

Review

Early origin of the bilaterian developmental toolkit

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Whole-genome sequences from the choanoflagellate *Monosiga brevicollis*, the placozoan *Trichoplax adhaerens* and the cnidarian *Nematostella vectensis* have confirmed results from comparative evolutionary developmental studies that much of the developmental toolkit once thought to be characteristic of bilaterians appeared much earlier in the evolution of animals. The diversity of transcription factors and signalling pathway genes in animals with a limited number of cell types and a restricted developmental repertoire is puzzling, particularly in light of claims that such highly conserved elements among bilaterians provide evidence of a morphologically complex protostome–deuterostome ancestor. Here, I explore the early origination of elements of what became the bilaterian toolkit, and suggest that placozoans and cnidarians represent a depauperate residue of a once more diverse assemblage of early animals, some of which may be represented in the Ediacaran fauna (c. 585–542 Myr ago).

Keywords: Cambrian explosion; evo-devo; cnidaria; Ediacaran; genetic toolkit

1. INTRODUCTION

A variety of transcription factors, signalling pathway genes and other regulatory elements once thought to be characteristic of bilaterians, and associated with their diverse cell types and more complex morphologies, have now been documented among more ancient clades that lack diverse cell types and complex morphogenesis. This raises questions about the current function of these elements (Srivastava *et al.* 2008) as well as their ancestral role during the early evolution of animals. The precursors of the bilaterian developmental toolkit have been documented in non-bilaterian animals and choanoflagellates by comparative studies and confirmed by whole-genome sequences of the choanoflagellate *Monosiga brevicollis* (the closest known relative of metazoans), the placozoan *Trichoplax adhaerens* and the cnidarian *Nematostella vectensis*. The whole genome of the sponge *Amphimedon queenlandica* is expected shortly. Table 1 shows the genome size, number of cell types and various features of developmental complexity in these groups, and includes *Drosophila melanogaster* for comparison.

This analysis requires a robust metazoan phylogeny (figure 1). Of note are the paraphyletic sponges, with demosponges-, calcisponges- and the homeoscleromorphs-independent clades (Sperling *et al.* 2007), and *Trichoplax* positioned between sponges and cnidarians (Srivastava *et al.* 2008). The acael flatworms are not included below the protostome–deuterostome

(P/D) divergence, following Philippe *et al.* (2007). The phylogenetic affinities of the acoels have long been contentious, with most studies supporting their placement as basal bilaterians (Ruiz-Trillo *et al.* 1999; Baguana & Riutort 2004; Baguna *et al.* 2008; Hejnlol & Martindale 2008a). By contrast, Philippe *et al.* (2007) employed a new model to overcome the long-branch attraction problems that have previously plagued phylogenetic positioning of this clade, suggesting that acoels are deuterostomes, and are secondarily primitive. I view the placement of acoels as basal bilaterians as the more likely, but it is not critical to the following discussion. Indeed, recent developmental studies of acoels (Hejnlol & Martindale 2008a,b) strengthen the arguments here. The tree in figure 1 is calibrated with the molecular clock results of Peterson *et al.* (2008), with metazoans originating during the Cryogenian and bilaterians during the Ediacaran.

2. THE PROTOSTOME–DEUTEROSTOME TOOLKIT

The similarity of a variety of complex morphogenetic pathways across protostomes and deuterostomes (based initially on studies of *Drosophila*, *Cenorhabditis* and *Mus*, but recently expanding to a more phylogenetically diverse array of animals) led to the conclusion that their last common ancestor not only possessed these developmental systems, but the complex morphologies that they produce in modern organisms (Slack *et al.* 1993; De Robertis & Sasai 1996; Ohno 1996; Valentine *et al.* 1999; Carroll *et al.* 2001; Erwin & Davidson 2002; Erwin 2006; De Robertis 2008; Raff 2008).

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Table 1. Genome size and the number of inferred genes are from the whole-genome studies cited in the text; that of *Amphimedon* is from Fahey *et al.* (2008). The number of cell types is from Valentine *et al.* (1994), except for *Trichoplax*, which is from Srivastava *et al.* (2008); and the number of miRNAs comes from Sempere *et al.* (2006), Gimson *et al.* (2008) and Wheeler *et al.* (2009). Note that according to Wheeler *et al.* (2009), the seven miRNAs in demosponges are not homologous to any eumetazoan miRNAs. The 10 transcription factors families are the homeodomain, forkhead, p53, Myc, Sox/TCF, ETS, HOX, NHR, POU and T-box, with the data for *Monosiga* from King *et al.* (2008), and other taxa from Larroux *et al.* (2008); true HOX class genes are missing from *Monosiga* and *Amphimedon*. The number of basic helix-loop-helix (*bHLH*) genes from Simionato *et al.* (2007) except for *Trichoplax* which is from Srivastava *et al.* (2008).

	<i>Monosiga brevicollis</i>	<i>Amphimedon queenslandica</i>	<i>Trichoplax adhaerens</i>	<i>Nematostella vectensis</i>	<i>Drosophila melanogaster</i>
genome size (Mb)	41.6	167	98	450	180
no. of genes	9100	?	11 514	18 000	14 601
no. of cell types	1	12	4	20	50
no. of miRNA	0	7	7	3	49
no. of metazoan transcription factors/families	?/5	57/26	35/9	min. 87/10	min. 87/10
no. of <i>bHLH</i> genes	0	16	27	68	59

The complexity of this toolkit varies as new studies and broader comparative studies are reported, but minimally includes the following: anterior/posterior (A/P) patterning with seven or eight *HOX* genes (de Rosa *et al.* 1999; Balavoine *et al.* 2002), and the associated microRNA responsible for inhibiting translation of *HOX* mRNAs (de Robertis 2008); the *HOX* genes part of a larger super cluster of at least eight other ANTP-class genes including the ParaHox and NK cluster genes (Butts *et al.* 2008); dorsal/ventral (D/V) patterning controlled by the *sog/chordin dpp/BMP2/4* system (Arendt & Nubler-Jung 1994; De Robertis & Sasai 1996); anterior patterning via *ems/Emx* and *otd/Otx* and a tripartite brain (Arendt & Nubler-Jung 1999; Reichert & Simeone 2001; Arendt *et al.* 2008) with posterior patterning via *evens-rippled/evx* and *caudal/cdx*; segmentation through *engrailed* and *Delta-Notch* (Holland *et al.* 1997; Balavoine & Adoutte 2003; Stollenwerk *et al.* 2003; Tautz 2004); eye formation controlled by a dense network of genes, including *Pax6* and *ey* (Quiring *et al.* 1994; Halder *et al.* 1995; Gehring 2004), but see Arendt *et al.* (2004) and Fernald (2000); endoderm formation and a regionalized through gut via GATA transcription factors, *brachyury* and *gooseoid* (Arendt *et al.* 2001); heart formation via *Nkx2.5/tinman* (Harvey 1996; Bodmer & Venkatesh 1998; Olson 2006); and *distal-less* involvement in appendage formation (Panganiban *et al.* 1997; Panganiban & Rubenstein 2002; Pueyo & Couso 2005). The pattern of acquisition of microRNAs tracks increasing morphological complexity and provides important information on the evolution of developmental control (Sempere *et al.* 2006; Gimson *et al.* 2008; Wheeler *et al.* 2009).

