A SIMPLE PLAN — CNIDARIANS AND THE ORIGINS OF DEVELOPMENTAL MECHANISMS

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Comparisons with cnidarians, long considered to be 'simple' animals, are providing crucial insights into the origins of conserved developmental mechanisms and the nature of the common metazoan ancestor. Traditionally, an extra germ layer and a second axis of body symmetry are the features that distinguish 'higher' Metazoa from lower animals such as cnidarians. Moreover, it was expected that 'lower' animals would have a simple gene set that corresponds to their simple morphology. Now, molecular genetic approaches are blurring the developmental divide between cnidarians and bilateral animals, and cnidarian sequencing projects are showing that the common metazoan ancestor was more genetically complex than was previously assumed.

BILATERIA
A monophyletic group of metazoan animals that is characterized by bilateral symmetry. This group comprises all of the Metazoa except for the Radiata (Ctenophores and Cnidaria) and the Parazoa (sponges).

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Thanks to the application of powerful molecular genetic tools to a few 'model' animals, a great deal is now known about the common principles of embryonic development in the BILATERIA. The availability of model animal genomic sequences has allowed inferences to be made about the gene complement of the common bilaterian ancestor, Urbilateria. However, little is known about the origins of animal patterning systems, and, as more genome sequences and EXPRESSED SEQUENCE TAGS (ESTs) have become available, some of the initial assumptions about the urbilaterian gene complement have been shown to be incorrect. To understand the evolution of HIGHER ANIMAL (higher metazoan) genomes and the developmental processes that they regulate, it is necessary to make comparisons with an appropriate outgroup. The Cnidaria, a group of LOWER ANIMALS (lower Metazoa) that includes corals and sea anemones, are the natural outgroup for comparative genomic and developmental studies (see BOX 1).

Cnidarians nominally develop from two germ layers, the ectoderm and endoderm, whereas the presence of a third germ layer, the mesoderm, traditionally characterizes higher animals. This apparently fundamental developmental difference led to these groups being referred to as diploblasts and triploblasts, respectively. The other important characteristic that is typically used to distinguish animals such as cnidarians from higher metazoans is the nature of their body symmetry. Higher animals have two obvious body axes, anterior–posterior (AP) and dorsal–ventral (DV), and are therefore bilaterally symmetrical (hence the term Bilateria). Cnidarians, by contrast, have a single overt axis (oral–aboral; OA) that is defined by the presence of the mouth (or BLASTOPORE) at one end. The OA axis has classically been thought to correspond to the AP axis of higher animals[1].

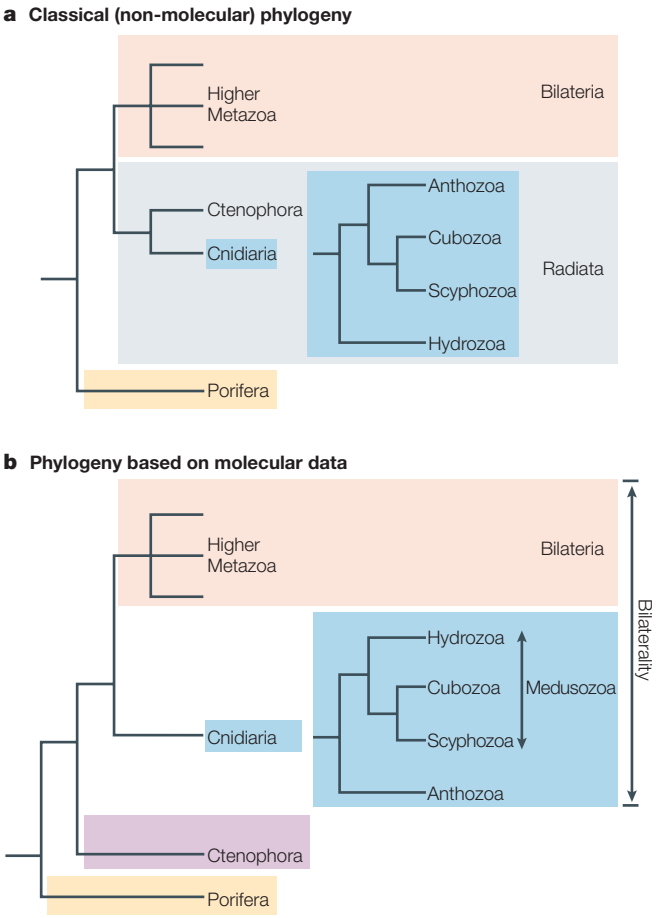
The single obvious axis and the near radially symmetric body plans of some of the MEDUSOZOANS led to cnidarians being grouped with ctenophores (comb jellies) as the Radiata, providing a neat division of the animal world into Bilateria, with three body layers and two axes, and Radiata, with two body layers and one axis.

However, some authorities have always regarded the features that distinguish these two groups as oversimplifications (summarized in REFS 2,3). Molecular techniques provide new ways to examine the validity of these distinctions as well as the reasonable expectation that the apparent morphological simplicity of cnidarians would be reflected in a simple genome. Molecular studies should also provide information on the genome composition of the common ancestor of cnidarians and bilaterians as any

Box 1 | **Changing views of relationships at the base of the tree of animal life**

According to pre-molecular phylogenies, the Cnidaria and Ctenophora were grouped either separately or together to form the RADIATA, whereas all of the 'higher' animals were grouped within the Bilateria (see figure part **a**). Within the phylum Cnidaria, the Hydrozoa were considered to be basal to the Anthozoa, Cubozoa and Scyphozoa. Under this view of phylogeny, the bi-radial or bilateral symmetry of anthozoans could be interpreted as having independently evolved within the Cnidaria.

Molecular data and some of the recent results summarized in the accompanying text have resulted in significant revisions to our views on how these groups of organisms are related (see figure part **b**). The position of the Ctenophora is uncertain[65,66], but here, we have followed the small subunit rRNA and combined analyses of Medina *et al.*[65]. The Ctenophora could be regarded as bilateral rather than radial, but we have left them in an uncertain category on the basis of the work summarized in Martindale *et al.*[3]. Within the Cnidaria, the Anthozoa are now generally regarded as basal on the basis of their possession of a circular mitochondrial genome, in common with the rest of the animal kingdom but in contrast to the other cnidarian classes[64]. This assignment is further strengthened by phylogenetic studies that are based on partial large-subunit[67] and complete small-subunit[24] rRNA sequences.



EXPRESSED SEQUENCE TAG (EST). A nucleic acid sequence that is derived from cDNA, usually from the ends of cDNA clone inserts as part of high-throughput sequencing projects.

HIGHER ANIMALS (Higher Metazoa). We use these terms as synonyms of Bilateria.

LOWER ANIMALS (Lower Metazoa). Here used to refer to Cnidaria, Ctenophora and Parazoa. Other authors include the Platyhelminthes (flatworms).

BLASTOPORE The opening of the archenteron in the gastrula.

MEDUSOZOA A clade comprising three of the four cnidarian classes, which produce a sexually reproducing medusa (jellyfish) as part of the life cycle.

RADIATA Animals that are traditionally considered to have radial symmetry. This group includes the ctenophores and cnidarians, and, according to some authors, the sponges.

TRANSVERSE FISSION A means of asexual reproduction seen in some sea anemones that involves division of the polyp into two or more parts with cleavage occurring in the transverse plane.

