Meeting report

Elucidating developmental gene networks

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Published: 2 June 2008

Genome Biology 2008, **9**:310 (doi:10.1186/gb-2008-9-6-310)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/6/310

A report on the Joint Meeting of the British Societies for Cell and Developmental Biology, Warwick, UK, 31 March-3 April, 2008.

This year's annual meeting of the British Societies for Cell and Developmental Biology focused on several aspects of signaling mechanisms and gene and protein networks in relation to cell architecture, animal and plant development and evolution. Here, we summarize some highlights on morphogen gradients, mouse embryo genetic manipulations, stem-cell biology and evolution and gene networks during evolution and development.

Tracking morphogen gradients

One way of establishing positional information within the developing embryo is through the graded distribution of a morphogen, a molecule that has the ability to specify cell fate. Gradients of transforming growth factor-β (TGF-β) family members such as activin are involved in the establishment of the three germ layers (endoderm, mesoderm and ectoderm). Jim Smith (Wellcome Trust/Cancer Research UK Gurdon Institute, UK) described the work of his group to visualize the formation of a gradient of TGF-β signaling in gastrulating embryos. They used the technique of biomolecular fluorescence complementation (BiMC) to follow the formation of the heterodimer of the transcription factors Smad2 and Smad4, the outcome of TGF-β-type signaling. The amino-terminal half of the fluorescent protein Venus is fused to Smad2 and the carboxy-terminal half of the protein is fused to Smad4. On addition of activin and formation of the Smad2-Smad4 complex the two parts of Venus come together, generating a complete fluorescent protein. In cultured cells, the more activin is added, the more fluorescence is accumulated in the nucleus, showing that the assay can provide a quantitative analysis. Using BiFC in *Xenopus* and zebrafish embryos, Smith and colleagues were able to visualize the formation of a Smad signaling gradient *in vivo* and the spreading of the gradient over time. Their results provide evidence that such gradients are required for the correct dorso-ventral patterning of the early zebrafish embryo.

The protein Sonic hedgehog (Shh) is indispensable for normal development and mutations in components of its signaling pathway are also linked to cancer. Shh acts as a morphogen in a concentration-dependent manner and regulates the dorso-ventral patterning of the neural tube and the antero-posterior patterning of the vertebrate limb. David Martinelli (Johns Hopkins University, Baltimore, USA) presented results on Gas1, a 37 kD membrane-anchored protein present in all vertebrates. Studies in NIH 3T3 fibroblasts in culture showed that Gas1 enhances Shh signaling activity, and genetic evidence from mouse mutants showed that Gas1 facilitates Shh signaling in all developmental contexts examined, such as the neural tube, limbs and craniofacial area. Studies in chicken neural tube showed that Gas1 acts in a cell-autonomous manner to fine tune the Shh activity gradient, and that in the dorsal neural tube Gas1 expression is restricted via Shh signaling, thus providing a self-limiting mechanism of Shh signaling. The results support a model in which Gas1 acts cooperatively with the receptor of Shh, Patched1, to transform the Shh protein gradient into an activity gradient.

The Fox proteins are a family of highly conserved transcription factors that control a wide spectrum of biological processes during development. Catarina Cruz (National Institute for Medical Research, London, UK) found *Foxj1* in a microarray-based expression screen for Shh-regulated genes in the chick neural tube. Foxj1 expression is restricted to cells of the ventrally located floor plate in the developing neural tube, and Shh is both necessary and sufficient for expression. Foxj1 is associated with the generation of long, possibly motile, cilia on floor-plate cells, which are morphologically

distinct from the primary cilia found elsewhere in the neural tube. Overexpression of Foxj1 in non-floorplate cells was sufficient to induce cilia with floor-plate-like characteristics. Foxj1 decreases the responsiveness of neural cells to Shh signaling, and consistent with this, Foxj1 repressed genes that require high levels of Shh signaling for their expression, such as *Nkx2.2*. These results indicate that *Foxj1* is a Shh-regulated gene in the neural tube that induces changes in the ciliogenesis program of neural cells and may modulate how these cells respond to Shh.

