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# Abstracts

## Wnt5a regulates ventral midbrain morphogenesis and the development of dopaminergic precursors in vivo

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Wnts, morphogens that have multiple and diverse functions during development, activate several signaling pathways including the Wnt/planar cell polarity (PCP) pathway activated by Wnt5a. Deletion of *Wnt5a* in mice results in typical PCP phenotypes including defects in inner ear hair cell orientation. However, no anomaly has been previously identified in the ventral midbrain. *In vitro*, Wnt5a is known to induce neuronal differentiation of midbrain dopaminergic precursors. Here we examined whether loss of *Wnt5a* affects the morphogenesis of the developing ventral midbrain and/or dopaminergic precursor differentiation *in vivo*. We found a broadened and flattened midbrain floor plate in *Wnt5a-/-* mice, with miss-oriented cells in the neurepithelium and increased proliferation. Also, while the absolute number of dopaminergic neurons was found to be normal, their distribution was broader laterally and shorter rostrocaudally. Interestingly, an increase in Nurr1+ progenitor cells was found, but their differentiation was impaired, resulting in a lower ratio of TH+:Nurr1+ cells in the *Wnt5a-/-* mice. Analysis of the activation of small GTPases in a dopaminergic cell line revealed that they are activated by Wnt5a. Moreover, inhibitors efficiently blocked Wnt5a-induced dopaminergic neuron differentiation in midbrain cultures, linking Wnt5a-induced differentiation with a known effector of Wnt/PCP signaling. In summary, our results show two novel functions of Wnt5a. Early in development, *Wnt5a* is broadly expressed in the ventral midbrain where it regulates morphogenesis. Later, *Wnt5a* expression becomes restricted to the ventral midline and promotes differentiation of Nurr1+ DA precursors. Thus, our results indicate a role for Wnt5a in the morphogenesis and differentiation of the midbrain *in vivo*.

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**Cooperation of NFκ-B and Notch signalling pathways induced by pigment epithelium-derived factor modulates neural stem cell renewal**

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Neurogenesis persists in two germinal regions in the adult mammalian brain, the subependymal zone (SEZ) of the lateral ventricles and the subgranular zone (SGZ) in the hippocampal formation. These brain areas contain adult neural stem cells (NSCs) that are characterised by self-renewal and multilineage differentiation. Specialised microenvironments or niches support the lifelong self-renewal of stem cells and their production of differentiated cells but the molecular mechanisms underlying these processes still remain largely unknown. Self-renewal is essential for stem cells because it is required for all stem cells to perpetuate themselves. We previously reported that pigment epithelium-derived factor (PEDF) is a niche signal secreted by endothelial and ependymal cells of the murine SEZ that promotes self-renewing symmetrical divisions in the NSC population of the adult SEZ both in vivo and in vitro. PEDF-treated NSCs express higher levels of Notch effectors Hes1 and Hes5 suggesting a possible role of PEDF in the regulation of Notch signalling. The receptor that can mediate PEDF actions on NSCs is unknown but it has been shown that PEDF could activate NFκ-B signal pathway on many cellular types. Here we suggest that PEDF actions on adult neural stem cells self-renewal are mediated by effects of NFκ-B signalling on the Notch pathway.

**The cyclin-dependent kinase inhibitors p21 and p27 differentially regulate neural stem/progenitor cell populations in the adult hippocampus**

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In adult mammals, neurogenesis persists in two specific brain regions: the subventricular zone that lines the lateral ventricles (SVZ), and the subgranular zone of the hippocampal dentate gyrus (SGZ). In these germinal niches, primary precursors or neural stem cells continuously generate new neurons in a process that is tightly regulated locally both by environmental factors and by intrinsic factors. In this work, we have characterised the role of the cyclin-dependent kinase inhibitors (CDKIs) p21 and p27 in the control of the proliferation and differentiation cascade of adult SGZ neuronal progenitor cells. Using knock-out mouse strains that are deficient in p21 or p27, and following immunohistochemical analysis, we have assessed the effect of the loss of these intrinsic cell cycle regulators in the SGZ. Our results show that both CDKIs regulate proliferation and neurogenesis in this area of the mature brain, acting differentially on lineage-related populations of precursor cells.

**Culture of male germ line stem cells from human testes for transplantation**

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Recently developed, highly effective, cancer treatments for children allow the majority of them to survive their cancer. However, one of the major adverse effects of anticancer therapy of prepubertal boys is permanent infertility due to azoospermia. Therefore, we now aim at translating our successful spermatogonial stem cells (SSCs) culture and autotransplantation in mouse and bovine to the human situation. As the final number of transplantable spermatogonial stem cells will influence the success rate of this technique, we first focus on establishing a spermatogonial stem cell culture from human testicular cells. For this, from five different patients undergoing bilateral castration as part of prostate cancer treatment, we received the testis samples. Testicular cells were isolated by enzymatic digestion and enrichment of germ cells was achieved by overnight preplating. The isolated floating cells were seeded in supplemented StemPro-34 medium. Characterisation of germ cells and spermatogonia in culture was performed by immunohistochemistry (IHC) using specific markers. The increase in MAGE A4 positive cells, as a marker for spermatogonial cells, after 1 and 2 weeks of culturing, suggested an increase in the number of spermatogonia in vitro. After about 4 weeks, germ cell containing colonies were formed in cultures of all five patients as indicated by anti-VASA, a marker for germ cells. These preliminary results suggest the feasibility of culturing human spermatogonia. However, more markers need to check as well as the stem cell capacity of these cells after transplantation to testis of busulphan treated mice. Moreover, in order to increase the number of spermatogonial stem cells for transplantation we will investigate the possibility of long term cultures.

**Epidermal stem cell homeostasis and cancer: role of Rac and Myc**

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Adult stem cells are potentially the only long-term tissue residents that can accumulate enough oncogenic mutations resulting in the development of neoplasias. Upon transformation, adult stem cells retain hallmarks of stemness such as self-renewal, high proliferation potential, and tissue-remodelling activities, among others, but lose the ability to follow the organisational cues that restrain uncontrolled growth and invasion in healthy tissues. We have previously shown that deletion of Rac1 GTPase in adult mouse epidermis stimulates stem cells to undergo irreversible differentiation, resulting in failure to maintain the entire epidermal unit. Upon deletion of Rac1, epidermal stem cells exit the niche through a by-functional mechanism, cell cycle entry and egression from the stem cell niche. Mechanistically, Rac1 exerts some of its epidermal effects via PAK2-mediated phosphorylation of the transcription factor c-Myc. Here we propose a novel function of Rac1 and c-Myc in epidermal stem cells and squamous tumours. Phospho-Myc,

downstream of PAK2, promotes quiescence and self-renewal of skin progenitors, inhibits the onset of differentiation, and induces strong homing to the stem cell niche. In addition, phospho-Myc enhances the invading and tissue remodelling potential of epidermal progenitors in 3D and 2D assays. Our data suggest that phosphorylation of Myc shifts its activity from a transcriptional activator to a repressor of transcription. Thus, we propose that the Rac/PAK2/Myc axis is required to sustain the equilibrium between epidermal stem cells and their progeny. Deregulation of this pathway might play a role in epidermal neoplasias.

