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TGF-β/nodal signalling can be regulated by post-transcriptional control of Arkadia RNAs

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The Transforming growth factor-β (TGF-β) family control diverse cellular processes and specify cell-fate/differentiation during embryogenesis in vertebrates and invertebrates. Mutations disrupting TGF-β signalling lead to developmental abnormalities and a range of diseases such as cancer. Nodal is a major TGF-β signal, responsible for gastrulation in embryogenesis. *Arkadia* (*Akd*) was discovered by mouse gene-trap mutagenesis and encodes a nuclear E3 ubiquitin ligase. Akd allows the Nodal signal to reach its maximum level and *Akd-null* mice lack mammalian organiser (MO) and mesendodermal tissues. Although *Akd* RNA is ubiquitously expressed, *Akd-null* mice lose a subset of Nodal-dependent functions. The specificity of Akd function is therefore most likely to be regulated post-transcriptionally or by co-factors. *Akd* possesses differentially spliced 5′ untranslated regions (UTRs) and large 3′ UTR. We have employed bioinformatics and developed a reporter system to address Akd post-transcriptional regulation. Akd RNA may initiate from different promoters and 5′ UTR differential splicing, upstream AUGs (uAUGs) and open-reading frames upstream (uORFs) may regulate protein translation. 5′ and 3′ UTRs can interact to either destabilise or decrease translational efficiency of RNA. The nature of this interaction is cell-type and signal level dependent. These data may represent mechanisms by which translational control of *Arkadia* is achieved and ultimately how TGF-β/Nodal signalling is regulated during embryogenesis.

A roof plate dependent enhancer controls Hop expression in developing cortex

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The current concept of cerebral cortex patterning involves transcription and paracrine factors that act in a gradual and concerted manner to set up area identities. In order to identify putative cortical patterning genes we performed microarray expression analysis comparing gene expression in five regions of the E16.5 mouse cortex. From the applied genes 114 fulfilled the criteria for regionalised expression. Here we present the cortical expression of selected candidate genes as determined by in situ hybridisation and discuss their putative involvement in corticogenesis. One of these genes, Homeodomain only protein (Hop), revealed a dynamic expression in developing cortex and other CNS structures. We identified a 418 bp enhancer element capable to drive reporter activity in Hop expressing regions of the CNS. Roof plate transplants were able to induce enhancer activity in slice cultures from E11.5 and E12.5, but not older cortices. Utilizing a novel in vitro approach we identified a 200 bp minimal enhancer not containing sequence elements that have been involved in cortical gene regulation so far. We propose that the CNS Hop enhancer is the target of a roof plate dependent pathway with yet unknown downstream mediators.

Investigating the potential functions of murine Angiogenin 4

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Human angiogenin is a member of the pancreatic ribonuclease superfamily initially identified as an angiogenic factor from tumour cell conditioned medium. However, it is now known to be expressed in a wide variety of normal tissues. The function of angiogenin in normal physiology is not yet understood. We are investigating the function of angiogenin by genetic and structure function based approaches using the mouse as a model system. The mouse has 4 angiogenins [Angiogenin 1 (Ang1), Angiogenin 3 (Ang3), Angiogenin 4 (Ang4) and Angiogenin Related Protein (AngRP)] as compared to one in humans. The initial part of our work has focused on mouse Ang4 which has been implicated in innate immunity but whose angiogenic potential is not yet determined. We have cloned and expressed this protein in a bacterial expression system and have developed a new modified *in vitro* assay of angiogenesis to investigate if Ang4 is indeed angiogenic. Site directed mutagenesis is being carried out to identify the key residues responsible for the innate immune function and angiogenic activity (if any).

Work funded by the Wellcome Trust and Medical Research Council, UK.

