

HYPOTHESIS

On the evolution of bilaterality

Grigory Genikhovich* and Ulrich Technau*

ABSTRACT

Bilaterality – the possession of two orthogonal body axes – is the name-giving trait of all bilaterian animals. These body axes are established during early embryogenesis and serve as a three-dimensional coordinate system that provides crucial spatial cues for developing cells, tissues, organs and appendages. The emergence of bilaterality was a major evolutionary transition, as it allowed animals to evolve more complex body plans. Therefore, how bilaterality evolved and whether it evolved once or several times independently is a fundamental issue in evolutionary developmental biology. Recent findings from non-bilaterian animals, in particular from Cnidaria, the sister group to Bilateria, have shed new light into the evolutionary origin of bilaterality. Here, we compare the molecular control of body axes in radially and bilaterally symmetric cnidarians and bilaterians, identify the minimal set of traits common for Bilateria, and evaluate whether bilaterality arose once or more than once during evolution.

KEY WORDS: Bilateria, Cnidaria, Bilateral symmetry, Body axes

Introduction

Most animals belong to Bilateria (see Glossary, Box 1), a group encompassing organisms with three germ layers (ectoderm, endoderm and mesoderm) and two body axes, i.e. an anterior-posterior axis and a dorsal-ventral (D-V) axis. Body axes can be thought of as systems of molecular coordinates (Niehrs, 2010), allowing different parts of the body to develop differently. For example, the central nervous system develops at the dorsal side of the vertebrate body, but ventrally in insects and many other animals. The anterior end is usually characterized by a concentration of sensory organs, such as eyes and the olfactory system. Bilaterality also favours the formation of left-right asymmetry in many animals, including vertebrates. However, among the non-bilaterian Metazoa (see Glossary, Box 1), other types of symmetry exist (Fig. 1). For example, sponges (Porifera), although missing a clear body symmetry in their modular, sessile adult state, have an obvious radial symmetry as larvae. Comb jellies (Ctenophora) are bi-radially symmetric, with an oral-aboral axis and two other planes of symmetry, one going through the bases of the tentacles and the other through the slit-like mouth. Placozoans are irregularly shaped, crawling animals that exhibit a dorsal and ventral surface, although how these surfaces arise is unclear as placozoan embryogenesis is unknown. These various types of symmetry and body axes raise the question of how and when bilaterality – a trait that likely contributed to the diversification of body plans (see Box 2) – might have arisen.

In the debate about the evolutionary origin of bilaterality, Cnidaria, which are robustly recovered as the phylogenetic sister

group of Bilateria (Cannon et al., 2016; Hejnol et al., 2009; Moroz et al., 2014; Philippe et al., 2011; Pisani et al., 2015; Whelan et al., 2015), are of particular interest. Cnidarian morphology does not permit one to distinguish a dorsal and a ventral side, and no obvious left-right asymmetry exists. However, while four cnidarian classes (Hydrozoa, Scyphozoa, Cubozoa and Staurozoa; uniting various jellyfish and hydroids) are combined into the Medusozoa, which consist of animals with radial symmetry, members of the fifth cnidarian class Anthozoa (encompassing hard corals, sea anemones, soft corals and sea pens) (Collins et al., 2006) are bilaterally symmetric (Fig. 1); in addition to the oral-aboral axis that is common to all Cnidaria, anthozoans have a second, so-called ‘directive’, axis running along the slit-like pharynx orthogonally to the oral-aboral axis. It is therefore particularly interesting to examine whether the bilaterality of Anthozoa and Bilateria was inherited from a bilaterally symmetric common ancestor and then lost in the radially symmetric Medusozoa, or whether it evolved convergently (Fig. 1). Furthermore, in the case of a homologous origin, we wish to know which of the cnidarian body axes correspond to the anterior-posterior and to the D-V body axis of Bilateria. In this review, we summarize and compare the molecular regulation of body axes in Bilateria and in cnidarians, as well as in Porifera and Ctenophora, and evaluate different scenarios of how bilaterality may have emerged. In order to do so, we first define the sets of features that are typical for the body axes of bilaterians, cnidarians and the evolutionary outgroups to the bilaterian-cnidarian clade: the ctenophores and sponges.

Reconstructing the bilaterian ancestor

Axis formation has been studied most thoroughly in several vertebrates (i.e. mouse, zebrafish, *Xenopus*) and especially in the fruit fly *Drosophila*, which became the textbook example. In *Drosophila*, maternally established gradients of the transcription factor Bicoid and the RNA-binding protein Nanos define the anterior and posterior ends, and activate a complex cascade of gene regulatory interactions that eventually lead to segmentation and regional specification of the anterior-posterior axis (Gilbert, 2010). However, in other arthropods, such as beetles, centipedes and spiders, as well as in vertebrates, segmentation involves a Hairy/Notch/Delta-dependent clock-like oscillation mechanism, which is initiated and controlled by a gradient of Wnt signalling (and, in the case of vertebrates, also by FGF signalling) from the posterior end (Chipman and Akam, 2008; El-Sherif et al., 2012; Gomez et al., 2008; Janssen et al., 2010; McGregor et al., 2008; Schönauer et al., 2016; Stollewerk et al., 2003). Strikingly, the crucial developmental regulator *bicoid* turned out to be an evolutionary innovation within Diptera (the insect order that includes *Drosophila*) that arose through duplication and divergence of the *hox3* gene homologue *zerknüllt* (Stauber et al., 2002). This clearly shows how plastic the crucial aspects of the regulation of animal development can be, and also highlights the importance of broad phylogenetic sampling when defining ancestral molecular features.

Department for Molecular Evolution and Development, Centre of Organismal Systems Biology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria.

*Authors for correspondence (grigory.genikhovich@univie.ac.at; ulrich.technau@univie.ac.at)

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Box 1. Glossary

Acoela. A group of animals with a single gut opening previously thought to be members of flatworms, but now usually placed within the earliest branching bilaterian lineage Xenacoelomorpha.

Ambulacraria. Besides chordates, one of the two major clades of Deuterostomia. Ambulacraria includes echinoderms (sea urchins, starfish, etc.) and hemichordates. In contrast to chordates, ambulacrarians do not have a centralized nervous system and, similar to non-deuterostome Bilateria, possess a ventral BMP signalling minimum.

Amphistomy. The mode of gastrulation in which the lateral lips of the blastopore fuse in a slit-like fashion leaving two openings: an anterior mouth and a posterior anus connected by a U-shaped gut.

Bilateria. The phylogenetic group of bilaterally symmetric animals, consisting of three germ layers. Bilateria are subdivided into Xenacoelomorpha, Deuterostomia and Protostomia.

Chordata. The second major clade of Deuterostomia, including cephalochordates (amphioxus), tunicates (ascidians, larvaceans, etc.) and vertebrates.

Deuterostomia. An animal group consisting of Ambulacraria and Chordata. The name comes from the fact that their mouth forms separately from the blastopore.

Ecdysozoa. An animal clade uniting moulting animals (nematodes, priapulids, arthropods, etc.).

GLWamide-positive neurons. Neurons expressing neuropeptides carrying GLWamide on the C terminus.

Lophotrochozoa. An animal clade uniting groups with trochophore-like larvae (molluscs, annelids, ribbon worms, etc.) and lophophorate animals (bryozoans, brachiopods, etc.). Currently considered as a subclade within Spiralia, which include also Gnathifera (gnathostomulids, rotifers, etc.) and Rousphozoa (flatworms, gastrotrichs), and uniting animals with spiral cleavage.

Mesenteries. Endodermal folds of anthozoans harbouring longitudinal muscles and gonads.

Metazoa. The clade uniting all animal phyla.

Planula. A type of diploblastic ciliated larva typical for all cnidarian clades.

Primary polyp. A developmental stage following metamorphosis of the cnidarian planula. A *Nematostella* primary polyp has four tentacles. As it develops, further tentacles will intercalate between the first four.

Protostomia. An animal group well supported by molecular phylogenies and containing Spiralia and Ecdysozoa. The name comes from the notion that, in protostomes, the mouth forms directly from the blastopore, which is not always the case.

Urbilaterian. The last common ancestor of all Bilateria.

