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Evidence that testicular differentiation in the marsupial, *Monodelphis domestica*, begins, but does not proceed, *in utero*

P. J. BAKER, A. M. C. BURGESS AND U. MITTWOCH
Department of Anatomy, Faculty of Basic Medical Science, Queen Mary & Westfield College, Mile End Road, London E1 4NS, UK

The grey short-tailed opossum, *Monodelphis domestica*, is a South American marsupial in which the young are born 14·5 days after mating and have an average weight of 98·4 mg. We have studied the growth and histological appearance of the gonads in eight litters of embryos and eight neonatal litters. Two of the embryonic litters were judged to be postmature, since they had passed their expected date of birth and their body weights exceeded 110 mg. Signs of testis cord formation were first seen on the day before the expected birth. There was an abrupt increase in the growth rate of XY gonads after birth, but this was not seen in XX gonads. Mean gonadal volumes of XY individuals exceeded those of their XX litter mates in all neonate and in six out of eight embryonic litters examined. Growth as well as histological differentiation of XY gonads appeared to be blocked in postmature embryos, whereas the growth of XX gonads in postmature embryos was not affected. Since it is known that exogenous oestrogens feminize the gonads of genetic males in some species of marsupials, including *M. domestica*, we suggest that testicular differentiation may not be able to proceed in a maternal environment, and that the ability to develop hormonally functioning testes in such an environment could be a fundamental requirement for eutherian mammals that distinguishes them from marsupials.

Imprinting studies of the central region of mouse chromosome 7

JACKY BARR, JANET JONES, COLIN BEECHEY AND BRUCE CATTANACH
MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD

In humans, maternal and paternal 15q11–q13 deletions are associated with Angelman's Syndrome (AS) and Prader–Willi Syndrome (PWS) respectively. AS is also associated with paternal disomy and PWS with maternal disomy. These findings indicate 15q11–q13 is subject to imprinting. Studies with DNA markers have established 15q11–q13 is homologous with the central region of mouse chromosome 7. Imprinting phenomena comparable to PWS and AS should therefore be found in the mouse. Genetic complementation tests, using reciprocal translocations, have revealed imprinting effects associated with the proximal and distal regions of chromosome 7 but not the central region. We have now generated mice with a maternal duplication of the central region using the X-autosome rearrangement, Is(InX;7)1Ct. This duplication causes lethality within a week of birth, thus demonstrating an imprinting effect of the central region of chromosome 7. Such mice possibly provide a model for PWS. To generate a model for AS we have looked for postnatal effects associated with a paternal duplication for the region proximal to the T50H breakpoint on chromosome 7, this includes the central portion of the chromosome. The effects seen are reduced viability, small size and skeletal abnormalities, effects that could be consistent with AS. However, mice with paternal duplication of the region proximal to the T9H breakpoint were also found to exhibit these effects, suggesting that this region and not the central region, is responsible. Therefore a mouse model of AS has not yet been detected.

GABRB3, D15S9 and SNRPN map to human 15q11–13 and are therefore candidate loci for imprinting. Mice with maternal duplication for the central region of chromosome 7 or with paternal duplication for the larger regional proximal to the T50H breakpoint have therefore been investigated for expression differences at these

loci. *Snrpn* was not expressed in mice with the maternal duplication, suggesting the locus is imprinted. However, no evidence has yet been found that either *Gabrb-3* or D15S9 (*D7Hms1*) are subject to imprinting.

New insights into the man-mouse map of the X chromosome

YVONNE BOYD, STEVEN LAVAL, HELEN BLAIR, VIVIENNE REED AND GARETH MASLEN
Genetics Division, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, UK

In order to confirm and/or identify homologies between X-linked mouse mutants and human genetic disorders, we are currently extending the man-mouse comparative map of the X chromosome. Markers cloned in our laboratory and obtained from elsewhere have been positioned on the mouse X chromosome using an interspecific backcross panel and on the human X chromosome using a panel of well-characterized somatic cell hybrids.

Previous comparative mapping studies of the mouse and human X chromosomes have revealed the presence of five distinct homologous segments (Amar *et al.* (1988) *Genomics* **2**, 220–230; Searle *et al.* (1989) *Ann. Hum. Genet.* **53**, 89–140). We have identified a further two homologous blocks by mapping conserved sequences on the human and mouse X chromosomes. One block is associated with the centromere of the mouse X chromosome and the extreme proximal region of the X chromosome short arm. The second newly recognised region of homology is between a slightly more distal region of the X chromosome short arm and the distal region of the mouse X chromosome. Thus the region of the human X chromosome short arm which extends from the centromere to the CYBB locus is contained in three different regions of the mouse X chromosome.

Microsatellite markers for lethal developmental mutations in the mouse

ANDREW COPP AND IACOB CHECIU[1]
ICRF Developmental Biology Unit, Department of Zoology, Oxford and [1] Laboratory of Embryology and Teratology, Timisoara, Romania

Many developmental mutations in the mouse, which serve as models of human birth defects, are embryonic lethals in homozygous form. The loop-tail (*Lp*) mutant is an example; homozygous mutant embryos develop severe defects in which the neural tube is open from midbrain to tail. This defect resembles the human malformation cranio-rachischisis. To study the developmental mechanism that underlies the *Lp* defect, it is necessary to use mating between viable heterozygotes and then to identify the *Lp*/*Lp* embryos from their *Lp*/+ and +/+ littermates prior to the onset of morphological abnormalities. Here we describe the use of closely linked microsatellite DNA polymorphisms to identify embryo genotype. PCR assays are performed on DNA extracted from small embryonic biopsies. We will describe the use of this method in studies of the developmental effects of the *Lp* mutation.

