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***Hoxa1* and *Hoxb1* are indispensable for neural crest formation independently of initial pharyngeal arch patterning in the mouse**

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The pharyngeal arches are transient metameric facial structures which are composed of cells of different embryonic origin. They are formed by surface ectoderm and pharyngeal endoderm and are populated by paraxial mesoderm and cranial neural crest cells. The relative contribution of each cell population to the patterning of these structures is still a matter of debate. We address the role of neural crest in pharyngeal arch formation and patterning by genetically ablating the second pharyngeal arch neural crest. This is achieved by combining the *Hoxa1* null mutation with the mutation of the ectoderm-specific Retinoic Acid Response Element in the 3′ of *Hoxb1*. In the compound mutant embryos there is a cell-autonomous defect of the neuroepithelium to generate the second arch neural crest population as evidenced by DiI lineage tracing of mouse embryos in culture, molecular and histological analysis. However, the formation and initial patterning of the second pharyngeal arch are not affected. This is inferred by the correct expression patterns of molecular markers and the formation of the second arch placode-derived ganglion. The cells comprising the mutant second arch are mitotically active and there is no increase in cell death suggesting that the core of the arch is not the source of indispensable survival/proliferation signals. In summary, we provide evidence that Hox genes are important players in both neural crest formation and patterning and that initial pharyngeal patterning in the mouse is not dependent upon migration of the neural crest.

Limb defects caused by a 120 kb deletion at the 5′ end of the human *HOXD* cluster

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Synpolydactyly (SPD) is a dominantly inherited limb malformation caused by mutations in *HOXD13*. In a father and daughter with atypical SPD, and no detectable *HOXD13* mutation, haplotype analysis revealed a microdeletion involving the *HOXD* gene cluster. FISH studies, Southern blot analysis and direct sequencing across the deletion breakpoint showed that the deletion encompasses *HOXD9* to *EVX2* and extends 90 kb upstream of *HOXD13*. These patients' limb abnormalities are most likely due to haploinsufficiency for one or more of the six deleted genes, particularly *HOXD13*, the most important 5′ *HOXD* gene in autopod development. Mice lacking both copies of *Hoxd11–Hoxd13* have an SPD-related phenotype, but mice lacking one copy of *Hoxd11–Hoxd13* have virtually normal limbs, suggesting a greater sensitivity to reduced *HOXD* gene dosage in humans than in mice. Mice heterozygous for a deletion encompassing *Hoxd11* to *Evx2* and extending 28 kb upstream of *Hoxd13* exhibit premature activation of the remaining 3′ *HoxD* genes, due to loss of an element that controls timing of *HoxD* gene expression, resulting in vertebral and sternal abnormalities. No such abnormalities are present in our patients, suggesting that their remaining 3′ *HOXD* genes are

not expressed, perhaps due to loss of a more 5′ locus control region.

Different mechanisms for *Hox* gene induction along the A–P axis of the avian neural tube

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Understanding of how homeotic (*Hox*) gene expression domains are established and maintained during development is of critical importance since a slight change in their boundaries leads to dramatic alterations in segmental identity. Using grafting experiments and transgenic mouse approaches in mouse and chick embryos, our group has investigated the mechanisms that set up *Hox* gene expression domains in the neural tube. We discovered that the paraxial mesoderm is sufficient as a source of environmental signals to establish and/or reprogram *Hox* expression in the hindbrain. We found that a secreted 20–100 kDa somatic factor as well as retinoid signalling are essential for *Hoxb4* early induction in the hindbrain. Moreover, somites are not able to induce or reprogram more posterior *Hox* genes in the spinal cord, suggesting that another mechanism is responsible for the initiation of *Hox* genes in the posterior neural tube. When retinoid and FGF pathways were tested for their ability to induce *Hox* gene expression, it emerged that *Hox* genes are regulated by different mechanisms according to their position along the A–P axis of the neural tube.

***Sox2* interprets retinoid signalling in the developing ventral neural tube**

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Sox1, *-2* and *-3* form a unique group of genes within the SOX family (Group B1) expressed in an overlapping pattern throughout the prospective central nervous system, involved in the establishment of the early dividing precursors of the central nervous system. As retinoids are key regulators of the developing central nervous system, we analysed the expression of *Sox1*, *Sox2* and *Sox3* in a retinoic acid (RA)-deficient environment in mouse embryos that lack retinaldehyde dehydrogenase 2 (RALDH2), a key enzyme in embryonic retinoic acid synthesis. *Sox3* is unaffected by the absence of RA while *Sox1* is restricted dorsally within its regular expression domain. *Sox2* is the only gene affected in a spatially restricted manner along the A–P axis, with a lack of expression in the spinal cord posterior to the r6/r7 boundary, as well as dorsal restriction in the caudal neuroepithelium. Retinoid involvement in the regulation of *Sox* gene expression is evolutionarily conserved in vitamin A-deficient quail embryos. Analysis of molecular markers such as *Pax6* and *ngn2* revealed the absence of ventral patterning along the neuroepithelium while *Shh* signalling and paraxial mesoderm has not been affected in RALDH2−/− embryos. Electroporation of *Sox2* in the neural tube of mouse embryos induces ectopic expression of *Pax6*. Thus, *Sox2* interprets the retinoid signal to establish a retinoid-activated pathway of neurogenesis in the developing ventral spinal cord.

Regulation of programmed cell death during cardiac development

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Using a novel data summation technique, we have made a detailed, quantitative, spatio-temporal survey of programmed cell death (PCD) in the developing mouse heart using the TUNEL assay. This covers embryonic days 10·5–13·5 when the heart is being divided, by the formation of septa and valves, into a four-chambered structure. We observe a time-dependent variation in the occurrence of PCD foci. These particularly affect the interventricular septum, the outflow tract cushions and myocardium, the ventral aspect of the ventricles, the atrio-ventricular cushions and the septum spurium of the right atrium. In contrast to other developing systems where high levels of PCD are seen, we demonstrate that macrophages are not involved in the clearance of this PCD. Evidence from immunohistochemical analysis of Fas ligand shows that this important 'death ligand' is expressed in regions of PCD foci, with the exception of the endocardial cushions. To investigate downstream pathways of this ligand, we have surveyed and

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quantitated PCD in FADD dominant negative mutant mice. Our results suggest that FADD, and therefore activation of death receptors, may both enhance and protect against PCD in cardiac development in different regions of the heart.

