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Conserved methylation imprints in the human and mouse GRB10 genes with divergent allelic expression suggests differential reading of the same mark

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Genomic imprinting is a form of non-Mendelian inheritance in mammals, where imprinted genes are expressed depending on whether they are inherited from the mother or the father. The 'imprinting-control regions' at most imprinted loci are methylated on one of the parental alleles only. These regions are frequently referred to as 'germline' DMRs (differentially methylated regions) because their allelic methylation is acquired in either the male or the female germline, and is maintained throughout development. Ultimately, the epigenetic features at these DMRs are read in differing ways to ensure proper parental-allele-specific expression of the genes or imprinted clusters. How genes are differentially marked in the germ-line, in particular the sequence properties which direct methylation, remains to be elucidated. To address this question we undertook a comparative sequence and methylation analysis of the mouse and human *GRB10* genes. Mouse *Grb10* is imprinted with maternal-specific expression; in contrast, human *GRB10* is expressed from both the parental alleles in most tissues. This discrepant imprinting provided the opportunity to investigate features involved in acquisition of germline methylation imprints. Unexpectedly, results obtained suggest that this discrepancy is not due to acquisition of an imprint mark but to differences in reading this mark. However, epigenetic and genetic features we identified led us to propose a model of acquisition of imprinted DNA methylation that we shall present.

Disruption of the imprinted *Grb10* gene in mice leads to disproportionate overgrowth with effects on metabolism

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Growth factor receptor-bound protein 10 (Grb10) is a member of the Grb7 family of SH2 domain-containing adapter proteins. Murine *Grb10* is maternally expressed, located on proximal chromosome 11, and uniparental disomy (UPD) of this region results in reciprocal growth phenotypes. The equivalent human region is associated with Silver-Russell syndrome (SRS), which is characterized by pre- and post-natal growth restriction with mUPD7 occurring in 7% of cases. *Grb10* is therefore a candidate gene responsible for the imprinted growth effects of UPD11 in mice and for SRS in humans. *In vitro* interactions of Grb10 with a number of activated tyrosine kinase receptors including Insr and Igf1r

have been documented, although its function and signalling remain unclear. We have generated mice with disruption of the *Grb10* locus to investigate the role of Grb10 in growth and metabolism. Our experiments demonstrate that maternal transmission of the deletion results in overgrowth, persisting at birth with disproportionate effects on organ size. A difference in body weight remains apparent in the adult, and a decrease in fat is evident, compared with wild-type littermates. Despite Grb10 expression being reminiscent of the Igf2 expression pattern during development, our findings indicate that Grb10 acts via an Igf-independent growth axis.

Nespas: the emerging story of its function in the _gnas_ imprinting cluster

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Antisense RNA transcripts are implicated in the regulation of gene expression. *Nespas* is a paternally expressed transcript that lies antisense to the maternally expressed *Nesp* transcript in the *Gnas* imprinting cluster. The *Nesp* promoter is unmethylated on the maternal allele, but methylated on the paternal allele. The objective is to determine whether *Nespas* is a *cis*-acting control element. A 1·6 kb targeted deletion encompassing the promoter and exon 1 of *Nespas* on the paternal allele was made. Chimaeric mice were generated and mated with wild-type females. Deletion carrier offspring were used to analyse the effect of the deletion on gene expression from the paternal allele. RT-PCR on newborn brain from the deletion carrier mice showed that *Nespas* is no longer expressed from the paternal allele. Northern analysis of mRNA from 15·5 dpc embryos showed increased levels of *Nesp* in the deletion carrier mice. RT-PCR on newborn brain using a variant showed that *Nesp* was expressed biallelically. Analysis of the *Nesp* promoter revealed that the promoter was unmethylated on the paternal allele with the targeted deletion. Thus, the effect of the deletion is to convert the paternal imprint mark to a maternal one. The evidence implicates *Nespas* as a regulatory element within the *Gnas* cluster.

Investigating the role of cis-acting sequences in the imprinting of the human GNAS1 locus using transgenic mice

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Imprinted genes are only expressed from one allele, according to parent-of-origin. The GNAS1 locus includes the *GNAS1* gene encoding the $G_s\alpha$ subunit (predominantly biallelic) and three imprinted alternative first exons to *GNAS1*: *NESP55* (maternal), *XLαS* (paternal) and exon 1A (paternal). It also includes a non-coding transcript antisense to *NESP55*, AS (paternal), and remarkably has two germline methylation marks (differentially methylated regions, DMRs, at *XLαS*/*AS* and 1A). The AS promoter region is, in addition, highly conserved and contains paternal hypersensitive sites and is therefore a candidate regulatory region (CRR1). To investigate the role of this region, transgenic mice were made using a human PAC in which CRR1 can be deleted. Six out of seven lines show an imprinting effect on methylation at the *XLαS*/*AS* DMR but not the 1A DMR, suggesting that the PAC contains the sequences responsible for the methylation mark at *XLαS*/*AS* but not for 1A. One line shows brain-specific upregulation of *NESP55* expression after deletion of CRR1, compatible with a regulatory role of the *AS* transcript. However, antisense transcripts were still present after deletion and found to originate from other regions of maternal methylation.