**Asymmetric cell division within the human hematopoietic stem and progenitor cell compartment**

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Somatic stem cells are undifferentiated cells which can self-renew over a long period of time and give rise to progenitor cells which differentiate upon their further development. Since both uncontrolled expansion as well as loss of stem cells would be fatal for multi-cellular organisms the decision of self-renewal versus differentiation needs to be tightly controlled. Therefore, one important aspect in the field of stem cell biology is to elucidate the mechanisms that govern this decision. Studies on model organisms like Drosophila melanogaster and Caenorhabditis elegans revealed two different strategies to control the cell fate of stem cell progenies: i) the asymmetric cell division, in which intrinsic cell fate determinants segregate differently, resulting in the maintenance of the stem cell fate in only one of the daughter cells, and ii) the stem cell niche, a microenvironment which provides a special combination of extrinsic factors, sustaining stem cells in their primitive state. Although there is good evidence for the existence of hematopoietic stem cell niches, it is often predicted that primitive hematopoietic cells divide asymmetrically. This assumption is mainly supported by the finding that primitive human hematopoietic cells often realise different proliferation kinetics and give rise to daughter cells adopting different cell fates. Although these data generally fit into the model of asymmetric cell division, it cannot be ruled out that in those cases arising daughter cells are identical first and by mutually influencing each others cell fates adopt different developmental capabilities post-mitotically. To unequivocally demonstrate that primitive hematopoietic cells can divide asymmetrically, we searched for molecules, which - similar to cell fate determinants in model organisms - segregate differentially during mitosis. So far, we have identified four proteins (CD53; CD62L/Lselectin; CD63/lamp-3 and CD71/transferrin receptor) that segregate differentially in about 20% of primitive human hematopoietic cells which were cultured under stroma cell-free conditions. Our findings indicate for the first time that HSC/HPC indeed contain capabilities to divide asymmetrically. Since three of these proteins, the transferrin receptor and the tetraspanins CD53 and CD63, are endosome-associated proteins, it is tempting to speculate that the process of asymmetric cell division within primitive hematopoietic cells is linked to the endosomal compartment.

**Wnt-knockout mouse embryonic stem cells – a tool for understanding the role of Wnt pathway in dopaminergic neuron development**

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Wnt signaling pathway represents one of the most evolutionary conserved biochemical pathways and plays a very important role during the development of nervous system, where Wnts regulate proliferation, cell fate decisions, neuronal differentiation, and morphogenesis. Mouse embryonic stem cells (mESC) represent a unique tool to study basic cellular and molecular mechanisms of neural development. Here we present characterisation of our derived mESC lines deficient in components of Wnt/beta-catenin pathway in terms of signaling defects and differentiation capabilities. We demonstrate that mESCs lacking Wnt1 or receptor Lrp6 show enhanced neuronal differentiation. We also present biochemical evidence that absence of Lrp6 strongly affects ability to response to wnt ligand. In summary, we show that these cells represent a potent tool to study the Wnt signaling pathway in the differentiation of dopaminergic neurons and as such may contribute to the successful use of Wnts and stem cells in the cell replacement therapy for Parkinson's disease.

**uPFK-2 gene silencing decreases glycolysis, induces cell-cycle delay and inhibits anchorage-independent growth**

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PFK-2/FBPase-2 (PFK2) catalyses the synthesis and degradation of fructose-2,6-bisphosphate, an activator of phosphofructokinase-1. This property confers to this bifunctional enzyme a key role in the control of glycolysis. There are four PFK-2/FBPase-2 isoenzymes in mammals, each coded by a different gene (pfkfb1-4) that generates several isoforms of each isoenzyme by alternative splicing. We are interested in the isoforms coded by pfkfb3 (namely, ubiquitous and inducible), since the expression of this gene is implicated in the high glycolytic rate present in cancer cells despite adequate oxygen supply, a phenomenon known as Warburg effect. Pfkfb3 is overexpressed in human tumors and tumoral cell lines, a fact that links to long-standing observations concerning the apparent coupling of enhanced glycolysis and cell proliferation. The aim of this work is to investigate how the silencing of pfkfb3 and the consequent reduction of glucose metabolism can affect cell proliferation and survival. Also, the study of TIGAR, a newly discovered p53 target gene, which is known to have fructose-2,6-bisphosphatase activity, can shed light on the importance of glycolysis in cancer.

**Amnion plasticity: lessons from the mouse**

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The amnion is the innermost of the extraembryonic membranes enveloping the embryo and fetus in utero. Almost a century ago, amniotic membrane transplantations were already used in wound

dressing to treat severe skin burns. For more that 60 years, amnion transplantations have been used in ophthalmological surgical procedures. Recently, several research groups have reported that human amniotic fluid but also amniotic epithelial cells from term placenta express several stem cell surface markers that are commonly found on pluripotent stem cells such as embryonic stem cells. In culture, these human amniotic cells differentiate into cell types from endoderm, ectoderm and mesoderm germ layers. We have shown that Smad5 mediated signaling is essential for amnion homeostasis in the mouse. Smad5 is an intracellular mediator of Bmp signaling. Its deficiency results in local thickening of the amnion, which is followed by ectopic vasculogenesis and haematopoiesis, and ultimately even ectopic primordial germ cell-like development. Here, we highlight the similarities and dissimilarities in the early development of the human and mouse amnion; and discuss data from genetic mouse models that may shed light on the underlying molecular mechanisms of amnion plasticity.

**Computer modelling of the spermatogonial stem cell niche**

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Spermatogonial stem cells are single cells situated on the basal membrane of the seminiferous tubules. Differentiation includes the formation of a pair and subsequently of chains of interconnected cells. For rodents detailed information is available on the numbers of these cells present, proliferative activity during the cycle of the seminiferous epithelium, cell cycle times and the topographical arrangement of the singles, pairs and chains of spermatogonia on the tubule basal membrane. Based on the knowledge of the cell kinetics of the spermatogonia a computer model was developed to simulate spermatogonial stem cell renewal and differentiation and the behaviour of the pairs and chains of early spermatogonia. In a first model it was assumed that the stem cells had a 50 % chance of self-renewal and differentiation. Interestingly, this always led to a depletion of the epithelium as in areas where by chance most of the stem cells differentiated, hardly any recovery took place. In a second model, the existence of a niche was assumed in which the stem cells had a 90-100% chance of self-renewal while outside of the niche the chance of differentiation was 90%. This model rendered steady state kinetics in which the numbers of stem cells remained constant and in which the production of differentiating cells was close to what was observed in the in vivo situation. We expect that further fine-tuning will render a model that will give us detailed insight into the characteristics of the niche and stem cell behaviour.

