Meeting report

New animal models for evolution and development

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A report on the annual UK Evolutionary Developmental Biology meeting, Oxford, UK, 13 September 2004.

To resolve the puzzle of metazoan evolution and development, bioinformatic and experimental approaches must be applied to a wider range of species than just the standard model organisms. This was reflected in the list of phyla covered in this year's 'Evo-Devo' meeting organized by David Ferrier and Peter Holland (both at the University of Oxford, UK). Much of our understanding of metazoan evolution and development (evo-devo) is based on comparisons of the model organisms *Caenorhabditis elegans* and *Drosophila melanogaster* with the vertebrates - but such comparisons have two main limitations. First, they cannot tell us where metazoan genes come from, or what their original role was in our unicellular ancestors. To try to answer this question, the unicellular choanoflagellates have now come on to the molecular stage. Second, evidence is accumulating that both *Drosophila* and *C. elegans* are fast-evolving organisms, have lost many ancestral genes, and have modified development more than other bilaterans. Needless to say, this complicates evolutionary studies. The meeting drew our attention to studies on crustaceans, the wasp *Nasonia* and the polychaete *Platynereis* that have shed new light on the evolution of segmentation, axis formation and eye development, respectively.

Many genes in multicellular animals, such as those for cell adhesion proteins, intercellular signaling proteins, extracellular matrix components and certain families of transcription factors, have no similarities to any known genes in plants or fungi. Their evolutionary origin is thus entirely unclear. In his talk, Holland reasoned that in order to gain more insight into the origins of metazoan proteins and body plans, it is crucial to sequence the genomes of animals that have branched off the evolutionary tree immediately before, or immediately after, the advent of multicellularity. Species thus selected for genome sequencing by the Joint Genome Institute in the USA and for the generation of vast collections of expressed sequence tags (ESTs) are unicellular choanoflagellates (two unrelated *Monosiga* species), the amorphous, basal metazoan *Trichoplax*, the sponge *Reniera* (Demonspongia) and the polyp *Nematostella* (Anthozoa, Cnidaria).

Holland focused on progress in studying the unicellular choanoflagellates, which are considered the sister group (the closest relatives) of all metazoan animals, a view initially proposed on the basis of morphological comparisons dating back to the nineteenth century. This key phylogenetic position of choanoflagellates has now been confirmed by Holland and co-workers using a total of 30,000 amino acids of concatenated protein sequence for phylogenetic tree construction. Choanoflagellate EST sequences are currently yielding nice examples of how knowledge of the genes and proteins present in basal groups can further enhance our understanding of the origins of metazoan genes and proteins. Besides helping us to understand the origins of metazoan genes, the sequencing of choanoflagellates, placozoa, sponges and anthozoans will also reveal general mechanisms acting in gene evolution.

Distinguishing one end from the other

Claude Desplan (New York University, New York, USA) reported on an apparent example of convergent evolution in the patterning of the anterior-posterior axis in insects. His group compared anterior-posterior patterning in *Tribolium* (a beetle), a so-called short germ-band insect that forms the segments sequentially, and in *Drosophila* and *Nasonia* (a parasitic wasp), both of which are long germ-band insects (forming the segments simultaneously). Desplan argued that an ancestral mode of anterior-posterior patterning acting in short germ-band insects involved the anteriorly acting genes otx and hunchback (hb), which counteracted the action of *caudal* at the posterior end. The developing *Drosophila* embryo has departed considerably from this scenario by establishing an anterior morphogenetic center that uses a

gradient of maternally supplied *bicoid* (*bcd*) mRNA, which establishes the correct sequence of anterior-to-posterior identities all at once. This is possible because *Drosophila* is a long germ-band insect with simultaneous segment formation. However, *bicoid* is a phylogenetically young gene that evolved late and only in flies. It does not exist in other long-germ band insects, not even in the mosquito *Anopheles*, a close relative of flies. How then do other long germ-band insects establish their anterior-posterior axis?

Desplan reported that in order to answer this question Jeremy Lynch in his lab investigated the role of *hb* and *otx* in anterior axis specification in *Nasonia*, a long germ-band hymenopteran whose lineage departed from that of the flies 220 million years ago (Mya). Interestingly, *otx* mRNA in *Nasonia* is localized at both the anterior and posterior poles of the egg. Inactivation of the *otx* gene by parental RNA interference (RNAi) produces headless embryos, indicating that *otx* has a *bicoid*-like role in patterning the anterior of *Nasonia*. RNAi of *otx* also causes significant disruptions in posterior segmentation.

In the beetle *Tribolium*, *hb* is not required for the development of the anterior-most head segments, but in *Nasonia*, in contrast, a zygotic mutation in *hb* (generated by Mary Anne Pultz, Western Washington University, Bellingham, USA) deletes almost the entire anterior end of the animal. This indicates that in *Nasonia*, *hb* functions as a major player, and that the *otx-hb* interaction in anterior patterning in *Nasonia* is as strong as, or stronger than, the analogous interaction of *bcd* and *hb* in the fly. Desplan proposed that, like *Drosophila*, *Nasonia* has independently evolved an anterior morphogenetic center by localizing *otx* at the anterior (and the posterior) end of the embryo to take advantage of, or to make possible, its long germ-band mode of development.