Imprinting along the *Kcnq1* domain is developmentally regulated and involves histone methylation

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Genomic imprinting is a developmental mechanism whereby the expression of certain genes depends on whether they are inherited from the mother or the father. DNA methylation is essential for imprinting. However, DNA methylation cannot be the sole determinant of imprints. Recently, we showed that the 'imprinting-control regions' (ICRs) of the Igf2r, Snrpn and U2af1-rs1 mouse imprinted loci are marked by hypermethylation on lysine 9 of histone

H3 on the DNA-methylated maternal allele, whereas histone H3 on the unmethylated allele has lysine-4 methylation and is acetylated. The same modifications were detected on the KvDMR1, the ICR that regulates a large imprinted domain (the Kcnq1 domain) on chromosome 7 comprising several genes that are essential for extra-embryonic development. The KvDMR1 ICR has DNA methylation on the maternal allele, and produces a non-coding transcript (Lit1) from the paternal allele. We explored whether the paternal repression in cis, mediated by the ICR, involves the acquisition of specific histone modifications. Histone methylation and acetylation were analysed along the domain. Our data show that on the paternal chromosome, specific histone H3 modifications (including K27 trimethylation and K9 dimethylation) become established along the imprinted domain during pre-implantation development. These paternal modifications are maintained in an extra-embryonic-lineage-specific manner.

Igf2: mechanisms of imprinting in the choroid plexus

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The *Igf2* gene is imprinted at most sites of its expression, but escapes imprinting in the choroid plexus and leptomeninges of the brain. *Igf2* also frequently undergoes loss of imprinting in some overgrowth disorders and in cancer. Imprinting of *Igf2* has been shown to be dependent upon a differentially methylated imprinting control region (ICR) that lies in the 5′ flank of the neighbouring *H19* gene and acts as an insulator to block enhancers that are situated further downstream. We show that enhancers for brain expression of *Igf2* reside within a 2 kb region located centrally between *Igf2* and *H19*. These elements permit biallelic expression of *Igf2* because of their location upstream of the ICR, avoiding its influence on the maternal as well as the paternal chromosomes. We have also identified other enhancers that drive expression in choroid plexus epithelial cells. We have created immortal choroid plexus epithelial cell lines to dissect the function of these regulatory elements and also investigate epigenetic factors *in vitro*. In summary, understanding how cis-regulatory elements and epigenetic mechanisms mediate the switch from imprinted to biallelic *Igf2* expression in the brain may give an insight into loss of imprinting in certain pathologies.

Evidence for X-linked imprinted genes affecting cognition in the mouse

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Imprinted genes are monoallelically expressed in a parent-of-origin dependent manner. Data from Turner's syndrome have implicated X-linked imprinted gene effects on cognition. We exploited a 39,XO mouse model in which the parental origin of the single X chromosome could be paternal (39,X^pO) or maternal (39,X^mO) to test the hypothesis that X-linked imprinted genes may affect cognitive, and underlying brain phenotypes. We found that 39,X^mO mice were impaired relative to 39,X^pO (and 40,XX) mice with respect to reversal learning, showing an inability to inhibit responding to an incorrect, but previously correct stimulus and to make a new stimulus–reward association. 39,X^mO mice were also impaired relative to 39,X^pO (and 40,XX) mice with respect to discriminative response accuracy under attentionally demanding conditions on a five-choice serial reaction time task. These behavioural effects were accompanied by highly specific changes in dopamine levels in the frontal cortex. The most plausible explanation for the data is the presence of one or more X-linked imprinted genes that are only expressed from the paternally inherited allele, and that mediate behavioural inhibition and attentional processes via effects mediated by frontal cortex function. This idea predicts sex differences in cognition, as males (being 40,X^mY) will not benefit from the expression of such genes; we have obtained some evidence that this may be the case.

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Fraser Syndrome and mouse blebbed mutants

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Fraser syndrome (FS) is a multisystem malformation usually comprising cryptophthalmos, syndactyly and renal defects; other defects occur more rarely. The condition is genetically heterogeneous with two disease loci identified, genes at both encoding a protein with similarity to the sea urchin extracellular matrix (ECM) blastocoelar protein *ECM3*. The domain structure of FRAS1 and FRAS2 suggests a structural role within the extracellular matrix as well as in cell signalling. Mouse blebbed mutants provide a model for FS, with mutations in five blebbed loci giving similar phenotypes to FS. Mutations in *FRAS1*, and *Fras1* in the *bl* mouse, have been identified that result in the premature termination of the protein. A missense mutation has been identified at a second locus, *FRAS2*, which has homology to a gene closely linked to the *my* (myelencephalic blebs) locus. Loss of the cytoplasmic multi-PDZ domain protein glutamate receptor interacting protein 1 (GRIP1) results in a phenotype similar to that of FS. In the *eye blebs* mouse we have detected a deletion covering two coding exons that disrupts the (*Grip1*) gene, resulting in a premature termination. These mouse models for FS should provide important insights into the development of epithelial structures, as well as eye and kidney development.