Expression of the *CRX* gene in human retina during early eye development

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Expression of the *CRX* transcription factor in the developing human retina was investigated. *CRX* is expressed from 10·6 weeks post-conception (pc) by RT-PCR analysis, which is relatively much earlier than in mouse eye development (20 weeks human equivalent). At 13 weeks pc by *in situ* hybridization, expression of *CRX* is localized to the newly born photoreceptors. At 15 weeks pc expression is detected throughout the outer neuroblastic layer (ONBL). In adult retina, expression is observed in both the outer nuclear layer (ONL) and the inner nuclear layer (INL), suggesting *CRX* is not photoreceptor-specific. Mutant *rdcl* mice, lacking outer segments (OS) and ONL, were included in this study. RT-PCR analysis revealed that *CRX* expression is maintained in these photoreceptor-degenerate retinas and its expression in the INL was confirmed by *in situ* hybridization. The expression of a number of retina-specific genes, postulated to be transcriptionally regulated by *CRX*, was also tested by RT-PCR in human and mouse fetal cDNAs. Each of the genes tested was expressed either at the same time as, or after, *CRX*. *PDEB*, however, was shown to be expressed 2 weeks before *CRX* in the human retina (8·6 weeks pc) and may have an as yet undetermined role in human eye development.

Identification and characterization of specific genes which are involved in actively dividing tissues by using testis as a model

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Testis is an interesting organ, compared with other organs in the body, because it contains many dividing cells in stages of development which range from undifferentiated stem cell populations of germ cells to highly differentiated cell populations, and ultimately sperm. The expression of a wide variety of genes is developmentally regulated during human spermatogenesis. By the identification and isolation of cDNAs expressed specifically in the human testis we aimed to identify novel genes involved in spermatogenesis and/or spermiogenesis. Using Differential Display Reverse Transcriptase-PCR (DDRT-PCR) on RNA from a range of tissues which were selected to be logically related to testis functions, we isolated a number of short cDNA fragments. Tissue-specific expression was confirmed by using RT-PCR with gene-specific primers. Entire transcripts were sequenced by primer walking along cDNA templates generated by 5′ RACE and the size of full-length cDNAs was confirmed by Northern analysis. In addition, the chromosomal locations of the genes were determined by two methods: firstly, by PCR amplification using gene-specific primers, of a panel of rodent somatic cell hybrids that each contain a single human chromosome; secondly, genomic clones (BAC or a PAC) were hybridized *in situ* to human metaphase chromosomes (FISH). In this way we have isolated and characterized two novel genes that are expressed in testis but not in a range of functionally related tissues, nor are they expressed in testes of two infertile patients. The genes have varying levels of expression in actively dividing fetal tissues. In addition, we observe that human ESTs homologous to these transcripts have been predominantly isolated from libraries constructed from human testis, fetal tissues and different sorts of tumours. The common factor here is that all sources contain a large number of dividing cells. Therefore, it is possible that patterns of gene expression in the dividing cells of the testis in may be a good model to compare with uncontrolled cell division in cancers.

Examining the regulation of the *Gnas* imprinting cluster

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Imprinted genes are unusual in that only one copy of the gene is expressed depending on the parental origin of the chromosome. The distal region of mouse chromosome 2 contains a number of imprinted transcripts, including *Nesp* and *Gnasxl*, expressed from the maternal and paternal alleles respectively.

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Uniquely, these two transcripts splice onto and share exons with a third, predominantly biallelically expressed gene *Gnas* (α-stimulatory G protein subunit). The function of these alternative gene products remains unresolved. In order to ascertain how this complex locus is regulated, we have analysed the region for both sequence and epigenetic features. We have determined the physical organization of the *Gnas* cluster and analysed the sequence for CpG island-like elements, and associated direct repeats, as association identified in control regions at other imprinted loci. Allele-specific methylation and chromatin organization have been determined, in both mouse tissues and embryonic stem (ES) cells. Striking hypersensitive sites were identified in androgenetic ES cells that were absent in parthenogenetic ES cells and these mapped to the location of a recently identified imprinted antisense transcript. It is possible that these elements may be important for imprinting control. We are producing large construct transgenic mice to study the regulatory effects of putative elements on imprinted expression.

Bi-allelic expression of ESTs within a cluster of imprinted genes

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Imprinted genes are silenced according to their parental origin. Mouse distal chromosome 2 contains a cluster of imprinted genes. Three of these, *Nesp–Gnasxl–Gnas*, are transcribed in the sense direction and a fourth, *Nespas*, in the antisense direction. *Nespas* starts just upstream of *Gnasxl*. *Nesp*, *Gnasxl* and *Gnas* form a single transcription unit with *Nesp* using maternally active promoters and *Gnasxl* using paternally active promoters. In both cases the promoters lie within differentially methylated regions and the active promoters are unmethylated. Analysis of the 14 kb genomic sequence separating *Nesp* and *Gnasxl* has revealed two new ESTs. Remarkably, neither of these two ESTs appear to be imprinted for they have been shown to be bi-allelically expressed. Furthermore both ESTs are transcribed in the sense and antisense direction and also in a tissue-specific manner. At the very least these ESTs must be part of two new transcription units, one in the sense and one in the antisense direction. Future studies are directed towards investigating whether the ESTs form part of the same transcripts, and whether they represent genes whose expression is regulated independently of the mechanisms controlling the imprinting of the cluster.

Detailed FISH analyses of Silver–Russell syndrome (SRS) patients with cytogenetic disruptions of chromosome 7p11.2–p13 define a candidate region for SRS

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SRS is a congenital disorder primarily involving lateral asymmetry and both intrauterine and postnatal growth restriction. This disorder is genetically heterogeneous, but maternal uniparental disomy for chromosome 7 (mUPD7) has been demonstrated in approximately 7% of cases. Consistent heterodisomy for the full length of chromosome 7 in five mUPD7 subjects indicates that at least one gene on this chromosome is imprinted and involved in the pathogenesis of SRS. We have previously reported a *de novo* duplication of 7p11.2–p13 on the maternal homologue in a patient with classical SRS features. This duplication was shown to include the GRB10, IGFBP1 and 3 genes, but not EGFR. A similar case of a maternally inherited duplication of 7p11.2–p13 has also been described by another group. These two cases suggest that SRS may result from overexpression of a maternally expressed gene involved in growth suppression. Mice with maternal disomy for proximal chromosome 11, the orthologous region to human 7p11.2–p13, demonstrate prenatal growth failure, further implicating this region in SRS. We have performed more detailed fluorescent *in situ* hybridization analysis on the two patients with duplications, to locate the breakpoints more precisely. In addition, we describe a group of SRS patients with other cytogenetic abnormalities involving 7p11.2–p13. The breakpoint mapping data presented here define a candidate region for genes which may contribute to the SRS phenotype in patients with a genetic aetiology involving chromosome 7.