In recent years, careful gene expression studies have shown stunning similarities in the patterning processes between vertebrates and invertebrates. The most important similarities are: (1) anterior expression of the ‘anterior brain genes’ *six3/6*, *foxq2*, *rx*, *iroquois* and *fgfr* (Marlow et al., 2014; Steinmetz et al., 2010; Tosches and Arendt, 2013; Vopalensky et al., 2012); (2) posterior expression of *caudal* and *wnt* (Gomez et al., 2008; Janssen et al., 2010; Wu and Lengyel, 1998); (3) oscillating expression of *hairy/hes* downstream of Wnt and Delta/Notch signalling in the developing segments (Gomez et al., 2008; McGregor et al., 2008; Schöner et al., 2016; Stollewerk et al., 2003); (4) patterning of the anterior-posterior axis by the staggered expression of Hox genes (Krumlauf et al., 1993; Mallo and Alonso, 2013; Montavon and Soshnikova, 2014); (5) repression of BMP signalling on the side of the D-V axis where the central nervous system will form (Mizutani et al., 2006; Sasai et al., 1995; Smith and Harland, 1992); (6) similar expression of the foregut and hindgut marker genes in the larvae of Deuterostomia and Protostomia (see Glossary, Box 1) (Arendt et al., 2001); and (7) similar mediolateral expression of the transcription factor encoding

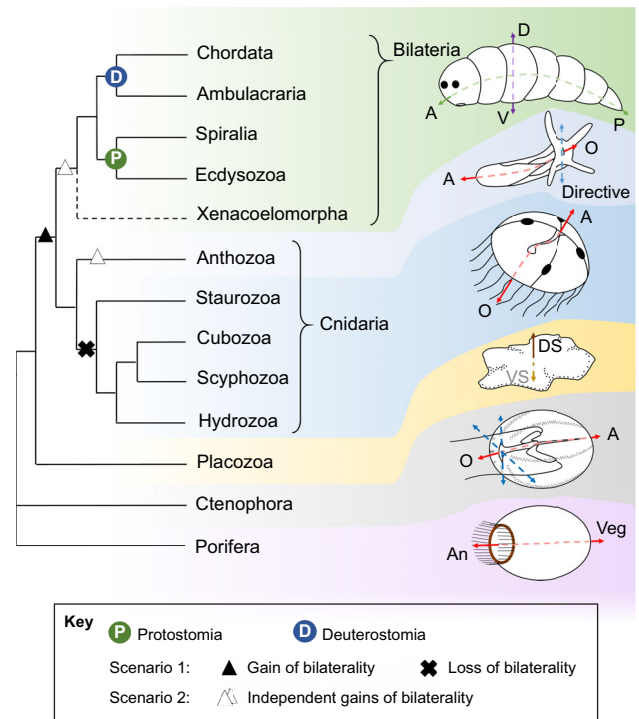
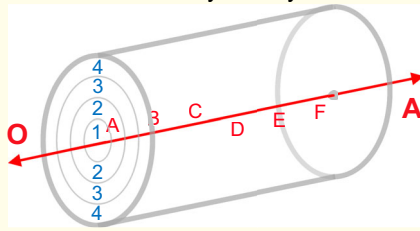


Fig. 1. The distribution of different body symmetries among animals.

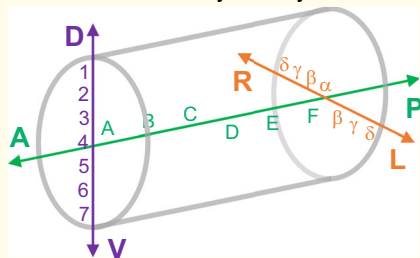
Alternative scenarios that can explain the emergence of bilaterality are depicted. An-Veg, animal-vegetal; DS-VS, dorsal surface-ventral surface; O-A, oral-aboral; A-P, anterior-posterior; D-V, dorsal-ventral.

genes *nk2.2*, *nk6*, *pax6* and *msx*, which pattern the ventral nerve cord of flies and worms, and the dorsal neural tube of vertebrates (Arendt et al., 2008; Denes et al., 2007; Tessmar-Raible et al., 2007).

As it does not seem likely that these similarities evolved multiple times independently, one can reconstruct a relatively complex hypothetical common ancestor of all Bilateria – the urbilaterian (see Glossary, Box 1) – resembling an annelid worm: i.e. with an anterior brain, a central nervous system in the trunk, a segmented body and a through gut (Tessmar-Raible and Arendt, 2003). However, this hypothesis (the ‘complex urbilaterian’ hypothesis) has been challenged in a recent review by Hejnol and Lowe (Hejnol and Lowe, 2015). The authors argue that the highly complex spatial expression of the transcription factors known to pattern the nervous system along the anterior-posterior axis does not necessarily imply the presence of a morphologically complex central nervous system in the animal. As an example, they illustrate this with the hemichordate *Saccoglossus kowalewski*, which exhibits a simple nervous system and expression of nervous system-patterning genes similar to that in Chordata (see Glossary, Box 1; Hejnol and Lowe, 2015). They show that the genes patterning the forebrain (*rx*, *six3*, *foxg*, *nk2-1*), midbrain (*emx*, *otx*, *pax6*, *lim1/5*) and hindbrain (*en*, *gbx*) in chordates are expressed in, respectively, the anterior, middle and posterior domains in *S. kowalewski*, yet not in the nervous system, but throughout the ectoderm. Based on this, they argue that the initial role of these genes might have been to pattern the ectoderm, not only the neuroectoderm (Hejnol and Lowe, 2015). It is difficult, though, to exclude the possibility that the deuterostome ancestor had a complex central nervous system that was lost at the base of Ambulacraria (see Glossary, Box 1). The main challenge to the ‘complex urbilaterian’ hypothesis comes, however, with their second argument, which we outline below.

Box 2. Bilaterality and the diversification of body plans**Radial symmetry**

$6 \times 4 = 24$ unique coordinates

Bilateral symmetry

$6 \times 7 \times 4 = 168$ unique coordinates

With the emergence of bilateral symmetry, the amount of positional information in a system is multiplied. If we assume, for example, a coordinate system of six positional values along the oral-aboral (O-A) body axis and four positional values from the centre line to the periphery, then a radially symmetric organism has 24 unique arbitrary coordinates (top). By contrast, a bilaterally symmetric organism with similarly spaced positional values has six positional values along the anterior-posterior (A-P) axis, seven along the dorsal-ventral (D-V) axis and four along the left-right (L-R) axis, which makes 168 unique coordinates (bottom). Hence, the emergence of bilaterality has likely contributed to the diversification of body plans among Bilateria, which make up the vast majority of the described animal phyla.

In order to reconstruct ancestral features, one must rely on phylogenetic trees. During the past 20 years, views on evolutionary relationships among animals have experienced several major changes: from the ‘new animal phylogeny’ splitting Bilateria into Lophotrochozoa (currently, part of a larger clade Spiralia), Ecdysozoa and Deuterostomia (see Glossary, Box 1) (Adoutte et al., 2000; Aguinaldo et al., 1997; Halanych et al., 1995; Struck et al., 2014), up to the recent disputed placement of Ctenophora as the earliest branching animal group (Cannon et al., 2016; Dunn et al., 2008; Hejnol et al., 2009; Moroz et al., 2014; Philippe et al., 2011; Pisani et al., 2015; Simion et al., 2017; Whelan et al., 2015). One other such major change was the proposed placement of the Acoela (see Glossary, Box 1), previously nested within the spiralian phylum Platyhelminthes (flatworms), as a sister to all other Bilateria (Cannon et al., 2016; Hejnol et al., 2009; Ruiz-Trillo et al., 1999). In all recent trees (Cannon et al., 2016; Hejnol et al., 2009; Philippe et al., 2011; Pisani et al., 2015; Struck et al., 2014; Whelan et al., 2015), annelids occupy a position within Spiralia, and several spiralian clades encompassing unsegmented animals with non-centralized trunk nervous systems diverged earlier than annelids (Struck et al., 2014). This would imply multiple independent losses of segmentation and multiple independent decentralizations of the trunk nervous system outside annelids, arthropods and chordates (Hejnol and Lowe, 2015) – an event arguably as improbable as the independent emergence of the similarities in the patterning

mechanisms we mentioned above. Moreover, if we accept that Xenacoelomorpha (acoels, nemertodermatids and xenoturbellids) represent a sister group to all remaining Bilateria, and that the absence of a through gut in them is not a result of a secondary reduction, the urbilaterian might have looked quite different. This unsegmented ‘simple urbilaterian’ with a non-centralized nervous system in the trunk might have had a blind gut and resembled an acoel worm (Hejnol and Martindale, 2008a; Srivastava et al., 2014). In future, it will be essential to establish whether some traces of the complex genetic regulation suggested for the ‘complex urbilaterian’ are recognizable in the unsegmented members of Ecdysozoa and Spiralia with non-centralized nervous systems (Hejnol and Lowe, 2015).

