Developmental diorama

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The field of developmental biology has expanded in recent years to cover a huge range of topics and ideologies, a journey that has taken it into the central well-spring of modern biological science. The field is no longer the domain of the individualist, interested in the pattern and growth of a few model systems and the genes that controls these events. Rather, the issues that so beguiled pioneering developmental biologists have now become crucial to the understanding of such disparate fields as cancer biology, cloning and stem cell totipotency. This situation has arisen because of the fact that the signal transduction pathways and genes that have been defined in model developmental systems have now been shown to regulate many aspects of biology, including those that directly impinge on the issues of human health.

This new 'broad church' of developmental biologists was certainly in evidence at this year's Spring meeting of the British Society for Developmental Biology (BSDB), at the University of Warwick, UK. The 3-day meeting held in April was divided into five, loosely themed, sessions with topics ranging from embryonic inductive mechanisms to developmental models of human disease. Although the topics covered were disparate in nature, a few central themes resonated through a number of sessions and are the focus of this review.

Inductive cues in specifying cell fate

The most obvious of the recurrent concepts to emerge from the meeting was the central role that similar inductive cues play across a variety of organisms and tissues in specifying cell fate. This emphasis almost certainly reflects the fact that one of the major recent advances of modern developmental biology has been the elucidation of many of the inductive cues that pattern plants and animals. Investigators have now turned their attention to the events downstream of these initial signals and how they are integrated to co-ordinate growth, pattern and cell fate. Judith Kimble (Howard Hughes Medical Institute, Madison, WI, USA), provided an example of this in her presentation of recent work from her laboratory, which focuses on inductive events that occur within the germline of the nematode, *Caenorhabditis elegans*. The gonad of the *C. elegans* hermaphrodite consists of two arms. A single migratory somatic cell termed the distal tip cell (DTC), which is positioned at the tip of each arm, acts as a specialised signalling centre to control the development and the shape of the gonad through a series of inductive events. Previous work has shown that this cell promotes the adjacent cells of the nascent germline to undergo mitosis through the use of the Notch signal transduction pathway, so preventing their entrance into meioses (reviewed by Kimble and White, 1981; Schedl, 1997). Surprisingly, the DTC appears to effect these decisions by regulating a series of different RNA-binding proteins, mutations of which alter the onset of mitosis and, consequently, germline proliferation. Two proteins, FBF-1 and FBF-2, which are related to the *Drosophila* Pumilo protein (similarly required to promote germline proliferation in *Drosophila*), stimulate mitosis (Crittenden et al., 2002), whereas the GLD proteins (1, 2 and 3) promote meiosis. FBF proteins appear to drive mitosis by directly binding to the 3′UTR of GLD1 to inhibit its translation. GLD-1 itself is known from recent work to bind mRNA encoding the *C. elegans* Notch receptor GLP-1, thereby also inhibiting the translation of this protein (Marin and Evans, 2003). Kimble proposes that it is via this interplay of different RNA-binding activities that proliferation and self-renewal of the germline are maintained appropriately.

The inductive functions of the Nodal-related factors of the TGFβ superfamily in the control of basic axis formation in vertebrate embryos were the focus of the presentations by Liz Robertson (Harvard University, Boston, MA, USA) and Alex Schier (Skirball Institute, NYU School of Medicine, New York, NY, USA). By manipulating the timing and position of *nodal* expression in the mouse embryo, Robertson and her co-workers have shown that the Nodal signal transduction pathway is required to control polarity in the early embryo (Brennan et al., 2001). This work showed that Nodal acts to promote posterior cell fates in the epiblast and to maintain pattern in the adjacent extra-embryonic ectoderm. Robertson described at the meeting how individual downstream effectors of Nodal signalling, such as the Smads, control the specification of these different fates. By generating conditional knockouts in the mouse of both *Smad2* and *Smad4*, this group have discovered that it is the combinatorial activity of different Smads that specifies different cell fates in the early mouse embryo. Alex Schier discussed recent data on

