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# *Abstracts of papers presented at the sixth Mammalian Genetics and Development Workshop held at the Wellcome Trust Building, Euston Road, London on 20–22 November 1995*

## The 6 megabase, X chromosomal, deletion breakpoint in a patient with DMD, CGD, Macleod phenotype and X-linked retinitis pigmentosa involves an Alu repeat

DOUG LESTER[1,2], JOHN BROWN[2], FIONA PRYDE[2], SANDY EDGAR[2], MARIA MUSARELLA[3] and ALAN WRIGHT[2]

[1] Present address: *Roslin Institute, Edinburgh EH25 9PS, UK*; [2] *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*; [3] *Department of Ophthalmology, University of Toronto, Ontario M5G 1X8, Canada*

The famous X chromosomal deletion patient BB helped spark off a positional cloning revolution in human genetics. This patient, with a visible cytogenetic deletion in band Xp21, suffered from four diseases, namely DMD, CGD, Macleod phenotype and X-linked retinitis pigmentosa (XLRP). Three of the genes involved in this patient's phenotype, namely Dystrophin, CYBB (CGD) and Xk (Macleod phenotype), were subsequently isolated by other laboratories using this patient's DNA (Royer-Pokora B. *et al. Nature* 1986; **322**: 32–38; Monaco A. P. *et al. Nature* 1986; **323**: 646–650; and Ho *et al. Cell* 1994; **77**: 869–880). To date the gene for the fourth disease from which this patient suffered, namely XLRP (RP3), has not been isolated. However, several genetic studies in XLRP families indicate that the gene for RP3 probably maps very near the BB proximal deletion breakpoint. Our laboratory therefore created a *Hin*d III phage library using patient BB's DNA and isolated a 10 kb phage clone that spanned the BB breakpoint. This clone was then shown by PCR analysis to contain dystrophin exons 33, 32 and 31 but not exon 30. Sequence analysis of both this deletion breakpoint phage and of a normal genomic phage clone that spanned the proximal breakpoint proved that the BB deletion breakpoint involved an Alu repeat. However, further PCR-vectorette and sequence analysis proved that there was no significant homology to the Alu repeat on the distal or dystrophin side of the deletion.

## *Wnt-7A* is involved in neuronal maturation

FIONA R. LUCAS and PATRICIA C. SALINAS

*Developmental Biology Research Centre, The Randall Institute, King's College London, 26–29 Drury Lane, London WC2B 5RL, UK*

*Wnt* genes encode secreted proteins implicated in cell fate changes during development. They are found associated with the plasma membrane and extracellular matrix, and can act in an autocrine and paracrine fashion. To begin to define the specific cell populations in which *Wnt* genes act, we chose to examine *Wnt* expression in the cerebellum, which has a relatively simple structure and contains well-characterized cell populations. We found that *Wnt-7A* is expressed in the adult granular cell layer. *Wnt-7A* starts to be expressed from postnatal day 6 to 22 (P6–P22) and then declines to low levels in adult cerebellum. The timing of enhanced *Wnt-7A* expression coincides with mossy fibres of pontine origin making synapes with granule cells. Purification techniques have been developed in which individual cerebellar cell types can be isolated for culture *in vitro*. We can exploit the *in vitro* system to study the molecular events underlying the maturation of the cerebellum, focusing on *Wnt* genes. Using this system we have isolated granule cells and co-cultured them on stable precursor cell lines transfected with *Wnt-7A*. We found that granule cells exposed to *Wnt-7A*-expressing cells show an increase in the spreading of neurites. These results suggest a role for *Wnt* genes in the maturation of granule cell neurons.

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**A multiple copy RNA-binding motif gene family on the mouse Y chromosome: mapping, expression and deletion analysis**

SHANTHA K. MAHADEVAIAH[1], TERESA ODORISIO[1], ÄINE RATTIGAN[1], STEPHEN H. LAVAL[2], BRUCE M. CATTANACH[2] and PAUL S. BURGOYNE[1]
[1] *Laboratory of Developmental Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*; [2] *Genetics Division, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, UK*

An RNA-binding motif (*RBM*, formerly *YRRM*) gene family has been identified on the human Y chromosome which maps to the same deletion interval as the 'Azoospermia Factor' (AZF). 'Zoo-blotting' indicated that related genes were present on the Y chromosome of other mammals and in the mouse the gene was thought to be single copy. We have shown that, contrary to expectation, the mouse *Rbm* is present in multiple copies, located proximally on the Y short arm between *Sry* and the centromere. Each copy of *Rbm* forms part of a larger repeating unit which includes the 2·6 kb *Eco*R I fragment detected by the probe pSx1, some copies of which lie within the Y short arm derivative *Sxr*. These *Rbm* sequences are expressed in tests in a germ-cell-dependent fashion, peaking around 14 days after birth. Mice with deletion of all but one copy of the gene are XY females (due to non-expression of *Sry*); sex-reversing such mice with an *Sry* transgene produces males with almost undetectable *Rbm* expression but of normal fertility.

**Molecular characterization of murine Nfe211**

JUDITH McKIE, KAREN JOHNSTONE, MARIE-GENEIEVE MATTEI and PETER SCAMBLER
*Molecular Medicine Unit, Division of Biochemistry and Genetics, Institute of Child Health, 30 Guilford Street, London, UK*

The murine homologue of the human *NFE2L1* basis leucine-zipper gene was isolated from an early embryo library. The *Nfe211* gene shows strong sequence similarity to the haematopoietic p45 subunit of the NF E2 transcription factor. Murine p45 NF E2 was cloned as a required activity at the locus control regions upstream of the globin gene complexes. Knockout analysis has shown that p45 NF E2 subunit is essential for megakaryocyte maturation and platelet production. The human *NFE2L1* gene was isolated using a complementation assay designed to detect proteins activating transcription at the NF E2/AP1 binding site. Both p45 NF E2 and NFE2L1 are more closely related to the *Drosophila* CNC protein than to any other bZIP protein. Mutational analysis in *Drosophila* of *cnc* has shown it to be required for the identity of two cephalic segments. *Nfe211* has been sequenced and analysed. It has been mapped in the mouse using radioactive *in situ* hybridization to chromosome 11, and in the human using fluorescence *in situ* hybridization to chromosome 17. Northern analysis in both murine adult tissues and embryos shows a ubiquitous expression pattern. Whole mount and tissue section *in situ* hybridization of murine embryos also shows a fairly ubiquitous expression pattern.