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A new imprinted gene mutation on mouse distal chromosome 2

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A mutant called oedematous/small (*Oed-Sml*) is described which shows an imprinted mode of inheritance. The mutation lies within the distal chromosome 2 imprinting region and shows two distinct phenotypes according to the parental route of transmission. When transmitted maternally the mutant displays a gross neonatal oedema with microcardia resulting in early lethality. When transmitted paternally the mutant displays postnatal growth retardation. The mutation has been mapped by crossing mutant males with *M. m. castaneus* females, backcrossing the mutants to C3H/HeH and typing the backcross progeny for *M. musculus–M. m. castaneus* polymorphisms at *Mit* marker loci in the candidate region. The *Oed-Sml* mutation is located between the markers *D2Mit25* and *D2Mit504*, which lie within the distal chromosome 2 imprinting region. This region includes the *Gnas* locus from which oppositely imprinted transcripts are expressed. This locus is being analysed as a candidate for the site of the mutation.

Characterization of a putative control element that lies between the imprinted *Igf2* and *H19* genes in the mouse

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The insulin-like growth factor II gene is imprinted in a tissue-specific manner in both mouse and man. In the majority of embryonic and extraembryonic tissues during development there is a strong paternal allele-specific bias of *Igf2* gene expression. In contrast, this gene is expressed from both parental alleles in the choroid plexus and leptomeninges of the brain. A series of transgenic mice have been created in which a reporter gene is driven from *Igf2* promoter 3, in cis to a 2 kb candidate regulatory region from the *Igf2* locus. This region (the CCD) lies midway between the *Igf2* and *H19* genes, and has previously been shown to be hypersensitive to nucleases, relatively hypomethylated, and conserved in a number of mammalian species. Transgenic mice bearing this element express the reporter gene specifically in the choroid plexus of the brain. Examination of the mode of reporter gene expression from these transgenic mice, as well as identification of proteins that interact with CCD sequences, may elucidate the mechanisms by which the *Igf2* gene escapes imprinting in the exchange tissues of the brain. This knowledge in turn may lead to an understanding of how the *Igf2* gene becomes bi-allelically expressed in pathological conditions.

Identification and analysis of conserved transcripts upstream of the *H19* gene

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Genomic imprinting leads to mono-allelic expression of some genes in a parent-of-origin-dependent manner. Much interest has been focused on the reciprocally imprinted genes *H19* (maternally expressed) and *Igf2* (paternally expressed), located on distal mouse chromosome 7 and human chromosome 11p15. These genes form a coordinately regulated unit and are separated by a large intergenic region of 90 kb in the mouse, thought to contain regulatory elements. Comparison of human and mouse genomic sequence between the *H19* and *Igf2* genes revealed two regions of strong (> 80%) homology. These H19 Upstream Conserved regions (HUC1 and 2) are transcribed in a variety of tissue types and developmental stages which are not exclusively coincident with the pattern of *H19* expression. Human mutations in the 11p15 region affecting imprinting lead to the genetic disease Beckwith–Wiedemann syndrome (BWS). Interestingly some tissues in which HUC expression was detected are affected in BWS. Current mouse models cannot fully explain all the human BWS phenotypes. The most conserved region (HUC2) is bi-allelically expressed in the mouse and in human placenta. The discovery of these conserved regions has implications for the mechanistic and functional analysis of the *H19–Ifg2* region.

Comparison of the imprinted *Dlk/Gtl2* and *Igf2/H19* loci in the mouse

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Dlk and *Gtl2* are two reciprocally imprinted genes that reside close to each other on chromosome 12 in

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the mouse. *Dlk* shows similarity to the Notch ligand Delta which is involved in regulation of cell growth and differentiation in *Drosophila*. *Dlk* is paternally expressed like the insulin-like growth factor gene (*Igf2*) on mouse chromosome 7. *Gtl2* is probably a non-translated RNA and is maternally expressed, thereby showing similarities to the non-coding *H19* gene downstream of *Igf2*. Similarities between *Dlk/Gtl2* and *Igf2/H19* concern not only the regulation and possible function of these genes but also the physical structures of both loci. On the basis of the DNA sequence of the intergenic *Dlk/Gtl2* region we were able to identify putative regulatory elements that are also present in the *Igf2/H19* region in similar positions. The observed similarities suggest that imprinting mechanisms at both loci may have co-evolved, and future analysis may provide key insights into the evolution of imprinting mechanisms at the genomic level.

A lysine-residue-specific histone acetylation pattern is a contributory factor in the control of imprinted gene expression

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Genomic imprinting describes a subset of mammalian genes whose expression depends upon the parental origin of that chromosome. This now applies to over 30 genes in mice and man with wide-ranging functions, including many aspects of growth and development. The two alleles of imprinted genes are identical in DNA sequence so their differential expression must result from an epigenetic mark, possibly chromatin-based. Here we investigate the role of histone acetylation, already shown to play an important role in dictating both local and widespread chromatin configuration. We have used chromatin immunoprecipitation (CHIP), with residue-specific antibodies to acetylated histones, and single-stranded conformational polymorphisms (SSCP), to directly compare parental alleles. We have identified a histone- and lysine-residue-specific acetylation pattern at three distinct imprinted loci in the mouse (*U2af-rs1*, *snrpn*, *Igf2-h19*). This pattern may play a key part in directing a series of protein–DNA and protein–protein interactions that mark and distinguish the parental alleles, leading to their differential expression.

Allele-specific histone acetylation confers differential chromatin conformation to an imprinted locus in the mouse

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Correct regulation of imprinted gene expression is essential for normal mammalian development. How cells 'recognize' the parental origin of imprinted loci remains unclear. Epigenetic features associated with genomic imprinting are parental allele-specific DNA methylation and differential replication timing. The latter may reflect chromatin conformation differences between parental alleles. Previously, we showed the imprinted, splice factor-encoding *U2af1-rs1* gene displays differential sensitivity to nucleases, throughout the region of differential methylation. In an attempt to characterize modifications responsible for the conformational difference between the repressed maternal and the active paternal allele, we performed chromatin immunoprecipitations using antibodies to specific acetylated lysine residues of H3 and H4. We detected allele-specific patterns of histone acetylation confined to the domain of differential nuclease sensitivity. Interestingly, for H4, both the parental alleles had high levels of acetylation except at lysine 5, which was hypoacetylated when inherited from the mother. The allelic differences for H3 were present at all lysines analysed, the maternal allele being hypoacetylated. Treatment with the histone deacetylase inhibitor Trichstatin-A resulted in an altered conformation, associated with a gain of acetylation on the maternal allele. Using a genetic approach, we established that CpG methylation is partly, but not entirely, responsible for the complex allelic patterns of histone acetylation.