Regardless of which of the two hypotheses may be correct, comparison of the ‘complex urbilaterian’ and the ‘simple urbilaterian’ hypotheses allows us to define a minimal set of ancestral bilaterian characters, which we use in the cnidarian-bilaterian comparison below. This comparison suggests that an urbilaterian seems to have been a worm-like animal with an anteriorly localized neural plexus or brain, which expressed apical organ/forebrain genes such as Wnt antagonists, *six3/6*, *foxq2*, *iroquois*. It had a posterior-to-anterior gradient of Wnt signalling. Its anterior-posterior axis was patterned by staggered Hox gene expression, and its D-V axis was regulated by a gradient of BMP signalling. Whether it had a through or a blind gut is uncertain, and we thus address both possibilities separately, when comparing the relation between the cnidarian and bilaterian body axes below.

The bilaterally symmetric non-bilaterian

Owing to their sister relationship to Bilateria, the Cnidaria are highly informative for addressing the question of how bilaterality might have arisen. All cnidarians have a main body axis, termed the oral-aboral axis, which in medusozoans (Hydrozoa, Cubozoa, Scyphozoa, Staurozoa) is also the only body axis. All molecular evidence from *Hydra*, *Hydractinia*, *Clytia* and *Nematostella* suggests that the oral-aboral body axis is patterned by Wnt/ β -catenin signalling (Kraus et al., 2016; Lapébie et al., 2014; Lee et al., 2007; Momose et al., 2008; Momose and Houliston, 2007; Röttinger et al., 2012; Wikramanayake et al., 2003). Suppressing Wnt/ β -catenin signalling results in the failure to gastrulate and form an oral-aboral axis both in the hydroid *Clytia* and in the anthozoan *Nematostella* (Leclère et al., 2016; Momose et al., 2008; Momose and Houliston, 2007; Wikramanayake et al., 2003). In line with this, ectopic activation of Wnt/ β -catenin signalling results in the formation of ectopic heads in the hydroids *Hydra* and *Hydractinia*, as well as in *Nematostella* (Broun et al., 2005; Guder et al., 2006; Kraus et al., 2016; Plickert et al., 2006). In terms of embryonic axial patterning, the best-studied cnidarian models are the radially symmetric hydroid *Clytia hemisphaerica* and the bilaterally symmetric sea anemone *Nematostella vectensis*. As mentioned above, anthozoans have an additional, second body axis perpendicular to the oral-aboral axis, and thus are particularly informative when examining the evolution of bilaterality. We therefore concentrate on the anthozoan *Nematostella* (Fig. 2) and provide a brief overview of the establishment and molecular control of its body axes.

Formation of the oral-aboral body axis in *Nematostella*

In Bilateria, gastrulation generally takes place at the vegetal pole of the embryo, where yolk-rich blastomeres give rise to the endodermal lineage (Brusca et al., 1997). Strikingly, with the few exceptions when endoderm is formed in an axial position-

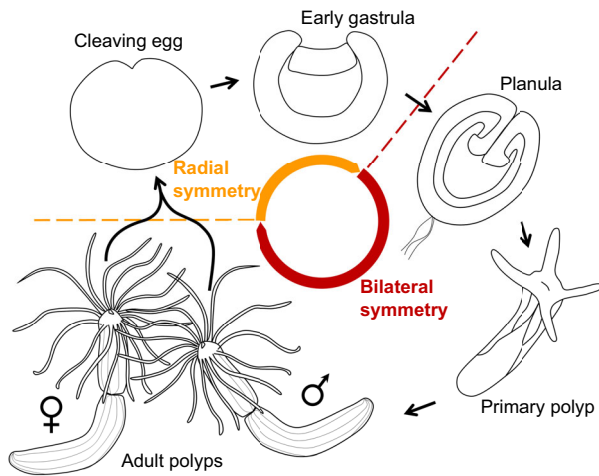


Fig. 2. The life cycle of *Nematostella*. The life cycle of *Nematostella vectensis* is shown (not to scale). The cleaving egg and early gastrula exhibit radial symmetry, which is then broken at late gastrula stage.

independent manner, cnidarians, including *Nematostella*, gastrulate from the animal pole (Byrum and Martindale, 2004; Fritzenwanker et al., 2007; Lee et al., 2007; Tardent, 1978) and their animal blastomeres form the endoderm. Gastrulation from the animal pole is also a feature of the earlier branching phylum Ctenophora. It has been postulated that an animal-vegetal axis inversion leading to gastrulation from the vegetal pole is likely to have happened at the base of Bilateria (Martindale and Hejnol, 2009). This notion is supported by similar developmental capacities of the cnidarian animal and bilaterian vegetal hemisphere material. Bisection experiments of cleaving or gastrulating *Nematostella* embryos (Fritzenwanker et al., 2007; Lee et al., 2007) showed that fragments of the embryo containing animal hemisphere material are capable of forming normal, although proportionally smaller, primary polyps (see Glossary, Box 1; Fig. 3A), whereas fragments containing only vegetal hemisphere material develop, in most cases, into ciliated spheres of aboral tissue without any obvious polarity. The same experiment (Fig. 3B) performed with sea urchin embryos yields similar results; however, it is the vegetal halves of the embryo that are capable of giving rise to larvae, while the animal halves, like the vegetal halves in *Nematostella*, give rise to ciliated spheres (Hörstadius, 1939). Body axis inversions, like the suggested D-V axis inversion at the base of chordates (Arendt and Nübler-Jung, 1994; Geoffroy St.-Hilaire, 1822), further complicate the concept of the body axes homology. However, we consider cnidarian and bilaterian gastrulation poles to be homologous based on: (1) their similar developmental capacity to form endomesoderm; (2) similar morphogenetic movements necessary for gastrulation (Tardent, 1978); and (3) similar regulatory events leading to the specification of the endomesodermal territory (Henry et al., 2008; Lee et al., 2007; Logan et al., 1999; Schneider et al., 1996; Wikramanayake et al., 2003) and driving gastrulation (Arendt et al., 2001; Kumburegama et al., 2011; Lee et al., 2007; Tada and Smith, 2000).

The axial organizer in *Nematostella*

The bisection experiments discussed above suggest the presence of a signalling centre in the animal hemisphere of the *Nematostella* embryo capable of instructing naïve cells in the vegetal hemisphere. Transplantation of minute fragments from different parts of the *Nematostella* mid-gastrula into the aboral ectoderm showed that only the blastopore lip implants (Fig. 3C), but not the pre-

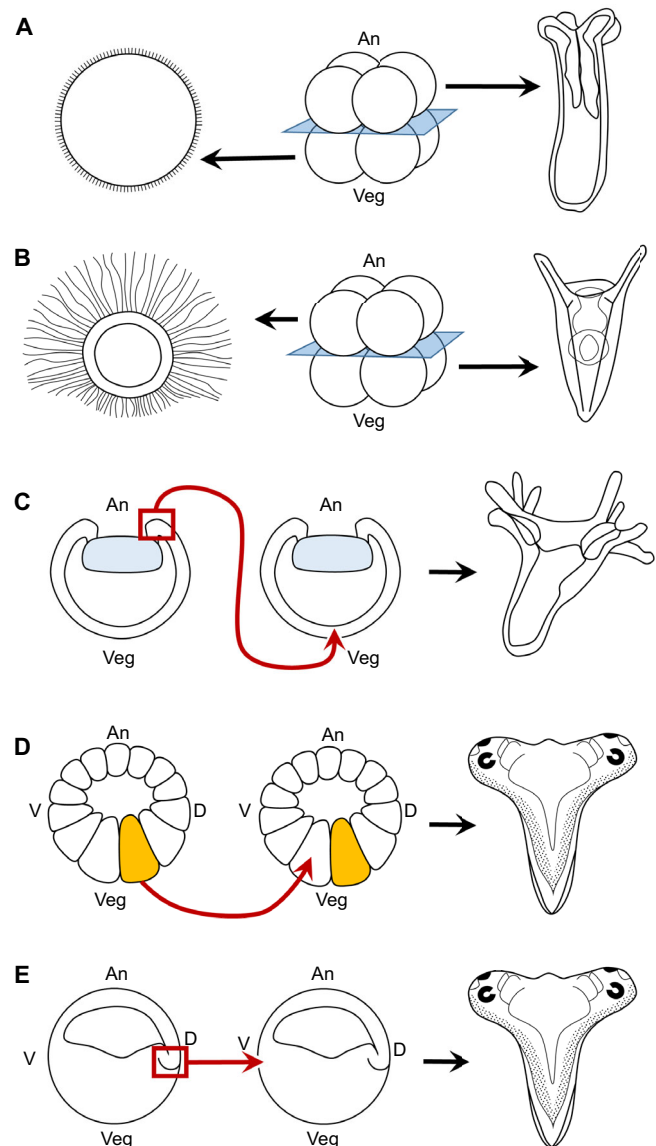


Fig. 3. β -Catenin signalling-dependent axial organizer capacity. (A,B) Bisection experiments in *Nematostella* (A) and sea urchin (B) demonstrate the presence of axial determinants in the cytoplasm inherited by cells giving rise to the gastrulation pole. For both *Nematostella* and the sea urchin, a primary polyp (A) or a pluteus larva (B) with normal body axes develops from only the half of the embryo where gastrulation would normally take place. (C,D) Transplantations of the blastopore lip of *Nematostella*, just like transplantations of the amphibian Nieuwkoop centre (D) or Spemann-Mangold organizer (E) into a ventral position, result in the formation of the Siamese twin embryos. An, animal pole; Veg, vegetal pole; D, dorsal; V, ventral.

endodermal plate or the aboral ectoderm implants, are able to induce the formation of an ectopic body axis in the recipient (Kraus et al., 2007). Similar to the situation in the famous Mangold-Spemann experiment (Spemann and Mangold, 1924), this twin new body is mainly built by recipient cells, and only the tip of the axis contains donor cells (Kraus et al., 2007).

