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New roles for Gsk-3β and Wnts during skull vault osteogenesis

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The timing of frontal bone ossification is critical for protection of the newborn brain. Defects in timing can lead to human disorders such as craniosynostosis (premature fusion of the cranial sutures). We hypothesize that changes in Wnt/β-catenin signalling are important for skull ossification, and are using genetic approaches to test this hypothesis. In both GSK-3$\beta^{-/-}$ and Axin2$^{-/-}$ embryos, we observed reduced osteogenesis in the frontal bone. Loss of either of these genes leads to up-regulation of the Wnt pathway, via nuclear localization of β-catenin. Compound mutant phenotypes suggest that GSK-3β and Axin2 synergize during ossification of the skull. Furthermore, we find differential expression of Wnt inhibitors between the frontal and parietal bone. These preliminary data suggest that Wnt antagonists in the frontal bone are critical for setting the rate of osteogenesis.

Imaging of melanoblast migration in embryonic skin

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Melanoblasts, the embryonic precursors of melanocytes, are derived from the neural crest and migrate along the dorsolateral pathway. They populate the epidermis around embryonic day 12 (E12) and localize to embryonic hair follicles between E13·5 and E16·5. Historically, imaging of melanoblast migration during mouse development has been hindered by the difficulty in culturing embryonic skin and the lack of good fluorescent markers. To overcome this, we labelled the melanoblast lineage by crossing transgenic mice that express Cre recombinase under the control of the tyrosinase promoter with ROSA26-EYFP reporter mice. Using a novel culture system, we were then able to perform live-cell imaging of melanoblast migration in embryonic skin for the first time. Movies generated by time-lapse confocal microscopy reveal that melanoblasts are highly mobile and allow measurement of the dynamics of their migration. It is anticipated that this technique will allow us to unpick the molecular mechanisms that control melanoblast dispersal throughout the embryonic dermis and subsequent localization to the developing hair follicles.

RASSF7: a new possible therapeutic cancer target?

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The N-terminal (NT) RASSF subfamily represents a distinct and evolutionary conserved group of Ras association domain containing proteins RASSF7, RASSF8, RASSF9 and RASSF10 (reviewed in Sherwood *et al.*, 2009). Although their biological function is still unclear, at least some of these proteins may play a role in oncogenesis. Therefore, the study of this family may be particularly interesting for

understanding tumour progression. Our objective is to understand the function of RASSF7. Previously, we found that in *Xenopus* embryos RASSF7 knockdown provokes cell mitotic arrest, nuclear fragmentation and ultimately cell death (Sherwood *et al*., 2008). We are now elucidating the function of mammalian RASSF7. We found that RASSF7 is broadly expressed during mouse embryogenesis and in adult tissues. It is also expressed in every human cell line tested so far. Knockdown of RASSF7 in HeLa cells causes a reduction in cell numbers, an arrest in mitosis and extensive DNA fragmentation. Finally, we show that consistent with a role in mitosis RASSF7 localizes to centrosomes. Thus, RASSF7 represents a new regulator of the fundamental process of mitosis.

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The origin of asymmetry: Pkd1l1 as a mechanosensor of nodal flow

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The early vertebrate embryo is bilaterally symmetrical, but gives rise to a markedly asymmetric body organization, with various internal organs positioned and patterned in a left–right asymmetric manner. Specification of the mammalian left–right axis is controlled by motile cilia-driven fluid flow in the embryonic node, a pit-like structure located at the anterior tip of the primitive streak. How the embryo perceives this nodal flow remains the subject of much debate. During an N-ethyl-N-nitrosourea (ENU)-driven forward genetic screen, we isolated a developmental mutant, *rks*, which displays striking defects in left–right patterning; no left side was specified, resulting in right isomerism. The *rks* mutation disrupts a conserved motif within an extracellular polycystic kidney disease (PKD) domain of the polycystic kidney disease gene family member *Pkd1l1*. Other members of this family are involved in flow-induced calcium signalling, and we hypothesize that *Pkd1l1* plays a similar role at the embryonic node, where symmetry is first broken and *Pkd1l1* expression is enriched. The *rks* mutant largely phenocopies the *Pkd2* null mouse, leading to the notion that Pkd1l1 and Pkd2 act together to sense nodal flow: we are currently investigating the biochemical basis behind this possibility. Taken together, our results indicate that Pkd1l1 is the long-undiscovered sensor of nodal flow, required for the determination of the left–right axis.

