Meeting report

Big science meets small embryos

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A meeting report from the Santa Cruz Developmental Biology Meeting, University of California at Santa Cruz, USA, July 21-25, 2000.

Recent advances in genomics and molecular biology have led to an explosive increase in the scale and speed of isolating and characterizing new genes involved in developmental processes. This was evident at the Santa Cruz Developmental Biology meeting. We focus here on the descriptions of two new model organisms being used for developmental studies, new results using various kinds of gene inactivation techniques and the impact of microarray techniques on developmental biology.

New model organisms

David Kingsley (Stanford University, USA) demonstrated in his talk that genome mapping projects will not necessarily bias research towards only a few developmental systems. His interest in the evolution of the vertebrate skeleton has led him to study the stickleback - a powerful system because it allows the study of evolutionary changes in morphology over a relatively short time period defined by geological events. A number of lakes in North America and Northern Europe were created after the end of the last ice age, approximately 10,000 years ago. Sticklebacks colonized many of these lakes, and the fish have subsequently evolved into a whole range of different morphological types in response to differing food sources, predators, and other ecological conditions present in the lakes. In the space of a year and a half, Kingsley's group have already sequenced 4 megabases of the stickleback genome and assembled several hundred microsatellite repeats and expressed sequence tags into a genome-wide linkage map. In contrast to classical models, in which evolutionary change is thought to involve small changes in many genes, the initial genetic mapping studies in sticklebacks suggest that many of the most dramatic morphological differences between the fish are controlled by a small number of major genes. This work shows that modern genomics techniques can be applied to new organisms relatively easily, and the speed in which Kingsley's lab have set this system up is impressive.

In the past few years, attention has moved towards tunicates as a model system. The advantages of these organisms for study include their small number of genes (like *Drosophila*, the tunicates studied have only one *hox* gene cluster and one *hedgehog* gene) and the ease of performing genetic manipulations on them (large numbers of transgenic animals can be generated simply by electroporation). William Smith (University of California, Santa Barbara, USA) described the use of the tunicate *Ciona savignyi* to study notochord development through genetic screens searching for notochord disruptions. The notochord is particularly prominent in *Ciona*, and the transparency of the organism makes it an ideal system in which to monitor the morphogenetic movements leading to extension of this structure. Two mutants that he described (*chongmague* and *chobi*) form a notochord that does not extend correctly; the genes that are defective in these mutants have not yet been identified.

Genomic and genetic approaches in traditional model organisms

RNA-mediated inhibition (RNAi) allows sequence-specific genetic interference through the introduction of double-stranded RNA (dsRNA). The technique is well established in nematodes and also works in *Drosophila* and mouse blastocysts, but it is proving more difficult to get it to work in other organisms. How RNAi interferes with transcription and is inherited by offspring was the focus of a screen carried out

in *Caenorhabditis elegans* by Craig Mello (University of Massachusetts, Worcester, USA). By screening for RNAi-deficient mutants he has isolated nine genes that are involved in this process, of which at least one, *rde1*, has homologs in both humans and *Drosophila*. Both *rde1* and *rde4* encode genes that are required for the formation of the inherited interfering agent, whereas *rde2* and *mut7* are required downstream in the RNA degradation process. Experimental RNAi appears to exploit a natural process called post-transcriptional gene silencing, in which an RNA trigger causes the post-transcriptional degradation of homologous cellular RNAs. Post-transcriptional gene silencing is also implicated in plants as an anti-viral mechanism. Mello described one possible model in which the double-stranded RNA that is introduced into the embryo is converted into small but long-lived pieces (perhaps analogous to the 23 nucleotides small RNAs detected in related silencing mechanisms in plant and *Drosophila*). These small pieces, complexed with specific proteins, might then mediate mRNA targeting and degradation, perhaps leading to a replenishment or amplification of the small RNAs in the process. Mello's work thus sheds light on this natural phenomenon as well as on experimental RNAi.

The grand scale of techniques available to developmental biologists was also demonstrated by Julie Ahringer (Wellcome/CRC Institute, Cambridge, UK), who has undertaken an RNAi analysis of chromosome 1 of *C. elegans*. Her group screened a library of the 2,500 predicted genes on Chromosome 1 by simply feeding bacteria expressing the relevant double-stranded RNA to the nematodes. They identified 339 genes that have an RNAi phenotype and characterized the overall spectrum of loss-of-function phenotypes of each. Interestingly, 70% of the genes that give a phenotype when inactivated by RNAi have sequence similarity to a gene in other organisms. By contrast, only 5% of genes with no known homolog have a phenotype. The phenotypes observed could be divided into groups: sterile (no embryo formed at all), embryonic-lethal, and post-embryonic defects. These correlated with functional classes and also, more surprisingly, with chromosomal location of the inhibited gene. For example, genes giving sterile phenotypes are enriched for protein metabolism genes, those giving embryonic-lethal phenotypes are enriched for genes involving RNA metabolism as well as those involved in the cell cycle and chromosome dynamics, and those giving post-embryonic phenotypes contained the highest number of unknown classes of genes. Genes giving sterile and embryonic-lethal phenotypes are evenly distrubuted along the chromosome, whereas those giving post-embryonic phenotypes are clustered at the more rapidly evolving arms. Descriptions of screens usually concentrate on just a few interesting phenotypes, so it was refreshing to see Ahringer's more systematic approach.

