overlapping expression of an *anterior-Hox* gene and a *posterior-Hox* gene at the same end of the body axis is not reminiscent of the bilaterian Hox code. However, none of the developmental contexts examined in this study (adulthood, regeneration, or asexual budding) are directly comparable to bilaterian embryogenesis.

The expression of three Hox genes has been studied during embryogenesis in the hydrozoan jellyfish, *Podocoryne carnea* (Masuda-Nakagawa *et al.*, 2000; Yanze *et al.*, 2001). Our own phylogenetic analyses indicate that *cnox1-Pc* and *cnox2-Pc* are *anterior-Hox* genes, while *cnox4-Pc* is a *posterior-Hox* gene (Finnerty *et al.*, 2003). Each of these genes is expressed in a restricted domain along the oral-aboral axis of the planula larva. *Cnox1-Pc* and *cnox2-Pc*, the *anterior-Hox* genes, are strongly expressed at the apical end of the planula larva (Masuda-Nakagawa *et al.*, 2000; Yanze *et al.*, 2001). The apical pole will become the aboral end of the polyp (Masuda-Nakagawa *et al.*, 2000). *Cnox4-Pc*, the *posterior-Hox* gene is expressed at the blastoporal end of the larva. The blastoporal end of the larva becomes the oral end of the adult polyp. The expression of *Podocoryne* Hox genes is reminiscent of bilaterian Hox genes in that each gene is expressed broadly (*i.e.*, not in a highly restricted subset of cell types) within a restricted region of the primary body axis. Collectively, these three Hox genes could play a role in patterning both the oral and aboral ends of the *Podocoryne* primary polyp.

The expression of five Hox genes, three *anterior-Hox* genes and two *posterior-Hox* genes, has been studied during larval

development of the sea anemone, *Nematostella vectensis* (Finnerty *et al.*, 2003). One of the *anterior-Hox* genes, *anthox6*, is expressed most strongly in the pharyngeal endoderm, near the oral extremity of the polyp (Fig. 3). This result is surprising in that *anthox6* is expressed at the opposite end of the body plan as the *anterior-Hox* genes of *Podocoryne* (Masuda-Nakagawa *et al.*, 2000; Yanze *et al.*, 2001). One of the *posterior-Hox* genes, *anthox1*, is expressed in ectodermal cells at the extreme apical end of the larva (Fig. 3). The apical end of the larva gives rise to the foot or physa of the adult anemone. The other three Hox genes display nearly identical patterns of gene expression. *Anthox1a*, a posterior hox gene, *anthox7* (anterior), and *anthox8* (anterior) are expressed in endodermal tissue of the pharynx and the body wall (Fig. 3). Unexpectedly, the expression of these genes is limited along two axes: the primary body axis and the secondary body axis. In cross-section these Hox genes are seen to be expressed in a limited sector of the pharyngeal endoderm, perhaps corresponding to the location of the single siphonoglyph (see below). Collectively, the expression domains of these five Hox genes account for practically the entire O-A axis of *Nematostella*, though one of the genes is expressed exclusively in the ectoderm (*anthox1*) and four are expressed in the endoderm (*anthox1a*, *anthox6*, *anthox7*, and *anthox8*).

In bilaterians, Hox gene expression has provided compelling evidence of homology in the A-P patterning mechanisms of deuterostomes and protostomes. Cnidarians appear to possess fewer Hox genes than bilaterians, but they appear to be expressed in a similar developmental context. The staggered Hox expression domains along the oral-aboral axis of cnidarians suggest that Hox genes might be responsible for patterning the axis. If so, the axial patterning mechanisms responsible for regionalization of the primary body axis may have a monophyletic origin in the cnidarian-bilaterian ancestor.

