SPRING MEETING

of the British Societies for Developmental and Cell Biology University of York, 20-23 March 2006

BSDB

BSCB

BSCB (Stem Cells)

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Roger PATIENT (UK)
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Cheryl TICKLE (UK)
David TOSH (UK)
Jerry TURNBULL (UK)
Tanya WHITFIELD (UK)

Early registration and abstract deadline: 20th January 2006.
BSDB travel grant deadline: 9th December 2005.
BSCB Honor Fell travel awards available on application.
For meeting registration and infomation visit: www.bscb.org *or* www.bsdb.org

BSDB/BSCB Annual Spring Symposium

York, 2006

The following are reports of selected sessions at the recent Spring Symposium.

Developmental Signals

Can you imagine development without its inductive or permissive signals? I do not know if you are of the permissive or inductive nature, but sessions like the one I was induced to report on have clear fate outcomes and maintain the BSDB Spring meeting as a high point of every developmental biologist's calendar.

What a great kick-off by **Richard Harland** (Berkeley, USA), giving us a taste of the diploid frog *Xenopus tropicalis*. Its diploidy permitted the dissection of BMP antagonism and established its importance from early organizer stages in the formation of dorsal structures, which had not been yet possible to establish in other model systems. Switching gears, Richard went on to illustrate how the same signalling molecule can have opposite roles in both mesoderm formation and neural induction. FGF8 turns out to have two spliceforms (FGF8a and FGF8b) that differ in an eleven amino acid deletion. FGF8b is a potent mesodermal inducer and has little effect in neural development, while FGF8a promotes the development of neural structures and ectopic neurons.

We had then the opportunity to listen to this year's winner of the prestigious Beddington Medal. **Marc Amoyel** (London) told us about his work on the role of Wnt1 in the establishment of boundary cells in zebrafish, which in turn serve as a signalling centre regulating neuronal differentiation in the hindbrain through the expression of proneural genes. He then went on to draw some similarities with the establishment of the well-studied dorsoventral boundary in the *Drosophila* wing imaginal disc.

In a field that is flooded of animal models it was very nice to listen to two exceptional studies in plants, from **Ottoline Leyser** (York, UK) and **Enrico Coen** (see Evolution and Development session). In conceptual terms Ottoline's talk could tell us a lot about mechanisms that can account for long range signalling yet to be explored in animals. She described the MAX pathway of auxin signalling in *Arabidopsis*, where auxin long range signalling is responsible for the control of shoot branching. We learned of a mechanism in which limited capacity of signal transport from its source was important for the phenotypic outcome of the signal.

The second half of the afternoon brought Wnt signalling into the play, first in the mammalian urogenital system by **Andrew McMahon** (Cambridge, USA) and then by **David Strutt** (Sheffield, UK) using the fly. McMahon focused on the role of canonical Wnt signalling during several epithelial to mesenchyme transitions occurring in the development of the urogenital system. As Wnt9b$^{-/-}$ mutant mice do not develop a kidney, McMahon showed us that Wnt9b is both necessary and sufficient to promote pronephric and metanephric development. Moreover, Wnt9b acts upstream of Wnt4 and is a common denominator in regulating epithelium invasion of the mesenchyme at different times throughout the organogenesis of the urogenital system

Strutt started with an overview of the non-canonical Wnt signalling pathway (e.g., *frizzled, strabismus, prickled, dishevelled*) in the well-characterized *Drosophila* wing imaginal disc. He then presented us a study on the role of such signalling molecules in migratory cells and an example of how the establishment of polarity is important for migration. In the Drosophila oocyte, border cells migrate as a cluster with two central cells on top of the cluster. These top two cells establish their polarity by the use of the non-canonical Wnt pathway and in turn are required to guide border cell migration.

Ricardo Costa, Wellcome Trust/Cancer Research UK Gurdon Institute, r.costa@gurdon.cam.ac.uk

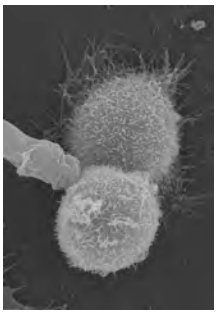
Haematopoietic Stem Cells

The HSC session kicked off with **Sten Erik Jacobsen** presenting an alternate model for haematopoietic stem cell and blood cell lineage commitment. He presented compelling data indicating that the earliest lineage commitment of the pluripotent HSC does not result in the strict separation into a common lymphoid and common myeloid progenitor as previously thought but that the LTR HSC, defined as a KSL Flt3$^{-/low}$ cell, divides asymmetrically to give rise to a megakaryocyte/erythroid (Mk and E) progenitor and a LSK CD34+Flt3 $^{+}$ HSC which maintain G, M, B and T cell potential with little or no Mk and E potential. This model fits well with the kinetics of blood lineage development in which myeloid lineage commitment precedes lymphoid in evolution, ontogeny and in transplantation experiments and also with recent HSC multi-lineage priming data.