**Embryonic stromal clones reveal developmental regulators of definitive hematopoietic stem cells**

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Hematopoietic stem cells (HSCs) have the ability to self-renew and to give rise to all mature hematopoietic cells. These decision fates are closely regulated by extrinsic signals produced by HSC microenvironment. In the mid-gestation mouse embryo, the first adult-type HSCs

emergence autonomously in the AGM (Aorta-Gonads-Mesonephros) region. To identify novel factors involved in the biology of HSCs when they appear in the embryo, we compared the expression profile of two closely-related stromal clones which differentially support AGM HSCs. We identified three putative HSC regulatory factors, b-NGF (a neurotrophic factor), MIP-1g (a C-C chemokine family member) and BMP-4 (a TGF-b family member). In vivo transplantation experiments reveal that these three factors enhance the repopulating potential of AGM HSCs. Interestingly, we show that AGM HSCs express BMP receptors and that BMP-4 is expressed in the mesenchyme underlying HSC aortic clusters. Furthermore, the addition of gremlin, a BMP antagonist, to AGM explants completely abolishs HSC activity. Since gremlin does not induce apoptosis or decrease the absolute numbers of hematopoietic cells, we suggest that endogenous BMP-4 regulates HSC development in the AGM in vivo.

**Role of uPAR in stem cells mobilisation**

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Introduction: The transplantation of hematopoietic stem cells (HSC) is a treatment of patients suffering from malignant proliferative blood diseases, particularly leukemia. Therefore HSC have to be mobilised from the bone marrow (BM) to the blood of a suitable donor. Here we report that the urokinase plaminogen receptor (uPAR) - which is known to be involved in the migration, adhesion and proliferation of different kind of cells - plays a role in the mobilisation of hematopoietic stem cells (HSC) from bone marrow to blood.
Materials and Methods: uPAR -/- mice and WT were treated i.v. with 5-Fluorouracil, a chemotherapeutic drug, which leads to the mobilisation of HSC from bone marrow to the blood. After designated time points, blood and bone marrow were isolated and HSC were sorted by flow cytometry. Four HSC-markers were used: Sca1, ckit, Lin-, AA 4.1. Furthermore we performed microarrays of control and 5-FU treated WT and uPAR KO hematopoietic stem cells, isolated from bone marrow to compare the gene expression difference due to the uPAR gene in the settings of HSC mobilisation.
Results: • After 5-FU injection: • BM cellularity decreases in WT and uPAR -/ mice • Living white blood cells disappear (mobilisation to blood) from the BM of wt mice, while they are retained in the BM of uPAR -/- mice. • uPAR -/- mice do not mobilise their HSC from BM to blood • Microarrays: a. Under baseline conditions, in the HSC from uPAR -/- mice cell cycle and apoptotic genes are overexpressed suggesting that these cells cycle more than wt but tend to die. b. After 5-FU-Injection, in both WT and uPAR-/- mice the cell cycle genes are stimulated. c. In uPAR-/- mice adhesion genes are down-regulated more than in wt, suggesting that HSC have a decreased capability of adhering to the stromal cells of bone marrow. In particular, since the adherence of HSC to bone marrow seems to be due to the alpha4beta1 Integrin (preliminary results of Marc Tjwa), the absence of an upregulation of the alpha4beta1 in uPAR -/- HSC reinforces this assumption.
Conclusion: uPAR plays a role in the mobilisation of hematopoietic stem cells from the bone marrow to the blood. UPAR seems to guarantee the quiescence and restoring capability of HSC, as uPAR-/- mice cycle more and show a higher degree of apoptosis in baseline conditions. The adherence of HSC to bone marrow stromal cells might be due to Integrin alpha4beta1, whose function is sustained by uPAR.

**Alfa-chemokines regulate proliferation and dopaminergic differentiation of ventral midbrain precursors and neurospheres**

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Increasing evidence suggests that α-chemokines serve several important functions in the nervous system including regulating neuroimmune responses, modulating neurotransmission, and promoting neuronal survival and CNS development. In this study, we examined the function of two α-chemokines, CXCL6 and CXCL8, and their receptors, CXCR1 and CXCR2, in the developing rat midbrain. We found that one of these receptors, CXCR2, and one ligand, CXCL6, are regulated during ventral midbrain development. Moreover, we also found that CXCL6 promoted the differentiation of Nurr1+ precursors into dopaminergic neurons in vitro. Intriguingly, CXCL8, a ligand expressed only in homosapiens, enhanced precursor proliferation. These results prompted us to examine whether an endogenous CXCL8-like ligand could regulate DA neuron development. We found that CXCL1, the murine ortholog to human CXCL8, is both expressed in the ventral midbrain of mouse/rat and increases the precursor proliferation in a similar manner as CXCL8. In conclusion, our results show that α-chemokines could be applied to expand and differentiate ventral midbrain neurospheres. These data also suggest that α-chemokines may find an application in cell replacement therapy for Parkinson's diseases, as a tool to enhance the number of dopaminergic neurons in precursor/stem cell preparations.

**Characterisation of Wnt signalling components in human embryonal carcinoma cells**

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The Wnt signalling pathway has been shown to be important both for stem cell maintenance and for neuronal differentiation. The goal in our laboratory is to use purified factors, small molecules and gene silencing technology to drive dopaminergic differentation of human stem cells. In our initial studies we have used the NTERA-2 human embryonal carcinoma (hEC) cell line, which shares many characteristics with human embryonic stem cell lines and also the SH-SY5Y neuroblastoma cell line. NTERA-2 cells and SH-SY5Y differentiate into neurons when treated with retinoic acid (RA) and can be further differentiated into dopaminergic neurons. We have examined the expression of Wnt and secreted Wnt antagonist genes during differentiation. In addition, we have conducted transcription assays using b-catenin/Tcf and tyrosine hydroxylase promoter gene reporters. Our results suggest significant crosstalk between Wnt and retinoic acid signalling pathways during neural differentiation.