An *SRY*-related gene expressed during spermatogenesis in the mouse encodes a sequence-specific DNA binding protein

PAUL DENNY, SALLY SWIFT, FRANCES CONNOR AND ALAN ASHWORTH
Chester Beatty Laboratories, The Institute of Cancer Research, Fulham Rd, London SW3 6JB, UK

SRY, the testis-determining gene, encodes a member of a family of DNA-binding proteins characterized by an amino-acid sequence motif known as the HMG box. This motif has been found in several transcription factors, such as the T cell-specific factors TCF-1 and TCF-1α, and the RNA polymerase I transcription factor UBF. Using degenerate primers and the polymerase chain reaction, we isolated *SRY*-related cDNAs from adult murine testis RNA. One of these, *Sox-5*, is derived from a testis-enriched mRNA which encodes a 43 kDa HMG-box protein with similarities to transcription-activating proteins. Anti-Sox-5 antibody was used to analyse expression of Sox-5 in pre-pubertal testis and in fractionated spermatogenic cells. Sox-5 is restricted to post-meiotic germ cells, being found at highest levels in round spermatids. Sox-5 is a DNA-binding protein and binding-site

selection assays suggest that it can bind specifically to oligonucleotides containing the consensus motif AACAAT. Sry can also bind to this motif, indicating that the Sry family may have overlapping sequence specificities.

This work suggests that Sox-5 may be an important regulator of gene expression in post-meiotic germ cells. Currently we are attempting to identify possible downstream targets of the *Sox-5* gene product.

Functional analysis of the human β-globin locus in transgenic mice

NIALL DILLON, JOHN STROUBOULIS, MEERA BERRY, PETER FRASER, SJAAK PHILIPSEN, JAMES ELLIS AND FRANK GROSVELD

Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

The human β-globin locus consists of five developmentally regulated genes, 5′εγγδβ3′, which are arranged in the order in which they are expressed during development. High level expression of all of the genes is driven by the locus control region (LCR) which consists of a series of four hypersensitive sites located upstream from the ε gene. The LCR potentiates expression of linked genes in transgenic mice which is position independent and copy-number dependent.

The developmental regulation of the locus has been studied using single and multi-gene constructs and by introducing the complete locus into transgenic mice. Current evidence suggests that developmental specificity is shared between the LCR and the individual promoters and that switching is fine-tuned by polar competition between the genes for activation by the LCR.

Variable expression of sheep β-lactoglobulin within a line of transgenic mice

KENNETH W. DOBIE[1,2] AND MARGARET McCLENAGHAN[1]

[1] AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS; [2] AFRC Centre for Genome Research, The University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JQ

We are examining factors involved in the stable expression of the sheep milk protein, β-lactoglobulin (BLG), in the mammary gland of transgenic mice. Generally, lines of mice carrying this transgene express the protein at consistently high levels; however, instability of expression has been observed in one established line (line 7), resulting in up to 6-fold variation of BLG levels in these mice. In an initial experiment, BLG levels in milk were measured at the same point (day 11) in consecutive lactation cycles and were shown to be stable within the individual mouse. Subsequently, the effect of genetic background was examined; the founder transgenic line was generated from C57BL/6 × CBA cross. It was found that backcrossing into each strain for 3 generations did not affect the variability within line 7. We conclude that something other than a strain effect is causing inconsistent expression of the transgene within this line.

Deletion mapping of the Sxr region of the mouse Y chromosome

INGRID EHRMANN, DIANE SCOTT, TOM KING, DERRY ROOPENIAN, EVA EICHER, AND ELIZABETH SIMPSON

Transplantation Biology, CRC and Jackson Labs, Maine

The gene encoding the male specific minor histocompatibility antigen, H-Y, has been localized to the region of the Y chromosome deleted in Sxr^b mice. In order to construct a physical map of the ΔSxr^b region of the mouse Y chromosome, Abelson transformed B cells from a (B10 × CBA) F_1 $XOSxr^a$ mouse were subjected to mutagenesis and tested for expression of the H-Y antigen. H-Y variant cell lines were selected with CTL clones specific for $H\text{-}YD^k$ or $H\text{-}YD^b$. The fourteen variant cell lines which were obtained were tested for the presence of different H-Y epitopes using a T cell hybridoma ($H\text{-}YK^k$ specific), a T cell clone ($H\text{-}YIa^b$ specific) and bulk cytotoxic T cells ($H\text{-}YD^b$ and $H + YD^k$). The Abelson variant cell liens express some, but not all of the H-Y

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epitopes allowing construction of a tentative map of this region. Additionally, it appears that there are at least four independently expressed H-Y epitopes.

Influence of a new MTV-encoded superantigen in T cell development

SUE FAIRCHILD, OLIVER ROSENWASSER AND KYUHEI TOMONARI
Transplantation Biology Section, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

Mouse mammary tumour virus (MMTV) superantigens in association with MHC class II molecules delete immature T cells bearing certain Vβ chains in the thymus. These superantigens are the products of the open reading frame in the 3′ long terminal repeat (LTR) of MTVs. We have identified a new MTV in NZW mice which deletes Bβ3$^+$, Vβ6$^+$, Vβ8.1$^+$ and Vβ9$^+$ T cells. Deletion of these T cells by one MTV is unusual, as previous reports have shown that deletion of this set of cells is mediated by two different MTVs. We have examined both sequence and function to elucidate its novel specificity.

Cloning and mapping of *Gli2* – a candidate gene for dominant hemimelia

MARTIN FARRALL, GARRY MORLEY, KATE SUTHERLAND, MARIE-GENEVIEVE MATTEI[1], GRAHAM KAY[2], DAVID HUGHES[3] AND ROBERT HILL[3]
MRC Molecular Medicine Group, RPMS, Du Cane Raod, London, W12 0NN; [1] Centre de Génétique Médicale, Hôpital d'Enfants de la Timone, Marseille; [2] Section of Comparative Biology, MRC Clinical Research Centre, Harrow; [3] MRC Human Genetics Unit, Western General Hospital, Edinburgh

The GLI gene family of Krüppel-related zinc-finger-encoding genes includes the mammalian genes GLI, GLI2 and GLI3; similar zinc-fingers are found in the *Drosophila* segment polarity gene *ci*^{D} and the *C. elegans* sex-determining gene *tra-1*. GLI3 is disrupted in Greig cephalopolysyndactyly syndrome (GCPS) which results from abnormal craniofacial and limb development. The murine homologous mutant extra-toes (Xt) is caused by partial deletion of *Gli3*.

