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Organization of casein kinase 2 α- and β-subunit genes in the mouse genome

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Protein kinase CK2 (CK2) is a widely distributed and highly conserved serine/threonine protein kinase which is closely associated with proliferation (e.g. in permanent cell lines, in tumours or during embryogenesis). It consists of two catalytic subunits (α and/or α') and two non-catalytic subunits (β). Elucidation of the genomic organization of the CK-2 genes should help to understand the regulation of CK-2 in tumours and during embryogenesis. Southern blot analyses of mouse genomic DNA using CK-2 α-cDNA as a probe suggested that either additional highly related copies of the gene are present in the genome and/or that the CK-2 α-gene comprises a large genomic region with several exons. By screening of a genomic mouse library positive clones were isolated which represent four different genomic loci. Three loci were identified as processed pseudogenes and sequenced completely. The fourth locus obviously contains the active CK-2 α-gene. So far 11 exons coding for amino acids 1–208 and 242–391 (last amino acid) were sequenced. The whole CK-2 α-genomic region is at least 25 kb in length.

Is *Sry* responsible for the more advanced development of male embryos compared to females embryos prior to gonadal sex differentiation?

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In a number of mammalian species, including mouse and man, it has been shown that male embryos are developmentally more advanced than female embryos well before gonadal sex determination has taken place. Some have assumed that this is due to an accelerating effect of the Y chromosome and have speculated that the testis-determining factor *Sry* may be responsible. By utilizing a range of sex-chromosomally variant genotypes it has been possible to show that this early XX–XY developmental difference is in fact due to both Y and X chromosome effects: (1) In the preimplantation period the Y chromosome has an accelerating effect on development. Evidence will be presented that this accelerating effect occurs in the absence of *Sry*. (2) In the postimplantation period the XX–XY developmental difference is magnified due to a retarding effect of two X chromosomes. Since it is known that a failure to inactivate the second X chromosome in the epiblast rapidly leads to embryonic death, it is suggested that in the period immediately preceding X inactivation the activity of two X chromosomes is suboptimal for development.

A Crouzon-like craniofacial dysmorphology in the mouse is caused by insertional mutation in the FGF4 region

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Retroviral vector insertional mutagenesis via ES cells has resulted in a new autosomal dominant mutation causing craniofacial dysmorphology in the mouse (*Bulgy-eye Bey*). Heterozygous *Bey* mice are viable and fertile but show facial shortening with increased inter-orbital distance and precocious closure of the sagittal and coronal sutures. These features are highly comparable with human craniofacial dysmorphologies in particular the Crouzon's syndrome. In the homozygous state the *Bey* mutation is lethal. Homozygous foetuses die from approximately 10·5 days of development with severe cranial abnormalities including absence of the maxillary component of the first branchial arch. The retroviral integration responsible for this mutation is inserted 6 Kbp upstream of FGF4 in the intragenic region between FGF3 and FGF4. Some of the Crouzon mutations have now been shown to be associated with an alteration in the extracellular domain of FGFR2 which implicates that this signal pathway is essential for normal craniofacial development. Our results now indicate that FGF4 is most likely the ligand responsible for the *Bey* phenotype.

'Germ-cell specific expression of YRRM and TSPY in the human testis'

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mRNA *in situ* hybridization studies have been performed to determine the normal gene expression patterns of YRRM and TSPY, two Y chromosome gene families involved in spermatogenesis, in the human testis. Expression of both appears to be principally confined to spermatogonia and/or early primary spermatocytes lining the walls of the seminiferous tubules in adult testes. Signal is not detectable over spermatogonia prior to puberty, in Sertoli cells, or in spermatids. The window of expression for both gene families appears to extend to late primary spermatocytes in men with spermatogenic impairment. Failure of condensation of the Y axis in meiotic prophase, as observed cytologically in many cells of such individuals, correlates with this extended period of expression.

Pax3 expression in the mouse heart

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Sp^{2H}/Sp^{2H} is a radiation-induced mutation involving a 32 bp deletion in the homeobox region of the Pax3 gene. Sp^{2H}/Sp^{2H} embryos show a range of abnormalities including spina bifida and/or exencephaly, the cardiac defect persistent truncus arteriosus and skeletal defects. The expression pattern of truncated Pax3 mRNA in Sp^{2H}/Sp^{2H} embryos mirrors the mutant phenotype, with high levels in the neural tube, head, migrating neural crest and myotomal cells. Surprisingly Pax3 expression has not previously been reported in the embryonic heart, although the Sp^{2H}/Sp^{2H} embryos die *in utero* of cardiac defects.

