

Showcase on Research

Evo-devo: Insights into the Evolution of Developmental Mechanisms from Non-Standard Animals

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To date, the search for common molecular principles of animal development has concentrated on only a few species, notably *Drosophila*, *Caenorhabditis* and the mouse. Although this focus has yielded great advances, it has also introduced strong biases into our ideas about the generality of developmental processes. Good laboratory animals (and hence model organisms) are not necessarily representative of their phyla, and comparisons of a small number of such highly specialised animals are unlikely to tell us very much about the evolution of developmental genes and processes.

One of the most exciting aspects of the era of high-throughput DNA sequencing and transgenic expression is that, over the next few years, we will be able to learn more about the limits to conservation. We are focusing on non-standard organisms – a cnidarian (coral), molluscs (snails and clams), polychaetes (marine worms)

and ascidians (sea squirts) – in order to address issues about the evolution of specific developmental genes and the evolution of body plans.

Acropora – a model lower animal

To understand the course of evolution we need to establish in what kind of organism a specific gene first appeared and what its original role is likely to have been (1). To shed some light on the early evolution of specific gene classes, we are studying the reef-building coral, *Acropora* (see Fig. 1).

Amongst the diploblastic (i.e. those with two body layers) lower animals, cnidarians occupy a key position in the evolution of animal complexity - they are probably the most closely related group to the “higher” (triploblastic) animals. The textbook representative cnidarian is *Hydra*, but *Acropora* has many advantages for comparative developmental studies (2). The most obvious of these is that *Acropora* reproduction is a predictable and accessible event (the spectacular annual mass coral spawning).

In contrast, many strains of *Hydra* do not reproduce sexually; for the few which do so, reproduction is unpredictable and early development occurs in a cyst that may be dormant for months. Cnidarians are a very diverse animal group and, whereas *Hydra* is a highly derived animal, *Acropora* belongs to the basal Class (the Anthozoa). This means *Acropora* is more likely to reflect the characteristics of the common ancestor of all animals than is *Hydra*.

There are also practical problems caused by the extreme (A+T)-bias of the *Hydra* genome; the more ‘normal’ base composition of the *Acropora* genome means that

there are no corresponding difficulties in this case.

Over the last few years, in collaboration with Eldon Ball and David Hayward at ANU, we (David Miller, Julian Catmull and numerous PhD and Honours students) have established most of the standard molecular methods (including *in situ* hybridisation technology) and tools (including genetic libraries) for *Acropora*. This has led to increasing recognition of its value as a comparator by the international community.

Insights into Pax gene evolution

Acropora has provided new evolutionary perspectives on several groups of regulatory genes, including the Pax family. The Pax genes encode a complex family of transcription factors with multiple DNA-binding domains and diverse functions, and for these reasons many aspects of their evolution remain speculative. Pax genes are defined by the presence of the paired box, first identified in the *Drosophila* pair-rule gene *paired*, which encodes a large (128-amino acid) DNA-binding domain (the Paired domain).

Some Pax genes also encode either a complete or partial homeodomain. Nine Pax genes are known in mammals, and eight in *Drosophila*. In addition to complex DNA-binding behaviour, alternative splicing and multiple roles during development complicate the identification of ancestral interactions and functions.

Most of the arthropod and chordate Pax genes fall into four classes on the basis of comparisons of domain structure and sequences, but the Pax-6 class, which includes *Drosophila* *eyeless*, is the only unequivocal case of conservation of function (3). The Pax-2/5/8 class is viewed as that most closely related to the Pax-6 group; the ‘supergroup’ comprising Pax-6/2/5/8 is clearly distinct from the other ‘supergroup’, which comprises the Pax-3/7 and Pax-1/9 clades.

In addition, many atypical Pax genes are known (for example, the *Drosophila*



Fig. 1. The ‘Evo-Devo’ approach. Surprisingly, the coral (*Acropora millepora* - lower) shares many genes with the large chordate shown (*Homo sapiens* - upper). The coral is likely to provide insights into the evolution of genes central to development of higher animals, because in the former case, gene duplication and co-option phenomena are probably less significant. Hence ancestral functions are likely to be more clearly seen (Photo – Ken Anthony)

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eyegone/lune gene) with more restricted distributions.

