Meeting report

Developmental genomics reaches new heights

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A report on the EMBO/SNF symposium 'The Genomics of Development', Arolla, Switzerland, 21-27 August 2006.

The recent EMBO/SNF symposium in Switzerland brought together 100 scientists from different disciplines from around the world. The scope of the meeting was to discuss the use of new tools for answering old questions and the review of new techniques and methods from a wide variety of perspectives in the area of developmental genomics. Here, I focus on a few presentations that illustrate different approaches, such as phenotype-based forward genetic screens and microarrays, to the understanding of the functioning of genes and gene networks during development.

Forward genetic screening by mutagenesis

Almost four years have passed since the Mouse Genome Sequencing consortium published a high-quality draft sequence and analysis of the mouse genome. Nevertheless, the functions of an estimated 20,000 genes still remain elusive, and in such cases phenotype-based forward genetic screens can be used to assign function to mammalian genes.

Kathryn Anderson (Memorial Sloan-Kettering Cancer Center, New York, USA) described a genetic screen for recessive mutations that affect morphogenesis and patterning of the mouse embryo. This approach has led to the molecular identification of 22 novel genes. Anderson described the analysis by her group of the mutants *wimple* and *kahloista*, which reveal the importance of microtubule- and actin-based structures during early development. *Wimple* mutants exhibit an open brain, as a result of the incomplete closure of the neural tube, and the ventral cell types of the neural tube are absent due to a major defect in signaling via the secreted morphogen Sonic hedgehog (Shh), which is produced by the notochord and the floor plate of the neural tube. *Wimple* turns out to be a mutation in a mouse homolog of the *Chlamydomonas* intraflagellar protein IFT172, and affects the formation and function of cilia. *Wimple* mutants have no cilia in the node (the main embryonic organizer region) and cilia in the presomitic mesoderm, limb bud and neural progenitors are severely affected. Anderson's group has previously reported that cilia are rich in Shh signaling components. Their results strongly indicate that cilia may provide a microenvironment where signaling components concentrate and interact. *Kahloista* mutants have a deficiency in formation of the paraxial mesoderm (which gives rise to the vertebrae and skeletal muscles) and cell movement in the primitive streak (which represents gastrulation in avian, reptilian and mammalian embryogenesis) and the heart is severely affected. *Kahloista* is a single missense mutation in the *Nap1* gene, which encodes a component of a protein complex required for movement at the leading edge of migrating cells. Careful analysis established a genetic hierarchy that places Nap1 downstream of Rac1 in the specification of the structures of the mouse main body axis.

Otitis media is a chronic form of middle-ear inflammatory disease that is the most common cause of hearing impairment and surgery in children. Steve Brown (MRC Mammalian Genetics Unit, Harwell, UK) discussed a mutagenesis screen for mouse deafness mutants and the identification and analysis of two single-gene models of otitis media. *Junbo* is a mutation in the gene *Evi1*, which encodes a zinc-finger transcription factor previously implicated in myeloid leukemia. In the ear, Brown reported that this transcription factor binds to the signaling intermediate Smad3; this binding represses the transforming growth factor-β signaling pathway and is implicated in mucin production. The second mutant described, *Jeff*, carries a mutation in an F-box gene, *Fbox11*, which belongs to a large family of 74 proteins in the mouse. F-box proteins act as specificity factors for the ubiquitin ligase SCF. Interestingly, a study of 142 families in Minnesota with otitis media provides evidence of genetic involvement of the human homolog, *FBOX11*, in this condition.

Roland Dosch (University of Geneva, Switzerland) focused on germ-cell development and on a mutagenesis screen for

the identification of maternal factors that affect oocyte maturation in zebrafish. Germ cells are the first cells to be specified in zebrafish, and the existence of a germ plasm organelle in oocytes - the Balbiani body - has been known since 1845. RNA determinants of germ cells such as *vasa* are localized in the Balbiani body. The mutagenesis screen revealed 68 mutants, among which a significant number affect embryonic polarity. Dosch described an analysis of the *buckyball* mutant in which the morphological polarity of the oocyte is disrupted. The mutation is a premature stop codon in the *buckyball* gene, which encodes a protein that is evolutionarily conserved throughout the animal kingdom except in mouse and human but shows no domain similarity with other proteins. Dosch reported that *buckyball* mRNA is asymmetrically localized in the one-cell-stage fish embryo but is not exclusively present in the Balbiani body.

Forward genome-wide screens using RNA interference

Julie Ahringer (Gurdon Institute, University of Cambridge, UK) described the construction of an RNA interference (RNAi) feeding library that consists of 16,757 bacterial strains expressing a double-stranded RNA, each designed to silence a single predicted gene when fed to *Caenorhabditis elegans*. Ahringer's group identified 1,722 genes with a lethal or morphological phenotype, 1,200 of which are novel. In her talk, Ahringer focused on the use of the RNAi library to identify genes implicated in cell division and polarity in *C. elegans*. Following fertilization, the first cleavage is asymmetric, resulting in two unequally sized daughter cells. Asymmetric division is thought to be established by PAR polarity proteins that act to regulate phosphatidylinositol 4 phosphate 5 kinase type 1 (ppk-1), an asymmetrically localized enzyme. ppk-1 controls the levels of the gpr-1/2 protein complex, which activates G-protein signaling to affect spindle force. In this way, asymmetric localization of gpr-1/2 leads to asymmetric pull on the spindle and thus to asymmetric cell division. Another gene revealed from the RNAi screen is that for casein kinase 1 (csnk-1), a protein localized in the membrane. Knockdown of *csnk-1* causes the formation of an unstable spindle that is positioned with excessively violent movements, and a symmetrical rather than an asymmetrical first cleavage is observed. Using a combination of loss-of-function experiments, Ahringer's group placed csnk-1 in the pathway for spindle force regulation, downstream of the PAR proteins and upstream of ppk-1.