Paying close attention to the embryonic hearts, we have examined the expression of Pax3 in control and Sp^{2H}/Sp^{2H} embryos by RT-PCR and *in situ* hybridization. Pax3 is found at low levels in the hearts of control and Sp^{2H}/Sp^{2H} embryos before possible neural crest invasion. At 10·5 days when neural crest cells begin to migrate into the outflow tract of the heart, Pax3 expression is greatly increased in hearts from control embryos. There is no concomitant increase in the expression of Pax3 in hearts of Sp^{2H}/Sp^{2H} embryos. Examination by *in situ* hybridization localizes Pax3 expressing cells to the outflow tract, and suggests that the neural crest cells which migrate into the heart are Pax3 positive. It appears that these cells do not reach the hearts of homozygous mutant embryos. We therefore suggest that the failure of Pax3 positive neural crest cells to migrate into the hearts of mutant animals causes the failure of outflow tract septation and results in persistent truncus arteriosus.

Paracrine regulation of stem cell renewal in DIA/LIF-deficient ES cell cultures: detection of a new ES cell regulatory pathway

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The propagation and differentiation of murine pluripotential embryonic stem (ES) cells is controlled by specific cytokines. The self-renewal of ES cells *in vitro* is sustained through the activation of intracellular processes associated with the signal transducer gp130. In an attempt to define the relative contributions of different cytokines to self-renewal in ES cell cultures we generated ES cells lacking a functional gene for the cytokine Differentiation Inhibiting Activity (DIA/LIF). These cells show a significantly reduced capacity for stem cell renewal when induced to differentiate, indicating that DIA/LIF is the major regulatory cytokine present in normal ES cell cultures. However, undifferentiated ES cell colonies are still produced in the complete absence of DIA/LIF. This is due to the secretion of a soluble, macromolecular, activity by differentiated ES cell progeny. In addition to DIA/LIF, the cytokines ciliary neurotrophic factor (CNTF), interleukin-6 in combination with soluble interleukin-6 receptor (IL-6/sIL-6R) and oncostatin M can each activate gp130 and support ES cell propagation. The involvement of either CNTF or IL-6/sIL-6R in our system has been precluded through the use of neutralising antisera against these factors. Most significantly, the effect of all the aforementioned cytokines on ES cells is abolished in the presence of neutralising antibodies against mouse gp130, whilst the activity in differentiated ES cell conditioned media is unaffected. These findings establish that ES cell self-renewal can be sustained via a gp130-independent signalling pathway and that differentiated ES cells secrete a factor which activates this pathway. We name this new factor JAC.

Expression and assembly of MHC class I products and associated molecules during murine pre-implantation development

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Defining where and when Major Histocompatibility Complex (MHC) is assembled is essential to understanding the function of these molecules during early development and in the establishment of fetal immunity. Using RT-PCR, immunoprecipitation and confocal scanning laser microscopy techniques class I mRNAs of both maternal and paternal haplotypes were shown in mouse pre-implantation embryos from the late one-cell stage. However, no translation of the message into 45 kDa H-2 class I heavy chain protein was detected until the uncompacted 8 cell stage of development. Analysis of the expression of B2 microglobulin and the peptide transporter molecules TAP1 and TAP2 showed protein expression of B2 microglobulin at the 8-cell stage and TAP2 (but not TAP1) at the two-cell stage of development. This indicates the likely involvement of TAP2 in the assembly of mature MHC at the blastomere surface, whilst regulation of expression of the heavy chain protein appears to be at the translational stage.

Minisatellite loci as a new tool for radiation-genetic studies

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Estimating the genetic hazards of radiation and other mutagens in humans depends on experimental systems for mutation scoring. The shortcoming of current approaches for genetic monitoring is the necessity to use very large numbers of individuals (more than 100000) to detect increases of mutation rate. A very high rate of spontaneous mutations altering the length of minisatellite loci has been found in humans and mice, often 1000 times more

than in most protein-coding loci and therefore of potential use for detecting induced mutations in relatively small population samples. Minisatellite mutations were scored in the progeny of γ-irradiated and unirradiated (101/HY × C3H/SnY) F_1-hybrid male mice. The frequency of mutation in the offspring of irradiated males was 1·72 times higher than in the control group. Evidence for germline mutation induction was obtained from a relatively small population sample (232 offspring in 26 irradiated and control families) at radiation doses substantially lower than can be monitored by standard genetic techniques in mice. The doubling dose computed from our data is 0·5 Gy, a value close to that obtained in mice using other monitoring systems. The potential use of minisatellite loci in monitoring radiation induced germline mutations in human populations will be discussed.

