

THE EVOLUTION OF METAZOAN AXIAL PROPERTIES

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Abstract | Renewed interest in the developmental basis of organismal complexity, and the emergence of new molecular tools, is improving our ability to study the evolution of metazoan body plans. The most substantial changes in body-plan organization occurred early in metazoan evolution; new model systems for studying basal metazoans are now being developed, and total-genome-sequencing initiatives are underway for at least three of the four most important taxa. The elucidation of how the gene networks that are involved in axial organization, germ-layer formation and cell differentiation are used differently during development is generating a more detailed understanding of the events that have led to the current diversity of multicellular life.

METAZOAN

A multicellular animal.

RADIAL SYMMETRY

The presence of multiple planes of mirror symmetry running through the longitudinal axis.

GASTRULATION

The process during which cells move from the outer regions of the embryo to the inside, to give rise to the endodermal (gut) and mesodermal (for example, muscle and blood) germ-layer derivatives; the cells that remain on the external surface of the embryo give rise to the ectodermal derivatives (skin and nervous system).

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Some of the most fundamental unanswered questions in biology revolve around the origin and tremendous diversification of the multicellular animals that inhabited the Earth's oceans more than 500 million years ago. Despite the great variation in form and function of METAZOANS the body plan of most of these species is organized along a single longitudinal axis, the anterior–posterior axis (A–P axis). This axis is often associated with the direction of locomotion, with the mouth and associated neural structures located at or near the anterior pole, and with the anus located at or near the posterior end. In addition, all animals with an A–P axis have a second, dorso–ventral (D–V) axis that runs perpendicular to it, and that, in turn, defines a plane of bilateral symmetry. In most of these 'bilaterian' animals it is easy to identify the 'top' and 'bottom', and the 'front' and 'back' of the body, but in others, such as some sessile marine invertebrates, these axes have become modified from their ancestral condition, or even change at different times in their life through metamorphic reorganization. The evolutionary ancestry of these metazoan axial properties is revealed by ancient molecular patterning mechanisms that operate during the embryonic period. Hox genes, for example, are expressed along the A–P axis of every adult metazoan that has been studied so far^{1–4} and a pathway that regulates D–V patterning — including

the proteins decapentaplegic or bone morphogenetic protein (Dpp/Bmp) and short gastrulation or chordin (Sog/Chrd) is shared by animals that are as distantly related as vertebrates and flies^{5–8}.

Although there is no shortage of alternative theories (see REF. 9 for an historical review), bilaterally symmetrical animals are thought to have evolved from hollow, ciliated, RADIALY SYMMETRICAL organisms (FIG. 1) that were themselves derived from flagellated protozoan ancestors¹⁰. Regardless of the exact evolutionary scheme, two of the most important design changes that have occurred in the evolution of metazoan body plans include the establishment of axial properties (such as the A–P and D–V axes) and the formation of multilayered animals through the embryonic process of GASTRULATION. Gastrulation is perhaps the most important event in metazoan evolution: it is the reason that animals are not all a hollow ball of cells, and provides the opportunity for the interaction of different tissue layers to give rise to complex structures and organ systems. Gastrulation and the establishment of embryonic polarity are intimately linked processes. The developmental events that generate axial organization during the early cleavage stages lead to the site of gastrulation, and therefore determine the onset of differential gene activity that is responsible for the specification of distinct mesodermal and endodermal germ-layer fates. Indeed, the site

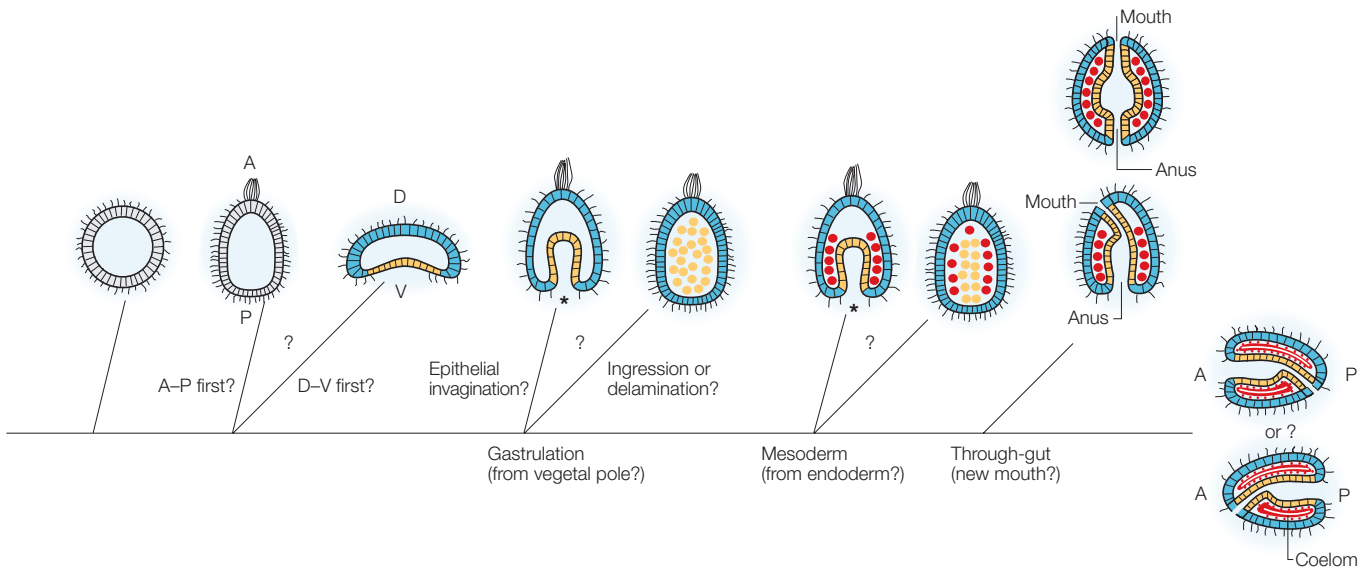


Figure 1 | How did the metazoan body plan evolve? The figure shows one of several evolutionary scenarios⁹ for how the body plan evolved in the plankton⁴⁶. Like most theories on this topic, this one proposes that the increase in body-plan complexity that occurred over evolutionary time is loosely based on the developmental stages of metazoan embryogenesis. A simple, hollow, ciliated ball of cells (left) is transformed by gastrulation into a multilayered embryo that consists of an outer ectodermal layer of cells (blue) that surrounds an internal endodermal layer (the gut, yellow). Gastrulation might occur by epithelial invagination or by individual cells **INGRESSING OR DELAMINATING** by a process of epithelial–mesenchymal transitions. Note that these models have implications for the origins of axial properties — that is, the transition from radial symmetry to bilateral symmetry, and the development of anterior–posterior (A–P) and dorso–ventral (D–V) axes — and for the relationship of landmarks, such as the site of gastrulation (*), to the mouth or anus of modern-day animals. For example, in this hypothetical model, the site of gastrulation corresponds to the posterior end of modern bilaterian animals and a new mouth opens on the ventral side, anteriorly. (Recent work in anthozoan cnidarians (a basal metazoan; see main text) contests this proposal⁴⁰, and argues that the site of gastrulation corresponds to the oral pole of bilaterians.) A suite of ‘gastrulation-specific’ genes would respond to the axial asymmetries that are set up during early embryogenesis, and generate new germ layers (in this case mesoderm, red) and their novel cell types. It is assumed that muscle (red circles) was the first form of mesoderm to appear and was followed by coelomic mesoderm.

