

## Dispatches

# Animal Evolution: *Trichoplax*, Trees, and Taxonomic Turmoil

The genome sequence of *Trichoplax adhaerens*, the founding member of the enigmatic animal phylum Placozoa, has revealed that a surprising level of genetic complexity underlies its extremely simple body plan, indicating either that placozoans are secondarily simple or that there is an undiscovered morphologically complex life stage.

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With the recent or imminent release of the whole genome sequences of a number of key animal species, this is an exciting time for the ‘evo-devo’ community. In the last twelve months, whole genome analyses of the cnidarian *Nematostella vectensis*, the choanoflagellate *Monosiga brevicollis* and the cephalochordate *Branchiostoma floridae* (commonly known as amphioxus) have been published, each providing significant new perspectives on animal evolution. The whole genome sequence of the enigmatic placozoan *Trichoplax adhaerens* was published recently [1] and several other important genomes are presently being analysed. The next few months should see release of the first whole genome sequence from a sponge (*Amphimedon queenslandica*), as well as those of several representatives of the second major protostome lineage, the Lophotrochozoa. When these key genomes are available for comparison, the placozoan data can perhaps be more unequivocally interpreted, but what is most striking about this work is that the whole genome sequence has not definitively resolved the interrelated questions of where *Trichoplax* fits in animal evolution and whether its morphological simplicity is ancestral or derived.

Churchill could have been thinking of *Trichoplax* when he said “It is a riddle, wrapped in a mystery, inside an enigma”. The history of placozoan research has been told several times [2]. *Trichoplax* is the simplest known animal in terms of cell-type diversity — just four types have been described — and structure — it comprises a flat bilayer of cells with distinct upper and lower surfaces,

but no other axial differentiation, resembling an amoeba. Grell [3] who formally described these common but inconspicuous marine organisms as belonging to a new phylum, assumed that their simplicity is primary, and that they therefore must represent a key stage in animal evolution. This view is still held by several prominent *Trichoplax* biologists, but has always been contentious; the view that it is derived from a more complex ancestor has recently been gaining momentum and is favoured by Srivastava *et al.* [1]. However, the *Trichoplax*-basal scenario cannot be firmly rejected based on their analyses.

### General Properties of the *Trichoplax* Genome

At around 98 Mb, the *Trichoplax* genome is amongst the smallest known from any animal; but although the total number of protein-coding genes is not large (approximately 11,500), it contains many genes whose homologs have conserved roles in complex patterning processes in ‘higher’ animals (Bilateria). Components of most of the animal-specific and developmentally-regulated signalling pathways are present. Although the Hedgehog pathway appears to be absent, as in the sponge, complete Wnt and TGF $\beta$  pathways are present. Additionally, the *Trichoplax* genome encodes most, though not all, Notch and JAK/STAT pathway components, the apparently missing parts being a canonical Notch ligand and a Janus kinase.

Most of the animal-specific transcription factors — members of the Sox, Pax, T-box, Fox and Ets families — are represented at a similar level of complexity to that found in the sponge. For example, the six *Trichoplax* Sox genes appear to fall

into the same major classes (C, E/F and B) as do those described from *Amphimedon* [4]. Consistent with a more derived position, however, *Trichoplax* has a number of *Antp* superclass Hox genes that are absent from the sponge *Amphimedon*. These include the ‘ParaHox’ gene *Trox-2* [5] and the extended Hox family gene *Not* [6] known from previous work. Particularly intriguing is the discovery in *Trichoplax* of many genes associated with neuroendocrine function across the Bilateria; in common with *Amphimedon* [7], many elements of the post-synaptic scaffold are present, but so too are channel and receptor proteins not known from sponges.

### Are We Missing Something?

Much of the *Trichoplax* life cycle is still unknown. The established means of reproduction are fission, usually in half, and production of ‘swarmers’ during which up to hundreds of small fragments, are simultaneously budded off. Molecular markers from a small wild-caught population indicate the existence of sexual reproduction [8], yet there is no reliable evidence of sex in the presently known life form. To quote Pearce and Voight [9] “not only do gametogenesis, embryogenesis, and metamorphosis remain undescribed, but also meiosis, sperm, and fertilization”.

If there is indeed a missing life stage then it may hold the key to the genomic complexity reported by Srivastava *et al.* [1]. So, what is this missing life stage and where is it hiding? If Pearce and Voight [9] are correct and the sexually reproducing life stage is missing, then this implies that the eggs and embryos that have repeatedly been reported [1] must be something else, perhaps a survival phase analogous to a sponge gemmule. But if that is the case why do these structures always degenerate and die? This is just one of the many mysteries that help *Trichoplax* to maintain its enigmatic status.