Non-random distribution of tetraploid cells in tetraploid ↔ diploid chimaeric mouse blastocysts

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The distribution pattern of tetraploid cells in 7·5 and 12·5 day tetraploid ↔ diploid (4n ↔ 2n) aggregation chimaeras has revealed the limited developmental potential of these cells. Tetraploid cells only successfully contributed to the trophectoderm and primitive endoderm lineages and not to the primitive ectoderm lineage from which the foetus itself is derived. Several explanations have been offered to account for this pattern of distribution. It is possible that selective pressures act which cause the 4n cells of the primitive ectoderm to die prior to 7·5 days. Alternatively 4n cells may be preferentially allocated to the trophectoderm and primitive endoderm lineages at the blastocyst stage. In an attempt to clarify the situation, 4n ↔ 2n mouse chimaeras were produced and the distribution of 4n cells was assessed at the early blastocyst stage (90 hr *p.c.*). 4n cells were hemizygous for a reiterated β-globin transgene (*Tg*/*Tg*/−/−) and could therefore be distinguished from diploid cells (−/−) by DNA-DNA *in situ* hybridization. Serial sections of 4n ↔ 2n and 2n ↔ 2n blastocytes were scored. Analysis of the distribution of 4n cells in the 4n ↔ 2n chimaeric blastocysts revealed an over-representation of cells carrying the *Tg* in the trophectoderm ($P < 0{\cdot}01$) rather than the inner cell mass. Further analysis revealed this difference to be due to significantly greater numbers of tetraploid cells in the mural trophectoderm ($P < 0{\cdot}001$) than in the other areas of the blastocyst. Such a pattern was not evident in the 2n ↔ 2n (*Tg*/− ↔ −/−) controls. It would appear, therefore, that 4n cells are preferentially allocated to the mural trophectoderm by this early stage (90 hr *p.c.*) of development. Further work is in progress to determine the cause of this non-random distribution in the blastocyst and how this affects the contribution of 4n cells to various tissues later in development.

Epigenetic modifications in the imprinted *Igf2* and *H19* genes

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The mouse *Igf2* gene is predominantly expressed from the paternal allele whereas the neighbouring *H19* gene is expressed exclusively from the maternal allele. We have analysed DNA methylation and other features of chromatin in these two imprinted genes. Two regions in the *Igf2* gene, one comprising upstream sequences and one in the 3′ part of the gene, were found to be more methylated on the expressed paternal allele (Feil *et al. Development*, in press). Genomic sequencing of individual chromosomes (Feil *et al.* 1994, *NAR* **22**, 695) showed that the allelic methylation in the upstream region is highly mosaic. Intriguingly, in the 3′ region, the level of methylation on the paternal allele is tissue-specific and correlates with expression of the gene. In adult choroid plexus, where *Igf2* is expressed from both alleles and *H19* is not expressed, both genes adopt a paternal type methylation pattern on both parental alleles. To understand when allelic methylation arises, we studied androgenetic and parthenogenetic ES cells. The *Igf2* and the *H19* methylation patterns were found to be very similar in these monoparental cells whereas derived teratomas had acquired the appropriate allelic methylation patterns. While for the *H19* promoter allelic nuclease sensitivity has already been demonstrated (Ferguson-Smith *et al.* 1993, *Nature* **362**, 751), we have not detected allelic chromatin features in *Igf2* so far. However, we detected

various DNase-I hypersensitive sites, some of which are present in ES cells already, whereas others are tissue-specific and arise during development.

Mapping and cloning of the X-linked cleft palate locus

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We have studied a large family from Iceland which displays a phenotype of cleft palate including ankyloglossia (tongue tied) in an X-linked Mendelian fashion. Linkage and recombination analysis has shown the X-linked cleft palate locus (CPX) to be between DNA polymorphisms (DXS1002, DXS95) and DXYS1X at Xq21.3. A total of 98 YACs were isolated using sequence tagged sites (STSs) for DXS1002, DXS95, DXS110, DXS1169, DXS472 and DXYS1X. Alu PCR fingerprinting and end clone hybridization analysis was used to establish overlaps between the YACs. One additional YAC was isolated using an end clone to complete the contig between DXS472 and DXYS1X. The size of the contig between DXS1002–DXYS1X is approximately 3 Mb. DXS1196 has been precisely mapped using this contig and was found to be the closest proximal flanking marker by recombination analysis in the Icelandic family. YACs spanning the remaining region of 1·9 Mb are now being used to isolate candidate genes from human craniofacial fetal cDNA libraries.

The behaviour of androgenetic cells in fetal mouse chimeras

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In a detailed analysis of androgenetic (ag) → wt chimeras using *in situ* detectable cell lineage markers we have observed non-random distribution of the uniparental cells. Ag cells were often present in large numbers in brown adipose tissue, perichondrium, and peripheral nerves as well as in heart, cartilage, pancreas and the epithelium of the gut. In some instances, the distribution of ag cells was exactly opposite of that observed with parthenogenetic (pg) cells, that are mainly observed in tissues of ectodermal origin as brain, dorsal root ganglia and the epidermis.

In several tissues, namely brain, cardiac muscle, skeletal muscle and the intestinal epithelium, the rate of proliferation of ag cells was higher than that of normal cells. This is again in contrast to the potential of pg cells which in pg ↔ wt chimeras showed decreased proliferation compared with wt cells. When the expression pattern of *Igf2* was analysed in tissue sections of ag → wt chimeras, ag patches exhibited higher levels of IGF2 mRNA than surrounding wt patches in tissues that normally express *Igf2*, indicating that both paternally derived alleles are expressed in ag cells. However, we also detected expression of *Igf2* in ag patches in tissues that do not normally express this gene, i.e. the brain, the epidermis and the epithelium of the tongue.