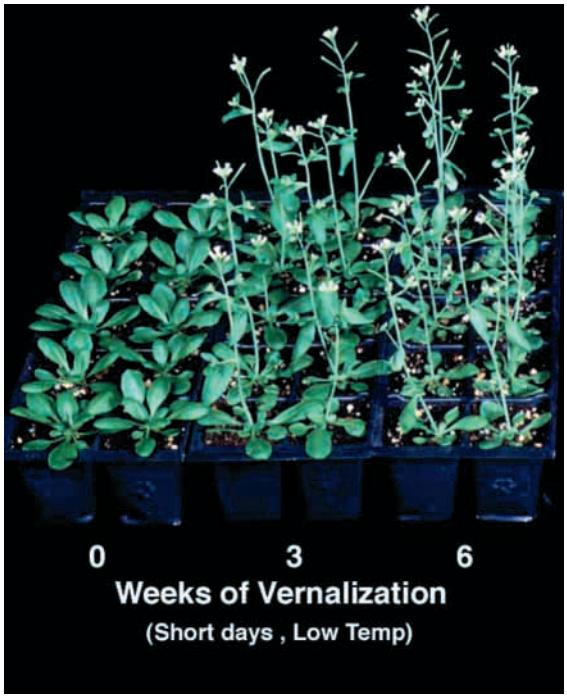

Fig. 1. Vernalization in plants. The cold induced flowering of plants, or vernalization, is an example of a developmental process that is regulated at the level of chromatin dynamics. Photo courtesy of Caroline Dean, John Innes Centre, Norwich, UK.

the inductive functions of two Nodal-related factors in zebrafish, Squint and Cyclops, in organising early axial development in zebrafish embryos. By transplanting cells into zebrafish embryos that have disrupted Nodal signalling, Schier has shown that Squint and Cyclops operate in zebrafish embryogenesis to specify cell fate via different mechanisms (Chen and Schier, 2001). Squint acts directly at long range and in a concentration-dependent manner to activate its target genes, whereas Cyclops acts only at short range. Furthermore, the range of signalling of Squint is attenuated by a secreted antagonist, Lefty (Chen and Schier, 2002), in a manner proposed to encapsulate elements of a classical reaction-diffusion model, which was postulated many years ago by Alan Turing to regulate patterning in fields of nascent cells (Turing, 1952).

Growth and pattern are also induced via external environmental stimuli, an example of which is the cold-induced flowering of plants. Caroline Dean (John Innes Centre, Norwich, UK) gave an intriguing talk that illustrated how the control of winter induced flowering, or 'vernalization', is an epigenetic process that regulates expression of the floral repressor gene, *FLC* (Fig. 1). The identification of mutant plants with a defective vernalization response (*vrn* mutants) has revealed that *FLC* transcription is controlled by proteins that show homology to the *Drosophila* Polycomb group proteins, which function in the control of chromatin structure in a wide variety of organisms (reviewed by Wagner, 2003). An examination of chromatin structure at the *FLC* locus within wild-type and *vrn* mutants revealed that vernalization results in histone methylation, and that chromatin-mediated silencing of *FLC* is lost in *vrn* mutants. Dean proposed a model whereby cold-induced factors initially repress FLC expression, and then chromatin regulators act to maintain the suppressed state of the *FLC* locus.

Transcriptional readouts of inductive cues

How specific transcription factors act downstream of particular inductive cues to engender cell fate was the general subject of a number of talks, several of which focused on the role of the basic helix-loop-helix (bHLH) transcription factors in controlling specific cell fates and the timing of their differentiation (Ryoichiro Kageyma, Kyoto University, Kyoto, Japan; Roger Patient, Nottingham University, Nottingham, UK; Andrew Jarman, University of Edinburgh, Edinburgh, UK). Andrew Jarman emphasised the central role that bHLH factors play in regulating 'node control points', where different differentiation pathways must be activated in response to individual inductive cues. He examined the paradigm of the *Drosophila* pro-neural bHLH transcription factors Achaete/Scute and Atonal, which control sensory organ formation in the developing fly (Fig. 2). The overexpression of these different pro-neural genes leads to the activation of different sensory cell differentiation programs. Achatete/Scute is responsible for the induction of sensory bristle formation, and Atonal controls the formation of chordotonal organs, the stretch receptors of the fly. The Jarman laboratory is interested in the difficult, but crucial, question of how closely related transcription factors impart two separate cell identities on nascent cells. To analyse this question, they have identified novel target genes for Atonal and are comparing how they are regulated with the known target genes of Achaete/Scute. Both the DNA-binding specificity of the proteins themselves, as well as their interactions with specific co-factors, appear to play crucial roles in the control of promoter activation.

