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Role of apoptosis in mammalian neurulation

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Dying cells were first observed in the neuroepithelium during neurulation over 30 years ago. More recently, these cells were shown to be undergoing apoptosis. Several animal models that develop neural tube defects (NTDs) have been associated with either a decrease or increase in the extent of apoptosis in the embryo, in the neuroepithelium and/or in underlying tissues. Studies of chick embryos have implied that apoptosis is required for neural tube closure to take place, but the requirement in the mouse is not well established. In the present work, we first evaluated the spatio-temporal distribution of apoptotic cells throughout the process of neural tube closure in mouse embryos. We then inhibited apoptosis during specific developmental time-windows in order to clarify whether cell death is required for the neurulation process to successfully occur. Cultured mouse embryos were exposed to chemical inhibitors in order to prevent cells from dying at specific stages of neural tube closure. Morphological evaluation showed that there was no difference between treated and control embryos. Our data indicate that, although dying cells are present during three main events of embryonic development (i.e. folding and fusion of the neural plate and migration of neural crest cells), apoptosis is not essential for neurulation to occur in the mouse.

Orientation of cell division is not a mechanism for axis elongation in mice

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Circletail is one of the handful of mouse mutants that display craniorachischisis, the most severe form of neural tube defect. *Circletail* carries a mutation in the *Scribble* gene which plays a role in the planar cell polarity (PCP) signalling pathway. Analysis of neurulation-stage *circletail* embryos has shown that the midline of the embryo is broader than normal, with widely spaced neural folds. The neural folds fail to oppose and fuse at the midline, giving rise to the neural tube defect. Studies with zebrafish demonstrate that PCP signalling is involved in the regulation of the orientation of cell division during gastrulation. In zebrafish, cells divide predominantly along the anterior–posterior axis, thus contributing to axis elongation and midline narrowing of the embryo. As *circletail* and the other mouse mutants that exhibit craniorachischisis affect genes in the PCP pathway, we investigated the orientation of cell division in the neuroepithelium of *circletail* embryos. Homozygous and wild-type embryos were collected at the 2–5 somite stage and the DNA were stained using OliGreen. The neuroepithelium of flat-mounted embryos was visualized with a confocal microscope. Our preliminary results indicate that the orientation of mitosis is not an important mechanism for axis elongation in mice. However, proliferation in *circletail* may be affected; further experiments are in progress to verify these results.

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Modelling fetal programming in a dish: from nephrogenesis to cystic kidneys

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We demonstrated that maternal low protein diet (LPD) deletes mesenchymal precursor cells and alters gene expression at inception of murine metanephrogenesis. Furthermore, maternal LPD and 'stresses' cause fetal exposure to increased corticosteroid levels. We hypothesized that excessive levels directly perturb kidney development. Mouse metanephroi isolated at embryonic day 11 (E11; ureteric bud stage, ~5 weeks human gestation) and E13 (when branching and primitive nephrons have initiated, ~7 weeks human gestation) were cultured for 6 days with/without a 'supraphysiological dose' of hydrocortisone (HC; 14 μM). Control E11 explants underwent branching and nephrogenesis, whereas HC-exposed organs showed failure of nephron initiation, reduced and distorted branching. Compared with controls, HC-exposed E13 explants showed impaired growth. Strikingly, HC-exposed E13, but not E11, explants showed polycystic kidney disease (PKD). HC has glucocorticoid and mineralocorticoid actions; we showed that both receptors were expressed in E11 and E13 explants. Metanephrogenesis is exquisitely sensitive to increased corticosteroid levels, but the type of induced perturbation (block in differentiation or cystogenesis) depends on metanephric age. These effects may be glucocorticoid and/or mineralocorticoid receptor-mediated. We suggest that 'programming' of fetal kidneys initiated by maternal stress may not only reduce nephron number but also be an environmental factor modifying the severity of PKD in later life.