The support for a complex P/D ancestor is not as strong as many proponents suggest. While A/P and D/V patterning seem inescapably part of the P/D ancestor, the assumption that all conserved elements were necessarily involved in the same morphogenetic roles as today may not be valid. The ongoing debate over the extent of nervous system centralization in the P/D ancestor illustrates the problem. In both flies and vertebrates, the boundaries of the tripartite brain reflect patterning by *Otd/Otx*, *Pax 2/5/8* and the

anteriormost *Hox* genes, leading to the widespread view that these divisions were present in the P/D ancestor (Arendt & Nubler-Jung 1999; Reichert & Simeone 2001; Ghysen 2003; Hirth *et al.* 2003; Lichtneckert & Reichert 2005; Denes *et al.* 2007; Mizutani & Bier 2008). Yet, the hemichordate *Saccoglossus kowalevskii* lacks a centralized brain, possessing a diffuse nerve net, albeit with some degree of anterior neuronal concentration. The ectodermal patterning of these three genes (and 19 others) is similar to that in animals with a tripartite brain (Lowe *et al.* 2003). Lowe *et al.* concluded that ancestral deuterostomes, and probably the P/D ancestor, similarly had a diffuse nerve net rather than a centralized brain (a conclusion that anatomists had previously reached based on comparative morphology). More recent studies, particularly comparisons between the annelid *Platynereis* and *Saccoglossus*, have revealed a more complex situation. Arendt *et al.* (2008) illustrate the conservation of the mediolateral patterning of the neurectoderm, as well as a conservation of neuron types, suggesting a 'conservation of molecular architectures' (p. 1527). But can we infer ancestral morphologies from these conserved architectures? Arendt *et al.* do not consider the issue within a phylogenetic context, which favours a diffuse nerve net, as Lowe originally argued. Lowe's continuing studies of *Saccoglossus* nerve system development confirm that the networks of regulatory interaction have been conserved, but he cautions that this information provides little insight into ancestral morphology (Lowe 2008). It is clear, however, that inferring ancestral morphologies from conserved genes, even from conserved networks of regulatory interactions, is not straightforward. Homology of a regulatory pattern does not demonstrate homologous morphologic structures.

Davidson and I (Erwin & Davidson 2002) made the more general argument that many of these conserved processes were responsible for cell specification and regional patterning rather than initiation of complex morphogenetic development. We suggested that pattern formation developmental control was subsequently intercalated into these simpler networks to produce specific patterning systems within different clades.

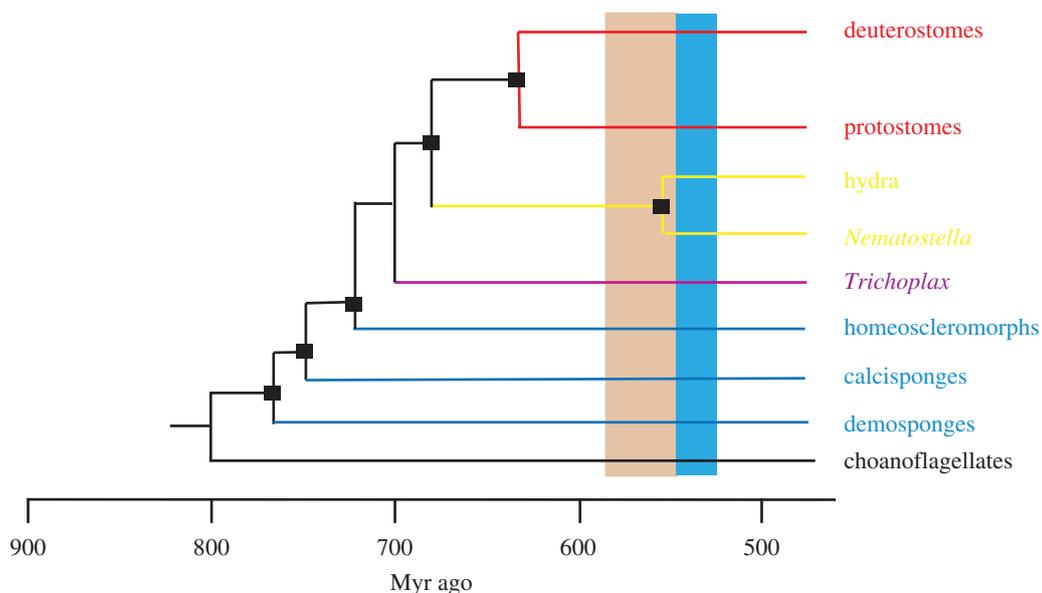


Figure 1. A phylogenetic tree for metazoans scaled against the molecular clock dates of Peterson *et al.* (2008). The ages of nodes are well calibrated by the molecular clock results of Peterson *et al.* and are shown as black boxes.

From this perspective, the P/D ancestor would have been much simpler than suggested by others, composed of a suite of regionally patterned specialized cell types, but not necessarily with the more complex structures observed today. One way of addressing this issue is to trace these highly conserved systems back into animal clades that arose earlier in evolution, but that lack complex morphology.

3. GENOMIC PRECURSORS

There are many cnidarian model systems, but the development of the sea anemone *N. vectensis* has received considerable attention and the entire genome sequence was recently released (Ryan *et al.* 2007b). Other cnidarians have also been studied, including the coral *Acropora* and *Hydra*, a hydrozoan. Anthozoans split from hydrozoans perhaps 548 Ma (figure 1), and the development of the medusoid life-history stage in hydrozoans was accompanied by the origin of a number of cell-type-specific genes (Hwang *et al.* 2007). Thus, anthozoans such as *Nematostella* may be more informative about the last common ancestor of cnidarians + higher bilaterians. Developmental studies of *Nematostella* have revealed unexpected complexity in its developmental toolkit. Orthologues of many genes previously considered characteristic of higher bilaterians arose before the origin of cnidarians. It is now clear that different lineages of higher bilaterians lost many genes present in ancestral metazoans, even as the diversity of particular gene families expanded through gene duplication.

