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A novel zinc finger gene expressed in male germ cells

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From a mouse testis cDNA library we have isolated a clone which codes for a zinc finger protein. The complete sequence of this clone reveals that it has twelve fingers and is a member of the kruppel family with the C2H2 format. The non-finger N-terminus of the clone has 25% basic residues and the C-terminus is acidic. The clone has been shown by Northern blotting to be expressed in germ cells and expression is not detected until around day 20 of spermatogenesis. When used as a probe on a Southern blot of mouse genomic DNA the full length cDNA (1·4 kb) detects an *Eco*R 1 fragment of 1·7 kb and no other hybridizing sequences. A genomic clone for the cDNA has been isolated and the 1·7 kb *Eco*R 1 fragment contained within it has been sequenced. As expected there are no introns in the coding sequence and the extra 300 nucleotides in the genomic fragment is upstream and downstream 'flanking' sequence. In situ hybridization of the cDNA with sections of mouse testis has confirmed the Northern data but also revealed that there is heterogeneity of hybridization of the probe; some seminiferous tubules are clearly positive whilst other are clearly negative. Experiments are under way to determine the binding site for the zinc finger protein.

X chromosome inactivation may explain the difference in viability of XO humans and mice

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Only about 1% of human XO conceptuses survive to birth and these usually have the characteristics of Turner's syndrome with a complex and variable phenotype including short stature, gonadal dysgenesis and anatomical defects. Both the embryonic lethality and Turner's syndrome are thought to be due to monosomy for a gene or genes common to the X and Y chromosomes. These genes would be expected to be expressed in females from both active and inactive X chromosomes to ensure correct dosage of gene products. Two genes with these properties are *ZFX* and *RPS4X*, both of which have been proposed to play a role in Turner's syndrome. In contrast to humans, mice that are XO are viable with no prenatal lethality and are anatomically normal and fertile. We have devised a system to analyse whether specific genes on the mouse X chromosome are inactivated, using an F1 interspecific hybrid between *Mus spretus* and laboratory mice (*Mus musculus domesticus*) carrying the T(X;16)16H translocation. Using this system we have demonstrated that three genes (*Zfx*, *RPS4* and *A1s9X*) that escape X-inactivation in humans undergo normal X-inactivation in mice. Thus the relative viability of XO mice compared to XO humans may be explained by differences between the species in the way that dosage compensation of specific genes is achieved.

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Identification of chromosomes in pig flow karyotype

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As part of the EC pig gene mapping (PIGMaP) programme, our aim is to use flow sorted pig chromosomes for gene localization and construction of chromosome libraries. The 20 peaks seen in the male pig flow karyotype were tentatively correlated with the 18 pairs of autosomes and the X and Y chromosomes of the standard pig karyotype. The peaks corresponding to the X and Y chromosomes could unequivocally be identified since in the female flow karyotype, a peak was noted with twice the number of events than in the male flow karyotype, and another peak present in the male flow karyotype was absent. The remaining peaks were tentatively assigned on the basis of their relative DNA content compared to the relative length of pig metaphase chromosomes. These tentative assignments need confirmation using direct methods of chromosome identification after sorting.

This work was supported by the EEC Bridge Programme and the AFRC Pig Science Programme.

Assignment of cDNAs to the European interspecific backcross

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In collaboration with the Pasteur Institute and Comparative Biology at the CRC, a mouse backcross resource comprising 1000 animals has been produced as part of the UK HGMP. Efforts are under way to assign anchor loci both by hybridization and by PCR-based techniques. This will be completed by April 1992 when new human and mouse markers will be assigned as a service. The Resource Centre in conjunction with the community is producing a large number of human and mouse ESTs. High throughput ways in which these could be assigned to the backcross are discussed.

Close linkage of the olfactory marker protein gene and the deafness mutation shaker-1

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1066 progeny have been generated from a backcross [(C57BL/10 × *sh-1/sh-1*) × *sh-1/sh-1*] segregating the mouse deafness mutation shaker-1 (*sh-1*). Backcross progeny were analysed for the *sh-1* phenotype and flanking genes tyrosinase (*c*) and β-globin (*Hbb*). Analysis of 1008 backcross progeny for a RFLV for the tyrosinase gene identified 54 mice recombinant between *c* and *sh-1*. Analysis of 1052 backcross progeny for a protein polymorphic variant of *Hbb* identified 13 mice recombinant between *sh-1* and *Hbb*. This panel of 67 recombinant mice in the vicinity of the *sh-1* locus enables the identification of markers closely linked to *sh-1*. A RFLV for the Olfactory marker protein (*Omp*) was analysed through the recombinant panel and only 1 recombinant with *sh-1* identified indicating that *Omp* lies only 0·1 cM from the *sh-1* gene. Haplotype analysis demonstrates that *Omp* lies proximal to *sh-1*, giving a genetic map in the vicinity of the *sh-1* locus: *c*-5·3 ± 0·7 cM-*Omp*-0·1 ± 0·1 cM-*sh-1*-1·2 ± 0·3 cM-*Hbb*. The close linkage of *Omp* to *sh-1* indicates that, on average, *Omp* lies only 200 kb from the *sh-1* locus and is a suitable start-point for a chromosome walk to the *sh-1* gene. Primers to *Omp* have been used to screen the Princeton YAC library and a positive YAC has been identified that will be used to initiate a YAC chromosome walk across the *sh-1* non-recombinant region delineated by the backcross panel.

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X chromosome imprinting effects on the prenatal development and viability of mice with single X chromosomes

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It is generally believed that XO mice have a high prenatal mortality. Evidence will be presented that X chromosome monosomy *per se* does not lead to reduced viability. Instead, it will be argued that a paternally-derived X chromosome, by causing developmental retardation, places X^pO (and X^pY) conceptuses 'at risk', but preferential elimination of these 'at risk' conceptuses only occurs if they are further compromised by the deleterious effects of maternal X dosage deficiency. As a consequence X^pO, but not X^mO, conceptuses are preferentially eliminated in XO, but not XX mothers. This is in marked contrast to man, where almost all X^pO and X^mO conceptuses in XX mothers fail to survive to term.