Placental growth in mouse interspecies backcrosses

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We have produced F1 crosses between various mouse species and subspecies in an attempt to analyse parent-specific expression of genes expressed in the placenta. In pregnant laboratory strain (*Mus musculus* = MUS) × *Mus spretus* (SPR) F1 females that had been backcrossed to MUS males, we consistently observed F2 offspring exhibiting enlarged placentas. Placenta weights of up to 480 mg were determined on day 18 of gestation. Enlarged placentas were also observed in MUS × *M. spicilegus* and MUS × *M. macedonicus* F1 females, but not in MUS × *M. m. musculus* and MUS × *M. m. castaneus* F1 females. The phenotype was not

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observed in the F1 conceptuses derived from MUS × SPR matings. In SPR backcrosses, the phenotype was associated with increased and considerably prolonged proliferation in the placenta. Accordingly, it became more pronounced in late gestation conceptuses. The phenotype segregated in the F2 generation, with approximately 50% of the F2 females containing F3 conceptuses with large placentas. At present, attempts are being made to map the responsible gene to a specific chromosome and to identify the target gene that causes the growth enhancement.

Positional cloning of the shaker-1 deafness gene

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In a 1066 progeny backcross [(C57BL/10 × *sh-1/sh-1*) × *sh-1/sh-1*] *sh-1* is non-recombinant with the Olfactory Marker Protein (*Omp*) gene. *Omp* has been used as a start-point to construct a 1·4 Mb YAC contig extending over the *sh-1* region. Two approaches to the analysis of the YAC contig in the vicinity of the *sh-1* gene are being followed. First, microsatellites and end-clones from the YAC contig are being used to identify the closest recombination breakpoints flanking the *Omp* and *sh-1* loci thus narrowing the search for the *sh-1* gene. Secondly, exon trapping and cDNA selection of YACs is being used to uncover expressed sequences for screening against inner ear cDNA libraries. Microsatellites isolated from the YAC contig and spanning 450 kb in the vicinity of *Omp* are non-recombinant with *sh-1* and a number of conserved sequences have been derived via exon trapping in this region. cDNAs for exaon trap sequences have been recovered. One cDNA derived from exon trap ET58 is expressed in mouse inner ear and in rat outer hair cell and encodes an unconventional myosin type VII. At the same time, a number of genes from the region, such as *Omp*, have been ruled out as candidates for *sh-1* either by sequencing of *sh-1* mutants or by demonstrating they lie outside the non-recombinant region around *sh-1*. The current status of candidate genes isolated from the shaker-1 region will be discussed.

Kyphoscoliosis – a new mouse neuromuscular mutation

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The kyphoscoliotic mouse (*ky*), a murine model of neuromuscular disease, exhibits a degenerative muscle disease resulting in a chronic deformation of the spinal column. At the cellular level the disease is characterized by pronounced motor nerve sprouting, accompanied by polyinnervation of muscle fibres and the severe and maintained disruption of neuromuscular junctions (NMJs). Using an interspecific backcross segregating the *ky* mutation we have mapped the *ky* locus to a small region of mouse chromosome 9. Fine mapping utilizing available microsatellites within this region shows that *ky* is non-recombinant with the microsatellites *D9Mit24* and *D9Mit169* and lies in a conserved linkage group that encompasses human chromosome 3. These microsatellites have been physically linked on a 400 kb YAC from the St. Mary's *rad52* YAC library. Narrowing of a 1·8 cM non-recombinant region between the closest proximal (*D9Mit36*) and distal (*D9Mit77*) markers to *ky* is in progress in order to employ a positional cloning strategy to identify the gene that causes kyphoscoliosis. Putative candidates s-laminin (*Lams*) and the gene for dystrophin associated glycoprotein 1 (*DAG1*) which map to human chromosome 3 are both recombinant with *ky* ruling them out as candidates. We have also eliminated other putative candidates which function at the NMJ. These include utrophin, syntrophin 2 and the acetylcholine receptor molecule 43 K.