**Targeting haematopoietic stem cells: the *Ly-6E.1* (*SCA-1*) gene in transgenic mice**

C. G. MILES, A. M. HOLMES and E. A. DZIERZAK
*National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

The *Ly-6E.1* gene encodes a murine antigen recognized by the Sca-1 antibody. As this antibody has been used for the enrichment of haematopoietic stem cells from mouse bone marrow, we sought to examine the potential of the gene to target haematopoietic stem cells. We demonstrate that the cloned *Ly-6E.1* gene recapitulates the expression pattern of the endogenous gene and can direct heterologous gene expression to transplantable bone marrow stem cells in transgenic mice. In addition, we show that *Ly-6E.1/lacZ* expression in mid-gestation embryos coincides spatially and temporally with a region that gives rise to intraembryonic haematopoiesis. We are currently utilizing this *Ly-6E.1* expression cassette to direct expression of heterologous genes, such as c-*myc* and *bcl*-2, to *Ly-6E.1* positive haematopoietic stem cells in transgenic mice. The manipulation of *Ly-6E.1* positive cells with these proto-oncogenes will provide a valuable insight into the nature of haematopoietic stem cells and the events of haematopoietic differentiation.

**The human *HOXD-4* retinoid-responsive enhancer directs spatially-restricted gene expression in the developing mouse neural tube**

ALASTAIR MORRISON[1], MARIA CRISTINA MORONI[2], LINDA ARIZA-McNAUGHTON[1], ROBB KRUMLAUF[1] and FULVIO MAVILIO[2]
[1] *Division of Developmental Neurobiology, Medical Research Council, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*; [2] *DIBIT, Istituto Scientifico H. S. Raffaele, Milan, Italy*

Expression of vertebrate *Hox* genes is regulated by retinoids in cell culture and in early embryonic

development. We have identified a 185 bp retinoid-responsive transcriptional enhancer 5′ of the human *HOXD4* gene which regulates inducibility of the gene in embryonal carcinoma cells through a complex pattern of DNA–protein interaction on at least two distinct elements. One of these elements contains a direct repeat (DR5) mediating ligand-dependent interaction with retinoic acid receptors (RARs), and is necessary though not sufficient for the enhancer function. The *HOXD-4* enhancer directs expression of a *lacZ* reporter gene in the neural tube of transgenic mouse embryos in a time-regulated and regionally restricted fashion, reproducing part of the anterior neuroectodermal expression pattern of the endogenous *Hoxd-4* gene. Administration of retinoic acid to developing embryos causes alterations in the spatial restriction of the transgene expression domain, indicating that the *HOXD-4* enhancer is also a retinoid-responsive element *in vivo*, Mutations in the DR5 or in a second (region X) element in the enhancer affect both retinoid response in culture and developmental regulation in embryos, suggesting that cooperative interaction between different factors mediates the enhancer activity. These data suggest a role for endogenous retinoids in the regulation and spatial restriction of *Hox* gene expression in the central nervous system.

**Chromosome pairing in female mouse meiosis**

C. O'KEEFFE[1], M. A. HULTÉN[2], S. J. ARMSTRONG[2] and C. TEASE[1]
[1] *Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxon OX11 0RD; UK;* [2] *LSF Research Unit, Regional Genetics Services, Birmingham Heartlands Hospital, Birmingham B9 5PX, UK*

The initiation and progression of homologous chromosome pairing at meiosis was investigated in female mice using fluorescence *in situ* hybridization (FISH) with repeat sequence probes and chromosome-specific paints. The behaviour of the proximal end of the X chromosome was observed using a repeat copy probe for the *DXWas70* locus. No significant amount of pairing was seen until late zygotene, by which time most of the chromosome was synapsed. Using dual-colour FISH, combining probes for both the minor satellite and telomere regions, both ends of all chromosomes could be studied. Observations with the minor satellite probe showed the centric ends of the chromosomes to associate in varying numbers of clusters from pre-meiotic stages onwards. However, the results from *DXWas70* demonstrated that the proximal ends of the X chromosome remained apart until late into zygotene, suggesting the clusters were non-specific and probably played no role in the initial phases of homologue alignment. Preliminary observations with whole chromosome paints supported this conclusion, as homologues were usually in different domains in leptotene to early zygotene cells. Telomere clustering was also observed upon entering zygotene. This may be interpreted as a process to bring telomeres together and could suggest a role for distal telomeres in initiating homologue synapsis.

**An analysis of the role of *Xist* in X chromosome inactivation using targeted mutations in embryonic stem cells**

G. D. PENNY, G. F. KAY, S. A. SHEARDOWN, S. RASTAN and N. BROCKDORFF
*MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK*

X chromosome inactivation is dependent on a major *cis*-acting switch gene, the X inactivation centre (*Xic*). The recently discovered *Xist* gene is a candidate for the *Xic*, since it maps to the minimal *Xic* region, is expressed from the inactive X and its expression during development precedes X inactivation. To determine whether *Xist* is the *Xic* it is necessary to generate targeted mutations of the locus. Since *Xist* is expressed in the male germ line it may be impossible to transmit any mutations generated in XY embryonic stem (ES) cells through the male germ line. Fortunately, XX ES cells provide a useful *in vitro* model because undifferentiated cells maintain both X chromosomes in the active state but undergo random X inactivation upon differentiation. The aim of our project is to target mutations to one *Xist* allele in an XX ES cell line and ask whether, upon differentiation, X inactivation can occur. To facilitate the analysis we have generated an XX ES cell line with marked X chromosomes carrying Pgk-1 isozyme variants in which the two *Xist* alleles are also distinguishable at the DNA and RNA levels. Preliminary results will be presented.