INGRESSION OR DELAMINATION

Two forms of cell movement at gastrulation. Ingression involves individual cells actively migrating into the blastocoel, whereas delamination occurs when a cell divides perpendicularly to the surface of the embryo, to generate one internal and one peripheral daughter cell.

ORGANIZER

A small dorsal region of the blastopore of a vertebrate gastrula-stage embryo that has the remarkable capacity to organize a complete embryonic body plan.

AMNIOTE

An animal, such as a reptile, bird or mammal, whose eggs contain an amnion — an extra-embryonic membrane that surrounds the embryo and helps retain fluids and store waste products.

BASAL METAZOAN

A term given to animal phyla that diverged from more derived groups early in animal evolution.

of gastrulation is associated with embryonic patterning in many different systems: one example is the ‘**ORGANIZER**’ role of the dorsal lip, shield or node in amphibian, fish and **AMNIOTE** embryos, in corresponding order (FIG. 1).

The origins of embryonic and organismal polarity, and the formation of multiple germ layers, are just two of the mostly unanswered questions that surround the origins of the Metazoa (some others are listed in **BOX 1**). Developmental studies (**BOX 2**) — including more traditional as well as molecular studies — such as the evolutionary analysis of genes that are involved in body-plan formation, are crucial for unravelling the origins of the basic bilaterian characteristics, such as the A–P and D–V axes. Many of the genetic programmes that are associated with body-plan formation in bilaterian model systems are in place well before overt morphological bilateral symmetry is recognized in adults, which is consistent with the view that metazoan animals arose from a common molecular and developmental platform^{11,12}. The diversity in deployment of these genes in different organisms begs the question of which, if any, of the current model systems most closely corresponds to the ancestral bilaterian developmental programme. The appropriate selection and sampling of **BASAL METAZOAN** models, along with advances in rigorous phylogenetic inference, will also be invaluable in establishing the events that occurred

at the dawn of the Metazoa, and, importantly, in determining the direction of evolutionary change. We therefore need a renewed emphasis on the functional interactions of complex gene-regulatory pathways in a phylogenetic context to unravel the legacy of morphological complexity that is seen in the animals of today.

This review investigates some of the implications of recent data that have emerged from new model systems of representative basal metazoan taxa. These studies are starting to shed light on the origin of fundamental bilaterian characteristics, the relationship of the site of gastrulation to the embryonic and organismal axes, and the establishment of novel cell types that are associated with embryonic germ layers; however, they also highlight the difficulties in reaching a consensus on a comprehensive theory of the molecular basis of body-plan evolution.

Model taxa: evolution of bilaterian features

Most of the invertebrate and chordate model organisms that are studied are highly **DERIVED** members of their own **CLADES**, possessing short generation times and rapidly evolving genomes^{13–19}. Although even highly derived taxa might still possess some features of the ancestral stock, if we are to understand the evolutionary origins of bilaterian characteristics it is necessary to identify potential **OUTGROUP TAXA** that are derived from ancestors that did not possess the features for which we are trying

DERIVED

Evolved to a state that is distinct from the primitive condition.

CLADE

A lineage of organisms or alleles that comprises an ancestor and all its descendants.

OUTGROUP TAXON

A closely related taxon that is used for comparison; for example, to infer the ancestral versus the derived state of character evolution.

ACOELOMATE

An animal that does not possess a mesodermally lined body cavity in which the internal organs (gut and derivatives) are suspended. The coelom is a design character that is often used in evolutionary arguments about the origin of body-plan complexity.

TRIPLOBLAST

An animal that has derivatives of all three germ layers (ectoderm, mesoderm and endoderm). A diploblast has derivatives of two germ layers (ectoderm and endoderm (also known as endomesoderm)).

PLACOZOA

A 'group' (currently defined by one species, *Trichoplax adhaerens*) that has been proposed to be an intermediate between protozoans (single-celled animals) and metazoans (multicellular animals).

MESENCHYMAL

Describes an individual migratory cell or populations of cells that are not part of an epithelial sheet.

ARCHENTERON

The primitive gut that forms during embryogenesis at gastrulation.

BLASTULA

The stage of animal development that follows the early cleavage programme but precedes gastrulation.

GASTRULA

The stage of animal development during which the formation of distinct germ layers occurs; that is, gastrulation.

to determine the origin. A growing body of evidence argues that acoelomorph flatworms — simple ciliated acoelomate animals with A–P and D–V axes and derivatives of all three germ layers — represent a basal clade of triploblastic bilaterians^{20–22}. Unfortunately, there are not many extant 'pre-bilaterian' metazoan groups to sample. Only four relevant animal groups reside at the base of the metazoan tree: cnidarians, ctenophores, sponges and the single described species of the placozoa, *Trichoplax adhaerens*. The adult body plans of each of these taxa are fundamentally different from one another (FIGS 2,3), but all show features that could be interpreted as being incipient bilaterian characteristics, as discussed below.

Trichoplax adhaerens. This placozoan is an epithelial animal that consists of an outer epidermis and a small population of internal mesenchymal (fibre) cells²³ (FIG. 3d). It shows no evidence of an A–P axis or preferred direction of movement, but it does have a clear D–V axis, with a ventral (nutritive) surface along the substrate that is histologically distinct from the upper dorsal (protective) surface; however, it is not clear whether this axis is

homologous to the D–V axis of bilaterians. Genes that are involved in the bilaterian D–V patterning system (such as *dpp/bmp*, *sog/chrd* and *tolloid (tld)*) have yet to be recovered in *T. adhaerens*, and candidate genes that could be involved in endomesodermal patterning — orthologues of two T-box DNA-binding transcription factors *brachyury (bra or T)* and T-box containing transcription factor (*tbx2/3*) (REF. 24), and the homeo-domain-containing gene notochord-related homeobox (*not*)²⁵ — are also not differentially expressed along the D–V axis, as might be expected if the ventral surface corresponded to the presumptive archenteron or gut. These molecular studies have clearly shown that *T. adhaerens* has more than the originally described four cell types; however, viable embryos from *T. adhaerens* have never been recovered, so it has not yet been possible to study the development of this interesting organism.

Sponges. Adult sponges have an apical–basal organization relative to the substrate that is difficult to relate to any bilaterian axis, and there is no evidence of distinct adult germ layers (FIG. 3c). It is not even clear whether adult sponges possess true epithelial tissues, as they

Box 1 | Origin and evolution of metazoan body plans: unanswered questions

Were there constraints on early metazoan patterning?