**Expression of angiogenic proteins by neural stem cells**

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Using microarray analysis of human fetal midbrain tissue we recently detected differential gene expression for angiogenic proteins. Some of these genes have been described as involved in stem

cell self-renewal regulation. We hypothesised that such proteins could be constituents of the neurovascular niche, communicating vascular cell components with neural progenitors and providing instructive signals to modulate their self-renew and/or differentiation. To analyse this hypothesis we are evaluating the expression of angiogenic proteins during mouse midbrain embryonic development and establishing temporal relation between gene expression profile and neurogenesis. RT-PCR results confirmed a temporal regulation during development. Complementary studies showed the expression of several angiogenic genes, including Vegf, Kdr1, Tie2, Scl, NP1. Similar gene expression analysis has been performed in ventral midbrain primary cultures, neurospheres, neural stem cells and embryonic stem cells. Our results show that several angiogenic genes are produced by these cells. We are currently evaluating their effects on neural progenitors proliferation and/or differentiation in vitro. In the future we would like to evaluate whether these genes/proteins could be important targets to regulate neural progenitor cells function and develop therapeutic strategies for Parkinson's disease.

**Cell-cycle independent action of p21WAF1/Cip1 on adult neural stem cells self-renewal and multipotency**

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Relative quiescence and self-perpetuation are defining characteristics of many tissue-specific stem cells (Morrison et al., 1999; Weissman, 2000). Adult stem cells can fulfill their function in tissue homeostasis ensuring physiological cell turnover during lifetime because they undergo self-renewing divisions in which one or two daughter cells retain the same replication potential and plastic properties of the parent stem cell (Morrison and Kimble, 2006). Self-renewal is inherently linked to cell division and it appears that proliferation must be tightly regulated to prevent expansion and depletion of stem cell reservoirs. In the natural endogenous niches, stem cells often produce large number of progeny through an intermediate transient cell type with high mitotic activity, a strategy that helps preserve the original slowly-dividing long-lasting stem cell population. It is still poorly understood how stem cells undergo a self-renewing vs. a differentiative division but derived progenitors generally have cell cycle kinetics that differ from those of their parental stem cells. We have observed that p21-deficient NSCs have defects in self-renewal capacity, resulting in a decrease in the number of secondary neurospheres formed with passages and in the appearance of a senescent phenotype in vitro. P21-deficient neurospheres exhibited reduced multipotency as most clones could only generate GFAP+ cells and lack of p21 resulted in increased proportions of GFAP+ terminally differentiated astrocytes in the intact SEZ. Therefore, it appears that the lack of p21 reduces multipotency and increases differentiation of NSCs towards a terminally differentiated astrocytic phenotype. Reduced self-renewal and multipotency could be reverted by addition of noggin suggesting that the phenotype of p21-deficient NSCs is independent from the role of p21 on cell cycle regulation and involves the BMP pathway.

**Activity-dependent signaling in the control of adult hippocampal neurogenesis**

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Neural stem cells continuously generate new hippocampal dentate granule neurons throughout adulthood. Adult hippocampal neurogenesis is controlled by multiple environment-derived signals including growth factors and neurotransmitters. The latter are likely to be released in an activity-dependent fashion and control the differentiation of new hippocampal neurons. At present the intracellular signalling pathways downstream of neurotransmitter signaling in hippocampal neurogenesis are unknown. Here, we investigate the role of a neuronal activity-dependent transcription factor in the control of adult hippocampal neurogenesis using a retroviral approach. Our results indicate that this pathway is highly active in immature newborn neurons. Moreover, we found evidence that this pathway is involved in the regulation of survival and maturation of newborn hippocampal neurons. Future experiments will address the question of the upstream activators of this pathway and the downstream targets of this pathway in adult hippocampal neurogenesis.

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**Acquiring otic neural fate by Sox3 and FGF signalling**

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The inner ear is a complex sensory structure responsible for the senses of hearing and balance. Three main cell-types, hair-cells, supporting cells and sensory neurons compose the main functional sensory unit. It is still not well understood whether hair-cells and sensory neurons share a common progenitor. For this reason, we have compared the expression of Sox2 and Sox3 genes in the inner ear and studied the role of Sox3 in neural commitment. The first cells to be specified in the otic placode are neuronal precursors that emerge in an anterior subdomain of the placode, the proneural domain. By expression studies we have observed that the otic proneural field appears before otic placode formation as a broad neural competent region expressing Sox3 and Sox2 that also encompasses the epibranchial placodes. Progressively, the shared ectodermal neural territory is split into the otic neural region and the epibranchial placodes. In the otocyst when sensory patches emerge, Sox3 is down-regulated while Sox2 expression is maintained, suggesting that Sox3 and Sox2 are mainly required for neuronal and sensory lineage respectively. Overexpression experiments of Sox3 in the preotic ectoderm revealed that the non-neural region was respecified to neural fate, while this competence declines after otic placode formation. Few ectopic Delta1 cells transit into NeuroD cells indicating that high levels of Sox3 inhibit neuronal differentiation and that other factors are required for full neurogenesis. It has been well shown that Sox3 expression is dependent on the activity of the FGF signaling pathway. We have explored which FGF could be responsible for the restriction of Sox3 in the anterior otic field. We analysed the expression of several FGF and found that FGF8 appeared in the chick ectoderm at 7 somites in a band similar to the Sox3 ectodermal expression. Ectopic expression of

FGF8 by electroporation and bead implantation experiments indicated that FGF8 was responsible for enhancing Sox3 in the anterior otic field. Furthermore, FGF8 also induced FGF10, suggesting that sustained FGF signaling in the anterior ectoderm drives ectodermal progenitors into neural fate and Sox3 activity. Finally, manipulation of the Notch pathway has indicated that Notch is required for otic regionalisation into a neural and non-neural domain but not for the establishment of a proneural field.

**Eph receptors and ephrins regulate proliferation in multiple adult stem cell niches**

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More than 1010 cells are generated every day in the human intestine. Wnt proteins are key regulators of proliferation and the only previously described mitogens for intestinal progenitor cells. We report that B ephrins and their EphB receptors, in addition to directing cell migration,regulate proliferation in the intestine. EphB forward signaling promote cell cycle reentry of progenitor cells and account for approximately 50% of the mitogenic activity in the adult mouse small intestine and colon. These data establish EphB receptors as coordinators of migration and proliferation in the intestinal stem cell niche.