**Crosses between translocation homozygotes with semi-identical breakpoints. II. Genetic consequences of repeated non-homologous meiotic pairing and the development of runted phenotypes**

A. H. F. M. PETERS, J. WESTERLAKEN, F. A. VAN DER HOEVEN and P. DE BOER
*Department of Genetics, Wageningen Agricultural University, Dreijenlaan 2, 6703HA Wageningen, The Netherlands*

In a mouse cross between the T(1;13)1Wa, +/+, + and homozygous *fuzzy* T(1;13)70H, Rb(11.13)4Bnr genotypes, female descendants carrying both reciprocal translocations were selected and subsequently

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backcrossed to the T70H,Rb4Bnr,fz homozygous karyotype. *Fuzzy* (*fz*) is a recessive coatmarker and is situated on the chromosome 1 part of the T70H $1^{13}$ small translocation chromosome. Smooth-coated (*fz*/+) offspring were karyotyped and checked for unwanted cross-overs in order to select for the T1Wa $1^{13}$ small translocation chromosome in all subsequent backcrosses. The G-banded karyotype was determined at B14, from B23 on routinely, indicating a T1Wa,Rb4Bnr/T70H,Rb4Bnr karyotype. Over the years a number of observations have been made: (a) meiotic chromosome pairing of the $1^{13}$H;$1^{13}$Wa small marker bivalent in B5 and B19 generations of males and in the B21 generation of females, (b) fertility of females over all generations and of males at the B5, B15 and B19 generations, and (c) the development of a class of runted offspring in a family-dependent manner since the B20 generation. These observations show this stock to be unstable in chromosome behaviour and male fertility. This instability will be discussed in the light of the chromosome pairing of particularly the $1^{13}$H;$1^{13}$Wa bivalent at zygotene/pachytene, and some preliminary microsatellite typings.

**Analysis of mouse aggregation chimaeras reveals multiple functions for *Pax6* in eye and nasal development**

JANE C. QUINN[1], JOHN D. WEST[1] and ROBERT E. HILL[2]

[1] *Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK*; [2] *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*

Several semi-dominant mutant alleles of the mouse *Small eye* (*Sey*) locus have been described. Molecular studies have shown that defects in *Pax6* are responsible for *Sey* mutants and that *Sey* and *Pax6* are identical. The *Sey*/+ heterozygote exhibits microphthalmia and anterior segment defects similar to the human ocular disorder aniridia. The *Sey*/*Sey* and $Sey^{Neu}/Sey^{Neu}$ homozygotes and the $Sey/Sey^{Neu}$ compound heterozygote are lethal postnatally, with a complete lack of eyes and nasal cavities. In these mice, development of the eye is characterized by a failure of lens formation and abnormal optic cup development. As progression of eye development requires the interaction of lens and optic vesicle, the anophthalmic *Small eye* phenotype could result from any defect in ocular development that affects these interactive events. To elucidate a primary role for *Pax6* in eye development, aggregation chimaeras were created utilizing two genetically identifiable *Small eye* alleles: *Sey* and $Sey^{Neu}$. Analysis of 12·5 day chimaeric fetuses showed that $Sey/Sey^{Neu}$ compound heterozygous cells were unable to contribute to lens and nasal epithelium and so were not rescued by wild-type cells. This indicates that *Pax6* acts directly and cell-autonomously to control the differentiation of lens and nasal placode from surface ectoderm. In compound heterozygous chimaeras, the size of these ectoderm-derived tissues was inversely related to the percentage of mutant cell contribution. *Pax6* was also found to play a role in development of retinal tissues with highly abnormal optic cup morphology observed in some $Sey/Sey^{Neu}$ chimaeras. Marked segregation between areas of $Sey/Sey^{Neu}$ and wild-type optic cup was observed, suggesting that *Pax6* produces cell surface change in the optic vesicle.

**An investigation into the effects of pairing and Y gene dosage on spermatogenesis of XYY mice**

TRISTAN A. RODRIGUEZ and PAUL S. BURGOYNE

*Laboratory of Developmental Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

'Meiotic quality control' is a mechanism that acts to eliminate those cells that have an unpaired sex chromosome at the end of pachytene. XYY mice are sterile and two hypotheses have been proposed to explain this sterility, one suggesting pairing disruption as the reason for spermatogenic failure and another implicating excess Y gene dosage. These models are not mutually exclusive. To see whether excess Y gene dosage will lead to sterility when pairing requirements are fulfilled, mice with four sex chromosomes (1X, 2Y and a $Y^{*X}$ chromosome that comprises only the pseudoautosomal region and no other Y-specific DNA) were produced. These mice have excess Y gene dosage as well as the potential for satisfying pairing requirements by forming two bivalents. All these males were found to be sterile, but SC analysis revealed that the pairing requirements were not being fulfilled. As a second model two types of mice with three sex chromosomes (XYY and $XYY^{*X}$ males) differing in the amount of Y DNA were compared. The $XYY^{*X}$ mice (with the lesser amount of DNA) proved to be fertile, while the XYY males were sterile. However, SC analysis revealed that the $XYY^{*X}$ males had an increased rate of trivalent formation (63%) compared with XYY mice (8%).

**A new mutation in mice causing polydactyly**

JAMES SHARPE and ROBB KRUMLAUF

*National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

In the course of making transgenic mice to study regulation of the *Hox* genes, we have created an

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insertional mutant line which displays polydactyly. In heterozygotes the forelimbs are normal. Digits 2–5 of the hindlimbs also appear normal; however, in place of the first digit (which is biphalangic in a wild-type) are one, two or three digits, each of which may be bi- or triphalangic. In this respect the phenotype appears to be extremely variable between individuals – a single litter can produce one pup with only one extra claw and another with two whole extra triphalangic digits – but there is much less variation between left and right sides of the same animal. In homozygotes all four limbs are more severely affected, with up to eight claws per limb, and shortening of the tibia and fibula. Apart from these skeletal deformities, the mice appear normal. We will describe analysis of the mutant line with respect to: expression of the reporter gene from the transgenic construct, *in situ* analysis of the expression of *vhh1* in the developing limb bud, skeletal preparation of the limb phenotype, and FISH analysis of the mutant chromosomes to localize the insertion site. The possibility that this line represents a new allele of a known classical mutation will be discussed.

