Abstracts of papers presented at the first Mammalian Genetics and Development Workshop held in the Linnean Society Rooms, Burlington House, Piccadilly, London on 6–9 November 1990

Loss of heterozygous DNA markers in testicular tumours

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Most testicular teratomas have a modal chromosome number in the near triploid range. Higher numbers are found in seminomas, and tumour progression is thought to result from net loss of chromosomes from a near tetraploid carcinoma *in situ* cell [Oosterhuis *et al.* (1989) *Lab. Invest.* **60**, 14]. Regions of the genome can be lost by chromosome rearrangement or loss of both copies of the same homologue. Certain chromosomes 4, 5, 11, 13, 16 and 18 are consistently *under*-represented in our teratomas while other such as 3, 7, 8 and 12 are always present at least *three* times. We have investigated 22 testicular tumours and four tumour cultures derived from three separate individuals and their corresponding normal tissue for loss of constitutional heterozygosity using 35 probes located on 15 different chromosomes. Loss of alleles on 1p in two cultures confirms previous studies using enzyme and chromosome polymorphisms [Parrington *et al.* (1987) *Hum. Genet.* **77**, 269]. Losses of 1q were also found in two of the cultures and individual losses were found on 11p, 11q, 9q, 14q and 20q. In the tumours, losses were found on the following chromosome arms: 1q (1 tumour out of 22), 5p (1/19), 5q (3/22), 7q (1/17), 11q (1/14), 13q (1/4), 16p (2/15), 18p (1/4), 18q (1/20), 20q (1/19). No losses were found on 1p (20 informative), 12p (4), 12q (20), 14q (17), 15q (13), 7p (19), 9q (9), 11p (19), 3p (10), 3q (4), 4 (6) and 17p (14). Additional sized bands were detectable with λMS32 (two individuals), 621 (one individual), and λMS31 (four individuals). In one of these patients the extra band detectable with λMS32 was present in both the seminoma and teratoma parts of the tumour thus suggesting a common origin for both histological types of tumour. In contrast with findings of Lothe *et al.* (1989) (*Genomics* **5**, 134) and Radice *et al.* (1989) (*Cytogenet. Cell Genet.* **52**, 72) we did not find any loss of alleles on 3p and the only loss on 11p was in a single tumour culture.

Genetic mapping in Meishan/Large White pig families

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We are engaged with others in a European collaborative project (PiGMaP) to derive physical and genetic maps of the porcine genome [Haley *et al.* (1990) *Proc. 4th World Congr. Genet. Appl. Livest. Prod.* **XIII**, 67–70]. The linkage studies for the genetic map will be performed on crosses of divergent pig stocks. The reference families in Edinburgh will take the form of several three-generation families. Four F1 litters have been produced from reciprocal crosses of purebred Meishan (Chinese) and Large White (European) pigs. These F1 pigs, which were born in June 1990 will be intercrossed to yield at least 200 F2 individuals. It is anticipated that these families will be of similar value to the *Mus domesticus/Mus spretus* interspecific crosses used to good effect in genetic mapping in mice.

The efficacy of cloned human sequences for detecting polymorphic differences between the purebred founders is being assessed. DNAs from the four founder Meishan and the four founder Large White pigs have been digested with a panel of twelve restriction enzymes and the resulting Southern blots are being screened with a selection of human and porcine probes. Human locus-specific VNTR probes reveal poor quality polymorphic DNA fingerprints when used at reduced stringency on pig DNA and probably will be of limited value for the pig project. A number of loci, fixed for different alleles in the founder breeds have been identified with cDNA probes, including cDNAs for human α1-antitrypsin and porcine albumin. The use of these diallelic loci as anchor points for genetic mapping and for comparative mapping will be discussed.

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Relationship between rate of embryonic development and the preponderance of cranial neural tube defects in females

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Female embryos develop cranial neural tube defects (NTD) more often than males, in both humans and the *ct* mouse mutant. One hypothesis to explain this phenomenon proposes that females develop more slowly than males, and so spend longer in the 'sensitive period' for the induction of NTD. To test this hypothesis we compared rates of development of male and female *ct*/*ct* embryos, collected at intervals between 8·5 and 10·5 days gestation. DNA was prepared from extraembryonic membranes of each embryo and examined for the presence of Y-specific sequences (Zfy) using the polymerase chain reaction. Although male embryos were found to be larger and more advanced in development than females throughout the neurulation period, the rates of growth and development of the two sexes were closely similar. Males must become developmentally advanced compared with females at an earlier stage of gestation and, during neurulation, the two sexes develop in parallel. This finding does not support the hypothesis that females develop cranial NTD more frequently than males because of a slower developmental rate.