As part of a strategy to analyze the other GLI family members during mammalian development, we have cloned the murine *Gli2* gene which shows multiple regions of protein similarity to GLI3. We have localized *Gli2* to mouse chromosome 1, very close to the mouse mutation, dominant hemimelia (Dh). We have mapped *Gli2* in a pre-existing cross segregating for Dh and have detected no recombination in 8 meioses that are recombinant with either the marker EN-1 or Emv-17 which are tightly linked (1.8 cM) and flank Dh. We are currently searching for mutations in *Gli2* in Dh mice.

Irradiation hybrids from chromosome 9q34 which span the interval containing the *TSC1* gene

JUDE FITZGIBBON, JOSEPH NAHMIAS, KAREN WOODWARD, DARREN GRIFFIN, NICK HORNIGOLD, SUSAN POVEY AND JONATHAN WOLFE
The Galton Laboratory, Wolfson House, 4 Stephenson Way, London, NW1 2HE, UK

The irradiation fusion approach of Goss and Harris (*Nature* **255**, 680–684, 1975) was used to generate hybrid lines retaining small fragments of human chromosome 9q in order to enrich for the region containing the gene for *tuberous sclerosis*, *TSC1*, on 9q34. The source of chromosome 9q material was a Chinese Hamster–Human somatic cell hybrid line, 64063a12, retaining the long-arm of chromosome 9 as its only human material. 39 independent radiation reduced hybrid lines were analysed for the retention of 25 marker loci, previously assigned to the chromosome arm. Fragment hybrids, particularly those retaining markers close to the TSC1 interval on 9q34, were also characterized by fluorescent *in situ* hybridization (FISH), to determine the number, distribution and stability of the fragments retained in each cell. Several hybrids which retain markers near the *TSC1* gene but with little or no other human material have been identified These have been used to construct a tentative deletion map of the region around the *TSC1* gene and have provided a good resource for cloning DNa from the area.

Novel cytogenetic techniques diagnosing genetic disease in preimplantation human embryos

D. K. GRIFFIN[1], A. H. HANDYSIDE[2], L. J. WILTON[3], R. M. L. WINSTON[2] AND J. D. A. DELHANTY[1]

[1] The Galton Laboratory, Dept of Genetics and Biometry, UCL, 4 Stephenson Way, London, NW1; [2] RPMS, Hammersmith Hospital, DuCane Road, London, W12; [3] Institute of Zoology, Regent's Park, London, NW1

We have used four approaches for 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 and could lead to males being diagnosed as females because of failure of hybridization (Y probe) or because of tetraploid nuclei (X probe). 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 but it takes 24 h to perform alone and thus does not allow biopsy, spreading, diagnosis and transfer within the 24 h period. The fourth approach is a rapid dual FISH sexing strategy taking 6–7 h and giving more reliable results than the above approaches. It is this method which has been put into clinical practice.

Eleven patients, each carrying X-linked disorders have had preimplantation diagnosis of sex in a total of 15 treatment cycles. In the first eight cycles biopsy was on day 2 post-fertilization and cells were allowed to divide overnight prior to FISH diagnosis. In the final seven, biopsy, spreading, FISH diagnosis and transfer were all on day 3. It is the latter strategy which has proven to be the most effective in terms of number of nuclei positively diagnosed. There is currently one ongoing pregnancy from these treatment cycles. In some cases, X-chromosome signals in female embryos display a pattern which may suggest that X-inactivation is occurring during early cleavage divisions in humans.

FISH can be used to distinguish whole chromosomes or specific regions of chromosomes. We have hence made progress towards the possible screening of chromosomal anomalies at the preimplantation stage using similar strategies.

DNA polymorphisms in the lactase gene

C. B. HARVEY, P. H. JOHNSON AND D. M. SWALLOW

MRC Human Biochemical Genetics Unit, University College London, The Galton Laboratory, 4 Stephenson Way, London, NW1 2HE

Intestinal lactase is responsible for digestion of dietary lactose. In the majority of the world's population lactase activity does not persist into adult life but declines after weaning. In Northern Europe however, lactase activity persists into adulthood in most individuals. This polymorphism is genetically determined but the molecular basis is not yet known. Population studies [Ho *et al.* (1982) *Am. J. Hum. Genet.* **34**, 650–657; Flatz (1984) *Am. J. Hum. Genet.* **36**, 306–310] and linkage analysis in families [Sebastio *et al.* (1990) *Ped. Res.* **27**, 532] suggest that the relevant genetic element(s) are *cis*-acting, i.e. they reside within or close to the lactase gene. Sequence analysis of the promoter region and the cDNA has shown single base variations but none of these are obviously associated with lactase persistence or non-persistence. However recent evidence suggests that there is more than one type of lactase non-persistence.

To make the LCT gene more informative for such studies we have been using the single strand conformation polymorphism (SSCP) technique [Orita (1989) *Genomics* **5**, 874] and denaturing gradient gel electrophoresis (DGGE) [Myers (1987) *Methods in Enzymol.* **65**, 358]. Both techniques were adapted to use silver staining to detect the DNA.

Seven distinct SSCP and five DGGE patterns have so far been found in 103 unrelated individuals. Mendelian inheritance has been demonstrated and the allele frequencies determined in two different population of European decent (from France and Utah).

The haplotypes (i.e. combination of alleles on each parental chromosome) are being analysed and the observed frequencies of the different haplotypes indicate that there is significant association between the alleles.

These newly described polymorphisms can now be used to determine whether specific haplotypes are associated with the lactase persistence phenotype, by analysis of families and also of individuals characterized with respect to their lactase persistence status.