**Molecular basis of three semi-dominant spotting loci on mouse chromosome 5**

DENNIS A. STEPHENSON[1,2], EDWARD NOVAK[2] and VERNE M. CHAPMAN[2]
[1] *Department of Genetics and Biometry, University College London, 4 Stephenson Way, London NW1 2HE, UK*; [2] *Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263.001, USA*

Genes affecting mouse pigmentation are rapidly being identified at the molecular level. The albino (*c*) locus was the first to be identified, partly because the biochemical basis of the mutant defect had been known for a long time (i.e. tyrosinase activity). The brown locus followed shortly afterwards, because it was found to encode a tyrosinase-related protein. Other loci for which the gene product was unknown have been identified by painstaking positional cloning techniques or by serendipity. Serendipity played a part in defining the molecular basis of the first of three semi-dominant spotting mutant (dominant spotting, *W*; patch, *Ph*; and rump-white, *Rw*) loci on mouse chromosome 5. Analysis of interspecific $F_1$ hybrids and backcross offspring was used to demonstrate a relationship between the *W* locus and the tyrosine kinase cell surface receptor *Kit*. Subsequent studies confirmed this relationship by demonstrating that a number of alleles had specific defects which abolished receptor function. A similar approach was used to establish that *Ph* was a deletional mutation encompassing the *Kit*-related tyrosine kinase receptor *Pdgfra*. Although the deletion does not appear to extend into the coding sequences of the *Kit* gene itself, it has been postulated that the spotting phenotype is probably due to aberrant regulation of KIT expression during fetal development. However, the observation that the patch-extended ($Ph^e$) allele appears to be a *Ph* mutation that has acquired a *Kit* mutation which abolishes tyrosine kinase activity, might suggest otherwise. Incorporation of the *Rw* mutation in molecular linkage studies resulted in significant suppression of recombination for loci mapping to the proximal third of the chromosome. Using *in situ* hybridization techniques, it was shown that this suppression was due to a large inversion associated with the *Rw* mutation. Further refinements in the physical linkage studies have established that the distal breakpoints of the inversion falls between *Pdgfra* and *Kit*. This presentation will focus on the contribution Verne Chapman's laboratory has made in this area.

**Cell recycling of a single human cell for preimplantation diagnosis of X-linked disease and dual sex determination**

ALAN THORNHILL and MARILYN MONK
*Molecular Embryology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

We have recently described a new procedure, called 'cell recycling', which combines the two powerful techniques of polymerase chain reaction (PCR) and fluorescent *in situ* hybridization (FISH) on the same single fixed cell. The dual procedure was developed to single cell sensitivity using single blastomeres of preimplantation mouse embryos. We have now extended the procedure to single human cells and demonstrated its potential use in preimplantation diagnosis to detect Duchenne muscular dystrophy (DMD) by PCR in addition to sexing the same single cell by both PCR and FISH. Should the PCR diagnosis of the DMD mutation fail, cell recycling provides two opportunities to identify the sex of the embryo, allowing selection of only the female embryos for transfer. As well as increasing efficiency and accuracy of single cell diagnosis, cell recycling may also prove to be invaluable for improving the pregnancy rate following preimplantation diagnosis procedures. After analysing a specific gene defect in a single blastomere, the same blastomere may be analysed for common chromosomal anomalies and defective embryos eliminated from the transfer procedure.

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**Evolution of DNA methylation patterns**

SUSAN TWEEDIE, JILLIAN CHARLTON and ADRIAN BIRD
*Institute of Cell and Molecular Biology, University of Edinburgh, Darwin Building, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK*

Mammalian genomes are predominantly methylated, apart from CpG islands which are found at the 5′ end of some genes. Furthermore, DNA methylation is known to be essential for mouse development. In sharp contrast, organisms such as *Drosophila* survive without any apparent DNA methylation. At some point in evolution methylation must have spread over the genome, in spite of the fact that it can result in both mutation and repression of transcription. We would like to understand what selective advantage could outweigh these potentially detrimental effects. We are also interested in the evolution of DNA methylation patterns. Has there been a gradual increase in the levels of DNA methylation or is there a sharp transition from essentially unmethylated to methylated genomes? To answer this question we have examined both overall levels of methylation and the methylation status of genes in a number of organisms at the vertebrate/invertebrate boundary.

**Excluding mutations in the transmembrane region of FGFR3 as the cause of dominantly inherited Dexter dwarfism (achondroplasia) in cattle**

A. P. USHA, DOUG LESTER, GEORGE RUSSELL and JOHN WILLIAMS
*Roslin Institute, Roslin, Edinburgh EH25 9PS, UK*

The short-legged, diminutive heterozygote Dexter cattle are thought to be the bovine equivalent of dominantly inherited achondroplasia (*ACH*/+) dwarfism in man. Homozygous Dexter cattle (*ACH*/*ACH*) or so-called bulldog calves, are often aborted before the eighth month of gestation, have very short legs and a distinctive craniofacial defect which causes the tongue to protrude grotesquely. Achondroplasia in humans has recently been shown to be caused by either one or two single amino acid changes in the transmembrane (TM) region of FGFR3. Our laboratory therefore isolated and sequenced most of the FGFR3 TM region in normal (+/+), heterozygous (*ACH*/+) and bulldog (*ACH*/*ACH*) cattle. This was achieved by using human oligonucleotide primers, specific for the TM domain of FGFR3, to PCR amplify and sequence cDNA from an activated bovine (+/+) macrophage cell line. The normal bovine DNA sequence showed 89%, 85% and 63% homology with human, mouse and chicken FGFR3 respectively. Genomic DNA from Dexter carrier (*ACH*/+) and bulldog cattle (*ACH*/*ACH*) was then PCR amplified and sequenced using human- and bovine-specific oligonucleotides for the TM region. No DNA sequence difference was observed between +/+, *ACH*/+ and *ACH*/*ACH* cattle in the TM region of FGFR3. Moreover the two amino acids that are changed in humans to give an achondroplastic phenotype was shown to be both conserved and unchanged in *ACH*/+ and *ACH*/*ACH* Dexter cattle.

**Chromosomal studies on Medi-Cult cultured human oocytes and embryos derived from assisted conception procedures**

M. B. WALL[1], K. MARKS[2], T. A. SMITH[1], C. M. GEARON[1] and A. L. MUGGLETON-HARRIS[1]
[1] *UMDS, Division of Obstetrics and Gynaecology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK*; [2] *Department of Medical Genetics and Cytogenetics, St George's Hospital Medical School, London, UK*