Michalis Averof (Institute of Molecular Biology and Biotechnology (IMBB), Crete, Greece) presented an analysis of the posterior patterning gene *caudal* (*cad*) in crustaceans and a comparison of its function across different phyla. In the brine shrimp *Artemia*, *cad* expression specifically localizes to the posterior growth zone. Moreover, Averof reported that Tijana Copf in his lab had shown that *cad* inactivation by RNAi in the larva can abolish all trunk segments, depending on the time of the interfering RNA injection. At the molecular level *cad* inactivation leads to severe perturbation of expression of the early segmentation genes *engrailed* and *even-skipped*. Averof's group, together with Reinhard Schröder's group at the University of Tübingen (Germany), found that interference with *cad* function similarly abolishes trunk segment formation in the beetle *Tribolium*. This implies that, in the most recent common ancestor of crustaceans and insects, segments were already being formed from a posterior growth zone under the control of *cad*.

As Averof pointed out, this role of *cad* also extends beyond the protostomes (the wider phylogenetic branch to which insects belong). In vertebrates, which are deuterostomes, *cad* orthologs (*cdx1-cdx4*) are expressed in the caudal presomitic mesoderm, where they are required for the segmental generation of the somites and the specification of somite identity. Mutations in the vertebrate *cdx* genes also compromise the self-renewing capacity of the presomitic mesoderm. Therefore, it appears that the function of *cad* in the growth zone and in posterior segmentation is ancestral for bilaterians (animals with bilateral symmetry, including both insects and vertebrates).

Averof also reported work of Tassos Pavlopoulos in his group who has succeeded in establishing transgenesis in the amphipod crustacean *Parhyale hawaiiensis* by injecting DNA into one-cell and two-cell embryos. He used a vector containing the *minos* transposable element from *Drosophila hydei* (developed by C. Savakis, Institute of Molecular Biology and Biotechnology, Crete, Greece). This vector appears to have the potential to function in a wide range of phyla.

Tracing origins

To determine homology between organs in different species, morphologists have in the past mainly focused on the structure of the organ as a whole. Organs are, however, composite structures of distinct tissues, each of which is composed of one or more cell types, and each cell type will have its own evolutionary history. The comparison of cell types from different species is thus a novel and useful approach to determining the evolution of organs. One of us (D.A.) described work from our lab that takes this approach to bilaterian eye evolution, comparing the photoreceptor cells of vertebrates with those of the polychaete *Platynereis dumerilii*, a marine invertebrate selected for genome and large-scale EST sequencing by the French national sequencing centre Génoscope. On the basis of evidence from sequence analysis, developmental gene expression and cellular ultrastructure we proposed that the rods and cones, the ciliary photoreceptors of the vertebrate retina, derive from a population of ciliary photoreceptor cells that was present in the brains of ancient bilaterians.

It has been estimated that more than half of all known animal species are parasitic at some stage of their life cycle and Tim Littlewood (Natural History Museum, London, UK) addressed the question of how the complex life cycles of parasites evolve, focusing on tapeworms and liver flukes. To be a successful parasite, the interaction between host and parasite must be finely balanced. Parasites have to circumvent the host's immune response and to adapt their life cycles to attach to or enter their hosts, as well as to use the resources provided optimally. Parasites may influence the host's behavior and development for their own advantage, but the balance between cost to the host and benefit to the parasite

is often finely tuned as, for example, expressed in the Red Queen hypothesis of host-parasite coevolution. Some of these interactions are especially interesting because of the very complicated parasite life cycles, where up to seven different hosts may be used.

Littlewood has started to achieve a better resolution of the phylogeny of the main platyhelminth groups and the constituent parasitic taxa, from which ancestral life cycles, and therefore evolutionary development, of the parasites can be inferred. The evolution of obligate parasitism in the phylum was a major single event but unpicking the way in which it arose and how each major parasitic lineage and the various life history and developmental strategies radiated requires a combination of phylogenetics, comparative life-cycle data and the need to perceive these life cycles in a more developmental, rather than ecological, sense.

It is clear that the morphological changes that occur during evolution are ultimately encoded at the DNA level. But where exactly in the genome do evolutionarily significant mutations occur? So far, enhancers have been the main focus of attention. Claudio Alonso and Adam Wilkins (University of Cambridge, UK) moved away from what they called "the enhancer cult", and instead emphasized the possible evolutionary importance of changes in regulatory elements other than enhancers. They presented a synopsis of the many different mechanisms now known to control mRNA and protein levels, including the tissue-specific expression of components of the basic transcriptional machinery, the different sequences of core promoters of different genes, and the control of gene expression via mRNA untranslated regions. Alonso and Wilkins also stressed the possible evolutionary importance of alternatively spliced transcripts. Their examples showed that ratios between the splicing isoforms of certain genes could be critical for normal development, and that unique protein domains introduced by tissue-specific alternative splicing could lead to distinct gene functions in different cell types. They argued that mutations affecting all these alternative regulatory points could potentially be as important as those that affect enhancers in regulating developmental gene activity. It will be interesting to investigate whether, for example, certain splicing variants of developmental genes are more evolutionary conserved than others, and if and how these differences might cause changes in the morphology of different species.

Although short, the meeting reflected well how information from non-model organisms leads to exciting questions and results that yield a broader comprehension of development and evolution. It has become clear that this understanding cannot be achieved by relying only on the classical model organisms.