Physical and genetic mapping of the class II genes of the rat major histocompatibility complex (MHC)

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It is known that the rat MHC contains 2 loci which control primary mixed lymphocyte responses, RT1.B and RT1.D. They contain genes with high sequence similarities to mouse H-2A and H-2E genes, respectively. We have used pulsed field gel electrophoresis to map this region, linking and orienting the RT1.B and RT1.D gene clusters. The gene order and distance between clusters are very similar to those for the homologous mouse H-2A and H-2E genes. The map is being extended to the 'left' of the RT1.B/D region. Genetic mapping using congenic and recombinant rat strains indicates that this region contains single HLA-DPA- and HLA-DPB-like sequences, which map provisionally within 400 kb of RT1.B. An additional HLA-DPA-hybridizing sequence segregates away from the MHC.

Role of the 3′ end in HPRT gene expression

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The X-chromosome linked HPRT gene encodes an enzyme involved in the purine salvage pathway in mammalian cells. HPRT is a member of the large group of housekeeping genes which are expressed constitutively in all cell types. In addition to this, HPRT has a characteristic tissue-specific pattern of expression, with elevated mRNA levels in the brain. We have obtained no evidence to suggest that these tissue-specific variations in HPRT gene expression result from changes in transcription initiation. Consequently, the role of 3′ untranslated region (UTR) is controlling HPRT expression, possibly at the level of mRNA stability is under investigation. No tissue-specific difference in the site of HPRT mRNA polyadenylation could be detected. Gene targeting is now being used to make precise alterations to the 3′ UTR of the endogenous HPRT gene in mouse embryonic stem cells. This permits the expression of the modified genes to be studied both in cell culture and in intact animals, since these cells retain the ability to develop normally when reintroduced into a host embryo.

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Transmission in male and female single sex mouse chimaeras between a chromosomally normal and homozygous translocation component

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Three males and three female single sex chimaeras between T(1;11.13S)70H/Tid and +/+ were followed through reproductive lifespan when caged with T(1;13)70H homozygotes of the opposite sex. All six animals were germline chimaeras. The *fz* gene marks the reciprocal translocation. Ratios between *fz*/*fz* and *fz*/+ offspring did not change during ageing of the males, but dramatically dropped in two of the three females. Within the males, there was generally good agreement between the ratios of translocation and non-translocation germ cells from spermatogonial mitoses to the first and second meiotic division. In one male, this ratio was also reflected in the offspring. In the other two, there was very significant selection during haplophase, from which both types of spermatozoa could benefit. The results are discussed against the effects of homozygosity for T(1;13)70H and Rb(11.13)4Bnr on gametogenesis.

Do co-adapted gene systems create barriers to gene flow in hybrid zones? The case of the *Mus musculus* X chromosome

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Secondary contact between two genetically differentiated populations often results in the formation of a hybrid zone in which gene flow is reduced. Insight into the nature of these genetic barriers can be gained by studying the gene flow in the hybrid zone between *M. musculus domesticus* and *M. m. musculus*. An earlier study showed that although there is no major hybrid inviability, the introgression of the Y chromosome is virtually non-existent. This led us to propose that the introduction of a foreign Y chromosome perturbs specific co-adapted gene combinations that have evolved in each sub-species. If this is the case, the X chromosome is likely to be involved in such a system given its interaction with the Y chromosome in sex determination. In order to test this hypothesis the distribution of three diagnostic X chromosome markers across a transect of the hybrid zone in Denmark was analysed. The markers used covered about half the X chromosome and it was shown that this region introgresses much less than the nine autosomal markers analysed previously. Furthermore, the few cases of introgression observed were always accompanied by recombination. Therefore, like the Y chromosome, there appears to be strong selective pressure on this part of the X chromosome that prevents it from penetrating the hybrid zone. As putative sex determination genes map towards the centre of the region covered, the results suggest that co-adapted gene systems involved in sex determination play an important role in maintaining the partial barrier to gene flow between the two sub-species.

A testis expressed gene mapping to the distal inversion of the mouse *t*-complex

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From a testis cDNA library a clone (pBs13) has been isolated which maps to the distal inversion of the mouse *t*-complex, as shown by *in situ* hybridization and partial haplotype analysis. The mRNA corresponding to this

clone has only been detected in male germ cells and is first expressed around the pachytene spermatocyte stage of spermatogenesis. Using the partial haplotypes t^{w18} and t^{h20} the gene maps either to a position between the deletions associated with these two haplotypes or distal to the t^{h20} deletion. Comparison of the nucleotide sequence of the *t* and wild type forms of clone pBs13 reveals that over 1·5 kb there are only 3 nucleotide changes, leading to one amino acid substitution (glycine→arginine). Analysis of the pBs13 protein reveals leucine heptad repeats and an RGD sequence, suggesting that a receptor for the protein may exist.