**Analysis of the pluripotent capacity of HNF1b-expressing cells in the embryonic and adult pancreas**

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Pancreas arises from gut endoderm at 9.5 days of embryonic development (E9.5) and all pancreatic epithelial cellular lineages derive from the pluripotent Pdx1+ cell population that constitutes the pancreatic buds at E9.5. Recently, a multipotent Cpa+ progenitor population has been described in embryonic pancreas, localised in the tips of the pancreatic epithelium branches. However, the steps between this Cpa+ multipotent population and the determination of the pro-endocrine Ngn3+ progenitors from which all endocrine cells derive, and the relationship of Cpa+ precursors with earlier pancreatic precursors, are still unknown. Previous work in the laboratory has strongly suggested that embryonic HNF1b/Tcf2/vHNF1 expressing cells might be precursors of pro-endocrine Ngn3+ cells. This work also suggested that Hnf1b+ cells might represent a pluripotent population which gives rise to other endocrine and non endocrine cells and it has been previously proposed that pancreatic duct Hnf1b+ cells might be the origin of the inflammatory dependent metaplastic ductal lesions. To conclusively study these hypotheses we have used BAC recombineering to generate transgenic mice expressing tamoxifen-inducible Cre and EGFP in pancreatic Hnf1b+ cells, and crossed this line with Rosa26R lines to carry out lineage tracing analysis. Our data resulting from the characterisation of this double transgenic mice model demonstrates that the lacZ reporter gene is selectively activated in most ductal Hnf1b+ cells after tamoxifen administration. We are currently carrying out lineage tracing studies to conclusively determine the pluripotent nature of Hnf1b+ pancreatic duct cells during embryogenesis and adult pancreas regeneration.

**Signalling through BMPR-IA regulates neural stem cell maintenance and neurogenesis in the adult hippocampus**

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New neurons arise from slowly-dividing neural stem cells (NSCs) in the adult hippocampus, but molecules regulating NSCs in vivo are scarce. Signalling molecules regulating embryogenesis may play a role in the modulation of adult NSC behaviour and, amongst those, BMPs are attractive candidates for brain niche signals, since they regulate a wide range of developmental decisions both in neural and non-neural lineages and are important components of numerous stem cell compartments across species, from invertebrates to mammals. Here we show that BMP signalling, a major regulator of stem cells across phylogeny, is active in hippocampal NSCs downstream of BMPR-IA. Although BMPs are considered as gliogenic molecules in the adult brain environment, BMPs enhance neuronal production from cultured adult hippocampal NSCs in a cell-autonomous manner. In vivo, conditional ablation of Bmpr1a in hippocampal NSCs after injection of Bmpr1a flox/null mice with a lentivirus expressing Cre under the control of the Sox2 promoter reduces neuronal birth by decreasing the number of progenitors. Acute brain infusion of the BMP antagonist Noggin bursts proliferation in the hippocampus, indicating that blockade of BMP signalling mobilises NSCs and leads to their loss. Taken together, our data identify BMP signalling as an in vivo component of the adult hippocampal neurogenic niche, demonstrating its implication in the maintenance of the stem cell pool that is necessary to guarantee continuous neuronal production in this brain structure during adulthood.

**Spermatogonial stem cells and their robust potentials**

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Studies to achieve reproducible and straightforward techniques for obtaining adult pluripotent stem cells [embryonic stem cell-like cells (ES-like cells)] are becoming highly imperative for regenerative medicine. In the future autotransplantation applications of more differentiated derivatives of ES-like cells to cure diseases will evade the risk of immunorejection. Moreover adult ES-like cells are considered to overcome the ethical and technical problems related to ES cells. Since the early 2000s, mouse spermatogonial stem cells (SSCs) have been shown to be induced to become ES-like cells and furthermore the transdifferentiation into various cell types have also been shown. The indications of strong prospective of male germ cells in terms of pluripotency are becoming evident. We describe herein the ES colony formation of human SSCs and induction of transdifferentiation of SSC derived ES-like cells *in vitro*. In human SSC cultures, we observed colony formation after four weeks of culture. The cells, forming the compact and sharp edged colonies, showed AP activity. We identified the cells with showing mRNA expressions of POU5F1, Nanog, Sox2, DNMT3B and Cripto genes by RT-PCR and

protein localisations of Nanog and SSEA4 by immunocytochemistry. We are at present inducing the differentiation of these cells into different cell lineages *in vitro*. We localised neural lineage specific proteins after neural induction of these cells, such as GFAP and Neurofilament protein. We also observed smooth muscle specific protein (α-smooth muscle actin) localisation after myogenic induction of our ES-like cells. Differentiations of these cells into other lineages are currently ongoing.

**Bone marrow adipocytes: a novel regulator of the hematopoietic microenvironment**

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In the bone marrow (BM), osteoblasts and endothelium constitute functional niches providing positive or negative signals for hematopoietic stem cell (HSC) self-renewal. In addition to hematopoietic cells, endothelial cells, and osteoblasts, adult BM contains numerous adipocytes. Interestingly, the number of adipocytes correlates inversely with the gross hematopoietic activity of the marrow. Whether adipocytes have a direct effect on hematopoiesis or whether they act as mere space-fillers in this context remains unclear. To determine the potential role of bone marrow adipocytes in hematopoiesis, we induced bone marrow-derived OP9 mesenchymal cells to differentiate into either osteoblastic or adipocytic stroma, and then tested their capacity to serve as surrogate HSC niches during in vitro hematopoietic culture. We found that the presence of BM-derived adipocytes suppresses the expansion of short-term hematopoietic progenitors. As an in vivo correlate, we compared the hematopoietic activity within the BM of the adipocyte-poor thoracic vertebrae and the adipocyte-rich proximal tail vertebrae. Indeed, we found that the percentage of HSCs and progenitors was decreased in fat-rich BM. Finally, we found bone marrow adipocytes to accumulate in great numbers upon bone marrow ablation, a process that is hindered in genetically adipocyte-deficient mice. Since early BM transplant survival depends on the rapid accumulation of short term hematopoietic progenitors, we were interested to explore whether the absence of adipocytes in the context of BM transplantation would foster faster recovery of lethally irradiated mice. As predicted, circulating leukocyte counts on the third week post-transplant were 3-5 times higher on the recovering fatless mice.

**In utero stem cell therapy in BrtlIV, a knock in murine model for Osteogenesis Imperfecta**

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Osteogenesis Imperfecta (OI) is a bone disorder characterised by bone fragility and deformity. BrtlIV is a murine model for dominant OI carrying the typical molecular defect, a Gly substitution in the α1(I) chain, and the clinical outcome of the moderate severe OI patients. In the attempt to develop a cell therapy treatment for this disorders we decided to act in utero both to avoid marrow ablation and because OI is an inborn disorder. We isolated bone marrow from long bones of 1-2 month-old GFP transgenic mice and injected the cells in the liver of BrtlIV and

WT E14.5 embryos. Mice were analysed at 2 m, the age corresponding to the worst BrtlIV phenotype with respect to WT. Engraftment was detected by fluorescent microscopy in different organs, and was quantitated by confocal microscopy in bone cryosections (5-10%) and by FACS in spleen and bone marrow (6%). Green cells were detected in trabecular and cortical bone, being more abundant in the region surrounding the trabeculae. Growth curve, femur length, X-Ray, BMD, and Micro CT analysis of trabecular and cortical bone were performed. Improvement was detected in terms of BV/TV, trabecular number and trabecular thickeness in the treated mice with respect the WT.