Identification of putative neural tube defect-causing mutations in planar cell polarity genes

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Neural tube defects (NTDs) are a common group of birth anomalies affecting approximately 0·1% of pregnancies worldwide. NTDs arise when the neural tube, the embryonic precursor of the brain and spinal cord, fails to close during embryo development. Craniorachischisis, the most severe form of NTD, results from failure of closure throughout the hindbrain and the entire spinal neural tube. Genetic factors are known to play a major role in determining NTD risk, but few genes for human NTDs are known.

The generation of NTD mouse mutants and the subsequent identification of NTD-causing genes have shown that mutations in genes associated with a non-canonical Wnt signalling pathway – the planar cell polarity (PCP) pathway – can cause the craniorachischisis phenotype in mouse. In this study, a number of PCP and PCP-related genes have been screened for mutations in an attempt to investigate their potential pathogenicity in human craniorachischisis. DNA sequence analysis involving 36 cases led to the identification of a number of unique, non-synonymous sequence changes in two genes: *SCRB1* and *CELSR1*. Of particular interest was the finding of five non-synonymous sequence changes in *CELSR1* and three non-synonymous sequence changes in *SCRB1*, all of which were absent from control chromosomes.

Investigation into the effect of the identified putative *SCRB1* and *CELSR1* mutations on protein function is currently underway. One hypothesis being tested is that *SCRB1* and *CELSR1* mutations may disrupt protein function by altering interaction with known binding partners. To date, CELSR1 protein interactors are not known. However, SCRB1 is known to interact with the PCP protein VANGL2, a guanine nucleotide exchange factor (GEF) protein

β-PIX and an apical–basal cell polarity protein lethal giant larvae 2 (LGL2). Immunoprecipitation experiments suggest that putative *SCRB1* mutations do not affect interaction with VANGL2, β-PIX or LGL2, indicating that *SCRB1* mutations may affect protein function in another way, not known as yet. Additional functional assays are currently ongoing in an attempt to elucidate the pathogenicity of these genetic variants. Confirmation that *SCRB1* and *CELSR1* mutations are causative in humans with severe NTDs would be a step towards identifying the genetic basis of human NTDs.

Scribble is required for lumen formation in the mammalian lung

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Lung development results in the production of a network of branches specialized for gas exchange. Correct lumen formation is essential for lung function; however, the mechanism of lumen formation in the lung is poorly understood. *In vitro* studies have shown cell polarity, cell–cell adhesion and establishment of an apical membrane are pre-requisites for lumen formation. Scribble has a role in apical–basal polarity in *Drosophila* and is required to maintain tight junctions, thus we sought to determine the role of Scribble in mouse lung lumen formation.

Scribble^{Crc} lungs appear smaller, with fewer, malformed branches compared to wild-type. In culture, *Crc*/*Crc* lungs are denser, and buds are less visible compared to controls. E14·5 *Crc*/*Crc* lungs display a 46% reduction in the number of 'normal' airways with an organized lumen. Immunostaining highlighted severely disorganized airways in *Crc* homozygotes. Consistent with previous studies, both adherens junctions and tight junctions are disrupted in *Crc*/*Crc* lung epithelia, yet apical–basal polarity appears largely unaffected.

We therefore propose that Scribble is required for normal lumen formation in the mammalian lung. Mutations in Scribble lead to defects in lumen formation as a result of disrupted cell adhesion and loss of cell–cell contacts during epithelial tube formation.

Characterization of imprinted genes in mouse: *Grb10* and *Dlk1*

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Genomic imprinting provides an exception to the Mendelian rule of inheritance. Imprinted genes are preferentially expressed from only one of the parental alleles and play important roles in the development of embryonic and extra-embryonic lineages and postnatally in the maintenance of glucose homeostasis. The conflict theory predicts that maternally expressed genes act as growth suppressors, limiting the usage of maternal resources, and paternally expressed genes function in an opposite manner. We propose the hypothesis that the *Grb10* (maternally expressed, inhibitor of foetal growth) and *Dlk1* (paternally expressed, growth promoter) genes act antagonistically in a common genetic pathway. To test this hypothesis, we have generated Grb10/Dlk1 double knockout mice and performed a phenotypic characterization. Results obtained from allometric and metabolic analyses, together with histological studies, reveal strong similarities between the phenotypes of Grb10 and Grb10/Dlk1 knockout mice. According to our hypothesis, these results imply that the *Dlk1* and *Grb10* genes are indeed involved in the same genetic pathway, in which Dlk1 is an inhibitor of Grb10 which is in turn acting as a growth suppressor. Our finding provides an insight into a novel gene network which involves two imprinted genes known to play significant roles in normal growth, development and metabolism.

