

# Acoel development supports a simple planula-like urbilaterian

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Molecular approaches to the study of development and evolution have had profound effects on our understanding of the nature of the evolutionary process. Developmental biologists became intoxicated with fanciful notions of reconstructing genetic pathways of morphogenesis while evolutionary biologists were sobered by the fallacy of reconstructing organismal relationships along increasing grades of morphological complexity. Increased taxon sampling and improvements in analytical techniques are providing a new approach and are forcing biologists to move past historical biases to allow more accurate mapping of morphological and developmental characters through evolutionary time. Here, we discuss the possible developmental and morphological features of the ‘urbilaterian’, the triploblastic animal with anterior–posterior and dorsoventral axes and predecessor of the protostome–deuterostome ancestor. We argue that this animal, with features resembling acoelomorph flatworms, was far simpler morphologically than the protostome–deuterostome ancestor despite possessing a nearly complete eubilaterian genome. We show that the deployment of some genes expected to pattern the protostome–deuterostome ancestor is not deployed in acoels in the predicted manner and thus might have been co-opted after the evolution of the urbilaterian. We also identify the developmental changes related to gastrulation that gave rise to the urbilaterian from a simpler cnidarian-like ancestor.

**Keywords:** Acoela; *Convolutriloba longifissura*; development; Urbilateria; *vax*; *Pax6*

## 1. INTRODUCTION

One of the most important contributions the field of evolution and development (‘evo–devo’) has made to understanding animal evolution is resolving the developmental basis for morphological features of stem species at distinct nodes in metazoan phylogeny. One hypothetical animal that has provoked a great deal of speculation is the ‘urbilaterian’, the first triploblastic animal that possessed definitive anterior–posterior and dorsoventral axes (figure 1). The reconstruction of this animal by evolutionary developmental biologists is primarily based on speculations of common molecular patterning programmes in such diverse animals as fruitfly and mouse. It presents a very different scenario of animal evolution than previously thought (Salvini-Plawen 1978): because many of the genes involved in bilaterian developmental patterning were already present in the protostome–deuterostome ancestor, this organism must have had complex morphological traits, e.g. body segmentation, centralized nervous system, coelom and circulatory system (Shenk & Steele 1993; Slack *et al.* 1993; Carroll *et al.* 2001).

## 2. THE CASE FOR A COMPLEX URBILATERIAN

The overwhelming similarity of several molecular components of complex developmental pathways of

the genetic model organisms (fruitfly, soil nematode, mouse and zebrafish) gives strong evidence that these pathways were already present in the most recent common ancestor of these animals. Examples include the anterior–posterior patterning mechanism using HOX genes, the *sog/chordin dpp/BMP2/4* in dorsoventral patterning (Arendt & Nübler-Jung 1994; De Robertis & Sasai 1996) and *PAX6/eyeless* for eye development (Gehring & Ikeo 1999), and were extended through the discovery of NK-class gene function in ‘heart’ tissue of both *Drosophila* and mouse (Harvey 1996), finding common members of an immune system (Hoffmann *et al.* 1999) and potential similarities in the process of body segmentation (Balavoine & Adoutte 2003; Tautz 2004). Soon afterwards, specific anterior patterning genes (*empty spiracles/EMX* and *orthodenticle/otx*) responsible for the formation of a tripartite brain (Reichert 2005) and posterior patterning genes, *even skipped/evx* (Patel *et al.* 1992) and *caudal/cdx* (Wu & Lengyel 1998) were added to the list of shared features. Together these studies supported the notion of a molecular ‘zootype’ defining a common plan for the construction of all animals (Slack *et al.* 1993). The extension of gene expression studies to members of the third ‘superclade’, the Lophotrochozoa, primarily members of the Annelida, appears to confirm the notion of a morphologically complex urbilaterian (Shankland & Bruce 1998; Arendt *et al.* 2001, 2002; Prud’homme *et al.* 2003; Seaver *et al.* 2005; Fröbisch & Seaver 2006; Seaver & Kaneshige 2006; Denes *et al.* 2007; Steinmetz *et al.* 2007). This rather complex bilaterian ancestor would