Evidence for somatic mutation during early development at highly unstable mouse minisatellite loci

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A highly unstable mouse minisatellite locus, *Ms6-hm*, has been identified in mouse DNA fingerprints produced by cross hybridization with the human minisatellite probe 33.6. *Ms6-hm* has been cloned, sequenced and mapped to chromosome 4. Analysis of an extended C57BL/6 pedigree showed a high germline mutation rate to new length alleles at *Ms6-hm* (2·5% per gamete) causing multiallelism and heterozygosity even within inbred strains of mice. Mice mosaic for cells carrying a non-parental allele in somatic tissue are also seen (2·8% of mice). In some cases mosaicism extends to the germline leading to three-way segregation of alleles into offspring. At reduced stringency *Ms6-hm* detects other loci, one of which, *Hm-2*, has been cloned and further characterized. Using the same C57BL/6 pedigree the germline mutation rate at *Hm-2* has been measured at 3·4% per gamete, similar to the rate of *Ms6-hm*. However, the incidence of somatic mutation leading to mosaicism is much greater (10% of mice are mosaic), making it an ideal locus for further study of somatic mutation events. Analysis of *Hm-2* in embryonic and extra-embryonic tissues has shown that many mutation events occur early in development, to produce in some cases divergence in *Hm-2* genotype between the embryo and the trophoblast. In at least one case, mosaicism was shared between the embryo and the trophoblast suggesting that this mutation arose very early in development, before the fifth cell division following fertilization.

Regulation of the human β-globin gene

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The human β-globin gene family is located on the short arm of chromosome 11 and contains five functional genes. These are arranged in the same order as they are expressed during development, i.e. $5'$-ϵ-γ_G-γ_A-δ-β-$3'$ over a distance of 55 kb. The embryonic ϵ-globin gene is active when the yolk sac is the hematopoietic tissue, the γ-globin genes are active in the liver during the foetal stage, and the δ- and β-globin genes in the adult stage bone marrow (for review, see Collins & Weissman, 1984). Each gene contains a number of tissue- and developmental stage-specific regulatory regions and the entire locus in controlled by the so-called Dominant Control Region (DCR). This DCR consists of four strong hypersensitive regions (HSS) upstream of the ϵ-globin gene. Addition of these regions confers copy number dependent expression on the human β-globin gene in murine erythroleukaemia cells and transgenic mice, at levels comparable to the endogenous mouse globin genes. Addition of the DCR to globin genes also results in altered developmental expression patterns of an individual gene. By using multiple globin genes, we show that the combination and order of genes is important for their expression. A model for the regulation of this multigene locus will be presented.

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Irradiation–fusion gene transfer hybrids from human chromosome arm 9q

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Tuberous sclerosis is an autosomal dominant disease, seeming to affect principally ectodermal cells. Attempts to map the disease locus have proved complicated, but many families have shown a strong linkage to markers in 9q34. With the aim of cloning the disease locus *TSC1* we have constructed a panel of 63 irradiation-fusion gene transfer (IFGT) hybrids containing fragments of 9q in a hamster background. The protocol used to construct the hybrids has been described [Benham *et al.* (1989) *Genomics* **4**, 509].

We have screened for the presence or absence of 18 loci (identified by 16 probes), scattered throughout the chromosome arm. Hybrids which contain very little human DNA, but contain markers closely linked to three disease loci, *FRDA* (Friedrich's ataxia), *TYD1* (Torsion distonia) and *TSC1*, have been identified. Human probes are being isolated from them, either as conventional genomic or linking clones, or by Alu primed PCR. These will be used to improve the linkage map and to make physical maps.

Control of the mouse α-foetoprotein gene expression

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Expression of the α-foetoprotein (AFP) gene is a typical onco-foetal trait, subject to tissue-specific and developmental controls. The AFP gene is expressed before birth (endodermal tissues: yolk sac, liver, etc.), is silent in the adult and is generally active in hepatomas. Using chimeric AFP-CAT constructions, we showed that the AFP promoter region contains a tissue-specific enhancer, highly active in AFP-producing hepatoma cells, and inactive in non-AFP-producing hepatoma cells and in non-liver cells like fibroblasts. This proximal enhancer has been localized to a 124 bp sequence, upstream position -79. This sequence contains several sites recognized by known transcription factors. However, evidence has been obtained indicating that the enhancer activity is dependent on a new transcriptional factor, present in AFP-producing hepatoma cells. On the basis of these observations, a genetic approach aimed at cloning the gene coding for this factor has been developed. On the other hand, the AFP promoter region also contains a tissue-specific 'silencer', active in fibroblasts, and inactive in hepatoma cells. This silencer is able to act on an heterologous promoter, is the target of negative *trans*-acting factors ('extinguishers') and appears to be at least partially responsible for extinction of the AFP gene expression, observed in hepatoma × fibroblast hybrids.