RNAi provides an effective loss-of-function approach for assaying gene function, but RNAi tools for the chick embryo have been less than perfect up to now, as they were adapted from vectors designed for mammalian cells. Nick Van Hateren (University of Sheffield, UK) first described a plasmid-based RNAi system specifically developed by his group for gene silencing in chick cells. They then used this system to focus on the developing neural tube. DNA microarray analysis identified 41 genes encoding cadherin domains in the neural tube. Van Hateren reported that a systematic knockdown allowed him to categorize cadherins on the basis of the phenotype generated. Knockdown of cadherin-encoding genes led to a decrease or an increase in the size of the neural tube, premature differentiation of electroporated cells (containing an RNAi construct to knockdown a cadherin gene) introduced into the mantle zone, and defects in neural tube closure.

Norbert Perrimon (Harvard Medical School, Boston, USA) commented on the organization of cellular networks in developmental signaling and stressed that, although there are only a few signaling pathways defined as canonical cassettes, there is a huge complexity of biological outcomes. A pathway cannot, therefore, be associated automatically with an outcome. Perrimon's group investigates networks of pathways using *Drosophila* cell-based genome-wide RNAi screens in order to identify core signaling pathway components that are characterized as 'canonical', receptor specific, or cell-type specific. RNAi screens are also an effective way to identify cross-talk between signaling pathways. In addition, they assign phenotypic signatures (known as phenoprints) to every gene of the fly. Phenoprints are used for clustering functionally related genes (a phenocluster) in a signaling network context. As an example, Perrimon described the results of a high-content screen for cytoskeletal signaling mediated by the small GTPases Rho and Rac that leads to cell shape changes; in this screen, cell images are automatically collected following transfection with an RNAi library.

Searching for new players in development using microarrays

Segmentation of the vertebrate body is established during embryogenesis by somitogenesis, the process in which blocks of mesoderm - the somites - are sequentially generated from the unsegmented presomitic mesoderm, in a periodic fashion. The underlying mechanism involves a Notch- and Wnt-dependent oscillator, the segmentation clock, that drives the dynamic expression of genes in the presomatic mesoderm. Monitoring the gene-expression periodicity in living embryos is important for drawing conclusions about somitogenesis. To this end, Olivier Pourquié (Stowers Institute for Medical Research, Kansas City, USA) described the generation of a mouse reporter line in which the yellow fluorescent protein Venus is under the control of the promoter of the gene *lunatic fringe*. Pourquié's group has developed a mouse tail culture technique to identify somite formation and has acquired high-quality movies of cyclic gene expression. They are currently developing mathematical tools for analyzing periodicity. To identify new cyclic genes, Pourquié's group chose a microarray approach, generating datasets from both chicken and mouse embryos,

and using special statistical algorithms and performing cross-species analysis. Their candidate cyclic genes belong to three major functional clusters, associated with Wnt, Notch and FGF (fibroblast growth factor) signaling. After validation of the results by *in situ* hybridization, 30 new cyclic genes were revealed. Nevertheless, there may be as many as 50 to 100 genes that constitute a large temporally controlled network. The core component or pacemaker of the molecular oscillator also remains to be definitively identified.

Shh plays a critical role in organizing the dorso-ventral patterning of the developing spinal cord, as noted earlier. It is also involved in limb patterning. Shh is expressed in the zone of polarizing activity in posterior limb-bud mesenchyme, forming a posterior-to-anterior gradient that is important for establishing digit patterning. Andrew McMahon (Harvard University, Cambridge, USA) described the generation of a transgenic line of mice with a Shh-GFP fusion gene, which is particularly useful for the visualization of Shh protein distribution and localization. High-resolution analysis using this transgenic line shows that Shh protein is co-localized along with γ-tubulin in cilia of the neural tube lumen. He emphasized the idea, mentioned also by Anderson, of microtubule-mediated trafficking of Shh protein. McMahon also discussed progress towards the identification of transcriptional targets of the Shh pathway. Using microarrays, his group has identified, among others, Cdo and Boc, two immunoglobulin domain-containing proteins. With the results of the microarray experiment they generated 'educated' genomic arrays that they used together with chromatin immunoprecipitation (ChiP-chip) to identify genomic regions upstream of several Shh target genes that are sufficient to function as enhancers in transgenic mice.

A recurring theme of the meeting was the assembly of gene networks that function as developmental cassettes, temporally and spatially coordinated with other networks for normal progression of the developmental program. Overall, the conference highlighted the main directions of developmental genomics research in the post-genomic era and it was apparent that by combining traditional genetics, new innovative approaches and advanced technology we can answer old questions in developmental biology.

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