**The role of LRP6 in midbrain development**

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Wnts are secreted lipoproteins that regulate many developmental processes, including the development of ventral midbrain (VM) dopaminergic (DA) neurons. The Wnt pathway is transduced by a ternary complex containing Wnts, the frizzled receptors (Fz) and the low density lipoprotein receptor (LDLR)-related protein (LRP) -5 or -6. We decided to investigate the role of LRP6 in the regulation of ventral midbrain dopaminergic neurogenesis. We examined its expression profile during VM development and analysed the LRP6 mutant homozygous mice. Our results show that LRP-6 has a dynamic pattern of expression in the developing VM. Analysis of the LRP6 -/- mice showed a 50% reduction of dopaminergic neurons at E11.5, the peak of the DA neurogenic period in mice, while proliferation and patterning where not affected. However, we observed a recovery in the number of DA neurons at later stages. These results highlight LRP6 role as a regulator of midbrain neuron development.

**Notch cooperates with β-catenin to activate a specific gene program**

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Wnt/β-catenin signaling pathway is essential to promote tumorigenesis in intestinal cells. We now show that Notch activation reverts the tumor suppressor effect of blocking β-catenin activity in a xenograft model. By microarray experiments we identified a group of genes that are simultaneous targets of both Wnt and Notch pathways in Ls174T cells including *hes1*, *EpHB3*, *CD44*, *PCGF4*, *EREG*, *SOX9* and *DSCR2*. We further demonstrate that β-catenin and Notch cooperate to activate specific gene transcription by two mechanisms: 1) transcriptional activation of the Notch-ligand Jagged1 by β-catenin/TCF and 2) at the chromatin level by direct binding to specific promoters. This mechanism likely operates in Familial Adenomatous Poliposis (FAP) patients where detection of nuclear β-catenin absolutely correlates with the presence of increased Jagged1 protein levels and activated Notch1 and Notch2. Moreover, several Notch/Wnt common targets are upregulated in the FAP intestinal tissue. We propose that nuclear β-catenin, through Jagged1, induces the activation of Notch in colorectal tumors and that both factors cooperate at the chromatin level to regulate a cancer-specific gene program.

**Molecular dissection of the conversion of adult exocrine pancreatic cells into exocrine precursors in vitro**

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The existence and nature of stem cells in the exocrine pancreas is a matter of debate. The steps for exocrine progenitors to differentiate to acinar cells have not been characterised. Isolated exocrine acinar cells from adult mouse pancreas undergo extensive phenotypic changes upon culture, mimicking changes occurring in disease states. To unravel the mechanisms involved, we have performed a detailed analysis of this process and have identified distinct steps therein. Exocrine cell isolation results in an immediate increase of Hes1 mRNA and a decrease of the acinar-specific p48/Ptf1a mRNA; by contrast Mist1 and RBP-L, two additional acinar factors, are unaffected. Culture of cells in free-floating conditions is associated with up-regulation of Pdx1, further down-regulation of p48 mRNA, and loss of expression of RBP-L. Concomitantly, mRNAs for keratins 7 and 19 and Hnf1-b are up-regulated. This is accompanied by loss of expression of most acinar enzymes (i.e. elastase). However, Carboxypeptidase A (CPA) mRNA - a marker of pancreatic exocrine progenitors- remains expressed in cultured cells at low levels. Using chromatin immunoprecipitation we show that, at this stage, p48/Ptf1a is preferentially associated to the CPA promotor versus the elastase promotor. Given the lack of RBP-L, we propose that maintenance of CPA expression results from binding of a PTF1 complex containing RBP-Jκ rather than its paralogue RBP-L, to which p48 binds in differentiated acinar cells. This is the first detailed characterisation of the step-wise reversal of acinar cells to a precursor-like state in culture.

**Directed-differentiation of embryonic stem cells into pancreatic acinar cells**

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Embryonic stem cells (ES) have been shown to differentiate into pancreatic endocrine cells but little information is available concerning the ability of these cells to adopt an exocrine phenotype. Our focus has been to generate specifically pancreatic acinar cells in vitro, as these cells play a crucial role in pancreatitis and pancreatic cancer and there are no models to study normal acinar cell differentiation in vitro. Reporter ES cells (Elas-puro) were generated that stably expressed both beta-galactosidase and puromycin resistance genes under the control of the elastase I promoter. Directed differentiation was achieved by incubation with conditioned media of cultured foetal pancreatic rudiments and adenoviral-mediated coexpression of p48 and Mist1, two bHLH transcription factors crucial for normal pancreatic acinar development and differentiation. Selected cells expressed the reporter gene and multiple markers of acinar cells, including digestive enzymes and proteins of the secretory pathway, at higher levels than in cells isolated after spontaneous differentiation, indicating activation of a coordinated differentiation program. Cells displayed transient agonist-induced Ca2+ mobilisation and exhibited a typical response to physiologic concentrations of secretagogues, including enzyme synthesis and secretion. These effects did not imply the acquisition of a mixed acinarductal phenotype.

Importantly, the ability of the Elas-puro cells to proliferate was significantly reduced in comparison to spontaneously differentiated cells. These studies allow for the first time to generate almost pure acinar-like cells from ES cells. This model will be extremely useful to study the acinar differentiation program.

**Is Wnt signaling involved in AGM hematopoiesis?**

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Hematopoietic stem cells (HSC) are responsible for life-long generation of blood cells of all lineages. The intra-embryonic AGM region, which include aorta surrounded by gonad and mesonephros tissues has been described as one of the first embryonic hematopoietic organs where the HSC appear. Defining the signals that maintain the balance between self-renewal and differentiation of HSC is crucial to understand stem cell biology. There are evidences that Notch, Hedgehog and Wnt signaling pathways regulate different aspects of HSC behavior. We are currently characterising the pattern of expression of Wnt molecules in the AGM region by RT-PCR and in situ hybridisation. We have found expression of both canonical and non-canonical Wnt molecules in the endothelium of the AGM region. Moreover, activation of the canonical Wnt pathway by LiCl leads to an increase in the number of CFC progenitors in cultures from E10.5 AGM. This result also agrees with the finding that CFC progenitors are increased in the AGM of the APCmin/+ embryos. On the other hand, inhibition of JNK, which is one of the downstream non-canonical Wnt pathways, lead to a decrease of CFC progenitors, however the number of erythroid and mixed colonies were maintained. Thus, our results suggest that both, canonical and non-canonical Wnt pathways, are favoring hematopoietic CFC formation in cells from AGM.