The branchial arches are distinguished by Hox 2 expression

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There is evidence to suggest that the neural crest is the most important tissue in patterning the vertebrate head. Some cranial crest is derived from an area of the hindbrain known to express Hox 2 genes in an overlapping, segment-restricted pattern. We have found that the cranial crest that will form the mesenchyme of the branchial arches expresses Hox 2 genes strongly. At 8·5 days of mouse development we have found expression of Hox 2.8 in crest emerging from the neural plate. At 9 days, after migration is complete, each branchial arch has a distinct pattern of Hox 2 expression. Arch 1 mesenchyme has no expression, arch 2 expresses Hox 2.8 alone, arch 3 expresses Hox 2.7 and Hox 2.8. The expression of some genes is retained from hindbrain to branchial arches in the crest. However Hox 2.9, expressed in the hindbrain when crest is migrating, is not expressed in the mesenchymal crest, but only in the later emerging, ganglionic crest. Given the role of the homologous genes in insects is in positional specification, and the suggestion that neural crest is imprinted before migration, it is possible that a step in the development of this part of the body is the specification of positional values in the neural crest and hence the arches by Hox 2 genes.

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PCR-based chromosome-specific probes for the mapping of mouse chromosome 16

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We have utilized an oligonucleotide primer from the 3′ end of the mouse L1 repeat element for amplification of mouse-specific PCR products from Chinese hamster/mouse somatic cell hybrids. The 3′ end of the mouse L1 element is present in approximately 100000 copies per genome. PCR of a chinese hamster/mouse somatic cell hybrid, 96AZ2, containing only mouse chromosome 16, produced a range of mouse-specific bands. Hybridisation analysis of a number of these PCR-generated bands through a panel of somatic cell hybrids demonstrated that, as expected, they originated from mouse chromosome 16. Furthermore, a number of the PCR products from 96AZ2 contain $(CA)_n$ microsatellites. Several of these PCR products have been isolated and mapped on mouse chromosome 16 using interspecific backcrosses between *Mus domesticus* and *Mus spretus*. For some PCR products generated from the 96AZ2 hybrid equivalent PCR product was not found in the *spretus* genome, presumably due to a variation in repeat sequence location between the two genomes. The mapping of these PCR products was facilitated by the use of an interspecific cross that employed *Mus spretus* as the backcross parent. PCR based chromosome-specific probes generated from somatic cell hybrids are an easy source of new markers to specific mouse chromosomes.

Analysis of human alpha-1-antitrypsin (AAT) variants by denaturing gradient gel electophoresis (DGGE)

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DGGE permits the resolution of DNA fragments which differ by as little as a single base pair change [Myers *et al.* (1985) *Nucleic Acids Res.* **13**; 3111]. In combination with the polymerase chain reaction (PCR) and the use of high melting point GC clamps at the end of the oligonucleotide primers the system has even greater applicability [Sheffield *et al.* (1989) *Proc. natn Acad. Sci, USA* **86**, 232]. As a preliminary to using this approach to detect cryptic variation in non-coding DNA sequences as markers for human linkage analysis, we have carried out studies on the polymorphic variants of human AAT: M1Ala and S in exon III, M3 and Z in exon V and M2 in exon II [Brantley *et al.* (1987) *Am. J. Med.* **84**, 13]. Amplified DNA was subjected to electrophoresis at 61 °C in TAE buffer pH 7·4 in 6·5% (>260 bp) or 10% (<260 bp) polyacrylamide gels which contained a linearly increasing gradient of chemical denaturant (100% denaturant = 7 M urea, 40% formamide). DNA fragments were detected with ethidium bromide staining. The exon III amplification (365 bp) comprised a single melting region. The two common point mutations, M1Ala and S (C→T and A→T, respectively) were readily detected due to the differing mobilities of their homoduplex bands. Furthermore heterozygous individuals also gave the two earlier melting heteroduplex products. However, the M1Ala polymorphism was resolved only after the incorporation of the GC clamp onto the 3′-terminus, whereas the S mutation was detected only when a 5′GC-clamp was used. This indicates that the nature and position of the GC clamp may profoundly influence the range of detectable base changes. The longer exon V (442bp) fragment, on which the M3 and Z single base changes are located (A→G and G→A), also constitutes a single melting domain. In this case the M3 mutation, which occurs in an AT-rich region, was readily detected but the Z mutation was only resolved after *Alu* I digestion of the fragment or by the use of different oligoprimers to amplify the Z but not the M3 mutation site. Analysis of the M2 mutation in exon II is in progress. The ease with which common variants of AAT were detected by this procedure is encouraging for the future analysis of cryptic variation in non coding DNA sequences.

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Sequences conserved between human and mouse X chromosomes

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Whilst X linkage of loci is preserved throughout the placental mammals the X chromosome has undergone intra-chromosomal rearrangements during the divergence of mammalian orders. Comparative mapping of the mouse and human genomes provides insights into the way in which the mammalian genome has evolved and can aid in the identification of animal models for human genetic disease loci. We have identified and mapped a number of novel human X chromosome probes which are conserved in the mouse genome and have mapped these back to the mouse X chromosome using an interspecific backcross panel. Through this strategy we aim to further characterize the blocks of X-linked loci conserved between mouse and man. As the conserved probes are potential coding sequences, we are examining their DNA sequences and patterns of transcription.