Sensory deprivation is associated with a mutation in the rat chaperonin delta subunit

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Using positional cloning techniques, we have identified the genetic defect associated with an autosomal recessive rat mutant (mutilated foot, *mf*) displaying early-onset sensory neuropathy (HSN). The mutation, which maps to the distal end of rat chromosome 14 in a region syntenic to human 2p13–p16 and proximal mouse 11, involves a G>A transition at nucleotide 1349 encoded by exon 12 of the chaperonin (delta) subunit 4 (*Cct4*) gene. This change results in the substitution of a highly conserved cysteine for tyrosine at amino acid 450. Although we have yet to demonstrate how this mutation leads to the specific phenotype associated with the *mf* mutant, it is know that the delta subunit of the chaperonin complex does participate in the folding of actin. Actin plays a crucial role in the cytoskeleton and, in particular, the movement of the axonal growth cone. Failure to make suitable contact can lead to neuronal loss. A pathological hallmark of the mutant phenotype is excessive apoptosis in the dorsal root ganglia during late fetal development leading to significant reduction in the number of neurons. Thus, a relationship between the *Cct4* gene product and the production of functionally active actin provides a likely explanation for this phenotype.

Characterization and genetics of a mouse model of hypophosphatasia

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The study involves characterization of a mutant mouse line first identified in the biochemical screen of the Harwell ENU Mutagenesis Programme. GENA line 328 mutants display significantly lowered levels of plasma alkaline phosphatase (ALP) and elevations in ALP substrates. Genotyping revealed linkage to Mmu 4. A splice site mutation in the candidate gene (*Akp2*) was subsequently identified. The mutation causes exon skipping and results in a truncated protein. GENA 328 is of particular interest for the study of the late-onset forms of the human condition hypophosphatasia, a disorder characterized by skeletal defects resulting from a deficiency of tissue non-specific ALP. Clinical presentation in humans is highly varied and correlation of genotype and phenotype is not clearly understood. GENA 328 homozygous mutants have no visible external phenotype and are viable >12 months after birth. Aged mice develop restricted movement of the large joints due to a late-onset skeletal abnormality. X-ray images show various skeletal abnormalities including joint mineralization, bone proliferation and areas of bone lucency. Cartilage hyperplasia was observed during initial histological investigations. GENA 328 contributes to the mouse

phenome database and may further our understanding of hypophosphatasia in humans.

Ocular coloboma: new genes and old

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Ocular coloboma is the incomplete closure of the embryonic optic fissure resulting in abnormal eye development affecting the iris, retina, choroid, optic nerve or eyelids. The molecular mechanisms orchestrating this fundamental process are not understood; however, disease genes can give clues to the molecular events leading to optic fissure closure. Approximately, 1 : 10 000 births are affected and OMIM lists 69 diseases with an ocular coloboma phenotype. To date 24 chromosomal loci have been mapped, 11 chromosomal aberrations verified and 17 of the genes identified. To identify genes involved in coloboma we have been investigating families from our Genetic Ophthalmology in London Database (GOLD), and searching for the causative genes. A family that had ocular coloboma segregating with Hirschsprung disease and high myopia were studied and we identified a missense mutation in the *ZFHX1B* gene. This gene is a transcriptional repressor which is expressed in a spatiotemporal manner commensurate with closure of the optic fissure. A second family was identified that segregated ocular coloboma with oto-dental dysplasia. We are currently investigating a candidate gene for this phenotype. These studies suggest there is a complex interaction of at least 35 genes that are involved in optic fissure closure in man.

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Mechanisms regulating the initiation of neural tube closure

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Initiation of neural tube closure occurs at the 5–6 somite stage, in the future cervical region of the embryo. Failure of this event leads to the most severe form of neural tube defect, termed craniorachischisis, in which almost the entire brain and spinal cord is affected. Insight into the molecular and cellular mechanisms involved comes from a handful of mouse mutants that exhibit craniorachischisis: *loop-tail*, *circletail*, *crash* and a double knockout of *dishevelled 1 & 2*. Intriguingly *loop-tail*, *crash* and the *dishevelled 1 & 2* mutants all appear to function in the planar cell polarity (PCP) signalling cascade, which may regulate neuroepithelial convergent extension cell movements. *Loop-tail* and *crash* exhibit a broadened midline of the neural plate, consistent with a defect in convergent extension. This midline abnormality may prevent neural tube closure simply by placing the neural folds too far apart to come into apposition. The *circletail* mutant is not an obvious component of the PCP cascade. However, *circletail* mutants exhibit a broadened midline, genetically interact with *loop-tail* and *crash*, and also exhibit a defect in PCP in the inner ear. The *circletail* gene, *scribble*, is therefore likely to have direct or indirect effects on the PCP pathway.