**Regulation of midbrain dopaminergic neuron development by Nurr1 and β-catenin**

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One of the major challenges of neuroscience remains the understanding of how signaling molecules and transcription factor networks interact to regulate the development of specific neurons. Several transcription factors and signaling pathways have been implicated in the developmental regulation of dopaminergic (DA) neurons. We have previously shown that Wnt treatment or stabilisation of β-catenin in VM precursors increase their differentiation into tyrosine hydroxylase neurons, a phenotypic marker of DA cells. Also nurr1 plays a fundamental role in the differentiation of VM neurons, as in its absence, midbrain DAergic neurons undergo an abnormal development, while its overexpression in different cell models results in neuronal differentiation. In addition, nurr1 is thought to play a key role in maintenance of a DAergic phenotype via regulation of DA neuron specific genes. Thus, nurr1 serves as a DA neuron-specific transcription factor whose activities could be regulated to modulate the DAergic neurotransmitter phenotype. However, the mechanisms regulating the differentiation process of VM neurons remain elusive, and in particular the transcriptional events induced by nurr1 and β-catenin activation that take place specifically in DA precursor neurons are unknown. β-catenin

and nurr1 are transcriptional regulators and, even though their importance for the development of DA neurons is indisputable, no studies have been conducted yet on the potential direct interaction of these transcription factors and their possible synergistic role at specific developmental stages. Therefore, our aim is to evaluate these interactions and clarify the molecular mechanisms controlling their nuclear function in neuronal precursor cells.

**Enhanced survival of IKVAV-attached neural stem cells on DHA-modified supported lipid bilayers**

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Model biological membranes prepared from synthetic phospholipids are well-defined structures of interest for delivery of hydrophobic molecules to cells. It was previously shown that supported lipid bilayers functionalised with a laminin-derived IKVAV-containing peptide provide appropriate substrates for attachment and culture of adult neural stem cells. We hereby investigated whether it is possible to influence neural stem cells attached to IKVAV-bilayers by incorporating the fatty docosahexaenoic acid (DHA) in the bilayer. DHA is known to exert a local influence on lipid properties of the cell membrane and has been found to promote cell survival. The effect of DHA on supported phospholipid bilayers was analysed with quartz crystal microbalance with dissipation monitoring (QCM-D), which revealed that DHA induced a stable mass increase of IKVAV-functionalised bilayers without compromising their resistance to protein adsorption. Neural stem cell line, c17.2, attached to IKVAV functionalised supported membranes in quantities comparable to glass, which was used as positive control surface. During the first 24 hours, impaired cell survival was observed and some recovery was detected after 4 days on both substrate types. The IKVAV-modified bilayers did not allow neurite extension and promoted growth of cell clusters. Treatment with low DHA doses (20 µM) in the presence of a mitogenic factor, promoted the survival of c17.2 cells and, at 4 days, the cell counts on glass and peptide functionalized bilayers were comparable. In reference to 1 day data the cell number increase was more effective on the supported membranes than on glass. Higher DHA dose (138 µM) did not have a positive effect on cell survival. In conclusion, supported membranes can be simultaneously engineered to provide both anchoring points and present functional triggers to stem cells. Such results broaden the spectrum of applications for supported bilayers to the delivery of lipophilic factors of importance in stem cell biology and for future therapeutic applications.

**Levels of Dyrk1A control self-renewal divisions in the Subependymal Zone (SEZ)**

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Neural stem cells (NSC) of the mammalian SEZ can self-renew while continuously producing intermediate fast proliferating precursors that differentiate into neurons. Our group and others have contributed to elucidate some of the signals present in the SEZ environment that control this lineage progression (Ramírez-Castillejo et al., 2006). Much less is known about the

integration of the signaling pathways activated by these niche factors, however the final response of the cells (self-renewal, proliferation, survival or differentiation) depends on this interplay. The human DYRK1A gene is included in the Down Syndrome Critical Region of chromosome 21 and inactivation of its orthologues in mice results in abnormal brain development (Fotaki et al., 2002) suggesting that the kinase Dyrk1A could regulate neuronal differentiation and/or proliferation of embryonic progenitors (Yang et al., 2001; Hämmerle et al., 2002). Interestingly Dyrk1A is widely expressed in the adult brain, staining the nucleus and the cytoplasm of both neurons and astrocytes (Martí et al., 2002). However the specific postnatal functions of this kinase have not been described so far. We have recently observed that Dyrk1A is strongly expressed in the mouse SEZ so we decided to explore if it could be controlling adult progenitor behavior in this germinal niche. Our data demonstrate that Dyrk1A haploinsufficiency results in reduced numbers of NSCs, in vivo and in vitro, due to impairment in their self-renewal in response to EGF but not FGF. The results indicate that normal levels of Dyrk1A protein are necessary to maintain EGFR expression which determines long-term persistence, but not generation, of adult NSCs and subsequent defects in self-renewal. Given that Dyrk1A has been related to several SEZ factors, like Shh or FGF, this kinase might serve to interprete the input received by the NSCs in this adult germinal zone.

**Balance between BMP4 and activin signaling regulates FGF3 expression and epithelial stem cells in mouse incisors**

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In a continuously growing mouse incisor epithelial stem cells are located in cervical loops. Stem cells proliferate within stellate reticulum and part of the progeny invades the basal epithelial cell layer. These cells differentiate into enamel forming ameloblasts only on the labial side. Labial side cervical loop differs from the lingual side also because of large stellate reticulum. Bone morphogenetic protein4 (BMP4) is known to induce the differentiation of ameloblasts while the proliferation of the epithelium is stimulated by Fibroblast growth factor10 (FGF10) and FGF3. Follistatin inhibits signalling of several Transforming growth factorβ superfamily members. We analysed mice overexpressing *Follistatin* in epithelium (*Keratin-14 Follistatin*) and noticed that they had small incisors with a hypoplastic cervical loop on the labial side. In contrast, *Follistatin*$^{-/-}$ mice had enlarged stellate reticulum on the lingual side. The expression of *Fgf3* was down regulated in the *Keratin-14 Follistatin* mice but in the *Follistatin*$^{-/-}$ incisors there were ectopic expression on the lingual side. In tissue culture experiment BMP4 was able to inhibit *Fgf3* expression while ActivinA induced ectopic expression of *Fgf3* on the lingual side and stimulated epithelial cell proliferation in the cervical loops. This effect was reversed by inhibiting Activin receptor-like kinase receptors. The enamel formation and growth of incisors were more affected when there was less FGF signaling.