Characterization of a cytoplasmic malate dehydrogenase (MOR-2) deficient mutant in *Mus musculus*

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A heterozygous mouse mutant with approximately 55% malate dehydrogenase (MOR) activity in blood compared to wild type was recovered in mutagenicity experiments with ethylnitrosourea [Charles & Pretsch (1987) *Mut. Res.* **176**, 81; Ehling *et al.* (1985) *Mut. Res.* **150**, 393]. Homozygous mutants exhibiting 6% residual activity were found in progeny of intercrosses. Altered physicochemical properties of cytoplasmic brain MOR suggest that the deficiency is a result of a point mutation at the *Mor-2* structural locus. Therefore the allele was a designated *Mor-2*$^{a\text{-}m4Neu}$. In comparison to the wild type the a-m4Neu allele codes for an enzyme which as a homodimer is characterized by a markedly decreased heat stability, increased electrophoretic mobility, but normal catalytic activity. Beside blood the deficiency is also expressed in the cytoplasm of all tissues. Despite this marked enzyme deficiency especially in homozygous mutants, neither heterozygotes nor mutant homozygotes show any effects on viability or fertility. The mutant is the first genetic variant for cytoplasmic MOR in the mouse and may be useful in the analysis of the structural locus of this enzyme.

Absence of sex-chromatin bodies in the amnion of the marsupial, *Monodelphis domestica*

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The grey short-tailed opossum *Monodelphis domestica*, has a karyotype of $2n = 18$, with a small X chromosome and a minute Y. In order to see whether embryos could be sexed by sex-chromatin analysis of amniotic cells, amniotic membranes stained with carbol-fuchsin were examined. No Barr bodies were seen, but a small proportion of cells from XX embryos contained an apparent chromosome-like structure in the centre of the nucleus. These findings could be related to the reduced level of X-inactivation in marsupials compared with eutherian mammals, although other explanations need to be considered.

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Preimplantation diagnosis – detection of heterozygosity in a single cell

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Genetic analysis of the first polar body, the small cell lying next to the oocyte following the first meiotic division of the human oocyte, gives information on the genetic status of the oocyte itself. Hence the unfertilized oocyte of a female carrier heterozygous for a genetic disease may be classified as normal or mutant for the allele under test. Genetic disease in offspring may therefore be avoided by replacing in the mother only the normal oocytes fertilized *in vitro*. However, the possibility of a cross-over between the mutant and normal alleles and the centromere can complicate the procedure. In this case both the polar body and the oocytes will be heterozygous and therefore the diagnostic test on the polar body must be capable of detecting both the normal and the mutant alleles in a single cell. By using PCR techniques we have investigated the efficiency of amplifying a β-haemoglobin sequence encompassing the site of the sickle cell mutation in a single buccal cell from an individual heterozygous for the sickle cell mutation. A restriction enzyme digest of the amplified product distinguishes the successful amplification of both alleles in the single cell. Such tests of accuracy and reliability are essential before oocyte diagnosis can be used in a clinical setting.

Sequence analysis of a repeat sequence island within a Giemsa-positive band on the mouse X chromosome

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The mouse genome contains fifty copies of a long complex repeat unit (LCRU) that are localised as a repeat sequence island at the A3 Giemsa-positive (dark) band on the mouse X chromosome. The repeat units are not tandemly arranged but are juxtaposed and inserted by unrelated sequences of high repetition. The repeat sequence island possesses two notable features that have been suggested as diagnostic features of mammalian Giemsa-positive bands. Firstly, the repeat sequence island encompasses a one-megabase region devoid of CpG islands and, secondly, it features a high concentration of L1 long interspersed repeat sequences [Nasir, Fisher, Brockdorff, Disteche, Lyon & Brown (1990) *Proc. natn Acad, Sci., USA* **87**, 399–403]. Sequencing of extensive portions of the LCRU has highlighted one region that shows loose homology to a portion of a dispersed low copy mouse repeat. Another region features a dinucleotide repeat that is highly polymorphic between individual LCRUs within the island. Furthermore, we have characterized the L1 repeat junctions within the LCRU. The sequencing data provides a clearer picture of the evolution of sequences within this region of the genome and suggests that the repeat sequence island consists of an active patchwork of sequences with a variety of origins from within the mouse genome.

Differentiation inhibiting activity/leukaemia inhibitory factor and the early mammalian embryo

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The regulatory factor Differentiation Inhibiting Activity/Leukaemia Inhibitory Factor (DIA/LIF) inhibits the differentiation of embryonic stem (ES) cells in culture, and obviates the need for feeder cells. ES cell lines have been derived in the absence of feeders using medium supplemented with DIA/LIF. Karyotypically normal males ES cells were injected into genetically dissimilar blastocysts to examine their contribution to adult tissues. Test breeding of overt male chimaeras established that the ES cells derived in DIA/LIF could contribute to the

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germline. RNase protection studies demonstrated that DIA/LIF is produced by the extra embryonic region of the egg cylinder. Together, these results suggest that DIA/LIF plays an important part in early embryogenesis. Further work is in progress to localise more precisely the regions and timing of DIA/LIF expression in the embryo.

