Meeting report

Developments in developmental genomics

Holly A Field* and Kevin P White†

Addresses: *Department of Biochemistry and Biophysics, University of California San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143, USA. †Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA.

Correspondence: Kevin P White. E-mail: kevin.white@yale.edu

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A report on the 62nd Annual Meeting for the Society for Developmental Biology (SDB), Boston, USA, 30 July to 3 August 2003.

Developmental biologists from around the world converged on Boston for the SDB annual meeting to discuss topics that ranged from embryonic development in invertebrates to mammalian stem cells. Genomic approaches are driving many new discoveries in developmental biology, as was reflected in several plenary-session talks and a special workshop that featured genomic and proteomic applications. A few of the highlights are described here.

Fast forward with reverse genetics

The first few cell divisions in an organism's life are a complex affair. Mutational approaches in the embryo of *Caenorhabditis elegans* have identified genes involved in asymmetric cell division, coordinated centrosome movements and proper segregation of chromosomes. Many - in fact most - of the genes required for normal early embryonic development are only now being identified, however, thanks to high-throughput RNA interference (RNAi) screening. Fabio Piano (New York University, USA) described how large-scale functional genomics has been merged with careful developmental analysis of the events of early *C. elegans* embryogenesis. Piano, in parallel with other groups such as those of Julie Ahringer (The Wellcome Trust/Cancer Research UK Institute, Cambridge, UK) and Tony Hyman (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany), has been using RNAi combined with high-resolution embryo imaging to investigate the functions of predicted *C. elegans* genes. Piano presented an evaluation of his own and the other groups' datasets, comparing them by the RNAi delivery technique used: soaking worms in double-stranded RNA (dsRNA), feeding them bacteria expressing dsRNA, or injecting dsRNA directly. Each group scored embryos for a standard set of phenotypes. Although there was significant overlap in the genes identified, Piano found that a large proportion of genes identified by each screen were not found by the others, indicating that multiple iterations of RNAi screening will be necessary to approach truly comprehensive coverage of the genome.

Piano also described a novel method his group is using to organize the large amounts of data produced from the RNAi screens. Genes are clustered on the basis of a standardized vocabulary that describes the phenotypes observed from the screen; this reveals functionally related genes solely on the basis of the constellation of phenotypes they affect. For example, Piano and colleagues identified genes involved in DNA replication and repair that are associated with 'exaggerated asynchrony' of the early cell divisions that produce the four-cell embryo. This 'pheno-clustering' approach promises to be of great utility for interpreting data from the increasing number of RNAi screens being carried out in *C. elegans* and other model systems.

RNAi is also a screening tool of choice in *Drosophila*. Amy Kiger (Harvard Medical School, Boston, USA) presented her RNAi loss-of-function screen in *Drosophila* cells, in which double-stranded RNA transcribed *in vitro* is used directly in cultured S2 embryonic cells. Kiger has screened approximately 91% of the predicted genes in the *Drosophila* genome for their effects on cytoskeletal components that influence cell morphology and cell size, digitally recording the effects of each RNAi soaking treatment after fixing and staining the cells to visualize actin, tubulin and DNA. Kiger has detected RNAi phenotypes for over 400 genes in her screen. Some of these genes, such as *cdc42*, have alleles already known to cause cell-morphology defects in animals, but the majority (81%) of the genes she has found have not previously been studied in *Drosophila*.

Craig Hunter's group (Harvard University, Cambridge, USA) is investigating the mechanisms that produce systemic spread of RNAi throughout the organism. Through a cleverly designed screen, they have identified two transmembrane proteins that are needed for dsRNA to get into and out of cells. Hunter's group has tested the function of one of these *C. elegans* proteins, SID-1, in *Drosophila* cell culture, and he described how the efficiency of RNAi in *Drosophila* cells can be increased by several thousand-fold by supplying SID-1 expressed from a transgene; SID-1 appears to be required for cells to take up dsRNA efficiently. In contrast to *C. elegans*, systemic RNAi does not occur naturally in *Drosophila*, but if this method of expressing *C. elegans* SID-1 works in whole flies, it could prove a powerful addition to the fly-researcher's toolbox.

Another version of RNA-based silencing was the focus of a talk by Bonnie Bartel (Rice University, Houston, USA). Her group has cloned a number of potential microRNAs (miRNAs) from a pool of small endogenous RNAs in *Arabidopsis* by searching for sequences similar to those of known miRNAs that form the characteristic hairpin precursors processed by the enzyme Dicer. Using data mining of the sequenced *Arabidopsis* genome, Bartel's group has made predictions about the regulatory targets of miRNAs and their mechanism of action in development. For example, on the basis of perfect sequence complementarity, a single miRNA, miR171, is expected to regulate three genes that code for Scarecrow-like transcription factors. In a comparison of the *Arabidopsis* and rice genomic sequences, Bartel's group has also found that half of these miRNAs, as well as a number of the potential target sites, are absolutely conserved. Although Bartel did not present direct evidence for the function of these novel miRNAs during development, she mentioned previous work by Steven Jacobsen and colleagues (University of California, Los Angeles, USA), who have shown that disrupting the machinery used to process miRNAs does lead to developmental defects in *Arabidopsis*, such as cell proliferation in floral meristems. Determination of the specific developmental function of each of the predicted miRNAs is eagerly anticipated.