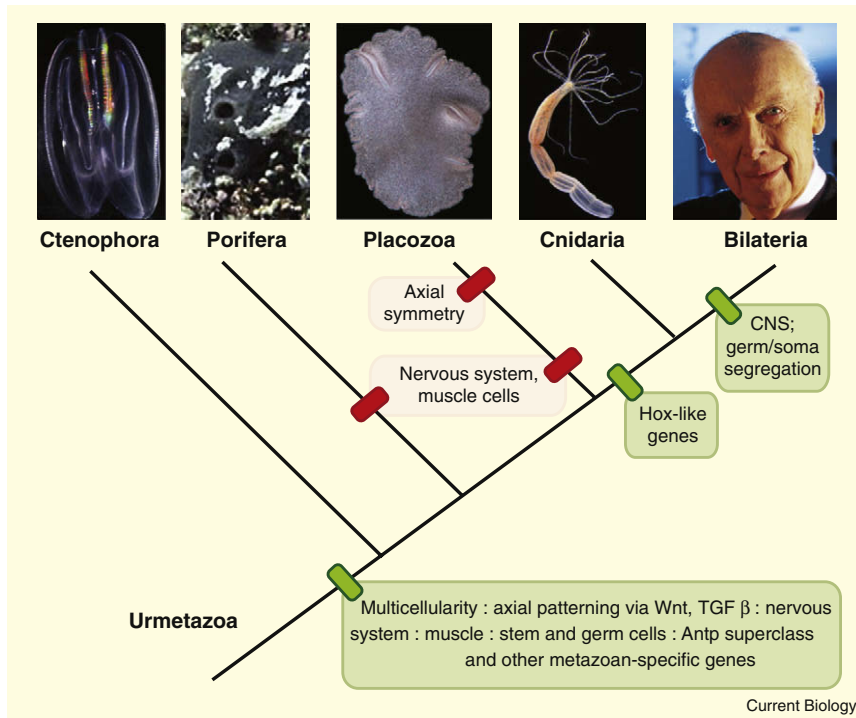


Figure 1. Possible evolutionary relationships at the base of the Metazoa.

Whilst the Ctenophora are morphologically complex, a recent large-scale phylogenomic analysis [12] places them at the base of the Metazoa, implying that the morphological simplicity of both sponges and placozoans is derived. The Dunn *et al.* [12] study did not include Placozoa; the whole genome sequence analysis [1] and qualitative aspects of its nuclear genome, including the presence of members of the extended Hox gene family, support the position shown here. However, the mitochondrial genomes of Placozoa are more 'primitive' in structural terms than would be expected under this scenario. Green bars and associated text indicate gains, and red bars and text losses during evolution.

### Lower Metazoan Relationships

Whilst the Bilateria is a well-defined phylogenetic group, both the branching order of the 'lower' animal phyla — Porifera, Placozoa, Cnidaria and Ctenophora — and their relationships to the Bilateria remain contentious. Their branching order is often assumed to be reflected in their relative morphological complexity, which increases from Placozoa, through Porifera and Cnidaria, with the Ctenophora branching immediately below the Bilateria (for example [10]). Some aspects of this scheme hold up: the Cnidaria are unquestionably more complex, in having many metazoan-specific gene families, than are either Placozoa or Porifera. For example, whereas *Nematostella* has members of most or all of the 12 Wnt subfamilies [11], very few Wnts are encoded by the *Amphimedon* or *Trichoplax* genomes. Whilst placozoans and sponges are unquestionably less morphologically complex than cnidarians and ctenophores, several lines of evidence, including a recent large-scale

phylogenomic analysis [12], support a very different view of lower animal relationships (Figure 1), with ctenophores as the basal animal group, implying that both sponges and placozoans are secondarily simple.

Although it is true that the ctenophores are not yet well represented in the sequence databases, there are no obvious molecular criteria that contradict this view; for example, as in the case of sponges (but unlike cnidarians), ctenophores appear to lack Hox-like and 'ParaHox' genes [13]. Although lacking Hox-like genes, *Trichoplax* has a 'ParaHox' gene — *Trox-2* [5] is clearly related to the *cnx2* genes of cnidarians, *Drosophila Ind* and the vertebrate *Gsx* genes. Both the presence of this gene and its expression pattern are consistent with a derived position for placozoans as shown in Figure 1; homologs of *Trox-2* have roles in the nervous system from Cnidaria to mammals, and its expression in marginal cells suggests a possible sensory role [5].