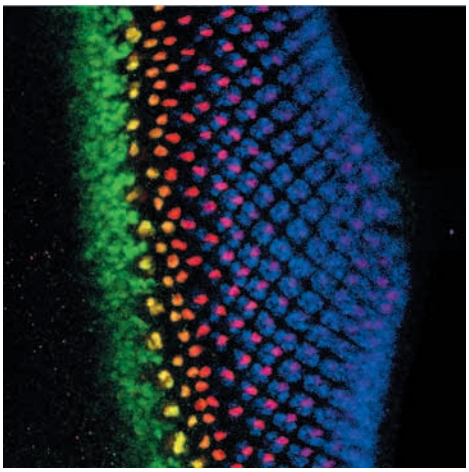

Fig. 2. Atonal expression in the morphogenetic furrow of the *Drosophila* eye. Atonal is initially expressed as a stripe (green). This expression pattern then becomes restricted to single, regularly spaced cells – the R8 founding photoreceptors of the eye. Atonal expression is shown in green, an R8 marker (Senseless) in red, and a photoreceptor marker (Elav) in blue. Photo courtesy of Emma Rawlings, The Jarman Laboratory, University of Edinburgh, UK.

Chromatin and development

The role of chromatin regulation in the control of developmental processes emerged again and again as a topic within different talks throughout the meeting. Nowhere was this more apparent than in the series of talks on nuclear and genomic reprogramming. The preferential localisation of Polycomb-like factors and other chromatin remodelling complexes to the mammalian female pro-nucleus was suggested by Azim Surami (Wellcome Trust/Cancer Research UK Institute of Cancer and Developmental Biology, Cambridge, UK) to be a crucial factor in the maintenance of pluripotency. He further suggested that the molecular differences between the environment of the female pro-nucleus of the egg and that of a somatic nucleus in terms of chromatin-associated factors and imprinted genes could underlie the low-level efficiency of nuclear transfer experiments. John Gurdon (Wellcome Trust/Cancer Research UK Institute of Cancer and Developmental Biology, Cambridge, UK) described how the serial transfer of a somatic nucleus can increase this efficiency dramatically, even when oocyte and nuclei are from different species, reinforcing again how oocyte-derived factors are crucial in controlling chromatin dynamics. Rod Scott (Bath University, Bath, UK) extended the analysis of genome programming to plants, where the balance of maternal and paternal genomes regulates endosperm or seed size. Scott discussed how the maternal genome actually inhibits endoderm size, promoting differentiation at the expense of proliferation. The imprinting of specific genes is thought to underlie the difference between paternal and maternal genomes, a specific imprinted target being the *medea* locus, which encodes a Polycomb-like factor, mutations of which result in a massive

over proliferation of endoderm. The use of related chromatin-associated factors in plant and animal cells again highlighted the surprising parallels between the epigenetic processes deployed to regulate both plant and mammalian genomes, an issue initially raised in Caroline Dean's talk on vernalization in plants.

Pluripotency, plasticity and stem cells

The examination of issues of cellular totipotency moved from the oocyte to the zygote in a talk from Austin Smith (Institute of Stem Cell Research, Edinburgh University, Edinburgh, UK). Mouse embryonic stem (ES) cells are pluripotent cells that are derived from the epiblast compartment of the pre-implantation embryo. The propagation of these cells is dependent on the provision of cytokines, such as leukaemia inhibitory factor (LIF). Essential roles have also been assigned to STAT3 and to the POU-domain-containing transcription factor OCT4 in the maintenance of ES cell pluripotency, although neither of these factors appears to possess the properties of a master controller of stem cell pluripotency. Smith outlined a clever series of, at the time, unpublished experiments that involved a functional cloning strategy using a line of ES cells that had been targeted for the LIF receptor. The transfection of cDNA libraries into these LIF-unresponsive ES cells resulted in the identification of a clone that encoded the transcription factor nanog, which was capable of inducing the clonal expansion of ES cells in a LIF-independent manner. Furthermore, nanog was found to be expressed in exactly the cells of the inner cell mass of the pre-implantation mouse embryo that exhibit totipotency (Chambers et al., 2003). These exciting findings point to a central role for nanog in the genetic hierarchy that defines ES cell identity.