A transcriptional silencer in the mouse *Igf2* gene is controlled by DNA methylation

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Imprinted genes are mono-allelically expressed and contain differentially methylated regions (DMRs). In some imprinted genes the inactive allele is methylated, but in others DMRs are methylated on the active

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allele, giving rise to the hypothesis that DMRs contain silencers which are epigenetically regulated. The DMR2 region in *Igf2* is located at the beginning of the last exon, and is highly methylated on the paternal allele in all tissues with *Igf2* expression. The effect of the DMR2 on transcription was examined using luciferase reporter gene assays. In these assays, DMR2 significantly reduced *Igsf2* promoter activity. Methylation of the luciferase constructs causes significant reduction of luciferase activity in the absence of DMR2, but not in the presence of DMR2. Results from deletion analysis suggest that the core (54 bp) DMR2 has separate silencing and silencing blocking regions. To test the silencer blocking function *in vivo*, the DMR2 core was deleted by Cre loxP in mice. These mice transcribe 50% less *Igf2* RNA than wild-type and are 30% smaller than wild-types. RNAse protection and nuclear run-on experiments are currently being performed to determine whether DMR2-mediated silencing is at the level of transcription elongation.

_G90_, an untranslated RNA predominantly expressed in post-mitotic cells

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The mouse *G90* gene is located on chromosome 6 and contains two exons and one intron. Its 1·5 kb transcript is polyadenylated but shows no long ORFs, indicating that *G90* is a non-coding RNA. *G90* orthologs found in rat and human also show characteristics of non-coding RNAs. *G90* shows a very specific expression pattern in the intestine and the testis of adult mice, its expression being restricted to non-proliferating cells in both tissues. Furthermore, *G90* is detected in various areas of the head during embryonic development, including the nasal and otic epithelia, and its expression often correlates with *p27Kip1* expression and lack of proliferation. Together, these findings suggest a role for *G90* in cell cycle control. High levels of *G90* are also detected in intestinal tumours from APC-deficient mice. To elucidate the role of *G90*, we have produced transgenic mice carrying the human *G90* plus extensive flanking sequences. The expression pattern of the transgene in the head is identical to the endogenous expression pattern. However, no obvious abnormalities due to the presence of the transgene have been identified in the head. Further experiments are presently being performed to determine the expression pattern and the possible effects of the human *G90* transgene in the intestine of adult mice.

Complex transcription of the utrophin gene: a study of short utrophin isoforms

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Utrophin is the autosomal homologue of dystrophin. Unlike dystrophin, utrophin is expressed ubiquitously and in skeletal muscle is located at the neuromuscular and myotendinous junctions. It has been shown that overexpressing utrophin in the muscle of *mdx* mice and mice deficient for both dystrophin and utrophin leads to amelioration of the muscle pathology. Thus upregulation of the endogenous utrophin gene in Duchenne muscular dystrophy patients might be useful as a therapeutic strategy. It is therefore important to establish a complete transcriptional map of the utrophin gene. Using 5′ RACE we have identified two novel transcripts of utrophin, Up71 and Up140, that appear to be homologues of two short dystrophin transcripts with unique first exons located in intron 62 and 44 respectively. These transcripts are subject to differential RNA splicing in a manner similar to dystrophin. RNA *in situ* hybridization to embryo sections from control and utrophin (targeted in exon 7) deficient mice show high levels of expression in neural tube, sensory ganglia and digits. Very recently we have identified a novel transcript in digit mRNA. Up71 and Up140 are ubiquitously transcribed; a further transcript, G-utrophin, is found only in neural tissue. Protein analysis suggests that these short utrophin isoforms are only translated in selected tissues, pointing towards a complex post-transcriptional regulatory mechanism.

Overexpression of human NAT1 in the mouse

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Arylamine *N*-acetyltransferase (NAT) exists as three isoforms in mice and two in human. Human NAT1 and mouse NAT2 are functional homologues and are

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likely to have a role in folate catabolism. To understand the importance of their role, we aimed to create a mouse transgenic model that has the human NAT1 under the control of the potent human cytomegalovirus promoter and targeted to disrupt the mouse NAT2. The litters from the first series of blastocyst injections were very small with signs of embryos dying at birth. None of the surviving pups were chimeric. To establish whether the transgene was deleterious, caesarean section was performed in subsequent experiments immediately before the expected time of birth. This revealed a lower than expected number of embryos. Some embryos were resorbing, whilst others were grossly enlarged and died at birth. These carried the transgene. One pup with minor coat chimerism survived but had retarded growth and a kinked tail. The constitutive expression of the transgene seems to have a deleterious effect on the developing embryos. Coat chimerism is very low in the surviving pups and the transgene is present in each abnormal embryo. Funded by SPARKS and the Wellcome Trust.

Dynamic regulation of the cdk inhibitor p57kip2 during embryo morphogenesis

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The cyclin-dependent kinase inhibitor (CDKI) p57kip2 is the only CDKI reported to be required for normal embryo morphogenesis. In humans, it is implicated in Beckwith–Weidemann syndrome (BWS). The complete developmental expression pattern of this gene has not been reported. We examined the localization of p57kip2 during mouse organogenesis (E10.5–E17.5). p57kip2 is in most organs at key stages of their genesis. It is in all three germ layers and neural crest cells. P57kip2 is found earliest (E10.5) in the heart and in neural tissues, notably developing pituitary. In parenchymal organs (e.g. lung and kidney) p57kip2 levels are strongest at E13.5. As differentiation is completed, p57kip2 levels decline. p57kip2 persists in the musculoskeletal system up to E17.5 and is also found extensively in the placenta and extraembryonic membranes. In many tissues we observe mosaicism of staining consistent with a cell cycle regulation of expression. In others, p57kip2 is present in groups of cells suggesting their regulation during initiation or execution of differentiation. In lung, kidney and gonad p57kip2 is coincident with shape changing and tubule formation. In kidney p57kip2 is associated with apoptosis. P57kip2 may regulate morphogenesis in tissues affected by BWS (e.g. kidney) and those which are not (e.g. lung).

Characterization of the human *CRX* gene which is essential for normal retinal development

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The human *c*one-*r*od homeobo*x* gene (*CRX*) is essential for normal retinal development. Mutations in *CRX* have been associated with a number of retinal dystrophies. We have further characterized the *CRX* gene and now present data showing the complexity of the *CRX* transcript. We have identified two upstream exons termed exons 1Aα (559 bp) and IAA (446 bp). RT-PCR from Y-79 retinoblastoma-derived cDNA has shown that exon 1Aα is alternatively spliced to generate exons 1Aβ (322 bp), 1Aγ (144 bp) and 1Aδ (77 bp). All these upstream exons are spliced to the previously reported exon 1 except exon 1Aβ, which may have exon laγ spliced at the 3′ end. The new exons appear to be untranslated as there are numerous stop codons in-frame with the previously published sequence for *CRX*. In the genomic sequence there are excellent matches to the splice acceptor sequence at the 5′ end of the exons, indicating the presence of additional exon(s) upstream. We have also shown that *CRX* has a 3′ UTR of either 1184 bp or 3369 bp depending on which of two polyadenylation sites is used. The new exon sequence has been screened in families linked to the *CRX* gene which have no coding sequence mutations; however, no mutations were identified.