Molecularly, the capacity of the animal hemisphere material to drive the development of the oral-aboral axis relies on Wnt/ β -catenin signalling components. Dishevelled protein is localized at the animal pole of the unfertilized *Nematostella* egg and then forms a gradient in the animal half of the blastula (Lee et al., 2007).

β -Catenin is stabilized in the animal hemisphere nuclei from the 32-cell stage onwards and marks the gastrulation site (Wikramanayake et al., 2003). Sequestering β -catenin or knocking it down completely blocks gastrulation, and the embryo remains a perfect sphere without any trace of body axes (Leclère et al., 2016; Wikramanayake et al., 2003). However, besides its role in gastrulation, Wnt signalling is also involved in patterning the oral-aboral axis of *Nematostella*. Eleven out of 12 *Nematostella* Wnt genes are expressed orally in staggered domains along the oral-aboral axis of the embryo and larva, while the genes encoding the Wnt antagonists Dickkopf1/2/4 and SFRP1 are expressed at the aboral pole (Kusserow et al., 2005; Lee et al., 2006; Leclère et al., 2016; Marlow et al., 2013; Sinigaglia et al., 2015). Recent work has shown that the axis-inducing capacity of the blastopore lip of *Nematostella* gastrulae is conveyed by two Wnt ligands: Wnt1 and Wnt3 (Kraus et al., 2016). Moreover, co-expressing these two Wnt ligands in any region of the gastrula ectoderm is sufficient to transform it into an axial organizer (Kraus et al., 2016). The existence of a Wnt/ β -catenin-dependent axial organizer associated with the blastopore (Fig. 3C–E) appears to be a conserved feature found in cnidarians, vertebrates (Nieuwkoop centre functioning as an axial organizer and inducing the Spemann–Mangold organizer) (De Robertis et al., 2000; Gimlich, 1985; Heasman et al., 1994) and sea urchins (Logan et al., 1999; Ransick and Davidson, 1993), but possibly also in protostomes (Itow et al., 1991; Nakamoto et al., 2011), although the molecular underpinnings of the axial organizer capacity in protostomes are still understudied. Taken together, these studies highlight that the definitive oral-aboral axis of the *Nematostella* embryo corresponds to the animal-vegetal axis of the egg, and that its development is Wnt/ β -catenin signalling dependent.

Formation of a second body axis in *Nematostella*

Strikingly reminiscent of the situation in bilaterians, the second directive body axis in anthozoans is regulated by BMP signalling. However, in contrast to Bilateria, where definitive anterior-posterior and D–V body axes are usually established simultaneously and early in development, the directive axis in *Nematostella* manifests itself at the end of gastrulation, downstream of the maternally established oral-aboral axis. At this time, the initial, radially symmetric expression of the *bmp4* orthologue *dpp* and of the BMP antagonist *chordin*, which is established by β -catenin signalling (Kraus et al., 2016), shifts to one side of the directive axis (Rentzsch et al., 2006) in a BMP signalling-dependent symmetry break (Saina et al., 2009) (Fig. 4A). We are just beginning to understand how the regulatory systems of the two cnidarian body axes are wired and coordinated (Wijesena et al., 2017); however, some interesting details are already known. Owing to differences in the wiring of the gene regulatory network in *Nematostella*, *dpp* and *chordin* are expressed on the same side of the body axis, unlike in *Drosophila* or frog (Genikhovich et al., 2015; Rentzsch et al., 2006). Once the directive axis is established, the expression of *dpp* and *chordin* becomes independent of the β -catenin input (Kraus et al., 2016). Under the control of BMP signalling, two signalling centres are then established on opposite sides of the directive axis (Fig. 4A), each expressing its own set of BMPs (Dpp and BMP5–8, on one side; and GDF5-like, on the other) and BMP antagonists (Chordin on one side and Gremlin on the other). The interaction of these secreted factors facilitates and shapes a gradient of BMP signalling along the directive axis (Genikhovich et al., 2015; Leclère and Rentzsch, 2014). As in Bilateria (Mizutani et al., 2005), Chordin likely shuttles BMP ligands away from the *chordin*-expressing side, creating a

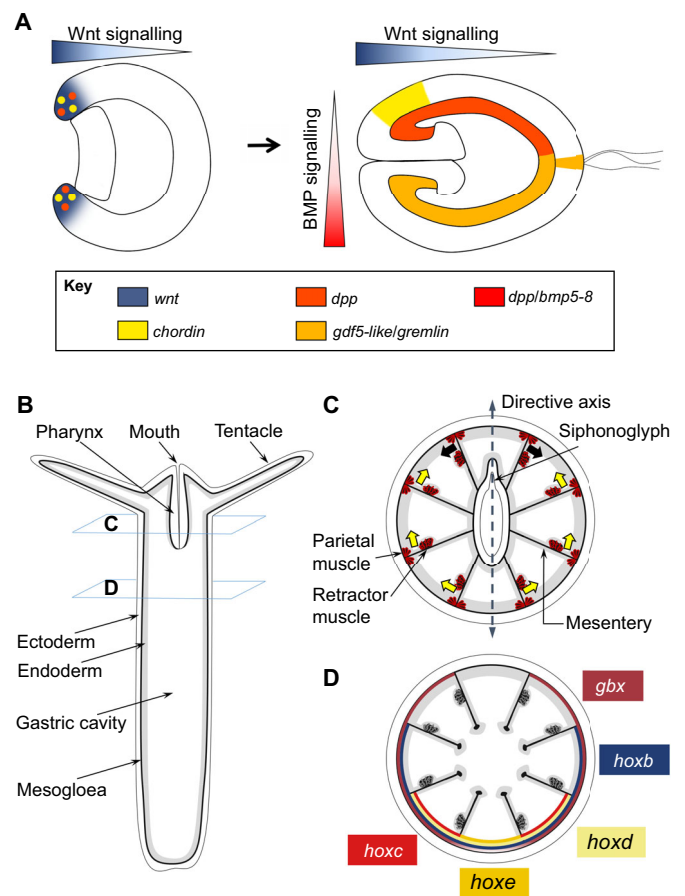


Fig. 4. Molecular and morphological manifestations of bilaterality in *Nematostella*. (A) In the early gastrula, the initial expression of *dpp* and *chordin* is radial and controlled by Wnt/ β -catenin signalling. After a symmetry break in BMP expression, the directive axis, with signalling centres on its opposing ends, is established. (B) General anatomy of the *Nematostella* polyp, highlighting its key structures and tissues. (C) Cross-section of the polyp in the pharynx region. The orientation of the directive axis is morphologically obvious throughout the body column from the orientation of the retractor muscles on the mesenteries (black and yellow arrows) and also from the location of the ciliated groove, termed siphonoglyph, in the pharynx. (D) Cross-section of the polyp below the pharynx. The expression domains of *gbx* and four *Hox* genes are shown, highlighting their staggered expression along the directive axis.

BMP signalling maximum on the opposite side of the directive axis (Genikhovich et al., 2015; Leclère and Rentzsch, 2014). Suppression of BMP signalling leads to the disappearance of the directive axis and the radialization of the embryo both at the molecular and morphological level: the eight endodermal folds, termed mesenteries (see Glossary, Box 1, Fig. 4B,C), fail to form and the endoderm remains uncompartimentalized (Genikhovich et al., 2015; Leclère and Rentzsch, 2014).