Effects of a truncation in the imprinted *Gnas* cluster: an emerging bone and growth phenotype

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The imprinted *Gnas* cluster contains a complex group of paternally, maternally or biallelically expressed genes. Within this cluster are paternal *Gnasxl*, and *Gnas*, which is maternally expressed in specific tissues.

Previous mouse studies have shown that ablation of paternal *Gnasxl* results in severe growth retardation and reduced adiposity, while ablation of maternal *Gnas* results in an opposite phenotype of increased adiposity.

Comparing two paternally inherited transgenic mouse lines we show that one line results in over-expression of *Gnas* in imprinted tissues. These mice exhibit the inverse phenotype to the maternal *Gnas* knockout with moderate growth retardation and reduced adiposity. Our second line results not only in over-expression of *Gnas* but also in truncation of *Gnasxl*, expected to result in a non-functional protein. These mice exhibit severe growth retardation and reduced adiposity. Moreover, it is only when *Gnasxl* is truncated that a reduction in bone mineral density is observed. To our knowledge, this is the first time that a bone phenotype has been reported in whole animal *Gnas* cluster mutants and we hypothesize that *Gnasxl* may provide a new link between bone and fat metabolism.

Impaired thermogenesis and persistent hypothyroidism caused by altered dosage of imprinted genes

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Neonatal survival in mammals is crucially dependent upon the maintenance of body temperature. Thermogenesis by brown adipose tissue (BAT) comprises the majority of neonatal heat production. BAT develops perinatally in mice, and requires the integration of adipogenic and thermoregulatory gene pathways. We describe a regulatory mutation in the imprinted cluster on mouse chromosome 12 that results in animals that are severely compromised in the early postnatal period due to failure to maintain body temperature. This cluster contains the genes encoding Delta-like homologue 1/Preadipocyte factor (Dlk1/Pref1) and iodothyronine deiodinase type 3 (Dio3), the expression of which is perturbed in mutants. We show that Dlk1 and Dio3 functions converge on the development of brown fat at the transition to independent life. Surviving mutants exhibited persistent alteration to the thyroid hormone axis, increased adipose mass and glucose intolerance. This confirms that imprinted loci can act as critical determinants in establishing and maintaining metabolic set points in mammals.

Endothelial thymosin β4 acts during vascular development to facilitate mural cell recruitment to the vessel wall

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Thymosin β4 is an endogenously occurring 43-amino acid peptide, which has been shown to be secreted by the myocardium during embryogenesis. In this setting, T β4 stimulates epicardial progenitor cell invasion of the myocardium in order to form the coronary vasculature. We expanded these investigations to determine whether T β4 plays a role in the development of the systemic vasculature.

In situ hybridization and immunofluorescence studies revealed expression of T β4 in the endothelium from E9·5 onwards. Initially, a T β4 knockout mouse was used to assess the effect of global loss of T β4 function on vascular development. A proportion of T β4 null embryos display pericardial and coelomic cavity haemorrhage at E10·5, caused by reduced mural cell recruitment to the dorsal aorta. In order to further understand the mechanism through which T β4 acts, T β4 was knocked down specifically in the endothelial cell lineage using a Tie2-Cre T β4 shRNA mouse. This recapitulates the phenotype of the global knockout and suggests a model by which T β4 acts in a paracrine fashion to stimulate the differentiation, migration or maturation of mural cells recruited to developing vessel walls.

DKK1 and GPCRX, two new transgenic mouse models for innovative Alzheimer's disease drug discovery

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Alzheimer's disease (AD) may arise from the deposition of a small peptide called Amyloid-Beta (Aβ) that is normally found in healthy individuals, but is abnormally abundant in the brains of AD patients. *In vitro* studies have shown that primary rat cortical neurons challenged with Aβ peptide up-regulate a set

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of genes. Among these up-regulated genes two drug-targetable candidates, DKK1 and a G-protein coupled receptor (GPCRX), have been chosen to develop inducible *in vivo* models to investigate correlations between their up-regulation and AD features and to screen new compounds against this common form of neurodegenerative disorder.