BLASTULA The early stage of animal development in which a single layer of cells surrounds a fluid-filled cavity, forming a hollow ball.

PLANULA The free-swimming, ciliated larva of a cnidarian.

ZOOID An individual specialized unit of a colonial cnidarian.

genes that are found in both groups were presumably present in the common ancestor. The surprising findings of such studies are the topic of this review.

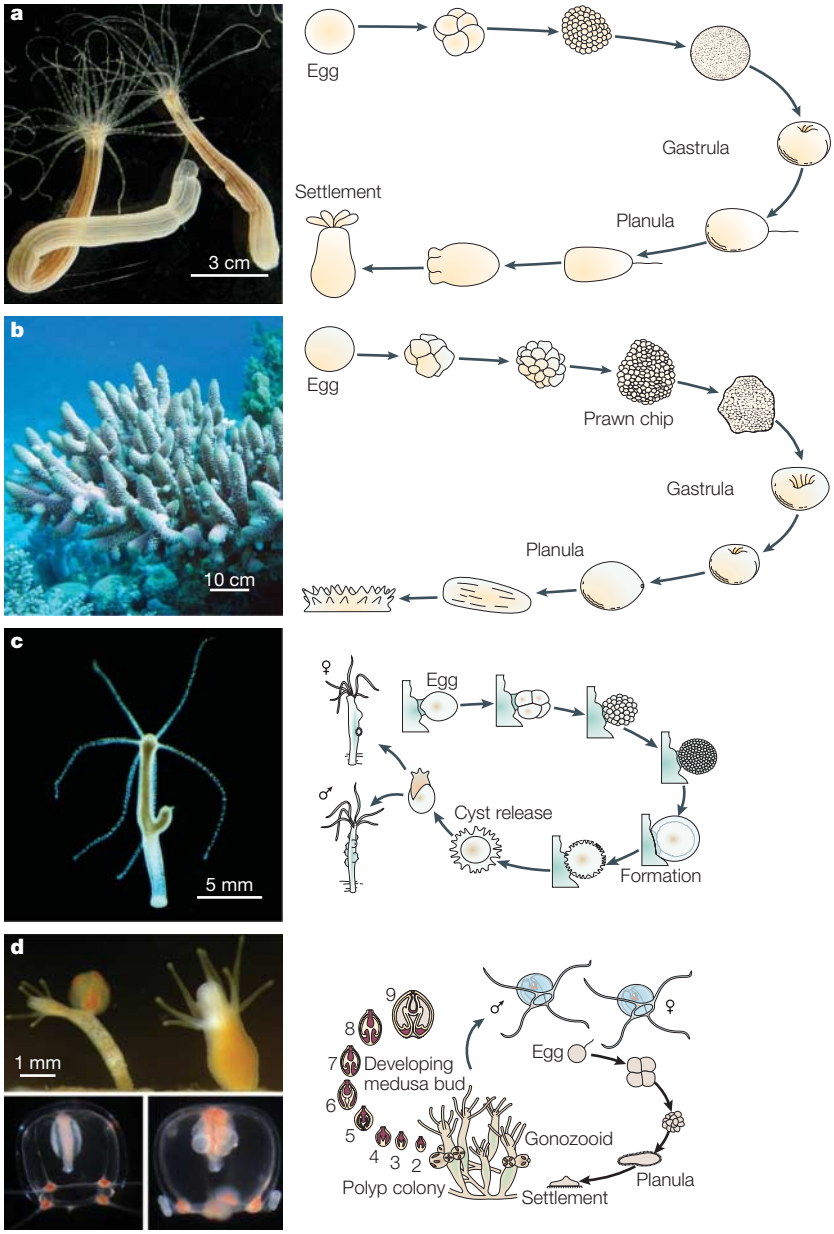
In terms of morphology and cellular diversity, cnidarians are the simplest animals at the tissue level of organization. But as we learn more about their genomes and gene-expression patterns, these simple animals reveal levels of complexity and striking similarities with higher animals to an extent that challenges many of the traditional assumptions about animal evolution. The expression pattern of, for example, the *snail* gene in the coral *Acropora millepora*[4] is strikingly similar to its counterparts in the fly and mouse. However, some cnidarian genes show highly divergent patterns of expression — for example, the expression pattern of an *Otx* gene in the jellyfish *Podocoryne carnea* indicates functions that are unrelated to those seen in higher animals[5]. Moreover, in some cases, closely related genes are expressed in seemingly unrelated ways in different cnidarians[6]. Understandably, much is made of the similarities in gene use between cnidarians and bilaterians, from which gene use in the common ancestor can be inferred. Here, we argue that the differences can also be informative.

In this review, we focus on recent data from four cnidarians (BOX 2): the anthozoans *Nematostella vectensis* and *A. millepora* and the hydrozoans *P. carnea* and *Hydra* spp. The last of these, however, receives relatively little attention because we focus on early developmental processes for which there are few molecular data that are directly comparable to data available for *N. vectensis*, *A. millepora* and *P. carnea* (see also BOX 2). First, we review recent findings from the Cnidaria that emphasize their similarity to the Bilateria and that question the validity of classical distinctions, such as those between diploblasts and triploblasts. Second, we argue that although cnidarians are in many respects mainstream animals, they might lack some key genes that characterize higher animals. Third, we discuss the paradoxical genetic complexity of, and molecular variation within, the Cnidaria.

Blurring the diploblast/triploblast boundary

In higher animals, there is an intimate connection between the process of gastrulation and the creation of mesoderm, to the extent that some authors have even defined gastrulation as the process by which mesoderm is formed (see, for example, REF. 7). In their valuable review of this topic, Technau and Scholz[8] use the much broader definition, which they trace back to Haeckel[9], that gastrulation "means the formation of a gastric cavity, the archenteron, in which food can be digested

Box 2 | **The model cnidarians**



Nematostella vectensis (Anthozoa; see figure part **a**) is a small burrowing sea anemone that is found in coastal estuaries on both the Atlantic and Pacific Coasts of the United States, as well as south east England[68]. It has separate sexes but can also reproduce asexually by TRANSVERSE FISSION. Its life cycle is simple. Gametes are released by the separate sexes, and after fertilization, development begins, producing a hollow BLASTULA. Gastrulation occurs by a combination of invagination and ingression. After gastrulation, a tear-drop-shaped, ciliated PLANULA is formed with the blastopore becoming the mouth. Eventually, tentacles are formed and the polyp sinks to the bottom, after which tentacle and septum formation continue[20,68,69]. Photograph in part **a** courtesy of U. Technau. Drawings in part **a** are modified with permission from REF. 68 © (1992) Marine Biological Laboratory and from REF. 69 © (2002) Springer.

Acropora millepora (Anthozoa; see figure part **b**) is a branching reef coral, widely distributed in shallow water throughout the tropical Indo-Pacific. Features of *A. millepora* embryonic development that differ from those seen in *N. vectensis* include formation of a flattened bilayer stage ('prawn chip') immediately before gastrulation and the complete closure of the blastopore to produce a sphere after gastrulation. The sphere then elongates first into a pear-shaped, ciliated planula and then into a spindle shape. The planula can survive for months before settling, but when it does so, it undergoes a much more pronounced reorganization than in *N. vectensis*, totally destroying its nervous system and markedly reorganizing its tissues to form a flattened crown-like structure within which production of the calcareous skeleton begins. From this structure, the first polyp arises, with subsequent branching giving rise to the colony. Photograph in part **b** courtesy of M. van Oppen. Drawings in part **b** are modified with permission from REF. 70 © (2002) Wiley.