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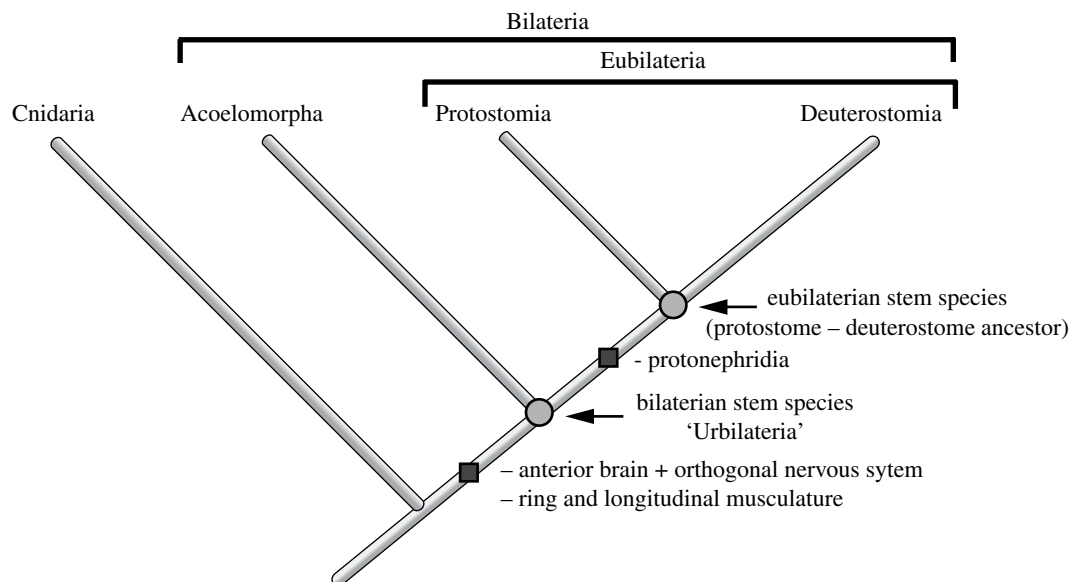


Figure 1. Phylogenetic position of the Acoela. The phylogenetic position of the Acoelomorpha based on molecular and morphological evidence (monophyletic Acoelomorpha: Ehlers 1985; sister to Eubilateria: Ruiz-Trillo *et al.* 1999, 2002, 2004; Jondelius *et al.* 2002; Telford *et al.* 2003). The position of the 'urbilaterian'—the stem species of the Bilateria—is distinct from the eubilaterian stem species (protostome–deuterostome ancestor). Synapomorphies are indicated by filled squares.

have possessed serially reiterated body segments, a mesodermally lined coelomic space, a through gut with separate mouth and anus, lateral appendages, heart, eyes, epigenetic germ-cell determination (Extavour & Akam 2003; Rebscher *et al.* 2007), a tripartite brain with a centralized nervous system and a biphasic life cycle with a feeding larvae (Arendt *et al.* 2001; Carroll *et al.* 2001; see figure 2).

Compared with the predictions of the adult morphology of the urbilaterian, few statements have been made about its developmental features. Since the early development and morphogenetic processes of the ecdysozoan genetic models *Caenorhabditis elegans* and *Drosophila* and the deuterostomian mouse are so different from one another, and not even representative of their own clades, it has been difficult to predict the ancestral patterns of early development and gastrulation patterns. Some workers have suggested that patterns of development such as spiral cleavage; a pattern of gastrulation described as amphistomy (Arendt & Nübler-Jung 1997; Shankland & Bruce 1998; Arendt 2004), with features of convergent extension and a posterior terminal growth zone involved with later growth and morphogenesis (Jacobs *et al.* 2005); and cell cycling regulated by a *notch/delta* system (Tautz 2004) might represent ancient characteristics of bilaterian development.

### 3. THE PHYLOGENETIC POSITION OF THE ACOELOMORPHA AND THEIR IMPACT ON THE GROUND PATTERN OF THE BILATERIA

The subdivision of the Metazoa into three major clades, the Ecdysozoa, Lophotrochozoa and Deuterostomia, focused the hunt for the 'Urbilateria'; however, the lack of resolution within these three clades prevented rational statements about the characteristics of each clade's stem species and thus the direction of evolutionary change. With recent improvements in animal

phylogeny, mainly by the increase of the number of genetic loci and improved taxon sampling ('phylogenomics'), characters found in animals such as polychaete annelids, like segmentation, trochophore larvae, spiral cleavage and amphistomic gastrulation, might now be viewed as derived features of specific clades (Giribet 2008). The placement of the unsegmented Chaetognatha as the sister group to the Lophotrochozoa (Matus *et al.* 2006a) has a huge impact on our understanding of the lophotrochozoan stem species, and recent work placing the enigmatic *Xenoturbella* inside the Deuterostomia as sister to the Ambulacraria (Bourlat *et al.* 2003, 2006) indicates that both protostomes and deuterostomes have rather simple unsegmented worms with only one body opening and a non-centralized nervous system near their base. In particular, the placement of the Acoela and Nemertodermatida as the possible sister group of the remaining Bilateria (Ruiz-Trillo *et al.* 1999, 2002, 2004; Jondelius *et al.* 2002; Telford *et al.* 2003; Baguñá & Riutort 2004) allows a more critical evaluation of the statements made about the morphology of the urbilaterian. The first obvious result of this placement is that the stem species of the Bilateria does not correspond to the protostome–deuterostome ancestor, but to the last common ancestor of the Acoelomorpha and the remaining Bilateria (the Eubilateria or Nephrozoa; figure 1). If acoelomorphs do occupy this pivotal position, they will provide important insight into estimating the morphological complexity of the urbilaterian.