DKk1 could contribute to the neurodegenerative pathology through the activation of Gsk3-β and may lead to a hyperphosphorylation of Tau protein that in turn is responsible for the breakdown of microtubules in affected neurons contributing therefore to the process of neuronal loss. Direct or indirect Pharmacological modulation of tau hyperphosphorylation might therefore represent a therapeutic strategy for such disorders.

The GPCRX receptor could be involved in astrocyte proliferation leading to astrogliosis. Blocking these two pathways might ameliorate the process of neuronal loss. This talk will focus on strategies adopted to develop these transgenic models and experiments planned to characterize these animal models.

The E3 ubiquitin ligase Arkadia2C regulates BMP signalling in motor neurons

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TGF-β ligands act as morphogens in the vertebrate embryo, eliciting different responses dependent on concentration; additional regulation of signalling thresholds is provided by intracellular inhibitors. The nuclear RING-domain E3 ubiquitin ligase Arkadia/Rnf111 enhances transcription of Nodal/TGF-β target genes by degrading promoter-bound complexes of the effector Smad2/3 and its co-repressors SnoN and Ski. Although these co-repressors also act on BMP signalling, Arkadia appears to be specific to Smad2/3.

BMP signalling has been shown to be involved in patterning of the spinal cord; however, little is known about cellular responses and threshold levels or its role in later stages of neuronal development such as connectivity. We have characterized Arkadia2C, a novel homologue of Arkadia, which is specifically expressed in neurons and found evidence that Arkadia2C enhances BMP signalling in a neural context where it is involved in motor neuron connectivity.

The Arkadia proteins are highly conserved within the substrate interaction domains and the RING domain that is essential for ubiquitin ligase activity. Arkadia2C mutant mice exhibit abnormal limb posture and movement and mild hypoxia/cyanosis; they die during the first postnatal weeks from dehydration/starvation most likely due to difficulty in feeding. Analysis shows defects in motor innervation of the forelimb and diaphragm.

Sprouty gene function is required for normal sensory cranial nerve morphology

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Sensory neurons located in the sensory cranial ganglia innervate the head, heart and gut, and are derived from the placodes (localized regions of thickened ectoderm) and late migrating neural crest cells. Fibroblast growth factors (FGF) have been implicated in both placode and neural crest development. The Sprouty (Spry) gene family encodes feedback antagonists of FGF signalling. *Spry1* and *Spry2* are transiently expressed in the region of the developing epibranchial placodes and late migrating neural crest cells prior to cranial nerve formation. Embryos lacking both *Spry1* and *Spry2* demonstrate a requirement for Spry in cranial nerve morphogenesis, with defects in cranial nerves V, VII/VIII, IX and X. Aberrant *Sox10* expression in these mutants imply a role for the *Spry* genes in late migrating neural crest cells. However, normal sensory cranial ganglia are found in neural crest-specific conditional *Spry1/2* double knockout ($Wnt1cre;Spry1^{flox-};2^{flox-}$) embryos, indicating that *Spry* expression within neural crest cells is not required for correct development. Furthermore, the expression of placodal and early neuronal markers is altered in *Spry1; 2* double knockouts, suggesting a possible role in placode formation and differentiation.

Wellcome Images

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Wellcome Images is the Wellcome Library's picture library and consists of an extensive collection of images on the history of medicine, biomedical science and clinical medicine. Wellcome Images is composed of two main collections: historical and biomedical. The historical collection is made up of images taken from existing material in Wellcome Library. The biomedical collection exists thanks to the contributions of external scientists and teaching hospitals.

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Role of antisense transcripts at the DIRAS3 locus

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Imprinted genes are monoallelically expressed in a parent-of-origin-dependent way. This is regulated by differentially methylated regions of DNA known as DMRs. Loss of imprinting (loss of monoallelic expression) occurs in cancer along with associated changes in DMR methylation.