Hydra (Hydrozoa; see figure part **c**) has been studied for more than 200 years, but is an atypical cnidarian in several important respects. First, unlike most cnidarians, *Hydra* is a freshwater animal. Second, it usually reproduces by asexual budding: when sexual reproduction does occur, there is no free-swimming stage. The egg develops on the side of the female. A cyst eventually surrounds the developing embryo and is released from the female's side. Once the cyst hatches, it develops into a polyp. *Hydra* is probably the most studied cnidarian and has provided many important insights into the evolution of both conserved developmental pathways (see, for example, REF. 43; reviewed in REF. 73) and peptide signalling (see REF. 74 for a review). However, owing to the technical challenges posed by the fact that early development occurs within a cyst, *Hydra* embryology has only recently been described[75], and, with the exception of the paper of Fröbius *et al.*[71], there are few molecular data directly comparable to that available for *N. vectensis*, *A. millepora* and *Podocoryne carnea*. Photograph in part **c** courtesy of T. Bosch. Drawings of developmental stages in part **c** are modified with permission from REF. 71 © (2003) Springer. Drawings of male and female *Hydra* in part **c** are modified with permission from REF. 72 © (2004) Brookes/Cole–Thomson Learning.

P. carnea (Hydrozoa; see figure part **d**) is a colonial marine hydroid that has the typical medusozoan alternation of generations between the motile medusa and sessile colony forms. The latter consists of morphologically distinct polyps that are specialized for feeding (gastrozooids, right) and reproduction (gonozooids, left). The gonozooids have medusa buds that increase in size through a series of developmental stages until they are liberated as free-swimming medusae (bottom photographs: male, left; female, right). The blastula is elongated and gastrulation is by unipolar immigration. As in *A. millepora*, the motile ciliated *P. carnea* planula undergoes a pronounced reorganization on settlement before colony development begins. *P. carnea* has been especially valuable for studying processes that are unique to medusae and might yield insights into the genetic basis for the alternation of generations and the production of differing types of ZOOID (see REF. 76). Photographs in part **d** courtesy of V. Schmid and T. Momose. Drawings in part **d** are modified with permission from REF. 76 © (2002) University of Basque Country Press and from REF. 77 © (2000) Institut de Ciències del Mar de Barcelona (CSIC).

Box 3 | **Organizers and germ layers: Brachyury and the Wnt pathway**

Genes that are related to the mammalian Brachyury (*T*) gene have roles in both mesoderm specification and axis elongation throughout the Bilateria[78]. In *Podocoryne carnea*, *Brachyury* is a maternal gene with widespread initial expression, which becomes stronger and more focused at the late blastula–early gastrula stage in ingressing tissue[17]. It is not expressed straight after gastrulation but it is expressed later in the anterior ectoderm of the planula. In the polyp, it is weakly expressed at the tip of the HYPOSTOME, whereas in the medusa, it is found wherever cell proliferation is occurring. Expression of *HyBra*, the *Hydra Brachyury*, is consistent with that seen in *P. carnea* to the extent that it is associated with the mouth, which arises from the blastopore, from the time of its earliest emergence[79,80] — a pattern that is initially difficult to relate to expression in vertebrates. However, Technau[80] has proposed that "the mesoderm arose during animal evolution by segregating a subset of cells from the blastoporal region." A study of *Nematostella vectensis Brachyury* revealed an early pattern similar to that seen in *Hydra* with initial expression around the blastopore, moving into the MESENTERIES as they started to develop, and then persisting in the mesenteries, but not their muscles, in the adult polyp[81]. This expression pattern indicated to the authors that the ancestral function of *Brachyury* was "in specifying the blastopore and its endodermal derivatives".

Components of Wnt signalling systems are also differentially expressed along the cnidarian oral–aboral (OA) axis. The study by Wikramanayake *et al.*[82] implies that during *N. vectensis* gastrulation, β-catenin is differentially stabilized along the OA axis, translocated into nuclei at the site of gastrulation and used to specify entoderm[82]. However, this last point has been questioned by Primus and Freeman[83] on grounds that include the Wnt/β-catenin pathway having many roles in different animals, which do not always include either endoderm specification or gastrulation. The finding that *Wnt* and *Tcf/Lef* (an effector in the Wnt signalling pathway) are expressed in the *Hydra* hypostome[43] is subject to a similar caveat in its interpretation because Wnt signalling has been implicated in patterning along the anterior–posterior, dorsal–ventral and proximal–distal axes in different animals.

later in adults". According to this definition, cnidarians gastrulate without forming mesoderm, and gastrulation is the process by which one or more internal cell layers are created from a single cell layer that is exposed to the surrounding environment. The genetic mechanisms that specify which cells will become internalized are still unclear, although there have been indications that Brachyury and the Wnt pathway are involved in this process in Cnidaria (BOX 3).

One of the most obvious derivatives of mesoderm is muscle. Over the past 20 years, the molecular events that underlie the specification of both mesoderm and muscle have been established in several model systems, which has allowed the identification of common principles (see, for example, REF. 10). In *Drosophila melanogaster*, two genes have key roles in the differentiation of mesoderm — *twist*, which encodes an activator of mesoderm-specific genes such as the muscle and heart determinants *Mef2* and *tinman*, and *snail*, which encodes a repressor of non-mesodermal genes. In vertebrates, closely related genes seem to have similar roles; *Twist* is central to early mesoderm patterning and muscle specification[11], and *Snail* genes have central roles in mesoderm formation[12].

The expression of mesodermal marker genes and muscle development in P. carnea. Although cnidarians lack obvious mesoderm, many jellyfish have musculature with similarities to striated muscle in triploblastic animals at both ultrastructural[13,14] and molecular[15] levels. Over the past few years, in a seminal series of papers, the laboratory of V. Schmid has reported the presence and expression patterns during jellyfish (medusa) muscle development of *P. carnea* genes related to those involved in mesoderm and muscle specification in triploblastic animals[16–18]. The early medusa, initially consisting of only ectoderm and endoderm, is formed by the proliferation of dedifferentiated POLYP cells. Later, a third layer, the ENTOCODON, separates from the ectoderm and ultimately forms the SUBUMBRELLAR musculature of the medusa bell[16–18] (FIG. 1). During medusa development, the helix-loop helix (HLH) gene *twist* is expressed early and transiently in the entocodon before muscle differentiation[16]. However, it should be noted that unlike its homologue in *D. melanogaster*, which is expressed only in mesoderm and mesodermally derived tissues, the *P. carnea twist* gene is expressed in several areas of high cell proliferation in addition to the dedifferentiating ectodermal and endodermal cells from which the entocodon arises.

Mef2 is expressed in a similar pattern to *twist*, whereas *snail* is expressed initially in dedifferentiated endoderm and ectoderm, with expression that is strongest in the entocodon during the formation of presumptive striated muscle and disappearing once this has differentiated[17]. Several other HLH genes related to those involved in myogenesis in higher animals are also expressed in the entocodon of *P. carnea*[18]. This series of studies indicates a sequence of events in the entocodon that mirrors, at the molecular level, the emergence of mesoderm and its differentiation into muscle in higher animals. So, *P. carnea* has muscle and seems to use the same genes in its specification as triploblasts do. How these results should be interpreted, however, continues to be a hotly debated topic to the extent that definitive statements are probably inappropriate. The strong molecular evidence that Anthozoa, which lack a medusa stage, are the basal cnidarians (BOX 1) implies that all of the similarities in the control of muscle formation between medusae and bilaterians reflect either convergence or coincidence. An alternative interpretation is that the formation of the entocodon might represent a form of delayed gastrulation; this view is favoured by Schmid and his collaborators (at the Zoologisches Institut der Uni Basel; see online links box), and is based

HYPOSTOME
The terminal region of a polyp, on which the mouth is situated.