The taxon Acoelomorpha (Acoela + Nemertodermatida; Ehlers 1985) comprises a group of relatively simple, small marine worms with bilateral symmetry, mesoderm and only one opening of the digestive system (figure 3). The development of acoels is direct. No larval form is generated and a miniature adult is formed at hatching (figure 3). The nervous system consists of an orthogonal array of nerves with a variable number of pairs of longitudinal nerve cords with a concentration

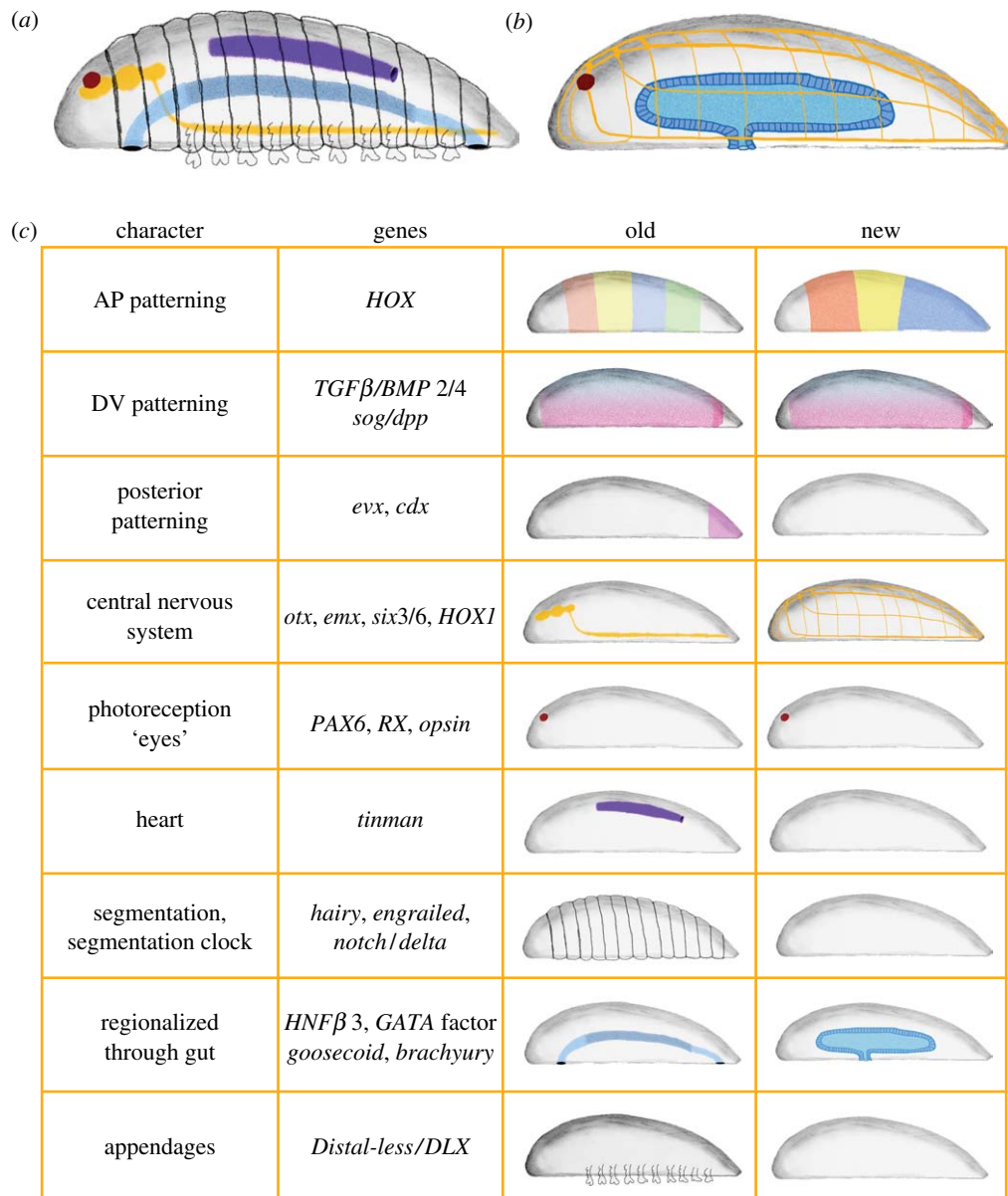


Figure 2. Old (protostome–deuterostome ancestor) and new urbilaterian. (a) The old depiction of the stem species of the Bilateria based on gene expression data from both protostomes and deuterostomes. (b) The ground pattern of the Bilateria in the light of recent molecular phylogenies suggests that the urbilaterian was far less complex than thought before. (c) Comparison of the gene expression data and organs present in the hypothetical ancestor.