Genetic basis of neural tube defects: the mouse gene *loop-tail* (*Lp*) maps to a region of chromosome 1 syntenic with human 1q21-22

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The mouse mutant *loop-tail* (*Lp*) is a model of the most severe form of human neural tube defect, craniorachischisis. Homozygous (*Lp*/*Lp*) embryos fail to initiate neural tube closure in the cervical region and die at around the time of birth. Heterozygous (*Lp*/+) mice are both viable and fertile but most have looped tails and enlarged brain ventricles. Previous studies using biochemical and phenotypic markers have linked the *loop-tail* gene to the distal portion of chromosome 1. We have been using a number of microsatellite and RFLP markers to generate an accurate genetic map of this region to enable *Lp* to be positionally cloned. Linkage analysis was carried out using an intraspecific backcross between LPT/Le (which carries the *Lp* mutation) and CBA: (CBA × LPT/Le) F1 × CBA. Only loop-tailed offspring were used as the partially penetrant phenotype means that about 10% of the animals with normal tails are genetically *Lp*/+. In an initial set of 191 loop-tailed backcross progeny, 15 informative markers were analysed, generating a map spanning 25·0 cM (*D1Mit14-D1Mit17*) and positioning *Lp* within an 8·9 cM interval between *Fcgr2*/*Mpp* and *D1Mit151*. To narrow this region, 7 markers within the interval were analysed in an expanded set of 612 mice. This has positioned *Lp* in a 1·46 cM interval between *D1Mit113* and *Crp*, several cM distal to its previously published position, in a region that has synteny with human chromosome 1q21-22. By generating a YAC-contig over this interval and carrying out cDNA selection we aim to clone and characterize the gene for *Lp*. This will allow a human homologue to be identified and assist in the understanding of neural tube closure.

Modelling down syndrome in mice

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Down syndrome (DS) results from a trisomy of chromosome 21, and is the most common human chromosomal abnormality, and the most common cause of mental retardation. However, neither the genetic, nor the biochemical basis of the syndrome are known. To investigate the molecular genetic basis of Down syndrome we wish to isolate the genes responsible for the phenotypic characteristics of DS and create a mouse model for features of the syndrome. To achieve this, we propose transferring megabase regions from the Down syndrome critical region (DSCR), on human chromosome 21, into embryonic stem cells (ES cells), thus creating a range of cell lines carrying human DSCR subregions in a mouse background. We intend to use a combination of the techniques of microcell-mediated chromosome transfer, and irradiation reduction to achieve the transfer of specific subfragments of human chromosome 21 into ES cells. We can subsequently use these cell lines to make chimeric mice by injecting blastocysts, and after bleeding of appropriate chimeras, obtain mice carrying human chromosome 21 genes. The first step in our experiments was to 'tag' human chromosome 21 with a dominant selectable marker (neo) at a specific locus on the DSR, by homologous recombination. These experiments were performed in the cell line HT1080. After trying two different constructs we have isolated two independent recombination events, thus creating two lines with neo selectable markers integrated near the D21S55 locus of human chromosome 21. The second step involves the transfer of megabase fragments of human chromosome 21 into ES cells. Experiments are now underway to establish the best conditions for: induction of microcell formation in HT1080 cells, the fusion of cells and micorcells to ES cells, the appropriate selection conditions for the recovery of transgenic lines, and the adequate amounts of irradiation to produce fragments of chromosome ranging from 3–6 Mb. Progress in this area, and preliminary results will be discussed.

Characterisation of the S4 gene, a member of a highly conserved gene family involved in ubiquitinated degradation of proteins

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A gene family has been identified recently that is highly conserved and whose conservation extends between prokaryotes and eukaryotes. We refer to this family as the TAB (*t*wo hundred amino acid *A*TP *b*inding domain) gene family in which members possess one or two copies of the ATP binding domain, allowing division into two subfamilies: TAB I and TAB II. Although the TAB proteins are highly conserved across their entire length, they are functionally distinct, with involvement in cellular processes as diverse as membrane trafficking and protein degradation. The S4 gene is a member of the TAB family. The S4 protein is a subunit of the 26S protease, a multimeric complex responsible for degrading ubiquitin targeted proteins. We have isolated and cloned the mouse and Drosophila S4 cDNAs. The expression pattern of S4 and its mapping position have been determined in Drosophila, and we find a developmentally regulated pattern of transcription. Mapping is underway to localise the S4 genes and pseudogenes in the mouse and human genomes, for comparative mapping and mammalian expression studies.

cDNA selection from human chromosome 21

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We have carried out a liquid phase cDNA selection (as developed by Korn *et al. Hum. Molec. Genet.* **1**; 235 (1992)) aiming to isolate and map cDNAs from human chromosome 21. The selection was performed using 3 courses of cDNA (fetal brain, fetal liver and adult muscle) and 9 cosmids from different regions of human chromosome 21. The cosmids were chosen because amplification products from a PCR based cDNA cloning protocol hybridised to them, suggesting the cosmids contained transcribed sequences. From the subsequent cDNA selection experiment approximately 400 partial cDNAs of greater than 400 bp were picked and gridded. From this point we followed two strategies: The first allowed more detailed description of single clones by their individual analysis for (i) mapping back to the cosmids; (ii) mapping back to human chromosome 21; (iii) sequencing. The second approach allowed rapid processing of large numbers of clones in pools. Sets of cosmid-specific partial cDNAs were then hybridised to either gridded cDNA filters from human adult liver cDNA, or to a human fetal brain cDNA library. We present our results with respect to the cDNAs and where they map.

