Meeting report
Signaling in development
David Chambers

Address: MRC Centre for Developmental Neurobiology, King's College London, Guy's Campus, London, SE1 1UL, UK.
E-mail: david.2.chambers@kcl.ac.uk

A report on the 'Integration of Signaling Pathways in Development' Keystone Symposium, Keystone, Colorado, USA, 27 January to 1 February 2001.

It is a common theme in developmental biology that the fates of cells are often determined by interactions at the cell surface, where an incoming ligand meets its complementary receptor. In order to construct a complex organism, several different signaling systems and pathways are employed by the embryo. Scientists at this Keystone Symposium discussed some of the latest progress in the study of some of the major signaling pathways used in construction of the embryo. Several key questions ran through the Symposium, notably the following. How do multiple signals co-operate and converge to produce the correct fate? What are the molecular events controlling propagation of the signal from the cell surface and how can we go about finding new players in signal transduction pathways? Microarray analysis, sophisticated screens, and more conventional genetic and cellular techniques have given exciting new insights into these areas.

Coordinating multiple signals

The limbs arise from small outgrowths (the limb buds) of the body wall of an embryo. It has long been known that the apical ectodermal ridge (AER), a specialized epithelial region located at the distal tip of a limb bud, is critical for normal limb outgrowth. This is best demonstrated by the surgical removal of the AER, which causes a complete failure of limb outgrowth. It has been established that fibroblast growth factors (FGFs), ligands which are expressed in the AER, can ectopically substitute for its outgrowth-induction characteristics. Juan Carlos Izpisua Belmonte (Salk Institute, La Jolla, USA) shed light on how signaling by ligands of the Wnt and bone morphogenetic protein (BMP) families may cooperate to control FGF-dependent limb initiation in the chick embryo. This work demonstrates that Wnts 2b and 8c, which

are expressed in the mesenchymal tissues adjacent to the prospective AER of the forelimb and hindlimb, respectively, act upstream of the FGFs, via the adhesion-associated protein β-catenin, to initiate the signaling cascade required to direct limb outgrowth. Izpisua Belmonte provided preliminary new evidence for the role that BMPs play in this process, showing that a secreted modifier of the Wnt pathway, Dickkopf, is under control of BMP in the developing limb. In addition, Alan Michelson (Harvard Medical School, Boston, USA) showed how refined such studies can be, using the specification of muscle and cardiac progenitors in *Drosophila* as a model system. He presented evidence indicating that mesoderm-specific expression of the progenitor-identity transcription factor gene *even-skipped* (*Eve*) was a direct result of combinatorial and convergent signaling from many pathways on a single small enhancer region downstream of the *Eve* coding region. His group has shown that this enhancer integrates information from the Ras GTPase and the Wnt and BMP pathways and from two tissue-specific transcription factors, Twist and Tinman. Thus, these studies provided good examples of how developmental specificity is achieved by multiple signals acting either in concert or sequentially to restrict cell fates.

New concepts in limb development

The use of the developing limb as a model system to study signal transduction was a recurring theme in the Symposium. It was, therefore, quite exciting to see an established model of limb patterning challenged by several of the attendees as a result of such studies. A current model in limb formation is that the positional identity of a cell along the proximo-distal axis - that is, from shoulder to fingertip - is acquired according to the amount of time spent in the progress zone (PZ), an area located at the distal tip of the growing limb bud. Such a model dictates that cells at the distal tip of the limb are continually respecified as limb outgrowth proceeds. Lineage analysis and tissue transplantation studies of the PZ from the lab of Cliff Tabin (Harvard

Medical School, Boston, USA) suggested that the proximo-distal pattern of the limb is set up very early and that this is later elaborated upon by proliferation. For example, when cells at the limb bud tip (the PZ) were labeled with a lipophilic dye (DiI) at early stages, the dye remained in that cellular population and did not become distributed among various limb segments, as would be predicted by the old model. The PZ model was also questioned by Gail Martin (University of California, San Francisco, USA) who had used conditional gene inactivation mediated by the Cre recombinase to disrupt FGF4 and FGF8 signaling in the AER of developing limb buds. Such studies have demonstrated that the likely role of these signaling molecules is to promote the proliferation of a prespecified proximo-distal pattern. The new model has been called the 'early allocation and progenitor expansion' model.