The planar cell polarity pathway is required for branching morphogenesis

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Many organs, including the lung, develop by branching morphogenesis. This involves changes in the cytoskeletal architecture and cell–cell adhesion. Thus epithelial cells can form tubules that continue to branch and give rise to the respiratory tree. The planar cell polarity (PCP) signalling pathway is capable of controlling this type of morphogenetic movement via the downstream effectors Rho GTPase and Rho kinase. Analysis of three PCP mouse mutants revealed disrupted branching morphogenesis in several branched organs; further analysis revealed cytoskeletal defects in the lung. When cultured with an inhibitor of Rho kinase, a dose-dependent inhibition of branching morphogenesis was observed in both lung and lacrimal gland, along with enlarged terminal buds producing a remarkably similar phenotype to the PCP mouse mutants. Furthermore, the mutant phenotype was rescued by addition of the Rho activator CNF-1. Asymmetric localization of the PCP protein Scrb was observed in groups of cells, suggesting the involvement of PCP proteins in local cell interactions during morphogenesis, and the mutant endoderm was unable to respond to the chemoattractant FGF-10. Our data suggest a novel role for the PCP signalling pathway in controlling polarized cell movements necessary for branching morphogenesis.

Hair-loss mutation (*dep*) caused by a mutation in palmitoyl transferase zdhhc21 – a role in maintenance of epidermal homeostasis

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Palmitoylation is a post-translational modification that involves the addition of the fatty acid palmitate onto specific cysteine residues. Recently, several members of a family of transmembrane proteins containing a zinc finger and a DHHC motif have been shown to be palmitoyl acyltransferases (PATs) in yeast and mammalian cells. The recessive hair loss mutant, *dep*, contains a mutation (del-233F) at the C-terminal of Zdhhc21. Wild-type Zdhhc21 has been shown to enhance palmitoylation of several specific substrates in a transfected cell assay. Zdhhc21 localizes to the *cis*-Golgi, whereas the mutant protein is mislocalized and is inactive in palmitoylation. We verified the candidacy of *Zdhhc21* by transgenic BAC rescue. *dep* is characterized by progressive hair loss, hyperplasia of the sebaceous glands, the

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interfollicular epidermis and the outer root sheath. *In situ* hybridization and immunohistochemistry show that both wild-type and *dep* mRNA and protein are present in the inner root sheath (IRS). Phenotypic characterization using molecular markers in cell culture and on skin sections reveals abnormalities that suggest a lack of correct hair shaft differentiation in *dep*. We speculate that *dep* may effect the function of one of four members of the Wnt family – essential regulators of hair shaft differentiation – because of their co-expression in the IRS and because *dep* exhibits a Wnt-deficient phenotype. This hypothesis may provide an example of how local signalling centres may be established to allow for spatio-temporal gene expression. Furthermore, *dep* is the first mouse model that provides direct evidence of PAT activity of the Dhhc.

Different mechanisms cause loss of imprinting of the *IGF2* gene in congenital growth disorders and cancer

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Aberrant imprinting of *IGF2* is important in the pathogenesis of the Beckwith–Wiedemann syndrome (BWS), the Silver–Russell syndrome (SRS) and various cancers including Wilms tumour (WT). Imprinted gene expression is regulated by differentially methylated regions (DMRs). The *IGF2*/*H19* locus contains a primary germ-line DMR 5′ to *H19*, which acts as an Imprinting Control Region (ICR1), and three somatic DMRs associated with *IGF2*. In the mouse, the *Igf2*/*H19* locus is regulated by interactions *in cis* between the *Igf2* DMRs and ICR1. We compared the methylation status of DMRs at the *IGF2*/*H19* locus in patients with congenital growth disorders (BWS and SRS) and cancer (colorectal cancer and WT). Our data show that in congenital growth disorders, methylation imprints affect a single allele coordinately, leading to both alleles looking like either a paternal allele (BWS) or a maternal allele (SRS). In the tumour samples, however, the methylation imprints are uncoupled. This indicates that epigenetic changes in WT and congenital growth disorders are distinct. Our model proposes that different epigenetic reprogramming occurs in tumorigenesis and early development.