Evidence of a role for Igf-2 in skeletal muscle fibre type selection

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During primary (E10.5–E13.5) and secondary (E14.5–E17.5) myogenesis two morphologically distinct myotubes are formed. These primary and secondary myotubes are also distinguished by their respective initial expression of slow and fast myosin forms. During subsequent maturation both may switch myosin expression, a poorly understood process essential for establishing fast and slow muscle groups with distinct function. Here we present data suggesting that Igf-2 plays a central role in establishing the fast muscle lineage. Between E15.5 and E17.5 Igf-2 localises to a subset of fast myosin positive secondary fibres and to primary fibres which have switched to fast myosin. Transgenic over-expression of Igf-2 during early differentiation (mCK promoter – MIG transgene) resulted in over-production of fast myosin myotubes *in vitro* and *in vivo* and in a failure to down-regulate fast-myosin in secondary myotubes in MIG mice. Fast myosin positive fibres were over-represented in a wide range of hypaxial and epaxial derived muscles and in all muscle groups examined. RNAi knock-down of Igf-2 *in vitro* reduced the proportion of Fast myotubes formed and concomittantly increased the formation of slow-myosin positive myotubes. To further illustrate the central role of Igf-2 in fast fibre formation we show that genetic disomy mutants Thp and MatDi which respectively over- and under-express Igf-2 also show an decrease (MatDi) and increase (Thp) in the proportion of fast-fibres in wide range of fast, slow and mixed muscle groups examined compared to wild-type litter mate controls.

Function of the placenta-specific *Igf2* transcript (P0) in development: A stereological perspective

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Placental development is paramount to the survival of the growing fetus, and so understanding its regulation is important for an appreciation of placental dysfunction in intra uterine growth retardation (IUGR). To elucidate factors involved in placental development the role of insulin-like growth factor 2 (*Igf2*) was investigated in the mouse placenta. The P0 transcript of *Igf2* is expressed exclusively in labyrinthine trophoblast, and when absent results in late gestation IUGR. Stereological analysis revealed that the E19 P0 mutant placenta volume was reduced compared to the wild-type littermate placenta. The labyrinth (Lz) and junctional zones were reduced in volume. In the P0 mutant Lz, maternal blood space and fetal capillary surface areas were reduced to half that in the wild-type, although given Lz volume, there is the correct amount of vascularisation. The harmonic mean thickness of the exchange membrane (LIM) was greater in P0 mutant thus the theoretical diffusion capacity of the P0 mutant placenta was significantly decreased compared to the wild-type placenta. Placenta-specific Igf-II appears to be important in controlling placental size and plays a role in remodelling of the LIM that is important for feto-maternal exchange. Altered placental morphology and size is therefore implicated as a key factor limiting fetal growth.

Functional and expression analyses in enlarged placentas of interspecies hybrids

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IHPD (Interspecific hybrid placental dysplasia) was observed in interspecific hybrids between different Mus species. Depending on the sex of the parental species in crosses and backcrosses, IHPD placentas may resemble cloned hyperplastic placentas even in morphology and gene expression patterns, as many genes were found to be commonly deregulated in these two hyperplastic placental models. Some up-regulated genes thus identified were further analysed for their roles in normal placentation and for their ability to rescue the placental hypersplasia of the AT24 congenic strain, which has a M. spretus derived proximal part of the X chromosome, by using knockout mice for the genes *Cacnb3*, *Car2*, *Fbln*, *Ncam* and *Lhx3*. A downregulated gene, *Cpe*, was also analysed for its role in normal placentation. Our results indicate that reduced expression levels of none of the up-regulated genes alone are sufficient to rescue the AT24 phenotype. However, homozygous mutation of some genes produces subtle placental phenotypes like increased giant cell population. In addition, detailed expression analyses of these genes reveal ectopic expression patterns in hyperplastic placentas.

Does Nicotinamide nucleotide transhydrogenase have a role in insulin secretion?

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Insulin release from pancreatic beta-cells is stimulated by closure of ATP-sensitive potassium (K_{ATP}) channels by ATP, resulting in membrane depolarisation. This leads to activation of voltage-gated Ca^{2+} channels, influx of calcium and exocytosis of insulin-containing vesicles. The C57BL/6J mouse strain exhibits glucose intolerance suggestive of human type-2 diabetes. K_{ATP} channels in C57BL/6J beta-cells show impaired closure in response to glucose metabolism which results in impaired insulin secretion. However, C57BL/6J K_{ATP} channels retain normal ATP sensitivity suggestive of a problem further upstream at the level of beta-cell metabolism. Using an F2 intercross (C57BL/6J × C3H/HeH control mice), three genetic loci were identified which mediate impaired glucose tolerance, underlying the chromosome 13 locus is a strong candidate gene Nicotinamide nucleotide transhydrogenase (Nnt). *Nnt* is a mitochondrial gene catalysing the reduction of $NADP^+$ by NADH. An adequate supply of NADPH into the mitochondrion is necessary to generate an electrochemical gradient resulting in ATP synthesis. Defects in this gene could influence insulin release through perturbation of beta-cell mitochondrial metabolism. C57BL/6J mice have a multi-exon deletion and >7-fold lower expression of *Nnt* compared to control mice. We have results indicating down-regulation of *Nnt* in beta-cells produces an impaired response of intracellular calcium to glucose.