Establishment and initial characterization of long-term cultures of mouse genital ridge cells

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We have established long-term cultures of mouse genital ridge cells explanted between 10·5 and 12·5 days *post coitum* (d.p.c.) from a transgenic mouse carrying a temperature sensitive SV-40 large T antigen regulated by an H-2K promoter. Cells from the genital ridge of individual embryos were dissociated and plated at the permissive temperature. Stimulation of T-antigen expression by growth in the presence of gamma interferon results in maintenance of explants over a period of at least 6 months in culture. Expression of *sry*, the gene which is believed to trigger the initiation of testes development, occurs between 10·5 and 12·5 d.c.p. in male embryos. Some of our explant cultures continue to express this gene over the 6 month culture period. Individual sublines from these cultures are being cloned in order to establish pure lines of pre-Sertoli and other supporting cell lineages. During this period, these gonadal cell cultures undergo many of the organizational features which are seen in short-term *in vitro* explants of this tissue. When male cells which have been maintained on plastic dishes for 4 months or longer are transferred to Matrigel, a reconstituted basement membrane, a complex structure resembling branching testes cords forms within 12 h. We are hopeful that this *in vitro* system will allow us to relate the expression of *sry* to other downstream genes which lead to the organogenesis of the testes.

Construction of a mouse YAC library in a yeast strain carrying the recombination-deficient *rad52* mutation

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Instability of YAC clones has been reported in YAC libraries built with the host AB1380 [Albertsen *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4256–4260]. Yeast strains carrying the *rad52* mutation have been demonstrated to stabilize some YAC clones containing tandem repeat sequences [Neil *et al.* (1990) *Nucl. Acids Res.* **18**, 1421–1428]. We have completed the construction of a partial *Eco*R I YAC library in the yeast 814-7/3a strain that contains the *rad52* mutation (814-7/3a: *rad52::TRP1, ade2.1, trp1, ura3, ilv, lys2.1, his3.11, his3.15, his5.2*). C57BL/10 female DNA was partially digested with *Eco*R I. Size selection fragments (> 300 kb) were ligated to pYAC4 and size selected again prior to transformation into 3a spheroplasts. In total, 43000 clones have been

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picked to microtitre plates. Sizing of 100 clones chosen at random within the first 10000 clones picked indicates an average insert size of 350 kb and constitutes the first genome equivalent of the *rad52* YAC library. Preliminary sizing of the 33000 clones recently produced indicates an average size of around 200 kb, providing a further two genome equivalents. The *rad52* library may provide a useful addition to the available YAC libraries for the recovery of genomic inserts that are unstable in AB1380. In addition, this library may aid the cloning and characterization of chromosome regions dense in repeat sequences.

SRY-related genes are expressed in the developing nervous system

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Sry has been shown to be genetically and functionally equivalent to *Tdy*, the testis determining gene in the mouse. We have found that *Sry* is a member of a new family of embryonically expressed genes. In addition to *Sry* we have cloned four other genes of this family. These genes are characterized by the presence of a highly conserved putative DNA-binding domain which shares homology to HMG proteins class 1 and 2, human UBF and the Mc mating type protein of *S. pombe*. The conserved motif has been termed the SRY box, and the four autosomal *SRY*-related genes are called *Sox-1* to *4*. Sequence analysis shows that the hypothetical gene products of three of them are almost identical in their DNA-binding domain but contain differences in their 3′ domains (presence or absence of polyalanine stretches) which could specify the effect these proteins have on their target sequences. *In situ* hybridization analysis of the expression of *Sox-1*, *Sox-2* and *Sox-3* in the embryo reveals that they are expressed in different patterns in the central and peripheral nervous system. One of them is also expressed in endodermal derivatives.

Identification of genes in the mouse TLA region encoding potential class I histocompatibility antigens

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The murine Q/TL region lies telomeric to the classical transplantation loci H-2K, D and L, and encodes class I antigens that share structural homology with them. The vast majority of class I genes lie within this region. Our approach to identifying novel class I genes involves screening a cDNA library made from a B10.BR (H-2^k) spleen mRNA preparation. An initial screening involving probing with an entire class I cDNA (Q7) at low stringency isolated about 2000 class I plaques. The plaques containing D^k and K^k, which represented over 95% of the total class I message, were eliminated by rescreening with gene specific oligonucleotides. A selection of the remaining plaques containing non-classical genes were subcloned into pKSM13 and partially sequenced. Comparison with published sequence data allowed us to select novel genes. From a pool of 15 partially sequenced genes, four were found to be identical and these were further studied using *in vitro* transcription and translation. Initial results suggest that these 4 clones may contain the gene encoding Qa-1^a and we are now analysing these clones in more detail.

Genetic and physical mapping of the mouse *Xist* gene

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The mouse X chromosome inactivation centre (Xic) lies between the T(X:16)16H and HD3 breakpoints [Rastan & Brown (1990) *Genet. Res.* **56**, 99–106] and comparative mapping of the human XIC region has suggested its

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most likely location within this region to be between *Phkα* and *Pgk1* (Brown *et al.* (1991) *Nature* **349**, 82–84]. In order to characterize this region we have undertaken genetic and physical mapping of linking clones and other genetic markers. The recently isolated mouse *Xist* gene [Brockdorff *et al. Nature* (1991) **351**, 329–331; Borsani *et al. Nature* (1991) **351**, 325–328] mapped between *Rps4X* and *Pgk1* and cosegregated with *Phkα* and linking clone loci DXCrc177 and DXCrc318 in our interspecific backcross. However, physical mapping using pulsed field gel electrophoresis has enabled us to establish both the order of the loci and the physical distance between them, which is Xcen-*Phkα*-175 kb-DXCrc177-585 kb-DXCrc318-210 kb-*Xist*-DXCrc13-*Pgk-1*. The linking clone probes detected, as expected, clusters of rare-cutter restriction enzyme sites, indicative of CpG-rich islands which are associated with the 5′ end of genes. As yet we have not found an island associated with the *Xist* gene.