MESENTERIES
Longitudinal sheets of tissues that extend radially from the body wall into the body cavity.

POLYP
The sessile form of life history in cnidarians; for example, the freshwater *Hydra*.

ENTOCODON
The mass of cells on the end of a medusa bud that becomes the velum and subumbrella surface.

SUBUMBRELLAR
The oral surface of a medusa.

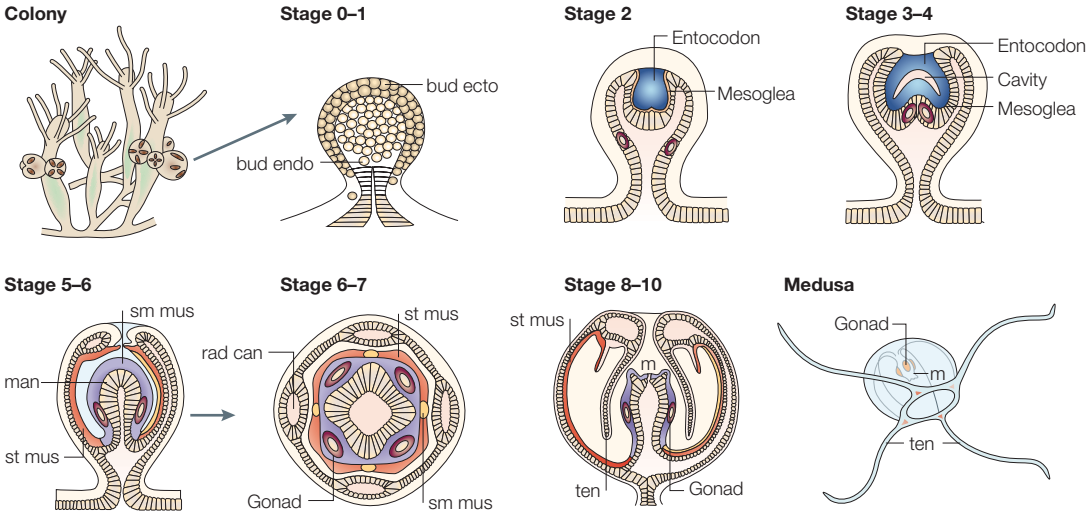


Figure 1 | **Muscle development in the medusa of *Podocoryne carnea*.** Mesodermal and muscle marker genes are expressed within the bell of the developing medusa, which arises from a bud on the gonozooid (reproductive polyp) of the *P. carnea* colony. The tissue that expresses the genes, and gives rise to the muscle, is the entocodon (blue), which is demarcated from the overlying cells by an acellular layer known as the MESOGLEA. As the stalk on which the mouth and gonads will be borne (the manubrium; man) pushes upwards at stages 5–6, it is covered by tissue of the entocodon differentiating into muscle. A cross-section of the developing medusa at the level indicated by the arrow is shown at stages 6–7. By stages 8–10, the entire inside of the bell of the medusa is lined with muscle. When the medusa is mature, it breaks loose from the colony, the tentacles flip outwards from within the bell and the medusa swims away, powered by the pulsing of the bell. Red indicates striated muscle, yellow and purple indicate smooth muscle. ecto, ectoderm; endo, endoderm; m, mouth; man, manubrium; rad can, radial canal; sm mus, smooth muscle; st mus, striated muscle; ten, tentacle. Modified with permission from REF. 18 © (2003) Elsevier Science and from REF. 76 © (2002) University of Basque Country Press.

on the assumption that the medusa is the ancestral cnidarian body plan[19]. Acceptance of this latter interpretation requires either rejection of the strong molecular evidence that Anthozoa are basal, or that the Anthozoa have lost the basal medusoid generation.

Irrespective of whether the entocodon is true mesoderm, the work on muscle specification in *P. carnea* does clearly illustrate the claim of Willmer[2] that "there is a continuum of degrees ... of triploblasty, and on no logical grounds can the cnidarians and ctenophores be set widely apart from other metazoans".

Mesodermal markers in anthozoan embryonic development. If the molecular phylogeny shown in BOX 1 is correct, then the polyp is probably the ancestral cnidarian body plan, as anthozoans lack a medusoid stage. For this reason, it might be more revealing of both the cnidarian and ancestral conditions to examine whether the mesoderm/muscle suite of genes is expressed at the time of anthozoan embryonic gastrulation, as it is in higher animals.

We identified a member of the snail family of transcription factors in *A. millepora* that is expressed in a pattern that closely resembles that of its *D. melanogaster* counterpart during the early stages of gastrulation[4] (FIG. 2). In both systems, expression is first seen on one side of the pre-gastrulation embryo, after which the expressing cells invaginate and eventually become internalized.

In *N. vectensis*, two *snail* genes are expressed in indistinguishable patterns[20] that seem to correspond to that of the *A. millepora* gene shown in FIG. 2, within the limits imposed by the different gastrulation patterns of the two species (BOX 2). A *twist* gene is also expressed during *N. vectensis* development, but after gastrulation has begun: it is expressed in a ring of endodermal cells around the presumptive mouth and, more broadly in the endoderm following opening of the mouth[20]. This pattern is different from that seen in *D. melanogaster*, in which the proteins that *dorsal* and *twist* encode interact to control *snail* expression (reviewed in REF. 21), so it will be interesting to see how the expression of these two genes is controlled in cnidarians. In addition to *twist* and *snail*, Martindale *et al.*[20] examined the expression of *forkhead*, *mef2*, a GATA transcription factor and a LIM transcription factor. With the exception of *mef2*, which was localized entirely in the ectoderm, gene expression was largely restricted to the endoderm, as would be predicted if it corresponds to the mesendoderm of higher animals, from which mesodermal as well as endodermal lineages can arise[22].

The common ancestor of Cnidaria and Bilateria. Studies on *P. carnea*, *A. millepora* and *N. vectensis* all show that genes associated with mesoderm and muscle formation are not only found in cnidarians, but that their expression is consistent with their roles in higher animals. As Technau and Scholz[8] have pointed out, the ancestral roles of these genes have probably been in regulating the proliferation, adhesion and motility of cells — processes that are essential in any multicellular organism. *snail* genes, for example, might effectively act as the key regulators of epithelial-to-mesenchymal transitions

MESOGLEA
(Also known as mesogloea). The body layer between ectoderm and endoderm in cnidarians, ctenophores and acoelomates, which is traditionally distinguished from mesoderm on the basis of the former being acellular and the latter cellular. However, in reality, enormous variation is seen across the Cnidaria in the extent to which the matrix of the mesoglea is invaded by various cellular and fibrillar components, and only in some hydrozoans does it approach true acellularity.