DIRAS3 is a paternally expressed tumour suppressor gene coding for a ras homologue with growth suppressor activity. DIRAS3 undergoes loss of expression in various cancer types including breast, ovarian, pancreatic, oligodendrogliomas and ovarian cancers. Most imprinted genes exist in clusters including at least one non-coding RNA, often at least partially antisense to the imprinted gene. However, no other transcripts, imprinted or otherwise, have been identified in the region of DIRAS3. I have recently identified an antisense transcript that by virtue of its genomic location could have a role in regulating DIRAS3 expression and I am working to understand its function in normal development and in cancer. I have identified the promoter region and several different splicing variants of this transcript. I am working to understand whether it has a function in regulation of DIRAS3 using *in vitro* assays to mimic the genomic organization of the locus.

A case-by-case analysis of imprinted retrogene evolution

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Retroposition is a widespread phenomenon resulting in the generation of new genes that are initially related to a parent gene via very high coding sequence similarity. We examine the evolutionary fate of four retrogenes generated by such an event; mouse *Inpp5f_v2, Mcts2, Nap1l5* and *U2af1-rs1*. Being subject to parental imprinting unites these genes. We first provide new data on the age of these retrogene insertions. Using codon-based models of sequence evolution, we show these retrogenes have diverse evolutionary trajectories, including divergence from the parent coding sequence under positive selection pressure, purifying selection pressure maintaining parent-retrogene similarity and neutral evolution. Examination of the expression pattern of retrogenes shows an atypical, broad pattern. Protein 3D structure modelling reveals that a positively selected residue in *U2af1-rs1*, not shared by its parent, may influence protein conformation. Our case-by-case analysis of the evolution of four imprinted retrogenes emphasizes that retrogenes are a widely varied group of genes, in terms of expression pattern and evolutionary path.

Variable methylation of the imprinted gene, SNRPN, supports a relationship between intracranial germ cell tumours and neural stem cells

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Germ cell tumours (GCTs) are a diverse group of tumours found in the midline of the body. GCTs

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found within and outside the gonad are generally believed to derive from a common origin, primordial germ cells. The evidence in favour of this model is that extragonadal GCTs, including intracranial GCTs, share molecular features, such as the degree of methylation of imprinted genes and the expression of GCT markers, with other GCTs. However, the origin of extragonadal GCTs is still contentious. We have proposed that in the brain, endogenous neural stem cells (NSCs) are a more plausible origin of so-called 'intracranial GCTs'. We showed that methylation was variable in NSCs derived from embryonic mouse brains. Consistent with this observation, a human NSC line exhibited remarkably variable methylation of *SNRPN*. Together, these observations suggest that, at an early developmental stage, NSCs exhibit features consistent with a role as the cell of origin of some GCTs, such as teratomas and yolk sac tumours. These findings undermine the assumption that all GCTs arise from germ cell progenitors, equally supporting the alternative suggestion that NSCs may give rise to some so-called 'intracranial GCTs' during early development.

Is inappropriate Y Gene expression in XY females a cause of their impaired fertility?

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XY females in mouse and man are typically sterile or nearly so. We have been studying two XY female mouse models that differ markedly in their fertility. The first, XY^{Tdym1}, carries a Y chromosome with an 11 kb deletion removing *Sry*, and are near sterile. The second, XY^{d1}, has a Y chromosome with a 3–4 megabase deletion removing the majority of copies of *Rbmy* and bringing *Sry* close to the centromere where it is no longer transcribed; these mice are of good fertility. We surmised that the near sterility of XY^{Tdym1} females was due to 'inappropriate' expression of *Rbmy* in oocytes. Although *Rbmy* is indeed expressed in XY^{Tdym1} oocytes, addition of an *Rbmy* transgene to XY^{d1} females did not impair their fertility. We then made the chance discovery that XO females carrying a *Zfy2* transgene are sterile. *Zfy2* is located just proximal to *Sry* so it seemed possible that it may be repressed in XY^{d1} oocytes but expressed in XY^{Tdym1} oocytes and this proved to be the case. *Zfy2* is a putative transcription factor so the challenge now is to try and identify the downstream genes that when expressed in oocytes result in severely impaired fertility.