Growth factor rescue of early kidney development in an organ culture model of the Fraser syndrome

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FRAS1 is a basement membrane-associated protein mutated in humans with the Fraser syndrome and Fras1 is mutated in blebbed (bl) mice. The human disease and mouse model are characterized by fatal kidney agenesis. It is already established that Fras1 is expressed by the ureteric bud but, beyond that, the cell biological bases of nephrogenic failure in the syndrome are unknown. We show in this paper that, *in vivo*, bl/bl ureteric buds fail to invade the metanephric mesenchyme, a phenotype replicated during culture of mutant primordia. Renal expression of growth differentiation factor 11 and glial cell line-derived neurotrophic factor was defective in bl/bl embryos, and addition of either recombinant protein to explanted mutant renal primordia restored ureteric bud invasion into metanephric mesenchyme which became molecularly induced, with fibronectin downregulation and upregulation of integrin α8 and B-cell lymphoma/leukaemia-2 proteins. *In vivo*, bl/bl mutant mesenchymes showed deficient expression of homeobox d11 and sine oculis *Drosophila* homologue 2 genes, whereas expression of several other mesenchymal transcription factors was unaffected. These data implicate Fras1 in modulating growth factor signalling at the start of metanephrogenesis and the observations also make it possible to conceive of novel, but biologically rational, therapies which may encourage normal differentiation of severe human renal tract malformations.

The evolution of an imprinted domain in mammals

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A comprehensive domain-wide comparative analysis of genomic imprinting between mammals that imprint and those that do not can provide valuable information about how and why imprinting evolved. The imprinting status, DNA methylation and genomic landscape of the *Dlk1-Dio3* cluster were determined in eutherian, marsupial and monotreme mammals. Imprinting across the whole domain evolved after the divergence of eutherian from marsupial mammals. The marsupial locus at 1·6 megabases is double that of eutherians due to accumulation of LINEs. Comparative sequence analysis of the domain in seven vertebrates determined evolutionary conserved regions common to particular sub-groups and to all vertebrates. The evolution of imprinting in eutherians has changed the behaviour of the maternally inherited chromosome and is associated with region-specific resistance to expansion by repetitive elements and the local introduction of non-coding transcripts including microRNAs and C/D snoRNAs. A recent mammal-specific retrotransposition event led to the formation of a completely new gene only in the eutherian domain, which may have driven imprinting at the cluster.

The relationship between British surnames and Y chromosomal haplotypes

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In Britain, surnames are paternally inherited and they are thus analogous to the paternally inherited Y chromosome. Men who share surnames might therefore be expected to share Y chromosome types. However, this simple relationship is complicated by the multiple origins of many surnames, non-paternity and mutation. Y chromosomal DNA polymorphisms provide us with a set of tools to directly test, at a molecular level, this hypothetical link. Initially, 150 pairs of men were recruited, each sharing a British surname, and Y-haplotype sharing was assessed within each pair. The clear signal of co-ancestry exhibited constituted powerful evidence for common origins of men sharing surnames. It also has forensic implications, showing that the prediction of surname from crime-scene samples may be possible. A set of 40 surnames, with an average sample size of 42 apparently unrelated men, was then investigated using Y markers. Significant correlations between surname frequency and degree of Y-diversity exist. While five surnames show evidence of having had a single founder, overall the picture is one of complex surname histories. Descent clusters within surnames have ages consistent with the known time of surname establishment, and historical non-paternity rates are between 1 and 3% per generation.

Morphogenesis of the mammalian spleen

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The mammalian spleen is located on the left side of the body cavity. This positioning is determined by the left–right asymmetry pathway and is, along with early splenogenesis, dependent on Bapx1/Nkx3-2. We examined other genes that may have a regulatory role in early murine spleen formation and found that the canonical Wnt signalling pathway is active in the spleen at E11.5–14.5. Prior to this, at the splenic condensation stage, Nkx2-5 is a very early marker of splenic precursors and its expression overlaps considerably with that of Bapx1. An Nkx2-5 gut regulatory sequence (NGRS), containing evolutionary conserved regions, was identified and used to drive LacZ expression in transgenic reporter lines (NGRS-LacZ). The NGRS confers expression in the spleen, posterior stomach and pyloric sphincter, with little of the cardiac expression associated with endogenous Nkx2-5. The NGRS was found not to require Bapx1 for its activity. Finally, the NGRS was demonstrated to mark abnormal spleen development in a previously unreported splenic mutant. A novel gut organ culture system was established and used in conjunction with NGRS-LacZ to dissect spleen development. These studies suggested that splenic precursors move along the stomach, and that an inhibitory 'anchor' effect is exerted by the posterior spleno-pancreatic mesenchyme.