Methylation status of CpG-rich islands on active and inactive mouse X chromosomes

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Single copy probes derived from CpG-rich island clones from EagI and NotI linking libraries and nine rare-cutter restriction endonucleases were used to investigate the methylation status of CpG-rich islands on the inactive and active X-chromosome of the mouse. Thirteen of the 14 probes used detected CpG-rich islands on genomic DNA. The majority of island CpGs detected by rare cutter restriction endonucleases were methylated on the inactive X chromosome and unmethylated on the active X chromosome, but some heterogeneity within the cell population used to make genomic DNA was detected. The CpG-rich islands detected by two putative pseudautosomal probes remained unmethylated on both the active and inactive X chromosomes. Otherwise, distance from the X-chromosome inactivation centre did not affect the methylation profile of CpG-rich islands. We conclude that methylation of CpG-rich islands is a general feature of X-chromosome inactivation and not restricted to housekeeping genes.

Transfer of Down's Syndrome critical region to mouse embryonic carcinoma cells

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Down's Syndrome (trisomy 21) is of major clinical importance with an incidence of 1 in 600 live births. We suggest that Down's Syndrome is caused by the over-expression of a few genes on chromosome 21. 21q22 is the 'critical region' or 'Down's Obligate Region'. We have introduced human chromosome 21 material into mouse embryonic carcinoma cells by chromosome mediated gene transfer. These cell lines will be used to isolate human sequences expressed during *in vitro* differentiation. Neuronal differentiation is of particular interest as mental retardation and neurodegeneration are major features of Down's Syndrome. Having established embryonic carcinoma cells as a model system we intend to transfect embryonic stem cells made in the same way as a route to the creation of mice transgenic for groups of genes within the Down's critical region.

The chromatin structure of the beta-2-microglobulin gene during differentiation

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The expression of both class I (H-2) and Beta-2-microglobulin (β_2m) genes are developmentally regulated. While a number of regulatory elements have been defined in the upstream region of β_2m and class I genes, it is not clear that these can fully explain the complex developmental regulation of these genes. We, therefore, decided to look for novel regulatory elements of the β_2m gene by analysing DNAase I hypersensitive (DH) sites. Five DH sites have been found within the vicinity of the β_2m gene. One of these sites (DH1), located around the promotor region, may correlate with the transcriptional activity of β_2m, since it is diminished in both the undifferentiated

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embryonic stem cell line CCE and embryonic carcinoma cell line F9. The remaining DH sites (2-5) are located downstream from the β_2m gene. The most proximal downstream site (DH2) located 5·5 kb from the last exon, appears only to be present in F9 and CCE cells, indicating that this site may be involved with the down regulation of β_2m. In addition, this site is markedly diminished in differentiated F9 cells. The third and fourth (DH3 and DH4) sites are present in all cell types examined so far. We are presently performing functional studies by linking these DH sites with a reporter gene.

Transcripts from an inherited HSR in chromosome 1 of *Mus musculus*

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Several populations of the house mouse (*Mus musculus*) are polymorphic for the presence (+) or absence (−) of an inherited homogeneously staining region (HSR) in chromosome 1. The HSR consists of amplified DNA sequences, present in low copy numbers in the HSR(−) genome. A cloned HSR-derived genomic sequence detected transcripts of about 1·3 and 4·5 kb on blots of poly(A)$^+$ RNA from liver of HSR(+) mice, but not of HSR(−) mice. A cDNA library from polyadenylated RNA of HSR(+) mice was screened with the HSR-derived genomic clone. Positive clones were isolated and shown to be complimentary to the 1·3 kb RNA species and to amplified DNA sequences in the HSR(+) genome. The sequence of four overlapping cDNA clones, 959 nucleotides in length, includes an open reading frame encoding a putative protein of 208 amino acids. The putative coding region for the 4·5 kb RNAs starts about 300 nucleotides downstream from the 3′ end of the 1·3 kb RNA homology region in the same genomic fragment.