Bilaterians have six major signalling pathways: *Wnt*, TGF- β , Notch, hedgehog, Jak/STAT and RT; all are present in cnidarians (Technau *et al.* 2005; Matus *et al.* 2008), as are a diversity of transcription factors (Putnam *et al.* 2007). All 12 of the known *Wnt* subfamilies occur in cnidarians, with overlapping expression along the oral–aboral axis, involvement in cell-type specification and gastrulation (Kusserow *et al.* 2005).

Fifty-six homeodomain families are present in *Nematostella* (Ryan *et al.* 2007a) and representatives of four different *Pax* gene classes (Piatigorsky & Kozmik 2004; Matus *et al.* 2007). More remarkably for an animal with only two tissue layers, at least seven genes associated with the formation of mesoderm in bilaterians are expressed in the developing endoderm of *Nematostella* (Martindale *et al.* 2004). Thus, germ-layer specification was already present in the ancestral forms. The conservation of these elements in cnidarians does not necessarily mean that they had the same morphogenetic role as in more complex bilaterians, rather, new or more enhanced roles may have developed later (Ball *et al.* 2004). The staggered expression of Hox genes along the body axis and the asymmetric expression of *dpp* (a TGF- β family gene), presumably reflecting a D/V axis (Finnerty *et al.* 2004), have been associated with bilateral symmetry. Comparison of synteny patterns led Hui *et al.* (2008) to propose that *Nematostella* has both a Hox and a Parahox cluster, indicating their duplication before the divergence of cnidarians. A suite of TGF- β genes and antagonists as well as homeodomain proteins are expressed asymmetrically along the A/P axis, and are interpreted by Matus *et al.* (2006) as the precursors of a bilaterally organized central nervous system.

As emphasized by Baguna *et al.* (2008), this distribution of characters challenges traditional views of metazoan evolution, in particular, by separating the origin of a D/V axis from the origin of mesoderm. Taken as read, it suggests that the last common ancestor of cnidarians and higher bilaterians had A/P and possibly D/V polarity, muscle cells of mesodermal affinities and a simple eye. But perhaps the most critical issues are proposals that triploblasty originated *before* the cnidarian divergence, and thus that the diploblastic condition of *Hydra* and some other cnidarians is a derived condition (Boero *et al.* 1998; Martindale *et al.* 2004; Seipel & Schmid 2005, 2006; Boero *et al.* 2007). Evidence in favour of this position includes the

histologically identical striated muscles in the entocodon of hydromedusae, the expression patterns of 'mesodermal' genes in cnidarians, cited above and the coelom-like hydromedusan subumbrellar structure. If these conclusions are correct, then the last common ancestor of cnidarians plus higher bilaterians possessed the toolkit for bilaterality and triploblasty, and possessed at least some elements of mesodermal muscle development. By contrast, Ball *et al.* (2007) critically reexamined the evidence for axial patterning and specifically the homology of the cnidarian oral–aboral axis with the eumetazoan A/P axis. As with mesoderm formation, Ball *et al.* (2007) make the provocative suggestion that the diversity of cnidarian body plans may represent an independent derivation of axial patterning and other regional patterning systems from those of bilaterians. For interpreting the fossils of the Ediacaran, the important point is that cnidarians possess the developmental tools for axial patterning and the production of triploblastic body plans, whether or not body axes and mesoderm of higher bilaterians actually are homologous to cnidarian structures.

Placozoans are 1–2 mm, disc-shaped animals with two epithelial layers and four cell types, but they lack specialized nerve, sensory or muscle cells. Their phylogenetic affinities have been controversial, but appear to have been resolved by the recent sequencing of the whole genome of *T. adhaerens* (Srivastava *et al.* 2008; see commentary by Miller & Ball 2008). Both Bayesian and maximum likelihood analyses strongly support placozoans as the sister group to cnidarians + bilaterians (figure 1), and reject previous suggestions that *Trichoplax* is either a derived cnidarian or a bilaterian. The repertoire of transcription factors and signalling pathways is remarkable: 35 homeobox TFs, including ANTP, PRD, POU and SIX-class genes that are associated with regional patterning in bilaterians; a variety of factors associated with cell-type specification; the components of a Wnt/ β catenin signalling pathway, responsible for axial patterning in bilaterians, cnidarians (Lee *et al.* 2007) and demosponges (Adamska *et al.* 2007) and the TGF- β pathway, active in D/V patterning in bilaterians, and evidently in cnidarians (Matus *et al.* 2006) and demosponge larvae (Adamska *et al.* 2007) as well. The hedgehog pathway is evidently absent and the Notch and JAK/STAT pathways are incomplete, although these were evidently lost as they have been identified in sponges. Despite their lack of a nervous system, placozoans respond to stimuli and have genes associated with synapse formation, for neurotransmitters, and photoreception (Srivastava *et al.* 2008). Given their developmental complexity, Srivastava *et al.* (2008) and Miller & Ball (2008) raise the issue that there may well be as yet unidentified body plans generated by *Trichoplax* that may make use of this complexity. This is an interesting possibility to explore, but here I am concerned with the attributes of the ancestral node rather than their current expression.

Sponges seem to be developmentally unsophisticated organisms, lacking true muscle or nerve cells, tissues or regionalization of discrete cell lineages, and more akin to a colony of cells than true animals. However, developmental studies of the demosponge

A. queenlandica have revealed a suite of developmental attributes including cell specification, patterning of distinct cell layers and morphogenetic gradients. These attributes must have arisen between the split from choanoflagellates and the origin of metazoa. Moreover, molecular phylogenetics has now shown sponges are not a single clade (Phylum Porifera), but three different clades (figure 1). Many details of the sponge developmental toolkit have been identified (Fahey *et al.* 2008; reviewed in Muller *et al.* 2004). The Wnt/catenin and TGF- β pathways provide axial and D/V patterning (Adamska *et al.* 2007). Other transcription factors have been identified, including a variety of ANTP (HOX), *Pax*, *POU*, T-box, *Sox*, *Mef2*, PRD and LIM class genes; some are expressed in specific tissues and thus establish regionalized domains of gene expression (Muller *et al.* 2004; Larroux *et al.* 2006, 2008). Complicating the issue is that demosponges have almost certainly lost a number of transcription factors, including NK and ANTP genes (Peterson & Sperling 2007). There are, however, important differences between demosponges and eumetazoans: there are relatively few genes within each class of transcription factors; the expansion of most of classes occurred after the origin of the demosponges (Kusserow *et al.* 2005; Simionato *et al.* 2007). Moreover, based on a comparison of the intergenic regions within the NK homeobox cluster of *Amphimedon*, Fahey *et al.* (2008) propose that demosponges possess more limited regulatory machinery than eumetazoans. Thus, the last common ancestor of all metazoa was able to specify multiple cell types, establish body axes and array different cell types along these axes and produce multicellular structures (Larroux *et al.* 2006), but evidently lacked the regulatory complexity and depth of transcription factors and microRNAs required to produce complex gene regulatory networks (GRNs), and thus more complex morphological structures.