Mapping candidate control elements of the Gnas imprinted locus

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The Gnas locus contains a number of imprinted promoters which splice onto common downstream exons and encode maternal-specific, paternal-specific and biallelic transcripts, including an antisense transcript and tissue-specific imprinted transcript. Three differentially methylated regions (DMRs) have been identified, two of which have been shown to be gametic methylation marks. However, we suspect that other regulatory elements exist at the locus. I have used cross-species sequence comparison to identify conserved non-coding regions, which might represent regulatory elements. Species studied include vertebrates with no evidence of imprinting, such as chicken and pufferfish, as well as a number of mammalian species including human, mouse, chimp and dog. The DNA methylation status and chromatin organisation of these conserved regions have been investigated in the mouse. A number of strong DNaseI hypersensitive sites have been identified in intergenic regions, including a pair located in the centre of the locus, close to an extensive tandem repeat, which is well conserved in mammals. Based on these analyses, potential regulatory regions have been assessed for enhancer, insulator and silencer activities in vitro. Preliminary results suggest a combination of different elements may be responsible for the regulation of the locus.

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Reciprocal imprinting and functioning of two gene products from the *Gnas* locus: XLαs and Gsα

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The mouse *Gnas* locus on distal chromosome 2 gives rise to several oppositely imprinted transcripts. Genetic evidence suggests that among these, *Gnas* and *Gnasxl*, expressed in specific tissues from the maternal and paternal alleles respectively, have opposite physiological effects. *Gnas* encodes Gsα, the alpha subunit of the stimulatory G protein, and *Gnasxl* encodes XLαs, an N-terminally extended form of Gsα with uncertain biochemical properties. By intercrossing mutant mice we have generated offspring lacking both maternal Gsα and paternal XLαs, which provide evidence for functional antagonism between these gene products. Phenotype comparisons with littermates lacking just maternal or paternal function have shown that loss of maternal Gsα effectively compensates for absence of XLαs, and for a subset of phenotypes, including survival, the reverse rescue effect is also shown. On the other hand, molecular measurements taken in brown adipose tissue show opposed functions in which the effects of maternal Gsα loss are epistatic. The different and conflicting (causal) functions inferred for *Gnas* and *Gnasxl* dovetail with the opposed (evolutionary) functions ascribed to maternal and paternal alleles under the conditions described in conflict theory.

A novel imprinted candidate gene for X-linked parent-of-origin effects on cognitive functioning in mice

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Imprinted genes are epigenetically marked so that they are monoallelically expressed in a parent-of-origin dependent manner. Whilst early work emphasised the role of imprinted genes in growth and development, more recent analyses have implicated imprinted gene function in brain and behavioural phenotypes. We have shown that the cognitive profile of 39,XO mice depends upon whether their single X chromosome is of paternal (39,X^pO) or maternal (39,X^mO) origin, a finding consistent with the presence of one or more X-linked imprinted genes; specifically, 39,X^mO mice displayed more perseverative behaviour on a serial reversal learning task relative to their 39,X^pO (and 40,XX) counterparts. Through comparing gene expression in 39,X^pO and 39,X^mO embryonic brains by microarray analysis, we have identified a candidate X-linked imprinted gene; this gene is expressed preferentially from the maternally inherited X chromosome throughout development and throughout all tissues examined. Investigations into the epigenetic basis of this parent-of-origin dependent expression and into how this gene may influence neurodevelopment and cognition are ongoing. Work supported by the BBSRC.