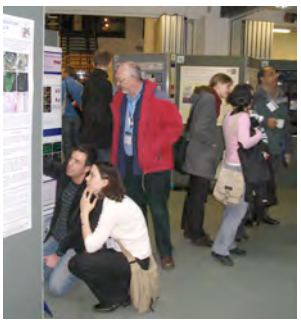
The next talk focused specifically on the role of the transcription factor c-myb in HSC development. In the c-myb knockdown there is a decrease in the total number of bone marrow cells but no change in the number of HSCs (KSL). However on closer analysis it appears that there is a reduction in the side population of HSCs, quiescent HSCs and cobblestone forming cells indicating that there is a loss of the most primitive HSCs and a phenotypic shift towards the more mature cells in the stem cell compartment. The dynamics of this process support Sten Erik's alternative model for HSC development.

The next talk moved away from mammalian systems to show how the Glia cells missing related proteins (Gcm and Gcm2) and the Runx homologue, Lozenge, function in the differention of bi-potential embryonic blood cell progenitors (prohemocytes) in *Drosophila*. The analysis suggests that the ancestral cell type produced by the prohemocyte appears to be the plasmatocyte, with expression of Lozenge promoting an alternative crystal cell fate.

"Despite the great interest and excitement that surrounds embryonic stem cells, it is surprising that we know relatively little about the mechanisms that regulate their earliest fate choices."



Scanning electron microscope image of extraembryonic endoderm (Xen) cells (Courtesy Tilo Kunath: see Kunath et al Development 132 (7))



Ana Cumano returned to the mammalian system presenting an overview of haematopoiesis in the mouse embryo, suggesting that the sub-aortic patches found ventral to the dorsal aorta may not only play a role in HSC emergence but may also be a source of HSC themselves. **Roger Patient** took us back down the evolutionary ladder to show how data from *Xenopus* and zebrafish have contributed to our understanding the origins of haematopoiesis and haematopoietic ontogeny. These model systems allow us to dissect precisely the requirement for specific transcription factors or signalling molecules during haematopoiesis using small chemical inhibitors or morpholinos. Hedgehog signalling was shown to be required for definitive but not primitive haematopoiesis and the transcription factor SCL was shown to have a critical role in the dorsal aorta formation in zebrafish. The role of Runx-1 and Tel1 in *Xenopus* were shown to be important in programming in the dorsal aorta prior to stem cell emergence in *Xenopus*.

The session was closed by **Utpal Banerjee,** who presented some beautiful data showing that *Drosophila* haematopoiesis shares a number of features with that of higher vertebrates. Haematopoiesis takes place in two distinct waves: the first primitive-like wave occurs in the head mesoderm while the second phase takes place in the larval lymph gland and provides the pupa with the haematopoietic cells it requires to progress into adulthood. He suggested that the lymph gland contains a haemangioblast-like cell, a prohemocyte niche and quiescent progenitor cells which differentiate in a manner that resemble the temporal and spatial emergence of haemtopoetic cells in higher vertebrates.

Claire Fernandez, Weatherall Institute of Molecular Medicine, Oxford

Mouse ES cells and embryo germ layer specification

Despite the great interest and excitement that surrounds embryonic stem cells, it is surprising that we know relatively little about the mechanisms that regulate their earliest fate choices. Although there has been some success at generating differentiated cells from ES cells, the intermediate steps along the way are, as pointed out by **Gordon Keller**, simply a "black box". Both Keller and **Shinichi Nishikawa** are finally opening up this black box by developing reporter ES cell lines in which either fluorescent markers or cell surface tags are expressed under the control of the regulatory sequences of genes that mark early mesendoderm or endoderm populations.