Issues of cellular plasticity and pluripotency were also explored within the context of adult stem cells. Helen Blau (Stanford University School of Medicine, Stanford, CA, USA) spoke about recently published work from her laboratory that highlights the tremendous plasticity of bone marrow-derived stem cells and their ability to contribute to different tissues in both humans and mice (Weimann et al., 2003; LaBarge and Blau, 2002). A central issue for adult stem cell research is whether or not stem cells can give rise de novo to new cells or contribute to existing tissue by the formation of stable heterokaryons via cell fusion. By examining the cerebellar tissue of human females who had received bone marrow transplants from male donors, Blau provided perhaps the strongest evidence to date that cell fusion occurs in vivo and is a physiologically relevant process. Her findings show that male chromosomes derived from bone marrow are detectable in female Purkinje cells neurons in human brains. Furthermore, the frequency with which both female and male sex chromosomes were observed in a single neuron, as well as the finding of more than two sex chromosomes per Purkinje cell, suggest that the chromosomal composition of these cells may well be caused by the fusion of the bone marrow-derived cells with these particular neurons.

Organogenesis: redeployed signalling

Jonathan Slack (Bath University, Bath, UK) introduced a session on organogenesis and paralleled the events that lead to patterning of the early embryo with those that coordinate the patterning and growth of different organ rudiments. Indeed, in his talk and the talks that followed, it became clear that many embryonic signal transduction pathways are redeployed during organogenesis to specify and to pattern individual organs. It was also clear from these talks that the study of the induction, patterning and growth control of organ systems is a fast growing and important area of future developmental biological research. Slack concentrated on the genes that specify pancreas formation and investigated the ability of the known pancreas-specifying transcription factor Pdx1 to re-specify liver cells into pancreatic tissue. By overexpressing a constitutively active form of *Pdx1* both in *Xenopus* tadpoles and in human hepatoma cells, Slack showed convincingly that pancreatic differentiation could be induced in liver cells. Ken Zaret (Fox Chase Cancer Centre, Philadelphia, PA, USA) also focussed on pancreatic development, concentrating on the issue of how the pancreas is induced in the early embryo. He reviewed the role of fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signalling in pancreas induction, and highlighted the role of the vasculature in specifying pancreatic bud development. By using knockout mice that lack blood vessels, the Zaret laboratory has been able to show that, yet to be defined, signals from the aorta are responsible for specifically inducing the formation of the dorsal pancreatic bud. Kidney development and the role of the Wilm's tumour (*WT1*) gene was the focus of talks from Nick Hastie (MRC Human Genetics Unit, Edinburgh, UK) and Jamie Davis (University of Edinburgh, Edinburgh, UK). *WT1* mutations can lead to childhood kidney tumours and to developmental defects of the kidney and gonad, and homozygous *Wt1* knockout mice fail at the earliest steps of kidney development. Nick Hastie discussed recent work on defining the molecular basis of WT1 function, revealing its role as both a transcription factor and RNA-binding protein, echoing the earlier talk of Judith Kimble, which highlighted the role of RNA binding proteins in controlling certain aspects of organogenesis in *C. elegans*. Jamie Davis explored the use of RNAi in mouse kidney organ culture to examine the later aspects of WT1 function, which are obscured by the severe and early defects in kidney formation in *Wt1*-null mice. Results from his laboratory convincingly show that RNAi-mediated knockdown of the *WT1* transcript can be achieved in both cell and organ culture. By knocking down *Wt1* in mouse kidney cultures Davis' group have discovered that that WT1 might have a role in regulating cell proliferation later on in kidney development.

Conclusions

More philosophical, but no less weighty issues, were discussed by the invited plenary speaker, Henry Sun (NYU School of Medicine, New York, NY, USA), who gave a lecture entitled 'How much can you trust your PhD supervisor'. As the talk wound to its inevitable conclusion that little trust could, or perhaps more correctly should, be invested in supervisors by students, group leaders were seen to hang their heads, and younger members of the audience were heard to mutter phrases, such as 'I told you so'. Luckily Professor Sun was proscriptive in providing us with an alternative approach to the management of student-supervisor relationships that left us all with much food for thought.

Overall the scientific content of the meeting served as a reminder of the generality of mechanisms that can often emerge from disparate avenues of analysis. The validity of

studies in genetic and embryologically tractable model organisms in providing such consensus was again reinforced throughout the course of the meeting. Although transposed onto many different cells, organs and even human disease states, a central issue appears to remain at the forefront of developmental biological research: exactly how are the fates of nascent cells engendered in response to defined inductive stimuli?

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