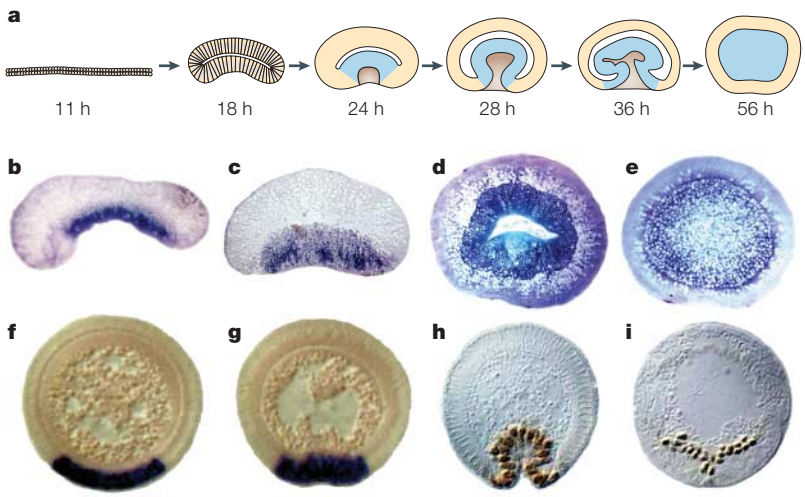


Figure 2 | **Expression of the mesodermal marker *snail* during early gastrulation in *Acropora millepora* and *Drosophila melanogaster*.** The snail protein has a key role during gastrulation throughout the higher Metazoa, and the remarkable similarity between early snail-expression patterns in *A. millepora* and *D. melanogaster* emphasizes the mechanistic, and probably evolutionary, links between endoderm formation in *A. millepora* and mesoderm formation in *D. melanogaster*. **a** | Schematic of the process of gastrulation in *A. millepora*. Although the starting point (a flattened bilayer, rather than a spherical blastula) is unusual, by the 28-h stage, there is little to distinguish *A. millepora* gastrulation from that in many other animals. (**b**–**e**) *In situ* hybridization reveals the distribution of *snail* mRNA in *A. millepora* embryos cut in transverse section to show the extent of staining cells. **b** | *snail* mRNA is first detectable in the apex of cells on the concave side of the embryo as it thickens and shrinks in circumference at approximately 16 h post fertilization. **c** | At approximately 21 h, mRNA is found throughout the cells on the inside of the concavity. **d** | This off-axis section of the blastopore of an embryo approximately 30-h old shows strong expression in an endodermal layer that is still maintaining its integrity. **e** | After approximately 50 h, the blastopore has closed and the cells have reorganized into a uniform-appearing endoderm, all of which express *snail*. **f**,**g** | During the early stages of gastrulation, the distribution of *snail* mRNA in transverse sections of *D. melanogaster* embryos closely resembles that seen in *A. millepora*. However, in *D. melanogaster*, early *snail* expression ceases immediately after the stage shown in (**g**), whereas in *A. millepora*, *snail* expression continues beyond the corresponding time (**d**,**e**). In **h**,**i**, the fate of the cells that had expressed *snail* earlier in gastrulation is shown by staining for the twist protein. A pattern of *snail* expression similar to that shown here for *A. millepora* has recently been reported in *Nematostella vectensis* [20]. Part **a** modified with permission from REF. 4 © (2004) Springer and from REF. 37 © (2002) National Academy of Sciences. Parts **b**–**e** modified with permission from REF. 4 © (2004) Springer. Parts **f**–**i** are reproduced with permission from M. Leptin.

(EMTs)[23], as they are also expressed during the EMT that leads to NEURAL CREST formation[7]. It now seems clear that in the common ancestor of cnidarians and bilaterians, these genes must have performed many of these same basic functions.

So, to what conclusions do the molecular data lead us about the common ancestor of Cnidaria and Bilateria? Martindale *et al.*[20] and Collins[24] provide extensive discussions of this topic. Both stress the need for more data, and with that caveat, we concur with their principal conclusions that "the mesodermal genes originated prior to the origin of mesoderm" and "were involved in the specification of endoderm in diploblasts, and that as mesoderm evolved from the primordial endoderm, their expression became associated with presumptive mesoderm"[20] and that "presently available information just slightly favours the idea that the medusa is a SYNAPOMORPHY of Medusozoa rather than of Cnidaria"[24]. The ability of early mesendodermal cells to give rise to both mesodermal and endodermal lineages was first described in zebrafish[25] and *Caenorhabditis elegans*[26], but seems to be a common feature of animal development[27,28].

NEURAL CREST
Cells at the dorsal midline of the vertebrate neural tube, which undergo an epithelial-to-mesenchymal transition and migrate to many locations, contributing to the development of a wide variety of structures, including the peripheral nervous system and craniofacial features, therefore essentially enabling a second wave of development.

SYNAPOMORPHY
A derived state that is shared by several taxa.

Which way up?

The evidence summarized above implies that the classical distinction between diploblasts and triploblasts is arbitrary, as the molecular mechanisms that underlie mesoderm specification might pre-date the origins of the Cnidaria. What about the issue of one axis versus two? As with mesoderm specification, a great deal is known about the molecular bases of the systems responsible for patterning both the AP and DV axes of higher animals. BOX 4 provides a summary of the expression domains of key components of the AP and DV patterning systems, including those discussed here. The *Hox* genes, the role of which in patterning the AP axis is considered to be a defining characteristic of (bilateral) animals[29], are among the most extensively studied genes. The question of whether true *Hox* genes are present in cnidarians is controversial. Based on sequence analysis, several authors (for example, Finnerty and Martindale[30], Martinez *et al.*[31], Gauchat *et al.*[32]) have argued for the presence of anterior and posterior *Hox* genes in cnidarians, and more recently Finnerty *et al.*[33] have presented expression data in support of this claim. Much, of course, depends on the definition of '*Hox*' gene, but in the absence of evidence of chromosomal linkage and correlated patterns of expression, we believe it more appropriate to refer to these cnidarian genes as '*Hox*-related' or '*Hox*-like'. However, cnidarians do have clear homologues of several of the other genes that have central and conserved roles in patterning along both of the obvious body axes of bilateral animals, as summarized below.