Choanoflagellates are the nearest sister group to the metazoa, a relationship long recognized by their similarity to sponge choanocytes. Recent comparative genomic studies and the recent sequencing of the genome of *M. brevicollis* have identified a remarkable developmental diversity of cell adhesion, extra cellular matrix, signal transduction and cellular differentiation elements, including 78 protein domains shared by choanoflagellates and metazoans but no other groups (King *et al.* 2003, 2008; King 2004). The *wnt* and TGF- β signalling pathways are absent, and there is only a single gene representing what develops into the JAK/STAT pathway. Some metazoan transcription factor families such as p53, Sox/TCF and Myc are present, but the HOX, ETS, POU and T-box families are all missing (King *et al.* 2008). Those families that are present are less diverse than in metazoa. For example, five immunoglobulin domains were identified in *M. brevicollis* versus the 150–1500 that may exist in vertebrates. More importantly, many of these protein domains do not have the characteristic architectures of metazoan adhesion proteins.

This comparative framework reveals a progressive increase in the regulatory complexity from choanoflagellates to sponges, placozoans, cnidarians and finally,

complex bilaterians. The four primary signalling pathways appeared relatively early, but there was an approximately threefold increase in homeobox and bHLH genes between sponges and cnidarians, although with a different amount of gene duplication in different lineages (Simionato *et al.* 2007; Larroux *et al.* 2008). Cell-type specification, regional pattern formation and some degree of axis formation are evident in sponges, and all three have increased substantially by the appearance of cnidarians, although some of these tools may have been acquired independently in sponges and cnidarians. The presence of many 'bilaterian' developmental tools in morphologically simpler organisms reinforces the view that the original role of these genes and regulatory networks was in the formation of specialized cell types in specific regions of the body, not necessarily in producing complex multicellular structures (Erwin & Davidson 2002). Evidence from acoels further substantiates this view (Hejnal & Martindale 2008a). The increasing diversity of microRNAs provides further evidence of increasing regulatory control (Sempere *et al.* 2006; Gimson *et al.* 2008; Wheeler *et al.* 2009). Gene loss is correlated with the increasing morphologic complexity of body plans, with deuterostomes retaining more of the ancestral gene families than ecdysozoans, although it may be premature to claim, as De Robertis (2008) does, that such losses play a fundamental role.

4. THE EDIACARAN FAUNA IN LIGHT OF DEVELOPMENT

Rocks from the Ediacaran Period (635–542 Ma) immediately preceding the explosion of animals diversity in the Early Cambrian, contain a diversity of centimetre to metre-long fronds, discs and more complex forms, some of them superficially similar to modern animal groups. Although some members of this assemblage of fossils, known as the Ediacaran biota (575–542 Ma), display apparent bilateral symmetry, none has evidence of appendages, eyes, a mouth (with one exception) or other characteristics of the higher bilaterians. This puzzling array of features has led to persistent controversy over what sorts of organisms these fossils represent. Paleontologists have allied particular fossils with the arthropods, annelids and other bilaterian clades, while others believe they represent early animals, but not bilaterians. Others have suggested they are not even animals, but rather lichen, fungi or even prokaryotes (for recent reviews see Gehling *et al.* 2005; Narbonne 2005; Fedonkin *et al.* 2007a; Xiao & Laflamme 2009).

Within the Ediacaran fauna, several different morphological and constructional clusters can be distinguished, although the extent to which they represent monophyletic clades is unclear. One group, known as the rangeomorphs from the genus *Rangaea*, includes a variety of fronds and brush-like forms, composed of alternately arranged frodlets with a fractal structure (figure 2a,b). The erniettomorphs include *Ernietta* (figure 2e) and *Pteridinium*, and have been interpreted as having quilted tubes, much like an air mattress, alternately arrayed along a midline. They appear to have been constructed of a reasonably

tough and tear-resistant material. *Dickinsonia* (figure 2f) is similar to the erniettomorphs but has a higher degree of bilateral symmetry, A/P differentiation and evidence of muscular tissue. It is, in some ways, an intermediate to a more heterogeneous assemblage of roughly bilaterally symmetrical forms, some of which appear to have segmentation, such as *Spriggina* and *Yorgia* (figure 2d). There are also a host of other forms that are more difficult to categorize, including a variety of discs that probably represent the holdfast of fronds, and forms with other symmetries. The one probable bilaterian in the Ediacaran assemblage is *Kimberella*, which apparently had a rasping mouthpart structure similar to the radula of a gastropod (figure 2c).

My concern here is assessing the variety of developmental strategies. Many Ediacaran organisms exhibit strong axial growth, as seen in a variety of frondose forms, including the rangeomorphs, in fronds such as *Charnia* and *Charnodiscus*, in the erniettomorphs and in *Dickinsonia* and its allies. Anterior–posterior differentiation is evident in *Dickinsonia*, and expands to include anterior specialization in *Kimberella*, *Marywadea*, *Parvancorina*, *Spriggina* and *Yorgia*. Dorsal–ventral differentiation and alternating growth along a midline have been described from all of the above taxa, as well as the erniettomorphs such as *Ernietta*. A more complicated issue is that of segmentation. Segmentation is distinct from serial repetition, and the Delta–Notch signalling system is involved in defining segmental boundaries in vertebrates and spiders (where Notch patterns the ectoderm, rather than the mesoderm, as in vertebrates) (Stollenwerk *et al.* 2003; Tautz 2004; Pueyo *et al.* 2008; reviewed in Erwin 2006). While the Notch signalling pathway is present in cnidarians, the Delta–Notch cascade appears to be a later innovation. Thus, if true segmentation could be shown in Ediacaran organisms, it would be evidence of developmental sophistication beyond that of cnidarians. The rangeomorphs exhibit a fractal rather than a segmental growth pattern, and in the suite of forms that appear most 'segmented', there is rarely evidence of internal repetition of structures. Clear evidence of internal structures occurs in only a few forms. With the exception of the apparent anterior proboscis of *Kimberella* (Fedonkin *et al.* 2007b), it is not obvious that any Ediacaran organisms possess developmental attributes that require their placement within the higher bilaterians. The presence of *Kimberella* in rocks dated to 555 Ma, suggesting that the P/D split must have occurred by this time. This does not necessitate that other Ediacaran organisms may have been complex bilaterians, although it is certainly possible. However, even the association of *Kimberella* with bilaterians could be spurious if cnidarians actually possessed the developmental tools for triploblasty, as discussed earlier.