A small family of LTR retrotransposon-derived genes in mammals and their relation to genomic imprinting

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Ty3/Gypsy retrotransposons are rare in mammalian genomes despite their abundance in invertebrate and other vertebrate classes. The conservation between human and mouse of the few identified elements suggests that they may have evolved endogenous functions in their mammalian hosts. We identified a family of nine mammalian loci with homology to Ty3/Gypsy retrotransposons. Five map to the X chromosome while the remaining four are autosomal. Comparative phylogenetic analyses show them all to have strongest homology to the sushi-ichi element from *Fugu rubripes*. Two of the autosomal gene members, *Peg10* and *Rtl1*, have previously been shown to be imprinted, being expressed from the paternally inherited chromosome homologue. This suggests, consistent with the host-parasite response theory of the evolution of imprinting, that parental-origin specific epigenetic control may be mediated by genomic parasitic elements such as these. However, we found *Rtl1* to be highly methylated on both parental alleles throughout development. Alternatively, these elements may have preferentially integrated into epigenetically regulated domains. We also assess the imprinting status of the remaining autosomal members of this family and show them to be biallelically expressed. This indicates that the ability to undergo genomic imprinting is not an inherent property of all members of this family of retroelements.

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The role of the imprinted gene delta like 1 (*Dlk1*) in mouse development

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Dlk1 is a transmembrane protein that belongs to the *Notch/Delta/Serrate* family of signalling molecules. Four splice forms have been described; two of them release the extracellular domain and two remain membrane bound. *Dlk1* is highly expressed during embryonic stages in a wide range of tissues, being downregulated after birth. *In vitro* studies showed that *Dlk1* is important in the development of hematopoietic, adipose and neuroendocrine tissues. *Dlk1* null mice are viable but growth retarded, catching up in weight due to obesity. *Dlk1* is an imprinted gene, being expressed by the paternal chromosome only. Overexpression of *Dlk1*, transcribed from both paternal alleles, is likely to contribute to the lethal phenotype and developmental abnormalities shown in mice with paternal uniparental disomy for chromosome 12 (*pUPD12*). In order to better understand the extent to which the *pUPD12* phenotype is caused by *Dlk1* overexpression and to learn about *Dlk1* gene regulation and imprinting, *Dlk1* transgenic mice were generated. Two transgenes, a long and a short, encompassing the whole *Dlk1* gene and differing amounts of surrounding sequence were generated and 4 founders for each transgene were generated, giving rise to 4 lines of mice for each transgene. In all four lines, the shorter transgene was not expressed. However, all four transgenic lines made with the longer construct express the transgene whether it is transmitted by the mother or by the father. Phenotypic consequences of transgene expression in heterozygote and homozygote animals will be presented, tissue specific expression of transgenic *Dlk1* analysed, and comparison with the developmental abnormalities reported in *pUPD12* conceptuses described.

Epigenetic stability of human embryonic stem cells

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Embryonic stem (ES) cells are derived from a stage of development characterised by global epigenetic remodeling. The epigenetic stability of some mouse ES cells has been shown to be unstable during derivation and culture. Loss of imprinting and methylation marks has also been implicated in certain human diseases. Therefore an assessment of epigenetic stability in human embryonic stem (hES) cells is an essential step to their future clinical applications. We have used allele-specific RT-PCR to reveal generally monoallelic expression of imprinted genes in four hES cell lines. As length of time in culture increased, all the paternally-expressed genes remained monoallelic. In contrast, some higher passage hES cells transcribed a maternally-expressed gene from the previously silent allele. We have used bisulphite sequencing to analyse the methylation status of key imprinting control regions, thereby clarifying the basis of these observations. This work provides the first insights into the epigenetic stability of human blastocyst-derived pluripotent cells. Future studies will focus on the role of epigenetic regulation on hES *in vitro* differentiation.

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Isolation, expression and regulation of the *Cdx1* gene from the pufferfish *Fugu rubripes*

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The *Cdx1* gene is a *Caudal* type homeobox gene and is expressed in the ectoderm and mesoderm during mouse gastrulation and in the developing and adult intestinal epithelium where its expression is restricted to the proliferating cells of the crypts. *Cdx1* plays a critical role in axial skeletal development and in intestinal epithelial cell proliferation and differentiation. We have used a comparative genomics approach to begin to understand the regulation of this gene during normal development and in adult tissue using the compact genome of the puffer fish, *Takifugu rubripes* (*Fugu*). Orthologues of *Cdx1*, *Cdx2 and Cdx4* were identified by sequence comparison in *Fugu*. The predicted protein sequence of *frCdx1* showed a high level of identity with the mammalian *Cdx1*. There was also a strong conservation of gene organization between the human, mouse and the *frCdx1*. Comparison of the genomic environment of the human, mouse and *frCdx1* showed synteny between the mouse, human and *frCdx1*. The tissue distribution of *frCdx1* was similar to human and mouse *Cdx1* with high expression in the gut and early embryos. Comparison of the upstream sequences as well as the intronic regions of mouse, human and *frCdx1* showed conserved regions.