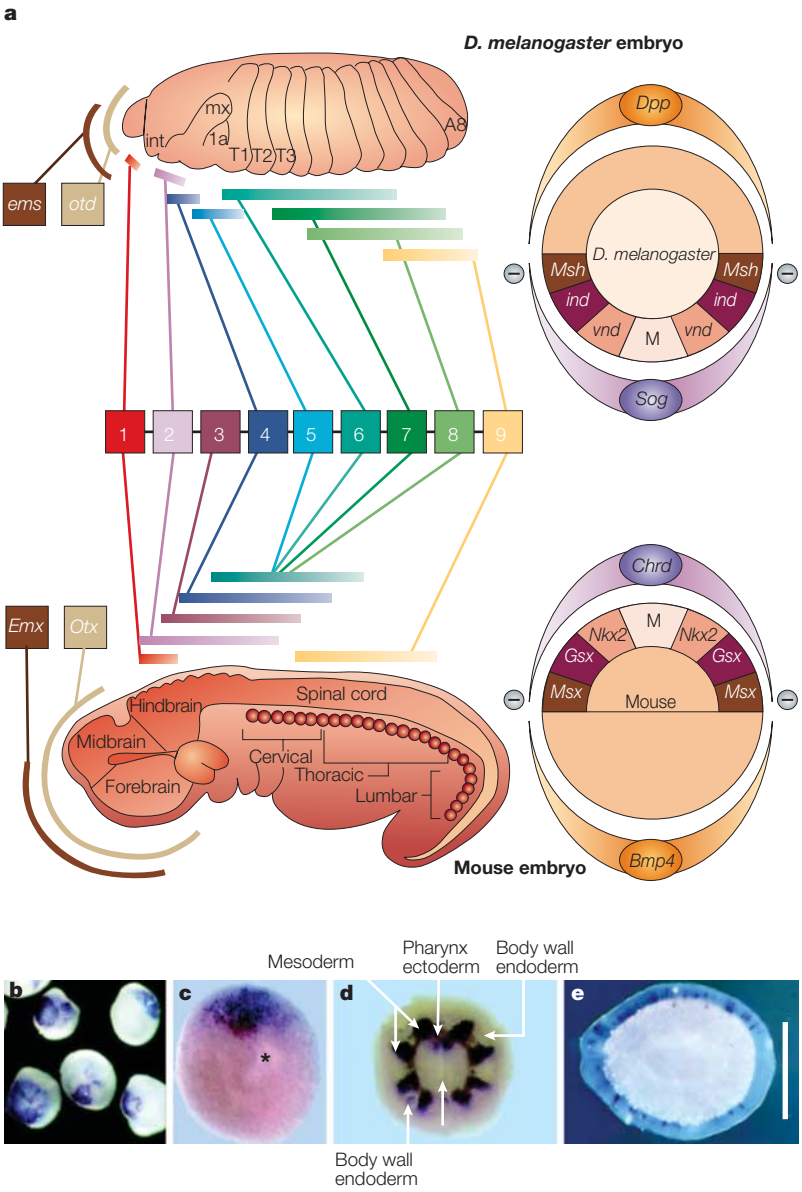
Genes involved in patterning the dorsal–ventral axis. The DV axis of the ancestral bilateral animal is thought to have been specified in part by a gene related to *decapentaplegic* (*dpp*) in the fly and its vertebrate counterpart, *Bmp4* (REFS 34,35). DPP/BMP4 is a member of the transforming growth factor-β (TGF-β) superfamily, and has strongly anti-neurogenic properties in both animals, and therefore, the nerve cord can only develop where DPP/BMP4 activity is inhibited or absent. In the fly, the ventral expression of the DPP antagonist Short gastrulation (SOG) allows the development of the neuroectoderm, and in vertebrates, the same effect is achieved but on the dorsal surface by the SOG-related molecule Chordin. Later, the neuroectoderm is patterned along the DV axis by directly interacting homeobox genes in both systems[36] (BOX 4).

The expression pattern of a gene clearly related to *dpp/Bmp4* — the key determinant of the DV axis in bilateral animals — has been seen during the embryonic development of *A. millepora* using *in situ* hybridization[37] (BOX 4). Expression is first detected in early gastrulating embryos and becomes localized to an ectodermal region close to the closing blastopore. Intriguingly, this expression is not distributed in a radially symmetrical way around the blastopore, but is localized to a sector. Therefore, although the embryo at this stage is morphologically radially symmetrical, the symmetry is broken at

Box 4 | **Axis-determining genes in *Drosophila melanogaster*, mouse and cnidarians**

The *Hox* genes (homeobox-containing genes) pattern the anterior–posterior (AP) axis of the fly and mouse and are arranged in the same chromosomal order in both organisms (shown in part **a** of the figure as boxes 1–9; the embryos are shown in lateral view to the left and in transverse section to the right). The anterior limit of their expression along the AP axis (shown by the horizontal bars) is the same as their order on the chromosome. Two other genes that have a role in patterning the brains of both organisms are *otd/Otx* and *ems/Emx*. Genes with important roles in determining cell fates in the dorsal–ventral (DV) plane are similarly conserved, albeit inverted in their patterns of expression. So, fly *dpp*/*sog* are homologues of mouse *Bmp4*/chordin (*Chrd*), respectively, and occur in concentration gradients as shown. Similarly, *Msh* (*Drop*)/*Msx*, *ind*/*Gsh* and *vnd*/*Nkx2-1* (*Titf1*) are homologous gene pairs that respectively pattern the nervous systems of the fly and mouse along the DV axis. M, midline.

The expression patterns of the homologous genes in cnidarians show some similarities to the expression patterns of their bilaterian counterparts. The cnidarian homologue of *dpp/BMP2/4* is expressed in an asymmetrical patch at the edge of the blastopore (*) late in gastrulation in *Acropora millepora* (see figure part **b**) and *Nematostella vectensis* (**c**). There is only a single plane that can pass symmetrically through the area of gene expression and the blastopore, therefore fulfilling the definition of bilateral symmetry. Bilaterality is even more apparent in the polyp of *N. vectensis*, which has a slit-like mouth (between the vertical arrows), with *dpp* expression in the pharyngeal ectoderm at only one end (**d**). Similarly, *A. millepora cnox-2* is orthologous with *ind* and *Gsh* and is expressed in putative transectodermal neurons, except for those at the aboral end of the planula larva[38] (see figure part **e**; the zone that lacks expression is marked by the vertical white bar). The lateral views in part **a** are modified with permission from REF. 84 © (1997) Blackwell Science. The transverse sections are modified with permission from REF. 85 © (2001) The Royal Society. Part **b** is modified with permission from REF. 37 © (2002) National Academy of Sciences. Parts **c** and **d** are modified with permission from REF. 33 © (2004) AAAS. Part **e** is modified with permission from REF. 38 © Springer.



the level of gene expression. Moreover, a single mirror-image plane of symmetry can now be drawn through the *dpp*-expression domain, effectively defining a second polarized axis, in addition to the one defined by the blastopore. A similar distribution of *dpp* mRNA has been reported during the embryogenesis of the sea anemone *N. vectensis* with the significant addition of asymmetrical expression in the pharynx of the polyp (BOX 4)[33].

In addition to the expression of a DPP/BMP4-related molecule in a pattern that could define a second body axis, homeobox genes related to the three genes that subdivide the *D. melanogaster* neuroectoderm, *vnd*, *ind* and *Msh* (*Drop*)[36], have been detected in *A. millepora* (REF. 38; D. de Jong *et al.*, unpublished observations). Moreover, one of these genes — *cnox-2Am*, the cnidarian equivalent of the fly gene *intermediate neuroblasts defective* (*ind*) and vertebrate *Gsh1/2* — is expressed during coral development in a manner that is reminiscent of its fly and vertebrate counterparts (BOX 4). *cnox-2Am* is expressed in a subset of presumed neurons that is restricted along the OA axis, whereas *ind* (and its vertebrate orthologues *Gsh1* and *Gsh2*) are expressed in the central nervous system in a DV-restricted manner. In addition, orthologues of *vnd* and *Msh* are also expressed ectodermally, in spatially restricted patterns along the OA axis in the *A. millepora* planula (D. de Jong, unpublished observations). Although more information on the cnidarian genes, the fly and mammal counterparts of which interact with *ind*/*Gsh*, and those that correspond to SOG/Chordin, is urgently required, these results cast some uncertainty on the simple correspondence of OA and AP axes.