We have identified a total of four *Pax* genes in *Acropora* (4,5). *Pax-A* has clear orthologs in several other cnidarians, and can probably be considered to correspond to *Drosophila poxneuro*. This latter gene has often been considered to be a highly diverged *Pax-2/5/8* gene. However, the detection of probable orthologs in cnidarians, together with its relatively simple structure (it does not encode a homeodomain) indicate that *Pax-A* may correspond to the ancestral type of *Pax* gene.

The *Acropora Pax-B* gene also has probable orthologs in several other cnidarians and also in a single sponge species, and can be viewed as corresponding to an ancestral *Pax-2/5/8* gene. Like *Pax-2/5/8*, but unlike *Poxneuro/Pax-A* and *Pax-6* proteins, the *Pax-B* paired domain has Q-R-H at amino acid positions 42, 44 and 47, which are known to determine specificity (6). Consistent with its assignment as a precursor of the *Pax-2/5/8* class, we have recently shown that the *Acropora Pax-B* paired domain binds to consensus *Pax-2/5/8* binding sites (5).

Two other *Pax* genes have been identified in *Acropora*, but have not yet been detected in other cnidarians. Some speculation is involved in assigning other cnidarian *Pax* genes to the classes identified in higher animals, but *Acropora Pax-D* falls unambiguously into the *Pax-3/7* class on the basis both of the Paired domain and the homeodomain structures. Clearly, substantial divergence of *Pax* genes had already occurred by the time that the Cnidaria split off from the line leading to the higher Metazoa.

Finally, *Acropora Pax-C* most closely resembles *Pax-6*, which we interpret as indicating an early origin of the latter class. Although *Pax* genes have many specialised and diverse roles in higher animals, most are expressed in the nervous system during development. We used whole mount *in situ* hybridisation to examine the distribution of *Pax-A* and *Pax-C* mRNAs during *Acropora* embryogenesis (Fig. 2).

The *Pax-Cam* message is most abundant at approximately 48 h into development, by which time the embryo is pear-shaped (see Fig. 4 in ref. 2). *Pax-Cam in situ*

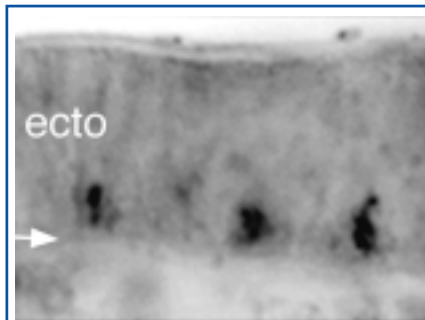


Fig. 2. Localisation of *Pax-A* mRNA by *in situ* hybridisation. The image is at high magnification, and shows staining of small cells at the base of the ectoderm of an *Acropora* planula larva. The morphology of these cells closely resembles those of interstitial cells in *Hydra*, although molecular confirmation of their identity is still lacking (Photo – Eldon Ball).

preparations show scattered labelled transectodermal cells, whose morphology is consistent with their assignment as neurons (5).

The *poxneuro* gene functions in neuroblast cell fate specification during embryonic development of *Drosophila*. The putative *Acropora* ortholog, *Pax-A*, is expressed in presumed interstitial cells of the embryo; such cells are the precursors of neurons, and hence the cnidarian equivalent of neuroblasts (Fig. 2; Reece-Hoyes *et al.*, in preparation).

If confirmed by double labelling, these preliminary results would appear to indicate that *Pax* diversification accompanied and facilitated elaboration of the nervous system (7). *In situ* hybridisation experiments aimed at localisation of other *Pax* mRNAs during *Acropora* embryogenesis are in progress.