- What was the genomic repertoire of the metazoan ancestor? Was morphological complexity driven by an increase in gene number, or by functional or regulatory interactions between early loci?
- How did novel cell types evolve? When did the germ line arise?
- Did any component of polarity that is observed in ancestral unicellular organisms relate to the evolution of embryonic, tissue or organismal polarity of extant multicellular metazoans?
- Do any extant metazoans show true radial symmetry? Were there ever any metazoans that did so?
- Was the first metazoan a deuterostome (for which the site of gastrulation corresponds to the anus) or a protostome (for which the site of gastrulation corresponds to the mouth)?

How did life history or ecology influence early metazoan development?

- Does ontogeny in any way 'recapitulate' phylogeny? That is, do the blastula and gastrula stages of development of modern organisms represent successive evolutionary stages towards increased body-plan complexity, or is this just the only way that we know how to think about this process?
- Did the foundations of metazoan body-plan evolution occur in free-swimming organisms in the plankton? Or were they established in organisms that were crawling around on the benthos?
- Do free-swimming larval stages of modern-day animals represent ancestral adult animals? Or are larvae novel forms that were inserted into the life history of ancestral, direct-developing benthic adults to allow dispersal over greater territories?
- When did separate mouth and anal openings evolve? Was this phenomenon related to increasing organismal size, or to burrowing or free-living life histories? How do changing patterns of gastrulation relate to the origin of both openings?

How many ways are there to make a multilayered organism?

- Which cellular behaviours were originally used to generate multilayered animals? Did the endoderm form as a result of cell-shape changes (epithelial folding) or from changes in cell adhesion (epithelial–mesenchymal transitions), or both?
- What was the nature of the first mesodermal tissue? Was it epithelial in nature? Did it generate a different compartment, such as the hydrostatic skeleton or coelom as seen in deuterostomes, or was mesoderm originally composed of muscular or contractile tissue and later expanded to give rise to parenchyma and mesenchyme, circulatory systems and coelom cavities?
- There are clearly conserved genes that are involved in axial patterning, gastrulation and germ-layer formation. Do these represent conserved networks of gene activity or the independent co-option of regulatory genes? Which parts of these pathways are the most highly conserved?

Box 2 | What can development tell us about the evolution of metazoan body plans?

PLANKTON

Small plants and animals that live and drift in the open ocean without associating with the ocean floor for at least part of their life cycle.

BENTHOS

The bottom of the ocean or body of water. The sea floor.

DIRECT DEVELOPMENT

A developmental strategy in which embryogenesis generates a juvenile adult without the formation of an intervening larval form.

HYDROSTATIC SKELETON

A means of maintaining body integrity by internal hydrostatic pressure, rather than by using a hard external exoskeleton (arthropods) or an internal endoskeleton (chordates).

PARENCHYMAL

Describes the loose tissue that fills the space between other tissues or organs.

LIFE HISTORY

The reproductive strategy of an organism.

DEMOSPONGE

The largest class of sponge (poriferans) that has siliceous (not calcareous) spicules and comprises roughly 95% of all sponge species.

ORAL-ABORAL AXIS

The body axis that runs from the oral side to the side that is opposite the oral (the 'ab' prefix means opposite). The term is used in animals that do not have an obvious anterior-posterior axis.

SHALLOW EST SCREENS

The sequencing of relatively small numbers (typically thousands) of random cDNA molecules from a given tissue to gain insight into the nature of gene expression, but is not intended to catalogue all rare transcripts.

PARAHOX GENES

Developmentally regulated transcription factors that are the evolutionary sister group to Hox genes and show collinear patterns of expression in chordate embryos.

The study of development contributes both a conceptual framework and experimental approaches for understanding body-plan evolution. As early as the middle of the nineteenth century, Haeckel⁹² proposed that the early stages of development (for example, the blastula and gastrula) represented the early phases of metazoan organismal complexity. Although some details have been refuted, the impact of his observations remains an important part of our thinking.

Early embryos and early metazoans share several attributes: they increase their cell number by cell division; show axial properties that lead to regional specification of adult body plans; and generate different tissues by the morphogenetic rearrangements of cells relative to one another.

The coordinated process of gastrulation and its specific relationship to the future adult body is also seen in our classification of living organisms. Despite the different patterns of gastrulation — which can occur at different developmental stages and can involve dozens or thousands of cells — bilaterally symmetrical animals are still categorized as being either protostomes or deuterostomes (FIG. 2a), depending on whether the site of gastrulation becomes the mouth or anus, respectively.

Genes that are involved in gastrulation in genetic model systems such as flies, nematodes and mice were present in the common ancestor of all bilaterians. It remains a challenge to determine which components of potentially conserved networks of gene activity are involved in particular aspects of the metazoan gastrulation process. For example, β -catenin and components of the Wnt signalling pathway seem to be involved in determining the normal site of gastrulation movements^{59–61}; the transcription factor Snail regulates the changes in cell adhesion that are required for epithelial to mesenchymal transitions^{93,94}; genes in the GATA, forkhead, and NK-class families of homeodomain transcription factors are involved in determining cell fates in the endomesodermal germ layer in various metazoan taxa^{65,95–99}. Therefore, the comparative functional analysis of these genes in various metazoans will reveal the degree of conservation of these genetic pathways.

essentially filter feed through PARENCHYMAL tissue. Sponge larvae have a preferred direction of swimming (possibly indicating the existence of an A–P axis) but have no apparent D–V organization. However, they are multilayered, which indicates that larval stages might be the most appropriate stages of the sponge LIFE HISTORY for gaining insights into the evolution of metazoan body plans in this group. Several interesting metazoan gene families have been recovered from sponges. These include those that encode tyrosine kinase and frizzled receptors, and T-box, Pax (paired homeobox) and homeodomain-containing transcription factors^{26,27}; the roles of these genes have not been studied during sponge development, primarily because an ideal sponge developmental model system has yet to be identified (although the larval development of the DEMOSPONGE *Reniera* sp. is currently being studied intensively (B. Degnan, personal communication).

Cnidarians and ctenophores. Of the four extant basal metazoan clades, the cnidarians — especially anthozoan cnidarians (such as corals and sea anemones), with their simpler life history and morphology — and ctenophores are the most promising for understanding the origin of the bilaterian axial properties and the origin of triploblasty (FIGS 2,3a,b). Cnidarians and ctenophores are the two most basal metazoan phyla to have nerve cells, true epithelial tissues and germ layers²⁸. Textbooks describe these animals as being radially or biradially (in the case of ctenophores) symmetrical along their main longitudinal body axis (the ORAL-ABORAL AXIS), diploblastic, and possessing a functionally blind gut (one that is lined by endoderm) with a single terminal opening — the mouth (which also serves as the anus) — located at the oral pole²⁹. These textbook descriptions are clearly over-simplifications however — ctenophores have definitive muscle

cells (mesodermal derivatives) and both ctenophores and some anthozoans have anal pores that could be viewed as evidence of a through-gut.