The importance of DNA repair gene Ercc1 in the response to ultraviolet radiation induced DNA damage in the skin

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Nucleotide excision repair is responsible for the removal of helix distorting lesions from DNA. These include cyclopyrimidine dimers and 6-4 photoproducts, caused primarily by ultraviolet radiation. Ercc1 acts in complex with XPF to make an incision 5′ of the lesions during nucleotide excision repair and as such is essential for the damage repair process. Ercc1 homozygous null animals die before weaning with a severe liver phenotype, so to study the function of Ercc1 in ultraviolet radiation induced DNA damage repair we have created a skin specific Ercc1 knockout mouse model. We have used the Cre-loxP system, where tissue specific recombination results in inactivation of the Ercc1 gene in epidermal cells. We have found our skin specific Ercc1 knockout animals to be markedly more sensitive to UVB radiation than control animals in both short and long term exposure experiments. With chronic UV exposure experiments we have seen many more tumours developing significantly sooner in experimental animals, as well as tumours induced at much lower doses than controls. After single UV doses our experimental animals display around a twelve-fold increase in sensitivity to UV induced erythema and photoimmunosuppression when compared with control littermates.

Mediation of Af4 function in the cerebellum by Siah proteins

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We have established that AF4, a putative transcription factor which had long been recognised as disrupted in childhood leukaemia, also plays a role in the CNS. *Af4* is mutated in the robotic mouse, a new model of autosomal dominant cerebellar ataxia characterised by adult-onset Purkinje cell loss and cataract. To determine the molecular basis of this mutation, we carried out a yeast two-hybrid screen and show that Af4 binds the E3 ubiquitin ligases Siah-1a and Siah-2 in the brain. Siah-1a and Af4 are expressed in Purkinje cells and colocalise in the nucleus of HEK293T and P19 cells. *In vitro* binding assays and coimmunoprecipitation reveal a significant reduction in affinity between Siah-1a and robotic mutant Af4 compared with wild-type, which correlates with reduced levels of ubiquitinated mutant Af4 and the almost complete abolition of its degradation by Siah-1a in HEK293T cells. As predicted, mutant Af4 accumulates in the robotic mouse due to a reduction in its normal proteasomal turnover. Another member of the Af4 family, Fmr2, which is involved in mental handicap in humans, similarly binds Siah proteins. These results provide evidence for a common regulatory mechanism that controls the levels and thereby the activity of the Af4/Fmr2 protein family.

Effects of novel β-catenin mutants on cell differentiation and proliferation

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β-catenin is an important component of the *Wnt/wingless* signaling pathway, which plays a critical role in development and cell differentiation. Perturbations in the Wnt/wingless signaling pathway due to mutations in *β*-catenin are also responsible for the development of colon cancers. We are investigating the effects of four newly identified mutant forms of *β*-catenin on cell differentiation, development and morphogenesis of intestinal cancer. Tetracycline inducible and constitutive expression constructs for the wild type and mutant *β*-catenins have been made which are epitope tagged. Transient and stable transfectants of intestinal epithelial cell lines and mouse ES cells expressing these constructs have been generated. The subcellular distribution of *β*-catenin and its association with other members of the complex have been studied by immunohistochemistry in these transfectants. Effects on cell cycle and differentiation of mouse ES cells is being investigated. These data will be presented.

Work funded by the Pathological Society of Great Britain and Ireland.

Characterization of holoprosencephaly in the *Zic2*$^{Ku/Ku}$ mutant

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Holoprosencephaly (HPE) is a common disorder involving the incomplete development and septation of midline structures in the CNS. It has an incidence as high as 1 : 250 in embryogenesis, but only 1 : 16,000 of live births due to intrauterine lethality. Clinical features vary widely, from a single brain ventricle and severe facial anomalies such as cyclopia with a proboscis, to mild facial dysmorphia such as a single front incisor and/or narrowly spaced eyes. A number of genes have been linked to HPE in humans and several are members of the *Sonic hedgehog* (*SHH*) and *BMP* signaling pathways. In addition, mutations in *ZIC2*, a gene homologous to the Drosophila pair-rule gene odd-paired, can also give rise to HPE in humans. We have previously isolated and partially characterized a murine *Zic2* mutant called Kumba ($Zic2^{Ku}$) which has a point mutation in the fourth C2H2 zinc finger domain. $Zic2^{Ku/Ku}$ homozygotes exhibit the clinical features of HPE. We have initiated a study into the embryological cause of the defect using *in situ* hybridization, with probes for midline markers. We are also working to establish whether *Zic2* can be placed into one of the HPE-associated genetic pathways.