Body plan evolution: molluscs, annelids and relatives as a case study

In my laboratory at the University of Queensland (B. Degnan) we have targeted the spiralian lophotrochozoan superphyletic group, a group of related phyla. Animals in these phyla exhibit a wide range of adult body architectures (e.g. molluscs, annelids, platyhelminths) that develop from an essentially identical larval form – the trochophore (Fig. 3).

The phylogenetic breadth of

this ontogenetic conservation is unparalleled in the animal kingdom and provides us with a tractable system to understand how changes in developmental genes cause animal evolution and generate novel morphology (8).

The initial goal of this research is to determine the developmental expression patterns of a set of key transcription factor genes in a number of spiralian lophotrochozoan phyla. This is the first study to compare systematically developmental gene expression in animals that possess unique body plans in related phyla. The transcription factor genes analysed are orthologs of known insect and vertebrate regulators of spatial patterning mechanisms (*Otx* and *Hox* genes), and the specification and differentiation of the mesoderm (*Mox* genes) and nervous system (*Pax* and *POU* genes). This spectrum of genes allows us to investigate and compare both early differentiation events associated with common trochophore development (e.g. *Mox*, *Pax-2/5/8* and *POUIII*) and later patterning events associated with construction of unique body plans (e.g. *Hox*) (see for example, refs 9, 10). Importantly, because only highly conserved genes are included in these analyses, the expression patterns we observe can be directly compared to those in insects, vertebrates and other animals.

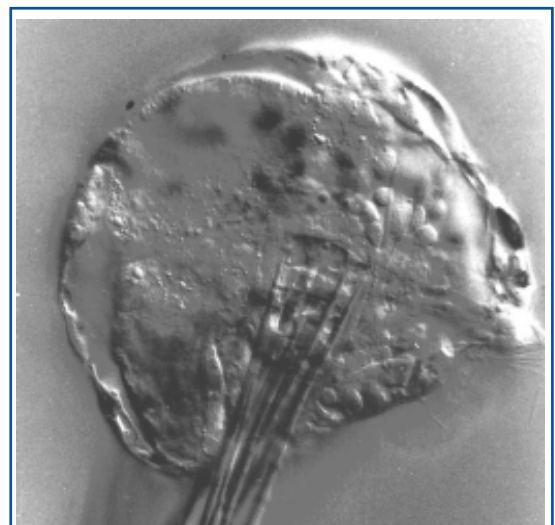


Fig. 3. The trochophore larva characteristic of spiralian lophotrochozoans. Typically this kind of larva is made of only a few hundred cells essentially organised into a single ectodermal layer with a set of ciliary bands, a fully to incompletely formed gut tube, a blastocoel filled with mesoderm derivatives, and possibly a simple sensory system linked to a simple ganglion.

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This will allow us to identify developmental genetic homologies that transcend radically different bilaterian body architectures.

Retinoic acid and homeobox genes in ascidians: insights into the chordate ancestor

Ascidians, as representative urochordates, provide a simple system in which to study a number of chordate-specific developmental processes. While the ascidian embryo displays determinative development and is comprised of a small number of cells, we and others have shown that the expression patterns of conserved regulatory genes (e.g. *brachyury*, *Hox1*, *Hox5*, *Lim*, *Otx*, *Cdx*, *HrPax-2/5/8*) are similar to those occurring in vertebrates (Fig. 4).