Nishikawa is exploiting these tools to search the genes that regulate lineage choice. After isolating the early specified subpopulations, he subjects them to microarray analysis and then uses bespoke bioinformatic tools to spot candidate regulators according to their characteristic expression profiles. These candidates are then tested using retrovirally-delivered shRNA. Both Keller and Nishikawa's groups have identified activin as a key inducer of endoderm differentiation, Keller further showed that activin acts in a dose-dependent manner together with Wnt to induce different mesendoderm and endoderm populations.

ES cells are often though of as a source of cells for drug screening or cell replacement therapies, but Keller and Nishikawa emphasized that these cells also give an insight into how early fate decisions are regulated *in vivo*. Work from **Liz Robertson** confirmed this assertion when she told us how nodal, (a molecule closely related to activin, and with similar activity) acts in a dose dependent manner as a pivotal regulator of germ layer specification in mouse embryos.

Janet Rossant is interested even earlier fate decisions: the choice of whether to contribute to the embryo itself or to one to one of the two extraembryonic lineages, trophectoderm or extraembryonic endoderm. Representative cell lines for both trophectoderm (TS cells) and extraembryonic endoderm (Xen cells) have been generated in her lab, whilst ES cells represent stem cells of the embryonic lineage. Remarkably, it seems that by manipulating a few key intrinsic determinants, Cdx2, GATA6, Oct4 or Nanog, it is possible to switch between the three lineages both in the cell lines and in the embryo.

Rossant used these key genes as markers to ask how lineages become segregated in vivo. GATA6+ cells, assumed to generate primitive endoderm, are initially intermingled amongst the cells of the inner cell mass, suggesting that they are pre-specified independently of their position and later segregate out to form a coherent tissue. In contrast, a different type of mechanism governs the even earlier decision to become trophectoderm, which is marked by Cdx2. The Cdx2+ cells appear to become segregated to the outermost cells of the embryo by polarised cell division, which suggests that this very first fate decision depends on simply being in the right place at the right time.

Sally Lowell, *Institute for Stem Cell Research, University of Edinburgh,*

Developmental Biology solving human disorders

This session was chaired by **Didier Stainier** (UCSF), who also made the first presentation. He addressed the developmental mechanisms of hepatopancreatic system. Didier suggested that foregut endodermal cells received the signal from adjacent cardiac mesoderm for the patterning of hepatopancreatic ducts and organs. *Fgf10* mutants showed mis-differentiation of the proximal pancreas into hepatic cells and liver to pancreatic cells, indicating the multi-potency of foregut cells, which highlights the novel role of Fgf10 in the development of hepatopancreatic system.

It is always encouraging to see fresh talent amongst the more "experienced" scientists within such venues. In this session, the next two presentations were from postgraduate students. **Rees** (University of Bristol) presented work on colorectal cancer. He showed the synergic interaction of Wnt and Shh signalling based on the fact that cyclopamine (Shh inhibitor) decreased the transcription activity of □eta-catenin/TCF while increasing E- cadherin expression, which may contribute to tumour invasion. **Clement** (University of Sheffield), addressed the questions underlining the role of the proteoglycans in skeletal development using zebrafish as model organism. She proposed the *dackel, boxer* and *pinscher* as candidate genes for the exostoses since they affect the stacking of chondrocytes in cartilage and their subsequent ossification of cartilage. Future work will help to elucidate the possible mechanisms of hereditary multiple exostoses – the most common musculoskeletal disorder.

Continuing the theme of using zebrafish as an animal model for the solving human disorders, **Tanya Whitfield** (University of Sheffield) presented interesting work relating to the causes of human deafness. She was able to demonstrate how deafness is linked to physiological irregularity, using homozygous vgo/tbx1 mutant embryos. Transcription factors eya, tbx, otx, and Shh signalling were shown to be responsible for morphological defects contributing to human deafness.

Following a brief, and well-deserved break for refreshments, **David Tosh** (University of Bath) presented work concerning endoderm development. He proposed the use of the transdifferentation pathway as a suitable approach to elucidate the mechanism via which developmental genes are identified and their role investigated. His group has developed powerful model for the transformation the pancreas to liver and *vice versa*, which will help in the understanding of the inter-changeability between these phenotypes.

The concluding presentation was made by **Ethan Bier** (University of California). He addressed the use of *Drosophila* as a powerful tool for prediction of the evolutionary conserved genes responsible for various human diseases, as well as the integration of different disciplines such as genetics, bacterial toxicology and developmental biology, in targeting these genes.