Molecular analysis of expression in human trisomies 13 and 18

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Human trisomies 13 (Patau syndrome) and 18 (Edwards syndrome) can be used as models for the analysis of the role that genes on chromosomes 13 and 18 play in fetal development. Trisomies 13 and 18 occur in approximately 1 in 4000 live births. They are compatible with life although prolonged survival is rare. Anomalies often involve the urogenital, cardiac, craniofacial and central nervous systems. Patau syndrome fetuses exhibit a wide range of abnormalities which include cleft lip and/or palate, congenital heart disease, holoprosencephaly, polycystic kidney and mental and growth retardation. Defects in trisomy 18 include congenital heart disease, rocker bottom feet, polyhydramnios and mental and growth retardation. It is possible that some of these abnormalities will be due to the over-expression of developmentally important genes on chromosomes 13 and 18. An investigation of the level of mRNA expression and the cell type localization of candidate genes on chromosome 13 and 18 has been compared, in tissues from trisomic and normal age-matched fetuses. The

candidate gene under study from chromosome 13 was esterase D and a candidate gene under analysis from chromosome 18 was transthyretin (prealbumin). Although differences in the cell type localisation of the mRNA and protein from trisomic compared to normal tissues has not been found, unexpected levels of esterase D and transthyretin mRNA in trisomy 13 and 18 tissues have been detected.

In vivo expression analysis of a cloned LY-6E.1 gene

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The blood system is continuously replenished by stem cells residing in adult bone marrow which have the ability to self-renew, expand their numbers and differentiate into all mature blood lineages. The frequency of haematopoietic stem cells (HSCs) in bone marrow is less than 1 in 50000. Enrichment procedures facilitating the study of murine HSCs have been developed based on the cell surface phenotype Thy-1(lo), Sca-1(+), Lin(−) – where Lin consists of a cocktail of antibodies to mature blood cells. The Ly-6E/A gene encodes Sca-1 and thus could serve as a useful molecular tool to assess HSCs. We have cloned the mouse Ly-6E.1 gene, mapped DNase1 hypersensitive sites and demonstrated that the cloned gene recapitulates the expression pattern of the endogenous gene in transgenic mice. We are currently analysing the regulation of the gene *in vivo* and utilising the cloned gene to direct the expression of heterologous genes to Sca-1 positive cells in transgenic mice. Data demonstrating the previously unknown expression pattern of Ly-6E.1/reporter constructs during embryonic development will be presented.

Targeted disruption of the *Sry*-related gene *Sox*-2 in the mouse leads to early embryonic lethality

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Sry is a prototype 'switch' gene, being responsible for choosing between male and female differentiation pathways in eutherian mammals. Along with *Sry* our lab isolated a family of embryonically expressed genes from the mouse, termed *Sox* genes (from *Sry*-related HMG box), related through a very similar DNA binding domain. We are investigating *Sox*-1, 2 and 3, all of which have the developing nervous system as their most prominent site of expression. *Sox*-2 is the most widely expressed of the *Sox* genes we are studying, being transcribed in blastocysts (in the inner cell mass) and in egg cylinder stages throughout the embryonic ectoderm. Expression is then progressively confined mostly to neural plate ectoderm, as well as to the sensory placodes and to gut endoderm. Later, *Sox*-2 expression is present in cells which are committed, but not differentiating yet (e.g. the ventricular zone in the neural tube), and is switched off with the onset of actual differentiation (e.g. in the motor neurons), *Sox*-2 is very conserved between mouse, man, chick and maybe frog. To investigate the function of *Sox*-2, a null mutation has been introduced into the gene via homologous recombination in embryonic stem cells. Chimaeras were obtained that transmitted the mutated allele through the germline, generating heterozygous animals that were normal and fertile. No homozygous mutant newborns were obtained from matings between heterozygotes, indicating that *Sox*-2 absence is lethal before birth. Initial observations 6·5 and 8·5 d.p.c. indicate that *Sox*-2 may already be necessary very early on, possibly around implantation.

Stem cell maintenance in the early mouse embryo

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Differentiation Inhibiting Activity (DIA) and the components of its receptor are shown by *in situ* hybridization to be present in the murine blastocyst. The expression patterns are consistent with DIA playing a role in maintenance of the pluripotential stem cells of the inner cell mass. However, the recent finding that homozygous DIA-deficient embryos are capable of development to term in heterozygous or wild-type females suggests that DIA is not essential to the very early embryo. The signal transducer for the DIA receptor, gp130, is a component

of several other cytokine receptors including interleukin-6 (IL-6), ciliary neurotrophic factor (CNTF) and oncostatin M (OSM). The possibility that any of these cytokines may be able to maintain the pluripotential state of the stem cells in the early embryo was investigated by attempting to establish ES cell lines *de novo* from blastocysts in culture. Germline-competent lines were obtained with high efficiency using OSM or IL-6 with its soluble receptor, but less effectively with CNTF. However, no significant expression has been seen in blastocysts by *in situ* hybridisation using a probe for the CNTF-specific receptor component. The possible involvement of another signal transduction pathway is considered.