Characterization of elements controlling the expression of myogenic regulatory factors during mouse embryogenesis

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The muscle-specific transcription factors Myf5 and Mrf4 are two of the four myogenic regulatory factors (MRFs) involved in the transcriptional cascade which initiate the myogenic process in vertebrate embryos. *Myf5* is the first MRF to be expressed. We have previously defined enhancers driving *Myf5* expression in particular subsets of muscle precursor cells within 14·2 kb encompassing *Mrf4*/*Myf5*, and indicated that

additional elements are required. Individual elements driving *Mrf4* expression have not been defined and only a subset of the pattern has been recapitulated using transgenic analyses. We have isolated six overlapping BAC clones constituting a *de facto* 5′ deletion series of the region. BACs were modified using homologous recombination to introduce alkaline phosphatase and nlacZ reporter genes into *Mrf4* and *Myf5*, respectively. We show that sequences up to 140 kb upstream of *Myf5* are sufficient to recapitulate the expression patterns of both genes. We define sequences involved in the regulation and maintenance of *Myf5* expression in different hypaxial muscle precursors and different branchial arches, reflecting the complexity of signals activating myogenesis. We also show that *Mrf4* regulation requires at least four elements one of which may be shared with *Myf5*, revealing a possible explanation for their linkage throughout vertebrate evolution.

Characterization of factors controlling the epaxial expression of *Myf5* during mouse embryogenesis

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Vertebrate myogenesis is controlled by a transcriptional cascade which includes the Myogenic Regulatory Factors (MRFs). *Myf5* is the first MRF expressed during embryogenesis. We have presented evidence that its role is to respond to the disparate signaling environments in the different anatomical regions by initiating the myogenic cascade. Using transgenic technology, we have shown that *Myf5* has distinct enhancers for each anatomical domain. The deletion of a 2·6 kb fragment close to the adjacent *Mrf4* gene eliminates expression of a reporter gene in the precursors of the epaxial musculature, white not otherwise affecting the expression pattern. 650 bp of this fragment are sufficient to drive epaxial expression. We built a series of reporter plasmids containing nested deletions of the epaxial segment and an additional enhancer directing branchial arch expression that we used as a control for transgenesis. This allowed us to determine two minimal sequences, of 90 and 140 bp, necessary for epaxial expression. We defined putative protein binding sites employing EMSA using protein extracts from 9·5 dpc embryos. We demonstrated the functional importance of two of these sites by mutation and transgenesis. This work constitutes the first step in the characterization of the factors controlling *Myf5* epaxial expression.

Fine mapping of the locus underlying the severe neural tube defect *loop-tail*

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The homozygous *loop-tail* (*Lp*) mouse has a severe neural tube closure defect, analogous to the craniorachischisis phenotype seen in humans. We have constructed sequence-ready bacterial clone contigs encompassing the *Lp* critical region in both mouse and the orthologous human region (1q22–23). Within these contigs, 21 genes, one EST and one pseudogene have been identified using a combination of EST database screening, exon amplification and genomic sequence analysis. Using several polymorphic markers identified in genomic mouse sequence, we have further reduced the *Lp* critical region to 450 kb, leaving the genes from *Slam* to *Atp1a2* as positional candidates for *Lp*. The comparative gene content and order are identical between mouse and human, indicating a high degree of conservation between the two species in this syntenic region of chromosome 1. Together, the physical and transcript maps serve as resources for the identification of the *Lp* mutation and further define the conservation of this genomic region between mouse and human.

***Circletail*, a new mouse mutant with a severe neural tube defect, maps to chromosome 15**

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Circletail (*Crc*) is a new mouse model for the severe form of neural tube defect, craniorachischisis. Homozygous (*Crc*/*Crc*) embryos exhibit an open neural tube from the midbrain throughout the spine, owing to a failure to initiate neural tube closure in the future

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cervical region (Closure 1). Homozygous embryos also show incomplete axial rotation. Heterozygous embryos exhibit a partially penetrant tail defect phenotype. The phenotype of *circletail* is very similar to the *loop-tail* (*Lp*) mutant, raising the possibility that *Lp* and *Crc* might be allelic. Indeed, an intercross between *Lp*/+ and *Crc*/+ mice produced fetuses with craniorachischisis, supporting this idea. However, genetic analysis shows that *Crc* is not linked to the markers that flank the *Lp* gene, and cannot, therefore, be an allele of *Lp*. A genome-wide scan shows linkage of *Crc* to the medial region of chromosome 15, between *D15Mit93* and *D15Mit68*. This region is not paralogous with the *Lp* region, suggesting that the *Crc* and *Lp* proteins do not have paralogous functions. Evaluation of candidates for both *Crc* and *Lp* is in progress. Identification of these genes will be essential for the elucidation of the molecular mechanisms required for Closure 1.

Protein kinase C isoforms in the prevention of neural tube defects

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Neural tube defects (NTD) are severe malformations of the central nervous system that can be prevented in up to 70% of cases by folic acid supplementation. The remaining 30% of NTD appear resistant to folic acid. The *curly tail* mutant mouse is a model of this folate-resistant category of NTD. We showed previously (Greene and Copp, 1997, *Nature Med* **3**, 60–66) that treatment of *curly tail* embryos with *myo*-inositol prevents many cases of spina bifida, raising the possibility of using inositol to prevent NTD in humans. The effect of inositol is mediated via activation of the protein kinase C (PKC) family of enzymes. In order to identify the PKC isoform(s) involved in the action of inositol, we applied chemical inhibitors of PKC to *curly tail* embryos developing in whole embryo culture. Inhibition of members of the 'conventional' PKC family abolished the preventive effect of inositol, whereas a structurally similar, inactive variant had no such effect. Chemical inhibitors are specific only for PKC families, whereas peptide inhibitors are becoming available with specificity for individual PKC isoforms. Use of peptide inhibitors with specificity for PKC βI and ζ was also able to block the preventive effect of inositol, whereas inhibition of the βII and ϵ isoforms had no such effect. We conclude that a subset of PKC isoforms is responsible for mediating the preventive effect of inositol on NTD. Supported by Wellbeing and the Wellcome Trust.

