

that FoxB1 regulates the establishment of the DV and AP axes in *Xenopus* ectoderm. Importantly, we show evidence that FoxB1 functionally interacts with its upstream factor XOct-25 and plays an essential role in induction and/or maintenance of neural tissue. Our results suggest that FoxB1 contributes to a mechanism that fine tunes and leads to the coordinated formation of the DV and AP axes during early development.

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## P25

### Specific expression of heparan sulfate glycosaminoglycan chains is crucial for local distribution of FGF signaling during mammalian embryogenesis

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Heparan sulfate (HS) proteoglycans comprising HS glycosaminoglycan chains (HS chains) and core proteins have been functionally implicated as co-receptors for multiple growth factors in the cell surface and extracellular matrix. However, the precise mechanisms by which HS chains regulate the activity of growth factors in complex mammalian morphogenetic processes remain to be elucidated. Here, we identified the transgene insertion allele of *Ext2*, a gene causative of multiple hereditary exostoses that catalyzes the elongation of HS chains. Marker expression analyses of *Ext2*-deficient embryos revealed that HS chains are essential for the response to Fibroblast growth factor (FGF) signaling and, in particular, for the local distribution of FGF ligands in the extraembryonic ectoderm. Moreover, the expression of HS chains attached to the cell surface is specific to those areas in which FGF signaling is potentially active. Additional fine mosaic studies with single-cell resolution involving chimeras suggested that the expression of cell surface-attached HS-chains is crucial for early embryonic development. Given that 22 FGF ligands, 4 FGF receptors, and 12 membrane-associated core proteins are redundantly expressed, we propose that the spatially and temporally localized expression of HS-chains attached to cell surfaces also contributes to FGF signaling distribution during mammalian morphogenesis.

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## P26

### Inhibition of phosphatidylinositol-3-kinase signalling promotes Activin A induced definitive endoderm differentiation of human embryonic stem cells

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Efficient definitive endoderm (DE) differentiation is essential for the generation of hepatocytes from human embryonic stem cells (hESCs), which are in demand for drug development and clinical application. However, this has been hindered as signaling pathways regulating the differentiation remain unclear. Therefore, understanding the signaling pathways controlling DE formation is important for differentiation of hESCs to hepatic lineages. Generation of DE through the initial formation of mesendoderm is activin-dependent differentiation. Further induction by higher concentration of Activin A gives rise to DE, whereas lower concentration favors mesoderm lineage. On the other hand, phosphatidylinositol-3-kinase (PI3K) signaling, which is important for ESCs self-renew shown to be potent inhibitors of the differentiation. It has been shown that suppression of PI3K signaling efficiently promotes differentiation of hESCs into mesendoderm and then DE in conditioned medium. However, the differentiation efficiency and molecular mechanism underlying the mesendoderm and DE differentiation cannot be clearly addressed in this culture condition. Therefore, in this study, we have used chemically defined medium to assess differentiation potential by suppression of PI3K in combination with Activin A. In this study, we demonstrate that suppression of PI3K signal in H1 hESCs causes downregulation of pluripotency marker (Oct4 and Sox2) and induces mesendoderm/endoderm gene expression (Brachyury, Gata 6 and FoxA2). Treatment of hESCs with combined Activin A (100 ng/ml) and PI3K inhibitor, Ly 294002 (20  $\mu$ M) increased expression of mesendoderm and endoderm markers (Brachyury, GSC, FOxA2, Sox17 and Wnt3a) several fold higher than that of Activin A (100 ng/ml) alone. As a result of improved DE formation, combined treatments also improved hepatocytes production, exhibited higher level of hepatic gene expression and increased Cyp3A4 activity (four-fold higher) than those derived from the treatment with activin alone. These results suggest that repression of PI3K is necessary for efficient DE differentiation and subsequently for the hepatocyte generation.

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## P27

### WNT-4 signalling regulates development of the ovarian follicle and is essential for integrity of the adult kidney

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WNT-4 is a member of the WNT family of secreted signaling molecules whose function is crucial for female sexual development. It regulates formation of the Müllerian duct and suppresses androgen synthesis during ovarian development. The conventional *Wnt-4* knock-out is lethal soon after the birth. In order to avoid the postnatal lethality and to study potential later functions of *Wnt-4* in the ovary and reproductive duct development we have generated a conditional *Wnt-4* knock-out allele by gene targeting technique. The *Wnt-4<sup>fllox/-</sup>* mice were crossed with the *Amhr2Cre/+::Wnt-4<sup>fllox/fllox</sup>* mice to obtain conditional *Wnt-4* knock-out mice. The efficiency of the average inactivation of the floxed allele was around 74% as judged by qRT-PCR. In case of conditional *Wnt-4* inactivation the ovarian size and weight were not affected after the birth but in the aging mice the ovarian weight increased by 40% mostly due to hypertrophied corpora lutea. In many of the cases the amount of follicles was not changed significantly. However when deletion efficiency was