Loss-of-function experiments have allowed the BMP signalling network that maintains the directive axis in the early planula (see Glossary, Box 1) of *Nematostella* to be deciphered. Topologically, this network resembles the BMP signalling network that patterns the D–V axis in *Xenopus*. However, strikingly, homologous molecules occupy different nodes, and different BMP signalling modulators are used in *Nematostella* and the frog to fine-tune the BMP signalling gradient (Genikhovich et al., 2015). This can either mean that the BMP signalling networks in vertebrates and *Nematostella* evolved independently, but using the same molecular components, or that patterning of the second axis by BMP signalling was a feature

of the cnidarian-bilaterian ancestor, but the networks became significantly diverged. Currently, it is impossible to choose confidently between these two scenarios. Nonetheless, this work highlights the importance of identifying which parameters of signalling networks are strongly constrained and which are not. Computer simulations using a ligand-shuttling model that describes the situation in *Drosophila*, *Xenopus* and *Nematostella* equally well (Genikhovich et al., 2015; Mizutani et al., 2005), demonstrate that if BMP ligand shuttling by Chordin is at work, the area of active BMP signalling is solely defined by the Chordin expression domain, while the precise location of the BMP expression domain is irrelevant (Genikhovich et al., 2015). Previously, experiments of Wang and Ferguson, who expressed *dpp* under the control of the *even skipped* stripe 2 enhancer in a *dpp*-null background in *Drosophila*, yielded exactly the same result (Wang and Ferguson, 2005). Hence, if there is no constraint on the spatial expression of BMPs, and as long as Chordin expression is confined, the BMP expression domain is expected to vary freely between different animal groups. In other words, similar or dissimilar expression patterns of BMPs in relation to *chordin* between different organisms carry little phylogenetic information. This observation is particularly relevant, as comparison of gene expression domains is routinely used to infer homologies in the evolutionary developmental biology (EvoDevo) field.

One important issue, which is still understudied, is the relationship between BMP signalling and the differentiation of the nervous system in *Nematostella*. In bilaterians with centralized nervous systems, the placement of the CNS is linked to the BMP signalling minimum (De Robertis, 2008), although BMP signalling is required for the differentiation of epidermal sensory neurons (Lu et al., 2012). *Nematostella* has a radially symmetric diffuse nervous system in which neurons accumulate in certain regions. Unlike in Bilateria, neurogenesis in *Nematostella* begins at the blastula stage and then continues both in the ectoderm and in the endoderm (Nakanishi et al., 2012). Expression studies on proneural genes suggest that certain neuronal subpopulations may originate in distinct locations along the oral-aboral axis, either directly or indirectly affected by Wnt/ β -catenin signalling (reviewed by Kelava et al., 2015; Watanabe et al., 2014a) independent of their position along the directive axis. The only known exception to this rule are the GLWamide-positive neurons (see Glossary, Box 1), which appear originally on the BMP signalling minimum side regulated by atonal-related protein 6, but later spread throughout the body of the animal (Watanabe et al., 2014a). However, the evolutionary relevance of this finding, as well as the proposed similarity of the molecular signature of the bilaterian CNS and the oral domain of the nervous system of *Nematostella* (Watanabe et al., 2014a), require further analyses. Currently, the most parsimonious explanation is that the common ancestor of Cnidaria and Bilateria developed its nervous system in a BMP signalling-independent way.

***Nematostella* Hox genes**

The co-linearity and homeotic function of Hox genes along the anterior-posterior axis in many bilaterians became a paradigm for the conservation of developmental mechanisms in animals (McGinnis and Krumlauf, 1992). Indeed, this feature was enthusiastically suggested to be a molecular hallmark of being an animal in the ‘Zootype’ concept (Slack et al., 1993). Interestingly, staggered expression of the Hox genes along the anterior-posterior axis is conserved even if the Hox cluster is atomized, as in the urochordate *Oikopleura* (Seo et al., 2004). However, early branching metazoans, such as sponges and ctenophores, lack Hox

genes altogether (Larroux et al., 2007; Moroz et al., 2014; Ryan et al., 2010, 2013; Srivastava et al., 2010), and only a single *Hox/ParaHox* gene has been reported in placozoans (Jakob et al., 2004).

Cnidarians are the only non-bilaterian animals with multiple Hox genes, hence the expression patterns of these genes in relation to the body axes are of particular interest. In *Nematostella*, two Hox genes are expressed at the pharynx and the aboral pole, respectively, while others are expressed in the body wall endoderm, leading to the hypothesis that Hox genes pattern the oral-aboral axis in a manner that is homologous to that patterning the anterior-posterior axis of Bilateria (Finnerty et al., 2004). However, except for the two aforementioned orally and aborally expressed Hox genes, all *Nematostella* Hox genes as well as a non-Hox homeobox gene, *gbx*, are expressed in staggered domains along the directive axis. Furthermore, their expression domains correspond to distinct numbers of mesenterial chambers (Fig. 4D) (Ryan et al., 2007), suggesting a role in positioning the mesenteries. In line with this, knockdown experiments with components of the BMP signalling network showed that BMP signalling is crucial for the formation of the eight endodermal mesenteries in *Nematostella* (Genikhovich et al., 2015). Surprisingly, these experiments also showed that, unlike in Bilateria, the staggered *hox* and *gbx* expression in *Nematostella* is dependent on BMP signalling (Genikhovich et al., 2015).

Regulation of Hox gene expression by BMP signalling, although not common, is not unknown in Bilateria. It has been suggested that early temporally co-linear *Hox* expression in the non-organizer mesoderm of the frog late gastrula is BMP and Wnt dependent (In der Rieden et al., 2010; Wacker et al., 2004). Others have shown that BMP signalling activates Wnt expression, which, in turn, initiates Hox gene and *cdx* expression during hematopoiesis in mouse and zebrafish (Lengerke et al., 2008). Therefore, the possibility cannot be excluded that BMP-dependent mesodermal expression of Hox genes in vertebrates represents a remnant of the endomesodermal expression of Hox genes in the cnidarian-bilaterian ancestor, while Wnt- and Cdx-dependent staggered expression along the anterior-posterior axis is a bilaterian novelty. However, the orthology of the cnidarian Hox genes is uncertain (Chourrout et al., 2006; Ryan et al., 2007; Thomas-Chollier et al., 2010). Cnidarian Hox genes do not appear to fall cleanly into the paralog groups as we know them from Bilateria. Instead there appears to have been cnidarian-specific diversification of the ‘anterior’ and ‘non-anterior’ (Chourrout et al., 2006) or ‘anterior’ and ‘central’ (Hudry et al., 2014; Thomas-Chollier et al., 2010) Hox genes. Overall, the unexpected mode of regulation of the staggered Hox gene expression in *Nematostella* as well as the unclear orthology of the cnidarian and bilaterian Hox genes raise the possibility of the independent involvement of Hox genes in patterning of body axes in Cnidaria and Bilateria (Genikhovich et al., 2015).

BMP signalling components in radially symmetric cnidarians

The section above has summarized the important role of the BMP signalling gradient in patterning the D-V body axis of Bilateria and the directive axis of anthozoans. Yet, all medusozoan classes of cnidarians (Hydrozoa, Scyphozoa, Cubozoa, Staurozoa) are radially symmetric, hence lack the directive axis. As mentioned above, their only body axis, the oral-aboral axis, is regulated by Wnt/ β -catenin signalling, as in Anthozoa. However, searches of publicly available transcriptomes and of the *Hydra* genome (Chapman et al., 2010) reveal the presence of BMP ligands and secreted antagonists, Tolloid proteases, BMP receptors and intracellular members of the BMP pathway in Medusozoa. The function of BMP signalling in

Medusozoa has not been elucidated: no information on where BMP signalling is active is currently available, and very few expression patterns of BMP signalling components in Medusozoa have been published. In the solitary polyp *Hydra*, a *bmp5-8* gene is expressed in a radially symmetric domain in the tentacle-formation zone and in the budding zone, as well as during head regeneration (Reinhardt et al., 2004), and a *bmp2/4* gene was suggested to be present (Watanabe et al., 2014a). However, whether *Hydra* BMP2/4, as well BMP2/4 from a colonial hydroid *Clytia hemisphaerica*, are highly derived BMP2/4 proteins or some other type of TGFβ molecules is unclear. Our phylogenetic analyses (Fig. S1A and S2) show that there is very weak support for hydrozoan BMP2/4 homologues. *Hydra* also has a *chordin-like* (*HyChdl*) molecule, which is expressed in a radially symmetric domain in the endoderm of regenerating heads and during budding, and its expression is downregulated in the steady state (Rentzsch et al., 2007). At its N-terminal end, the predicted HyChdl protein is missing the first cysteine-rich chordin domain present in other chordin molecules but instead has an insulin-like growth factor-binding protein (IGFBP) domain and a follistatin domain. Injection of *HyChdl* mRNA into zebrafish leads to dose-dependent dorsalization of the embryo, showing that HyChdl can antagonize BMP signalling. The dorsalizing effect was twice as weak when mRNA encoding HyChdl that lacked the IGFBP and follistatin domains was injected (Rentzsch et al., 2007). Interestingly, HyChdl might be either a very derived Chordin or not a bona fide Chordin at all. Phylogenetic analysis of Chordin molecules shows that, unlike *Nematostella* Chordin, which groups together with bilaterian Chordin/Sog proteins, HvChdl forms an outgroup to Chordin/Sog and Kielin molecules (Fig. S1B). No Chordin orthologue has been found in