Rho kinase is required for convergent extension and initiation of mouse neural tube closure

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Recent genetic studies in the mouse implicate a non-canonical Wnt/frizzled signalling pathway (the so-called planar cell polarity pathway) in initiation of neural tube closure. Mutation of three distinct genes – *Vang12* (*strabismus*), *Celsr1* (*flamingo*) and *dishevelled* – each yields embryos that exhibit severe neural tube defects owing to failure of initiation of

neural tube closure. Each gene appears to play a role in the transduction of Wnt/frizzled signals to downstream intracellular proteins including RhoA and JunN terminal kinase. The end result of this signalling pathway is the regulation of convergent extension, whereby cells move in a medial direction (convergence) and in a caudal–rostral direction (extension) to create an elongated, narrow shape of the neural plate. Convergent extension is essential for neural tube closure in *Xenopus* but this relationship has not been examined in mammals. To explore the role of planar cell polarity in mouse neurulation we analysed embryos treated with a specific inhibitor or Rho kinase, Y27632, during development in whole embryo culture. Embryos were explanted at E7.5 and cultured for 24 hours to the 8–12 somite stage. A significant proportion of treated embryos exhibited defects of convergent extension and failed to initiate neural tube closure, a close phenocopy of the *Vangl2* (*Lp*; *loop-tail*) loss of function mutation. Analysis of cultured loop-tail litters revealed an increased predisposition to the effects of Y27632 in *Lp*/+ embryos compared with their wild-type littermates. These findings support a critical role of planar cell polarity signally, via Rho kinase, in the onset of mouse neural tube closure.

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Convergent extension is defective in *loop-tail* (*Vangl2*) mouse embryos prior to failure of neural tube closure

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The recent identification of *Vangl2* (*strabismus*) as the gene mutated in the *loop-tail* (*Lp*) mouse raises the possible role of convergent extension cell movements in the onset of neural tube closure. Convergent extension has been extensively investigated in amphibian gastrulation and was recently demonstrated to depend on normal function of the planar cell polarity pathway, in which Wnt/frizzled signalling is mediated intracellularly by *dishevelled* and *strabismus*. Misexpression of either gene in the midline of the *Xenopus* neural plate leads to disturbed convergent extension and failure of neural tube closure. In order to investigate the role of *Vangl2*/*strabismus* in mouse convergent extension, we labelled small groups of cells with DiI in the neural plate midline of pre-headfold E7.5 embryos. Following 18 hours of culture, the position of the DiI-labelled cells was assessed, and correlated with embryo length and width, and the success/failure of neural tube closure. Wild-type embryos exhibited onset of neural tube closure and had an elongated narrowed body axis. In contrast, homozygous *loop-tail* (*Lp*/*Lp*) littermates failed to initiate neural tube closure and showed a short, wide body axis. Some heterozygous embryos showed the *Lp*/*Lp* appearance whereas others were more similar to wild-type. DiI-labelled cells became spread out along the midline, rostral to the initial site of injection, in all wild-type embryos and in most of the Lp/+ littermates. In contrast, the original clump of DiI-labelled cells remained coherent in *Lp*/*Lp* embryos with little or no extension in the midline. We conclude that the *Vangl2* genetic defect in *loop-tail* mice disturbs convergent extension cell movements, leading to failure of initiation of neural tube closure.

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Fate-mapping the mouse spinal cord and forebrain: a PAC transgenic approach

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During mouse development neurons and glia are generated from neuroepithelial precursor (stem) cells that line the central canal of the spinal cord and the ventricles of the brain. There are five stem cell domains in the ventral spinal cord, each of which gives rise to a single neuronal subtype. While the identities of some of the neurons generated from these precursor domains have been established, little is known about the origins and identities of glial cells. We are using phage-derived artificial chromosomes (PACs) to express Cre recombinase in defined precursor cell domains in the spinal cord and forebrain, in order to permanently mark them and identify their neuronal and glial progeny. Using this approach we demonstrate that the first oligodendrocyte progenitors to populate the developing cerebral cortex originate in the ventral forebrain and undergo long-range migration into the cortex. Future studies will allow us to determine the embryonic origins and migration patterns of other populations of neurons and glial cells in the spinal cord and brain.