Paleontologists who assigned many of these taxa to higher bilaterian clades (annelids, arthropods, etc) often did so because of apparent similarities in overall form, and from the presumption that cnidarians lacked the developmental processes to produce such morphologies. Our new understanding of the developmental tool kit of sponges, placozoans and cnidarians now raises the possibility that elements of the

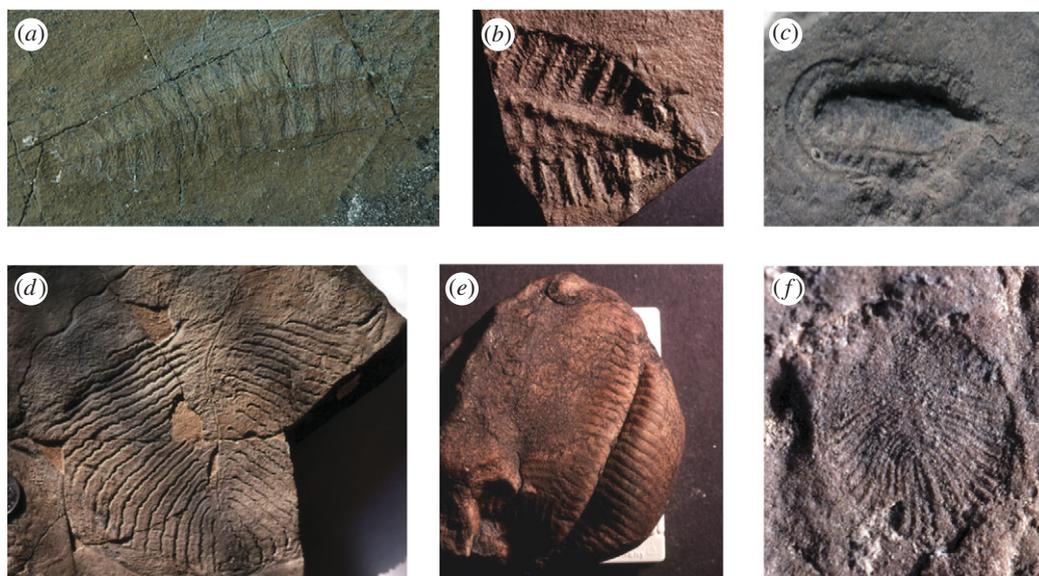


Figure 2. Ediacaran fossils mentioned in the text. (a) *Fracofusus* from Mistaken Point, Newfoundland, 14 cm. (b) *Rangea* from southern Namibia; 5 cm. (c) *Kimberella* from White Sea, Russia; anterior to right, with the anterior elongation, 9 cm. (d) *Yorgia* from the White Sea, Russia; anterior (?) up, about 18 cm. (e) *Ernieetta* from southern Namibia, approximately 15 cm. (f) *Dickinsonia* from Ediacaran Hills, south Australia, 6 cm.

Ediacaran fauna may belong to these clades, or represent now-extinct clades positioned between the sponges and the P/D split.

5. DISCUSSION

We have witnessed the boom and bust of several plausible metrics relating organismic complexity to various features of the genome, including genome size and gene content (Carroll *et al.* 2001; Copley 2008). Comparative developmental studies and whole-genome sequencing of more basal metazoans, as well as a choanoflagellate, have now demonstrated the difficulty in unambiguously identifying the role of even highly conserved genes. As Hejnal & Martindale (2008a) note, the critical question is how and when the various genes were assembled into the various developmental GRNs responsible for cell-type specification and regional patterning. Comparative developmental studies and whole-genome sequencing of early metazoans have demonstrated that cell-type specification, axial differentiation and regional patterning occur within sponges and cnidarians. Even when the phylogenetic patterning of these networks is known, however, problems of establishing homology between the developmental networks and the resulting morphology are likely to remain.

The very ancient divergence between the two cnidarian classes Anthozoa (including *Nematostella*) and Hydrozoa (including *Hydra*) revealed by molecular data raises the possibility that current cnidarian diversity may represent a depauperate sample of the original phylogenetic and morphologic diversity of the clade. Although some Ediacaran organisms evidently had more sophisticated morphogenesis than extant cnidarians, it appears that the clade possessed the tools to build such morphologies. A/P and D/V differentiation, anterior patterning, muscular contraction and response to sensory stimulation are all within the

scope of cnidarian-grade organisms. Placozoans are the basal-most eumetazoans, yet possess a number of key elements of the bilaterian toolkit, further emphasizing the developmental potential of the earliest nodes on the metazoan tree. Although *Dickinsonia* and some other Ediacaran organisms *could* have been members of the protostome or deuterostome clades, their developmental complexity, and our growing knowledge of the developmental complexity of basal metazoans, now make it possible, perhaps even likely, that most Ediacarans belonged to clades below the P/D split.

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REFERENCES

- Adamska, M., Degnan, S. M., Green, K. M., Adamski, M., Craigie, A., Larroux, C. & Degnan, B. M. 2007 Wnt and TGF-beta expression in the sponge *Amphimedon queenslandica* and the origin of metazoan embryonic patterning. *PLoS ONE* **2**, e1031. (doi:10.1371/journal.pone.0001031)
- Arendt, D. & Nubler-Jung, K. 1994 Inversion of dorso-ventral axis. *Nature* **371**, 26. (doi:10.1038/371026a0)
- Arendt, D. & Nubler-Jung, K. 1999 Comparison of early nerve cord development in insects and vertebrates. *Development* **126**, 2309–2325.
- Arendt, D., Technau, U. & Wittbrodt, J. 2001 Evolution of the bilateria larval foregut. *Nature* **409**, 81–85. (doi:10.1038/35051075)
- Arendt, D., Tessmar-Raible, K., Snyman, H., Dorresteyn, A. & Wittbrodt, J. 2004 Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* **306**, 869–871. (doi:10.1126/science.1099955)
- Arendt, D., Denes, A. S., Jekely, G. & Tessmar-Raible, K. 2008 The evolution of nervous system centralization.

