intracellular signalling pathways in neuron-like cells and for their ability to stimulate neurite outgrowth of neuron-like cells.

Milk proteins that have shown effects on neural-like cell lines are being tested in vitro using a whole gut culture system to examine their effects on the neonatal ENS development.

doi:10.1016/j.mod.2009.06.513

13-P041

The balancing action between activin A/TGFβ, FGF, Wnt and BMP signalling instructs human embryonic stem cell fate decisions

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Activin A/TGFβ, FGF, Wnt and BMP signalling are crucial for the self-renewal and early differentiation of human embryonic stem cells (hESCs). However, the mechanism of how they coordinate to regulate these two processes is unclear. To address this question, we treated hESCs with graded concentrations of these growth factors and their inhibitors separately and in combination and carried out comprehensive analysis on marker gene expression. We found that the expression of pluripotency marker genes and key lineage determination genes change in a highly coordinated manner depending on the combination and the dosage of growth factors. Similar to mESCs, inhibition of ERK1/2 reduced differentiation in hESCs. hESCs can maintain long-term self-renew in the presence of ERK1/2 inhibitor in a chemically defined medium. Our results suggest that ERK1/2 signalling enhances hESCs differentiation. We propose a model that the balancing action between Activin A/TGFβ, FGF, Wnt and BMP signalling determines hESC self-renewal or lineage choices.

doi:10.1016/j.mod.2009.06.514

13-P042

Dkk1 regulates patterning and neurogenesis of the zebrafish eye

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Visual information is processed by the retina, a highly organized sensory organ in vertebrates. Several signalling pathways are known to be involved in retinal patterning (including BMP4, FGF and retinoic acid) and neurogenesis (such as FGF and Hedgehog).

Here, we show that another signalling system, the Wnt/β-catenin pathway, regulates both patterning and neurogenesis in the eye. We found that the Wnt antagonist Dickkopf (dkk1) is required for normal retina development. dkk1 is expressed specifically in the dorsal retina from 6 somite until prim5 stage, defining a dorsal organizing centre responsible for the DV eye patterning. Loss of function embryos, obtained using a morpholino (Mo) knockdown approach, show a defect in dorso-ventral patterning: loss of the ventral marker vax2, and an expansion of dorsal identity.

We also observed a dramatic expansion of the activity of the signalling pathways Fgf8 and BMP4, raising the possibility that these pathways are also under the influence of dkk1 activity.

Several genes known to be involved in neurogenesis are also severely affected by the loss of dkk1 function, such as fgf8, pax2, and fog1. In fact, we observe a complete absence of neurogenesis in the retina of dkk1 Mo injected embryos, as shown by the absence of ath5 expression.

Our results suggest that dkk1 has a dual role in both patterning and neurogenesis of the retina, possibly by interacting with multiple signalling pathways.

doi:10.1016/j.mod.2009.06.515

13-P043

Unraveling the complex mechanisms of spatial and temporal Sonic hedgehog expression in the limb

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Sonic hedgehog (Shh) signaling, from a discrete region in the posterior mesenchyme of the developing limb bud, establishes a concentration dependent signal that regulates digit number and identity. We have recently identified a highly conserved 800 bp enhancer element, ZRS, located over 1 Mb from the Shh promoter that regulates expression in the limb. In order to understand the genomic organisation of long-range limb enhancers we created a posterior mesenchymal limb specific cell line to investigate insulator binding and epigenetic marks using targeted arrays.

The 800 bp ZRS demonstrates an exceptional level of conservation between species and suggests an important role for the entire sequence, substantiated by numerous single nucleotide mutations throughout the ZRS arising in patients with preaxial polydactyly (PPD). Analysis of binding sites within the enhancer revealed multiple sites for the FGF-signaling transducer, PEA3, we also uncovered a surprising interplay between FGF and Shh in regulating spatial restriction. We identified novel PEA3 binding sites generated by single nucleotide mutations in families with PPD that leads to the loss of spatial restriction in the posterior mesenchyme resulting in ectopic expression in the anterior mesenchyme. We also propose that spatial restriction of Shh expression, mediated by PEA3, is dependent upon co-regulators. In addition we identified, through a number of biochemical approaches, multiple transcription factors binding to the ZRS that has provided an insight into the workings of such a large enhancer element.