Mutational analysis of *HESX1* within seta-optic dysplasia (SOD)

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Septo-optic dysplasia (SOD) is a variable developmental abnormality of the midline structures of the brain, classically resulting in hyoplasia of the optic nerves, the septum pellucidum and the corpus callosum, as well as dysgenesis and dysfunction of the pituitary. The homeobox gene *hesx1* is expressed at gastrulation within the anterior midline visceral endoderm. Subsequent expression is seen within the prosencephalon and Rathke's pouch. The *hesx1* null mutant mouse shows abnormal development of these tissues and results in a phenotype similar to SOD in man. This led to the search for causative *HESX1* mutations in patients with SOD. Four hundred and sixty patients were screened for sequence variations within the coding region of the *HESX1* gene using SSCP and HPLC heteroduplex analysis. Here we report a novel *HESX1* mutation associated with SOD, a heterozygous point mutation Ser170Lue. This occurs within a conserved motif, has not been found in 140 control chromosomes and, *in vitro*, shows a 7-fold reduction in DNA binding. A number of other variants have also been found and are undergoing functional analysis. To conclude, mutations of *HESX1* in association with SOD are rare, but give valuable insights into normal development.

Generation of trans-chromosomal mice using ES cells containing freely segregating fragments of human chromosome 21 (Hsa21): a model of human Down syndrome

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A mouse model for characteristics of human trisomy 21 is being generated through the application of irradiation microcell-mediated chromosome transfer (X-MMCT) and ES cell technology. Using X-MMCT, we have created a panel of mouse ES cell lines of XY

origin and containing fragments of freely segregating Hsa21 by selection for neomycin resistance. Cell lines have been subsequently injected into mouse host blastocysts and resulted in the birth of chimeras of varying degrees of chimerism. The trans-chromosomal mice were extensively bred and progeny monitored for germ line transmission based on coat colour. Lack of germ line transmission was noted and alternative strategies implemented based on (i) subcloning the existing panel of XY ES clones for XO clones, known to go through the germ line and (ii) recreating a panel of trans-chromosomal ES cell lines containing Hsa21 portions with an XX ES cell line; both strategies use the fact that exogenous chromosomes go through the female germ line at a higher rate of success than through the male. Both techniques are currently being applied to generate chimeras and consequently entire families of trans-chromosomal mice to allow phenotype/genotype correlations in human Down syndrome, with the aid of the recently published Hsa21 sequence.

Mapping an ENU mutagenesis derived, low total cholesterol, low HDL cholesterol mutant mouse to chromosome 4

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Use of the mouse for extensive mutagenesis programmes and genetic crosses has given new insights into the understanding of gene function and provided new animal models of human disease. SmithKline Beecham Pharmaceuticals (SB), the MRC Mammalian Genetics Unit, Harwell, Imperial College, London and the Queen Mary and Westfield College, London have embarked on a 5 year large-scale mouse ENU mutagenesis programme. This research programme is aimed at generating large numbers of new mouse phenotypes, many of which carry disorders that model human genetic disease. Several phenotypes of interest have already been generated; 140 of them have been inheritance tested and more than 20 of those have been mapped on different regions of the mouse genome through linkage analysis. One of the mutant mice that attracts great pharmacological interest is GENA241. This mutant exhibits low HDL cholesterol and low total cholesterol levels. To map the GENA241 phenotype, backcross progeny were split into three groups: mice with total cholesterol levels more than 2 standard deviations lower than the F1 mean were classed as mutants, mice with total cholesterol levels within 1 standard deviation of the mean were classed as non-mutants, and all other mice as uncertain. A whole-genome scan using approximately 100 microsatellite markers was carried out in pooled DNA samples and the phenotype was mapped to mouse chromosome 4, between markers *D4Mit214* (21·9 cM) and *D4Mit178* (30·6 cM). Further mapping to narrow the critical interval is in progress; identification of the mutant gene should give an increased understanding of HDL and total cholesterol regulation in both mice and humans.

Extraembryonic tissue defects in mice lacking the choroideremia gene

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Choroideremia (CHM) is an X-linked recessive eye disease which is characterized by progressive degeneration of retinal pigment epithelium, choriocapillaris and retina. Targeted deletion of the *Chm* gene in mice resulted in parental lethality in hemizygous males and heterozygous females (*Chm*−/*Chm*+) after maternal transmission, whereas male chimeras and *Chm*+/*Chm*− females were viable and showed photoreceptor degeneration (van der Hurk *et al.*, 1997). Histological analysis of mutant placenta showed multiple layers of giant cells with reduced labyrinthine trophoblast and spongiotrophoblast. Mutant yolk sacs lacked normal vasculature. *Chm*−/Y males always exhibited a more abnormal phenotype than *Chm*−/*Chm*+ females. Mutant embryos could be rescued by aggregation with tetraploid wild-type mouse embryos. Together, these findings show that defective development of extraembryonic tissues is responsible for the lethality of *Chm*−/Y and *Chm*−/*Chm*+ genotypes, and that differential phenotypes of *Chm*+/*Chm*− and *Chm*−/*Chm*+ embryos are due to the imprinting of the X chromosome in the extraembryonic tissues. Thus, in the mouse *Chm* can be regarded as an essential gene for the development of extraembryonic tissues. Surprisingly, further studies showed that *Chm*−/*Chm*+ females were viable when their *Chm*+/*Chm*− mothers had been mated to *M. spretus* males.

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Investigation of the role of the *EFEMP1* gene in the developing retina and retinal disease

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A single mutation, Arg345Trp, in the human EGF-containing extracellular matrix protein-1 gene (*EFEMP1*), is associated with the retinal disorder dominant drusen. The expression pattern of *EFEMP1* in the retina remains to be determined. We have screened our cohort of dominant drusen families and sporadic patients for mutations in the *EFEMP1* gene, and investigated the expression of *EFEMP1* in the developing retina. Only 6 of the 9 families and 2 of the 18 patients had the Arg345Trp mutation. These patients were all found to have the same genetic haplotype across a 1 cM interval on chromosome 2p16, suggesting a founder mutation. Three families and 16 sporadic patients did not have any further coding sequence or splice site mutation in the *EFEMP1* gene and also had different haplotypes from the founder population. Recombination events in one family showed exclusion of *EFEMP1* as the causative gene. These data suggest that mutations in the *EFEMP1* gene only account for proportion of the disease phenotype, and that dominant drusen is genetically heterogeneous with at least two distinct loci. *EFEMP1* was found to be expressed in the human retina all ages tested from 8·6 weeks post-conception, suggesting a potential role in retinal development.