Sequence of the *t*-complex *Tcp-10a*^{t} gene and examination of the *Tcp-10*^{t} gene family

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Transmission ratio distortion (TRD) is a property of complete *t* haplotypes which results in the preferential transmission of the *t* haplotype chromosome from heterozygous *t*/+ males to the majority of the offspring. A candidate gene for one of the primary genetic elements in TRD, the *t*-complex responder locus, has recently been suggested to be *Tcp-10b*^{t}. There are multiple, functional *Tcp-10*^{t} genes but genetic data suggest the presence of the *Tcp-10a*^{t} gene alone is compatible with normal transmission ratios. Here we present the complete sequence and genomic structure of the *Tcp-10a*^{t} gene which is compared with sequence data from a number of cDNAs and genomic subclones representing all active *Tcp-10*^{t} family genes. We have clarified the extent of 5′ untranslated alternative splicing patterns exhibited by this gene family and discovered a 60 bp in-frame deletion from the 5′ end of exon 3 of the *Tcp-10a*^{t} gene compared to the equivalent regions of *Tcp-10b*^{t} and *Tcp-10c*^{t}. A search of the University of Edinburgh data base has revealed a significant homology between a region of the *Tcp-10b*^{t} open reading frame and several cytosolic filament proteins. Interestingly, the region of homology is involved in the deletion from the *Tcp-10a*^{t} gene.

An interaction between the curly-tail and splotch mutations in the mouse can lead to lethal neural tube defects

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Neurulation is an important developmental event during mammalian embryogenesis. To investigate the morphogenetic basis for neural tube defects (NTD) we are studying the interaction between mutations at the curly-tail (*ct*) and the splotch (*Sp*) loci in the mouse. Although both these mutations are recessive for NTD, 8·3% of double heterozygotes show tail flexion defects similar to those seen in homozygous curly-tail. When *ct*/+ *Sp*/+ animals are back-crossed to *ct*/*ct* a proportion of the offsping should be of the genotype *ct*/*ct Sp*/+. Our data show up to a 52% chance of lethality within this group. Examination of explanted embryos suggests that the lethality is due to severe lumbo-sacral spina bifida and that the time of death is at or immediately after birth. The late lethality and the localization of the defect to the lower spine suggests that what we are seeing is an exacerbation of the curly-tail type phenotype on a heterozygous splotch background. This will be discussed in greater detail.

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Mip: a candidate gene for hereditary cataract in the mouse

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The major intrinsic protein (MIP) of vertebrate eye lens fibre cell membranes is believed to function in the maintenance of lens transparency. Using immunoblot and immunocytochemical analysis, we have detected structurally modified forms of MIP associated with cataract development in the lenses of *Cat* (cataract Fraser), *Lop* (lens opacity) and *Nct* (Nakano cataract) mouse mutants. We have also detected RFLPs associated with the gene for MIP in the *Cat* and *Nct* mutants and have recently localized the *Mip* gene to mouse chromosome 10D1 by *in situ* hybridization. Currently the *Mip* gene represents a candidate for the *Cat* locus which has also been assigned to the distal end of mouse chromosome 10.

Preimplantation sexing of human embryos by fluorescent *in situ* hydridization using X and Y chromosome specific probes

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Preimplantation diagnosis of sex has been reported using the polymerase chain reaction (PCR) in families carrying sex-linked recessive disorders [Handyside *et al.* (1990) *Nature* **344**, 768–770]. This approach has a number of disadvantages not least of which is the reliance on the absence of an amplified fragment on a polyacrylamide gel to positively diagnose a cell as female and hence proceed with embryo transfer on this basis. In this study we describe four approaches to sexing human blastomeres utilizing the technique of fluorescent *in situ* hybridization (FISH). The first and second involve employing Y and X chromosome-specific probes respectively in single hybridization experiments. Although good efficiency of hybridization is obtained, each strategy has drawbacks. Sexing using a Y chromosome probe alone could lead to misdiagnosis because of failure of hybridization, use of an X probe alone could lead to females being diagnosed as males due to overlying signals or, more seriously, males being classed as females because of tetraploid nuclei. The third is a dual FISH approach using two Y chromosome probes and one X chromosome probe, here the possibility of diagnosing males as females is virtually eliminated. Ambiguous results are common however due to the appearance of autofluorescent foreign bodies. All the above methods have an inherent disadvantage in that they take 24 h to perform whereas the PCR method takes 5 h only. Finally, a rapid dual FISH sexing strategy is described. The procedure takes six to seven hours and the problem of signals being obscured by autofluorescent foreign bodies is kept to a minimum because signal amplification is not necessary in this approach as it is in the others. FISH is potentially a powerful tool in the preimplantation diagnosis of sex and chromosomal disorders.

Methylation of transgenes can be controlled independently of transcriptional activation

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Methylation of tissue specific genes correlated with transcriptional activity, but there is a question as to whether the methylation status of a gene is the result of its transcriptional status, or vice versa. There is a positive correlation between the expression of group one mouse major urinary protein (MUP) genes and hypomethylation in the tissues of adult mice. MUP genes are hypomethylated in foetal liver but are not expressed. Methylation of adult tissues and foetal liver has been analysed in lines of transgenic mice carrying one to three copies of 2·2 kb

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MUP promoter linked to the herpes simplex 1 thymidine kinase gene coding region. In two of these lines the transgene is hypomethylated but not expressed in foetal liver. In adult tissues the transgene is hypomethylated in both expressing and non-expressing tissues. In a third line, due to the chromosomal context, the transgene is neither hypomethylated nor expressed in foetal liver, while in adult tissues demethylation correlates with transgene expression. By studying these transgenic lines, we show that the methylation status of a gene can be at least partly controlled independently of the potential capacity of the tissue to transcribe the gene.