Complexity of basal metazoans

Comprehensive molecular analyses of the proteome of basal metazoans have revealed many surprises³⁰. Comparative genomics studies have already indicated that morphological complexity and gene content are not obviously related. SHALLOW EST SCREENS in a coral species³¹ and gene surveys in the sea anemone *Nematostella vectensis* (both anthozoan cnidarians, the most basal of the four cnidarian classes^{32,33}) indicate that morphologically simple anthozoans possess and express gene-family members that are present in vertebrates but that have been lost in other invertebrate genomes (such as those of the fly and nematode). For example, *N. vectensis* (the first cnidarian to have its entire genome sequenced) possesses orthologues of 11 of the 12 Wnt gene families, whereas *Caenorhabditis elegans* and *Drosophila melanogaster* possess only 5 and 6, respectively³⁴. Continued searches in basal metazoans whose genomes have been completely sequenced will be instrumental in understanding the molecular basis of evolutionary novelty.

Although cnidarians such as *N. vectensis* have more genes than one would expect given their simple appearance, molecular phylogenetics indicates that the placozoan *T. adhaerens* should have other developmental patterning genes. An anterior PARAHOX GENE, *trox-2* (REFS 35,36), has been recovered in *T. adhaerens*. One cannot have ParaHox genes without Hox genes as they arose simultaneously in evolutionary history from the duplication of a ProtoHox/ParaHox cluster consisting of four linked progenitor genes^{37,38}. However, no other Hox or ParaHox genes other than *trox-2* have been recovered from *T. adhaerens*; so, assuming that

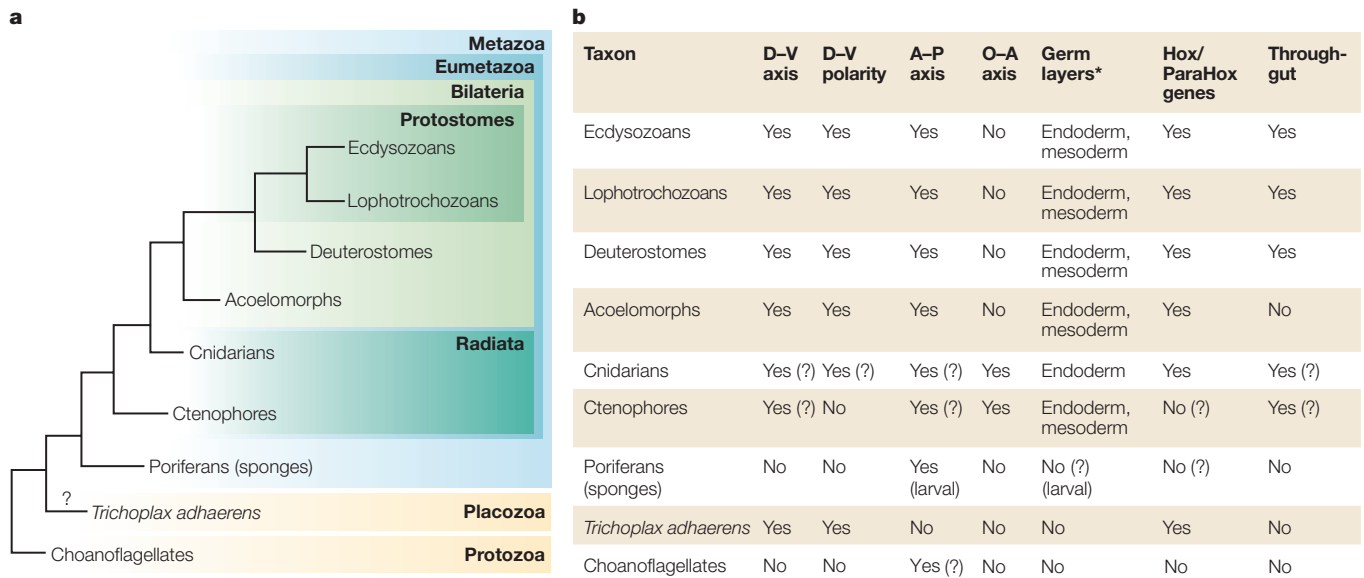


Figure 2 | **The phylogenetic relationships of basal metazoans.** The tentative tree (a) is based on information from molecular phylogenetic studies, and some pertinent issues of body-plan organization for each basal metazoan taxon are listed in the table (b). Although comprising the acoel and nematodermatid flatworms (comprising the Acoelomorpha), which lack a mesodermally lined coelomic cavity, are probably the basal extant group of triploblastic bilaterians^{20–22}, the phylogenetic position of the four ‘pre-bilaterian’ animal phyla (cnidarians, ctenophores, sponges and placozoans) is still controversial, depending on whether morphological or molecular characters are emphasized. Cnidarians and ctenophores have been categorized as diploblastic metazoans that are radially symmetrical along their oral–aboral axis (O–A). Ctenophores (and acoel flatworms) have definitive muscle cells — which are derived from endomesoderm, and a centralized nervous system (apical organ) — which tend to unite them with triploblastic bilaterians. However, molecular data argue that cnidarians are the sister group to the bilaterians, as shown here^{28,32,82–84}. The position of the placozoan *Trichoplax adhaerens* is still in doubt. Small subunit ribosomal RNA analysis^{28,82,100} indicates that *T. adhaerens* is a derived and secondarily simplified planulazoan or bilaterian. However, morphological and mitochondrial genome analysis indicates that it diverged from the rest of the Metazoa before the clade that contains sponges and eumetazoans (as shown here; S. Dellaporta, personal communication). A–P, anterior–posterior; D–V, dorso–ventral. *Ectoderm is present in all taxa.

they have not been simply overlooked, *T. adhaerens* might have lost as many as 7 genes (4 Hox and 3 ParaHox) over evolutionary time³⁹.

Alternatively, phylogenetic analyses might have misidentified *trox-2*. It has been suggested that it is the ancestral ProtoHox/ParaHox gene that gave rise to the progenitor of the Hox and ParaHox genes of all metazoans and that it merely retains similarity to anterior ParaHox genes³⁶. This is an attractive hypothesis if *T. adhaerens* is the most basal extant metazoan clade, as the mitochondrial data indicate (S. Dellaporta *et al.*, personal communication). No *bona fide* Hox and ParaHox genes have been recovered so far either from sponges (B. Degnan, personal communication) or ctenophores (M.Q.M., unpublished observations), which indicates that the expansion of the Hox and ParaHox clusters arose later in metazoan evolution (in the ancestor of cnidarians and bilaterians). Whole-genome sequencing of both *T. adhaerens* and a sponge in the *Reniera* genus are currently underway at the **Joint Genome Institute** and should help to determine the complement of axial patterning genes that are present in the basal metazoan toolkit and resolve the true phylogenetic position of extant metazoans

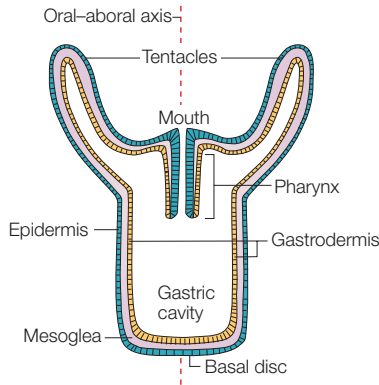
The presence of A–P and D–V axes and the possession of three embryonic germ layers are definitive characteristics of the Bilateria. Cnidarians and ctenophores share a more recent common ancestor with bilaterians

than any other extant group of animals; therefore these taxa are clearly pivotal for elucidating the origins of these important features.