Colinearity of *Nematostella* Hox expression domains with their bilaterian homologs is not readily apparent (Finnerty *et al.*, 2003). However, this is not unexpected. The cnidarian Hox genes appear to derive from only two of the four recognized Hox sub-groups (the anterior class and the posterior class; Fig. 2). The absence of the group 3 and central Hox genes would make colinearity more difficult to recognize. Furthermore, it appears that independent Hox gene duplications have occurred in the history of the Cnidaria. For example, the *anthox7-anthox8* duplication appears to have been restricted to anthozoan cnidarians (Finnerty and Martindale, 1999), so neither of these genes has a direct counterpart in bilaterians (Fig. 2). Hox genes have been known to evolve novel

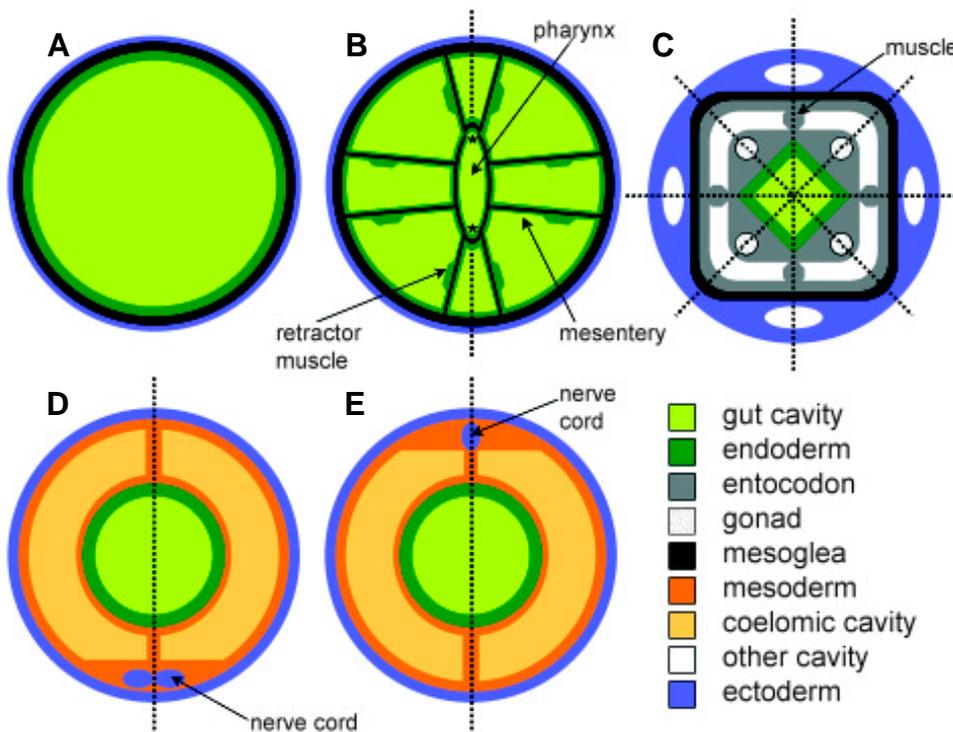


Fig. 4. Cross-sectional architecture and germ layer composition of cnidarians and bilaterians. (A) *Hydra*. (B) A sea anemone with eight mesenteries, one siphonoglyph (*), and bilateral symmetry about its directive axis (e.g., *Nematostella*; Stephenson, 1926; Stephenson, 1935). (C) A hydrozoan jellyfish exhibiting tetradial symmetry (drawing based on Spring *et al.*, 2002). (D) A protostome bilaterian. (E) A deuterostome bilaterian. Dotted lines indicate planes of mirror symmetry.

functions following gene duplication. For example, the group 3 genes of *Drosophila* (*zen1*, *zen2*, and *bicoid*) have lost their ancestral involvement in the Hox code (Damen and Tautz, 1998).