Morpholino oligonucleotides are another example of functional interference-based technology that takes advantage of the newly sequenced genomes. The classic use of morpholinos has been a reverse-genetic approach - to block translation of previously identified genes to monitor their role in development. Steve Ekker (University of Minnesota, Minneapolis, USA) presented a strategy in which morpholinos are used to screen for developmental regulatory genes in zebrafish. Morpholinos against zebrafish expressed sequence tags (ESTs) for secreted proteins with high similarity to mouse, *Fugu* and human ESTs were tested for their ability to induce developmental phenotypes. This strategy has identified new functions *in vivo* for both known and unknown genes. For example, Ekker described zebrafish 'morphants' in which the transmembrane heparan-sulfate proteoglycan Syndecan 2 has been targeted with a morpholino. These morphants have defects in angiogenic sprouting of blood vessels that can be rescued by expression of human Syndecan. Ekker described how a consortium of labs is taking advantage of the screen. For instance, Steve Farber (Thomas Jefferson University, Philadelphia, USA) is testing for genes involved in lipid metabolism, while Matthias Hammerschmidt's lab (Max Planck Institute for Immunobiology, Freiburg, Germany) is focusing on embryo morphology.

Proteomics in living cells

Developmental biologists like to see exactly where their favorite proteins are, but visualizing the location of each protein under study can require careful optimization of antibodies or green fluorescent protein (GFP)-tagged transgenes. Antibodies can only give limited information, however, as staining patterns in fixed tissue cannot reflect the dynamics of protein localization, and often, because of fixation artifacts, subcellular antibody staining patterns may not reflect the localization of the protein *in vivo*. For organisms without efficient homologous recombination (such as *Drosophila*, *C. elegans* and zebrafish), GFP tags cannot be specifically incorporated into the genome and GFP tagging has relied on transgenic approaches that can result in overexpression of a protein and can thus yield potentially unreliable results.

Ideally, one would like to do what has been done in yeast - tag each gene *in situ* with a GFP sequence and then visualize the protein as it is expressed from the gene's natural position in the genome. For a large proportion of the proteins in the *Drosophila* genome, Lynn Cooley (Yale University, New Haven, USA) may have found a way to do just this. She described how, in collaboration with Bill Chia (King's College, London, UK) and Allan Spradling (Carnegie Institution of Washington, Baltimore, USA), her group is using the well-established P-element system to mobilize a transposable element that contains a GFP open reading frame flanked by splice-acceptor and splice-donor sequences. Upon mobilization, this P element jumps semi-randomly throughout the genome, and when it lands in an intron (more than 75% of *Drosophila* genes have at least one intron) it has a one in six chance of making an in-frame fusion protein (one in three for being in the right frame and one in two for being in the right orientation). Because landing in an intron is rare enough in itself for a P element, correctly oriented intronic insertions only happen about once in 2,000 jumps. Thus, millions of embryos must be screened to get a reasonable proportion of the proteome represented. Cooley's solution is to assay 20 embryos per second for GFP fluorescence in a high-speed fluorescence-activated live-animal sorter. Cooley's group has already screened several million embryos this way, and has isolated more than 1,000 GFP-fusion insertions. They are following

up the primary screens with analysis of protein localization to cytoskeletal components of the *Drosophila* ovary, and several other groups have begun to screen the fly strains for protein localization patterns in other tissues. Cooley presented videos of the actin cytoskeleton that reveal dynamics and structure that had previously been undocumented using traditional fixation and antibody approaches.

Notably, most of the insertions do not affect normal protein function: several essential genes have been successfully trapped and made homozygous. This strategy promises to yield a valuable reagent for a large proportion of the more than 14,000 protein-coding genes in the *Drosophila* genome, and could potentially revolutionize cell biological studies of *Drosophila* development. Will zebrafish be next for this transposon-based approach?

Profiling embryonic stem cells

A great deal of research is currently directed to deciphering the signals that can induce stem cells to become differentiated cell types such as muscle, insulin-producing islet cells, skin or neurons. In a plenary session on recent progress in defining the molecular components of stem cells, Janet Rossant (University of Toronto, Canada) described transcriptional profiling results from mouse embryonic stem (ES) cells and trophoblast stem (TS) cells (the latter form the extraembryonic placental tissue). Gene-expression signatures from both ES and TS cells were identified, confirming many of the known markers for these cells and identifying novel genes, such as *Esg-1*, in ES cells. *Esg-1* appears to be associated with pluripotency, the ability to differentiate into any of the embryonic cell types derived from ES cells. In total, several hundred genes were newly identified as expressed in either ES or TS cell lines. Rossant also described the development by her group of powerful transgenic technology for RNAi in ES-derived embryos, which promises to allow the genes identified through the microarray experiments to be rapidly characterized.

In the same session, Nissim Benvenisty (Hebrew University, Jerusalem, Israel) described how human ES cells can be induced to produce embryoid bodies that contain differentiated tissue. His group has used microarrays to show that genes indicative of a large range of tissue types were induced in these embryoid bodies, depending on which signaling pathways were stimulated. This work could speed up the identification of the right combinations of stimuli required to produce desired tissue differentiation patterns for use in transplantation medicine. As Rossant's and Benvenisty's groups have used expression profiling in mice and human ES cells, respectively, it will be interesting, and hopefully informative, to compare these parallel datasets.

Developmental biologists are capitalizing on the rapid succession of whole-genome sequences that have become available for their model systems. Technologies that depend on knowing gene-sequence information, such as morpholino knock-down and RNAi, are now widespread. Mining the sequence data and the accumulating functional-genomic data is giving new understanding of previous discoveries, such as miRNAs, and driving the identification of novel molecules involved in a wide range of developmental processes. Old questions in developmental biology take on new dimensions, and new questions have appeared, as genomic approaches provide new impetus to this venerable field.