Adult phenotype in the mouse can depend on epigenetic events in the early embryo

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A number of observations in the mouse suggest that there are nucleocytoplasmic interactions in the early embryo that can affect epigenetic programming of later gene expression. It is possible that some influences of parental inheritance on offspring phenotype are caused by this type of mechanism. We have begun to examine the effect of parental inheritance of alleles on offspring phenotypes by analysing 2-dimensional protein patterns in adult livers of reciprocal F1 hybrids between C57BL/6 and DBA/2. To assess the effect of correct nucleocytoplasmic interaction on adult phenotypes, experimental zygotes were made that have a B6 type cytoplasm, DBA type maternal chromosomes and B6 type paternal chromosomes. Hence, the usual genotypic harmony between cytoplasm and maternal genome has been disrupted in these nucleocytoplasmic hybrids. Proteins were extracted from adult liver of these animals and their 2D electrophoresis patterns were compared to those of the parental strains and the natural F1 hybrids. A prominent group of liver proteins was found whose expression was virtually undetectable in the nucleocytoplasmic hybrids. A partial amino-acid sequence was obtained of one member of this group. Our results suggest that adult phenotype can depend upon epigenetic events in the early embryo.

Comparative mapping of the human and mouse X chromosomes

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Comparative mapping of mouse and human X chromosomes has identified a minimum of five blocks of loci which are homologous. In order to further investigate the organization of homologous loci between the X chromosomes of man and mouse we have mapped four murine homologues of human X-linked genes and shown that these map to the homologous regions of the mouse X chromosome. In addition we have cloned and mapped eight probes which detect homologous sequences in the mouse genome. Five of these probes have been shown to lie on the human X chromosome short arm and four of the five to map to the proximal region of the mouse X chromosome. This analysis has extended the known length of this conserved block to approximately twice its previous length. The comparative mapping of one conserved sequence indicates that an additional conserved block may be present close to the centromere of both the human and mouse X chromosomes. This information may be important in the context of the proposed homologies between the mouse mutants scurfy (*sf*) and Tattered (*Td*) with the human conditions Wiskott–Aldrich syndrome (WAS) and sporadic *incontinentia pigmenti* (IPI) respectively.

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Detection, by Hydrolink heteroduplex analysis, of a retinal binding site point mutation in rhodopsin, in a family with severe dominant retinitis pigmentosa

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Heteroduplex formation in the polymerase chain reaction has previously only been shown in heterozygous individuals with minor deletions ranging from 3 to 18 bp [Inglehearn *et al.* (1991) *Am. J. Hum. Genet.* **48**, 26–30]. However, our laboratory has found that single base-pair mismatches can be observed on non-denaturing ethidium bromide stained 24 cm D5000 Hydrolink gels (AT Biochem, supplied by Hoefer, UK) following PCR [Keen *et al.* (1991*a*) *Trends Genet.* **7**, 5]. In 1990, Dryja and others (*Nature* **343**, 364–366) first observed that a point mutation at codon 23, causing a proline to histidine change, was the putative cause of autosomal dominant retinitis pigmentosa (ADRP), a slow inherited retinal degenerative disorder, in a small proportion of ADRP patients. Subsequently our laboratory detected four new putative rhodopsin mutations in different ADRP patients, using Hydrolink heteroduplex analysis [Keen *et al.* (1991*b*) *Genomics* **11**, 199–205]. One of these mutations on sequence analysis proved to possess a 12 bp deletion, while the remaining three proved to be mutations. One of these point mutations occurred at the highly conserved, positively charged, amino acid lysine 296, changing it to a negatively charged glutamic acid. This was found in a family with a particularly severe ADRP phenotype. The lysine amino acid acts as the retinal attachment site in all vertebrate and invertebrate opsins, and even halobacteria rhodopsin. A change to glutamic acid in half of the rhodopsin molecules of an ADRP patient would therefore, almost certainly give rise to a protein which failed to bind retinal during its biosynthesis in the endoplasmic reticulum. How this and the other rhodopsin mutations cause ADRP is yet to be elucidated.

Spontaneous deviation towards malignant transformation. A cytogenetic study of rat liver cells derived from primary cultures

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Spontaneous malignant transformation of cultured liver epithelial cells as a result of prolonged standing at confluency was recently reported [M.-S. Tsao *et al.* (1990) *Cancer Res.* **50**, 1941]. Under our experimental conditions, liver primary cultures can resume proliferation after a ten week quiescent period in serum supplemented Ham F_{12} medium, without cell transfer or further manipulations. The cell clusters which start dividing in long-term primary cultures are malignant as shown by subcutaneous tumour induction *in vivo*. They actively synthesize γ-glutamyl-transpeptidase. After early passage, sub-cloning of a primary culture gave rise to a deviated cell line [J.-F. Houssais *et al.* (1988) *Gastro-enterol Clin. & Biol.* **12**, 2] phenotypically and caryotypically different from previously described hepatic cell lines [M.-S. Tsao *et al.* (1985) *Am. J. Pathol.* **118**, 306]. We here report some results of its cytogenetic study, using the chromosome R-banding techniques [B. Dutrillaux et J. Couturier (1981) In: *Technique de laboratoire*, Ed Masson 1981, Paris; E. Viegas-Pequignot *et al.* (1983) *Cytogenet. Cell Genet.* **35**, 269]. The analysed metaphases showed paratetraploid karyotypes (no. 77 chromosomes) with strong evidence for a monoclonal origin as indicated by the presence of a recurrent translocation involving chromosome 3, and of 3 identical markers in all the cases. Marker 1 resulted from a translocation of chromosome 3 and marker 2 was an isochromosome. Normal chromosome 3 was always absent. Markers 1 and 3 were usually present in two or more copies, whereas marker 2 was unique. It is therefore likely that, while the latter results from a post-endoreplicative event, the others represent pre-replicative changes. Of interest now would be a better understanding of the significance of these chromosomal alterations correlated to metabolic deviations in the reported transformed liver cells.