Identification of four genomic loci, highly related to CK-2α cDNA and characterization of a CK-2α pseudogene within the mouse genome

BRIGITTE BOLDYREFF, KLAUS WEHR, ROLAND HECHT AND OLAF-GEORG ISSINGER
Institute für Humangenetik, Universität des Saarlandes, D-6650 Homburg/Saar, Germany

Casein kinase-2 (CK-2) is a widely distributed and highly conserved serine/Threonine protein kinase which is closely associated with proliferation. cDNAs for all three subunits have been isolated and sequenced from several organisms. Southern blot analysis using highly related copies of the CK-2α gene encompasses a large genomic region with several exons. By *in situ* hybridization two chromosomal loci for CK-2α have been identified. Using the coding region of the human CK-2α cDNA as a probe for screening a genomic mouse library, positive clones representing four different genomic loci were isolated. Partial DNA sequences of these loci encompassing the first 120 nucleotides of the putative coding region are reported. Oee positive clone was further analysed by sequencing completely. This clone displays all characteristics of a processed pseudogene, e.g. lack of introns, several nucleotide insertions and deletions. In its 3′ region it contains a large purine rich stretch which consists of (CCTT) and (CT) repeats. In the 5′ region three (CCCCCT) and several (CT) repeats are found. Presumably these stretches were involved in chromosomal integration of an intronless mRNA intermediate of CK-2α.

Transgenic rescue of lethal albino mice

D. D. KELSEY, S. RUPPERT[1], A. SCHEDL, F. BEERMANN[2], E. SCHMIDT AND G. SCHÜTZ
Institute for Cell and Tumor Biology, German Cancer Research Center, 6900 Heidelberg, Germany; [1] HHMI, Dept. of Molecular and Cell Biology, University of California, Berkeley CA 94720, USA; [2] ISERC, CH-1066 Epalinges s/Lausanne, Switzerland

Mice homozygous for deletions such as *c14CoS* around the *c* locus die within a few hours of birth. They present a pleiotropic phenotype in the liver and kidney that includes severe reductions in the expression of enzymes of gluconeogenesis and the urea cycle; reduced expression of tissue-specific transcription factors; and induction of transcripts associated with detoxification, growth arrest and DNA damage. By positional cloning, we identified that the gene encoding fumarylacetoacetate hydrolase (FAH) is disrupted by the *c14CoS* deletion. FAH catalyses the final reaction in the metabolism of tyrosine, and inherited FAH deficiency in man predisposes to a severe liver failure of infants, hereditary tyrosinaemia type I. By creating lines of transgenic mice carrying an FAH cDNA, we have obtained complete phenotypic rescue of *c14CoS* homozygotes, which establishes FAH deficiency as the underlying cause of the phenotype. Present investigations are aimed at addressing the mechanisms by which potentially toxic tyrosine metabolites accumulating in the absence of FAH could bring about such profound changes in gene expression.

Motch A and *Motch B* – two mouse *Notch* homologues

MICHAEL LARDELLI AND URBAN LENDAHL
Department of Molecular Genetics, Medical Nobel Institute, Karolinska Institute, S-104 01 Stockholm, Sweden

Notch is one of the neurogenic genes of *Drosophila* controlling the decision between ectodermal and neural fate for cells in the early embryo. We have used a PCR-based strategy to identify cDNA clones representing two mouse homologues to the Drosophila *Notch* gene. One of the genes, *Motch A*, is the mouse orthologue to the previously cloned *Notch* genes in *Xenopus*, rat and man. The other gene, *Motch B*, is more distantly related to the characterized vertebrate *Notch* genes, but equally similar to the *Drosophila Notch* as is *Motch A*, and therefore represents a distinct branch of a vertebrate *Notch* gene family. The *Motch A* and *Motch B* branches probably arose by gene duplication early in vertebrate evolution. Both *Motch A* and *Motch B* retain the same principal structure, encode a mRNA of approximately 10 kb, are expressed during mouse embryogenesis, and have largely overlapping expression patterns in adult tissues. We discuss possible consequences of expressing two closely related *Notch* homologues in the same set of tissues in terms of cell-cell signalling and differentiation control.

Mouse tasting genes and some speculations about homologies

IAN LUSH, NICK HORNIGOLD AND JONATHAN STOYE
Genetics and Biometry, UCL and NIMR, Mill Hill

Soa (sucrose octoacetate bitterness-tasting) is now known to be a member of the cluster of bitterness-tasting genes on MMU6. The probable order of these genes is *Cyx–Qui–Rua–Glb–Soa*.

Sac (Sweetness-tasting) has been mapped to the distal end of MMU4. Our hypothesis is that all the bitterness and sweetness tasting genes have a common evolutionary origin. If MMU4 and MMU6 arose from a single chromosome by a tetraploidization event in an early vertebrate ancestor, one might expect to find other paralogous genes shared by these two chromosomes. We have found six such examples, plus some other candidates which are rather speculative. Homologies between segments of chromosomes in Man and mouse are well documented. Thus there is extensive homology between HSA1p and distal MMU4, and between HSA12p and distal MMU6. It is therefore arguable that these human and mouse chromosome segments all had a common origin. All the homologous genes shared by two or more of these chromosome segments have been tabulated. In total they present considerable support for our hypothesis. In the table below, the numbers of each of the

HSA		MMU		
1p	12p	4	6	No.
●	●	●	●	3
○	●	●	●	0
●	○	●	●	1
●	●	○	●	4
●	●	●	○	1
●	●	○	○	5
●	○	●	○	19
●	○	○	●	3
○	●	●	○	1
○	●	○	●	9
○	○	●	●	2

eleven possible combinations of homologous genes on the four chromosome segments are shown. A filled circle indicates the presence of a gene, an empty circle indicates the absence of a gene. It is particularly noteworthy that three genes have homologues on all four chromosomes.