Establishment of genomic imprinting in human spermatogenesis

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Genomic imprinting is a mechanism that regulates gene expression leading to monoalellic parental-dependent expression of imprinted genes. Imprinting

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marks – DNA methylation at CpG dinucleotides – are erased in primordial germ cells being re-established during gametogenesis, according to the sex of the germline. *H19* and *MEST/PEG1* are two oppositely imprinted genes: *H19* is methylated on the paternal allele and *MEST* is methylated on the maternal allele. We studied, by bisulphite genomic sequencing, *H19* and *MEST* methylation patterns in sperm from normozoospermic males and oligozoospermic patients. Our results suggest that imprinting errors, namely *H19*, including CTCF binding site 6, hypomethylation and complete unmethylation and *MEST* hypermethylation and complete methylation, are associated with abnormal spermatogenesis. The analysis of testicular spermatozoa retrieved from patients with azoospermia also revealed that disrupted spermatogenesis is associated with *H19* and CTCF binding site complete unmethylation. These results suggest that imprinting errors are associated with disturbances in spermatogenesis and could contribute to explaining the cause of the Silver–Russell syndrome in children born after assisted reproductive technologies (ART). Additionally, unmethylation of the CTCF binding site could lead to inactivation of the paternal *IGF2* gene and be linked to decreased embryo quality and birth weight in ART children.

Evolution and spectral tuning of vertebrate shortwave-sensitive visual pigments

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The shortwave-sensitive class (SWS1) of visual pigments in vertebrates show the widest variation in peak sensitivity, ranging from violet at >440 nm to ultraviolet (UV) at 360 nm. Phylogenetic evidence indicates that the ancestral pigment was UV-sensitive (UVS) and that the shifts between violet and UV have occurred several times in evolution. The spectral shift between UV and violet in these pigments is largely determined by substitution at a single amino acid site in the opsin protein. In avian pigments, Cys90 in UVS pigments is replaced by Ser90 in VS pigments, whereas in non-avian pigments, Phe86 in UVS pigments is replaced by a variety of other residues in violet-sensitive (VS) pigments. The objective of my project has been to extend our understanding of the molecular basis for this UV/violet shift and to trace the evolutionary route of these substitutions. In particular, I have shown that substitution at site 86 is also important in the avian lineage for the generation of VS pigments and that the variability in primate SWS1 pigments in the residue present at site 86, with Phe86 retained in one species, tunes within the violet but does not cause a shift into the UV.

The inevitable accession of Neo-XY systems

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In species with XO males and XX females, the fusion of an autosome with an X chromosome can give rise to a Y chromosome. The Neo-XY system could become established by the fixation of the fusion in part of the species range, either by genetic drift or by some forms of selection (perhaps due to the changed linkage). In either case the further spread of the Neo-XY system throughout the species range is more difficult to explain, yet evolutionary analysis provides many examples where it appears to have occurred. This talk presents a new explanation for the spread of the Neo-XY system, based on fitness regimes resulting from the evolution of sexually antagonistic effects on the Neo-Y chromosome, as predicted by sex chromosome evolution theory. Paradoxically, while an evolved Neo-XY system will not necessarily spread if introduced at low frequency in a new XO population, it will spread at the meeting point between large XO and Neo-XY populations. The reason is that selection on the Neo-Y chromosome creates an environment in which the fused X is favoured and so the Neo-XY system advances. Phylogeographic evidence for such an advance will be presented for the grasshopper *Podisma pedestris*.

