

NEWS AND COMMENTARY

Molecular phylogeny

My sister is a sea squirt?

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Sir Ray Lankester was lying on the deck of a research vessel in heaving seas, green with sea sickness. As the latest trawl of sea creatures was emptied on to the deck Lankester caught a glimpse of a small silvery animal, 'a semitransparent, oblong, flattened thing, like a small paper knife'. He wrote 'I recognised him and immediately felt restored to well being' (Lankester, 1915). This was the first living specimen he had seen of the lancelet or amphioxus; an animal of considerable fame, and sufficiently exciting to cure his affliction. Lankester's subsequent research on this animal, together with work by Willey, Goodrich and others, showed that amphioxus, although resembling a small fish in general form, is not a true vertebrate, but is an invertebrate relative of the vertebrates. It shares many anatomical characters with vertebrates, notably segmented muscle blocks along its flanks, dorsal nerve cord, notochord and gill slits. It lacks a vertebral column and an elaborate head region.

For a century, most zoologists have viewed amphioxus as the closest living relative of the vertebrates. (Here, I am using the term amphioxus to refer to all 20 or so species in the subphylum Cephalochordata.) With the publication of a new study by Delsuc *et al* (2006), it may be time to move amphioxus away from that position, slightly, and to give the honour to the tunicates. These are another well-known group, including *Ciona* and *Oikopleura*, two animals that have recently had their complete genomes sequenced (Dehal *et al*, 2002). Tunicates, although also long recognised as relatives of vertebrates, have fewer anatomical characters in common with amphioxus and vertebrates, so the new conclusion is certainly a surprise.

The study by Delsuc *et al* (2006), uses molecular phylogenetics to investigate the relationships between the three chordate groups (vertebrates, amphioxus, and tunicates). Of course, genes have been used to tackle this question many times before, but usually only one or a few genes have been employed, or a small sample of species has been included. For example, using the 18S ribosomal DNA gene, am-

phioxus is placed as the sister to the vertebrates, with tunicates a little more distant (Wada and Satoh, 1994), just as anatomical comparison suggests. In recent years it has become clear that use of more genes, and more species, provides more robust phylogenetic answers. Last year, two studies made important steps in this direction (Blair and Hedges, 2005; Philippe *et al*, 2005), and the new report takes this principle even further. A total of 146 protein-coding genes were used (comprising a sequence alignment of over 30,000 amino acids in length), from eight vertebrates, four tunicates, and one amphioxus species. From analysis of these data, the authors conclude that tunicates are the closest invertebrates to the vertebrates, this result being statistically significant over alternative hypotheses. One caveat is that only a single amphioxus species is used (*Branchiostoma floridae*); inclusion of other cephalochordates, notably the divergent genus *Epigonichthys*, would be interesting. A second, but much more tentative, result is that amphioxus may be closer to echinoderms than to tunicates and vertebrates, but this latter suggestion must be treated with extreme caution because only one species of echinoderm was used and no hemichordates. Furthermore, the echinoderm–amphioxus link is not statistically better than monophyly of

the chordates (for which there is more evidence from other sources).

If the first conclusion of Delsuc *et al* (2006), is correct, what are the implications? First, Ray Lankester was correct that cephalochordates (amphioxus) are close relatives of the vertebrates, but perhaps not quite as close as first thought. Many people (myself included) will have to stop referring to amphioxus as the closest invertebrate relative of the vertebrates! The phylogeny also seems compatible with the recent discovery of migratory neural-derived cells in some tunicates (Jeffery *et al*, 2004), perhaps homologous to vertebrate neural crest cells. Such cells have not yet been detected in amphioxus. However, what of the anatomical characters that were traditionally used to place amphioxus as the sister of vertebrates? The most obvious are the somites that develop into segmented axial musculature. We would have to conclude that these arose earlier in evolution (at the origin of chordates) and have been modified in tunicates. Such is the perennial problem of deep phylogenetics based on morphology.

There are also important implications for the use of tunicates or amphioxus as models for developmental biology or genomics research. It is already clear, from the genome sequences of *Ciona* and *Oikopleura*, that tunicate genomes can be very different to vertebrate genomes. Several sets of genes that are organised into linked arrays in vertebrates (for example Hox and ParaHox genes) are dispersed in tunicates (Ferrier and Holland, 2002; Ikuta *et al*, 2005) and individual genes can be highly divergent in sequence. The more limited information we have from the

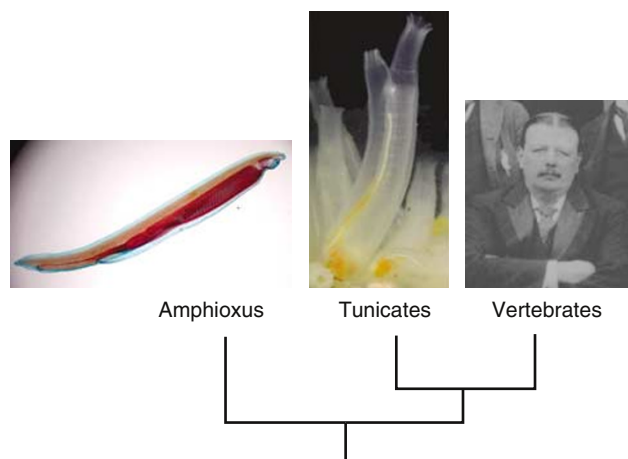


Figure 1 Proposed phylogeny of the chordates (*Ciona* image courtesy of Nori Satoh, Anna Di Gregorio, David Keys, and the US Joint Genome Institute).

amphioxus genome reveals more similarities to vertebrates, in the arrangements of gene clusters, gene sizes, and intron–exon boundaries (although, of course, vertebrate genomes are complicated by extensive gene duplication). If the revised chordate phylogeny is correct, we must conclude that the genomic similarities between amphioxus and vertebrates date back to the origin of the chordates. Consequently, every difference in tunicates is secondarily derived from that ancestral condition. The new phylogeny, therefore,

affirms the importance of amphioxus as being the key organism from which we will learn about the ancestral chordate genome. If sea squirts (and their allies) are the sister group to the vertebrates, then amphioxus is our more sensible cousin (Figure 1).

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Apologies to my sister Fiona. She can be reassured that *Ciona* – not *Fiona* – is a sea squirt; *Fiona* is a genus of sea slug.