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Developing a subtractive screening method to identify and clone embryonic-kidney-specific cDNAs

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The mouse kidney is an excellent model system for organogenesis. First because its development encompasses many of the characteristic events in embryogenesis. Secondly all of these events will take place *in vitro* and so are accessible for experimental manipulation, a situation unusual for a mammalian tissue. Although the physical development of the kidney is well understood, the molecular mechanisms underpinning these events remain largely unknown. To identify genes important in the control of kidney morphogenesis, we have set out to find cDNAs that are specifically expressed during its development. To do this we first made a mouse 14·5 day p.c. embryonic kidney cDNA library, then subtracted 14·5 day p.c. kidney cDNA against adult liver RNA and created a subtracted probe renewable by PCR. Thus far, Northern analysis has shown all clones satisfy the selection criteria of expression in the embryonic kidney, as well as other tissues, but not in the adult liver. We are currently analysing three cDNAs which encode novel proteins as well as other clones that contain the mouse B1 and other repetitive elements. We are also refining the substraction to find clones purely embryonic kidney-specific.

Sex chromosome pairing and fertility in XY, XXY and XYY female mice

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XY female mice have very few oocytes surviving to puberty, and consequently are rarely fertile. We have compared the breeding performance of XY, XXY and XYY females, with a view to assessing the relative importance of the lack of a second X as compared to the presence of a Y chromosome, in reducing fertility. Only 4 of 10 XY females tested were fertile, and they had small infrequent litters. All the XXY females were fertile with near normal litter size and reproductive lifespan. This suggests that the lack of a second X, rather than the presence of a Y is the major factor leading to the poor fertility of XY females. Nevertheless, the XYY females tested did appear to be more severely affected than their XY sibs. It has been argued that unpaired sex chromosomes in pachytene oocytes cause increased atresia, and hence oocyte deficiency. We have therefore assessed the level of sex chromosome asynapsis in XY, XXY and XYY females. X–Y pairing occurred in only 11% of XY and 15% of XYY pachytene oocytes, and the Y remained as a univalent in virtually all XXY oocytes. The frequency of pachytene cells with sex chromosome asynapsis decreased as pachytene progressed, suggesting that these cells were indeed being preferentially eliminated. Significantly, protection from atresia appeared to be afforded by self-synapsis of univalents as well as by normal bivalent synapsis. The relative fertility of XY, XXY and XYY females will be discussed in the light of these observations.

Multiple intestinal neoplasia (*Min*) in the mouse: developmental and genetical aspects

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Mice heterozygous for the *Min* mutation (multiple intestinal neoplasia) develop numerous spontaneous intestinal tumors. The tumors from B6 *Min*/+ mice are generally adenomas comprised of undifferentiated cells. Small foci of differentiated enterocytes, enteroendocrine cells and Paneth cells are found within the tumours. As judged by characteristic cellular markers, these differentiated cells are appropriate for the position along the

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intestinal tract. Other inbred strains carry alleles at loci unlinked to *Min* that modify the action of *Min*. On hybrid backgrounds the number of tumors is greatly reduced and *Min*/+mice can survive for over 300 days. A backcross analysis indicates that there is a small number of dominant modifier loci unlinked to *Min*. *Min* has been mapped to mouse chromosome 18 by linkage to Grl-1, Fgfa and MCC. This is in the region of the mouse homologue to human APC gene.

Structure and expression pattern of the mouse anti-Müllerian hormone gene

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Anti-Müllerian hormone (*Amh*) plays a role in the sexual differentiation of male embryos and is also found postnatally in the female. In order to learn more about the regulation and function of the gene in embryonic and postnatal development it was essential to clone the mouse gene. We have isolated a cDNA corresponding to *Amh* from a 14·5 days *post coitum* fetal testis library. A mouse genomic fragment containing the entire coding region of *Amh* was identified and sequenced. The structure of the gene and the predicted amino-acid sequence are highly homologous to that of the human and bovine *Amh* genes. We have established the pattern of *Amh* expression in both sexes by *in situ* hybridization. Correlation of the obtained pattern with cellular events occurring in gonadal development allowed us to speculate on its function and regulation.

LCR/MEL: a versatile system for high-level expression of heterologous genes in erythroid cells

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We have used the human globin locus control region (LCR) in assembling an expression system capable of copy number dependent, integration position independent expression of heterologous genes and cDNAs at high levels in murine erythroleukaemia (MEL) cells. Insertion of cDNAs between the β-globin promoter and a region of the β-globin gene containing the large (second) intron, then placing this expression cassette downstream of the LCR, enables expression of the cDNAs at levels comparable to those of murine β-globin in MEL cells. Linkage of heterologous genes containing intron sequences to the LCR and β-globin promoter enables expression of these genes to similar levels. We have demonstrated that after induction of terminal differentiation, MEL cells are capable of secreting heterologous proteins over a prolonged period.