Analysis of some mutant alleles at the pink-eyed dilution locus of the mouse

M. F. LYON[1], T. R. KING[2], Y. GONDO[3], J. M. GARDNER[3], Y. NAKATSU[3], E. M. EICHER[2] AND M. H. BRILLIANT[3]
[1] *MRC Radiobiology Unit, Chilton, Didcot, Oxon;* [2] *Jackson Laboratory, Bar Harbor, Maine, USA;* [3] *Institute for Cancer Research, Fox Chase Canter Center, Philadelphia, USA*

Many recessive mutant alleles at the pink-eyed dilution (*p*) locus on mouse chromosome 7 have arisen spontaneously, or in mutagenesis experiments. All alleles reduce pigmentation of the coat and usually also the eyes. Some alleles also affect reproduction, behaviour, or prenatal or neonatal viability. Complementation analysis of the various alleles has resulted in the linear mapping of at least four functional units. These involve (*a*) prenatal lethality, (*b*) male and female fertility, runting, and neuromuscular effects, (*c*) hypopigmentation and (*d*) cleft palate. Molecular analysis has shown that several alleles are deletions. Complementary DNA clones from the *p* locus have been obtained, and transcripts are missing or altered in some mutant alleles. The homologous human cDNA has been obtained and maps to the Prader–Willi–Angelman region of human 15q, and hence altered expression of this gene may be responsible for the reduced pigmentation seen in some affected individuals.

Targeting of domain deletions to the C-terminus of the mouse dystrophin gene

M. K. MACONOCHIE, A. J. GREENFIELD AND S. D. M. BROWN
Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK

Duchenne muscular dystrophy is an X-linked recessive myopathy caused by mutation in the dystrophin (DMD) gene. The *mdx* mouse mutant is due to mutation in the cognate murine gene (*Dmd*). Although in both cases the lesions result in dystrophin deficiency, man and mouse exhibit diverging pathologies. A better understanding of the function of the murine gene is required to further the utility of the mouse and the *mdx* mutant for studies and treatment of Duchenne muscular dystrophy.

Sequence homology delimits four putative functional domains in the dystrophin gene. In order to address the *in vivo* functional significance of such homologies and thereby delineate structure-function correlations, and to clarify the relationship between pathology and mutation, we are using homologous recombination in embryonic stem (ES) cells to direct mutations to the *Dmd* gene. We have targeted an exon at the junction of the triple-helical repeat domain and the cysteine-rich domain to incorporate an in-frame promoterless LacZ reporter gene. The mutation is designed to generate a stable transcript lacking the two terminal domains which should enable the function of these domains to be elucidated. Furthermore, the inclusion of the LacZ reporter may facilitate the monitoring of the temporal and spatial distribution of the mutant protein throughout development. Utilising a strategy of positive-negative selection together with analysis of individual clones by Southern hybridisation, we report here a targeting frequency of the dystrophin locus with this construct of approximately 1/1400 G418 resistant clones. We also describe *in vitro* differentiation studies of the targeted cell line which demonstrate that X-gal staining can be used to follow myogenesis and we are currently generating chimaeras for *in vivo* studies.

Hearing in mice with five different shaker-1 mutations

MARY T. MAHONY AND KAREN P. STEEL
MRC Institute of Hearing Research, University Park, Nottingham, NG7 2RD, UK

The development of cochlear responses in young shaker-1 mice has been investigated as part of a larger project to identify the shaker-1 gene and correlate its mutations with phenotypes. Both mice carrying the original spontaneously occurring shaker-1 gene and those carrying one of four new ENU-induced shaker-1 mutations were used in the study at 12, 15 and 20 days after birth (DAB). Cochlear responses were evoked using pure tone stimuli and recorded from the round window with a silver wire electrode. They consisted of cochlear microphonics (CM) and summating potentials (SP) both arising from sensory hair cells, and compound action potentials (CAPs) which represent the summed activity of the auditory nerve. A previous study of the original shaker-1 strain showed that homozygous mice began to develop some cochlear responses, but after 14 DAB these responses deteriorated until by 30 DAB they could only be obtained at very high intensities. Similar results were obtained in this study. The littermate controls of the new shaker-1 mutants showed normal development of cochlear responses, with CMs and SPs present at 12 DAB and CAPs appearing by 15 DAB. The thresholds for these response improved as the mice matured. However, the homozygous mice carrying ENU-induced mutations showed no responses at any of the three ages studied and in addition they appeared to have a more severe vestibular dysfunction at an early age (12 days), which later manifests as head-bobbing and circling. The possibility of shaker-1 being a model for Usher's syndrome type 1 is also being investigated by studying visual responses and retinal histology, but no anomalies have so far been identified in young mutants.

Polymorphism in the 3′ untranslated region of human phosphoglucomutase-1 detected by SSCP analysis

R. E. MARCH, Y. H. EDWARDS AND D. B. WHITEHOUSE
MRC Human Biochemical Genetics Unit, Galton Laboratory, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE

Genetic analysis of the 3′ untranslated region of human phosphoglucomutase-1 (PGM1) by a non-radioactive single-strand conformational polymorphism (SSCP) technique and direct sequencing demonstrated the presence of five new alleles. Analysis of 75 unrelated individuals and six CEPH families whose protein polymorphism

subtypes were known revealed a strong allelic association between the subtype alleles (+ and −) and the SSCP alleles. In contrast, there was no association between the SSCP alleles and the PGM1*1 and PGM1*2 alleles. There was a complete correlation between nucleotide sequence and SSCP phenotype. This study provides support for the model that the protein polymorphism is determined at two distinct sites, one coding for the PGM1*1 and PGM1*2 alleles and the other coding for the '+/−' alleles. These sites are separated by a third region, where intragenic recombination has occurred.

The expression of *Hox-B1* (Hox-2.9) in transgenic mice and its response to retinoic acid

HEATHER MARSHALL AND ROBB KRUMLAUF
Molecular Embryology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA

The genes of the *Hox-B* (Hox-2) cluster are collinear in that there is a direct correlation between the position of a gene within the cluster and its relative domain of expression along the embryonic anterior–posterior axis. Furthermore, the order of the Hox genes within the complex correlates with their temporal order of activation during development and also their responsiveness to retinoic acid (RA). We have isolated the murine *Hox-B1* gene from its complex and recreated its endogenous pattern of expression in transgenic mice. Deletion experiments have identified a *Hox-B1* fragment giving expression in a subset of the endogenous pattern which is restricted to rhombomere 4 (r4) in the developing hindbrain.