The 'meiotic quality control' acts via apoptosis in mouse spermatogenesis

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There is now overwhelming evidence for the existence of some form of meiotic quality control which acts to eliminate spermatocytes and oocytes in which there has been incomplete sex chromosome synapsis. We have used male mice with a single sex chromosome ($X^{Y^*}O$) and consequently no X–Y synapsis, to investigate the possibility that the 'meiotic quality control' acts via apoptosis. Features characteristic of apoptosis were assessed in cells from the seminiferous epithelium of pubertal and adult testes. The presence of a DNA ladder with oligonucleosome length fragments and the specific nuclear labelling by acridine orange indicate that the 'meiotic quality control' acts via an apoptotic process. By using a technique for detecting apoptotic cells *in situ* we show that the cells undergoing apoptosis in $X^{Y^*}O$ mouse testes are metaphase spermatocytes, the stage at which spermatogenesis is blocked in these mice. The identification of apoptosis as the cell death mechanism in the 'meiotic quality control' is a first step towards the understanding of the molecular mechanisms acting in this process. We are now investigating the possibility that apoptosis could be a more general cell death mechanism in male sterility.

Regulation of *Hoxb-1* expression in rhombomere 4 of the vertebrate hindbrain

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The vertebrate *Hox* genes are involved in the patterning of the hindbrain. Anterior expression limits of *Hox* family members located 3′ in the *Hox* complexes coincide with rhombomere boundaries. *Hoxb-1* expression in the hindbrain is restricted to rhombomere 4 in several vertebrate species. Sequence comparison between a previously identified rhombomere 4 enhancer of the murine *Hoxb-1* gene and sequences upstream of the chicken and puffer fish *Hoxb-1* genes identified a region of sequence conservation that falls within a 331 bp *Stu*I-*Hind* III fragment in mouse. The *Stu*I-*Hind*III fragment was sufficient to direct reporter gene expression to rhombomere 4 in transgenic mice. An interesting feature of the homologous region is the presence of four short highly conserved sequence blocks. Alignment of three of these blocks revealed a consensus that may represent a transcription factor recognition site. In order to assess the importance of these repeats, mutations were introduced into each motif in the context *Stu*I-*Hind*III fragment. None of the founder embryos obtained with the mutated version showed expression confined to rhombomere 4 demonstrating that the motifs are critical for rhombomere 4-restricted expression. We are now identifying and characterising proteins that bind to the repeats, using electrophoretic mobility shift assays.

Comparative mapping of YRRM- and TSPY-related sequences in man and hominoid apes

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Using chromosomal *in situ* hybridization (FISH) it has been demonstrated that specific members of the *YRRM* and the *TSPY* families are multicopy and Y chromosome specific in hominoids. After hybridization with the *YRRM*-related cosmid A5F and the *TSPY*-related cosmids cos36 and cY91 a reverse and complementary pattern of main and secondary signals are detected on the Y chromosomes of the human, the pygmy chimpanzee, and the gorilla, while the location of signals coincides on the Y chromosomes of the chimpanzee, both orangutan subspecies, and the white hand gibbon. Our *in situ* data of complementary distribution of *YRRM* and *TSPY* sequences on the Y chromosomes of hominoid species, including man, might be readily explained by assuming similar sequence motifs that are shared and evolutionarily conserved by certain members of both gene families and/or repeated elements flanking those genes. Otherwise this complementary signal distribution could go back to a common organization of these genes next to each other on an ancient Y chromosome which was disrupted by chromosomal rearrangements, and amplification of one or other of the genes at each of the locations. Double hybridization experiments with digoxigenin labelled *YRRM*- and biotinylated *TSPY*-related cosmid probes and subsequent two-colour detection on interphase nuclei of man and the pygmy chimpanzee lend support to the second alternative.

Identification of a gene from the short arm of the mouse Y chromosome T expression of an epitope recognized by H–Y-specific T cell

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The short arm of the mouse Y chromosome contains genes that control expression of the male-specific minor transplantation antigen, H–Y (*Hya*), spermatogenesis (*Spy*), a Y-linked ubiquitin activating enzyme, (*Ubely-1*) and two zinc finger containing genes (*Zfy-1 & Zfy-2*). Recently another gene, *Smcy*, has been identified from this region (Agulnik *et al.* 1994). This gene is expressed in all adult male tissues tested. It is conserved on the Y chromosome of mammals and marsupials, and the human Y homologue maps to the Yq deletion that contains the human *H–Y* gene. *Smcy* may therefore represent a good candidate for *Hya*. Our previous studies have shown that there is likely to be more than 1 gene that encodes H–Y; a finding that would be in keeping with that for other minor transplantation antigens. We have now transfected a cosmid containing most of the exons of the *Smcy* gene into recipient cell lines expressing the appropriate MHC molecules and examined their ability to stimulate our class I and class II restricted H–Y specific T cells. These transfectants strongly stimulate our H–YK^k restricted T cell clone and hybridoma but do not cause significant stimulation of our H–YA^b restricted clone. Cells transfected with a 10 kb subcloned fragment from this cosmid were shown to express the H–YK^k epitope. Further enzyme mapping studies indicated that the H–YK^k epitope is encoded by one of two approximately 3·5 kb EcoR-1 fragments. The results of experiments to further define this epitope will be presented.