Clytia; however, we were able to identify a true Chordin molecule in the transcriptome of a scyphozoan *Aurelia* (Brekhman et al., 2015). In contrast, while having a clear BMP5-8, *Aurelia* appears to have lost a BMP2/4 homologue. Finally, our comprehensive search of publicly available cnidarian genomic and transcriptomic datasets for the presence of Chordin, BMP2/4 and BMP5-8 (NCBI, www.compagen.org, reefgenomics.org) shows that Anthozoa appear to universally possess all three of these molecules; however, medusozoans seem always to lack some of them (Fig. 5). Computer simulations and experimental data suggest that Chordin, BMP2/4, BMP5-8 and Tolloid form the core of the BMP signalling network necessary for ligand shuttling (Genikhovich et al., 2015; Mizutani et al., 2005), although there are rare examples in which Chordin was lost in Bilateria without deleterious effects to the D-V axis (Kuo and Weisblat, 2011; Özüak et al., 2014). It is, nonetheless, tempting to speculate that loss of the functionality of one of these proteins might have caused the loss of bilaterality in Medusozoa. To test this hypothesis, sequencing additional genomes followed by a comprehensive analysis of the complement, time of expression and function of BMP signalling components across the whole range of Medusozoa, and an experimental test of whether *Hydra* chordin-like and *Aurelia* Chordin are capable of shuttling BMPs will be necessary. If it turns out that some of the BMP signalling components crucial for ligand shuttling are missing in all Medusozoa, it will be a strong indication, although by no means proof, for the bilaterality of the cnidarian-bilaterian ancestor. As the BMP signalling gradient along the directive axis also is required for the staggered expression of Hox genes in *Nematostella*, it is interesting to look at Hox gene expression in radially symmetric Medusozoa. Notably, until now,

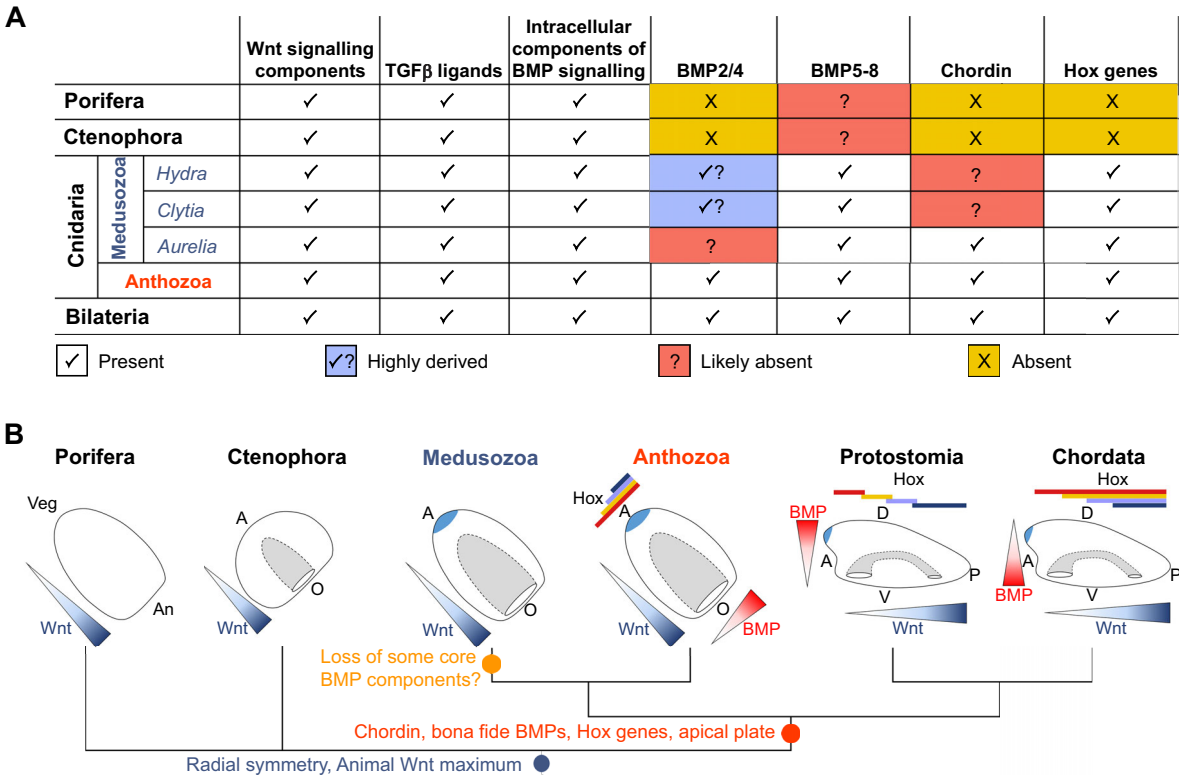


Fig. 5. The distribution of selected molecular traits amongst animals. (A) Table summarizing the presence or absence of several key regulatory components in the genomes and transcriptomes of Porifera, Ctenophora, Cnidaria and Bilateria. (B) The direction of the Wnt and BMP signalling gradients, and the expression of Hox genes, throughout Metazoa.

staggered axial expression of Hox genes has not been reported in any medusozoan (Chiori et al., 2009; Gauchat et al., 2000; Kamm et al., 2006; Reddy et al., 2015; Yanze et al., 2001).

Axis regulation in comb jellies and sponges

In the outgroups to the cnidarian-bilaterian clade, the biradially symmetric Ctenophora and the radially symmetric Porifera, Wnt/ β -catenin signalling components are expressed at distinct positions (Adamska et al., 2007; Borisenko et al., 2016; Leininger et al., 2014; Pang et al., 2010), suggesting a gradient of Wnt/ β -catenin signalling along the body axis of the embryo and larva. Searches in the two published ctenophore genomes (Moroz et al., 2014; Ryan et al., 2013) and a sponge genome (Srivastava et al., 2010), as well as in the available sponge transcriptomes (<http://www.compagen.org>), show that comb jellies and sponges also have components of BMP and TGF β signalling; however, they are expressed at distinct positions along the single body axis in a radially or biradially symmetric manner (Adamska et al., 2007; Leininger et al., 2014; Pang et al., 2011). We do not find support for any true sponge or ctenophore BMPs (Fig. S2). Notably, neither the ctenophores nor the sponges have a *chordin* orthologue or Hox genes (Fig. 4C) (Larroux et al., 2007; Moroz et al., 2014; Ryan et al., 2010, 2013; Srivastava et al., 2010). Taken together, these findings suggest that, among non-bilaterians, only anthozoan Cnidaria have bilateral symmetry, and there is no evidence for the presence of bilaterality in the earlier branching clades, in contrast to an earlier hypothesis (Jägersten, 1955).

The cnidarian-bilaterian ancestor

A phylogenetic comparison of the characteristic traits of bilaterians, cnidarians, sponges and ctenophores (Fig. 5) allows us to propose that the last common ancestor of cnidarians and bilaterians had a number of key features. It gastrulated from the animal pole at the location where Wnt/ β -catenin signalling was activated (Adamska et al., 2007; Borisenko et al., 2016; Henry et al., 2008; Lee et al., 2007; Leininger et al., 2014; Logan et al., 1999; Pang et al., 2010; Schneider et al., 1996; Wikramanayake et al., 2003). It had an axial organizer associated with the blastopore (Kraus et al., 2016). Around the blastopore, it expressed a set of conserved genes such as *brachyury*, *goosecoid* and *foxa* (Broun et al., 1999; Fritzenwanker et al., 2004; Martindale et al., 2004; Technau and Bode, 1999; Technau and Scholz, 2003). The gut was most likely blind, as bilaterian foregut and hindgut marker genes are co-expressed around the cnidarian blastopore (Arendt et al., 2001; Fritzenwanker et al., 2004; Martindale et al., 2004; Scholz and Technau, 2003; Technau and Scholz, 2003). At the aboral end, it had an apical plate expressing Wnt antagonists and the bilaterian brain markers *six3*, *foxq2* and *irx* (Chevalier et al., 2006; Lee et al., 2006; Marlow et al., 2014; Sinigaglia et al., 2013, 2015). Comparisons of anthozoans and Bilateria indicate that it also had BMPs and a complete set of intracellular molecules required for transducing a BMP signal, as well as Tolloid and Chordin (Genikhovich et al., 2015; Leclère and Rentzsch, 2014; Matus et al., 2006a, b; Rentzsch et al., 2006; Saina et al., 2009). It also had *gbx* and, considering the cnidarian-specific and bilaterian-specific diversification of the anterior and non-anterior Hox genes, a small Hox cluster probably consisting of a single anterior and a single non-anterior Hox gene (Chourrout et al., 2006; Ryan et al., 2007).