The *Hox* genes show an increased sensitivity to RA moving 3′ along the cluster, *Hox-B1* being the most responsive. To investigate the responsiveness of the *Hox-B1* transgene to RA, pregnant females carrying transgenic embryos have been treated with retinoic acid and their litters harvested at various stages. We find that the gene rapidly responds to RA at presomitic headfold stages, expanding the anterior domain of *Hox-B1* expression from the presumptive r4 region to the midbrain/hindbrain border. Examining embryos at later stages of development has revealed that patterns of expression characteristic of r4/5 are now found ectopically in r2/3. Moreover, these changes in expression are accompanied by alterations of the motor nerve derived from r2 (trigeminal) such that it now has the morphology of the r4 (facial) nerve. These results suggest that RA has induced changes in the hindbrain Hox code which result in the homeotic transformation of r2/3 towards an r4/5 identity.

YAC contig analysis of a repeat sequence island on the mouse X chromosome

P. MILEHAM AND S. D. M. BROWN
Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, London W2 1PG, UK

The mouse genome contains 50 copies of a long complex repeat unit (LCRU) that are localized as a repeat sequence island at the A3 Giemsa positive (dark) band on the mouse X chromosome (Nasir *et al.*, *Proc. Natl. Acad. Sci. USA* **87**, 399). This repeat sequence island encompasses a one megabase region. Within the island, the LCRUs are not tandemly arranged but are juxtaposed and inserted by a high density of L1 line elements (Nasir *et al.*, *Nucl. Acids Res.* **19**, 3255). We have screened a partial RI mouse YAC library prepared in a recombination-deficient strain (*rad52*) of yeast and recovered 20 stable YAC clones (Chartier *et al.*, *Nature Genetics* **1**, 132). Fingerprinting of the 20 YAC clones using *Taq* I site variation between individual LCRU repeat units has been used to determine overlapping YACs. Two major contigs have been identified amongst the 20 *rad52* clones. *Sfi* I restriction mapping of YAC clones is being used to determine the exact overlaps of individual clones and to construct a restriction map and complete contig covering the entire repeat sequence island region.

Two new ENU-induced mouse models of human genetic diseases: alcaptonuria and erythropoietic protoporphyria

X. MONTAGUTELLI, A. LALOUETTE, S. TUTOIS, Y. BEUZARD[1], J. C. DEYBACH[2], P. KAMOUN[3] AND J. L. GUÉNET

Unité de Génétique des Mammifères, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris cedex 15; [1] Laboratoire d'hématologie, Hôpital Henri Mondor, 94010 Creteil; [2] Laboratorie de Biochimie, Hôpital Louis Mourier, 92701 Colombes cedex; [3] Laboratoire de Biochimie, Hôpital Necker, 75743 Paris cedex 15

Two new autosomal recessive mutations have been identified in the mouse following a mutagenesis experiment using ethyl-nitrosourea (ENU). one is the homologue of alcaptonuria, a human genetic disorder characterized by the urinary excretion of homogentisic acid. This mutation has been mapped on mouse chr 16, using an interspecific backcross.

The second mutation is the homologue of the human erythropoietic protoporphyria, a disease caused by a deficiency in ferrochelatase, the last enzyme of haem biosynthesis pathway. In the human, it causes photosensitivity and, in some cases, a liver disease which can be fatal. In the mouse, jaundice is always observed, together with anaemia. Most of the biochemical features are the same in the two species. In the mouse, the mutation has been mapped to chr 18. The ferrochalatase cDNA was cloned by Taketani *et al.* [*J. Biol. Chem.* (1990) **265**, 16377–16380], thus opening the field for gene therapy experiments. We will present preliminary data obtained with reciprocal bone-marrow transplantations as well as with transgenic animals.

The adaptive function of mammalian X-chromosome inactivation

T. F. MOORE

MRC EETU, St George's Hospital Medical School, Cranmer Terace, London, SW17 0RE

Certain species which have heteromorphic sex chromosomes exhibit increased levels of transcription from the X-chromosome in the somatic cells of the heterogametic sex. The requirement for dosage compensation in these species, but not in others, may simply be due to differences in the relative proportion of the genome represented by the X-chromosome: absence of dosage compensation in birds and butterflies is associated with high chromosome numbers in the karyotype [Moore, T. F. (1992) PhD thesis, Univ. of London, pp. 165–194]. Mammalian X-inactivation has traditionally been viewed as an alternative strategy for achieving dosage compensation between sexes carrying different chromosome complements. However, a number of problems with this view may have been overlooked (Moore, 1992). In this proposal I suggest that the evolution of mammalian X-inactivation can best be understood in terms of that part of population genetics theory which deals with parent-offspring conflict. Specifically, X-inactivation may provide a two-fold advantage to maternally-derived genes by (i) reducing the nutrient demands of the foetus on the mother, and (ii) preventing sex ratio distortion due to unequal acquisition of nutrients by male and female embryos (Moore, 1992). This proposal may explain why the strategy of chromosome inactivation rather than increased transcription is used to achieve dosage compensation in mammals, and provides an adaptive explanation for the different patterns of somatic X-inactivation observed in marsupials and eutherians [Moore, T. & Haig, D. (1991) *Trends Genet.* **7**, 45–49].

Rat inbred strain homogenity approached in the view of DNA fingerprinting

M. MRUG, P. NÜRNBERG[1], K. BENDER[2] AND R. BRDIČKA

Institute of Haematology and Blood Transfusion, Molecular Genetics Laboratory U nemocnice 1, 128 20 Praha, Czechoslovakia; [1] Institute of Medical Genetics, Humboldt University, Berlin, Germany; [2] Institute of Human Genetics and Anthropology, Freiburg, Germany

In the second round of rat inbred strain typing 71 stocks (21 strains) from 170 stocks, collected in the cooperative programm initiated by Professor Bender, were tested using the method of DNA fingerprinting.