Romana Kucerova, University of Aberdeen

Heparan sulphate proteoglycans and development

In this session, the chair **Charles Emerson** gave a thorough and clear introduction to the topic. He introduced us to the different classes of HSPGs, and to the enzymes involved in their synthesis and modification. He then went on to describe the work of his lab on the *sulf* genes and the 6-O-endosulphatases they encode. The wide-ranging defects found in the *sulf1/2* double knockout mice, together with experiments using mouse embryonic fibroblast and satellite cell cultures, allowed him to present models of how the levels of HSPG sulphation (decreased by the sulphatases) are important for regulating FGF signalling (increased sulphation = increased FGF signalling).

This was followed by two short talks chosen from the abstract submission. First, **Steve Freeman** from Betsy Pownall's lab discussed the role of *Xenopus tropicalis* Sulf1 in inhibition of FGF and BMP signalling. Second, **Sally Stringer** showed us how the sulphation levels of HSPGs are important for regulating binding to VEGF. She has been looking in zebrafish at 6-O-sulphotransferases as well as sulphatases (i.e. the effects of adding as well as removing 6-O-sulphate groups), specifically focussing on the process of angiogenesis, which requires VEGF signalling.

After a quick tea break we heard from **Scott Selleck**, who gave an excellent talk on his work using the neuromuscular junction in *Drosophila* as a model for the cellular function of HSPGs. He showed how mutants in HSPG synthesis and sulphation have defective membrane dynamics and how by electrophysiological recording he could pin these defects onto problems with the reserve pool of neurotransmitter vesicles. He bravely showed us his newest data suggesting that HSPGs are important for mitochondrial distribution on the muscle side of the synapse.

Isabel Guerrero showed beautiful pictures of her *Drosophila* wing disc clonal analysis. She is investigating the roles of lipid modification of Hh, and of the HSPG synthesis and sulphation genes, in the movement of Hh and activation of subsets of target genes with different thresholds. She introduced the new player *shifted*, which encodes an orthologue of human Wnt inhibitory factor and in flies seems to be involved in restricting movement of Hh.

Finally, we had a double-act from **Jerry Turnbull** and **Tarja Kinnunen**. Turnbull described the changing structures of heparan sulphate during mouse neural development and showed the complexity of sulfotransferase interdependency. Kinnunen uses *C. elegans* to investigate the roles of HSPGs in neuron migration.

Catherine Moore, Centre for Developmental and Biomedical Genetics, University of Sheffield

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Imaging and temporal understanding of development

The ability to analyse cell movements *in vivo* is fundamental to our understanding of embryonic development, and this session contained talks from four laboratories making use of advanced imaging techniques to address developmental questions. The session was chaired by **Richard Adams** (University of Cambridge, U.K.), whose is studying the development of the zebrafish forebrain. Extensive cell rearrangements occur during neurulation, with the neural tube forming from a flat sheet of cells and two eyes arising from a single cyclopic eye field. Richard described the use of 3D confocal imaging and computational analysis to track the movements of hundreds of cells simultaneously and develop a model of forebrain folding during neurulation.

Dan Kiehart (Duke University, U.S.A.) is using *Drosophila* dorsal closure as a model for epithelial sheet morphogenesis. The balance of forces from the actin-rich leading edge and the tension in the lateral epidermis leads to a gradual closure of the epidermis; surgical ablations reveal that each of these forces is orders of magnitude greater than the net force required for closure. The regulation of this balance of opposing forces seems to be a key part of closure, and the embryo is able to adjust these to compensate for a variety of surgical and genetic manipulations.

Kat Hadjantonakis (Sloan-Kettering Institute, U.S.A.) described the use of multiple spectrally-distinct fluorescent proteins to simultaneously image multiple cell types – and cellular compartments – within the living embryo. In addition to the mouse, Kat is making use of *Ciona*, at the other end of the chordate clade, to investigate the morphogenesis of the paraxial mesoderm.

The session concluded with a talk from **Scott Fraser** (California Institute of Technology, U.S.A.), who has been making use of confocal imaging to rapidly image fluorescently labelled cells in the zebrafish heart. Computational analysis is able to compensate for movements during the scanning process, building up 4D renderings of the beating heart and providing new insights into the process of heart development.