The fact that the last common ancestor of cnidarians and bilaterians had all BMP signalling components including Chordin does not automatically imply that it was bilaterally symmetric. Even if all medusozoans prove to lack some of the crucial components of the BMP ligand shuttling machinery, this can only serve as

circumstantial evidence against the independent emergence of the two orthogonal body axes regulated by Wnt/ β -catenin and BMP signalling in anthozoan Cnidaria and Bilateria. Moreover, BMP signalling networks differ significantly between cnidarians and bilaterians (Genikhovich et al., 2015), and recent mathematical modelling has shown that the formation of two orthogonal body axes from an initial state when Wnt and BMP signalling networks act along the same axis can occur easily (Meinhardt, 2015); it requires the BMP signalling network to contain a negative-feedback loop regulating *chordin* expression, and to be under control of the Wnt signalling network (Meinhardt, 2015). If this was the case, the formation of the ur-bilaterian, as well as of the ur-anthozoan, was accompanied by the uncoupling of the initially collinear Wnt and BMP signalling gradients.

The relationship between cnidarian and bilaterian body axes

If the last common ancestor of cnidarians and bilaterians was radially symmetric, and bilaterian and anthozoan bilateralities emerged independently, it does not seem possible to homologize the cnidarian oral-aboral axis with any of the bilaterian body axes, except with the inverted animal-vegetal axis. However, if the last common cnidarian-bilaterian ancestor was bilaterally symmetric with two body axes, one regulated by Wnt and the other by BMP signalling, the definitive cnidarian and bilaterian body axes can be compared. Answers to two questions are crucial for this comparison. First, did the urbilaterian have a blind gut or a through gut? Second, to what extent does the regulation of two body axes in extant anthozoans represent the ancestral mode typical for the cnidarian-bilaterian ancestor?

Comparative embryology data show that a cnidarian is, in essence, a ‘stretched gastrula’, where the main body axis corresponds exactly to the animal-vegetal axis of the egg. The body plan of the bilaterian early gastrulae is very comparable. Therefore, we argue below that the body plan of the bilaterally symmetric early planula of *Nematostella* can be used as a proxy for the body plan of the hypothetical bilaterally symmetric cnidarian-bilaterian ancestor, considering the ‘blind gut’ and the ‘through gut’ urbilaterian hypotheses separately.

What if an urbilaterian had a blind gut?

If the phylogenetic position of Xenacoelomorpha as a sister group to Bilateria (Cannon et al., 2016; Hejnol et al., 2009; Ruiz-Trillo et al., 1999) is true, and their blind gut represents an ancestral bilaterian trait (Beklemishev, 1944; Hejnol and Martindale, 2008a,b; Hyman, 1951; Ivanov and Mamkaev, 1973; von Graff, 1891; von Salvini-Plawen, 1968), the comparison seems to be relatively straightforward. According to acoel phylogeny (Jondelius et al., 2011), the earliest branching acoel group Diopisthoporidae has a terminal mouth at the posterior end. Although a description of gastrulation and mouth development in these animals has not yet, to our knowledge, been reported, a terminal posterior mouth directly deriving from the blastopore might represent an ancestral xenacoelomorph trait. Based on the directions of the Wnt and BMP signalling gradients, it seems likely that the cnidarian oral-aboral (O-A) axis may be homologous to the posterior-anterior (P-A) axis, and the directive axis may be homologous to the D-V axis of the ‘simple’ urbilaterian (Fig. 6A). The BMP signalling maximum in this animal must have been located on the dorsal side, as in extant protostomes, ambulacrarian deuterostomes and, likely, acoels (Hejnol and Martindale, 2008b; Lambert et al., 2016; Lapraz et al., 2009; Lowe et al., 2006; Mizutani et al., 2005; Srivastava et al., 2014). Later in evolution, the mouth could have

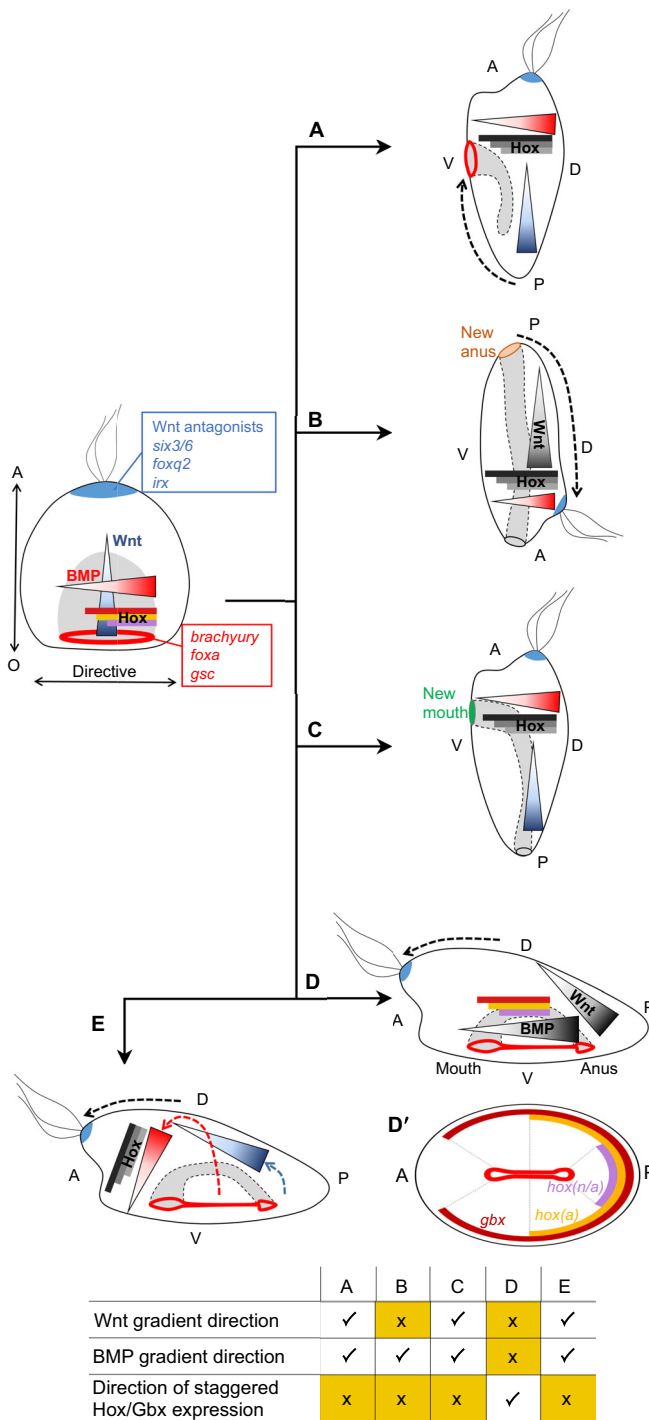


Fig. 6. Scenarios that could explain the evolution of a bilaterian from a hypothetical bilaterally symmetric cnidarian-bilaterian ancestor. The organization of the bilaterally symmetric *Nematostella* planula (with BMP-dependent Hox and Gbx expression) is taken as a proxy for the cnidarian-bilaterian ancestor. Staggered Hox expression is shown as coloured bars; Wnt and BMP signalling gradients are shown as coloured triangles. The circumblastoporal domain expressing *brachyury*, *foxa* and *gsc* is shown as a red ring; the apical plate expressing Wnt antagonists, *six3/6*, *foxq2* and *irx* is shown as a blue patch. If the direction of the Wnt or BMP gradient or of the staggered *hox* gene expression does not fit the situation observed in Bilateria, the corresponding pictograms are shown in grey. (A) O-A=P-A scenario of forming a 'simple urbilaterian' with a blind gut. (B-E) Scenarios of forming a bilaterian with a through gut: (B) O-A=A-P; (C) O-A=P-A; (D) directive=A-P; (D') ventral view of the animal from the scenario in D [*hox(a)*, anterior Hox gene; *hox(n/a)*, non-anterior Hox gene]; (E) modified amphistomy scenario.

become displaced to or formed *de novo* at the anterior/ventral position, as occurs in the development of Acoela and many Protostomia (Hejnol and Martindale, 2008b; Martín-Durán et al., 2016). If this scenario is correct, it is likely that Hox gene expression staggered along a body axis evolved independently in anthozoans and bilaterians, which appears plausible considering their unclear orthology (Chourrout et al., 2006; Ryan et al., 2007; Thomas-Chollier et al., 2010) and the different modes of regulation (Genikhovich et al., 2015) of the anthozoan and bilaterian Hox genes.