- Phil. Trans. R. Soc. B* **363**, 1523–1528. (doi:10.1098/rstb.2007.2242)
- Baguana, J. & Riutort, M. 2004 The dawn of bilaterian animals: the case of acoelomorph flatworms. *BioEssays* **26**, 1046–1057.
- Baguana, J., Martinez, P., Paps, J. & Riutort, M. 2008 Back in time: a new systematic proposal for the bilateria. *Phil. Trans. R. Soc. B* **363**, 1481–1491. (doi:10.1098/rstb.2007.2238)
- Balavoine, G. & Adoutte, A. 2003 The segmented *Urbilateria*: a testable scenario. *Int. Comp. Biol.* **43**, 137–147. (doi:10.1093/icb/43.1.137)
- Balavoine, G., de Rosa, R. & Adoutte, A. 2002 Hox clusters and bilaterian phylogeny. *Mol. Phyl. Evol.* **24**, 366–373. (doi:10.1016/S1055-7903(02)00237-3)
- Ball, E. E., Hayward, D. C., Saint, R. & Miller, D. J. 2004 A simple plan—cnidarians and the origins of developmental mechanisms. *Nat. Rev. Genet.* **5**, 567–577. (doi:10.1038/nrg1402)
- Ball, E. E., De Jong, D. M., Schierwater, B., Shinzato, C., Hayward, D. C. & Miller, D. J. 2007 Implications of cnidarian gene expression patterns for the origins of bilaterality—is the glass half full or half empty? *Int. Comp. Biol.* **47**, 701–711. (doi:10.1093/icb/icm028)
- Bodmer, R. & Venkatesh, T. V. 1998 Heart development in *Drosophila* and vertebrates: conservation of molecular mechanisms. *Dev. Genet.* **22**, 181–186. (doi:10.1002/(SICI)1520-6408(1998)22:3<181::AID-DVG1>3.0.CO;2-2)
- Boero, F., Gravili, C., Pagliara, P., Piraino, S., Bouillon, J. & Schmid, V. 1998 The cnidarian premises of metazoan evolution: from triploblasty, to coelom formation, to metamery. *Ital. J. Zool.* **65**, 5–9. (doi:10.1080/11250009809386722)
- Boero, F., Schierwater, B. & Piraino, S. 2007 Cnidarian milestones in metazoan evolution. *Int. Comp. Biol.* **47**, 693–700. (doi:10.1093/icb/icm041)
- Butts, T., Holland, P. W. H. & Ferrier, D. E. K. 2008 The urbilaterian super-hox cluster. *Trends Genet.* **24**, 259–262. (doi:10.1016/j.tig.2007.09.006)
- Carroll, S., Grenier, J. & Weatherbee, S. 2001 *From DNA to diversity*. Malden, MA: Blackwell Scientific.
- Copley, R. R. 2008 The animal in the genome: comparative genomics and evolution. *Phil. Trans. R. Soc. B* **363**, 1453–1461. (doi:10.1098/rstb.2007.2235)
- De Robertis, E. M. 2008 Evo-devo: variations on ancestral themes. *Cell* **132**, 185–195.
- De Robertis, E. M. & Sasai, Y. 1996 A common plan for dorsoventral patterning in bilateria. *Nature* **380**, 37–40. (doi:10.1038/380037a0)
- de Rosa, R., Grenier, J. K., Andreeva, T., Cook, C. E., Adoutte, A., Akam, M., Carroll, S. B. & Balavoine, G. 1999 Hox genes in brachiopods and priapulids and protostome evolution. *Nature* **399**, 772–776. (doi:10.1038/21631)
- Denes, A. S., Jekely, G., Steinmetz, P. R., Raible, F., Snyman, H., Prud'homme, B., Ferrier, D. E., Balavoine, G. & Arendt, D. 2007 Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129**, 277–288. (doi:10.1016/j.cell.2007.02.040)
- Erwin, D. H. 2006 The developmental origins of animal body plans. In *Neoproterozoic geobiology and paleobiology* (eds S. H. Xiao & A. J. Kaufman), pp. 157–197. Dordrecht, The Netherlands: Kluwer Press.
- Erwin, D. H. & Davidson, E. H. 2002 The last common bilaterian ancestor. *Development* **129**, 3021–3032.
- Fahey, B., Larroux, C., Woodcroft, B. J. & Degnan, B. M. 2008 Does the high gene density in the sponge NK homeobox gene cluster reflect limited regulatory capacity? *Biol. Bull.* **214**, 205–217.
- Fedonkin, M. A., Gehling, J. G., Grey, K., Narbonne, G. M. & Vickers-Rich, P. 2007a *The rise of animals*. Baltimore, MD: Johns Hopkins University Press.
- Fedonkin, M. A., Simonetta, A. & Ivantsov, A. Y. 2007b New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): paleontological and evolutionary implications. In *The rise and fall of the Ediacaran biota* (eds P. Vickers-Rich & P. Komarower), pp. 157–179. London, UK: Geological Society.
- Fernald, R. D. 2000 Evolution of eyes. *Curr. Opin. Neurobiol.* **10**, 444–450. (doi:10.1016/S0959-4388(00)00114-8)
- Finnerty, J. R., Pang, K., Burton, P., Paulson, D. & Martindale, M. Q. 2004 Origins of bilateral symmetry: Hox and dpp expression in a sea anemone. *Science* **304**, 1335–1337. (doi:10.1126/science.1091946)
- Gehling, J. G., Droser, M. L., Jensen, S. R. & Runnegar, B. N. 2005 Ediacara organisms: relating form to function. In *Form and function: fossils and development* (ed. D. E. G. Briggs), pp. 43–66. New Haven, CT: Peabody Museum of Natural History, Yale University.
- Gehring, W. J. 2004 Historical perspective on the development and evolution of eyes and photoreceptors. *Int. J. Dev. Biol.* **48**, 707–717. (doi:10.1387/ijdb.041900wg)
- Ghysen, A. 2003 The origin and evolution of the nervous system. *Int. J. Dev. Biol.* **47**, 555–562.
- Gimson, A., Srivastava, M., Fahey, B., Woodcroft, B. J., Chiang, H. R., King, N., Begnan, B. M., Rokhsar, D. S. & Bartel, D. P. 2008 Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature* **455**, 1193–1197. (doi:10.1038/nature07415)
- Halder, G., Callaerts, P. & Gehring, W. J. 1995 Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science* **267**, 1788–1792. (doi:10.1126/science.7892602)
- Harvey, R. P. 1996 NK-2 homeobox genes and heart development. *Dev. Biol.* **178**, 203–216. (doi:10.1006/dbio.1996.0212)
- Hejnol, A. & Martindale, M. Q. 2008a Acoel development supports a simple planula-like urbilaterian. *Phil. Trans. R. Soc. B* **363**, 1493–1501. (doi:10.1098/rstb.2007.2239)
- Hejnol, A. & Martindale, M. Q. 2008b Acoel development indicates the independent evolution of the bilaterian mouth and anus. *Nature* **546**, 382–386. (doi:10.1038/nature07309)
- Hirth, F., Kammermeier, L., Frei, E., Walldorf, U., Noll, M. & Reichert, H. 2003 An urbilaterian origin of the tripartite brain: developmental genetic insights from *Drosophila*. *Development* **130**, 2365–2373. (doi:10.1242/dev.00438)
- Holland, L. Z., Kene, M., Williams, N. A. & Holland, N. D. 1997 Sequence and embryonic expression of the amphioxys engrailed gene (*AmphiEn*): the metameric pattern of transcription resembles that of its segment-polarity homolog in *Drosophila*. *Development* **124**, 1723–1732.
- Hui, J. H. L., Holland, P. W. H. & Ferrier, D. E. K. 2008 Do cnidarians have a ParaHox cluster: analysis of synteny around a *Nematostella* homeobox gene cluster. *Evol. Dev.* **10**, 725–730.
- Hwang, J. H., Ohyanagi, H., Hayakawa, S., Osato, N., Nichimiya-Fujisawa, C., Ieko, K., David, C. N., Fujisawa, T. & Gohobori, T. 2007 The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of *Hydra*. *Proc. Natl Acad. Sci. USA* **104**, 14 735–14 740. (doi:10.1073/pnas.0703331104)
- King, N. 2004 The unicellular ancestry of animal development. *Dev. Cell* **7**, 313–325. (doi:10.1016/j.devcel.2004.08.010)