Involvement of the chromosome 11q13 region in human breast cancer: the search for the responsible gene(s)

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Activation of several oncogenes (*neu*, *myc* and *int*-2) has been described in human breast cancer and may be of interest as potential prognostic marker in breast cancer. We have studied amplification of genes within the chromosome 11q13 region at different stages of breast cancer development. We found that the *int*-2 oncogene in this region is amplified in 25/189 (13%) of the cases studied. Amplification of the 11q13 region was significantly correlated with estrogen receptor positivity ($P < 0{\cdot}04$) and with the presence of lymph node metastases ($P < 0{\cdot}01$). These data suggest that amplification of the chromosome 11q13 region is associated with a distinct tumour type and presumably is indicative, within the group of patients with estrogen receptor positive tumours, of poor prognosis. The mean follow-up time of our patients is, however, still too short to correlate amplification with tumour recurrence and with patient survival. Because we did not observe any RNA expression of the *int*-2 and *hst*-1 gene in tumours with an 11q13 amplification, we assume that another gene nearby *int*-2 is involved. Using a panel of different probes, mapped on chromosome 11q13, only *int*-2, *bcl*-1 and *hst*-1 were co-amplified in all cases with an 11q13 amplification, suggesting that the unknown gene in the amplified 11q13 region is close to one of these probes. With pulse-field-gel-electrophoresis we have identified the smallest fragment (±1500 kb), recognized by all three probes. Presently, this fragment is being cloned and will be used to identify transcriptionally active genes within the amplified 11q13 region. Differential cDNA cloning provided us with a clone which is amplified and overexpressed in cells with an *int*-2 amplification.

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Expression of HPRT mini-genes in embryonic stem cells

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Similar levels of HPRT mRNA are found in cultured mouse embryonic stem (ES) cells and mouse fibroblasts. However, our basic mouse HPRT mini-gene which works well in fibroblasts is inactive in gene transfer experiments into ES cells. This mini-gene contains a short promoter region with the coding region interrupted by the last two short introns (introns 7 and 8) from the gene itself. A bigger mini-gene containing, in addition, the larger introns 1 and 2 functioned more efficiently in fibroblasts and was now active in ES cells. The critical component for mini-gene expression in ES cells has been localised to a short segment within intron 1. This element does not have the properties anticipated of a conventional enhancer element, but rather appears to interact with the HPRT promoter in a position-dependent manner. These modified mini-genes are being evaluated for use in gene targeting in ES cells. The mini-genes can be used to provide either the positive or negative component in a positive–negative selection system when used with HPRT deficient ES cells.

Hereditary haemochromatosis: a search for expressed sequences on the short arm of chromosome 6

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Hereditary haemochromatosis (IH) is an autosomal recessive trait characterised by iron accumulation in parenchymal tissues as a consequence of chronic, excessive iron absorption by the small intestine. The abnormal gene is present in approximately 10% of caucasian individuals. Clinical manifestations include hepatic cirrhosis, hyperpigmentation, cardiac failure and diabetes mellitus.

The underlying cause of the excessive absorption has not been elucidated, but genetic analysis has demonstrated a tight linkage to HLA-A and -B phenotypes (cumulative lod score of 60, recombination fraction $<0{\cdot}01$). These data map the IH locus to the short arm of chromosome 6 (6p21.3) close to the HLA class I locus.

We plan to isolate and characterise expressed sequences from this region of chromosome 6, and towards this end have constructed a cosmid library from the mouse/human somatic cell hybrid MCP6 containing a human X-6 translocation chromosome as the only human component. Several unique sequences have been identified, isolated and mapped to either chromosome 6 or the X chromosome using a panel of rodent/human somatic cell hybrids. In addition, a chromosome 'walk' has been initiated from HLA-A.

Molecular analysis of the hairless mutation of mice

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To assess the genetic impact of the numerous retroviruses present in the germ line of mice we have developed a series of oligonucleotide probes which allow the unambiguous identification of all the endogenous murine leukemia viruses. A number of mouse mutations were examined with these probes including the hairless (*hr*) mutation which affects skin and thymus function. HRS/J *hr/hr* mice contain one extra polytropic virus compared to HRS/J +/+ mice. Genetic studies have shown perfect concordance between the mutation and the provirus. Analysis of a haired revertant proved the causal nature of the insertion since it revealed the excision of most of the provirus leaving behind a solo long terminal repeat. We have cloned 25 kb of genomic sequences surrounding the viral integration site. A CpG island, located within sequences conserved in humans, has been identified 8 kb upstream from the integration site. A transcript running from the CpG island toward the integration site was found in nuclease protection experiments and northern analyses using RNA from the skin of new born animals. Two potential exons were identified by sequencing genomic clones; PCR studies with cDNA confirm that they are spliced together. We are currently attempting to clone the 6·3 kb transcript.

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The rat gene map and comparative mapping with mouse and man

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In the last few years, considerable progress has been made in the gene mapping of the rat. Using a well-characterized panel of mouse × rat somatic cell hybrids that segregate rat chromosomes, we have localized over 100 rat genes and published the localization of about 60 of them. Genes have been assigned to each rat chromosome, except the 16 and the Y. Five of the 11 rat linkage groups (I, II, VII, IX, X) have been assigned to specific chromosomes. The new knowledge about the rat gene map renders it possible to make interesting comparisons of gene locations in rat, mouse and man. Close similarities in the chromosomal G-banding patterns of several rat and mouse chromosomes were detected earlier. This morphological homologies clearly extend to the gene level. In addition, for 10 rat synteny groups, all the mapped genes are also syntenic in the mouse. There is also evidence of rat–mouse genetic homology whereas no obvious chromosome morphological similarity is detectable. Not surprisingly, rat synteny groups have been dispersed to a much greater extent in the human genome than in the murine one. However, several synteny groups are conserved in rat and man and when the rat, mouse and human chromosome maps are compared, several conserved synteny groups are found.