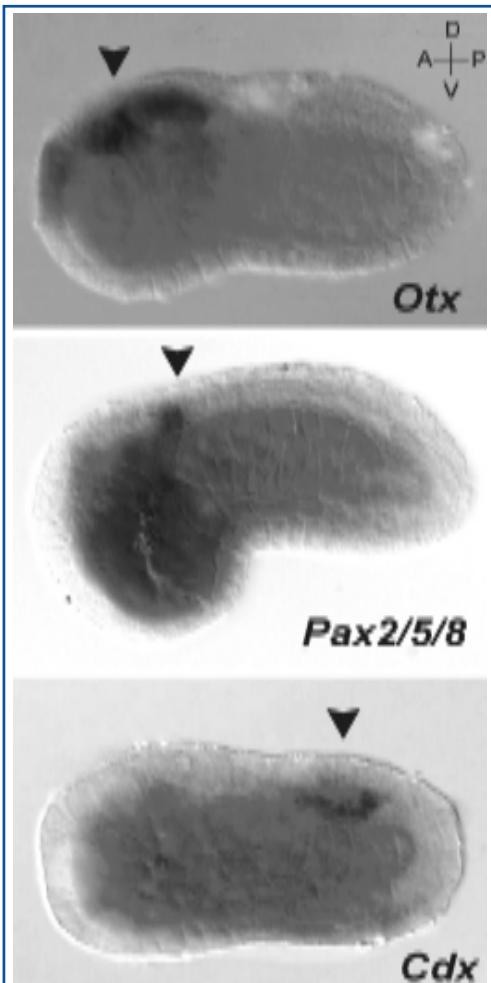


Fig. 4. Expression of *Otx*, *Pax2/5/8* and *Cdx* genes in the developing brain and nerve cord of the tailbud embryo of tropical ascidian *Herdmania curvata*. The expression of these and other homeobox and *Pax* genes in the ascidian closely match those of homologous vertebrate genes. Orientation key: D, dorsal; V, ventral; A, anterior; P, posterior.

Comparisons between vertebrates and ascidians suggest that a genetic regulatory system controlling the development and patterning of chordate-specific tissues was established prior to the divergence of the chordate subphyla and the evolution of the diverse embryological processes exhibited by chordates.

Unlike other chordate taxa, ascidians (and thaliaceans) have a biphasic life-history common among marine invertebrates (11). Embryogenesis results in a tadpole larva with notochord, dorsal hollow nerve cord and axial musculature.

During metamorphosis these tissues degenerate and morphogenesis of the ectodermal and endoderm primordia begins, forming, amongst other juvenile organs, the feeding apparatus which includes the mouth, pharyngeal basket, endostyle and posterior gut tube.

We and others have previously shown that ectopic all-trans retinoic acid (RA) affects embryonic and postlarval development in ascidians. In *Herdmania curvata*, RA induces the loss of the anterior pharyngeal basket in the juvenile, and appears to have a less significant impact on larval anterior CNS development (12).

We were interested to determine the molecular mechanism underlying these effects and if some of the same genes known to be regulated by RA during vertebrate embryogenesis are also regulated during ascidian development. In vertebrates, ectopic application of RA results in the aberrant expression of a number of homeobox genes, including *Otx*, which leads to defects including the loss of forebrain and midbrain, fusion or mis-patterning of rhombomeres, craniofacial defects and homeotic shifts and fusions.

We characterised the expression of the ascidian *Otx* and *Pax2/5/8* gene in normal and RA-perturbed embryos and postlarvae to determine the ancestral role of RA regulation of homeobox gene expression. The ascidian *Otx* is expressed in the anterior of the larval neural tube and juvenile pharynx in a manner similar to that observed in representative taxa of the other chordate subphyla.

RA impacts on *Otx* expression that is associated with pharyngeal tissues in both embryos and juveniles. Unlike, the situation in vertebrates, RA does not appear to affect either *Hec-Otx* or *Hec-Pax2/5/8* expression in the CNS or to result in a loss or mis-specification of CNS tissue.

These data imply that while the expression patterns of *Otx* and *Pax2/5/8* genes in vertebrate and ascidian neural tubes are strikingly similar (implying that this pattern existed in basal chordates), the process by which these patterns are regulated are likely to be different. RA regulation of *Otx* genes is probably an ancestral condition in chordates, but is predominantly associated with pharyngeal rather than neuroectodermal expression in the ascidian (13).

Exploration of the regulatory basis of development in organisms with such diverse body plans as those discussed here will not only produce insights into the details of evolution, but will also reveal how flexible conserved regulatory processes can be, in terms of the structures that they generate. In turn this is certain to yield important and enlightening information about the molecular nature and function of these pathways.

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