- King, N., Hittinger, C. T. & Carroll, S. B. 2003 Evolution of key cell signaling and adhesion protein families predates animal origins. *Science* **301**, 361–363. (doi:10.1126/science.1083853)
- King, N. *et al.* 2008 The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**, 783–788. (doi:10.1038/nature06617)
- Kusserow, A. *et al.* 2005 Unexpected complexity of the *Wnt* gene family in a sea anemone. *Nature* **433**, 156–160. (doi:10.1038/nature03158)
- Larroux, C., Fahey, B., Liubicich, D., Hinman, V., Guathier, M., Gongora, M., Green, K., Worheide, G., Leys, S. P. & Degnan, B. M. 2006 Developmental expression of transcription factor genes in a demosponge: insights into the origins of metazoan multicellularity. *Evol. Dev.* **8**, 150–173. (doi:10.1111/j.1525-142X.2006.00086.x)
- Larroux, C., Luke, G. N., Koopman, P., Rokhsar, D., Shimeld, S. M. & Degnan, B. M. 2008 Genesis and expansion of metazoan transcription factor gene classes. *Mol. Biol. Evol.* **25**, 980–996. (doi:10.1093/molbev/msn047)
- Lee, P. N., Kumburegama, S., Marlow, H. Q., Martindale, M. Q. & Wikramanayake, A. H. 2007 Asymmetric developmental potential along the animal-vegetal axis in the anthozoan cnidarian, *Nematostella vectensis* is mediated by Dishevelled. *Dev. Biol.* **310**, 169–187. (doi:10.1016/j.ydbio.2007.05.040)
- Lichtneckert, R. & Reichert, H. 2005 Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. *Heredity* **94**, 1–13.
- Lowe, C. J. 2008 Molecular genetic insights into deuterostome evolution from the direct-developing hemichordate *Saccoglossus kowalevskii*. *Phil. Trans. R. Soc. B* **363**, 1569–1578. (doi:10.1098/rstb.2007.2247)
- Lowe, C. J., Wu, M., Salic, A., Evans, L., Lander, E., Strant-Thomann, N., Gruber, C. E., Gerhart, J. & Kirschner, M. 2003 Anteroposterior patterning in hemichordates and the origin of the chordate nervous system. *Cell* **113**, 853–865. (doi:10.1016/S0092-8674(03)00469-0)
- Martindale, M. Q., Pang, K. & Finnerty, J. R. 2004 Investigating the origins of triploblasty: ‘mesodermal’ gene expression in a diploblastic animal, the sea anemone *Nematostella vectensis* (phylum, Cnidaria; class, Anthozoa). *Development* **131**, 2463–2474. (doi:10.1242/dev.01119)
- Matus, D. Q., Pang, K., Marlow, H., Dunn, C. W., Thomsen, G. H. & Martindale, M. Q. 2006 Molecular evidence for deep evolutionary roots of bilaterality in animal development. *Proc. Natl Acad. Sci. USA* **103**, 11 195–11 200. (doi:10.1073/pnas.0601257103)
- Matus, D. Q., Pang, K., Daly, M. & Martindale, M. Q. 2007 Expression of Pax gene family members in the anthozoan cnidarian, *Nematostella vectensis*. *Evol. Dev.* **9**, 25–38.
- Matus, D. Q., Magie, C. R., Pang, K., Martindale, M. Q. & Thomsen, G. H. 2008 The hedgehog gene family of the cnidarian, *Nematostella vectensis*, and implications for understanding metazoan hedgehog pathway evolution. *Dev. Biol.* **313**, 501–518. (doi:10.1016/j.ydbio.2007.09.032)
- Miller, D. J. & Ball, E. E. 2008 Animal evolution: *Trichoplax*, trees and taxonomic turmoil. *Curr. Biol.* **18**, R1003–R1005. (doi:10.1016/j.cub.2008.09.016)
- Mizutani, C. M. & Bier, E. 2008 EvoD/Vo: the origins of BMP signalling in the neurectoderm. *Nat. Rev. Genet.* **9**, 663–677. (doi:10.1038/nrg2417)
- Muller, W. E., Wiens, M., Adell, T., Gamulin, V., Schroder, H. C. & Muller, I. M. 2004 Bauplan of urmetazoa: basis for genetic complexity of metazoa. *Int. Rev. Cytol.* **235**, 53–92. (doi:10.1016/S0074-7696(04)35002-3)
- Narbonne, G. M. 2005 The Ediacara biota: neoproterozoic origin of animals and their ecosystems. *Annu. Rev. Earth Planet. Sci.* **33**, 421–442. (doi:10.1146/annurev.earth.33.092203.122519)
- Ohno, S. 1996 The notion of the Cambrian pananimalia genome. *Proc. Natl Acad. Sci. USA* **93**, 8475–8478. (doi:10.1073/pnas.93.16.8475)
- Olson, E. N. 2006 Gene regulatory networks in the evolution and development of the heart. *Science* **312**, 1922–1927.
- Panganiban, G. & Rubenstein, J. L. R. 2002 Developmental functions of the Distal-less/Dlx homeobox genes. *Development* **129**, 4371–4386.
- Panganiban, G. E. F. *et al.* 1997 The origin and evolution of animal appendages. *Proc. Natl Acad. Sci. USA* **94**, 5162–5166. (doi:10.1073/pnas.94.10.5162)
- Peterson, K. J. & Sperling, E. A. 2007 Poriferan ANTP genes: primitively simple or secondarily reduced? *Evol. Dev.* **9**, 405–409.
- Peterson, K. J., Cotton, J. A., Gehling, J. G. & Pisani, D. 2008 The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Phil. Trans. R. Soc. B* **363**, 1435–1443. (doi:10.1098/rstb.2007.2233)
- Philippe, H., Brinkmann, H., Martinez, P., Riutort, M. & Baguna, J. 2007 Acoel flatworms are not platyhelminthes: evidence from phylogenomics. *PLoS ONE* **2**, e717. (doi:10.1371/journal.pone.0000717)
- Piatigorsky, J. & Kozmik, Z. 2004 Cubozoan jellyfish: an Evo/Devo model for eyes and other sensory systems. *Int. J. Dev. Biol.* **48**, 719–729. (doi:10.1387/ijdb.041851jp)
- Pueyo, J. I. & Couso, J. P. 2005 Parallels between the proximal-distal development of vertebrate and arthropod appendages: homology without an ancestor? *Curr. Opin. Genet. Dev.* **15**, 439–446. (doi:10.1016/j.gde.2005.06.007)
- Pueyo, J. I., Lanfear, R. & Couso, J. P. 2008 Ancestral Notch-mediated segmentation revealed in the cockroach *Periplaneta americana*. *Proc. Natl Acad. Sci. USA* **105**, 16 614–16 619. (doi:10.1073/pnas.0804093105)
- Putnam, N. H. *et al.* 2007 Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science* **317**, 86–94. (doi:10.1126/science.1139158)
- Quiring, R., Walldorf, U., Kloter, U. & Gehring, W. J. 1994 Homology of the eyeless gene of *Drosophila* to the small eye gene in mice and Aniridia in humans. *Science* **265**, 785–789. (doi:10.1126/science.7914031)
- Raff, R. A. 2008 Origins of the other metazoan body plans: the evolution of larval forms. *Phil. Trans. R. Soc. B* **363**, 1473–1479. (doi:10.1098/rstb.2007.2237)
- Reichert, H. & Simeone, A. 2001 Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Phil. Trans. R. Soc. Lond. B* **356**, 1533–1544. (doi:10.1098/rstb.2001.0972)
- Ruiz-Trillo, I., Riutort, M., Littlewood, D. T. J., Herniou, E. A. & Baguna, J. 1999 Acoel flatworms: earliest extant bilaterian metazoans, not members of Platyhelminthes. *Science* **283**, 1919–1923. (doi:10.1126/science.283.5409.1919)
- Ryan, J. F., Burton, P. M., Mazza, M. E., Kwong, G. K., Millikin, J. C. & Finnerty, J. R. 2007a The cnidarian-bilaterian ancestor possessed at least 56 homeoboxes: evidence from the starlet sea anemone, *Nematostella vectensis*. *Genome Biol.* **7**, R64. (doi:10.1186/gb-2006-7-7-r64)
- Ryan, J. F., Mazza, M. E., Pang, K., Matus, D. Q., Baxevanix, A. D., Martindale, M. Q. & Finnerty, J. R. 2007b Pre-Bilaterian origins of the Hox cluster and the Hox code: evidence from the sea anemone, *Nematostella vectensis*. *PLoS ONE* **2**, e153. (doi:10.1371/journal.pone.0000153)