Genes involved in patterning the anterior–posterior axis. Although it is still unclear whether the Cnidaria have true *Hox* genes, other genes known to have central and conserved roles in patterning the AP axis of higher animals are present, including clear homologues of the head-patterning genes *otd/Otx* and *ems/Emx*. In higher animals, these genes are expressed in, and pattern, regions that are anterior to those in which *Hox* genes are expressed[39]. *Otx* genes have been cloned from both *Hydra*[40] and *P. carnea*[5], but it is difficult to relate their expression patterns to the conserved function of *Otx* genes in AP patterning in higher animals. So, the conserved role of *Otx* in anterior patterning might have arisen after the Cnidaria diverged from the higher Metazoa. However, the fact that many genes seem to have been independently duplicated in cnidarians (see below) and the derived nature of hydrozoan life cycles clearly highlight the need for expression data for other cnidarian *Otx* genes.

Although the available data for the *Hydra* and *P. carnea* *Otx* genes do not contribute to understanding the origins of axis specification, expression data for cnidarian *Emx* genes show clear parallels to their counterparts in higher animals. In gastrozooid polyps of the hydrozoan *Hydractinia symbiolongicarpus*, expression of *emx* is detected at the oral ('head') end of the OA axis[41]. During embryonic development of *A. millepora*, regionally restricted expression along the OA axis is also observed, although here, expression is excluded from the oral end (N.R. Hislop, D.C.H., P. Pontynen, D.J.M. and E.E.B., unpublished observations), which is, however, posterior as the animal swims. Although these results are in apparent contradiction, an inversion of the expression domain along the OA axis following larval settlement has previously been reported for the homeobox gene *cnox1* in *P. carnea*[42]. The simplest interpretation of the expression data for *emx-Am* and *cnox-2Am* is that the molecular bases for both AP and DV patterning systems pre-dated the cnidarian/bilaterian divergence, but became separated only in the latter after the split[43]. However, this model does not account for the *Bmp2/4-Am* data, as this gene is differentially expressed in what seems to be a second axis (at right angles to the OA axis) during the development of both *A. millepora*[37] and *N. vectensis*[33].

Seeing is believing: *Pax* genes in cnidarians

The above discussion emphasizes the similarities between cnidarians and higher animals. So, what are the developmental differences that distinguish them? Are there key absences from the cnidarian gene set? Elsewhere, we have argued that cnidarians might lack true *Hox* genes (D.J.M., D.C.H. and E.E.B., unpublished observations). Another gene that is believed to have been characteristic of Urbilateria and the involvement of which in photoreceptor specification might post-date the cnidarian/higher animal split is *Pax6*.

The demonstrations that there is a 'master control gene' for eye specification, and that the fly (*eyeless*; *ey*) and human (*PAX6*) genes are strikingly similar are among the most widely known experiments in modern biology[44]. One implication of this work is that, contrary to zoology textbook dogma, vision might have arisen only once during animal evolution. A crucial test of this hypothesis is whether genes related to *Pax6/ey* also specify the photoreceptors of cnidarians. Two recent papers[45,46] address this question, and, although their final interpretations differ in terms of the original question, the results are surprisingly consistent.

Photosensitivity is a general property of cnidarians, and photoreceptors vary in complexity from eye spots to highly sophisticated eyes (complete with retina and lens)[47,48]. Predictably, the most complex sense organs are present in cubozoans, the most active of all cnidarians. Anthozoans, which are sedentary and less active, lack specialized photoreceptors, although they clearly respond to light[49].

Genes that belong to the *Pax6/2/5/8* superfamily have been identified in a wide range of cnidarians, but are themselves extremely diverse. The widely distributed *PaxB* genes are most similar to the *Pax2/5/8* class in the paired domain, but are unlike them in that they also encode complete homeodomains. However, in terms of domain structure and homeodomain sequences, *A. millepora* PaxC more closely resembles the Pax6/EY type than do the other cnidarian sequences[50], although its paired domain more closely resembles a Pax2/5/8 protein. Moreover, *PaxC* is expressed in a subset of presumed neurons during early coral development[50], whereas the *PaxB* genes seem to have more general expression domains at the corresponding stages in jellyfish[51] and coral (E.E.B., unpublished observations).

One of the properties of bona fide *Pax6/ey* orthologues is their ability to induce ectopic eyes when expressed in *D. melanogaster* imaginal discs[52]. We recently demonstrated that, when fused to the EY transactivation domain, *A. millepora* PaxC is able to induce ectopic eyes in the fly[45]. Although this was also true of *A. millepora* PaxB, the effect was significantly weaker. Similarly, Kozmik *et al.*[46] showed that PaxB from the cubozoan *Tripedalia cystophora* is able to induce ectopic eyes in *D. melanogaster*. *In vitro* DNA-binding experiments indicate that PaxB and PaxC paired domains bind both *Pax6* and *Pax2/5/8* targets[45,46]. So, cnidarian genes do not directly correspond to either the *Pax6* or *Pax2/5/8* types in fly and man — rather, they have some properties of both classes. To a limited extent, *T. cystophora PaxB* can also substitute for the *D. melanogaster Pax2/5/8* gene *sparkling* (*shaven*)[46], which is again consistent with the cnidarian genes being intermediate in properties to the *Pax6* and *Pax2/5/8* types.

Relationships between the cnidarian *Pax* genes and the *Pax6* and *Pax2/5/8* classes of higher animals are therefore not simple, and the roles of specific genes in cnidarian photoreceptor development remain unclear. Kozmik *et al.*[46] interpret this to mean that vision arose independently in the Cnidaria. However, the implied regulatory interaction between *T. cystophora* PaxB and the jellyfish J3 crystallin promoter[46] parallels the activation of crystallin promoters by *Pax6* genes in vertebrates[53]. Therefore, it is probably premature to rule out some common principles of eye specification in the Cnidaria and higher animals.

So many genes, so little morphology

One of the most intriguing findings to emerge from preliminary EST projects on several cnidarians (*A. millepora*, *N. vectensis*, *Hydra*) is that the gene sets of cnidarians and, by implication, the common metazoan ancestor, are surprisingly rich and complex. This flatly contradicts traditional expectations — modern cnidarians are 'primitive' animals, with little obvious morphological differentiation, and this is often also assumed to have been the case with Urbilateria. The assumption has been that simple morphology equates to a simple gene set; fewer genes should be required to build a sea anemone than a fly, but this seems not to be true. This paradox is exemplified by the fact that, whereas anthozoan cnidarians have the simplest extant nervous systems, the *A. millepora* genome contains many of the genes known to specify and pattern the much more sophisticated nervous systems of vertebrates and the fly[54]. Moreover, this genome includes several genes that were previously assumed to have been vertebrate innovations, such as those that code for the transcription factors Churchill and Tumorhead. Although we are a long way from a definitive answer to this conundrum, the lessons from the comparative genomics of higher animals, mentioned below, are directly relevant.

How many genes? In some ways, the surprise of the complexity of these simple animals is reminiscent of that which greeted the equally counterintuitive finding of the relative simplicity of the human gene set. The human genome had been predicted to contain more than 100,000 genes, whereas the actual number is approximately one-third of that. The mammalian gene set is not substantially larger than those of the roundworm or fruit fly, the key differences being additional duplications[55,56] and some novel combinations of domains[57–59]. Why does it require almost as many genes to build a fly as it does a man? The answer in part is that gene number is a poor indicator of the sophistication of gene use; it is now widely accepted that alternative splicing and transcriptional regulation are generally more complex in mammals than in the fly and that it is this difference that accounts for the execution of more complex genetic programmes.