The results of band-pattern checking were used to: (1) create the tree diagram using the cluster analysis; (2) calculate genetic distances between rat strain stocks; (3) estimate genetic within-strain variability. Other views – sex, mutation rate, residual heterozygosity and genetic contamination with foreign gene were also taken into account.

Relationship among strains was demonstrated on maps constructed by projection of their locations from the *N*-dimensional space (where *N* is a number of different checked bands) into the two dimensional space. The real strain relationship is shown here less deformated than in case of other for presentation used methods (e.g. tree diagram).

The expression and effects of differentiation inhibiting activity (DIA/LIF) on early mouse embryos

JENNIFER NICHOLS, ANNETTE DUWEL, MORAG ROBERTSON AND AUSTIN SMITH
Centre for Genome Research, Edinburgh

Germline transmission has been achieved using embryonic stem (ES) cells derived and maintained in medium supplemented with DIA/LIF, and without the involvement of feeder cells. The implication that DIA/LIF may play a role in stem cell maintenance in the developing embryo has been reinforced by the results of RNase protection analysis, which indicates the presence of DIA/LIF transcripts in the egg cylinder at 6·5 and 7·5 days post coitum (dpc). Expression has also been detected in blastocysts by *in situ* hybridization.

The effects of increased amounts of DIA/LIF on developing embryos has been tested via blastocyst injection of stably transfected ES cells which over-express DIA/LIF. Resulting implantations were dissected at 8·5 dpc and assessed for levels of chimaerism by glucose phosphate isomerase electrophoresis. Various types of abnormality were obtained from two of these clones. These included complete disorganisation, blebs on the visceral yolk sac, abnormal allantois or head, additional membranes or duplications of the body axis. The variability in phenotype may be related to the colonisation patterns of the ES cells. Means by which the distribution of donor cells may be accurately mapped are being developed.

Spread of inactivation into the autosome in X;autosome translocations is associated with methylation of autosomal CpG islands

DOMINIC NORRIS, GAVIN KELSEY[1] AND SOHAILA RASTAN
Section of Comparative Biology, MRC Clinical Research Centre, Harrow, HA1 3UJ, UK; [1] Institute of Cell and Tumour Biology, German Cancer Research Centre, Heidelberg, Germany

We have previously shown that methylation of CpG islands is a general feature of the inactive X chromosome [Norris *et al.* (1991) *Mamm. Genome* **1**, 78–83]. It is known that inactivation can spread in *cis* from the X chromosome into autosomal material in X;autosome translocations. Analysis of a number of closely linked CpG rich islands around the *c* locus on chromosome 7 in two different X;7 rearrangements shows that spread of inactivation into the autosome is associated with specific methylation of these normally unmethylated autosomal CpG islands. However in the X;autosome translocation T(X;7)3Neu, 4 islands proximal to the visibly inactivated *c* locus (hence closer to the X inactivation centre) remain unmethylated, whilst an island distal to the *c* locus is methylated. This absence of methylation of certain CpG islands may reflect a region of chromosome 7 which has escaped inactivation, possibly due to the presence of a gene required to be expressed in two copies for survival during development. Alternatively, this may represent a region which is inactivated but not methylated.

Ube1y1: a candidate for the spermatogenesis gene, *Spy*

GRAEME D. PENNY, GRAHAM F. KAY, PAUL BURGOYNE[1], ALAN ASHWORTH[2] AND SOHAILA RASTAN
Section of Comparative Biology, MRC Clinical Research Centre, Harrow HA1 3UJ, UK; [1] MRC Mammalian Development Unit, London NW1 2HE, UK; [2] Chester Beatty Laboratories, Institute of Cancer Research, London SW3 6JB, UK

We [Kay *et al.* (1991) *Nature* **354**, 486–489] and others (Mitchell *et al.* (1991) *Nature* **354**, 483–486] have described a new mouse gene, *Ube1y1* (previously called *A1s9Y1*) which maps to the short arm of the Y chromosome in the region deleted in the Sxr^b mutation. *Ube1y1* is homologous to ubiquitin activating enzyme E1 and is

expressed exclusively in the testis. There is a ubiquitously expressed X-linked homologue, *Ube1X* (previously called *As19X*) which undergoes normal X inactivation (Kay *et al.*, 1991). The testis-specific expression of *Ube1y1* and its location in the Sxr^b region, suggests that it may be a candidate for the functionally defined spermatogenesis gene, *Spy*, which is responsible for spermatogonial proliferation [Sutcliffe & Burgoyne (1989) *Development* **107**, 373–380]. We present evidence on *Ube1y1* expression throughout testicular development that supports the hypothesis that *Ube1y1* is *Spy*.

Rapid allele-specific HLA-DQ genotyping from genomic DNA in a single-step PCR

JEAN K. PICARD
Transplantation Biology, Clinical Research Centre, Watford Road, Harrow, Middx, HA1 3UJ

A set of oligonucleotide primers has been designed which allows the direct amplification of 8 HLA-DQA alleles, and 13 HLA-DQB alleles, thus enabling the unambiguous identification of the 18 most common HLA-DQ haplotypes. It is anticipated that this PCR system will prove a value in the HLA matching of donor-recipient pairs, in studies of the associations of certain HLA-DQ haplotypes with certain diseases, such as a autoimmunity, and perhaps also in forensic applications. In addition, because of linkage disequilibrium between the HLA-DQ and DR regions, knowledge of the HLA-DQ haplotype allows prediction of the HLA-DR serotype.

The mitochondrial DNA of Irish house mice

A. W. RYAN, E. J. DUKE AND J. S. FAIRLEY[1]
Zoology Department, University College, Belfield, Dublin 4; [1] Zoology Department, University College, Galway

Examination of Irish house mice (*Mus musculus domesticus*) revealed a high degree of polymorphism and geographical localization of mitochondrial DNA types. A significant correlation was also observed between genetic divergence and geographical distance [A. W. Ryan, E. J. Duke and J. S. Fairley *Heredity* (in press)]. Patterns from some restriction enzymes were not only common to those in laboratory mice and other *Mus musculus domesticus*, but also to other subspecies, such as *Mus musculus molossinus*. The maximum observed sequence divergence in the present study was 1·166%. Such a divergence is unlikely to have arisen in Ireland since glaciation ended, approximately 10000 years ago, as too high a mutation rate would have been required. Dendrograms constructed from distance matrices suggest that genetic drift, in the form of random local extinction of clones, accounts for much of the differences in genetic make-up between sampling sites in Ireland. Gene flow within and between populations seems to be strongly influenced by the behaviour and ecology of the species, as well as by topographical features.