Characterization of a novel gene expressed in a segmental pattern during the development of the vertebrate hindbrain

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A series of bulges, the rhombomeres, appear during the development of the hindbrain in all vertebrates. Studies at the cellular level have shown that these repeated structures are compartments of cells and that they correlate with a segmental organisation of neurons in this region of the nervous system. These findings raise the question of the molecular basis of hindbrain segmentation. A zinc-finger gene, *Krox-20*, is a promising candidate for a gene involved in the formation of segments, since it is expressed in two alternating rhombomeres, a pattern that

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correlates with cellular properties that underlie rhombomere boundary formation. A detailed analysis of the function of *Krox-20* and of molecular mechanisms of hindbrain segmentation will require the identification of further genes potentially involved in rhombomere formation. Towards this end we have used a PCR-based method to clone cDNAs from the mouse hindbrain and have screened these for segmental expression by *in situ* hybridization. By this approach we have identified a novel gene that is expressed in two alternating rhombomeres. We will present studies of the expression of this gene and discuss its potential function in hindbrain segmentation.

Expression of human class I MHC and β2 microglobulin molecules in transgenic mice

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We have produced and analysed several mouse lines transgenic for the human major histocompatibility complex class I gene *HLA-A2.1*. Previously, we have shown that in transgenic adults the human protein, in association with mouse β2-microglobulin (β2m), is expressed normally and is functional in all respects except in stimulating an HLA-restricted cytotoxic T cell (CTL) response. When human β2m is introduced into the HLA transgenic line, cell surface expression of the human molecule in the adult mouse is increased to about 2 times that of the endogenous mouse H-2 molecule. However, there is still a lack of HLA-restricted CTLs. Immunocytochemical analysis of the fetal and neonatal thymus demonstrates that HLA is expressed in the epithelial cells of the cortex, where thymic education is thought to take place, only 13 days after H-2 is first expressed there. We propose that this developmental time lag in HLA expression leads to a defect in thymic learning and is at least partly responsible for the absence of HLA restriction seen in these mice.

A novel gene isolated downstream from the mouse β_2-*microglobulin* gene

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In an attempt to find novel regulatory elements of the mouse $\beta_2 m$ gene, by analysing DNase I hypersensitive (DH) sites, we found a total of five DH sites within the vicinity of the $\beta_2 m$ gene, one within the promoter region and the remaining four DH sites located 5–13 kb downstream of the gene. Our existing $\beta_2 m$ cosmid clone lacks some of these downstream DH sites. Using a 3′ end probe we isolated a cosmid clone which contained these four DH sites. Interestingly, around this region there are a number of rare-cutter restriction enzyme sites, suggesting the presence of CpG islands, and therefore possibly a gene, in this area. To test this a λgt10 cDNA library (from Con A spleen blasts) was screened (in the presence of competitor DNA) with a 9 kb fragment containing these four DH sites. Six cDNA clones have been isolated. We are currently sequencing these clones and studying their pattern of tissue expression.

Koala, *Koa*, is associated with an inversion on mouse chromosome 15

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Koala, *Koa*, was found among the progeny of a C3H/HeH male mouse treated with (3·0 + 3·0) Gy X-rays 24 h apart, such that the affected germ cell stage was spermatogonial stem cells. *Koa*/+ have a bushy muzzle and the

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pinna is densely covered with hair; in addition to these features *Koa/Koa* have open eyelids at birth and are smaller than their *Koa*/+ or +/+ litter mates. Genetic studies show that *Koa* is tightly linked to *Ca*, caracul, on chromosome 15 (0/400 recombinants) and suppresses recombination between *Ca* and *bt*, belted.

Cytogenetic investigations have been carried out and suggest that a paracentric inversion has occurred in the distal half of chromosome 15. Meiotic analyses of synaptonemal complex preparations showed part paired inversion loops in 8% of cells and mitotic studies of G-banded chromosomes have revealed that *Koa* is associated with an inversion extending from 15D2 to 15F. Another mutation *Eh*, hairy ears, affecting the amount of hair on the pinna had been described earlier and had also been shown to be associated with a paracentric inversion extending from 15D2 to near the end of chromosome 15 [Davisson *et al.* (1990) *Genet. Res.* **56**, 167–168]. No recombination has been found between *Koa* and *Eh* in 422 mice. Thus there are now two mutations, *Koa* and *Eh* which affect the amount of hair on the pinna and are associated with paracentric inversions with similar breakpoints on chromosome 15.

Amplification of a region of the beta-globin gene from the DNA of single human blastomeres

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Preimplantation diagnosis is a powerful new technique already being utilized in clinical practice for the sexing of embryos prior to their replacement in the mother. During this procedure embryos are biopsied by the removal of a single blastomere at the 4 to 8-cell stage and a short section from a Y chromosome specific repeat sequence is amplified up to 10^9 times in a few hours using the polymerase chain reaction (PCR) [Handyside *et al.* (1989) *Lancet* **1**, 347–349]. To extend preimplantation diagnosis to the analysis of genetic diseases involving mutations within a single gene it would be necessary to demonstrate that highly reliable amplification of a unique locus from the DNA of a single human blastomere was possible.

In this study, we have used a region of the human β globin gene carrying the sickle cell locus as our target sequence for PCR. Using standardized conditions of sample collection and amplification, we have compared the variability in positive amplification signals achieved using DNA from individual human blastomeres with that achieved using DNA from other single human somatic cells or groups of cells. Following visualization of chromatin using a fluorescent chromophore, we have also compared amplification rates in blastomeres with differing nuclear staining patterns. Successful amplification was achieved more frequently with DNA from intact embryos between the one cell and blastocyst stages (100%), single cumulus cells (83%), oocytes which had failed to fertilize and polar bodies (82%) than from single blastomeres disaggregated from intact embryos and treated in an identical manner (45%). Exposure of embryos and blastomeres to the nuclear chromophore. Diamidino-phenyl-indole (DAPI) did not interfere significantly with the amplification of the β globin gene fragment. The DAPI staining pattern revealed considerable interblastomere variation, but no clear correlation between staining pattern and successful amplification was observed. The reasons for this unreliable amplification of DNA from single blastomeres are unclear but the results have important implications for preimplantation diagnosis of genetic disease.