Gene targeting studies at the *Prnp*/*Prnd* locus

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The *Prnd* gene, which encodes the novel PrP-like protein Doppel (Dpl), has previously been identified 16 kb downstream of the mouse prion protein gene, *Prnp*. *Prnd* expression is upregulated in the central nervous system of two PrP null lines as a result of intergenic splicing between *Prnp* and *Prnd* that generates chimeric transcripts. This upregulation is associated with the late-onset ataxia observed in these mice. A variety of PrP null lines, which differ in the nature of the targeted allele, have been generated. The level of *Prnd* expression in the brain has been found to vary between these lines, with higher amounts of *Prnd* than that seen in the original PrP nulls being expressed in at least one of the new lines. In its normal context, *Prnd* is known to be expressed in the adult testis. We have generated a Dpl null line by gene-targeting and found null males to be sterile. This phenotype may result from increased oxidative stress. A possible link between Doppel and oxidative stress will be discussed. PrP has been shown to have an anti-oxidant function. A putative correlation between Doppel expression in the brains of PrP null mice, oxidative stress and Purkinje cell degeneration is being investigated.

A molecular analysis of the *wasted* mutation

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Wasted mice are normal until 21 days, whereupon they develop a neuromuscular and immunological phenotype. They provide a possible model for motor neuron disease (MND). The genetic defect responsible is a 15·8 kb deletion which removes the promoter and first exon of eukaryotic elongation factor 1A-2 (*Eef1a2*), abolishing its expression. This gene is highly homologous to elongation factor 1A-1 (*Eef1a1*). We have found neuronal vacuolation in the spinal cord of *wasted* mice. Our immunohistochemical analysis has demonstrated that substantially more anterior horn neurons in *wasted* mice are positive for GFAP and phosphorylated neurofilaments than in controls. We are using three transgenic approaches to investigate the functions of *Eef1a2* and potentially create improved MND models. Firstly, we are creating *Eef1a2* knockout mice to determine whether this gene alone is responsible for the *wasted* phenotype. Secondly, we are placing *Eef1a1* under the control of the *Eef1a2* promoter to establish whether the two genes have equivalent functions. Thirdly, we are driving *Eef1a2* expression from a muscle-specific promoter in *wasted* mice. We have also identified a second gene approxi-

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mately 15 kb from the wasted deletion which we are investigating as a candidate for the immune system abnormalities.

Mapping of *Loa*, a mouse motor deficit mutation, to distal chromosome 12

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Motor neuron disease is a progressive genetic disorder with 5 sufferers per 100000 people in Europe and the UK. Approximately 20% of these are familial cases with predominantly autosomal dominant inheritance but, with the exception of *SOD1*, the causal genes remain unknown. Our mouse model of motor neuron degeneration carries a mutation in one of these genes. The mutant mouse, legs at odd angles (*Loa*), has a dominantly inherited, early/mid-onset phenotype characterized by progressive loss of motor function in the hind limbs, with loss of anterior horn cells. Heterozygotes have a normal life span but homozygotes die within 24 hours of birth. To map and subsequently clone the *Loa* gene, an intraspecific backcross between the mutant mouse and the inbred strain C57BL/6 was set up. Over 1000 affected N2 animals were used to genetically map the *Loa* mutation to a 1·6 cM region of distal MMU12. A BAC contig spanning this critical region was constructed. We are using SNPs to reduce the critical region and sequence from the comparative interval on human 14q32 to identify additional candidate genes. Identification of the *Loa* gene will provide new insights into the genetic cause of dominant motor neutron degeneration.

Rapid genome scan reveals linkage to chromosome 15 for the ENU-induced circling mouse mutant *spin cycle*

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Mutant mouse phenotypes are important for characterizing mammalian gene function and identifying some of the genetic pathways that may be involved in human inherited disease. An ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis programme was established to increase the mutant mouse resource; presently mutant phenotypes exist for only 1–2% of all mouse genes. ENU is a powerful point mutagen. This programme is a genome-wide, phenotype-driven, large-scale screen for dominant mutations in the mouse. So far over 140 confirmed inherited phenotypes have been generated and linkage analysis has revealed the chromosomal localization of 20 of these phenotypes. One mutant mouse was identified with circling behaviour and a head tremor with the SHIRPA behavioural and functional assessment protocol and was designated *spin cycle*. Inheritance of the phenotype was confirmed by backcrossing. In order to rapidly identify the causative mutation, a genome-wide scan was performed on pooled DNA samples from mutant backcross animals, which had been standardized to an equimolar concentration prior to pooling. The mutant was mapped to mouse chromosome 15 between the markers *D15Mit235* (32·5 cM) and *D15Mit170* (49·2 cM). The 16 cM critical interval contains the gamma subunit 2 (*cacng2*) gene of a voltage-dependent calcium channel and the paravalbumin (*pva*) gene. These candidate genes are currently been sequenced in order to identify any mutations that may be associated with the phenotype.

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ENU-induced mutations causing vestibular and hearing dysfunction in mice

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A phenotypic approach has been adopted in the mouse to identify molecules involved in ear development and function by using *N*-ethyl-*N*-nitrosourea mutagenesis and screening for mice that show hearing and/or balance defects. Two of these mutants are presented. Slalom (*Slm*) mutants show subtle head weaving and shaking. Paint-filling of the inner ear revealed truncations of the superior and/or the posterior semicircular canals. Observation of the organ of Corti by scanning electron microscopy (SEM) revealed a patterning defect affecting the hair cells. We have mapped the mutation to chromosome 2 and identified a missense mutation in the *Jagged1* gene. Headbanger (*Hdb*) mutants display circling and head-tossing. Surface analysis of the organ of Corti by SEM has revealed defects within the stereocilia bundles of both inner and outer hair cells. The mutation has been mapped to chromosome 7. These mutants will contribute to our understanding of the genes involved in hearing and balance mechanisms.

Quantitative trait loci (QTL) for female reproductive traits on porcine chromosome 8

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Livestock breeding programmes are embracing molecular genetic techniques that offer the potential to significantly improve selective breeding through the use of marker-assisted selection. In this project we are interested in identifying markers for genes controlling reproductive performance in pigs. This study utilized crosses of the commercial Large White breed with the Chinese Meishan breed. The Meishan is one of the most prolific pig breeds known, displaying greater litter sizes than commercial breeds. This difference in litter size is attributed to the Meishan's superior levels of pre-natal survival. A three-generation Large White × Meishan cross ($n = 343$) was genotyped with 15 polymorphic markers distributed over chromosome 8. The phenotypic data collected for the F2 females ($n = 220$) and the genotypes were combined for QTL mapping. The trait data measured included ovulation rate (OR), litter size (LS) and pre-natal survival (LS/OR). Two related QTL were identified, both of which showed positive dominance effects from the Meishan breed. These QTL were for pre-natal survival and litter size and were both significant at the nominal level ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively). These suggestive QTL identified on chromosome 8 map close to the SPP1 locus in a region homologous to the region on sheep chromosome 6 which contains the *Booroola* gene (*FecB*). *FecB* is associated with prolificacy in sheep. We are pursuing a comparative genome mapping approach in order to locate the trait genes within these porcine QTL. This project is supported by a BBSRC Industrial CASE studentship. PIC International Group PLC are the industrial sponsors.