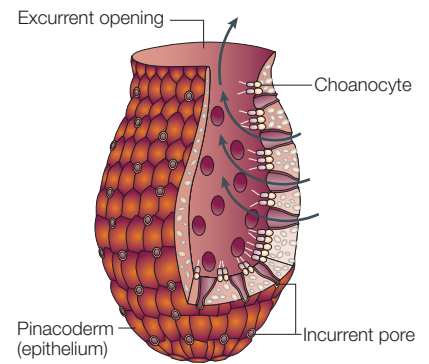
Origin of the A–P axis

Arguments have been made on the basis of gene-expression patterns in *N. vectensis* that the oral–aboral axis of cnidarians corresponds to the A–P axis of bilaterians⁴⁰. It can be problematic to give definitive orthology assignments to individual cnidarian Hox genes, particularly as these genes are also not expressed in the same kind of complex combinatorial code that is seen in more derived and morphologically complex bilaterians. Despite these obstacles, however, it seems likely that Hox and other genes were involved in patterning the main longitudinal axis of cnidarian and bilaterians before these two groups diverged about 600 million years ago. Although Hox genes are expressed in all three germ layers in bilaterians, the coordinated collinear expression that is the hallmark of A–P patterning in bilaterians is seen primarily in the neurectoderm and paraxial mesoderm^{1–4}. By contrast, in *N. vectensis* most Hox genes are expressed primarily in the bifunctional endomesodermal gastrodermis and not the ectoderm⁴⁰. Cnidarians possess a diffuse nerve net in both ectodermal and endodermal layers, which indicates that Hox genes were co-opted into patterning the CNS and mesoderm concomitant with their origin in bilaterian ancestors.

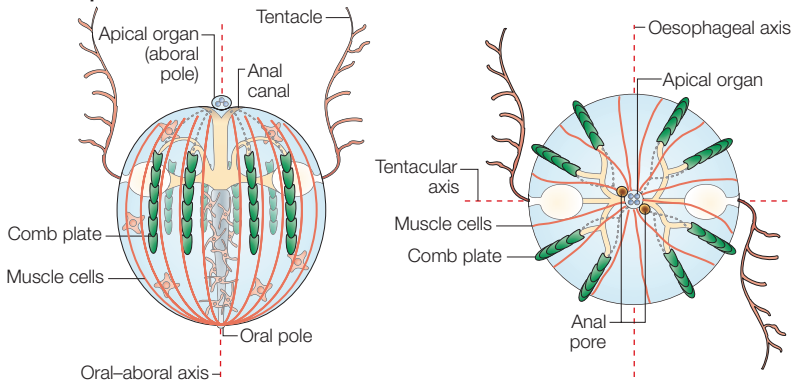
a Anthozoan cnidarians



c Sponges



b Ctenophores



d Trichoplax adhaerens

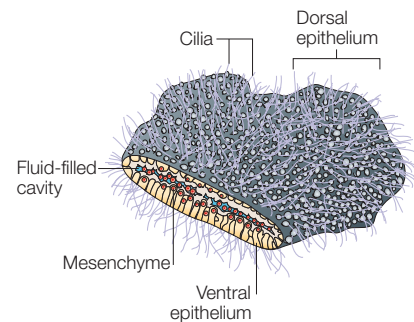


Figure 3 | Body plans of four basal metazoan taxa. a | Lateral (left) and cross-sectional (right) views of a benthic anthozoan (cnidarian) polyp. The main longitudinal axis is the oral–aboral (O–A) axis. Cnidarians are diploblastic, possessing only ectoderm (blue) and endoderm (yellow), separated by the largely acellular MESOGLEA (pink). Note the single opening to the gastric cavity, which is surrounded by tentacles. As can be seen in the cross-section, anthozoans show signs of internal bilateral symmetry in the positioning of their mesenteries, which connect the pharynx to the outer body wall. The siphonoglyph (a ciliated groove that runs along the length of the pharynx) marks polarity along the dorso–ventral (D–V) axis. **b** | Lateral (left) and aboral (right) views of the body plan of ctenophores. The lateral view shows the O–A axis, with the gravity-sensing statocyst (apical organ) located at the aboral pole. Ctenophores have two planes of symmetry that run through the O–A axis (right panel): the tentacular axis and an axis that is perpendicular to the tentacular axis and is called the oesophageal axis. Ctenophores have definitive muscle cells (red): these are located in distinct areas of the animal, which moves through the water column by the coordinated beating of eight longitudinal rows of COMB PLATES. Note that two small anal canals open at the anal pores that are adjacent to the apical organ. **c** | Sponges attach to a substrate and are composed of functional units or chambers that filter seawater. The chambers are lined with flagellated cells (choanocytes) that generate flow throughout the chambers and have remarkable similarities to choanoflagellate protozoans (FIG. 2a). Sponges do not possess true epithelial tissue; particles are trapped by phagocytic cells that can migrate throughout the choanocyte chambers. Sponges ‘larvae’ (not shown) have a preferred direction of swimming, with the anterior end corresponding to the part of the adult that attaches to the substrate, but it is not clear whether the apical–basal axis of sponges has any relationship to a bilaterian axis. **d** | Lateral view of *Trichoplax adhaerens*. Thought to be the simplest of metazoan animals, these placozoans are flattened epithelial balls with dispersed mesenchymal ‘fibre’ cells in the internal cavity. There is no anterior–posterior axis or preferred direction of movement. A ‘D–V’ axis is defined by the absorptive columnar surface, which faces the substrate, and an upper protective epithelium on the ‘dorsal’ surface. Up to four unique cell types have been defined morphologically. Panel **b** is reproduced, with permission, from REF. 58 © (2004) Cold Spring Harbor Laboratory Press.

MESOGLEA
The extracellular space between the outer ectoderm and inner endoderm of diploblastic animals that is composed primarily of extracellular matrix molecules and can house loose populations of cells.

COMB PLATES
‘Paddles’ that are composed of arrays of thousands of cilia and that are found only in the locomotory organs of ctenophores.

ANIMAL POLE
The position on the oocyte and embryo in which the meiotic reduction divisions occur, and that will give rise to the anterior region of the adult body plan. It is the side opposite to the vegetal pole, which generally gives rise to the gut.

The Hox expression data also address the long-standing question of whether the mouth of cnidarians and ctenophores (which also serves as the functional anus and arises from the site of gastrulation) corresponds to the anterior, posterior or ventral pole of bilaterians. Cell-labelling experiments indicate that the site of gastrulation occurs at the ANIMAL POLE of cnidarian and ctenophore embryos^{41–44}; indeed, the head (anterior) region of most animal taxa develops from regions around the animal pole, and the head does not derive from the opposite, or vegetal, pole in any metazoan. In *N. vectensis* anterior Hox⁴⁰ and

ParaHox³⁸ genes are expressed near the oral terminus and posterior Hox genes are expressed at the aboral pole⁴⁰; the bulk of evidence therefore indicates that the oral pole of anthozoan cnidarians corresponds to the bilaterian anterior pole. This interpretation differs markedly from traditional morphological arguments, which propose that the site of gastrulation in early metazoans corresponds to either the posterior^{20,45–48} or ventral^{49–51} pole of modern-day bilaterians (FIG. 1). Further molecular markers of axial position are needed to extrapolate the axial properties of cnidarians to bilaterians.