Genetic intervention in signaling pathways

The enteric nervous system controls the contractility of the gut and is a vast network of neurons derived from neural crest cells (NCCs). NCCs migrating from vagal and sacral regions of the embryo colonize the whole length of the gut. Hirschsprung's disease is a congenital disorder affecting 1 in 5,000 newborns that is characterized by the absence or reduction of NCC-derived enteric ganglia in a variable portion of the distal gastrointestinal tract. Most familial cases are related to mutations in the receptor-type tyrosine kinase RET. RET has two major isoforms, RET9 and RET51. Vassilis Pachnis (National Institute for Medical Research, UK) reported work by his group to determine the roles of these isoforms *in vivo* by generating mice that expressed either RET9 or RET51 only. RET9-only mice were viable and normal, but mice lacking RET9 did not develop enteric ganglia in the colon, phenocopying Hirschsprung's disease. Interestingly, these mice showed additional defects in neuronal differentiation in the gut, which have not been reported before and might have implications for the future treatment of the disease. Pachnis also reported the isolation, culture and molecular characterization of enteric nervous system progenitor cells. When transplanted into mouse embryos *in utero*, these cells are able to survive, migrate and integrate into the enteric nervous system.

The ability of current conditional knock-out and knock-in strategies to define the temporal requirements for a protein's function during development are limited. To lift this constraint, Karen Liu (King's College London, UK) has engineered a widely applicable technology based on fusing the protein of interest to an 89 amino-acid tag called FRB* that confers instability. Stabilization, and thus restoration of protein level and activity, can be induced by addition of rapamycin, which enables the dimerization of FRB* with the cytoplasmic protein FKBP, a member of the immunophilin family of proteins that function as protein folding chaperones. She reported that mice with the rapamycin-dependent allele GSK3β$^{FRB^*/FRB^*}$ displayed defects identical to GSK3β-null mice, and that these mutant phenotypes could be rescued if rapamycin was applied in the critical time window in which the protein function was needed. The rapamycin-induced protein stabilization can be reversed by a competitive inhibitor. Liu also reported an application of the technique to control the nuclear export of transcription factors: the transcription factor is tagged with FRB* and expressed together with a fusion of FKBP and a nuclear export signal (FKBP-NES). Dimerization of the FRB*-tagged transcription factor and FKBP-NES, and thus the cellular localization of the transcription factor, can be tightly controlled by the experimenter by the addition of rapamycin or a competitive inhibitor.

Advances in mammalian, invertebrate and plant stem-cell systems

Within a population of embryonic stem (ES) cells cultured under seemingly homogenous conditions, the majority will differentiate into neural cells upon withdrawal of self-renewal stimuli; the remaining cells, however, will either not respond to the differentiation cues or will respond differently, creating non-neural, mesodermal progenitors. Successful application of ES cell-based technology critically depends on suppressing such diversity and precisely controlling lineage commitment. Sally Lowell (University of Edinburgh, UK) focused on efforts to understand the mechanism that underlies inappropriate non-neural differentiation of cultured ES cells. Previous experiments have shown that Notch signaling, a conserved intercellular pathway that mediates lateral inhibition among neighboring cells, biases pluripotent ES cells towards the neural lineage. Lowell presented evidence that Notch signaling exerts its effect via its target, Hes1, by amplifying in a cell-autonomous manner the LIF/STAT3 signaling pathway, which is known to maintain pluripotency and self-renewal of ES cells in culture. In line with this, she reported that both Hes1 and phosphorylated STAT3 show a variable expression pattern in ES cell cultures. She also showed that active Notch signaling increases STAT3 promoter activity, and that overexpression of its direct target Hes1 increases the proportion of ES cells with activated STAT3; in this way pluripotent ES cells could be specifically blocked from entering the non-neural, mesodermal lineage.