A mouse stock with 38 chromosomes nullisomic for the centromere of chromosome 7 and the proximal and distal telomeres of chromosomes 7 and 15, respectively

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From the reciprocal translocation T(7;15) with presumed breaks in 7A1 and 15F3, induced in late spermatids by acrylamide treatment (5 × 50 mg/kg) of male (102/E1 × C3H/E1)F1 mice, a breeding stock with 38 chromosomes, homozygous for the long marker 15^7 and lacking the short 7^{15} marker, was established. The outcrosses of the original heterozygous, semisterile female generated among a total of 15 males analysed 9

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tertiary trisomic as well as 3 monosomic males for the short marker 7^{15}. The tertiary monosomic males had reduced litters of an average of $4{\cdot}5 \pm 1{\cdot}9$ and reduced testis weight. Males and females with 38 chromosomes, homozygous for the long marker 15^{7}, had normal litter size, when crossed inter se or outcrossed to chromosomally normal partners. At weaning the homozygous mice had the same body weight as the chromosomally normal mice of the same age, *in situ* hybridization with a centromere and a telomere DNA-probe showed off appropriate signals in the long 15^{7} and the short 7^{15} marker, indicating that both were fully intact chromosomes. On the other hand, the long marker showed a bright centromere signal at the fusion site, indicating that the breakpoint on chromosome 7 is within the pericentric heterochromatic block presumably immediately below the centromere. This is the first report of a homozygous mouse translocation stock with 38 chromosomes derived from a tertiary monosomy.

HYA expression in mouse B-cell clones

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Cloned B-cell lines from a female T16H/XSxr mouse in which *Tdy* expression was suppressed due to X inactivation and from a male X/XSxr mouse, both of the (kxb)F1 haplotype, were examined for H–Y expression. This was determined both by their ability to act as targets for H-2^{k} and H-2^{b}-restricted H–Y-specific cytotoxic T cells and by their ability to stimulate the proliferation of H-$2K^{k}$, H-$2D^{b}$ (class I) and A^{b} (class II)-restricted T-cell clones. In B-cell clones from the T16H/XSxr mouse, expression of H-Y/D^{b} exhibited partial X inactivation and only a proportion ($\simeq 30\%$) of the cells were targets for or stimulated H-$2D^{b}$-restricted H-Y-specific T cells. In contrast, H-Y epitopes restricted by H-2^{k} (H-Y/K^{k}, H-Y/D^{k}) and A^{b} (H-Y/A^{b}) exhibited no X inactivation. Furthermore, no inactivation of H-Y/D^{b}, H-Y/A^{b} or H-Y^{k} was observed in the male X/XSxr mouse. These results indicate that the T16H/XSxr female is a mosaic, as a result of the variable spread of X inactivation into the *Sxr* region. They further suggest that the H-Y antigen recognized in association with H-2^{k} and H-$2D^{b}$ class I molecules and A^{b} class II molecules may be the product of more than one gene. Methods being currently employed to clone *Hya* gene(s) will be discussed.

Targeted mutagenesis of the hexosaminidase *A* gene in murine embryonic stem cells

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Tay–Sachs disease is an autosomal recessive disorder caused by mutation in the β-*N*-acetylhexosaminidase α-chain (*Hex A*) gene. In affected infants the age of onset is within the first year of life leading to death in early childhood after progressive motor and mental deterioration. At present there is no animal model available to study this disease and most research is limited to the use of fibroblast cells obtained from patients. We have constructed a positive/negative selection vector containing a 4·3 kb portion of the *Hex A* genomic sequence interrupted in exon 8 by insertion of the *neo* gene (G418 resistance) and flanked at the 5′ end by the *HSVtk* gene (gancyclovir sensitivity). Murine ES cells have been transfected with this vector and homologous recombinant clones obtained. These will be used to generate the germ-line chimaeras required for the production of a mouse model of Tay–Sachs disease.

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Hyperphenylalaninemic mutants of the mouse: closer approaches to models for human PKU

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Among an initial set of recessive mouse mutations induced by ethyl-nitrosourea, one was shown to affect the activity of phenylalanine hydroxylase (PAH) and to map at or near the *Pah* locus [McDonald *et al.* (1990) *Proc. natn Acad. Sci. USA* **87**, 1965]. However, HPH-5 mice, homozygous for this mutation, showed no elevation of ambient serum phenylalanine (PHE), nor the effects upon fetal and neonatal development and adult pigmentation that are characteristic of human phenyl-ketonuria (PKU). A locus-specific screen has elicited two new mutant alleles at the *Pah* locus, each of which in homozygous form leads to elevated serum PHE, growth retardation, loss of pigmentation, and a PHE-dependent maternal effect.

Experimental prevention of neural tube defects in the curly tail mouse embryo

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In the curly tail (*ct*) mouse mutant, 60% of homozygous embryos develop neural tube defects (NTD) as a result of delayed closure of the posterior neuropore (PNP) in the lower spinal region. Increased ventral curvature of the caudal region is found in affected *ct/ct* embryos and there is a direct relationship between the extent of delayed PNP closure and the degree of ventral curvature. By inserting the tip of a human eyelash into the hindgut lumen, to act as a brace and provide mechanical support for the caudal region, we experimentally reduced the ventral curvature in *ct/ct* embryos and, concomitantly, found that the development of NTD was prevented. The increased curvature in affected *ct/ct* embryos may be due to imbalance of growth rates between dorsal and ventral tissues as a result of a cell-type specific proliferation defect. Evidence to support this hypothesis comes from an experiment in which we re-balance the growth rates, by *in vitro* growth retardation of the embryo. This resulted in significantly reduced ventral curvature and neurulation was normalized. We conclude, therefore, that a growth imbalance results from action of the *ct* gene and is responsible for the development of spinal NTD by increasing ventral curvature in affected embryos.