Dpp and the secondary body axis

Since Cuvier erected the «Radiata» as one of four metazoan «embranchments,» cnidarians have generally been described as radially-symmetrical animals (Cuvier, 1828). Indeed, many cnidarians exhibit radial symmetry. The body plan of *Hydra* is a simple cylinder, radially symmetrical about the primary body axis, the oral-aboral axis (Fig. 4A). However, careful morphological examination reveals instances of asymmetry, biradiality, tetra-radiality, or even bilaterality in the internal anatomy of cnidarians (Fig. 4 B,C; Stephenson, 1926; Willmer, 1990). Many sea anemones (class Anthozoa, order Actinaria) exhibit bilaterality (Fig. 4B). Like *Hydra*, the external appearance of a sea anemone is that of a simple cylinder with a single central axis of polarity, the oral-aboral axis. The external radial symmetry is nearly perfect, but it is disrupted by the slit-shaped mouth and external openings on the oral disk (Stephenson, 1926). In cross-section, many anemones exhibit bilateral symmetry in the orientation of the mesenteries about the pharynx (Fig. 4B). The mesenteries are membranous lamellae constructed of two layers of endodermal epithelium surrounding an inner core of mesoglea (Stephenson, 1926). The mesenteries provide structural support for the tubular pharynx, a structure not found in *Hydra*. In addition, the mesenteries carry muscle fibers and serve as the location for the developing gametes. The axis of bilateral symmetry in sea anemones is known as the directive axis (Stephenson, 1926), and it passes through the long axis of the slit-like pharynx. Typically, the pharynx will possess one or two ciliated grooves, or siphonoglyphs, located at opposite ends of the pharynx along the directive axis (see asterisks in Fig. 4B). In species with only a single siphonoglyph (*e.g.*, *Nematostella*, Stephenson, 1926), the polarity of the directive axis is even more obvious than in species with two siphonoglyphs. What is the relationship of the secondary axis in sea anemones (the directive axis) to the secondary axis in bilaterians (the D-V axis)? If these axes have a monophyletic origin dating from the CBA, then homologous genes may yet be responsible for patterning the axis in bilaterians and cnidarians.

A hallmark of dorsal-ventral polarity in many bilaterians is the asymmetrical location of the central nervous system. In vertebrates, the CNS is located dorsally with the gut and major vessels located more ventrally. In arthropods the CNS is located ventrally. This reversal in the relative location of the CNS led Geoffroy St-Hilaire to propose in 1822 that the dorsal-ventral axes of vertebrates and arthropods are homologous but reversed in their typical orientation to the substratum (Saint-Hilaire, 1822). In other words, dorsal in vertebrates corresponds to ventral in arthropods.

The «dorsal-ventral inversion» hypothesis failed to gain broad acceptance until 1995, when homologous D-V patterning mechanisms were found to be operating in vertebrates and insects (Holley *et al.*, 1995). In *Drosophila*, the TGF- β family member *decapentaplegic* (*dpp*) is expressed in the dorsal blastoderm and is required for patterning the dorsal region of the embryo (reviewed in Ferguson, 1996). *Dpp* is antagonized by *short gastrulation* (*sog*), which is expressed in the ventral-lateral blastoderm, in those cells that will give rise to the neurogenic

ectoderm. In *Xenopus*, the TGF- β family member *BMP4* is expressed in the ventral blastomeres where it acts to promote ventral fates in cells destined to become mesoderm. Therefore the expression of *BMP4* is reversed along the D-V axis relative to its *Drosophila* homolog *dpp*. (*BMP4* is not a direct ortholog of *dpp*. *BMP4* is the product of a gene duplication that also produced *BMP2*. The common ancestor of *BMP4* and *BMP2* appears to have been the *dpp* ortholog.) *Chordin*, the *Xenopus* homolog of *sog*, is expressed dorsally in cells that will give rise to dorsal mesodermal structures such as the notochord (reviewed in Ferguson, 1996). As *sog* antagonizes *dpp* in *Drosophila*, *chordin* is thought to antagonize *BMP4* in *Xenopus*. So, homologous mechanisms appear to pattern the D-V axis of vertebrates and insects, but the relative orientation of the axis has been inverted.