Amputated planarians show an amazing capacity to regenerate a perfect head or tail. Alejandro Sanchez Alvarado (University of Utah, Salt Lake City, USA) revealed β-catenin as a molecular switch for specifying and maintaining anterior-posterior polarity in planarians during both regeneration and tissue homeostasis. Decreasing Wnt signaling by RNA interference (RNAi) of β-catenin or Dishevelled (components of the Wnt signaling pathway) followed by simultaneous head and tail amputations resulted in the regeneration of two heads, whereas increasing Wnt signaling by RNAi of APC1 (which inhibits β-catenin activation) gave rise to animals with two tails. Remarkably, RNAi against β-catenin itself was sufficient to induce formation of ectopic head-like structures in unamputated animals. The regeneration potential in planarians resides in numerous stem cells, which are spread throughout the body and need to be tightly controlled for normal tissue homeostasis. Alvarado also showed that

depletion of the planarian homolog of the human tumor suppressor gene *PTEN* is lethal as a result of overproliferation of stem cells and the resulting formation of functionally disrupted tissues.

Ben Scheres (Utrecht University, the Netherlands) presented recent work on the positioning and specification of stem-cell centers during embryogenesis or regeneration in the plant *Arabidopsis thaliana*. In roots, the maximum response to the plant hormone auxin is in the stem-cell niche, whereas a low auxin concentration is observed in the transit-amplifying cells that derive from the stem cells and will go on to differentiate. Scheres reported that auxin induces four *PLETHORA* (*PLT*) genes that encode AP2-domain containing transcription factors essential for root stem-cell specification. The *PLT* genes regulate the expression of the PIN membrane proteins that are polarized on cells and facilitate polarized auxin transport in the root. During regeneration of the root stem-cell niche after laser ablation, PIN proteins continuously transport auxin into the area. The high levels of auxin drive neighboring cells to organize and regenerate the root stem-cell niche and growth of the plant continues. PIN-mediated auxin accumulation also positions the shoot organ primordia. Scheres also showed that shoot-specific *PLT* mutants affect shoot organ positioning, demonstrating that PLT and PIN regulatory loops are conserved and function in both roots and shoots.

Gene regulation and evolution

Binding of transcription factors to noncoding DNA sequences that act as *cis*-regulatory modules contributes to spatial and temporal regulation of gene expression during vertebrate development. Greg Elgar (Queen Mary, London, UK) combines *in vivo* functional assays, molecular biology and bioinformatics to explore the function of highly conserved noncoding elements (CNEs) in the vertebrate genome. He reported a comparison of the genome of the pufferfish *Fugu rubripes* with those of mammals that identified 1,400 CNEs that are likely to function as regulatory elements, many of them organized in clusters in the vicinity of genes encoding known developmental regulators. His group has developed functional *in vivo* assays to investigate the enhancer activity of these CNEs. Candidate CNEs were amplified by PCR and co-injected into zebrafish embryos with a green fluorescent protein (GFP) reporter construct under the control of a β-globin minimal promoter; the embryos were then screened at different developmental stages for GFP expression. Many of the CNEs tested were able to spatially and temporally up-regulate GFP expression, although their molecular mechanism of action remains elusive. The CNEs and the results of the *in vivo* assays can be accessed in the database CONDOR [http://condor.fugu.biology.qmul.ac.uk].

Sean Carroll (University of Wisconsin, Madison, USA) and colleagues want to understand the mechanisms contributing to the evolution of form. Extensive evidence shows that changes in animal form result from alterations in the spatial pattern of gene expression, but the way these changes have evolved is unclear. Carroll argued that evolution of *cis*-regulatory sequences is the major contributor to evolution of form. He pointed out that in contrast to alterations in coding sequences, effects of changes in modular *cis*-regulatory sequences are minimally pleiotropic, and illustrated this by considering the diversity of pigmentation in *Drosophila* species. For example, *Drosophila santomea* differs from *D. yakuba* in loss of abdominal pigmentation due to mutations in a *cis*-regulatory element controlling expression of the pigmentation gene *tan*. Similarly, a successive gain of *cis*-regulatory modules for both transcriptional repressors and activators has produced the pigment spot in the wing of *D. biarmipes*, a species closely related to *D. melanogaster* (which has no spot). These data demonstrate that changes in *cis*-regulatory sequences provide a mechanism to explain evolution of form.

Overall, the meeting highlighted many advances and approaches in different areas of cell and developmental biology, and we look forward to more.

Acknowledgements

We thank the speakers for comments on the manuscript and Cancer Research UK for providing us with funds towards the cost of attending the meeting.