Two new X-autosome Robertsonian translocations in the mouse

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Two new spontaneous Robertsonian (Rb) translocations have been found in a mouse stock being investigated for chromosome nondisjunction involving the translocation, Rb(X.2)2Ad. Surprisingly, both new rearrangements involved the X chromosome and an autosome: in the first, chromosome 9 and in the second chromosome 12 [Rb(X.9)6H and Rb(X.12)7H respectively]. In both, the translocation involved the X chromosome from the chromosomally-normal parent. The frequency of sex chromosome non-disjunction was found to be elevated in Rb/+ females and Rb/Y males. Unexpectedly, Rb(X.12)/Y males were found to be sterile; in contrast Rb(X.2)2Ad/Y and Rb(X.9)7H males are fertile although they have reduced sperm counts. Preliminary examination of pachytene spermatocytes indicated that synaptic anomalies involving the sex chromosomes did not appear to be responsible for the sterility of Rb(X.12)/Y males. Females mice carrying two different X-autosome Rb translocations, and therefore with monobrachial homology for the X chromosome, have been produced and these generate sex chromosome aneuploid progeny at a high rate. The fate of trisomy X conceptuses is under investigation.

Transcriptional control of β-lactoglobulin in the mammary gland

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The minimal 5′ proximal promoter/control element of the sheep β-lactoglobulin (BLG) gene is 409 bp, as defined by restriction analysis in transgenic mice (Whitelaw *et al.* in prep.). The DNA-binding factors which interact with this element are being defined using gel mobility shift assays and DNase footprinting. A number of short synthetic double-stranded oligonucleotides which span this region have been utilised for the assays in conjunction with defined restriction fragments. The results indicate that several different factors bind to this sequence *in vitro*, some of which occupy multiple sites. Using oligonucleotide competition analysis, the identity

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of two factors has been determined. These are a NF-I like activity and a NFKB-like activity. NF-I is known to exist in different forms in different cell types and the possibility of a mammary specific form is being investigated. Preliminary data also indicate that a factor, which binds to at least three sites in the BLG control region, is mammary specific.

The precise definition of binding sites will be determined by a variety of footprinting and mutational analyses. Oligonucleotide screening of expression cDNA libraries is also being undertaken to clone the mammary specific factor(s).

Analysis of gene regulation in the mouse Hox-2 cluster

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The Hox-2 genes are expressed in the neuroectoderm and mesodermal derivatives of the developing mouse embryo such that the gene order is colinear with the anterior boundary of expression along the A-P axis of the embryo. In order to dissect the molecular mechanisms by which regulatory elements are shaping the specific patterns of spatially and temporally restricted gene expression we have fused the lacZ gene in frame into large genomic clones of Hox-2.1, Hox-2.5 and Hox-2.6 genes flanked by 5′ and 3′ sequences of variable length. Such constructs have been used to generate transgenic lines of mice. Our results so far indicate that individual genes could be removed from the cluster and still retain the correct domains of expression (Hox-2.6). However, in other cases the flanking sequences are not able to recreate entirely the endogenous expression patterns. These data suggest that multiple regulatory elements spread throughout the cluster may act at a distance to control the process of pattern formation, the clustered organization being not essential for regulating all the genes in the mouse Hox-2 complex.

Controlling the expression of genes in transgenic animals: hybrid transcription circuits

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Elements from yeast and *E. coli* involved in the control of gene expression have been shown to work in eukaryotic cells. Our objective is to control the expression of heterologous genes introduced into transgenic animals by means of *cis* and *trans* regulatory elements from yeast and *E. coli.* The reporter constructs used consist of the chloramphenicol acetyl transferase (CAT) open reading frame fused to 348 or 31 bp of the human α1-antitrypsin promoter. The 348 bp fragment should provide tissue specific expression whilst the 31 bp fragment may function as a minimal promoter. Into these reporter constructs is inserted a trans regulatory element; either the lac operator from *E. coli* or the GAL upstream activation sequence from yeast. The effector constructs position either yeast *GAL4* (+ve transcriptional control) or *E. coli LACI* (−ve transcriptional control) under the influence of the 348 bp α1-antitrypsin promoter. The analysis of these constructs *in vitro* by transient expression assays in HEPG2 and HeLa cell lines and a microinjection programme creating transgenic mouse lines for *in vivo* analysis is underway.

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