- Seipel, K. & Schmid, V. 2005 Evolution of striated muscle: jellyfish and the origin of triploblasty. *Dev. Biol.* **282**, 14–26. (doi:10.1016/j.ydbio.2005.03.032)
- Seipel, K. & Schmid, V. 2006 Mesodermal anatomies in cnidarian polyps and medusae. *Int. J. Dev. Biol.* **50**, 589–599. (doi:10.1387/ijdb.062150ks)
- Simionato, E., Ledent, V., Richards, G., Thomas-Chollier, M., Kerner, P., Coornaert, D., Degnan, B. M. & Vervoort, M. 2007 Origin and diversification of the basic helix-loop-helix gene family in metazoans: insights from comparative genomics. *BMC Evol. Biol.* **7**, 33. (doi:10.1186/1471-2148-7-33)
- Sempere, L. F., Cole, C. N., McPeck, M. A. & Peterson, K. J. 2006 The phylogenetic distribution of metazoan microRNAs: insights into evolutionary complexity and constraint. *J. Exp. Zool. (Mol. Dev. Evol.)* **306B**, 575–588. (doi:10.1002/jez.b.21118)
- Slack, J. M. W., Holland, P. W. H. & Graham, C. F. 1993 The zootype and the phylotypic stage. *Nature* **361**, 490–492. (doi:10.1038/361490a0)
- Sperling, E. A., Pisani, D. & Peterson, K. J. 2007 Poriferan paraphyly and its implications for Precambrian palaeobiology. In *The rise and fall of the Ediacaran biota* (eds P. Vickers-Rich & P. Komarower), vol. Special Publication 286, pp. 355–368. London, UK: Geological Society.
- Srivastava, M. *et al.* 2008 The *Trichoplax* genome and the nature of placozoans. *Nature* **454**, 955–960. (doi:10.1038/nature07191)
- Stollenwerk, A., Schoppmeier, M. & Damen, W. G. M. 2003 Involvement of Notch and Delta genes in spider segmentation. *Nature* **423**, 863–865. (doi:10.1038/nature01682)
- Tautz, D. 2004 Segmentation. *Dev. Cell* **7**, 301–312. (doi:10.1016/j.devcel.2004.08.008)
- Technau, U. *et al.* 2005 Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet.* **21**, 633–639. (doi:10.1016/j.tig.2005.09.007)
- Valentine, J. W., Collins, A. G. & Meyer, C. P. 1994 Morphological complexity increase in metazoans. *Paleobiology* **20**, 131–142.
- Valentine, J. W., Jablonski, D. & Erwin, D. H. 1999 Fossils, molecules and embryos: new perspectives on the Cambrian explosion. *Development* **126**, 851–859.
- Wheeler, B. M., Heimberg, A. M., Moy, V. N., Sperling, E. A., Holstein, T. W., Heber, S. & Peterson, K. J. 2009 The deep evolution of metazoan microRNAs. *Evol. Develop.* **11**, 50–68. (doi:10.1111/j.1525-142X.2008.00302.x)
- Xiao, S. H. & Laflamme, M. 2009 On the eve of animal radiation: phylogeny, ecology and evolution of the Ediacara biota. *Trends Ecol. Evol.* **24**, 31–40. (doi:10.1016/j.tree.2008.07.015)