No molecular work has confirmed that the oral–aboral axis of ctenophores, like that of cnidarians, is homologous to the A–P axis of bilaterians (*bona fide* Hox genes have not been recovered in any basal metazoan taxon other than cnidarians). However, this is extremely likely to be the case, because the oral–aboral axis in ctenophores is set up developmentally in precisely the same way as it is in cnidarians⁴⁴, and behaviourally the adult animal swims mouth–first. Sponge larvae also show a prospective A–P (swimming) axis; therefore, if whole-genome sequencing confirms the current failure to detect Hox genes in ctenophores and sponges, this will indicate that patterning along the A–P (oral–aboral) axis preceded the origin and/or recruitment of Hox or ParaHox genes. This provides an exciting opportunity for studying the origins of the molecular basis of axial patterning at the base of the Metazoa.

Origin of the D–V axis

Molecular studies of cnidarian embryogenesis have focused on the colonial marine hydrozoan *Podocoryne carnea* (which has a complex life cycle) and the anthozoans *Acropora millepora* (the staghorn coral) and *N. vectensis* (sea anemone). Expression studies throughout the development of *N. vectensis* have unexpectedly revealed that at least five genes — three Hox genes (*hox7*, *hox8* and *hox1a*) and two transforming growth factor- β (TGFB) family members (*dpp* and *gdf5* (growth differentiating factor 5)) — are expressed asymmetrically along an axis (the directive axis) that runs perpendicular to the oral–aboral axis⁴⁰ (FIG. 3a). One of these genes, *dpp*, which was first described as being asymmetrically expressed during gastrulation in *A. millepora*⁵², is an orthologue of *dpp* and *bmp2/4*, which are known to have a causal role in establishing the D–V axis in both flies and vertebrates, respectively⁵³. Together, these results indicate that the directive axis might be evolutionarily related to the origin of the D–V axis.

Other components of the bilaterian D–V network (such as *sog/chrd*, *bmp5–7*, *noggin* (*nog*) and *gooseoid* (*gsc*)) have recently been recovered in *N. vectensis*. All of these genes show asymmetrical expression along the directive (D–V) axis, and the orthologue of *gbx* (gastrula brain homeobox) shows left–right expression (D.Q. Matus, K. Pang, J. Thomsen and M.Q.M., unpublished observations), further supporting a deep homology with the bilaterian D–V axis. The cnidarians that have the greatest degree of ‘radial-ness’ are medusae in the most highly derived taxa (Hydrozoa); this loss of overt bilaterality is likely to be an adaptation to a drifting lifestyle in a pelagic environment.

Ctenophores have been described as being biradially symmetrical along two planes, the oesophageal plane and the tentacular plane (FIG. 3b). It is unknown whether either of these axes conforms to the directive (D–V) axis of anthozoan cnidarians. Therefore, although not morphologically obvious, it is likely that A–P and D–V patterning mechanisms were in place at least before the common ancestor of cnidarians and bilaterians.

The evolution of germ-layer formation

As I mentioned in the introduction, the position, process and consequences of gastrulation have had an important role in understanding the origins of axial organization in animal evolution. The complicated process of gastrulation generally takes place in the same way in any individual species, but the details can be radically different in closely related species. The site of gastrulation relative to the primary egg axis has been noted in many types of embryo, and genes that are involved in the gastrulation process are being investigated in an increasing number of taxa. The results of these studies provide important clues about the molecular control of body-plan evolution.

Site of gastrulation in deuterostomes and protostomes.

The site of gastrulation in DEUTEROSTOMES is highly conserved: it occurs at the vegetal pole and corresponds to the position of the anus. Fate-mapping experiments in basal deuterostomes (echinoderms and hemichordates) have shown that the mesoderm arises from the vegetal pole⁵⁴, whereas in chordate deuterostomes it arises in equatorial regions, sandwiched between the prospective ectoderm and endoderm (BOX 3a).

In protostomes the site of gastrulation is more variable and often difficult to study, owing to factors such as small egg size, internal fertilization, developmental rates and extra-embryonic investments. However, it generally initiates at positions between the animal pole and the equator of the embryo, and gives rise to the mouth. Comparative studies on non-model protostomes indicate that an anterior terminal mouth is the ancestral condition^{55,56} in ecdysozoan protostomes (nematodes, priapulids, tardigrades, pycnogonids and onychophorans). Although there is a dearth of reliable data on this subject, at least in some ecdysozoans the site of gastrulation corresponds to the animal pole⁵⁷. Clearly more comparative work is needed to determine the ancestral pattern of gastrulation for different nodes in the metazoan lineage.

Site of gastrulation in ‘pre-bilaterians’ (ctenophores and cnidarians).

Fate-mapping experiments (BOX 3a) in ctenophores⁴⁴ and cnidarians^{41,42}, including anthozoans⁴³ such as *N. vectensis* (P. Lee and M.Q.M., unpublished observations), have shown that the site of first cleavage, gastrulation and the future oral pole normally occur at the animal pole. Although this is true for all ctenophores that have been studied, it is more problematic to make such a generalization in cnidarians owing to the wide range of patterns of gastrulation present in this group⁵⁸. It is likely that the coordinated movement of cells, either individually or as small groups, to a single pole is the ancestral mode of gastrulation in early metazoans¹². So, both the position of gastrulation relative to the primary egg axis (the animal pole in cnidarians, ctenophores and many ecdysozoans versus the vegetal pole in deuterostomes) and the site of gastrulation relative to organismal organization (the mouth in protostomes versus the

DEUTEROSTOME

A bilaterian animal in which the mouth forms as a secondary opening, separate from the blastopore. Deuterostomes include chordates, hemichordates and echinoderms. By contrast, the mouth of protostomes develops before the anus during embryogenesis. Protostomes include arthropods, molluscs and worms.