of sensory cells at the anterior end and a ‘brain’ in the form of a cerebral commissure (figure 3). The nervous system, like cnidarians, is located basiepidermally, although in some acoel species nerve fibres are located subepidermally (Rieger *et al.* 1991; Raikova *et al.* 1998, 2004), and is connected to photoreceptive cells (‘eyes’) and epidermal sensory cells. Thus, if acoelomorphs resemble the urbilaterian, a dorsally or ventrally centralized nervous system is not part of the ground pattern of the Bilateria.

The digestive system of acoels and nemertodermatids has only one ventral opening, the so-called mouth, which can occur at a variety of different locations along the anterior–posterior axis. Nemertodermatids have an epithelial blind gut, while the Acoela have reduced the epithelium to a digestive syncytium. Since an epithelial digestive endoderm is already present in Cnidaria, the condition in the Nemertodermatida represents the plesiomorphic condition.

Acoelomorphs show no signs of a circulatory system or nephridia, thus an excretory system is absent and seems to be an evolutionary novelty of the Eubilateria (or Nephrozoa). The primary derivatives of the mesoderm include circular, longitudinal and oblique musculature, and in some acoels a secondary peripheral parenchyme. Thus, coeloms are not part of the ground pattern of the Bilateria and as a consequence the ‘enterocoely hypothesis’ describing the transition of an adult cnidarian polyp into a coelomate annelid-like worm can be rejected (Sedgwick 1884; Remane 1963). The acoelomorph body shows no external segmentation or other reiterated structures along the anteroposterior body axis so that the presence of circular musculature is not sufficient to define segmentation. Acoelomorphs have tremendous capacities for regeneration and asexual reproduction through the actions of multipotent, mesodermally derived stem cells called neoblasts. Whether these cells are homologous with