WAMIDEX: a web atlas of murine genomic imprinting and differential expression

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Genomic imprinting refers to the distinct epigenetic programming of the parental genomes during gametogenesis, resulting in the parent-of-origin-specific expression of imprinted genes in the offspring. In mammals, imprinted genes are essential for growth

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and development. Uniparental chromosomal duplications (UpDp) and mutations leading to deficiencies in DNA methylation are proven tools for studying genomic imprinting in the mouse. Using microarrays, we interrogated gene expression in multiple tissues of UpDp mice and in maternal methylation imprint-free (Dnmt3L$^{-/+}$) embryos. These and other imprinting-relevant microarray data are now immediately explorable via our web atlas of murine genomic imprinting and differential expression (WAMIDEX: https://atlas.genetics.kcl.ac.uk). A comprehensive literature-derived catalogue of imprinted genes and a genome browser provide the essential context. The UpDp data cover Chrs 7, 11, 12 and 18 containing ~60% of the known murine imprinted genes, among which are the orthologues of many human disease-associated imprinted genes. The genome-wide Dnmt3L$^{-/+}$ data are relevant to the majority of imprinted genes that depend on maternal methylation imprints for transcriptional regulation. The parent-of-origin-specific expression of imprinted genes is often limited to particular tissues or developmental stages, a fact that WAMIDEX reflects in its design and content.

Biochemical and functional analysis of the chromatin remodeller Mi-2β and the associated NuRD complex in pluripotent cells

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Mi-2β, encoded by the Chd4 gene, is a chromatin remodeller belonging to the CHD (chromo-helicase-ATP-DNA binding) family of ATP-dependent nucleosome remodelling proteins and is a component of a 2 MDa co-repressor complex known as NuRD (nucleosome remodelling and histone deacetylase complex). Due to its histone deacetylase activities, the NuRD complex is considered to be a transcriptional repressor complex. However, NuRD requires interaction with DNA-binding proteins to allow the complex to be recruited to DNA and repress transcription. NuRD also plays key roles in mouse development, as deletion of the Mbd3 subunit results in embryonic lethality. This study reveals a role for Mi-2β in early embryonic development, as Mi-2β-null embryos are not evident in post-implantation development. *Ex vivo* analysis reveals a critical role of Mi-2β in the viability of early embryos. Additionally, a biotinylation tagging strategy reveals that the NuRD complex is not a static complex but instead a fluid complex. Furthermore, the biotinylation purification allowed the identification of interacting transcriptional regulators in mES cells, which included the pluripotency-associated protein Sall4. Overall, this study ascertains the importance of Mi-2β in development, reveals the biochemistry of the NuRD complex in ES cells and identifies the associated sub-proteome in ES cells.

The role of transcriptional events in establishing imprinted methylation

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Genomic imprinting in mammals results in allelic silencing determined by the parental origin of the allele. Such monoallelic expression results from imprints that comprise dense regions of DNA methylation imposed during gametogenesis, but the mechanisms responsible for imprint establishment are not fully understood. Using the mouse *Gnas* locus as a model, we are investigating the role of transcriptional events in determining germline methylation. The *Gnas* locus comprises maternal-specific (*Nesp*), paternal-specific (*Nespas*, *Gnasxl* and *exon1A*) and tissue-specifically imprinted transcripts (*Gnas*); there is an extensive oocyte-derived differentially methylated region (DMR) covering the *Nespas*/*Gnasxl* promoters and a separate maternal germline DMR at the *exon1A* promoter. From several lines of evidence, we hypothesized that the *Nesp* transcript, which initiates furthest upstream and transcribes through the remainder of the locus, could be involved in germline methylation establishment. We identified that *Nesp* transcripts are present in mature oocytes, where they are expressed from a start site distinct from that utilized in somatic tissues. By insertion of a transcription termination cassette we have generated an allele that truncates the *Nesp* transcript and now show that maternal transmission of this truncation allele results in loss of methylation of the maternal germline DMRs. Based on the common features of imprinted clusters, we propose that transcription events may be a general mechanism in the establishment of maternal germline methylation and we show that transcription occurs in oocytes across the germline DMRs of a number of other imprinted genes.