### The Mitochondrial Genome Sometimes Tells a Different Story

Whereas the presence of specific genes such as *Trox-2* and molecular phylogenetics based on large numbers of nuclear genes [1] imply that the simplicity of *Trichoplax* is likely to be secondary, features of its mitochondrial genome suggest otherwise [14]. Three additional placozoan mitochondrial genomes from divergent clades have now been sequenced [15]. Whilst all four of these placozoan mitochondrial genomes contain the 'normal' animal gene complement (although they all appear to lack *atp8*), they resemble *Monosiga* and other non-metazoans in two respects — they are all much larger (32–43 kb) than the vast majority of bilaterian mitochondrial genomes, and they also contain a number (three in the case of *T. adhaerens*) of large open reading frames of unknown function. Viewed in isolation, these characteristics would seem to point to placozoans as the basal metazoans. However, a recent Bayesian analysis, based on 13 protein sequences encoded by most mitochondrial genomes, found that placozoans are a sister group to the Bilateria [16].

It is most unfortunate that none of the recent large-scale studies have included all of the lower metazoan phyla. Thus, Dunn *et al.* [12] did not include Placozoa and Srivastava *et al.* [1] did not include Ctenophora. It would be particularly interesting to see the dataset run by Dunn *et al.* [12] rerun with the inclusion of the appropriate placozoan genes.

### Degeneration from a Complex State — Is There a Pattern?

Traditionally it has been assumed that, with the exception of a few aberrant organisms such as *Trichoplax*, extant levels of morphological complexity represent progressive steps in animal evolution — essentially, that what we see now is as 'advanced' as a particular lineage ever got. Other interpretations have always existed, however, and, intriguingly, a number of recent large-scale studies have breathed life into the old idea that loss of morphological complexity may be a common theme in animal evolution.

The consensus 'new view' of chordate evolution (for example [17]) has tunicates as the sister group to the vertebrates, and the cephalochordate amphioxus as representative of the

basal chordate lineage. As discussed above, there is now substantial molecular support for the idea that ctenophores branch most basally within the animal kingdom, implying that sponges are secondarily simple. The case for a morphologically complex common bilaterian ancestor (Urbilateria) has been eloquently made by de Robertis [18] and others, but these recent large-scale studies imply that this also holds for Urmetazoa and Urchordata. Hence, not only is genetic complexity ancestral [19,20], but the same may be true for morphological complexity as well.

### Outstanding Questions

As outlined above, these are interesting times for students of evolutionary genomics, with genome sequencing in progress for representatives of several missing key phyla. It is to be hoped that decreasing costs permit the determination of whole genome sequences for more representatives of each phylum in the near future, as gene losses in individual lineages obscure general patterns, and many animal phyla are very diverse.

With so much sequence data now available for *Trichoplax*, it will be fascinating to see where and when the homologs of many of the key development regulators are expressed. Very few expression patterns are yet available, but these imply much greater cell diversity than does morphology alone. It is possible, however, that many of these genes are not expressed in the presently known life form. Notwithstanding the importance of the forthcoming whole genome sequence for the sponge *Amphimedon*, the most intriguing question of all at the moment is where the ctenophores

fit — more sequence data from ctenophores are urgently required. Other outstanding questions include how placozoans have maintained large and complex mitochondrial genomes, and whether sponges are monophyletic or paraphyletic. Although sponges are classically lumped together, there is an emerging view that the phylum Porifera is a paraphyletic group, homoscleromorph sponges (such as *Oscarella*) being most closely related to the Eumetazoa. We live in interesting times indeed.

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## Visual Neuroscience: Retinotopy Meets Percept-otopy?

In the mammalian brain, the primary visual cortex forms a systematic spatial map of the visual field. A new study suggests that the representations on this map are affected by visual illusions that alter perceived size. Spatial patterns of activity may thus reflect perceived size.

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Retinotopic organization is a fundamental organizational element

in visual neuroscience. In the early visual cortices of many animals (including monkeys and humans), nearby neurons respond to adjacent

parts of the visual field. This ‘topographic’ relationship maintains a systematic structure on a large scale, so that each half of the visual field is mapped onto the contralateral hemisphere [1]. Figure 1 shows that, when a human observer views a high-contrast checkerboard ring, a corresponding ring-like swath of activity is generated upon the cortical surface. This isomorphic relationship is typically assumed to be inherited: so long as the wiring that connects the retina to the cortex is not