The quality and culture of early cleavage human embryos *in vitro* may depend on the procedures and culture conditions used by Assisted Conception facilities. Asynchronous cytoplasmic and nuclear maturation and cell cycle kinetics of human oocytes prior to fertilization may cause chromosomal imbalance. In addition, sub-optimal culture conditions may contribute to a failure in normal embryonic development. Studies by others have shown the incidence of chromosome anomalies to range from 11% to 23% for *in vitro* fertilization (IVF) oocytes, with a rate of 24% for the mature oocyte as opposed to 16·7% for non-inseminated pre-ovulatory oocytes. A rate of 20–70% has been reported for cultured cleavage-stage human embryos. In this study, results from the analysis of 105 IVF 'failed fertilized' oocytes (which had failed to show morphological evidence of normal fertilization) showed that 79% had $M_{II}$ metaphase plates. Of these, 66·3% were further analysed and 9·5% were found to contain sperm chromosomes in addition to $M_{II}$ oocyte chromosomes. Analysis of 16 'failed fertilized' oocytes following intracytoplasmic injection of a single sperm (ICSI), demonstrated that 18·7% contained sperm chromosomes, 12·5% decondensed sperm heads and 37·5% condensed sperm heads. Of the oocytes that gave $M_{II}$ metaphases (87·5%), 50% could be analysed. Cytogenetic analyses showed a 50% aneuploidy rate for the IVF $M_{II}$ oocytes and a 43% for the ICSI group. Of those that were karyotyped, chromosomes of the G group (21–22) were identified with the anomalies. Specific centromere, X, Y, 18 and 21 chromosome FISH probes are also being used to assess the status of developing embryos cultured in Medi-Cult media. The methods used and results obtained from our studies using oocytes/embryos (donated by patients for research) provide the basic information for

undertaking an informed programme of research of preimplantation genetic diagnosis on embryos derived in our facility.

**Developing a strategy for preimplantation diagnosis of familial adenomatous polyposis coli**

D. WELLS, A. AO, A. H. HANDYSIDE, R. M. L. WINSTON and J. D. A. DELHANTY
*Galton Laboratory, University College London, 4 Stephenson Way, London NW1 2HE, UK: Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK*

Preimplantation genetic diagnosis, in which blastomeres biopsied from human cleavage-stage embryos are tested for a genetic disorder, has been achieved for a variety of recessive diseases. The testing of embryos generated by *in vitro* fertilization with selective transfer of unaffected embryos represents an alternative to prenatal diagnosis. We are investigating strategies for the preimplantation diagnosis of familial adenomatous polyposis coli (FAPC), a dominant cancer predisposition syndrome caused by germline mutation of the *APC* gene. The patient we are working with is infertile and comes from a family with a severe manifestation of the disease. Efficient amplification of a region of the *APC* gene containing the causative mutation has been achieved by nested PCR performed on single lymphocytes and blastomeres. The mutation can be detected in PCR products from lymphocytes of patient origin by heteroduplex and single strand conformation analysis (SSCA). To reduce the risk of misdiagnosis we are investigating a linked polymorphism in addition to the mutation. Polymorphic and mutation sites are to be amplified separately following whole genome amplification by primer extension preamplification (PEP).

**X chromosome inactivation and imprinting: gametic methylation-imprint of the *Xist* gene and paternal X inactivation**

MAURIZIO ZUCCOTTI and MARILYN MONK
*Molecular Embryology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

X chromosome inactivation in female mammals occurs at the time of embryo implantation. It is random in the inner cell mass (ICM), whereas always the paternal X is inactivated in the trophectoderm and primary endoderm cell lineages. A gene, *Xist* (X inactive specific transcript), located at the X inactivation centre is a strong candidate for the *cis*-acting effector of the process of inactivation. Parental alleles of the *Xist* gene are imprinted such that exclusive expression of the paternal allele occurs from the 4-cell stage of embryonic development. We have shown that the imprint that differentiates the paternal and maternal alleles in the embryo originates as a differential pattern of methylation in the promoter region of the *Xist* gene in sperm and eggs. A total of 11 CpG sites in the promoter region and in the body of the gene were analysed; two CpG sites in the promoter region are unmethylated in sperm whereas the maternal allele is methylated at these sites in eggs. The differential methylation of the maternal (egg) and paternal (sperm) *Xist* alleles persists in the zygote until the blastocyst stage, when paternal X inactivation occurs. We are now extending these studies to the regulation of human *XIST* expression and X inactivation. Our current investigations follow two working hypotheses based on the existence of a binding factor capable of either binding to the unmethylated paternal allele and triggering the transcription or, alternatively, acting as a repressor that binds to the methylated maternal allele and blocks its transcription.

**Finding unknown genes on pig chromosomes**

DENISE V. ANDERSON DEAR and J. ROSS MILLER
*Laboratory of Genome Mapping, The Babraham Institute, Babraham Hall, Cambridge CB2 4AT, UK*

We are interested in isolating unknown genes on pig chromosomes or chromosome fragments. The method we have used depends on the presence of pig-specific repetitive sequences in unspliced immature RNA, i.e. heterogeneous nuclear RNA (hnRNA). hnRNA was prepared from the pig × hamster somatic cell hybrid 8990 which contains only the p arm of pig chromosome 12 in a hamster background. hncDNA was made from this using both a random hexamer and oligo dT as primer for first strand synthesis, followed by PCR amplification with two pig-SINE specific primers. hncDNA was then size selected above 600 bp and a library made using PCR-Script as the vector. The library containing 6000 clones, 21% of which had inserts over 600 bp. Clones with large inserts have and are being sequenced and homology searches performed using the Fasta program. To date, in 30 sequenced clones we have already found three hncDNAs which show strong homologies with sequences in the Genbank database. One of these is 95% homologous over 74 bp with a family of over 30 overlapping human unassigned ESTs. Further analysis suggests that this covers a region which has been subjected to coding pressure; one is 89% homologous over 95 bp with the *MLN50* gene which is amplified in human familial breast cancer and lies on the homologous human chromosome (17), and one showed 80% homology over 163 bp with mouse, rat and sheep mammary gland factor and human stat5.