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A new DEAD box RNA helicase isolated from a PCR-generated mouse notochord cDNA library

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In vertebrate embryos the notochord is an important organizer of developing midline structures including somites, floorplate and the spinal cord. We are interested in identifying and characterizing genes that might be important in the broad range of notochord function and have prepared a cDNA library from microdissected notochords of 9·5 dpc mouse embryos. We have analysed a selection of notochord cDNAs of which a high proportion appear to be novel expressed sequences. Here we describe the isolation and characterization of the embryonic RNA helicase, ERH, a new member of the DEAD box family of putative RNA helicase proteins. This gene maps to chromosome 1 and is expressed throughout the 9 dpc embryo and in later stages of development shows a more restricted pattern of expression in brain and kidney. ERH shows high sequence similarity to the testes specific mouse PL10 gene and to the maternally acting Xenopus An3 mRNA which localizes to the animal hemisphere of oocytes. Its expression profile shows similarities to that of An3 and suggests that ERH is the murine equivalent of the Xenopus An3 gene.

X and Y chromosome specific analysis of biopsied cells of the cultured preimplantation human embryo using FISH and PCR techniques

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We have successfully undertaken FISH and PCR analysis of both biopsied early cleavage stage and trophectoderm cells of the human preimplantation embryo (Muggleton-Harris *et al.* 1994, Human Reproduction in press). At present, we are developing and optimizing existing techniques with which to assess how representative the biopsied cell samples are of the remainder of the embryo. The efficiency of FISH methods are being developed using spent amniocyte and lymphocyte cell cultures (in collaboration with the Clinical Genetics and Cytogenetics Department, St. Georges Hospital Medical School). Results show that we obtain efficient hybridization when nuclei and metaphase chromosome spreads from these cultures have been probed with X and Y chromosome-specific markers. Subsequently, these methods were applied to biopsied cell samples from the preimplantation embryo. Initial results show that, following fixation using the method of Harper *et al.* 1994 (*Hum. Reprod.*, Vol. 9, pp. 721–724) and Coonen *et al.* 1994 (*Hum. Reprod.*, Vol. 9, pp. 533–537), 68% of blastomeres from disaggregated embryos are retained on the slide. Furthermore, using FISH on these blastomeres, results show a hybridization efficiency of 100%. Duplicate samples of the cells are utilized for PCR amplification using both X and Y specific primers.

The *T* gene regulates exit of mesoderm cells from the primitive streak of gastrulating mouse embryos

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Deletion of the *T* gene in *Brachyury* (*T*/*T*) homozygous mouse embryos causes a deficiency of mesoderm posterior to the seventh somite, resulting in axial truncation and embryonic death at 11·5 days post coitum. Cells ingressing through the primitive streak express the *T* gene during their transition from primitive ectoderm to mesoderm. Furthermore, overexpression of the *Xenopus* homolog of the *T* gene in animal caps redirects their fate to that of posterior/ventral mesoderm. We have examined the role of *T* using chimeras made between *T*/*T* or control *T*/+ embryonic stem (ES) cells and wild type embryos. Introduction of a ubiquitously expressed *lacZ*

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marker to these cells allowed their visualization in chimeras during gastrulation, where *T/T* cells accumulate in the primitive streak of early headfold stage embryos. In normal embryos, cells remaining in the primitive streak after gastrulation give rise to the tailbud, and it is in this site that a conspicuous block of *T/T* cells resides in later chimeric embryos. Thus, *T/T* cells accumulate in the locations where *T* would normally be expressed, suggesting that *T/T* cells are deficient in a cell adhesion or migration property which facilitates their normal exit from the primitive streak to lateral locations. The *Brachyury* deletion spans a large (180 kb) stretch of DNA, which may harbour genes other than *T*. Thus, to demonstrate that the *T* gene itself is involved in morphogenetic movement, we have introduced a genomic cosmid containing the *T* gene into the marked *T/T* ES cells, to attempt to rescue their morphogenetic behaviour. Four independent ES cell clones express *T* mRNA and protein in an *in vitro* differentiation assay. In chimeras, these cells express *T* protein appropriately in the primitive streak, while during later development they do not accumulate in the tailbud. These experiments demonstrate that *T* is indeed able to assist the lateral movement of mesoderm cells from the primitive streak.