Our results indicate that the epithelial stem cell niche in the incisor is redundantly regulated by FGF3 and FGF10, and BMP4 negatively controls the expression of *Fgf3* and epithelial cell proliferation. Activin counteracts the inhibition on the labial side and thus stimulates stem cell proliferation. Follistatin contributes to the formation of normal asymmetric incisor by inhibiting the function of Activin on the lingual side.

**Identification of endogenous LXR ligands and characterisation of the mechanism of LXR-dependent transcriptional regulation in the mouse ventral midbrain**

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Liver X Receptors (LXRs) are ligand-dependent nuclear receptors known to regulate cholesterol metabolism. This laboratory has recently shown that LXRs regulate the balance between DA neurogenesis and gliogenesis in the embryonic Ventral Midbrain (VM). Our work now focuses in identifying the endogenous ligands, such as oxysterols and ketocholesterols, that specifically activate LXR alpha and/or LXR beta in embryonic VM. E11 mouse VM oxysterols have been extracted and fractionated chromatographically by HPLC. Using a luciferase reporter gene assay, we have identified five HPLC fractions that contain candidate endogenous LXR ligands. Interestingly, different positive fractions come from neutral and acidic extractions, suggesting that the different fractions may represent different molecular entities. These are currently being subjected to HPLC-coupled Electrospray Tandem Mass spectroscopy in order to identify the molecular structure of the candidate endogenous LXR ligand. We also work on the characterisation of the mechanism of LXR-dependent transcriptional activation and repression by novel LXR ligands. The role of specific repressors such as the nuclear receptor co-repressor (N-CoR) are being examined. Work in SN4741 cells has shown that co-transfection of N-Cor with LXRa or LXRb reduces the expression of the luciferase reporter gene. Addition of the LXR ligand 22(R)-hydroxycholesterol to the cells partially rescues this inhibitory effect. Our results also show that both LXRa and LXRb are regulated in the same way by N-CoR, since the pattern of luciferase expression was the same for the two receptors. Our ultimate aim is the implementation of LXRs and the novel ligands identified to enhance DA differentiation and reduce gliogenesis in mouse neural stem cells as well as in embryonic stem cells.

**Expression of pluripotency marker, UTF1, is restricted to a subpopulation of early A spermatogonia in rat testis**

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The population of early A spermatogonia includes stem cells that possess spermatogonial stem cell properties. Recent reports suggest that these cells have the ability to regain pluripotent properties. Here we show that expression of the pluripotency marker undifferentiated embryonic cell transcription factor 1 (UTF1) is restricted to distinct germ cells within the testis. In

embryonic and neonatal testes, all gonocytes were found to strongly express UTF1. During further testicular development, expression of UTF1 was restricted to a subset of A spermatogonia which became smaller with increasing age. Ultimately, in the adult rat testis, only a small subset of the A spermatogonia expressed UTF1. Remarkably, even in testes of vitamin A deficient rats, in which the early A spermatogonia ($A_s$, $A_{pr}$ and $A_{al}$) are the only type of spermatogonia, only a subset of the spermatogonia expressed UTF1. In adult rat testis expression of UTF1 is restricted to a subpopulation of the PLZF positive early A spermatogonia. Furthermore, the observed distribution pattern of UTF1 expressing cells over the different stages of the cycle of the seminiferous epithelium suggests that the expression of UTF1 is restricted to those $A_s$, $A_{pr}$ and short chains of $A_{al}$ spermatogonia that are in the undifferentiated state and therefore maintain the ability to differentiate into A1 spermatogonia in a next round of the epithelial cycle or possibly even in other directions when they are taken out of their testicular niche.

**Smad interacting protein 1 (Sip1) is essential for neural differentiation of murine embryonic stem cell in vitro**

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Sip1 is a zinc finger/homeodomain containing DNA-binding protein of the Zfhx family of transcriptional repressors. äEF1 and Sip1, the only two members in vertebrates, have similar structural features and DNA binding specificity, and both bind to activated receptor-regulated (R-) Smads and the co-repressor CtBP (C-terminal binding protein) as well as to other proteins. However, only Sip1 binds directly to such Smads. Loss of heterozygosity of ZFHX1B (zinc finger homeobox 1B protein), encoding SIP1, is implicated in the etiology of Mowat-Wilson Syndrome (MWS). All patients with typical MWS present severe mental retardation, in addition to multiple additional congenital defects. Studies in mouse and Xenopus embryos further indicate a role for Sip1 in early neural tissue formation. In addition to our past and ongoing work with conditional knockout mouse models, we recently established gene-targeted Sip1-deficient mouse embryonic stem (ES) cells to address its role during neural differentiation in vitro. Analysis of neural-specific marker gene expression revealed that neural differentiation of these ES cells is strongly affected. Our findings therefore show that Sip1 is essential for neural differentiation of ES cells in vitro.

**Regulation of midbrain dopaminergic neuron development by Wnts**

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Wnts are secreted proteins that regulate processes such as cell fate specification, proliferation, and self-renewal. Wnt signalling appears to be involved in the majority of processes required to generate a fully functional neuron, participating in steps such as neural induction, precursor proliferation, differentiation, migration, axon guidance, synaptogenesis and dendritogenesis. Embryonic stem cells (ESC) differentiated into dopaminergic neurons (DN) are able to properly function in the adult brain in vivo and rescue histological, biochemical and behavioural parameters in animal models of Parkinson's disease, but tumor formation is still a risk in stem

cell-based replacement approaches. For this reason, we need a more extensive and profound knowledge about the mechanisms that control SC proliferation and differentiation. The strong effect of Wnt5a on midbrain dopaminergic precursor differentiation in culture, suggested a possible developmental defect in Wnt5a nulls. Preliminary results suggest deficiencies in differentiation and a convergence-extension in the midbrain Wnt5a nulls, but do not present a lack of DN. We hypothesize that Wnt5a function might be compensated for by another non-canonical Wnt such as Wnt11, which is also expressed in the midbrain. Thus, are currently examining whether there is a functional redundancy between these two non-canonical Wnts during mouse dopaminergic neuron development.

**Characterisation of hematopoietic clusters in the mouse embryo**

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During mouse embryogenesis, hematopoietic stem cells (HSC) activity is first detected in the aorta-gonad-mesonephros (AGM) region at E10.5 and increases afterward. Hematopoietic clusters, which are closely associated with endothelium of dorsal aorta, are thought to be putative HSC, but these are observed earlier than the emergence of HSC activity. To examine the relationship between HSC and hematopoietic clusters, we performed whole-mount immunohistochemistry and 3D confocal microscopic analysis, and analyzed clusters quantitatively at different stages. In contrast to HSC activity, number of c-Kit+ clusters in the dorsal aorta decreases between E10.5 and E11.5, suggesting that clusters are heterogeneous population and maturation into HSC might occur within the clusters.