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**BMP-7 mRNA localization and ectopic expression in the mouse embryo**

R. M. ARKELL and R. S. P. BEDDINGTON
*Division of Mammalian Development, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

Bone morphogenetic protein-7 (BMP-7), a member of the TGF$\beta$ superfamily, was isolated from a 7·5 dpc mouse primitive streak cDNA library. Investigation of the pattern of BMP-7 mRNA distribution in the gastrulating mouse embryo suggests it may play distinct roles in induction and subsequent patterning of the neurepithelium. It is expressed at early primitive streak stages in the anterior of the primitive streak. By 7·5 dpc transcripts are detected in the ventral node and notochord. At early headfold stages BMP-7 transcripts first became detectable in the neurectoderm itself, when they accumulate in the anterior, prospective floorplate region. At early somite stages BMP-7 begins to be expressed in the surface ectoderm of the embryo and this expression is elevated at the junction of the surface and neural ectoderm. To investigate the function of BMP-7 during neural axis formation we are using novel ectopic expression strategies which do not rely on the availability of specific promoters or recombinant protein. Transfected COS cells are used as a source of secreted molecules and aggregates of COS cells are grafted into mouse embryos at specific locations. The embryos are allowed to develop in culture for 24 h; thus the effects of the secreted molecule can be assessed in the context of the whole embryo. When an aggregate of COS cells is grafted into the embryo the COS cells remain at the site of the graft and no deformities associated with either the grafting procedure or the presence of wild-type COS cells have been detected. However, when COS cells transfected with BMP-7 are placed adjacent to the ventral neural tube in the hindbrain region of early somite embryos dramatic changes are seen in the adjacent neurectoderm after culture for 24 h. Current analysis of these changes suggests that at the site of the graft there is additional neurectoderm and this neurectoderm appears to be dorsal in nature.

**Notch function in early central nervous system**

PAUL BEATUS, U. LENDAHL, R. WILLIAMS, M. LARDELLI and T. MITSIADIS
*Department of Cellular and Molecular Biology, Karolinska Institute, S-171 77 Stockholm, Sweden*

The *Drosophila Notch* gene is involved in cell fate decisions in many cell types, including neural cells. The *Notch* gene encodes a large membrane-bound receptor consisting of an extracellular domain, composed of 36 EGF-like repeats, a transmembrane domain and an intracellular domain containing 7 cdc 10/ankyrin repeats. Multiple homologues of this gene have been identified in a number of species including *C. elegans*, *Xenopus*, mouse and human. In mammals there are three *Notch* homologues (*Notch 1*, *2* and *3*) which are highly conserved, both with respect to each other and to *Drosophila Notch*. The high degree of sequence conservation implies important functions for these gene products in most multicellular organisms. In mammals *Notch 1–3* have distinct patterns of expression during early embryogenesis and CNS development. During gastrulation *Notch 1* is expressed only within the forming notochord, *Notch 2* is expressed in the mesodermal cell layer as well as in the notochord, whereas *Notch 3* is widely expressed in both mesoderm and ectoderm but not in the notochord. Studies of null-mutant mice for *Notch 1* imply its importance in somitogenesis. During this stage *Notch 1* and *2* are strongly expressed in presomitic mesoderm and forming somites but are then down-regulated in the mature somites. *Notch 3* expression, however, does not appear until the somites are mature. The intracellular domain of *Notch* receptors transmit the signal to the interior of the cell. Here we report that mice transgenic for the intracellular domain of *Notch 3* under the nestin promoter, show major developmental disturbances in the CNS. The phenotype may be a consequence of increased cellular proliferation and block of neuronal differentiation. There is a great variation in the severity of the defects observed, probably due to different levels of expression of the truncated gene. Embryos expressing the mild phenotype show expansion of the hindbrain, irregularities in the spinal cord and reverse tail flexure. The severe phenotype is dominated by defects in the brain, characterized by non-closure of the anterior neural pore in the midbrain. These data show that a deregulated *Notch* function can affect early stages of CNS development.

**Elucidating the genetic bass of the 'bleb' mutations in the mouse**

E. BENTLEY[1], J. HOPKINS[1], A. GOSSLER[2] and S. M. DARLING[1]
[1] *Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK*; [2] *The Jackson Laboratory, Bar Harbor, Maine, USA*

There are four 'bleb' mutations in the mouse which map to different chromosomes and which are all characterized by the appearance of embryonic subepidermal blebs and by abnormalities of the eyes, limbs, kidneys and hair in newborn pups and adults. One of the 'bleb' mutations, myelencephalic blebs (*my*), had previously been mapped only with respect to biochemical and phenotypic markers on mouse chromo-

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some 3. Linkage analysis was carried out using an intersubspecific backcross between $my^{UCL}/my^{UCL}$ and the wild-derived strain *Mus musculus musculus* PWK: $(my^{UCL}/my^{UCL} \times \text{PWK})\ F_1 \times my^{UCL}/my^{UCL}$. One hundred and forty-six backcross progeny were analysed with 22 informative microsatellite markers generating a map spanning 59·9 cM (*D3Mit204–D3Nds9*) and positioning *my* within a 6·2 cM interval between *D3Mit134* and *D3Mit275*. To narrow this region, a second intersubspecific backcross was set up between $my^{UCL}/my^{UCL}$ and *Mus musculus castaneus*, and over 1000 backcross progeny have been generated. Markers within the interval between *D3Mit134* and *D3Mit275* will be analysed in this second backcross, generating an accurate genetic map of this region to facilitate the positional cloning of *my*. A second 'bleb' mutation, *blebbed* (*bl*) on chromosome 5, is also the subject of a positional cloning strategy; the $F_1$ progeny in the $[(bl/bl \times Cast)F_1 \times bl/bl]$ backcross have been generated so far. In conjunction with the molecular identification of the 'bleb' mutations, histological and ultrastructural studies on bleb formation during embryogenesis are under way and these studies may provide vital clues to the underlying developmental mechanism governing bleb formation in this family of mutations.

**Genetic and physical characterization of the candidate region for Coffin–Lowry syndrome (CLS) in Xp22.13**

H. BIRD, N. GODDARD, O. O'BRIEN and S. LINDSAY
*Molecular Genetics Unit, Department of Human Genetics, University of Newcastle upon Tyne, UK*

Coffin–Lowry syndrome (MIM 303600) is an X-linked disorder causing mental retardation and skeletal abnormalities. The disease is flanked distally by the marker DXS7161 (AFM291wf5) and proximally by DXS365, a region of approximately 2·2 cM (Nelson *et al. Cytogenet. Cell Genet.* 1995, in press; Biancalana *et al. Genomics* 1994; **22**: 617–625; Bird *et al. Am. J. Med. Genet.* 1995, in press). The latter was defined by recombination analysis in our panel of 22 unrelated patients. Using published data (Nelson *et al. Cytogenet. Cell Genet.* 1995, in press; Francis *et al. Genomics* 1994; **21**: 229–37; Alitalo *et al. Genomics* 1995; **25**: 691–700; Ballabio *et al. Cytogenet. Cell Genet.* 1995, in press) and our own observations we have completed a YAC contig across the region. Transcripts have been isolated from one of the YACs in the contig using end ligation–coincident sequence cloning (EL–CSC) and a candidate gene from another YAC in the region (Trump *et al. Cytogenet. Cell Genet.* 1995, in press) is being screened for mutations in CLS patients. Transcripts from the region will continue to be isolated and screened for their involvement in CLS using these techniques.