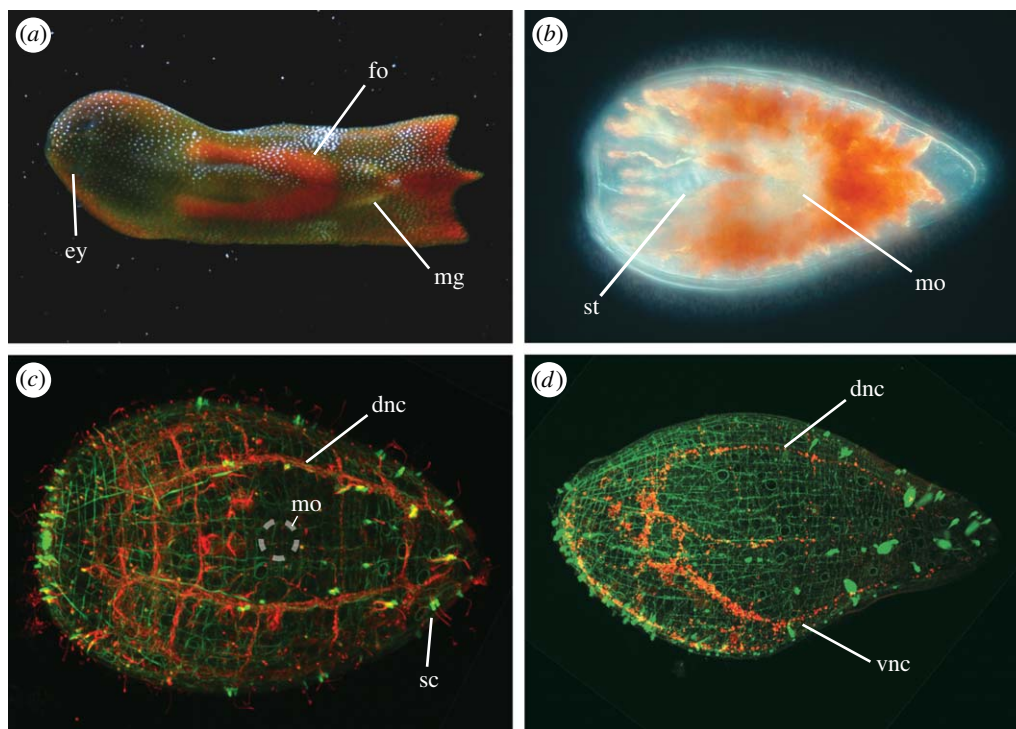


Figure 3. Morphology of the acoel *C. longifissura*. (a) Adult with ripe female (fo) and male genital (mg) organs. A pair of eyes (ey) is located at the anterior end. (b) At hatching, the juvenile possesses a statocyst (st) and a pair of lateral eyes. The mouth opening (mo) is ventral, anterior to the left. (c) Confocal image of a juvenile to visualize the nervous system. Actin is visualized with Alexa-488 phalloidin (green) and microtubules with anti-tubulin antibody (red). Dorsal view, the nervous system runs orthogonally with bilateral nerve chords on the dorsal and ventrolateral side (dnc, vnc), sensory cells (sc) are connected with the main nerve chords. The muscular system is composed out of longitudinal and circular musculature. The position of the mouth opening (mo) is indicated with a circle. (d) Lateral view of a juvenile (green phalloidin, red anti-serotonin). The serotonergic subset of the nervous system is labelled in red.

pluripotent cells in other bilaterian taxa, such as *Planaria*, is debatable (Gschwentner *et al.* 2001).

The cleavage pattern of acoels appears to be a unique 'spiral duet' cleavage programme (Henry *et al.* 2000) that is different from any other metazoan, including nemertodermatids (Jondelius *et al.* 2004). The ancestral cleavage for the Bilateria is likely to be a total 'radial' or chaotic cleavage pattern which is found in both branches of the Eubilateria as well as the Cnidaria (Siewing 1969). The unipolar cleavage present in both ctenophores and cnidarians appears to have been lost in the stem lineage of the Bilateria. These features support the acoeloid–planuloid hypothesis of bilaterian evolution of von Graff (1891), which predicted that an acoel flatworm-like ancestor evolved through paedogenesis from a cnidarian planula larva, or that both the cnidarians and bilaterians evolved from a planulomorph ancestor that showed traits of bilateral symmetry ('Planulozoa' hypothesis, see Wallberg *et al.* (2004)).

#### 4. THE URBILATERIAN VERSUS THE PROTOSTOME–DEUTEROSTOME ZOOTYPE

Despite initial optimism that an increase in genomic complexity and a cursory analysis of comparative gene expression of a handful of conserved genes in a few model systems might lead to a simple explanation of the evolution of organismal form (Carroll *et al.* 2001), recent evidence suggests that greater care needs to be exercised when performing these analyses. There are no such things as 'segmentation', 'eye', 'heart' or 'limb'

genes. Genes that are supposed to specify 'mesodermal' cell types are present in cnidarians, which do not even possess muscle or a mesodermal germ layer (Spring *et al.* 2002; Martindale *et al.* 2004), and animals that possess several different kinds of eyes do not possess 'the eye gene' *Pax6* (Matus *et al.* 2007). There are just molecules that can bind to DNA or interact with receptors, phosphorylate other molecules, etc. Furthermore, not even the most intimate understanding of a gene or genetic pathway can predict *a priori* its developmental/morphological outcome. The problems with homologizing structures using only the underlying regulatory developmental gene network have been discussed elsewhere and had led to the explosion of new ideas around the notion of 'homology' (Dickinson 1995; Bolker & Raff 1996; Nielsen & Martinez 2003; Scholtz 2005; Wagner 2007). Recent findings from the analysis of the genome of cnidarians show that conclusions based on the relationship of the morphological 'complexity' of an organism to its gene content are over simplified (Putnam *et al.* 2007).

The fact that a highly conserved 'toolkit' of genes exists in virtually all metazoans begs the question of how and when these genes were incorporated into interacting networks responsible for the formation of discreet regional, tissue and/or cell type-specific identities. By sampling key genes at different places in the metazoan tree, it should be possible to determine when and how these dynamic networks are put together. Although our molecular understanding of