Molecular basis of neural fold adhesion and fusion in closure of the spinal neural tube

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The molecular basis of neural fold adhesion and fusion in closure of the mouse spinal neural tube is a crucial process, since failure may result in spina bifida. Previous studies have shown that cleavage of glycosyl phosphatidylinositol (GPI) anchored proteins causes neural tube defects in the mouse. In this study, mouse embryos undergoing neural tube closure were treated with the phosphatidylinositol phospholipase C (PIPLC) enzyme, known to cleave GPI-anchored molecules, and then cultured for 8 hours. PIPLC treatment inhibits spinal neural tube closure, as shown by an enlarged posterior neuropore. EphrinAs are GPI-anchored cell surface proteins, and were considered as candidates for a role in adhesion. Blocking ephrinA ligand binding to EphA receptor by injecting EphA3 fusion protein into the amniotic sacs of cultured embryos inhibits neural tube closure. Further blocking experiments with EphA1 fusion protein also inhibits closure suggesting that the specific ephrinA ligand, ephrinA1, is required for spinal neurulation. Expression studies show localization of ephrinA1, ephrinA3 and the EphA2 receptor in the caudal neural tube. These findings suggest that EphrinA molecules are required for neural fold fusion during spinal neural tube closure.

Genetics and morphology of the developing middle ear

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The middle ear consists of three ossicles the malleus, incus and the stapes and two structural components the gonium and the tympanic ring. These elements are homologous to the bones and cartilage of the primary jaw joint in non-mammalian jawed vertebrates. We have been investigating how the joint region between the malleus and incus forms. Using β galactosidase type II collagen reporter mice, and from studying cartilage and bone markers (Sox9, Collagen type II and Runx2), we have shown that the malleus and incus at E13.5 form from one condensation. At E14.5 this splits to become two separate components. We are currently mapping the expression of genes involved in joint formation such as chordin, Wnt 14, Bapx1, Gdf5 and Gdf6 in the malleal incudo joint region. In the limbs formation of the joints involves apoptosis. To see if a similar process is occuring in the malleal incudo joint we are using TUNEL and investigating the expression of apoptotic markers such as fas and Bcl2. These studies should help us understand the process of how and when the malleal incudo joint forms.

Age-related changes in the maintenance of the corneal epithelium by coherent stem cell clones

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Maturation of the murine corneal epithelium occurs postnatally. Limbal stem cells maintain the corneal epithelium through a process of clonal growth and

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ordered migration. Because of their position millimetres away from the tissue they regenerate, limbal stem cells provide an excellent model system for the study of adult stem cells in general. Therefore an understanding of the dynamics of tissue maintenance by limbal stem cells is important. In female mice, that express a *LacZ* reporter gene on one of their X-chromosomes, random clumps of *LacZ*-positive cells are seen in the cornea at 6–8 weeks of life. This pattern resolves between 8–10 weeks forming radial stripes thought to represent chords of clonally related, inwardly migrating cells. By measuring the number and width of stripes around the epithelium's circumference and correcting for the effects of different proportions of *LacZ* positive cells, an estimate of the number of coherent stem cell clones maintaining the tissue can be derived. Analysis of X-inactivation mice at 5 ages demonstrated that the estimated number of coherent stem cell clones contributing to the corneal epithelium is reduced from ~95 at 15 weeks to ~50 at 39 weeks and is then stable at least until 52 weeks.