Paul Overton, MRC Developmental Neurobiology, London

Evolution and Development

Peter Holland – as both chairman and speaker – opened on a philosophical stance with the notion that the session was an opportunity to take stock of the field. He then went on to discuss his work on gene (cluster) evolution for Hox and ParaHox genes, arguing that the selective pressures maintaining clusters or permitting their break-up differ for different gene families. **Harv Isaacs** continued these lines of enquiry, focusing on the ParaHox genes in the frog *Xenopus tropicalis*. Through a combination of genomic organization and morpholino knockdown data, he concluded that what had been intra-cluster regulatory interactions in a Proto-Hox cluster have been retained, but are now manifest as inter-cluster interactions following cluster duplication and fragmentation.

Changing emphasis to cell lineages, **Andrea Pasini** described the development of the peripheral nervous system in the invertebrate chordate *Ciona intestinalis*. Fate mapping in a system with low cell number is combined with functional tests of whether inductive signaling pathways elucidated in vertebrates are conserved in this outgroup. **Nipam Patel** also presented early lineage and fate map work in a marine animal – the crustacean *Parhyale hawaiensis* – this time where the established point of comparison is segmentation in the fruit fly. One intriguing point is which germ layer(s) are involved in the segmentation process, and how these layers interact.

Comparison over a finer time-scale was explored in **Pat Simpson**'s discussion of proneural gene regulation in different *Drosophila* species. Her group's empirical work delves into nuances of enhancer function and evolution in the context of the *achaete-scute* genes and thoracic bristle patterning. Concluding the session, **Enrico Coen**'s exploration of flowering architecture and flower color was another instance of microevolutionary comparisons. However, empirical data were not the emphasis, but rather the starting point for modeling of adaptive landscapes. In graphically depicting three-dimensional phenotypic spaces, he was able to convey strikingly why some spaces, which in two-dimensional representations seem very different or even contradictory, are easily occupied. Here science met science fiction, as the audience was exhorted to think in higher dimensions, where there are connections via "evolutionary wormholes."

That is a fair consideration in taking stock of Evo-Devo research. Comparisons reveal both similarities and differences. The early work uncovered the similarities – surprising levels of conservation over vast evolutionary distances. Once conservation was established, descriptive work on differences afforded scope for speculation in this historically minded discipline. As Evo-Devo matures into adolescence, the tool kit for comparative work has been expanded and honed, and the biological scope is broad. As researchers take diverging paths of investigation, meeting sessions such as this one provide a forum for insightful discourse that makes higher-level connections. This will maintain Evo-Devo as a field of research with a common thread, rather than merely a catchy umbrella term for a medley of interesting studies.