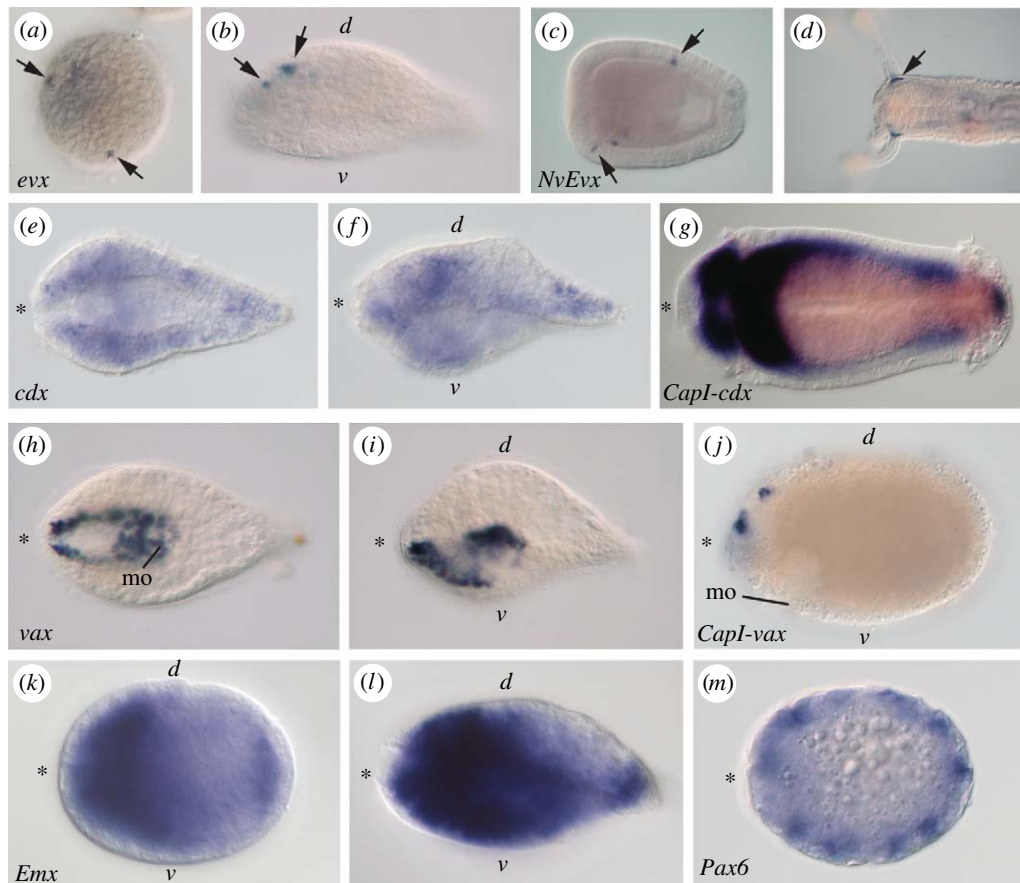


Figure 4. Gene expression of *ClEvx*, *ClCdx*, *ClVax*, *ClPax6* and *ClEmx* during acoel development and *NvEvx* in the sea anemone *Nematostella vectensis* and *CapI-cdx*, *CapI-vax* in the annelid *Capitella* sp. I. (a) Expression of *ClEvx* in an acoel embryo with approximately 250 cells. The expression seems to be sparsely distributed in isolated cell (arrows). (b) *ClEvx* expression in a hatchling of *C. longifissura*. The expression is found in median neurons anterior and posterior to the statocyst. Lateral view, anterior to the left. (c) *NvEvx* expression in a planula of the sea anemone *N. vectensis*. *NvEvx* is expressed in isolated neural cells in the ectoderm (arrows). Later in the polyp (d), *NvEvx* is expressed in cells at the base of each tentacle (arrow); oral pole to the left. (e, f) *ClCdx* expression in a *C. longifissura* hatchling. *ClCdx* is expressed in the nervous system along the whole body. (e) Ventral view, (f) lateral view; asterisk indicates anterior end at left. (g) *Cdx* expression in the annelid *Capitella* sp. I. is expressed all along the body axis, mainly in the mesodermal layer (dorsal view; Fröbuis & Seaver 2006). (h) Expression of the gene *ClVax* in a *C. longifissura* hatchling. The gene is expressed in the ectoderm ventral and anterior to the mouth opening to the anterior tip of the animal (ventral view). (i) Lateral view of (h). (j) *CapI-vax* expression in the annelid *Capitella* sp. I. *CapI-vax* is expressed in the ectoderm anterior to the mouth opening (mo) on the dorsal side and in the dorsal hemisphere of the prototroch (not shown); lateral view. (k) Expression of the 'anterior' gene *ClEmx* in the acoel *C. longifissura*. *ClEmx* is in the late embryo along the entire length of the body; lateral view. In the hatchling (l) it is expressed in the nervous system, primarily on the ventral side (lateral view). (m) *ClPax6* expression in a late embryo of *C. longifissura*. *ClPax6* is expressed in an ectodermal ring of cells, separating the dorsal and ventral hemispheres of the embryo, but not in the position of the eyespots (dorsal view).

acoelomorphs is in its infancy, it appears that they might display features expected for their position between cnidarians and eubilaterians. For example, the search for HOX genes in four acoelomorph species (Cook *et al.* 2004; Jiménez-Guri *et al.* 2006; own results) yielded a small number of HOX class genes: 1–2 anterior class, a single posterior class, and 1–2 central class (central class HOX genes appear to be absent in cnidarians; Chourrout *et al.* 2006; Ryan *et al.* 2006, 2007). In addition, a *Cdx* and *Xlox* orthologue has been found in acoels and nemertodermatids (Cook *et al.* 2004; Jiménez-Guri *et al.* 2006). A recent search for microRNAs in cnidarians, acoels and protostomes and deuterostomes shows that a subset of microRNAs common in eubilaterian species is present in the acoel *Childia* (Sempere *et al.* 2006). These data from acoels show that although the urbilaterian may have an increased genomic complexity relative to cnidarians,

this does not necessarily mean they showed the complex morphological traits predicted to be in the protostome–deuterostome ancestor.