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Wnt signalling in neural stem cell differentiation and tumorigenesis in the CNS

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To understand how brain tumours develop, it is essential to determine the cells of origin and the events that lead to this transformation. We have previously shown that inactivation of Rb, p53 and PTEN in the neural stem cell compartment in adult mice results in brain tumours. Here, we set out to investigate if activation of an oncogenic form of β-catenin in these stem cells can alter their proliferation and fate and whether this activation is sufficient to elicit brain tumours. Because the overexpression of oncogenic β-catenin in the CNS during development causes early postnatal lethality with gross morphological abnormalities, we generated transgenic mice in which a KT3-tagged β-catenin (ΔGSK β-catenin) can be activated by Cre-mediated excision of a preceding stop cassette. The construct was electroporated into ES cells, which were injected into blastocysts to generate chimeric mice. Adeno-Cre-mediated overexpression of β-catenin in neurospheres from germline expressing mice show significant increase in growth and self-renewal *in vitro*. In contrast, this overexpression is not associated with lethality or brain abnormalities *in vivo*, indicating dosage effects and the need of additional mutations. Therefore, additional pathways such as PTEN and p53 are now being inactivated in conditional transgenic/knock-out mice.

Mutation of *Tulp3*: a novel negative regulator of the Shh pathway

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During ongoing mouse ENU mutagenesis programmes, several new mutants have been created that exhibit neural tube defects. The *hitchhiker* mutant is characterized by multiple developmental abnormalities, including exencephaly, spina bifida, oedema and polydactyly. Using a positional cloning strategy, we have identified the causative mutation in *hitchhiker* as a splicing defect in *Tulp3*. Analysis of dorso-ventral patterning in the caudal neural tube reveals expansion of ventral markers, suggesting that *Tulp3* may be a novel negative regulator of Shh signalling. Epistasis experiments place *Tulp3* downstream of *Shh*. Experiments in progress aim to elucidate the precise function of Tulp3.

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Snai1 expression in early osteogenesis, *in vivo* and *in vitro*

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During mammalian development, the transcription repressor, Snai1, is expressed in various tissues including the primitive streak and the neural crest, where it plays a role in epithelial–mesenchymal transition. *Snai1* transcripts have been detected in the developing craniofacial region, but little is known about Snai1 protein and its potential role in osteogenic mesenchyme. Therefore, Snai1 protein expression was investigated in the murine craniofacial region (E13-P1) with a focus on secondary palate bone development, in the osteoblastic cell line, MC3T3, and in organotypic models of cleft palate. Immunohistochemistry showed Snai1 expression in preosteoblasts in the palate and its decrease with the progression of bone differentiation, as also observed in MC3T3 cells. This was consistent with changes in transcript levels assessed by qPCR. In MC3T3, *Snai1* expression was decreased by a short treatment with Tgfβ1, an inducer of osteoblast maturation and proliferation, whereas the differentiation marker, *Osteopontin*, decreased. Finally, inhibition of palatal shelf fusion in organotypic cultures, which reduced osteoblast differentiation, as indicated by increased *Twist1* and *Runx2* and decreased *Osteopontin* expression, also led to Snai1 expression increase as compared with fused palates. These findings indicate that Snai1 is a preosteoblast marker and may be a negative regulator of osteoblast differentiation during palate development.

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Gbx2: a modifier of Tbx1 happloinsufficiency phenotype

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Our long-term goal is dissecting pathways controlled by the T-box transcription factor Tbx1, the gene responsible for most of the DiGeorge syndrome haploinsufficiency phenotypes. A microarray screen in *Tbx1* mutant mice identified *Gbx2* as one of its potential downstream targets. *Gbx2* mutant embryos display a number of cardiovascular abnormalities associated with 22q11 deletion syndrome, some of which could potentially be linked to *Tbx1*. Our study showed that *Tbx1* and *Gbx2* interact genetically in the development of the 4th pharyngeal arch artery (PAA) and that conditional deletion of *Gbx2* in *Tbx1*-expressing domains was sufficient to recapitulate this 4th PAA defect. Conversely, the outflow tract phenotype found in *Gbx2* mutants is not associated with *Tbx1*. We also showed that *Gbx2* participation in the 4th PAA phenotype is *FGF8*-independent and involves the pharyngeal ectoderm as a key signalling centre. Despite neither *Gbx2* nor *Tbx1* being expressed in cardiac neural crest cells, both mutant mice displayed cardiac crest migration defects leading to abnormal 4th PAA development. We are now investigating a non-cell-autonomous pathway controlled by *Gbx2*-ectodermal expression and regulating cardiac crest migration.