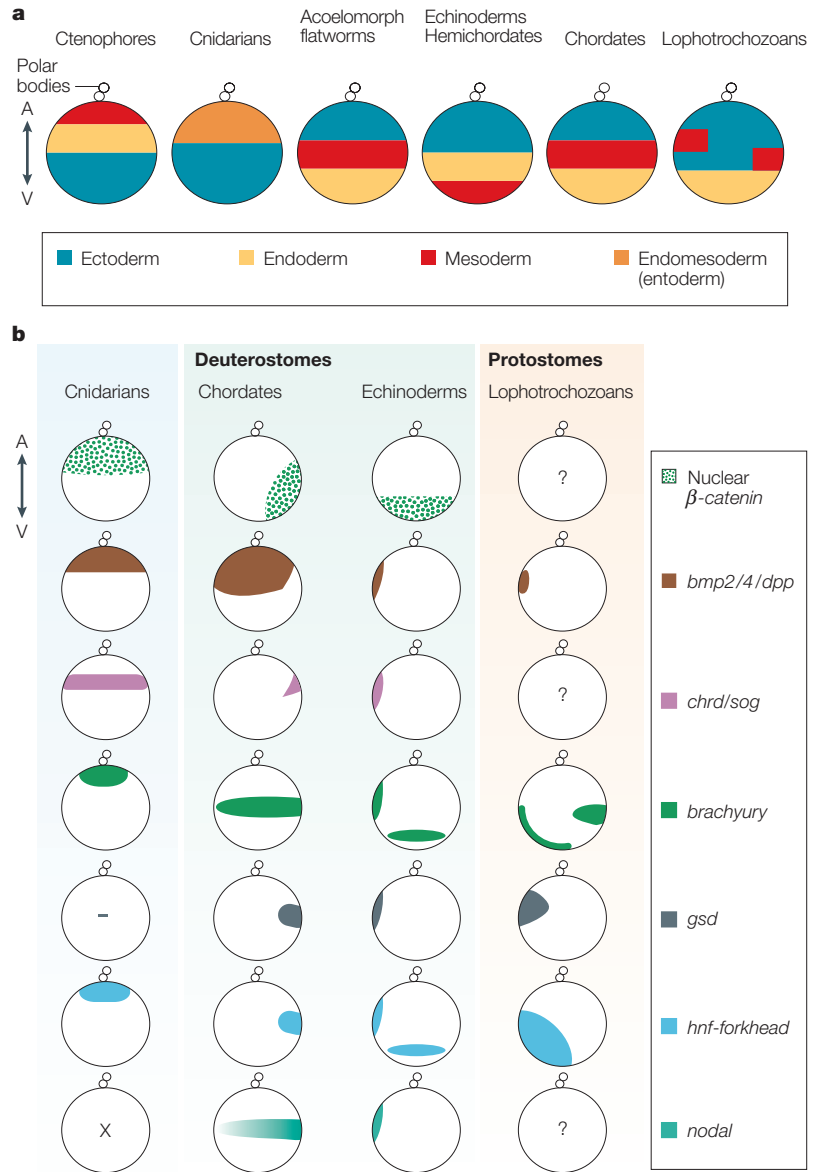
Box 3 | Changing axial positions of germ layers and associated gene expression at gastrulation

In cnidarians and ctenophores, the animal pole (A) corresponds to the site of the first cleavage, gastrulation and the future oral pole (see panel a). In ctenophores, mesoderm arises at the animal pole (polar bodies); by contrast, cnidarians fail to segregate mesodermal and endodermal germ layers, and instead a bifunctional endomesoderm arises in a similar location to the ctenophore mesoderm. Note that in echinoderms and hemichordates, mesoderm arises at the vegetal pole (V), whereas in chordates it arises equatorially.

Lophotrochozoans have dual sources of mesoderm, which is derived from both ectodermal and endodermal regions, but acoelomorph flatworms have only endomesoderm that arises from the vegetal pole. In most bilaterians, the animal pole corresponds closely with the anterior end of the adult, but the site of gastrulation and endomesoderm form at the vegetal region or subequatorial region; that is, opposite, or nearly opposite the animal pole. Changes in the site of gastrulation and patterns of cell movements relative to adult axial properties might partially explain how body plans change in metazoan clades.

Panel b shows the generalized patterns of gene expression at the start of gastrulation in different metazoan embryos, mapped onto the A–V axis. Although more thorough surveys need to be carried out, it is clear that some genes change their expression to track the changing site of gastrulation (for example, nuclear β -catenin), whereas other genes that are involved in gastrulation in some species are dissociated from the site of gastrulation in others (for example, *chordin* (*chrd*) and *goosecoid* (*gsd*) in echinoderms). Note that many of these genes are present in anthozoan cnidarians and are involved in gastrulation, and with further study it might be possible to understand how the molecular control of gastrulation and germ-layer specification affected body-plan diversification in metazoans. The polar bodies mark the animal pole.

'X' indicates that the gene is not present in the genome; '?' indicates that the gene has not been recovered in this clade; *bmp*, bone morphogenetic protein; *dpp*, decapentaplegic; *hmf*-forkhead, hepatocyte nuclear factor, a forkhead homologue; *sog*, short gastrulation.



anus in deuterostomes) has changed in various metazoan groups. However, the organismal axial relationships relative to the primary egg axis have remained largely the same; that is, the animal pole gives rise to the anterior of the animal, and the vegetal pole to the posterior. Unfortunately, experimental information on the relationship of axial properties during embryogenesis in sponges and *T. adhaerens* is not yet available.

Molecular remodelling of gastrulation. In all taxa, changes in egg size and the number of cells at the time of gastrulation have had marked effects on the cellular dynamics of gastrulation. For example, maternal nutritional contributions (in the form of yolk) to prospective gut lineages affect both the cleavage programme and the ability of cells to physically migrate, and the types of

signalling constraint on organizing thousands of cells during gastrulation in vertebrates, or a single pair of cells at the 28 cell-stage during *C. elegans* gastrulation, are obvious. However, molecular studies of gastrulation reveal even more variation than one would expect from these obvious morphological differences. Some genes seem to have highly conserved functions and are likely to have evolved early in metazoan development. Nuclear localization of the protein β -catenin is a robust and highly conserved marker that is causally involved with the position of gastrulation and endomesoderm formation^{59–61} (although there is a 180-degree reorientation of this location relative to the animal–vegetal axis in deuterostomes and pre-bilaterians; see BOX 3b). The molecular basis for β -catenin stabilization is an important problem in understanding the changing patterns of metazoan gastrulation.

The genes *twist* (*twi*) and *snail* (*sna*), which were first identified for their role in fly gastrulation, are expressed in mesodermal precursors and do not seem to be involved in gastrulation in vertebrates or lophotrochozoans⁶². However, several genes that are expressed in conjunction with gastrulation in chordates seem to be more broadly shared across the Metazoa (BOX 3b), although their spatial (and temporal) expression patterns can vary markedly among species. Even within the deuterostomes, many of the classic vertebrate dorsal-lip organizer genes (such as *chrd*, *gsd* and *nodal*) are expressed in the ectodermal oral field of sea urchins, which is the opposite side of the animal to the one we might expect^{63,64} (BOX 3b).

Many of the genes involved in gastrulation in both deuterostomes and protostomes are expressed at the site of gastrulation in anthozoan cnidarians (BOX 3b). Nuclear β -catenin⁶¹, *brachyury*⁶⁵, *sna*^{66–68}, *twi*⁶⁶, *dpp/bmp2/4* (REFS 40,52), *forkhead*^{66,67}, *gata*⁶⁶, *chrd/sog*, *otx* (*orthodenticle*), *bmp5–8*, *nog*, *gsd* (D.Q. Matus *et al.*, personal communication) and a large collection of Wnt genes³⁴ are expressed in association with the oral or animal pole. These data indicate that a common network of genes involved in gastrulation was present at least before the cnidarian–bilaterian divergence, and perhaps even earlier. In addition, these data support the idea that definitive mesoderm arose from endoderm, and that a segregation of myogenic factors from a common endomesodermal network of gene activity was responsible for the formation of a third germ layer during the gastrulation process.