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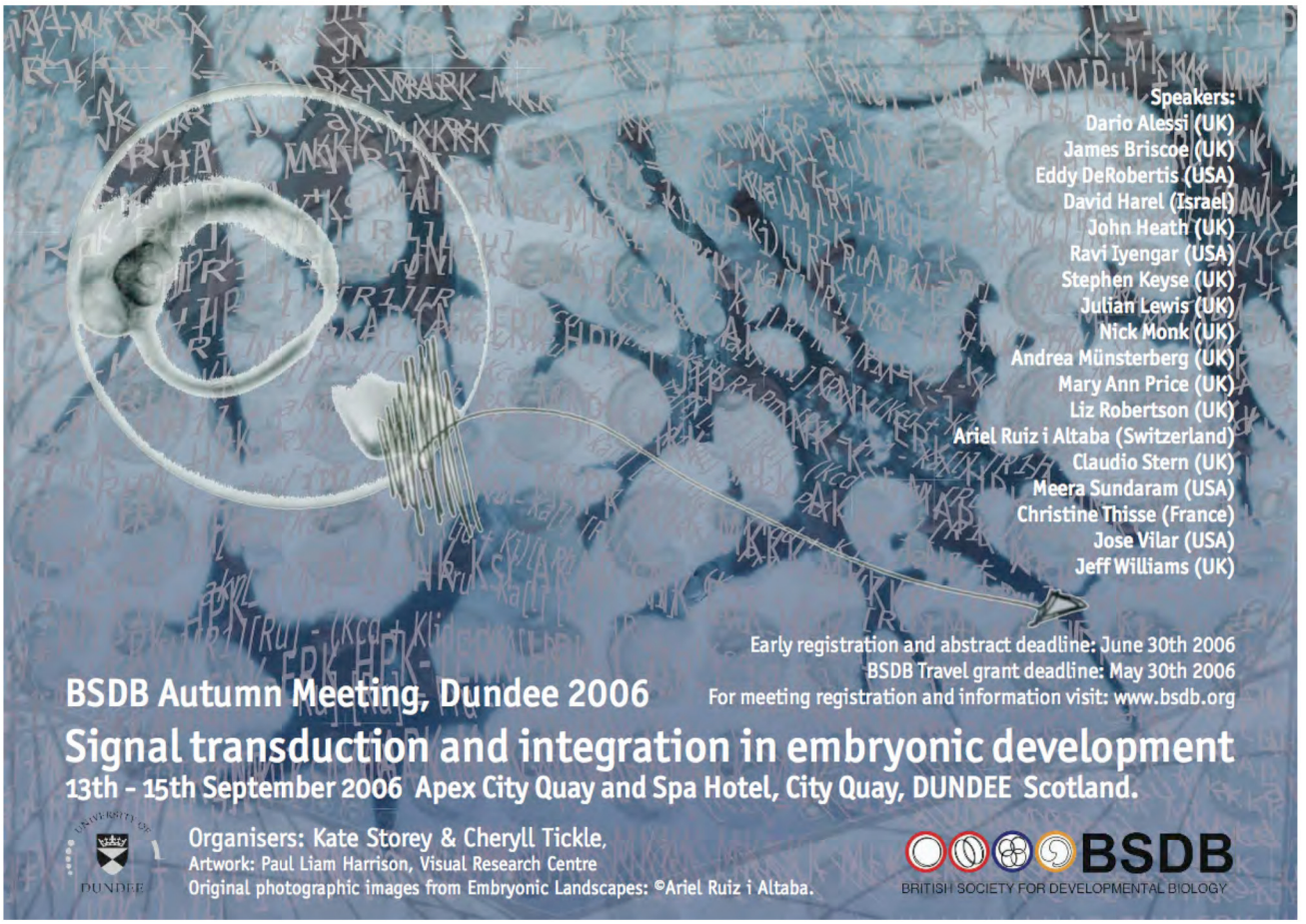
BSDB Autumn Meeting 2006



The aim of this meeting is to explore how the understanding of signal transduction pathways illuminates developmental mechanisms: in particular, how different signalling modes and patterns can influence cell responses and how different signals are integrated. The sessions will focus on specific developmental systems in embryos from a wide range of organisms with the aim of drawing out general principles. The conference will bring together developmental biologists, biochemists and mathematical modellers.

This meeting will not be held as usual in an academic venue, but in the Apex City Quay Hotel & Spa(!) which is a recently built, very modern hotel on the waterfront.

Speakers

John K. Heath (Birmingham) “Fibroblast Growth factor signalling dynamics: *in vivo*, *in vitro* and *in silico*”

Dario Alessi (Dundee) “PDK1 signalling”

Andrea Münsterberg (UEA) ERK MAP kinase signalling in somite patterning and differentiation

Stephen Keyse (Dundee) “Roles for dual-specificity protein phosphatases in regulating the physiological outcome of MAP kinase signalling”

Ravi Iyengar (USA) “Modelling MAPK signalling” (provisional title)

Christine Thisse (Strasbourg) “Revisting the concept of the organizer and the importance of integrated signalling by BMP, nodal and Wnt8”

Eddy De Robertis (USA) “Integration of signaling pathways during neural induction”

Claudio Stern (London) “The hypoblast (AVE): multiple roles in regulating embryo polarity”

Liz Robertson (Oxford) “TGF-b signalling in the early mouse embryo” (provisional title)

Jose Vilar (USA) “Computational modelling of TGF-b Superfamily Ligand-Receptor Network”

Mary Ann Price (Sheffield) “Casein Kinase I in Hedgehog and Wnt signalling”

James Briscoe (London) “Graded signals and the control of neural cell fate”

Ariel Ruiz i Altaba (Geneva) “SHH-GLI signalling in stem cells and cancer”

Julian Lewis (London) “Feedback, oscillations and noise in the Notch signalling pathway”

Nick Monk (Sheffield) “To be announced”

Jeff Williams (Dundee) “Transcriptional regulators of Dictyostelium pattern formation”

Meera Sundaram (USA) “EGFR/Ras/ERK control of renal development in C. elegans”

David Harel (Israel) “Some Thoughts on Comprehensive and Realistic Modeling”