In an effort to compare the molecular zootype of a potential proxy for the urbilaterian to that proposed for the protostome–deuterostome ancestry (Slack *et al.* 1993), we present the expression patterns of orthologues from two of the genes predicted to be 'posterior' patterning genes, *even-skipped/evx* (Patel *et al.* 1992) and *caudal/cdx* (Wu & Lengyel 1998; Copf *et al.* 2004; de Rosa *et al.* 2005) in the acoel species *Convolutriloba longifissura* (Cl; figure 4). We show (figure 4a–d) that *ClEvx* expression in the acoel is more similar to the pattern found in cnidarians and, at least in the hatchling, is expressed exclusively in distinct neurons of the brain anterior and posterior to the statocyst (figure 4b). *ClEvx* expression at earlier stages appears to play a role in sensory cell specification in both

cnidarians and acoels (Ryan *et al.* 2007). A similar pattern of *evx* expression in the brain is found in *Branchiostoma* (Ferrier *et al.* 2001) and thus suggests a neural function of *evx* in the urbilaterian and not a role in posterior patterning. Another posterior gene, the orthologue of the paraHOX gene *caudal*, *CiCdx*, is expressed in the nervous system in the hatchling along the entire body axis (figure 4e,f). *CapI-cdx* in the polychaete annelid *Capitella* sp. I (figure 4g) is expressed along the whole body axis of the larva (Fröbuis & Seaver 2006). Thus, the proposed role of *caudal* in posterior patterning must have been co-opted from an earlier function and was not likely to be involved in the gastrulation of the urbilaterian (Arendt 2004).

One of the most intensively discussed questions in animal evolution is the origin of eyes in the different lineages of the Metazoa (Salvini-Plawen & Mayr 1977). From developmental genetics, the gene *PAX6/eyeless* plays a major role in eye formation in different bilaterians (Gehring & Ikeo 1999). Many acoel flatworms have simple eyes, composed only of two cells, one pigment cell and one receptor cell, which has neither rhabdomeres nor cilia (Yamasu 1991). It is not clear if this is a derived condition in the Acoelomorpha, since photoreceptors have not been described for nemertodermatids. However, an acoel orthologue of the *Pax6* gene, *CiPax6*, is expressed along the body axis, most probably in sensory cells (figure 4m), but not in the eye spots. *Pax6* expression is seen in longitudinal columns flanking the dorsal midline in vertebrates and along the ventral midline in polychaete worms (Denes *et al.* 2007). Pax genes are also expressed in mechanosensory cells in bilaterians and it might be that neurospecification was an ancestral role of *Pax6* in Bilateria and Cnidaria (Matus *et al.* 2007).

The gene *emx/empty-spiracles* along with *otx/orthodenticle* and *HOX1* have been suggested to be anterior patterning genes in the zootype and used as an argument for the presence of a tripartite brain (Reichert 2005) in the urbilaterian. Interestingly, *CiEmx* is expressed all along the body axis in the acoel *C. longifissura* (figure 4k,l). The expression of 'anterior' genes along the whole anterior–posterior axis might support the hypothesis that the cnidarian body plan (derived from a planula larva) represents the anterior-most region of bilaterians and that the bilaterian body plan was elaborated by growth from the posterior terminus (Meinhardt 2002).

If acoels are an accurate proxy for the urbilaterian, it would appear that these genes have been redeployed prior to the origin of the protostome–deuterostome ancestor. Other genes, such as HOX or germ layer-specific genes, might give more insight into the molecular basis of body-plan organization. One interesting candidate is the homeobox gene *vax* (ventral anterior homeobox). This gene was named after the location and function in chordate embryos, where it is responsible for the ventralization of the retina in mouse and *Xenopus* (Barbieri *et al.* 1999; Mui *et al.* 2005). In acoels, *CiVax* is expressed in the ventral anterior ectoderm of the hatchling between the anterior tip and the mouth opening in a pattern remarkable similar to

that seen in vertebrates (figure 4h,i). Interestingly, in the polychaete *Capitella* sp. I, *CapI-vax* is expressed on the opposite (dorsal) side of the head (figure 4j). The fact that it is expressed on the ventral side of deuterostomes and the dorsal side of a protostome supports the notion of 'dorsoventral inversion' in which gene expression becomes localized to the opposite side of the embryo in protostomes from the urbilaterian starting condition (Arendt & Nübler-Jung 1994; De Robertis & Sasai 1996). Further investigations of *vax* expression patterns in protostomes will deliver more insight into this question.