The type 1 diabetes susceptibility locus *IDDM2* may have a multi-locus aetiology

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The insulin minisatellite is the best candidate for the type 1 diabetes (TIDM) susceptibility locus *IDDM2*. Large class III alleles associate with dominant protection against TIDM, with small class I alleles generally associating with susceptibility. We have analysed tandem repeat variation within the minisatellite in a population of TIDM-affected sib pair families to further investigate association of the locus with disease. Class I alleles divide by a combination of repeat composition and variation in the flanking haplotype into three sublineages: IC+, ID+ and ID−. All class I alleles associate with predisposition to TIDM except for ID− alleles, which protect against disease when transmitted from ID−/III heterozygous fathers. Similar results have been pre-

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viously identified within this cohort for alleles of 42 repeats in length, a subset of our ID− lineage. We found this protection may be a feature of all ID− alleles, irrespective of size. ID− alleles are only clearly distinguished from all other alleles by variation in the flanking haplotype. Variation both at the minisatellite and within the flanking haplotype may therefore contribute to TIDM susceptibility, suggesting that *IDDM2* has a multi-locus aetiology.

Sib-pairs and embryonic lethals

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The largest set of affected sib-pairs in the public domain are those of Mein *et al*. (*Nature Genetics*, July 1998), consolidating earlier claims of excessive sib-similarity at many loci in severe diabetes. Accompanying papers dismissed most claims as statistically inadequate. Simpler analyses based of counts by gamete in informative sibships confirmed substantial and highly significant excess in these segments, including many implicated in other disorders. The simplest explanation of these marked and consistent findings is that recessive lethals cause most of the extensive loss, of the order of 50%, between conception and birth. As with black holes in astronomy, offending or null alleles will only be recognized by disturbing the movement of neighbours. An exception is some A × BC matings where '.' is a null allele or deficiency. Self-sterility alleles would cause similar effects. Embryonic lethals may be old variants maintained by heterozygote advantage or new mutations maintained by recurrent deficiencies. Penrose (1935) advanced sib-pair analysis by ascertainment of sibships with one or more affected sibs, providing normal sib controls. He pointed out parental typing was inefficient compared with adding further families. His sib-pair papers, and some analyses of the limited sib-pair data available, can be seen on: http://www.bioch.ox.ac.uk/~jhe.

Minisatellite Variant Repeat (MVR) analysis of the tandem repeat region of the *MUC1* gene

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The mucin genes are characterized by their long domains of tandemly repeated coding sequence, which, in most cases, shows length polymorphism due to variation in the number of repeats. It is known that, in most cases, the repeats are not identical throughout the array, but the extent to which they vary from person to person is not known. *MUC1*, in contrast, was thought to show very little repeat sequence variation, although one of the early reports (Siddiqui *et al*., 1988, *PNAS* **85**, 2320), as well as our own unpublished data, showed several nucleotide changes. We have examined *MUC1* repeat sequence variation using MVR-PCR (Jeffreys *et al*., 1991, *Nature* **354**, 204). We can currently demonstrate three repeat classes, which are associated with three nucleotide changes. Two of these cause amino acid changes in the immunogenic region (PDTR to PESR). The published sequence (PDTR) is the most abundant repeat class, but there is considerable person-to-person variation in the position and frequency of the alternative repeats (PESR). Studies are in progress to compare the patterns in disease and control groups, to examine the evolution of the *MUC1* tandem repeat array and to study somatic mutations.

Comparative analysis of human 19p12–13 region in *Fugu* and mouse

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Fugu cosmid 116113 had been identified, via preliminary sequence scanning through the Fugu Landmark Mapping Project, as syntenic to a 1 Mb region of human chromosome 19p 12–13. This region is completely sequenced in both human and mouse and contains seven genes (*COMP*, *PNORF1* (*HUPF1*), *GDF-1*, *UOG-1 COPE*) and two putative genes (*R27090_2* and *R27090_3*). The *Fugu* cosmid was fully sequenced and analysed with regard to conservation of synteny and gene structure. It is 35 kb in length and contains 4 complete (*R27090_2*, *PNORF-1*, *BAA91015* and *LSM4*) and one partial gene (*R27090_3*). All these genes (with the exception of *BAA91015*, which is an unmapped EST) are present in the human chromosomal (and mouse syntenic) region 19p12–13. Comparison of gene content of the *Fugu* cosmid with human and mouse reveals there are genes not present on this cosmid. However, the conservation of *R27090_2*, *PNORF-1* and *LSM4* as a conserved syntenic block across three species, all of which are potentially involved in the same biochemical pathway, that of mRNA surveillance and degradation, leads to speculation that the positioning and gene order of these specific proteins may influence their interaction or regulation.

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UK Mouse Sequencing Consortium: one year on

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The UK mouse sequencing consortium was set up in October 1999 with the remit of underpinning the UK mouse genomics effort by obtaining 50 Mb of genomic sequence, targeting four regions which have been the subject of genomic and biological investigation in the UK: (i) The WAGR-homologous region on mouse chromosome 2; (ii) the *brown* deletion complex on mouse chromosome 4; (iii) the Del(13)Svea36H chromosome 13 deletion; (iv) *Dmd-Ar* region on chromosome X. The project will also complement the large-scale ENU mutagenesis programme under way at Harwell, as obtaining the finished genomic sequence will greatly improve the efficiency of mutation scanning. The principal interest of the project is the discovery of novel genes and features in the targeted regions. The other main rationale is to construct complete transcript maps with accurate gene models derived from bioinformatic and experimental analysis, ultimately allowing highly efficient mutation scanning for positional cloning of ENU-induced mutations. The first phase of the project required the building up of maps and minimal tiling paths from the PRCI23 C57BL/6J library (Osoegawa *et al*., 2000). This stage is nearing its completion and was carried out by the three mapping groups at HGU, Harwell and Imperial College. The sequence is placed in the public domain via Ensembl (http://www.ensembl.org/) and a project update will be presented.

Reference

Osoegawa, K. *et al*. (2000), Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. *Genome Research* **10**, 116–128. Project web-site: http://mrcseq.har.mrc.ac.uk

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