Moving onto a different axis, John Fallon (University of Wisconsin, Madison, USA) presented experimental evidence to suggest that the antero-posterior identity of developing digits is controlled, at least in part, by signals from the interdigital mesoderm, and it is likely that this is mediated by BMPs. Tissue transplantation and replacement strategies, as well as interference with BMP signaling using the secreted antagonist Noggin, demonstrated that identity is not a fixed property of the digit primordium *per se*, but rather can be altered by shifting the interdigital mesoderm between digits at least until stage 29 in the chick. This affects the current model of antero-posterior digit specification by signaling of the lipid-linked ligand sonic hedgehog (Shh) from the zone of polarizing activity in the limb, and we'll have to await further data to fully integrate the two models.

Signaling at a distance?

Construction of an entire complex embryo relies on the use of signaling centers, dedicated regions that secrete signaling molecules that 'diffuse' to influence cell fates at a distance (greater than a few cell diameters). One of the central questions of developmental biology is, therefore, just how far can a signaling molecule go? Andrew McMahon (Harvard University, Cambridge, USA) addressed this question using the polarizing properties of Shh in the developing mouse limb as a model. Using an allele of Shh containing a targeted disruption of the cholesterol-addition motif, his group showed that the cholesterol modification of Shh was absolutely required for long-range signaling activities, whereas short-range signaling is independent of cholesterol modification. There was also the suggestion that cholesterol-mediated long-range signaling was an 'active' process, requiring more than just diffusion. Other data that McMahon presented using the same model of Shh movement led to the speculation that the amounts of a given receptor (in this case Patched) were critical in sequestering the ligand and thus limiting the amounts of ligand available for movement. Thus, we now have good evidence that molecules can signal

over a larger distance but also that we may have to look beyond diffusion as the sole mediator.

Microarrays and more

A main theme running through the conference was the number of novel genes that have been implicated in playing a role in all of the major signal pathways. Almost invariably, it seems, there has been a wealth of microarray experiments on downstream targets of signaling molecules. For example, Hans Clevers (University Hospital Utrecht, The Netherlands) presented data on the identification of downstream targets of the TCF transcription factor family (mediators of Wnt signaling). Using mouse microarray gene chips 'loaded' with 25,000 sequences, in conjunction with the mouse *Tcf-1* knockout, approximately 40 genes were shown to dependent on TCF for their expression. These ranged from known genes, such as c-Myb and c-Myc, to unidentified expressed sequence tags, whose role in the transduction of TCF-signaling is not yet clear. Considering the breadth of microarray data presented, two factors were apparent. Firstly, arrays that were constructed from oligonucleotides (as opposed to longer cDNAs) seemed to have the edge with respect to reproducibility and sensitivity, and secondly, the number of gene expression changes observed is often far larger than would be predicted from what was known about the pathway previously; genes that have previously been categorized as 'ubiquitous' or 'housekeeping' may in fact have roles to play in some stages of cellular differentiation.

With the arrival of the 'post-genomic era' and its coupling to microarray technologies, it is easy to forget that many other methodologies exist to identify new genes in a given biological system. Edgar Pera (University of California, Los Angeles, USA) presented in a poster the results of an extremely elegant screen for secreted molecules that may influence signaling events in development. Termed 'secretion cloning', it allowed the unbiased detection of proteins solely on the basis of their ability to be secreted by transfected cells. Using this methodology, four previously identified secreted antagonists of the Wnt signaling pathway - Frzb-1, Sizzled, Sfrp-2, and Crescent - were shown to have distinct effects on their Wnt targets in the gastrulating *Xenopus* embryo. This represents a common theme in developmental biology: the cocktail of 'negative regulatory' molecules expressed by a cellular field is equally as important as the positive effectors themselves. In addition to the known molecules, screening of a *Xenopus* gastrula-stage cDNA library also identified at least 17 novel *bone fide* secreted proteins. One of these, called 'Isthmin', shared many expression domains (for example, at the mid/hindbrain boundary or isthmus) with FGF8, a secreted polypeptide signaling molecule known to have numerous roles affecting cell fate and proliferative status during embryonic development. As with other work presented, however, defining the precise role of these molecules in established signaling events is less

than straightforward. Even with the wealth of genomic data available, it is often impossible to predict gene function from sequence similarities alone, especially if the novel protein is devoid of any conserved functional domains. This leaves us with an exciting wait for information on the function of these novel proteins, while new molecules are tinkered with in the same old genetic ways.

As a whole, it was evident at this Symposium that unraveling the intricate web of signaling events needed to piece together a complex organism has relied on the deployment of the latest biochemical and genetic tools. It is impossible to ignore the impact of new 'gene screening' technologies such as microarrays, but it was also clear that they are at their most powerful when used in conjunction with more 'traditional' approaches. There appears to be good evidence for the cooperation of several of the pathways, but their interactions at the molecular level have still to be elucidated. With the breadth of molecular and organismal tools at our disposal, however, it is certain progress in this area will soon be made.