**Crosses between mouse translocation homozygotes with semi-identical breakpoints. I. Choice of translocations and meiotic chromosome behaviour in the $F_1$ hybrids**

P. DE BOER, A. H. F. M. PETERS, F. A. VAN DER HOEVEN and R. BAKKER
*Department of Genetics, Wageningen Agricultural University, Dreijenlaan 2, 6703HA, Wageningen, The Netherlands*

The T(1;13)70H and T(1;13)Wa translocations have cytologically identical breakpoints in the distal 13D1 band. The chromosome 1 breakpoints are A4 for T70H and C1·2 for T1Wa. In $F_1$ hybrids, two heteromorphic bivalents are formed, both with the chromosome 1 region between the breakpoints as the segment seeking ectopic homologous contact. This contact fails to form a synaptonemal complex. In the long $1^1$ bivalent, this leading to pairing adaptation in both males and females. In the $1^{13}$ short translocation bivalent, meiotic pairing saturation is not always achieved, rendering most males sterile. The independently segregating heteromorphic bivalents lead to four gamete types, of which the one with a deletion for 1A4-1C1·2 is an embryonic lethal and the one with a duplication for this region leads to a proportion of normal-looking offspring. Meiotic pairing and chiasma formation in the $F_1$ will be presented.

**Organization and regulation of the protein kinase CK2 β subunit gene**

B. BOLDYREFF[1], U. HOFFMANN and O.-G. ISSINGER
*Institut für Humangenetik, Universität des Saarlandes, D-66421 Homburg, Germany*; [1] *Present address: Zentrale Biotechnische Betriebseinheit, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany*

Protein kinase CK2 is a widely distributed and highly conserved serine/threonine protein kinase which is closely associated with proliferation (e.g. in permanent cell lines, in tumours or during embryogenesis). It consists of two catalytic subunits ($\alpha$ and/or $\alpha'$) and two non-catalytic subunits ($\beta$). In some kidney carcinomas asymmetrical expression of the two subunits was observed. Elucidation of the genomic organization of the CK2 genes should help to understand the regulation of CK2 in tumours and during embryogenesis. The CK2 $\beta$ gene seems to be a single copy gene. After screening a genomic mouse library with the CK2 $\beta$ cDNA several overlapping clones were isolated representing only one genomic locus. The locus was sequenced completely. The gene comprises about 7 kb in length and consists of seven exons, the first of which is untranslated. In the corresponding human gene a sequence in the promoter region had been identified which can bind the catalytic

$\alpha$ subunit and it has been shown that binding of the $\alpha$ subunit stimulates transcription of the $\beta$ subunit gene. No such sequence is present in the mouse CK2 $\beta$ promoter region, although the promoter regions are otherwise highly homologous. Regions responsible for transcriptional regulation of the mouse CK2 $\beta$ gene were investigated using CAT assays.

**Null mutants of the monoamine oxidase genes in man**

HAN G. BRUNNER[1], GRAEME EISENHOFER[2], NICO ABELING[3], DENNIS MURPHY[2], HENK KONINGS[4], LIESBETH BLEEKER-WAGEMAKERS[4], WOLFGANG BERGER[1], BERNARD VAN OOST[1] and JACQUES LENDERS[5]

[1] *Department of Human Genetics, University Hospital, Nijmegen, The Netherlands*; [2] *National Institutes of Health, Bethesda, MD 20892, USA*; [3] *Academic Medical Center, Amsterdam, The Netherlands*; [4] *Free University, Amsterdam, The Netherlands*; [5] *Department of Internal Medicine, University Hospital, Nijmegen, The Netherlands*

Monoamine oxidase (MAO) plays a pivotal role in the catabolism of neurotransmitters such as serotonin (5-HT), noradrenaline and dopamine. The two isoenzymes of monoamine oxidase, MAO-A and MAO-B, are encoded by separate genes that are close together in Xp11.3. We have recently reported selective MAO-A deficiency in a large Dutch kindred in which a syndrome of borderline mental retardation and prominent behavioural abnormalities was present in several males (Brunner *et al. Science* 1993; **262**: 578–580). Biochemical and genetic analyses demonstrated complex selective MAO-A deficiency due to a point mutation in exon 8 of the MAO-A gene which changes a glutamine (CAG) to a termination (TAG) codon. We have now extended our studies to include two brothers with selective deficiency of MAO-B due to a microdeletion as well as five unrelated males with combined deficiency of MAO-A and MAO-B, due to larger X chromosome microdeletions that also encompass the Norrie disease gene. The results allow comparison of selective and combined deficiencies of MAO-A and MAO-B, in terms of their effect on neurotransmitter metabolism and on behaviour and cognition (Lenders *et al.* submitted). The neurochemical as well as the behavioural and cognitive phenotypes are specific for each of these MAO deficiency states. Interestingly the behavioural phenotype of mice with genetic MAO-A deficiency has similar characteristics to its human counterpart (Cases *et al. Science* 1995; **268**: 1763–1766). MAO-A deficient mice have disordered morphology of certain brain areas, presumably as a consequence of prenatal exposure to raised levels of serotonin. This pathogenic mechanism may have relevance for the human phenotype associated with MAO-A deficiency as well. These combined results suggest that it may be worth while examining and comparing the phenotypic, morphological and biochemical consequences of the various MAO deficiency states between humans and other mammalian species such as mice.

**Microsatellite mapping of chromosome 2 deletion breakpoints associated with radiation-induced acute myeloid leukaemia**

D. CLARK[1,2], E. MEIJNE[3], S. BOUFFLER[1], R. HUISKAMP[3], C. SKIDMORE[2], R. COX[1] and A. SILVER[1]

[1] *NRPB, Chilton, Didcot, Oxon OX11 0RQ, UK*; [2] *School of AMS, University of Reading, Whiteknights, Reading RG6 6AJ, UK*; [3] *ECN, PO Box 1, 1755 2G Petten, The Netherlands*

Mouse models have a significant role to play in understanding the genetic lesions that result in the initiation and development of the neoplastic phenotype. The deletion and/or rearrangement of one copy of chromosome 2 (ch.2) is a consistent and early event in the initiation of murine acute myeloid leukaemia (AML) by radiation. More than 90% of AMLs induced in the CBA strain of mice express such cytogenetic alterations, with ch.2 breakpoints clustering in the C and F regions of the chromosome. The molecular resolution of ch.2 deletion breakpoints in highly inbred strains of mice is problematic. However, by using X-ray induced AMLs in $F_1$ progeny of genetically divergent CBA/H $\times$ C57B1 Lia we have been able to show region-specific loss of heterozygosity (LOH) for genetically linked sets of ch.2 microsatellite markers. This has allowed definition of the extent and parental origin of ch.2 losses, and identification of the genomic regions involved. A molecular map of ch.2 breakpoints has now been established in ten $F_1$ AMLs as a first step towards cloning of breakpoint sequences.

