Meeting report

# Developmental biology reaches new lineages

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A report on the Developmental Biology Annual Symposium and GENETICS 2004, Warwick, UK, 14-16 March 2004.

This joint meeting of the Genetics Society and the British Society for Developmental Biology (in association with the International Society for Developmental Biologists) juxtaposed functional and evolutionary studies spanning enormous timescales - many in emerging model organisms - with descriptions of cutting-edge molecular genetic techniques for fruit flies and mice. It became clear that new challenges in the post-genomic era are stimulating the development of novel strategies to understand gene function. Here, we focus on a few of the presentations that illustrate the multidisciplinary nature of current research in developmental biology and genetics.

## Evolution of patterning mechanisms

The winner of the Genetics Society Medal 2004, Peter Holland (University of Oxford, UK), opened the meeting with a lecture that took the audience back half a billion years to when the latest common ancestor of bilaterians was alive. Bilaterians - or triploblasts - are defined as all animals with a clear anterior-posterior axis, bilateral symmetry and three embryonic germ layers (ectoderm, mesoderm and endoderm). Comparative analyses of the genomic organization and expression patterns of homeobox-containing genes has led Holland and colleagues to hypothesize that this ancestor used one distinct homeobox gene cluster to pattern each of three germ layers. In the ancestral bilaterian, positional information along the anterior-posterior axis of the nervous system, a derivative of the ectoderm, was determined by the function of a single Hox gene cluster. The endodermal layer (the gut) was patterned by a ParaHox gene cluster, the evolutionary sister of the Hox cluster that includes homologs of the Cdx, Xlox and Gsx families. Finally, Holland proposed that this organism used an ancestral cluster of NK-class homeobox genes (including homologs of the fly genes *tinman*, *bagpipe* and others) to pattern its mesodermal layer. This NK-class cluster is suggested to be as ancient as the Hox and ParaHox clusters but - unlike the other two clusters - appears to have no colinearity between the expression patterns of the individual genes along the anterior-posterior axis and their positions on the chromosome. Holland stressed that gene duplication, which generated these diverse clusters of genes, has been crucial during the evolution of more complex organisms, and also that wide comparative studies, including groups of organisms in key phylogenetic positions, are essential for ascertaining the ancestral functions of a given gene.

Many of these points were returned to in the final session of the meeting, entitled 'Evolution of patterning mechanisms'. Seb Shimeld (University of Reading, UK) presented evidence on the evolution of a subset of genes encoding transcription factors of the forkhead-box (Fox) family, and their role in patterning the mesoderm. He concentrated on the Fox genes that are clustered in mammalian genomes: *FoxL*, *FoxC*, *FoxF* and *FoxQ*. By comparing the genomic organization of these genes among various organisms, he showed that the ancestral condition of this subset in the common ancestor of bilaterians was a clustered organization. He also proposed that the linkage between Fox genes might have been constrained over evolution because of their essential function in diversifying the mesoderm.

Victoria Prince (University of Chicago, USA) detailed experiments in which zebrafish was used as a model organism for teleosts, whose genomes are duplicated relative to other vertebrates. Using studies of the zebrafish duplicate gene pair *Hoxb1a* and *Hoxb1b*, she showed how gene duplication can facilitate shuffling and subdivision of functions between

paralogous genes. Furthermore, she described how duplicate genes are maintained in genomes by acquiring novel, essential functions after overcoming an initial phase of genetic redundancy. A central conclusion from this work was that having more genes does not always imply more functions; rather, complexity should be considered in terms of the sum of the sub-functions performed by the genes under consideration.

Michael Akam (University of Cambridge, UK) described segmentation mechanisms in an early-evolving arthropod, the centipede *Strigamia maritima*, and used this to illustrate how cyclic mechanisms of segmentation have been conserved throughout phylogeny. Such mechanisms involve periodic expression of key regulatory genes that is transformed into segmental expression of downstream genes along an axis. Segmentation genes that do not exhibit cyclic behavior in *Drosophila*, such as *odd-skipped*, do so in the centipede. Other genes, such as the ParaHox gene *caudal*, which is not expressed segmentally and does not exhibit cyclic behavior in *Drosophila*, is expressed segmentally and does cycle in the generation of segments in the centipede. Interestingly, *odd-skipped* has been shown to be a downstream target of the Notch signaling pathway, a key pathway that regulates segmentation of the lateral-plate mesoderm in vertebrates (among many other processes).

The paucity of direct, functional approaches applicable to organisms that are at key phylogenetic nodes - and are therefore useful models for studies of evolution - has frustrated researchers working on the evolution of development. Angelika Stollewerk (University of Cambridge, UK) described how her group is using RNA-interference (RNAi) techniques to investigate how developmental processes have been modified to produce the diversity of neural structures found in different arthropod groups. She showed that a conserved genetic network - lateral inhibition mediated by the Notch signaling pathway - is involved in the generation of neural precursor cells in insects, spiders and myriapods. But unlike the situation in *Drosophila*, in which neuroblast-type stem cells give rise to neural precursors, in spiders and myriapods there is direct recruitment of epidermal cells to form neurons. This difference may account for the morphological differences in neural structures between the different arthropod groups.