It will be interesting to know the ancestral functions of these genes (for example, in regulating cell adhesion, germ-layer specification or cell signalling) and whether their changing expression in different metazoan animals is correlated with respective changes in gastrulation patterns. For example, *brachyury* is broadly expressed as a panmesodermal marker before gastrulation in chordates⁶⁹, but not in other animals^{70–72} including basal deuterostomes such as echinoderms^{73,74} and hemichordates⁷⁵. *brachyury* is expressed at the site of gastrulation in sea anemones (mouth/anus)⁶⁵, echinoderms (anus)⁷³, hemichordates (anus)⁷⁵, CHAETOGNATHS (anus)⁷⁶, molluscs (mouth)⁷⁰ and polychaetes (mouth)⁷¹, which indicates that it is involved with gastrulation movements; however, it is also expressed in the mouth of echinoderms, hemichordates, and chaetognaths, at positions that are far from the site of gastrulation, and in the mouth/anus of asexual buds in adult *Hydra*⁷⁷, which does not undergo anything that is similar to embryonic gastrulation. In molluscs and polychaete annelids, *brachyury* is not expressed in either the mouth or the anus, but along the ventral midline that connects the two^{70,71}. So, understanding the role of genes that are involved in the evolution of gastrulation can be difficult because they can be involved in several aspects of the gastrulation process, including spatial positioning, cell behaviour and the initiation of gene networks that are specific to a germ layer.

Nerve and mesodermal cell origins

On the basis of traditional morphological analyses, ctenophores have been thought to be the sister group to bilaterally symmetrical animals^{78–81}. Little is currently known about the molecular basis of axial organization in ctenophores. However, two distinct morphological features set adult ctenophores apart from the other three basal metazoan taxa: the presence of definitive individual muscle cells, and a ‘centralized’ nervous structure (the apical organ) that is located at the aboral pole (FIG. 3b). Whereas cnidarians are essentially epithelial animals that possess only ectoderm and a bifunctional endomesodermal gastrodermis (which carries out both absorptive and contractile functions), ctenophores possess definitive contractile muscle cells that reside in the extracellular space between the ectoderm and endoderm (FIG. 3a,b). Previous workers have refused to consider ctenophores as being triploblastic because they are acoelomate and never form a definitive mesodermal ‘layer’²⁹, but the fact that asymmetrical cell divisions give rise to distinct lineages of muscle (mesoderm) and gut (endoderm) in ctenophores leaves little doubt that these organisms possess definitive mesodermal descendants. Ctenophores could therefore be morphologically and phylogenetically the most appropriate of the basal organisms for understanding the origin of muscles and the metazoan nervous system^{28,82–84}. Although molecular confirmation of homology is still lacking, fate-mapping studies have shown that ctenophore muscle cells are derived from endomesodermal lineages⁸⁵, as are most muscles in other bilaterians, including muscle cells in a basal triploblastic acoelomorph flatworm⁸⁶ (FIG. 2).

The ctenophore apical organ consists of a highly innervated ‘gravity amplifying’ statocyst that integrates sensory information and controls locomotory behaviour through the beating of the eight longitudinal comb plates⁸⁷. The presence of a primitive ‘brain’ and derivatives of the third germ layer (mesoderm) in ctenophores could represent primitive bilaterian features. However, recent molecular phylogenetic studies that are based on both large and small subunit ribosomal DNA (FIG. 2) have argued convincingly that cnidarians are the true sister group to the Bilateria (in a group that are called the Planulozoa) and that ctenophores are the sister group to cnidarians and bilaterians^{28,33,82–84}. If this were true, it would indicate that cnidarians have lost the entire mesodermal germ layer and retain only a single bifunctional endomesodermal gastrodermis. This finding is supported by molecular studies in anthozoan cnidarians, which show that genes that are causally involved in the site of gastrulation⁶¹ and those that are involved in both endodermal and mesodermal development (*gata*, *sna*, *twi*, *hepatocyte nuclear factor* (*hnf*), *forkhead*, muscle *lim* and *tropomyosin*) are expressed in the pharynx and gut^{66–68}. If the phylogenetic position of ctenophores is basal to Cnidaria and Bilateria it also indicates that the ctenophore apical organ is not homologous to the brain, an argument that is supported by the absence of serotonin staining⁸⁸ or other brain markers⁸⁹. Indeed, the embryological origin of nerve cells in the Metazoa

CHAETOGNATHS
An enigmatic phylum of marine animals that have features of both protostomes and deuterostomes and therefore defy phylogenetic placement.

MEIOFAUNAL

A class of small but highly diverse interstitial animals that live between grains of sand.

is not clear. Work in species of the freshwater hydrozoan *Hydra* indicates that nerve cells arise from endodermal 'T' stem cells⁹⁰, although cell-lineage work in ctenophores indicates that at least some components of the nervous system are derived from ectodermal precursors⁸⁵.

Future prospects

The results described in this article highlight the importance of knowing the true phylogenetic relationships among the basal groups of organisms for understanding the gain (or loss) of important bilaterian characters. Despite intense efforts, neither existing morphological nor molecular data have unambiguously resolved the phylogenetic relationships among the species that diverged deep in metazoan evolution, although increased taxon sampling seems to be quite promising⁸⁴. Luckily, the **Joint Genome Institute** is currently sequencing the genomes of several basal metazoans, including *N. vectensis*, a species of *Reniera* and *T. adhaerens* (a ctenophore that is currently under consideration for 2006; D. Rokhsar, personal communication). It is therefore likely that the precise phylogenetic relationships and the genomic complexity of these basal metazoans will soon be resolved, allowing for a detailed analysis of the molecular basis for body-plan organization.

Conclusions

The last common ancestor of all metazoan taxa (with the possible exclusion of *T. adhaerens*) was probably a free-swimming, bilayered, planula-like organism, and

the formation of the varied types of adult body plans (for example, sessile adults or free-moving bilaterians) and complex cell and tissue types probably evolved later in independent lineages^{45,91}. Axial patterning genes (for example, Hox or ParaHox and *dpp* or *sog*) that are deployed in bilaterians could have been recruited well after functional axes arose, perhaps to refine new domains of morphological complexity. In addition, the lack of selection for increased axial organization in adult organisms (such as in burrowing, MEIOFAUNAL, parasitic or colonial organisms) might have driven the loss of ancestral features and of some of the molecular components of the underlying developmental pathways (for example, Hox genes) that generated them over evolutionary time.

Genes that are involved in gastrulation (for example, *β-catenin*, *sna*, *forkhead*, *gata* factors, and *brachyury*) seem to be conserved at least as far back as cnidarians. However, the precise roles of these genes (for example, changes in cell shape, adhesion or behaviour, or mesodermal or endodermal cell-type specification) in early metazoans are unknown. It is also unclear how many genes were present in these early networks, at what stages of metazoan evolution new genes were recruited (or lost), and how the spatial deployment of these networks was modified in individual lineages. Unravelling the intricate details of the embryonic regulation of axial patterning and gastrulation events in embryos of basal metazoan taxa will yield a rich source of information about the evolution of animal body plans.

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Competing interests statement

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