Functional characterization of a novel paired box gene: *Ehox*

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The differentiation of murine embryonic stem (ES) cells *in vitro* is a valuable model of embryonic development. To identify genes that may be involved in promoting differentiation a day 5–day 3 subtracted embryoid body cDNA library was generated. We selected a novel homeobox gene (*Ehox*) from the subtracted library and analysed its function using loss and gain of function strategies in ES cells. Overexpressing *Ehox* sense or antisense transcripts in ES cells provided evidence that *Ehox* plays a role in the initial differentiation of a precursor cell type. *In vivo*, whole-mount *in situ* hybridization analyses have shown that *Ehox* is expressed in the trophoblast lineage and in pharyngeal pouches 2 and 3. These expression patterns suggest two distinct roles for *Ehox* during embryogenesis: (1) within the stem cell/precursor compartments of the developing placenta and (2) during development of the pharyngeal pouches and thymus.

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Genetic control of cell lineage specification in the chick ventral spinal cord

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We generated a cDNA library of embryonic chick ventral spinal cord enriched for sequences up-regulated between E5 and E7.5, looking for genetic markers for glial lineages and compartments of ventricular neuroepithelium. We screened the library for differentially expressed transcripts, examining expression of 128 candidates by *in situ* hybridization, and sequenced those of greatest interest. Three cDNA fragments were analysed in more detail: (1) cDNA for KIAA1102, a recently identified transmembrane protein with a LIM domain on C-terminal. *KIAA1102* is expressed in the ventral half of neuroepithelium complementary to *Mash1*. (2) cDNA for an axon-guidance molecule Semaphorin-3a, which we found to be involved in the compartmentalization of the spinal cord. Overlap between *Sema3a* and Nkx2.2 determines the domain that generates first oligodendrocyte progenitors. (3) cDNA for chick receptor tyrosine phosphatase zeta/beta (cRPTPζ/β). *cRPTP* ζ/β is expressed throughout the ventricular zone and in scattered cells outside it. *cRPTP*ζ/β is down-regulated in neurons or oligodendrocyte progenitors. The majority of scattered *cRPTP*ζ/β^+ cells co-expressed *glutamine synthetase* and *fibroblast growth factor receptor-3*, indicating that the cells are astrocyte precursors. Our data suggest that alternatively-spliced isoforms of *cRPTP*ζ/β, including phosphacan, are not present in the developing chick spinal cord.

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Expression of arylamine *N*-acetyl transferase in the developing heart

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Arylamine *N*-acetyltransferase 2 (NAT2), one of three arylamine *N*-acetyltransferases in mice, is expressed in many tissues in early development, suggesting that, although a drug-metabolizing enzyme, it may have an endogenous role in embryogenesis. To define this role, we have constructed *Nat 2* knockout mice (Cornish *et al. Pharmacogenomics J.* 2003; **3**, 169–177). *In vitro* acetylation assays show that tissues from Nat2 heterozygotes have half the activity of wild-type tissues, with no compensating increase in other NAT isoenzymes. Western blots of adult heart tissue do show, however, that the synthesis of certain cytoplasmic proteins is up-regulated in knockout mice. Whilst these knockout mice have no overt phenotype, the targeted insertion of the lacZ gene into *Nat2* provides a powerful tool for investigating its expression during development. Within the developing heart, expression of Nat2 is spatially restricted.

X-gal staining of neonatal hearts from knockout animals shows Nat2 strongly expressed in the crux of the heart, closely associated with the atrioventricular node, and in the valves of the heart. Immunohistochemical studies of human embryonic hearts show a similar timing and pattern of expression of the equivalent human NAT isoenzyme.

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The familial obesity protein BBS4 targets PCM1 to the pericentriolar region and is required for microtubule anchoring and cell cycle progression

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BBS4 is one of several proteins causing Bardet-Biedl syndrome (BBS), a multisystemic disorder of significant genetic and clinical complexity. Here we show that BBS4 localizes to the centriolar satellites of centrosomes and basal bodies of primary cilia, and that it likely functions as an adaptor of the $p150^{glued}$ subunit of the dynein transport machinery to recruit PCM1 (Pericentriolar Matrix 1 protein) and its associated cargo to the satellites. BBS4 silencing induces PCM1 mislocalization and concomitant de-anchoring of centrosomal microtubules, arrest in cell division, and apoptotic cell death. An equivalent effect on PCM1 and microtubules is observed upon overexpression of two truncated forms of BBS4 that are similar to those found in some BBS patients. Our findings indicate that defective targeting and/or anchoring of pericentriolar proteins, and microtubule disorganization, contribute to the multisystemic phenotype of BBS and potentially provide novel insights into the causes of familial obesity, diabetes and retinal degeneration.