The inactivation of the DNA repair gene ERCC-1 by gene targeting and germline transmission of the inactivated allele

JIM SELFRIDGE, ANGELA M. POW, JIM McWHIR, THOMAS MAGIN AND DAVID W. MELTON
Institute of Cell and Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR

Gene targeting provides a means of making specific changes to the mammalian genome. When used in combination with embryonic stem cells, gene targeting can be used to generate mouse models for human genetic diseases. We wish to produce mice with DNA repair deficiencies to serve as models for human disorders such as Xeroderma Pigmentosum (XP) where defects in the excision repair pathway for the removal of DNA damage predispose to skin cancers. We are working with ERCC-1, the first mammalian repair gene to be cloned, which fails to complement any of the nine known Xeroderma complementation groups. To facilitate this work a new $HPRT^-$ stem cell line, HM-1, was developed which contributed to the germ line with a high efficiency. This line enables the use of HPRT minigenes as selectable markers. As it is possible to select both for and against HPRT

activity this system will allow more subtle changes to the target locus to be made using a single marker gene. Using a replacement vector in a positive negative selection strategy, we identified embryonic stem cell clones containing an ERCC-1 targeted allele by means of a PCR assay. Targeting the ERCC-1 allele in the HM-1 cell line has enabled us to create chimaeric mice by injecting the cells into host blastocysts. Analysis of the offspring from these chimaeras has revealed germline transmission of the disrupted allele. The phenotype of ERCC-1 deficient mice is being studied.

Characterization of *Sox-1*, *-2* and *-3*: DNA-binding proteins expressed in the developing central nervous system of the mouse

SHANTHINI SOCKANATHAN, MICHEL COHEN-TANNOUDJI, JEROME COLLIGNON AND ROBIN LOVELL-BADGE
Laboratory of Eukaryotic Molecular Genetics, MRC National Institute of Medical Research, The Ridgeway, Mill Hill, London NW7 1AA

A new family of genes in the mouse, termed the *Sox* genes, have been identified based on a shared region of similarity to the testis-determining gene, *Sry*. This common region encodes an HMG box which has allowed further division of the *Sox* genes into subfamilies based on sequence comparisons both within and outside the box. *Sox-1*, *-2* and *-3* are three genes which belong to the subfamily closest in sequence to that of *Sry*. Analysis of the expression patterns of *Sox-1*, *-2* and *-3* RNA by Northern blots have demonstrated expression in foetal stages between 11–14 days post coitum in addition to expression in adult brain. A more detailed study by *in situ* hybridization has localized the expression of the three genes to the developing nervous system of the mouse embryo, a particularly interesting pattern of expression in the case of *Sox-1* and *Sox-2* being observed during development of the eye.

Results presented here will concentrate on the further characterization of the SOX 1 and SOX 2 proteins. We have demonstrated by Western blotting that similar profiles of expression of both SOX 1 and SOX 2 proteins as compared to the RNA are observed in foetal brain throughout embryogenesis. Further experiments as to the localisation of the proteins in embryonic sections by immunohistochemistry are currently in progress. Evidence that SOX 1, 2 and 3 are DNA-binding proteins will also be presented and possible mechanisms as to their mode of action will be discussed.

Evidence for transcription of paternal histocompatibility genes in the one cell preimplantation mouse embryo

MATTHEW T. SPRINKS, MARTIN H. SELLENS, GILLIAN B. DEALTRY AND NELSON FERNANDEZ
Department of Biology, Wivenhoe Park, Colchester, Essex CO4 3SQ

The expression of MHC products by the mammalian embryo is of developmental as well as immunological significance. We have used a highly sensitive polymerase chain reaction (PCR) assay in conjunction with oligonucleotide primers complementary for polymorphic regions of the mouse H-2D gene to detect the transcription of maternal and paternal class I mRNAs in gametes and preimplantation embryos. Using congenic strains of mice differing only at the major histocompatibility loci, class I (H-2D) mRNA could not be detected in vas deferens or *in vitro* capacitated sperm or in ovulated secondary oocytes, but mRNA of the maternal haplotype was present in embryos from the zygote to the blastocyst stage. Messenger RNA of the paternal haplotype was demonstrated in embryos from the zygote stage onwards, only 10 h after the presumed time of mating. These data clearly show that paternal MHC class I genes are transcribed from the earliest stages of embryonic development, and suggest that any developmental regulation of expression of their protein products is principally at the post-transcriptional level.

Targeted mutation of the mouse homeobox gene *Cdx I* in mouse embryonic stem cells

VASANTA SUBRAMANIAN[1] AND PETER GRUSS[2]

[1] *Department of Biochemistry, University of Bath, Bath BA2 7AY;* [2] *Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, D3400, Göttingen, West Germany*

The mouse *Cdx I* gene, the homologue of the *Drosophila* caudal was isolated from an 8·5 day mouse embryo cDNA library and is at present the only known homeobox gene expressed in endodermally derived cells. The first detectable expression of this gene by *in situ* hybridization is at 8·5 days of development and at day 14 onwards in the developing intestine. In order to assign a functional role for *cdx I* in mouse development we have successfully mutated this gene in embryonic stem cells by homologous recombination. A 4·1 kb genomic fragment was disrupted in the box region by the neo gene and flanked at the 3′ end by the HSV TK gene. The ES cell line E 14 was electroporated with this construct and cells subjected to double selection with G418 and gancyclovir. Single clones were isolated and analysed in pools by PCR. Correct targeting was confirmed by genomic Southern blots. Ten independent recombinant clones were isolated. Injection chimeras have been generated from these clones.

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