Recently, a *dpp* ortholog has been isolated from two species of anthozoan cnidarians, and its expression has been characterized during embryogenesis and larval development. In the coral, *Acropora millepora*, *dpp* mRNA accumulates near the blastopore of the gastrulating embryo (Hayward *et al.*, 2002). Its expression is not symmetrical about the primary body axis, which runs through the blastopore. Rather, expression is concentrated in one quadrant of the surface ectoderm next to the blastopore (Hayward *et al.*, 2002). In the sea anemone, *Nematostella*, *dpp* transcript is also expressed in an asymmetrical fashion adjacent to the blastopore at the onset of gastrulation (Fig. 5; Finnerty *et al.*, 2003). Later in development, *dpp* transcript is expressed in a bilaterally symmetrical fashion about the directive axis. It is expressed in each of the developing mesenteries, and it is expressed in the pharyngeal ectoderm at one end of the long axis of the pharynx (Fig. 5B).

The *dpp* expression patterns obtained for *Acropora* and *Nematostella* are consistent with *dpp* having a role in patterning an axis that is orthogonal to the primary body axis (Hayward *et al.*, 2002; Finnerty *et al.*, 2003). Other interpretations are plausible though (Hayward *et al.*, 2002). For example, in sea urchins, expression of BMP2/4 helps establish a boundary between presumptive endoderm and ectoderm (Angerer *et al.*, 2000). *Dpp*

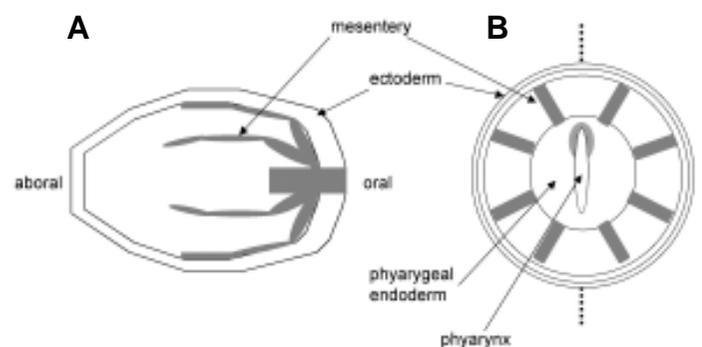
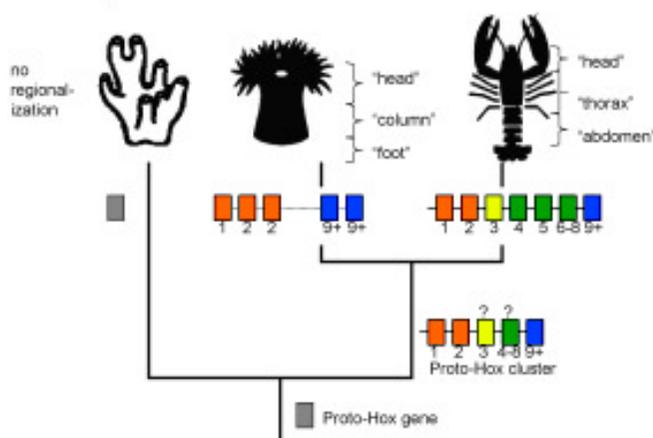


Fig. 5. Expression of *dpp* in *Nematostella* larvae (modified from Finnerty *et al.*, 2003). (A) A view of the internal anatomy of a half-animal sectioned along the primary body axis. Four of the eight developing mesenteries are visible. (B) A cross-section through the pharyngeal region. *Dpp* expression is indicated in dark gray. *Dpp* transcript accumulates in the eight mesenteries and in the pharyngeal endoderm at one end of the directive axis (gray shading).