Genetic mapping and microarray analyses to identify factors associated with a mouse obesity QTL region

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The incidence of obesity in the developed world has reached epidemic levels. However, despite the highly heritable nature of obesity, very few of these cases have been attributed to known monogenic causes. We are studying a polygenic model of obesity and have previously reported the discovery of four body fat quantitative trait loci (QTL), between the long-term divergently selected fat (F) and lean (L) lines of mice, differing 5-fold in total body fat. We have since generated congenic lines for the Chr15 *Fob3*-QTL region by backcrossing to the F-line. A more detailed QTL mapping analysis through progeny testing of recombinants, produced from crosses between the F-line and congenic mice, reveals that *Fob3* is composed of at least two smaller QTL: a proximal late-onset *Fob3a* and a distal early-onset *Fob3b*. To identify candidates for the *Fob3b* QTL, a microarray analysis was performed using the NIA-NIH 15K arrays, examining differential expression between the F-line and a congenic line. Several candidates have been identified that are being verified further by Northern or quantitative PCR analyses, and may reveal the causative mutation(s) underlying the QTL effects and identify metabolic pathway(s) responsible for the development of obesity in our models.

The BXSB *Bxs6* interval on chromosome 13 directs gp70 autoantigen levels and concomitant autoantibody production

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Spontaneous murine models of systemic lupus erythematosus (SLE) develop a high serum level of the retroviral envelope protein gp70. This is expressed at a low level in the serum of all murine strains; however, only SLE-prone strains produce anti-gp70 antibodies. These form immune complexes with gp70 (gp70IC) and contribute to nephritis. In the BXSB model, the overexpression of gp70 and the production of gp70IC have been mapped to a single locus on chromosome 13 (*Bxs6*) (LOD = 36, 19·6 respectively, variance = 0·85). Due to the recessive nature of these phenotypes, we have been able to use rare recombinant mapping of BXSB backcross mice to narrow down the region of interest from 35 Mb to 20 Mb. Analysis of a congenic line containing BXSB chromosome 13 has shown that this region is sufficient to cause the overexpression of gp70 and the formation of gp70IC on a C57BL/10 background. Preliminary data suggest that production of gp70IC is solely dependent on *Bxs6*, and does not require additional factors. It is therefore an ideal model of a single locus controlling autoantigen-driven autoantibody production.

Characterization of a gene trap integration into a novel endothelial expressing aminopeptidase (*Ecap*)

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Gene trapping is a powerful approach to characterize and mutate genes. Using this strategy in murine embryonic stem cells, we have isolated and characterised a novel gene, endothelial cell aminopeptidase (*Ecap*). Germline transmitting mice were generated and a detailed analysis of the β-galactosidase (β gal) reporter gene expression was carried out in embryos and adult mice. At 8·5 days post coitum (dpc), β gal staining was observed in the developing heart and dorsal aorta and at 9·5 dpc expression was seen in the heart, developing vasculature, intersomitic vessels and capillary plexus of the head. In adult spleen, kidney, lung and skeletal muscle expression is exclusive to endothelial cells whereas in the adult heart expression is also seen in the cardiomyocytes. No obvious phenotype has been observed in animals homozygous for this gene trap integration. This is probably due to the fact that the gene trap vector has integrated into the last intron of *Ecap* resulting in the generation of a fusion protein that retains the majority of the endogenous protein sequence and therefore much of its functional activity. The cDNA structure of the gene has been determined by 5′RACE and RT-PCR. A number of different isoforms have been characterized. One isoform contains a matrix metalloproteinase domain, and the whole protein sequence has weak homology to a protein called amino peptidase B. The gene maps to mouse chromosome 13 and there is a highly conserved human homologue on the syntenic region of chromosome 9. As Ecap is an aminopeptidase that is expressed almost exclusively in endothelial cells we predict that it may be involved in angiogenesis and could be a molecular target for cancer therapy.

A role for Igf-2 in fibre-type selection: insulin-like growth factor II (Igf-2) promotes the formation of fast myosin positive myotubes

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The peptide growth factor Igf-2 is expressed in newly formed myotubes during embryogenesis as well as in newly formed muscle fibres during post-natal muscle fibre regeneration. To investigate the function of Igf-2 in the formation of these myotubes and their subsequent maturation, we have used a bicistronic transgene to force the overexpression of Igf-2 in skeletal muscle cells. Results show that, under differentiation permissive conditions, forced expression of Igf-2 increases the proportion of fast myosin positive myotubes formed in normal skeletal muscle cells by 30%. Furthermore skeletal muscle cells stably transfected with Igf-2 produce higher numbers of myotubes than controls, suggesting that forced expression of Igf-2 promotes muscle cell fusion *per se*. These data support the hypothesis that Igf-2 plays an important role in the regulation of skeletal muscle differentiation and may also play a role in fibre type selection. To further dissect this role, we are now using RNAi constructs to target both endogenous Igf-2 and transgenically expressed Igf-2 in skeletal muscle cells. Thus, we will further establish the function of Igf-2 in skeletal muscle myotube differentiation by knocking out Igf-2 expression. We expect these data to shed light on the normal role of Igf-2 in myogenesis; however, this work also provides an important clue towards understanding the processes underlying the anti-dystrophic action of Igf-2 that we described previously (Smith *et al. Cell Death Differ*. 2000; **7**, 1109–1118).