These data taken together have started to reveal the complex mechanism of Shh expression in the limb.

doi:10.1016/j.mod.2009.06.516

13-P044

Sip1/Zfhx1b is a regulator of Wnt–β-catenin signaling during early midbrain and anterior hindbrain development

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The entire central nervous system develops from the neural plate, initially a single-cell layered epithelium. Anterior–posterior patterning is established by signals emanating from tissues outside the neural plate, while local signaling centers that are established within the neural plate itself will refine this initial patterning. One signaling center that plays an important role in the early development of midbrain and anterior hindbrain is the mid/hindbrain organizer (MHB). It is expresses *Wnt1* on its rostral side and *Fgf8* on its caudal side to exert its effects on future mid- and hindbrain.

We have previously shown that Sip1/Zfhx1b is a multi-domain transcriptional repressor that can interact with several protein partners, thereby exerting several different functions depending on cellular context. We report here a new function of Sip1 in midbrain and hindbrain patterning.

At E8.5, *Sip1* is expressed in almost the entire neural plate. Sip1 represses *Dkk1* expression in the early midbrain, leading to precocious *Dkk1* expression at late headfold stage and increased expression at 5S stage in *Sip1* knockout embryos. As Dkk1 is an inhibitor of Wnt signaling, the observed aberrant expression of *Dkk1* leads to disturbed Wnt–β-catenin signaling in the future midbrain and anterior hindbrain. Hence, this aberrant Wnt signaling in *Sip1* knockout embryos leads to early MHB patterning defects, as shown by the altered expression domain of MHB region genes, including *En2* and *Pax5*.

Sip1 is thus a regulator of Wnt–β-catenin signaling during early midbrain and anterior hindbrain development and thereby indirectly influences patterning of these tissues.

doi:10.1016/j.mod.2009.06.517

13-P045

Functional analysis of CHEP12, a novel gene involved in the differentiation of heart precursor cells

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We have identified a promoter element of chick *Cerberus* able to drive EGFP expression into the heart and the hemangioblast precursor cells. More importantly, these EGFP-positive H/HPC were able to be traced back to a population of cells that consistently exit from the anterior primitive streak region from as early as stage 4.

In order to identify and study novel genes expressed and involved in the correct development and differentiation of the vertebrate H/HPC lineages, a differential screening using Affymetrix GeneChip system technologies was performed.

Remarkably, this screening led to the identification of more than 200 new genes potentially expressed in these haematopoesis, angiogenesis or cardiogenesis precursor lineages.

One of the novel genes identified, was initially denominated CHEP12 (Chick Heart/Haemangioblast Progenitor #12) due to its expression in anterior cardiac mesoderm precursor cells, both in chick and in mouse, and encodes for a novel secreted protein. Using anti-*CHEP12* morpholino oligonucleotides resulted into several of heart tube malformations. We have also found that besides the canonical cCHEP12 transcript there are several alternative-spliced form variants, which indicates that several isoform proteins may be synthesized in response to different signals. By performing developmental, genetic, biochemical and functional studies in chick, *Xenopus* and mouse models we aim to unravel the roles and the mechanisms of this novel gene in early vertebrate heart/hemangioblast induction and organogenesis.

Currently, we are using this promoter in mouse ES cells, as a differentiation/commitment marker of H/HPC in order to take full advantage of the mouse genomics and genetics resources.

doi:10.1016/j.mod.2009.06.518

13-P046

The *Drosophila small wing* phospholipase Cγ acts as a bridge between the insulin and the MAPK pathways during development

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small wing (sl) codes for the sole PLCγ homolog in fruit flies. Mutations in this gene cause three different phenotypes: extra R7 cells and extra wing veins, both differentiation phenotypes, and smaller wings, due to smaller cells, a growth phenotype. Our experiments show that *sl* interacts with both the insulin and the MAPK pathways, acting as a positive regulator of growth and as a negative regulator of differentiation. Genetic interactions show that *sl* counteracts the MAPK pathway in R7 and extra wing veins, whereas it acts in concert with the insulin pathway in promoting wing growth. Moreover, *sl* needs insulin pathway input to effect its modulatory role in growth and differentiation, acting downstream of the insulin receptor. *sl* activates a PKC (protein kinase C) gene in these three processes via calcium release from intracellular stores (via the IP_3 receptor) and putatively diacylglycerol, and via the *RACK1* gene for the wing phenotypes. *RACK1* is not required for the eye phenotype. We have conducted a structure–function analysis of *sl*, and found that the SH2 domains are differentially required for these functions. Taken together, these results imply a higher complexity of PLCγ signaling than hitherto acknowledged.

doi:10.1016/j.mod.2009.06.519

13-P047

Role of the adherens junction in neurogenesis

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