Analogously, we suggest that gene numbers in cnidarians will probably be in the same range as the fly and roundworm — and in some cases, might be much higher (see below) — but that these genes will individually be used in much less complex ways. Perhaps one gene having one role might be an over-simplification, but we expect that there will be few cnidarian genes that show such complex patterns of expression as, for example, *even skipped* in *D. melanogaster*.

Independent gene duplications in cnidarians. One more subtle implication of various gene-characterization and EST-sequencing projects is that several key regulatory genes have been independently duplicated in cnidarians. Some examples of this phenomenon are listed in TABLE 1. The best-documented of these are the *Hydra nanos* genes, which phylogenetic analyses show to have been independently duplicated. One of these genes, *Cnos1*, seems to have retained the ancestral function in the germline, whereas *Cnos2* has acquired cnidarian-specific roles — expression in the endoderm of the hypostome, which indicates a (derived) role in head morphogenesis[60]. Several other examples of this phenomenon are listed in TABLE 1; note that this is not a comprehensive list, and the extent of duplication is probably much higher.

Given the period of time since divergence of the Cnidaria from the line leading to the Bilateria, it is not surprising that some genes have been independently duplicated in each lineage. The key question is whether these examples reflect a more extensive duplication phenomenon — has a genome-wide duplication event occurred following the Cnidaria/Bilateria split, or have these genes simply been independently duplicated? Moreover, it is not clear whether these examples of apparent duplication in specific cnidarians occur more generally throughout the phylum. Indications are that at least some of them do; for example, it is clear that *A. millepora* has homologues of both *Cnos1* and *Cnos2* (H. Go and D.J.M. *et al.*, unpublished observations), which indicates that the duplication of *nanos* genes occurred before the divergence of Anthozoa and Hydrozoa.

In addition to what might be phylum-wide duplications of at least some genes, it is now clear that marked changes in DNA content (and possibly gene number) have occurred within some cnidarian genera[61]. Most *Hydra* species have notoriously large genomes, including

Table 1 | **Examples of independent gene duplications in the Cnidaria**

Gene	Organism	Reference or accession number
paired-like (*Prdl-a*/*Prdl-b*)	*Hydra vulgaris*	86
nanos	*Hydra magnipapillata*	60
nanos	*Acropora millepora*	Go, H. and D.J.M., unpublished observations
Hox-related (*Anthox7*/*Anthox8*)	*Nematostella vectensis*	30
Hox-related (*Anthox1*/*Anthox1a*)	*Nematostella vectensis*	30
snail	*Nematostella vectensis*	20
mox	*Nematostella vectensis*	AAP88427/AAP88428
Nuclear receptor (*AmNR2*/*AmNR6*)	*Acropora millepora*	87
Nuclear receptor (*AmNR4A*/*AmNR8*)	*Acropora millepora*	87
Smad1/5	*Acropora millepora*	88
Doublesex	*Acropora millepora*	89; Go, H. and D.J.M., unpublished observations
Vnd/*Nkx2-1*	*Acropora millepora*	de Jong, D., D.C.H., E.E.B. and D.J.M., unpublished observations
msh/*Msx*	*Acropora millepora*	de Jong, D., D.C.H., E.E.B. and D.J.M., unpublished observations
Dmbx	*Acropora millepora*	Hislop, N. and D.J.M., unpublished observations
Not	*Acropora millepora*	D.C.H., Poon, C. and Knight, A., unpublished observations

PARALOGUE
Two genes are paralogous if they were a result of a duplication event.

SIPHONOPHORE
Any marine colonial hydrozoan of the order Siphonophora, including the Portuguese man-of-war.

the species that has been most intensively studied and for which a large EST collection is now available — *H. vulgaris* (1,250 Mb). However, the genome of *H. viridissima* is much smaller (380 Mb), despite the fact that all *Hydra* species seem to have a similar number of chromosomes (2n = 30). Few precise estimates of cnidarian genome sizes are available, but the estimate for *H. viridissima* is similar to that for *N. vectensis* (U. Technau, personal communication).

Not so simple: the challenge of cnidarian genomes. The fact that many genes have been independently duplicated within the Cnidaria and that large differences in DNA content occur even at the genus level might explain some of the observed differences in gene-expression patterns across the phylum — it is probable in some cases that PARALOGUES have been compared. Another contributing factor is the diversity of the phylum, which is reflected in several mechanisms of gastrulation and a morphological diversity that runs from the apparently simple design of an anthozoan polyp to SIPHONOPHORE colonies that are tens of metres in length with zooids so different that they would not be recognized as the same species if they were not physically connected. So, perhaps, rather than being surprising, substantial variation in patterns of gene use is to be expected.

Clearly, factors such as genome size must be considered in selecting appropriate representative cnidarians for genomic sequencing. Moreover, only once complete genome sequences are available for several cnidarians will it be possible to reconstruct the evolution of specific gene families with any confidence. Cnidarian genomes are potentially a key to understanding many aspects of animal evolution, but in the pre-genomic era, we need to beware of simplistic interpretations of sequence data and expression patterns of cnidarian genes.

Redefining the clade of bilateral animals

Although the Cnidaria probably diverged before some specific regulatory genes evolved, the cnidarian gene set is surprisingly complex and includes several genes that had previously been assumed to be vertebrate-specific[54]. Moreover, a growing body of gene-expression data, some of which are summarized above, emphasizes how similar cnidarians are to higher animals in many respects and make it appropriate to reconsider their relationship. Although they might lack the obvious mesoderm characteristic of the higher Metazoa, cnidarians undergo a process during embryonic development in which cells are internalized and genes that are clearly related to mesodermal markers are expressed in similar ways to their counterparts in higher animals. Moreover, cnidarians have genes that are responsible for patterning both of the principal axes of higher animals, and these are expressed in patterns that are consistent with axis specification. Taken together, these lines of evidence greatly narrow the gulf that supposedly separates the Cnidaria from the Bilateria. Although the issue of whether cnidarians have true *Hox* genes is still open, we agree with the suggestion of Finnerty *et al.*[33] that currently available data indicate a bilateral common ancestor for the Cnidaria and Bilateria.

The idea of cnidarians as essentially bilateral animals is not as revolutionary as it might at first seem. Many authorities, including Hyman[62,63], who was probably most responsible for the perception of cnidarians as radial animals, have recognized that anthozoans are often bilaterally symmetrical, but have interpreted this to be a state that had arisen independently within the Cnidaria because they viewed the (radially symmetric) medusa as the ancestral form. On the basis of molecular data[24,64], we now know that this view of phylogeny is probably incorrect, and that the Anthozoa are basal. So, rather than having independently evolved as a derived state, the bilaterality of anthozoans most probably reflects the ancestral character state within the Cnidaria. The common ancestor of cnidarians and higher Metazoa was probably a bilateral animal, and the near-radial symmetry of some jellyfish a derived state. There are many precedents for this evolutionary scenario. Within several animal phyla, there has been evolution away from a bilateral ancestral form — for example, we accept that the ancestral echinoderm was a bilateral animal, despite the fact that most modern representatives of the phylum are not.

The only alternative is to view cnidarians as having independently recruited the same genes to the processes of gastrulation and axis specification as are used by higher animals. This is clearly counterintuitive. The time has come to welcome cnidarians to the fold — these apparently simple creatures are bona fide bilateral animals.

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Competing interests statement

The authors declare that they have no competing financial interests.

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