	PORIFERA	CNIDARIA	BILATERIA
1° Axis	absent	oral-aboral	A-P
Presence of <i>Hox</i> genes	no	yes	yes
Staggered <i>Hox</i> expression along 1° axis	n/a	yes	yes
2° Axis	absent	directive	D-V
Presence of <i>dpp</i>	no	yes	yes
Asymmetric <i>dpp</i> expression along 2° axis	n/a	yes	yes

Fig. 6. Phylogenetic reconstruction of body axes and patterning genes in major metazoan lineages. Ancestral *Hox* gene complements are indicated along the branches using the color scheme presented in Fig. 2. Group 3 and central class *Hox* genes have so far not been convincingly identified in cnidarians (Finnerty, 2001). Therefore, their existence in the cnidarian-bilaterian ancestor is uncertain (?). Character state assignments in the table are based upon the results summarized in the text.



is expressed at or near the endoderm-ectoderm junction in both *Acropora* and *Nematostella*, and it may be involved in the specification of germ layers. However, the post-gastrulation expression of *dpp* in *Nematostella* occurs after the establishment of the germ layers. In fact, *dpp* is expressed in both endodermal structures (mesenteries) and ectodermal structures (the lumen of the pharynx). Furthermore, this late expression of *dpp* is clearly asymmetrical relative to the directive axis (Fig. 5B). In this case, a role for *dpp* in patterning the directive axis seems more likely than a role in germ layer specification.

Summary

Some cnidarians and most bilaterians are united by the possession two orthogonal axes of polarity. Together, these axes produce bilateral symmetry. While the bilateral symmetry of a sea anemone is far less overt than the bilateral symmetry of a mouse or a fruitfly, a comparison of gene expression patterns suggests that this fundamental body plan feature is homologous between cnidarians and bilaterians. The anterior-posterior axis of bilaterians is known to be patterned by staggered *Hox* expression domains. In cnidarians, *Hox* expression domains are similarly staggered along the oral-aboral axis. The dorsal-ventral axis of bilaterians is patterned partially by the asymmetrical expression of *dpp*/BMP4. Likewise, *dpp* is expressed asymmetrically relative to directive axis of sea anemones. From these findings, we may tentatively conclude that bilateral symmetry is a synapomorphy of bilaterians and cnidarians.

Attempting to infer structural homology from molecular evidence is fraught with difficulty (Abouheif *et al.*, 1997). However, the hypothesis presented here does not imply structural homology. Rather, the utilization of homologous developmental genes by cnidarians and bilaterians to pattern their primary and secondary body axes suggests homology at the level of the patterning mechanisms themselves, not the structures whose locations are governed by the patterning mechanisms. It may be the case that bilaterians and cnidarians

inherited the same basic axial ground plan from their common ancestor. Since their divergence, bilaterians appear to have elaborated upon this organizational scaffold to a greater degree than their cnidarian sister group (*e.g.*, deploying a greater number of *Hox* genes to encode a finer degree of regionalization), and they have invented numerous organs and structures to superimpose upon the scaffold such as brains, circulatory systems, skeletal systems, locomotory structures, digestive organs, and sense organs.

In the coming years, a different interpretation of the comparable *Hox* and *dpp* expression patterns of cnidarians and bilaterians may gain favor (see Erwin and Davidson, 2002). Supportive or contradictory evidence for the hypothesis that bilateral symmetry is homologous in cnidarians and bilaterians could come from any of the following

sources: (1) investigation of *dpp* expression during embryogenesis and larval development in more cnidarian taxa, those displaying bilateral symmetry as well as those displaying radial, biradial, or tetradial symmetry; (2) identification and characterization of a *sog*/*chordin* homolog in cnidarians; (3) characterization of *Hox* gene expression and *dpp* expression in the comb jellies (phylum Ctenophora), another so-called radiate phylum that appears to be an outgroup to the clade containing Cnidaria and Bilateria and that may display biradial or bilateral symmetry. As numerous recent reviews on bilaterian origins have emphasized (*e.g.*, Holland, 2000; Erwin and Davidson, 2002), there is an urgent need to study the non-bilaterian phyla for insights into early metazoan evolution. As a complementary approach, functional studies of cnidarian proteins in bilaterian systems may support hypotheses of homology by providing evidence of functional equivalency. However, failure of cnidarian proteins to mimic the function of bilaterian proteins in bilaterian embryos does not contradict a hypothesis of homology.

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