Chromobox-containing genes and the molecular mechanisms of epigenetic inheritance

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Modifiers of position-effect-variegation (PEV) in *Drosophila* encode proteins that are thought to modify chromatin, rendering it heritably changed in its expressibility. We have used a sequence motif, termed chromobox, that is shared between a suppressor of PEV, Heterochromatin protein 1 (HP1), and a repressor of homeotic genes, *Polycomb* (*Pc*), to clone mammalian and mealy bug homologues of both HP1 and *Pc*. The characterization of the genes and proteins is described. We also discuss the role of these proteins in epigenetic (yet heritable) changes in gene expressibility.

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Regulation of expression of carbonic anhydrase 1

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Three homologous carbonic anhydrase genes *CA1*, *CA2* and *CA3* are clustered within 200 kb on the long arm of chromosome 8 in humans [Lowe *et al.* (1991) *Genomics* **10**]. These genes each have a characteristic pattern of expression. *CA2* is ubiquitously expressed while *CA3* expression is confined to tissues of mesodermal origin such as muscle and notochord. *CA1* is unique in that it has two promoters separated by a large 36 kb intron [Lowe *et al.* (1990) *Gene* **93**, 277–283]. The distal promoter is active in erythroid cells and the proximal promoter in colon tissue; transcription gives rise to two alternative mRNA species differing in their 5′ leader sequences. The erythroid transcription unit is flanked by consensus binding sites for the erythroid-specific transcription factor GATA-1 and a number of constitutive factors [Brady *et al.* (1989) *FEBS Lett.* **257**, 451–456]. We have identified tissue-specific DNaseI hypersensitive sites around the *CA1* gene using cultured cells as a source of chromosomal DNA. Chimaeric genes were formed by linking *CA1* erythroid promoter fragments to a reporter gene. Transcription from the exogenous *CA1* promoter is specific to erythroid cells including the foetal-like K562 erythroid cells which do not normally express the endogenous *CA1* gene. This transcriptional activity is associated with the generation of hypersensitive sites in the transfected *CA1* promoter.

Mapping rhombotin gene expression in the CNS of transgenic mice using *lacZ* as a reporter gene

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The rhombotin gene was identified as a transcription unit adjacent to a T-cell tumour-associated chromosomal translocation, and is believed to be involved in tumourigenesis. It has two transcriptional promoters, designated 1 and 1a, which give rise to two distinct mRNA species encoding essentially identical proteins. To begin investigating the *in vivo* regulation of rhombotin expression, transgenic mice were generated expressing a rhombotin promoter 1–*lacZ* fusion construct. In a previous study, histochemical (HC) staining for β-galactosidase (β-gal) in these mice revealed expression in various regions of the developing CNS [*Nature* (1990) **344**, 158]. *In situ* hybridization in non-transgenic mice of equivalent ages, showed rhombotin to have a wider distribution of expression in the CNS, suggesting that additional sites of expression occur when both promoters are available. To compare directly the expression from the two rhombotin promoters, we have generated transgenic mice expressing a rhombotin promoter 1a-*lacZ* construct. Histochemical and immunohistochemical (IHC) procedures have been used to analyse β-gal expression in transgenic mice at a number of developmental and adult stages. We have found that IHC detection of β-gal shows greater sensitivity than HC staining and allows visualization of cell morphology, enabling not only distinction of neurons and glia but also identification of subpopulations of these cells. The use of immunohistochemistry to characterize further the expression of β-gal in the CNS of these transgenic mice has revealed that promoters 1 and 1a have distinct spatial and temporal patterns of activity. This observation suggests that the two promoters function to allow independent regulation of rhombotin protein expression in different CNS cell populations.

Reconstruction of the human β-globin genomic locus in transgenic mice

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The human β-globin genomic locus spans a region of approximately 70 kb on chromosome 11, and comprises five functional genes arranged in the order in which they are expressed during development i.e. $^{5'}$-ε-^{G}γ-^{A}γ-δ-β-$^{3'}$. The ε-globin gene is expressed in the blood islands of the embryonic yolk sac, the γ-globin genes are active

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in liver in the foetal stage, and the δ- and β-genes are expressed in bone marrow in the adult stage. The entire locus is regulated by the locus control region (LCR), which in transgenic mice has been shown to confer high levels of tissue-specific, integration site-independent and copy number-dependent expression of a human β-globin gene.

We have reconstructed the human β-globin genomic locus containing the LCR and all five functional genes in their native configuration. This was achieved by annealing together two cosmids using complementary, single stranded oligonucleotide 'tails'. The resulting 70 kb DNA fragment was gel-purified and microinjected into fertilized mouse ova. Three transgenic mouse lines that appear to contain the intact transgene as a single copy have been established. A preliminary analysis of the developmental regulation of the human β-globin genomic domain in these transgenic lines will be presented.

Analysis of β-lactoglobulin gene expression in transgenic mice

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The major whey protein of sheep, β-lactoglobulin (BLG), is expressed specifically in the mammary gland in a developmentally regulated pattern. To identify the *cis*-acting DNA regions involved in the regulation of BLG expression, resected and internally deleted constructs were analysed in transgenic mice. Transgenes containing the proximal 406 bp promoter, the entire transcription unit and 1·9 kbp of 3′ flanking sequences were expressed specifically in the mammary gland during gestation and lactation. Expression was seen in all mice/lines and correlated with transgene copy number. Further resection of this region or deletion of most or all introns resulted in a reduced frequency of expression. From these data, and complementary data from BLG-hybrid transgenes, we propose that efficient BLG expression requires (at least) two distinct regulatory regions. Studies designed to analyse the chromatin structure of both the endogenous sheep gene and the BLG transgenes support this hypothesis.

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