Identification of mitotic spindle abnormalities associated with chromosomal chaos in human preimplantation embryos *in vitro* by confocal laser scanning microscopy

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Despite recent technical improvements, many human preimplantation embryos fail to develop to the blastocyst stage or implant after transfer to the uterus. A possible cause for this is the high incidence of nuclear and postzygotic chromosomal abnormalities observed during cleavage, including chaotic chromosome complements, suggestive of defects in mitotic chromosomal segregation. The underlying mechanisms are largely unknown, but similarities with chromosome instability in human cancers led to the proposal that cell cycle checkpoints may not operate at these early stages. To investigate this and to examine whether spindle abnormalities contribute to chromosome malsegregation, we have used confocal laser scanning microscopy, following immunolabelling with antibodies specific for α-tubulin, γ-tubulin, acetylated tubulin, or EB1, combined with a DNA fluorochrome to visualize spindle and chromosome configurations in normal and arrested human embryos, from cleavage to blastocyst stages. We demonstrate for the first time that, in addition to frequent interphase nuclear abnormalities, spindle abnormalities including monopolar, tripolar and multipolar spindles as well as abnormally shaped spindles and chromosome lagging are a regular feature of preimplantation embryos. We conclude that spindle abnormalities and centrosome replication anomalies may be the primary mechanism leading to chaotic chromosomal segregation and the nuclear abnormalities observed in early human development.

Muc1 hypervariability in gastric and oesophageal disease

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The membrane mucin MUC1 contains a long tandem repeat (TR) array which is highly variable, both in length and in sequence. Despite this variability there is tight linkage disequilibrium across the region. We have previously shown that short tandem repeat alleles are associated with increased risk of gastric cancer (Carvalho *et al. Glycoconjugate J.* 1997; **14**, 107–111) and also an increased risk of developing H. pylori gastritis (Vinall *et al. Gastroenterology* 2002; **123**, 41–49 and Silva *et al. Eur. J. Hum. Genet.* 2001; **9**, 548–552). MUC1 is also aberrantly expressed in gastritis (Vinall *et al.*, 2002). Our aim is to further characterize the haplotype distribution of MUC1, particularly in gastric disease. Southern blot analysis was used to determine TR length, multiplex PCR for the flanking markers and minisatellite variant repeat (MVR) analysis (Fowler *et al. Hum. Genet.* 2003; 27 Aug) was used to generate maps of the PDTR-PESR changes in the tandem repeats. We have tested patients with *H. pylori* gastritis, oesophageal cancer, gastrointestinal clinic controls, and unrelated and unselected UK controls as well as gastritis patients from North Portugal. Preliminary haplotype analysis showed that in the UK gastritis group there is an over-representation of small alleles of rare haplotype when compared with other groups.

Virtual gene nomenclature: making the most of the genomic sequence

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Historically, the existence of genes was frequently inferred from work on proteins. Investigating isozymes or cell surface antigens in somatic cell hybridization, and more recently the *in situ* hybridization of cDNA probes to multiple genomic regions, led to the assignment of gene symbols to approximate chromosomal locations, but without gene sequence. The HUGO Gene Nomenclature Committee (HGNC) recently ran a virtual gene nomenclature workshop, which aimed to associate newly available genomic sequence to 1353 approved gene symbols with a

chromosomal location but without sequence. As a direct result of the workshop, 761 (56%) of the initial 1353 genes now have sequence associated with them. In many cases, the workshop also resulted in gene sequence being newly associated to the symbol approved for the mouse orthologue. We will discuss the bioinformatic methods used and present examples showing when these methods were successful and unsuccessful.

The work of the HGNC is supported by NIH contract N01-LM-9-3533 (60%) and by the UK Medical Research Council (40%).

Multimerin-1 and alpha-synuclein are deleted in C57BL/6JOlaHsd mice and lie in a paralogous cluster of unrelated genes

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We have recently discovered a spontaneous deletion of the murine *Snca* (alpha-synuclein) gene in C57BL/6JOlaHsd animals, a population of the inbred mouse strain C57BL/6J (Specht, C. G. and Schoepfer, R. Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neurosci.* 2001; **2**, 11). The human orthologue of alpha-synuclein has been implicated in the aetiology of Parkinson's disease. We have now characterized the exact nature of this deletion, Del(6)*Snca*1Slab. Detailed mapping followed by sequencing of the breakpoint region revealed the absence of a 365 kbp segment from mouse chromosome 6, encompassing the *Mmrn1* (multimerin-1) gene in addition to *Snca* (Specht, C. G. and Schoepfer, R. Deletion of multimerin-1 in alpha-synuclein-deficient mice. *Genomics* 2004; **83**, 1176–1178). The analysis of gene expression profiles by cDNA array technology and behaviour in a spatial learning task did not reveal any noticeable phenotypes in affected animals despite the lack of the alpha-synuclein and multimerin-1 gene products (Specht & Schoepfer, 2001; Chen, P. E., Specht, C. G., Morris, R. G. & Schoepfer, R. Spatial learning is unimpaired in mice containing a deletion of the alpha-synuclein locus. *Eur. J. Neurosci.* 2002; **16**, 154–158). Sequence comparisons revealed that the chromosomal organisation of the *Sncg* and *Mmrn2* loci is similar to the region containing *Snca* and *Mmrn1* (Specht & Schopfer, 2004). This suggests a duplication event of a cluster of apparently unrelated genes during evolution. Interestingly, none of these paralogous sets of genes have undergone pseudogenization but have acquired distinctive expression patterns, possibly related to the deletion or insertion of upstream regulatory sequences.