## 5. THE EVOLUTION OF GASTRULATION AND THE ORIGIN OF BILATERAL SYMMETRY

Confusion reigns regarding the role of gastrulation in body-plan evolution. Its importance in understanding the evolution of development hails back to the earliest days when animals were categorized as being either protostomes (the site of gastrulation becomes the mouth) or deuterostomes (the site of gastrulation becomes the anus; Grobden 1908). Gastrulation is a complicated process in all animals and is difficult to define and study. In its simplest sense, it is the formation of distinct germ layers (endoderm, endomesoderm and mesoderm) but the process is dynamic and can begin and end over highly variable periods (i.e. when embryos have 28 cells in soil nematodes to thousands of cells in vertebrate embryos), and precursors of these tissues can arise from multiple locations in the embryo (e.g. ectomesoderm and endomesoderm). In most embryos, the site of gastrulation has a definitive position relative to the primary egg axis, the animal–vegetal axis. Deuterostomes, for example, gastrulate at the vegetal pole and this site becomes the future anus with the mouth forming secondarily in the animal hemisphere. In most protostomes, the site of gastrulation (endomesoderm formation) also derives from the vegetal pole, but this location does not correspond to either the mouth or the anus. For example, in spiralian embryos (Boyer *et al.* 1998; Maslakova *et al.* 2004; Ackermann *et al.* 2005; Hejnoj *et al.* 2007), intracellular fate-mapping experiments clearly show that endomesoderm forms from 'macromeres' at the vegetal pole, but the mouth and foregut form from ectodermal derivatives in the animal hemisphere posterior to the prototroch (first and second quartet micromere derivatives) and the anus (when present) forms de novo from cells that are pushed posteriorly and ventrally by asymmetric growth on the dorsal side of the embryo. The same pattern is seen in acoel flatworms (Henry *et al.* 2000) where endomesoderm forms from both third duet macromeres at the vegetal pole and the mouth forms anteriorly as 1a micromere descendants expand around the posterior pole (although an anus never forms).

In contrast to the position of gastrulation in bilaterian embryos, ctenophores and cnidarians gastrulate (endomesoderm formation) at the animal pole. Thus, sometime after the origin of ctenophores and cnidarians but before the origin of the urbilaterian, the factors that control the site of gastrulation changed their position by

180°. It should be noted that the mouth still forms in the animal hemisphere in both bilaterians and ctenophores and cnidarians, but endomesodermal fates are segregated to the vegetal pole. The position of endomesoderm formation in deuterostomes (Weitzel *et al.* 2004) and an anthozoan sea anemone (Lee *et al.* 2007) is controlled at least in part by the regulation of downstream components of the Wnt signalling pathway, thus providing a foothold into the mechanistic understanding of the evolution of body-plan reorganization. Many, but perhaps not all, downstream targets of this pathway also changed their spatial pattern of expression (Lee *et al.* 2006). There is a considerable body of evidence accumulating that anthozoan cnidarians possess bilateral symmetry in both ectodermal and endomesodermal tissues centred around the blastopore/mouth (Finnerty *et al.* 2004; Matus *et al.* 2006b) indicating that asymmetries in gene expression evolved well before changes in the site of bilaterian gastrulation. If planuloid ancestors represented the anterior ends (heads) of bilaterian descendents, the changing site of gastrulation, with its ability to generate and pattern new germinal tissues, to the vegetal pole facilitated the expansion, growth and differentiation of the posterior end in bilaterian lineages.

## 6. CONCLUSIONS

To summarize the impact of understanding the morphology and development of the Acoelomorpha, we can draw conclusions on the constitution of the stem species of the Bilateria (summarized in figure 2). It was likely to be a small, non-sessile, marine, direct-developing, planula-like organism. This animal had an orthogonal nervous system as proposed by Reisinger (1925) with an anterior concentration of sensory cells and a simple brain. The third germ layer, the mesoderm, separated during the development from the endoderm and formed only musculature, mesenchyme-like tissue and maybe germ cells, but neither a coelom nor a heart-like structure or other mesodermal organs. The urbilaterian was unsegmented, small and most probably covered with cilia. The digestive system was composed of a blind, epithelial gut with a small lumen, similar to that found in nemertodermatids, with only one ventral opening with variable position along the anteroposterior axis. The bilaterian stem species had a radial, total cleavage programme probably with a strong regulatory potential (Boyer 1971) with gastrulation at the vegetal pole. Ectodermal cells residing at the vegetal pole following gastrulation moved towards the ventral side by the expansion of dorsal ectoderm thus driving the mouth anteriorly. This process of transition of radial-symmetric blastula to a bilateral gastrula already exists in anthozoan cnidarians and probably represents the major symmetry-breaking event in the evolution of a dorsoventral and anteroposterior axis.

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