What if an urbilaterian had a through gut?

If the Xenacoelomorpha are not the earliest branching bilaterian clade, or if their blind gut is a result of the secondary reduction of a through gut, the comparison of the cnidarian and bilaterian body axes becomes more complicated. Three scenarios of direct correspondence of the anthozoan and bilaterian body axes can be envisaged: (1) oral-aboral=anterior-posterior (O-A=A-P); (2) oral-aboral=posterior-anterior (O-A=P-A); and (3) directive=anterior-posterior (directive=A-P).

In the first (O-A=A-P) scenario (Finnerty et al., 2004; Martindale and Hejnol, 2009), a new anus forms at the aboral position and the existing mouth is kept as a mouth in a bilaterian (Fig. 6B). The anthozoan directive axis would then correspond to the bilaterian D-V axis. Although plausible at the time it was suggested (Finnerty et al., 2004), this scenario now appears to be less likely in the light of new data. Most importantly, it has been shown that a posterior maximum of Wnt/ β -catenin signalling is clearly ancestral for Bilateria (Gomez et al., 2008; Janssen et al., 2010; Srivastava et al., 2014), while in cnidarians Wnt/ β -catenin signalling has a maximum at the oral end (Lee et al., 2007; Momose et al., 2008; Momose and Houliston, 2007; Wikramanayake et al., 2003). If the cnidarian O-A axis corresponded to the A-P axis of the urbilaterian with a through gut, one would need to explain the inversion in the direction of the Wnt signalling gradient and the relocation of the apical plate from the aboralmost/posterior to the oralmost/anterior position (Fig. 6B).

The second possibility (O-A=P-A, Fig. 6C) is that the oral-aboral axis of the *Nematostella* planula-like cnidarian-bilaterian ancestor gave rise to the posterior-anterior axis of the primitive bilaterian (Meinhardt, 2008) with the blastopore forming the anus. In this scenario, a new mouth forms in the vicinity of the apical plate on the ventral side, where BMP signalling intensity is lower. The reported circumblastoporal expression of Wnt genes (Kusserow et al., 2005; Lee et al., 2006) and aboral expression of bilaterian brain markers (Sinigaglia et al., 2013) in *Nematostella* support this scenario. The directions of the Wnt and BMP signalling gradients in this case also fit the situation in *Nematostella*. The only clearly discrepant fact is the direction of the staggered Hox gene expression in Cnidaria and Bilateria, although this may be explained by independent involvement of Hox genes in axial patterning in these lineages.

The third possibility is that the cnidarian directive axis corresponds to the bilaterian A-P axis (directive=A-P). In this scenario, the blastopore closes in a slit-like fashion in the middle and produces both mouth and anus connected by a through gut (Fig. 6D) (Jägersten, 1955; Remane, 1950). In this appealing scenario called amphistomy (Arendt et al., 2015, 2016; see Glossary, Box 1), the *hox/gbx*-free region of the directive axis in the *Nematostella* planula corresponds to the bilaterian head, and the rest makes up the trunk subdivided by staggered *hox* and *gbx* expression into coelomic pouches derived from mesenterial chambers (Fig. 6D). This scenario assumes that the orthology of cnidarian and bilaterian Hox genes, and their involvement in axial

patterning, is a feature of the cnidarian-bilaterian ancestor, which, considering the single anterior and single non-anterior Hox gene hypothesis (Chourrout et al., 2006), would have had six mesenterial chambers (Fig. 6D'). It is not clear, however, whether these assumptions are correct, given the unusual BMP-dependent expression of the anthozoan Hox genes. Importantly, this scenario does not account for the direction of the BMP and Wnt/ β -catenin signalling gradients in Bilateria and *Nematostella*, which would both be required to 'switch places' to generate a posterior-to-anterior Wnt gradient and a dorsal-to-ventral BMP gradient (Fig. 6D). Thus, an alternative amphistomic scenario (Fig. 6E) can be envisaged, involving the coordinated rotation of the orthogonally oriented Wnt and BMP gradients concomitant with the shift of the apical plate towards the future anterior end, generating a ventral BMP signalling minimum typical for all Bilateria except chordates. Thus, the Wnt gradient becomes oriented from posterior to anterior and the BMP gradient becomes oriented from dorsal to ventral. If Hox genes in the cnidarian-bilaterian ancestor were under control of BMP signalling, as in *Nematostella*, the shift in the orientation of the BMP signalling gradient would lead to them being expressed in staggered domains from the ventral to the dorsal side. This does not fit the pattern observed in Bilateria, but can be explained by the independent involvement of Hox genes in axial patterning in Cnidaria and Bilateria. The requirement for the shift of the Wnt and BMP gradients leads us to conclude that in this scenario (Fig. 6E) neither of the definitive cnidarian body axes would directly correspond to any of the definitive bilaterian ones.

Overall, none of the scenarios described above can adequately explain the development of Bilateria from a bilaterally symmetric *Nematostella* planula-like ancestor without contradiction, although based on the direction of the Wnt and BMP signalling gradients, three scenarios (i.e. those depicted in Fig. 6A,C,E) seem more likely. Scenarios A and E gain further support due to the co-expression of the bilaterian foregut and hindgut marker genes around the cnidarian and acoel blastopore (Arendt et al., 2001; Fritzenwanker et al., 2004; Hejnol and Martindale, 2008b; Martindale et al., 2004; Scholz and Technau, 2003; Technau and Scholz, 2003). Notably, none of these three plausible scenarios account for the staggered expression of Hox genes along the directive axis of *Nematostella* under the control of the BMP signalling, which favours their independent involvement in axial patterning in Bilateria and Anthozoa.

Outlook

We are only beginning to appreciate the evolvability of the complex signalling and regulatory systems involved in body axis evolution. The question of the origin of bilaterality still remains unanswered; however, new molecular data from Cnidaria and other early branching metazoan clades allow us to formulate key questions (Box 3), which will bring us closer to finding a solution to this complex issue. Here, we showed that several scenarios of the correspondence of the anthozoan and bilaterian body axes are plausible; however, the independent evolution of bilaterality in Cnidaria and Bilateria cannot be excluded. Currently, taxonomic sampling is becoming broader, making phylogenetic trees more robust. In addition, not only the expression but also the function of genes can now be assayed in an ever-broader range of animal models, including early-branching metazoan phyla. Together, these approaches will provide us with a thorough understanding of the regulatory and morphological consequences of changes in the activity of genes responsible for the establishment and patterning of

Box 3. Key unanswered questions in the field

- Why are medusozoan cnidarians radially symmetric? Do all of them lack some of the central components of the BMP ligand shuttling machinery, which would be indicative of a derived state? How are the existing components expressed, where does BMP signalling take place and what is it regulating?
- What are the transcriptional targets of Wnt/ β -catenin and BMP signalling in cnidarians and bilaterians? Are the Hox genes and *gbx* direct transcriptional targets of BMP signalling in anthozoans? Is the wiring of the gene regulatory networks regulated by Wnt/ β -catenin and BMP in cnidarians and bilaterians similar or different?
- How is the staggered axial expression of the Hox genes and *gbx* regulated in anthozoans? Is the mechanism similar to that in Bilateria? Do anthozoan Hox genes regulate regional specification of the directive axis?
- To what extent do the Xenacoelomorpha body plan and the way in which they regulate their body axes represent the urbilaterian situation? How is gastrulation and mouth formation controlled in different Xenacoelomorpha? What is the role of Wnt/ β -catenin and BMP signalling in this process? What are their transcriptional targets? What is controlling staggered Hox gene expression along the A-P axis in acoels?

the body axes. Once we have this information, thinking in terms of regulatory networks rather than individual genes is bound to bring major new advances in our understanding of how bilaterality evolved.

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Competing interests

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Supplementary information

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