De novo UPIIIa mutations cause human urinary tract malformations which lead to severe renal failure

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Uroplakin (UP) proteins are components of the urothelial apical membrane and *UPIIIa* null mutant mice have malformed urinary tracts. Because of the complex relationship between urinary tract malformation phenotypes our strategy was a candidate gene screen of *UPIIIa* and its plaque partner *UPIb*, by direct sequencing, in 42 patients with a range of clinical presentations. We found *de novo UPIIIa* mutations in four such individuals with renal aplasia/dysplasia. Two individuals (one with a persistent cloaca) had a P273L missense change in the cytoplasmic domain of UPIIIa. This mutant protein was processed normally to the surface of COS-1 cells and so may act dominant-negatively. Two other individuals had 3′UTR mutations (963T→G; 1003T→C). The missense mutant genotype correlated with high grade primary vesicoureteric reflux, whereas the 3′UTR mutations did not. These results suggest that *UPIIIa* is important in the development of the urogenital sinus, ureters and kidney, and in support of this we observed UPIIIa and UPIb in human urothelium of the newly septate urogenital sinus, and later in the ureters and renal pelvis. Our findings begin to illuminate the pathogenesis of human sporadic urinary tract malformations and have important implications regarding the genetic counselling of affected individuals now reaching reproductive age.

The relationship between the Wilms' Tumor Suppressor, WT1, and Wnt4 during metanephric kidney development

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The formation of the metanephric kidney involves a complex series of interactions between mesenchymal and epithelial cells. The first stage of development is marked by the invasion of the epithelial ureteric bud into surrounding mesenchyme. Through reciprocal interactions, the bud is induced into bifurcation, in turn inducing the surrounding mesenchymal cells to undergo a mesenchymal-epithelial transition, eventually leading to the formation of the epithelial nephron. Many genes have been implicated in various stages of metanephric development, although the exact mechanism governing signalling and morphogenesis remains to be elucidated. We have previously shown that a novel siRNA based approach targeting the WT1 gene can result in reduced nephron formation in kidney explant culture, illustrating a function for WT1 at a stage in kidney development beyond which WT1 mutant kidneys survive. Using an siRNA base approach, we have investigated the role of Wnt4, a gene implicated in the mesenchymal-epithelial transition, and have shown that re-introduction of Wnt4 to WT1 siRNA-treated explants rescues the nephrogenic phenotype. In order to investigate signalling downstream of Wnt4, various studies have been employed to characterise frizzled receptor expression in the kidney, and pathways activated by interactions between these receptors and Wnt4. The relationship between WT1 and Wnt4 could provide valuable clues to elucidate the mechanism of Wilms Tumour formation, a paediatric kidney cancer thought to arise through mutations altering kidney development.

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A G3 recessive screen for mutations affecting left-right patterning

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Vertebrates have three major axes, anterior-posterior, dorsal-ventral, and left-right (L/R). We have established a G3 recessive screen for defects in left-right patterning and are screening G3 embryos at 9·5 dpc and 13·5 dpc. We are scoring 9·5 dpc embryos for defects in embryonic turning and heart looping, and 13·5 dpc embryos for the L/R position and patterning of the heart, lungs, liver, stomach and gut. To date we have screened over 40 lines at 9·5 dpc and 80 lines at 13·5 dpc. We have identified 9 mutant lines showing consistent defects in organ situs. Intriguingly 5 of these lines fall into a single class that shows multiple defects in addition to the L/R defects, including exencephaly, polydactyly and lung formation. We term these gasping (gsp) mutants. The other 4 lines show defects solely in L/R patterning. We present preliminary data on the characterisation and mapping of these lines.

A functional genomics approach to the investigation of human 6p gene function

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The region of human chromosome 6p telomeric to the major histocompatibility complex (6p21.3–6p25) has been associated with many disease loci and complex traits. Analysis of chromosomal imbalances has defined a 6p trisomy syndrome and two deletion syndromes. We have taken advantage of a large block of synteny between 6p22–25 and Mmu13 to functionally annotate genes in the region of these diseases and gene dosage syndromes. We used a mouse strain that carries an interstitial deletion of proximal MMu13 (*Del*(*13*)*Svea36H*). The deleted region contains the *Foxq1* gene and mice that are homozygous null for *Foxq1* have a glossy coat and distinguishable from the wildtype at 10 days post birth allowing the use of the strain in a two generation genetic screen for ENU induced recessive mutations in genes monozygous in (*Del*(*13*)*Svea36H*). High quality finished sequence has been generated and annotated using a comparative sequencing approach for a 12·7 Mb region that spans the deletion. The region is gene dense (201 genes and 91 pseudogenes), but most of the genes belong to five gene family clusters. 71 genes do not form part of these clusters. The screen has identified 13 lethal mutations. All phenotypes so far examined mimic aspects of the 6p gene imbalance syndromes.

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