Molecular characterization of *hitchhiker*, a mouse mutant exhibiting spina bifida

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Hitchhiker (*hhkr*), a mutant exhibiting spina bifida, exencephaly and polydactyly, was identified through an ENU G3 recessive screen at Harwell. The mutation was mapped to *Tubby like protein 3* (*Tulp3*), and is predicted to cause loss of all functional domains. Detailed examination of *hhkr* mutant embryos has revealed a failure to complete neural tube closure in the head or spine, and we are trying to identify the primary cellular defect leading to this phenotype. Reduced dorsolateral hinge points were observed in the caudal neural tube, and this is likely the result of increased Shh pathway activity. In contrast, in the head, Shh pathway activity appears to be unaffected. Examination of cellular processes reveals no change in apoptosis or proliferation in this region. The rate of apoptosis in *hhkr* hindbrain is normal; however, there is a significant increase in proliferation in the hindbrain of mutant embryos.

Examination of markers of dorso-ventral patterning in the neural tube reveals a ventralization of the caudal spinal cord, in hitchhiker. This phenotype suggests that *Tulp3* normally acts as a negative regulator of the Shh signalling pathway. Crosses with other mutants demonstrate that *Tulp3* acts genetically downstream of Shh and Smoothened, but upstream of Gli2. Whether *Tulp3* acts directly or indirectly on the pathway is unknown. We are investigating the interacting protein partners of *Tulp3*, using affinity co-purification combined with data generated from a yeast-2-hybrid screen, in order to determine the molecular function of *Tulp3* and help to define how *Tulp3* promotes neural tube closure.

A new mouse ciliopathy with a deletion in the Talpid3 gene, first identified in chicken

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We have generated a Talpid3 mutant mouse by deleting a highly conserved region of the Talpid3 gene using the Cre-loxP system. A mutation in Talpid3 is responsible for the chicken talpid[3] mutant phenotype, which includes embryonic lethality, polydactylous limbs, dorsalized neural tube and polycystic kidneys. Similar phenotypes are seen in human syndromes with mutations in genes encoding centrosomal or intraflagella transport proteins leading to defects in ciliogenesis. Chicken Talpid3 localizes to the centrosome and cells of chicken talpid3 mutants lack primary cilia. Analysis of the chicken Talpid3 protein identified a highly conserved region, required to rescue ciliogenesis and sufficient for centrosomal localisation. Constitutive removal of this highly conserved region in mouse results in lack of primary cilia, embryonic lethality, pericardial oedema and abnormal heart looping. Conditional removal of this region in the developing mouse limb bud results in abnormal expression of Hh target genes, leading to short polydactylous limbs with abnormal ossification. We can use the talpid3 mutant mouse mutant to conditionally remove cilia in different tissues or at different times to further our understanding of the role of cilia in development and cross with other ciliopathy mutants to test genetic interactions between Talpid3 and other genes required for ciliogenesis.

Retinal repair by photoreceptor precursor transplantation

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Degenerative disease of the retina is the leading cause of untreatable blindness in the developed world, with inherited degeneration affecting 1 in 3000 of the population. Visual impairment is often caused by the loss of photoreceptor neurons and central visual acuity deteriorates rapidly once the cone-rich macula is affected. Repair of such damage by cell transplantation is a feasible approach, as photoreceptor degeneration initially leaves the inner retinal neurons intact. A transplanted photoreceptor need only make one synaptic connection to contribute to the retinotopic map.

Transplantation of enriched post-mitotic photoreceptor precursors from embryonic mouse retinae to the non-neurogenic adult retina led to integration of grafted cells in the outer nuclear layer, where photoreceptor nuclei reside. The majority of photoreceptor precursors developed into rod photoreceptors with mature morphology, displaying synapses and light-sensory structures (inner and outer segments). A small proportion of integrated cells exhibited cone photoreceptor features and expressed mature cone markers. In further transplantation experiments, photoreceptor precursors also integrated into the retina of two models (*RetGC1*$^{-/-}$, *Crb1*$^{-/-}$) of human Leber's congenital amaurosis, an early onset retinal degeneration.

The UniProt Knowledgebase (UniProtKB): a freely accessible, comprehensive and expertly curated protein sequence database

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100 other databases including organism-specific, domain, family and structural databases. Recent developments to UniProtKB include: the release of a new unified website providing added functionality including a new interface and search engine, the release of the first draft of the complete human proteome in UniProtKB/Swiss-Prot, and the addition of additional bibliography information in UniProtKB by extracting literature citations from external databases. UniProt is updated every 3 weeks and can be accessed or downloaded from http://www.uniprot.org.

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