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Conductive hearing loss and the Treacher Collins syndrome

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The Treacher Collins syndrome (TCS) is an autosomal dominant disorder of craniofacial development that results from loss-of-function mutations in the *TCOF1* gene. Anomalies in jaw and temporal bone development are commonly associated with TCS and approximately 50% of patients also exhibit conductive hearing loss resulting from the abnormal development of the outer and middle ear apparatuses. In heterozygous *Tcof1* mutant mice, many of the defects observed phenocopy those exhibited by the human syndrome, although the severity of the phenotype is highly dependent on genetic background. The more severe phenotypes result in embryonic or neonatal lethality, precluding analysis of anomalies associated with later development such as the development of the murine middle ear. The *Tcof1* heterozygotes on the DBA/1 mouse strain background are however viable and we have identified anomalies in middle ear development causing deafness in mice carrying the loss-of-function allele. This finding identifies a mouse model for conductive hearing loss and provides a means for further study of the mechanisms at work during middle ear development.

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Morphological, stem cell and myosin abnormalities in the cav-3$^{-/-}$ and mdx dystrophic embryo reveal an embryonic basis for muscular dystrophy

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Examination of embryonic myogenesis of two distinct but functionally related, skeletal muscle dystrophy mutants (mdx and cav-3$^{-/-}$) establishes for the first time that key elements of the pathology of duchenne muscular dystrophy (DMD) and limb-girdle muscular dystrophy (LGMD) type 1C originate from disruption of the embryonic cardiac and skeletal muscle patterning processes. Novel elements of the phenotype suggest that compromised stem cell function has an important role in the aetiology and severity of both diseases. Hyperproliferation and apoptosis in myf-5^{+} embryonic myoblasts and attrition of the pax-7^{+} stem cell population *in situ* occur earlier in mdx (E11·5) than in cav-3$^{-/-}$ (E15·5), but result in depletion of total pax-7 protein, respectively, in mdx and cav-3$^{-/-}$ embryos by 15 and 60%. mdx embryos have severalfold elevation of caveolin-3, which may contribute to the aetiology of mdx/DMD pathologies with reciprocity with cav-3$^{-/-}$. In mdx, myotube numbers are reduced and myotubes misaligned, hypotrophic and branching. The more restricted phenotype of cav-3$^{-/-}$ comprises excess, malformed hypertrophic myotubes and 2-fold increase in myonuclei. Myosin heavy chain (MyHC) content is disrupted in both mutants as is timing of secondary myogenesis. We conclude that caveolin-3 and dystrophin have important embryonic roles and act together

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to ensure the normal progression of embryonic muscle formation. Muscular dystrophy (MD) pathology originates from embryonic myogenesis through early failure of the dystrophin-glycoprotein complex (DGC) and disruption of the emerging skeletal muscle stem cell population.

Neural stem cells differentially govern tumorigenesis within the brain dependent on their early genetic profile

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A population of stem cells (NSCs) persists within the adult brain localized to the subventricular zone. Using Adenovirus-Cre-mediated conditional gene recombination/inactivation, targeted to the neural stem cell compartment (NSCC), we have shown that we can induce tumorigenesis and that the resultant tumour phenotype is largely determined by the initial genetic mutations. For example, $PTEN^{-/-}$ $p53^{-/-}$ mice exhibit glial tumours (malignant glioma) after 8 months, while $Rb^{-/-}$ $p53^{-/-}$ and $Rb^{-/-}$ $p53^{-/-}$ $PTEN^{-/-}$ mice exhibit primitive neuroectodermal tumours (PNETs) after a much shorter latency. Furthermore, NSCs recombined *in vitro* and then grafted into recipient mice resulted in a very similar phenotypic profile, supporting the putative relationship between initial genetic lesion and tumour phenotype. Our *in vitro* system has enabled us to use growth, differentiation and gene expression assays to delineate the processes involved in the progression from recombined NSCs, to tumorigenic neurospheres, and fully developed brain tumours following engraftment. The expression profile of these tumours will be compared with *in vivo* generated neoplasmas. To examine the role $CD133^{+}$ 'cancer stem cells' play in the formation and maintenance of brain tumours, we are generating $CD133^{+}$ and $CD133^{-}$ populations of NSCs to compare their growth and differentiation patterns *in vitro* and *in vivo*.

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