Gene inactivation was also the focus of much attention in other systems. The use of morpholinos appears to be making both zebrafish and *Xenopus* more tractable as genetic systems. Morpholinos are undegradable antisense oligonucleotides that function by blocking translation. Traditional antisense oligonucleotides interfere with gene function by mediating RNA degradation, but have frequently given inconsistent phenotypes. The use of morpholinos to study early developmental processes was demonstrated in *Xenopus laevis* by Janet Heasman (University of Minnesota, USA). In addition, it was apparent that more sophisticated and refined zebrafish mutant screens are revealing many new mutants. Didier Stainier (University of California, San Francisco, USA) has uncovered a surprising role for lysosphingolipid signaling in heart formation, through the identification of the gene responsible for the *miles apart* (*mil*) mutant phenotype. Mil turned out to be a sphingosine-1-phosphate (S1P) receptor required for the migration of cardiac mesoderm.

In the mouse, Gail Martin (University of California, San Francisco, USA) is elegantly combining the use of hypomorphic alleles and conditional null mutants of one or more fibroblast growth factor (*Fgf*) genes, taking advantage of the growing number of mouse lines carrying the Cre recombinase expressed from tissue-specific promoters. For example, conditionally removing *Fgf4* in the apical ectodermal ridge (AER) of the limb revealed that this molecule is not essential in the classic 'feedback loop model' for *sonic hedgehog (shh)* expression in the zone of polarizing activity, and hence not for limb outgrowth either. In contrast, a knockout of *Fgf8* in the AER reveals that it is required for normal limb development, and further suggests that there is a critical dosage requirement for the combined activites of Fgfs in the AER.

William Skarnes (University of California, Berkeley, USA) and Kathryn Anderson (Memorial Sloan Kettering Research Institute, USA) both described mouse mutant screens. Anderson's ethylnitrosourea-based screen was used to search for mutations that alter dorso-ventral patterning in the mouse neural tube. Skarnes used an elegant gene trap strategy that targets genes encoding secreted or membrane-spanning molecules. His 'secretory trap' vector produces an insertional mutation by creating a fusion with a *LacZ* gene including sequences encoding a transmembrane domain. If this construct inserts into genes encoding a signal sequence, the β-galactosidase protein inserts into the endoplasmic reticulum (ER) membrane with its catalytic domain in the cytosol, so the enzyme is active. Insertion into genes lacking a signal sequence leads to expression the other way round in the ER membrane, so the β-galactosidase is exposed to the lumen of the ER, where it is inactive. This screen has revealed a number of interesting molecules, including a protein related to low density lipoprotein receptors that is required for signaling by multiple Wnt proteins during embryonic development. The screen was further described by Marc Tessier-Lavigne (University of California at San Francisco, USA), as together with Skarnes he is using a

modified version to trap molecules involved in axon guidance. Addition of an alkaline phosphatase reporter gene to the construct enables the paths of axons in the mutants to be traced. Details of mutants isolated in this screen are available at the University of California Resource of Gene Trap Insertions [www.genetrap.org].

Expression arrays

Roel Nusse (Stanford University, USA) described the use of microarray technology to examine gene expression on a global scale. By performing a differential screen between human embryonic carcinoma cells incubated with either Wnt3a-conditioned medium or control medium, he was able to isolate genes regulated by the secreted signalling molecule Wnt3a. Of 24,000 genes screened, 50 were up-regulated and several were repressed. Among these were known target genes, and we await with interest the identity of the new targets.

In *Drosophila*, members of the Wnt family signal through both the canonical β-catenin pathway and also through a second pathway that is involved in planar polarity and uses Jun N-terminal kinase (JNK) signaling. Richard Harland (University of California, Berkeley, USA) presented evidence for signaling through this second Wnt pathway in vertebrates during the convergent extension movements occurring in gastrulation. He suggested that receptor specificity determined which pathway was activated. Nusse extended these observations by demonstrating the complexity of the Wnt signaling pathway: in *Drosophila*, in which the total number of *Wnt* genes is known to be seven, the different Wnt proteins bind with a complex pattern of affinities to five receptors, eliciting different biological responses.

Ira Herskowitz (University of California, San Francisco, USA) reported the use of expression microarrays to study the response of yeast cells to high osmolarity, with the broader aim of investigating how cross-talk between signaling pathways is restricted. He showed that high osmolarity induces expression of stress-response genes rather than only the expected set of genes. He also uncovered the possible existence of an osmoresponse pathway independent of a component that was thought to be central, Hog1 (a mitogen-activated protein kinase). A plausible model for restricting cross-talk presented by Herskowitz was that one pathway is activated so transiently that it has no time to activate any parallel pathways. These results show the power of microarray experiments in testing hypotheses as well as providing new data; Herskowitz stressed the importance of testing hypotheses and not being distracted by the wealth of new data these kinds of methods can produce.

It was clear from the meeting that the genome sequencing projects and new technologies for gene inactivation and large-scale expression analysis are changing developmental biology quite radically. An increasing number of organisms can be studied, and insights into developmental mechanisms can be gained much more quickly. The resulting rapid accumulation of information is allowing the dissection of increasingly complex genetic cascades of patterning mechanisms. With so much new information at our fingertips, the next few years will provide exciting times for us to reap the benefits.