The novel ras/MAPK antagonist, *Sprouty1*, is essential for normal renal development

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The *sprouty* (*spry*) gene was identified in *Drosophila* as a regulator of branching morphogenesis of the trachea. SPROUTY proteins act as general, intracellular feedback inhibitors of ras/MAPK signalling downstream of growth factor receptors. In order to investigate the role of the first mammalian homologue of this family, *Sprouty1*, during embryogenesis, the *Sprouty1* gene was inactivated by gene targeting in the mouse. *Spry1* knockout mice exhibit severe defects in kidney and genito-urinary tract development, resembling the human condition CAKUT (congenital abnormalities of the kidney and urinary tract). These include duplex kidneys, multiple ureters, hydronephrosis and cystic dysplasia of the kidney. The embryonic origin of these defects was investigated and shown to be the result of ectopic ureteric buds appearing along the Wolffian duct. We propose that *Sprouty1* restricts ureteric bud outgrowth to the caudal portion of the Wolffian duct by antagonizing growth factor receptor activation along the duct.

Striking dosage and genetic background effects on eye phenotype in PAX6 transgenic mice

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'PAX77' yeast artificial chromosome transgenic mice, carrying five to seven copies of the human PAX6 gene and two mouse *Pax6* genes, have small, abnormal eyes (Schedl *et al*., *Cell* 1996; **86**, 71–82). We crossed PAX77 mice (outbred CD-1 genetic background) to inbred CBA/Ca mice to compare the phenotype with CBA/Ca-*Pax6*$^{+/-}$ mice. At 12 weeks, PAX77, CD-1 eyes were much smaller than eyes from non-transgenic littermates: $5{\cdot}62 \pm 0{\cdot}37$ mg ($n = 37$ eyes) versus $25{\cdot}17 \pm 0{\cdot}34$ mg. ($n = 29$); $P < 0{\cdot}0001$. The small-eye phenotype disappeared in the first generation (CD-1 × CBA/Ca)F1 eyes and there was no significant weight difference: $22{\cdot}18 \pm 0{\cdot}62$ mg ($n = 43$) versus $23{\cdot}24 \pm 0{\cdot}50$ mg ($n = 24$); $P = 0{\cdot}249$. However, a significant difference reappeared after five backcrosses to CBA/Ca: $13{\cdot}89 \pm 1{\cdot}54$ mg ($n = 20$) versus $22{\cdot}54 \pm 0{\cdot}25$ mg ($n = 16$); $P < 0{\cdot}0001$. PAX77, CD-1 eyes had severe abnormalities affecting the lens (subcapsular epithelial cell proliferation; thickened and folded capsules), retinas (folded and dysplastic with rosettes; adherent to the lens), irides (malformed and adherent to the cornea and/or lens capsule) and ciliary body (disorganized). Sometimes the vitreous cavity and/or anterior chamber was absent. PAX77, (CD-1 × CBA/Ca)F1 eyes had disorganized ciliary bodies and flattened cyst-like irides but were otherwise normal. These striking differences provide a further warning of the importance of standardizing the genetic background for phenotype comparisons of different mutants in an allelic series.

Lack of Chx10 causes embryonic retinal progenitors to persist in the adult retina

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The six types of retinal neuron and Muller glial cells derive from retinal progenitor cells (RPCs) of the embryonic optic cup. A full complement of retinal neurons is born by postnatal day 11 in the mouse when neurogenesis is complete. Little is known about how RPCs regulate the timing of the decision to continue dividing or to differentiate. In mice lacking the *Chx10* homeobox gene, proliferation of RPCs is reduced during development and adult mice have microphthalmia. In this study we investigate the hypothesis that *Chx10* plays a critical role in regulating the number of RPCs. We compared the distribution of RPCs in the postnatal *Chx10* null and wild-type retina, using incorporation of the thymidine analogue BrdU to label dividing cells. We report that lack of *Chx10* causes a significant number of cycling cells to persist in the adult neural retina. Immunostaining with nestin and neuronal marker β3-tubulin indicates that the BrdU-labelled cells can give rise to neurons within the adult retina. This is the first report of neurogenesis in the mature mammalian central retina. More dividing cells were also detected in the mutant ciliary epithelium (CE) than in the wild-type CE (which is known to harbour cells with stem cell properties).

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