## Exploiting the *Drosophila* genome sequence

In a session entitled 'Genomic technologies in *Drosophila* whole genome analysis', Eileen Furlong (European Molecular Biology Laboratory (EMBL), Heidelberg, Germany) demonstrated the use of a variety of genomic and bioinformatic approaches, in combination with the genetic techniques available for the fly, to identify genes required for various aspects of muscle development in *Drosophila*. Using an embryo sorter her group has developed, they can isolate pure populations of mutant embryos on the basis of their expression or lack of expression of green fluorescent protein (GFP) from a reporter gene disrupting the gene of interest. This provides source material for comparisons of expression profiles between wild-type embryos and embryos that are defective in various aspects of muscle development, using whole-genome microarrays. The strategy has identified many novel genes: for example, *gleeful*, which is expressed in the developing muscles and encodes a transcription factor related to vertebrate Gli proteins and to the *Drosophila* Cubitus interuptus protein. (This gene has also been identified independently by the group of Michael Bate (Cambridge University, UK) and named *myoblasts incompetent*, *minc*.) Disruption of *gleeful* function using RNAi produces an embryo with no somatic muscle but with normal heart-muscle and gut-muscle development. To understand muscle diversity and morphogenesis better, the same techniques have also been used in more refined screens to identify genes differentially expressed in muscle founder cells or fusion-competent myoblasts.

Renato Paro (EMBL, Heidelberg, Germany) described the work of a consortium of groups who are using microarray technology to study the cellular memory mechanisms that maintain stable and heritable gene-expression patterns in the developing *Drosophila* embryo. The consortium has developed a novel transcriptome array that contains over 21,000 potential ORFs (The Heidelberg FlyArray [http://hdflyarray.zmbh.uni-heidelberg.de/]). Attempts to validate these arrays revealed the existence of at least 2,000 genes that had not been found using other analyses. Developmental expression profiling of a subset of these by *in situ* hybridization showed that they have temporally and spatially restricted expression patterns, and whole-genome RNAi screens have also been undertaken (in collaboration with Norbert Perrimon, Harvard Medical School, Boston, USA) to explore gene function.

## Vertebrate development and organogenesis

Sigolene Meilhac (Pasteur Institute, Paris, France) described an innovative study that used retrospective clonal analysis to look at cell lineage in the mouse heart. The method uses an inducible *lacZ* reporter gene targeted to the *cardiac actin* locus; spontaneous recombination, at low frequency, occasionally leads to loss of *lacZ* expression in some cells, and this property is inherited by all subsequent daughter cells. The results, which challenge the textbook 'segmental heart' model of cardiac morphogenesis, indicated the existence of two myocardial lineages: the primitive left ventricle and outflow tract are derived exclusively from one lineage, whereas all other regions of the heart are contributed to by both lineages. Detailed analysis of the shape of clones seen at later stages of heart development suggested that oriented cell growth of clones within certain regions of the heart is critical for the formation of normal cardiac morphology.

A session entitled simply 'Vertebrate development' was held in honor of the mammalian developmental biologist Chris Graham, with contributions from former colleagues and students. John Gurdon (Cambridge University, UK) described the use of *Xenopus* oocytes to investigate the factors and mechanisms that control reprogramming of the nucleus by the cytoplasm; he demonstrated that human and mouse blood-cell nuclei can be reprogrammed in *Xenopus* oocytes to express *Oct4*, a marker of stem cells. Talks from Elizabeth Robertson (Harvard University, Cambridge, USA), Andrew McMahon (Harvard University) and Frank Costantini (Columbia University, New York, USA) illustrated the power of mouse molecular genetic techniques to address a range of questions in developmental biology. Robertson has analyzed an allelic series of compound mouse *Smad2/Smad3* mutants, showing that signaling by Nodal (a member of the transforming growth factor β family) is responsible for anterior-posterior patterning at the primitive streak in a dose-dependent manner.

McMahon reported the phenotype of a mouse mutant in *Wnt9b*, an essential component of the inductive signals between epithelium and mesenchyme during early development of kidney tubules. In *Wnt9b*$^{-/-}$ kidney tubules, branching morphogenesis is disrupted and does not continue beyond the first T-branch. He also showed that the related gene *Wnt1* could rescue the *Wnt9b* phenotype, suggesting that *Wnt9b* signals via β-catenin, through the canonical Wnt pathway. Continuing the focus on the development of the kidney, Costantini explained the use of mouse lines expressing GFP together with organ culture to produce time-lapse movies of the repeated branching, growth and remodeling that characterizes formation of the epithelial ureteric buds of the kidneys. These methods have been used to study development of the ureteric bud in mice mutant for the Ret tyrosine kinase and in chimeras containing a mixture of wild-type and Ret mutant cells. Ret mutant cells were always absent from the growing tip of the ureteric bud and were present only in elongating tubule trunks. These results suggested that Ret is required for the proliferation of cells at the tip of the kidney tubule and for the formation of the specialized branched morphology of these cells but is not required for elongation of the tubule trunk.

A recurring theme throughout the meeting was that genomic sequence information from an increasing number of model organisms and a burgeoning set of bioinformatics tools are being harnessed to address fundamental biological questions. Comparative genome analysis can provide tantalizing clues about the regulation, organization and evolution of genes. The development and application of novel techniques are beginning to elucidate the details of gene function and regulation and to reveal the subtle differences between conserved genetic networks that can account for morphological diversity.