**Differential control of transgene expression in oocyte and in maternal or paternal genome of early mouse embryos**

ELIZABETH CHRISTIANS[1] and JEAN-PAUL RENARD[2]

[1] *Service d'Histologie et Embryologie de la Faculté de Médecine Vétérinaire, ULg, Liège, Belgium*; [2] *Unité de Biologie du Développement de l'LRNA, Jouy en Josas, France*

Transgenic lines were produced by pronuclear injection of a construct including *firefly* luciferase gene under the control of HSP70.1 promoter. Various experiments have demonstrated that the pattern of embryonic expression of such a transgene transmitted by the male gamete is similar to that of endogenous HSP70.1 gene. This makes these transgenic lines a

powerful tool for studying zygotic gene regulation (Thompson *et al. Mol. Cell Biol.* 1994; **14**: 4694–703; Christians *et al. Development* 1995; **121**: 113–22). Taking advantage of this model, we investigated early transcriptional activity of the maternal genome using embryos that received the transgene from the female gamete. Conducted in the $F_2$ transgenic line, this analysis showed that transgene expression is significantly reduced at the 2-cell stage following female transmission in comparison with male transmission. In several other transgenic lines this study was not feasible due to two unexpected features of transgene expression in the oocytes before fertilization. First, the HSP70.1Luc transgene is expressed in oocytes during growth and maturation, in contrast to the silent endogenous HSP70.1 gene. Secondly, luciferase activity is dramatically increased in these oocytes following their maturation whereas transcriptional activity is arrested at the beginning of the maturation process. Such a high level of luciferase in oocytes makes it difficult to discriminate between maternal and zygotic transgene expression once these oocytes reach the 2-cell stage following fertilization. From our results in the $F_2$ line, it is confirmed that maternal and paternal genomes exhibit different levels of early transcriptional activity. The peculiar pattern of transgene expression in oocytes in other lines indicates both transcriptional and post-transcriptional regulatory mechanisms. First attempts towards the understanding of these different mechanisms will be described as well as their consequence for experimental approaches using reporter gene constructs.

**Expression of runt box proteins in primitive and definitive haemopoiesis**

MARIA TERESA CORSETTI and FRANCO CALABI
*Developmental Biology Unit, Division of Cell and Molecular Biology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

The *runt* loci encode a family of DNA binding factors which are likely to play a role in several developmental events. In man, one of the *runt* loci (also known as *AML1*) is involved in the 8;21 translocation associated with acute myeloid leukaemia. This suggests a specific role in controlling haemopoietic differentiation. To address this question we have studied the pattern of expression of runt products in primitive and definitive haemopoietic tissues in the mouse. Using an affinity-purified anti-runt-box antiserum, we find expression in a perinuclear location in both erythroid cells of early blood islands (day E7.5) and circulating primitive erythrocytes (day E11.5). At the latter time, positive erythroid cells are also seen developing in the liver. In day E16.5 liver, runt expression in erythroid cells is hardly detectable, while maturing granulocytic blasts are strongly positive. Adult bone marrow shows a pattern substantially similar to that of E16.5 liver. The intracellular distribution of runt box proteins in liver and bone marrow cells appears different from that in erythroid cells originating from the yolk sac. By western blotting analysis we have identified several runt polypeptides, some of which appear to be lineage specific. Thus, runt box proteins may have a role in the differentiation of more than one haemopoietic lineage. Furthermore, distinctive forms of *runt* may be associated with separate erythroid differentiation stages.

**Onset of embryonic gene transcription in early pre-implantation human development**

ROB DANIELS and MARILYN MONK
*Molecular Embryology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

We have examined the expression of the myotonin protein kinase (MPK), hypoxanthine phosphoribosyl transferase (HPRT) and $\beta$-globin genes in individual preimplantation human embryos. Previously, using an allele-specific reverse transcription polymerase chain reaction assay (RT-PCR), we have shown *de novo* transcription of the MPK gene at the 1-cell to 4-cell stages. We could not attribute a significance to the transcription of a predominantly muscle-specific gene at this early stage of development. We have addressed the questions of illegitimate transcription and possible derepression of transcriptional control in the early stages of preimplantation development. Monitoring the transcription of a gene expected to be expressed (HPRT) and a gene one would not expect to be expressed ($\beta$-globin) at this developmental stage will provide evidence as to the level of transcriptional control exerted, and the significance of detecting transcripts by sensitive RT-PCR techniques, in individual preimplantation embryos.

**Variegated expression of ovine β-lactoglobulin in the mouse mammary gland is determined by the transgene locus**

KENNETH W. DOBIE[1,2], MURIEL LEE[3], JUDITH A. FANTES[3], ELIZABETH GRAHAM[3], A. JOHN CLARK[2], ANTHEA SPRINGBETT[2], RICHARD LATHE[1] and MARGARET McCLENAGHAN[2]
[1] *Centre for Genome Research, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JQ*; [2] *Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK*; [3] *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK*

Lines of mice carrying an ovine $\beta$-lactoglobulin (BLG) transgene secrete large quantities of BLG protein into their milk. To explore the stability of transgene

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expression, we entered into a systematic study of expression levels in three BLG transgenic mouse lines (lines 7, 14 and 45). Unexpectedly, only line 14 expressed BLG in a predictable fashion while lines 7 and 45 exhibited variable levels of transgene expression. Copy number within lines appeared to be stable and there was no evidence of transgene rearrangement. Studies on line 7 showed that BLG production levels were stable within individual mice in two successive lactations. Backcross experiments showed that the genetic background did not contribute significantly to the variation of expression levels. Tissue *in situ* hybridization experiments revealed mosaic patterns of expression within individual mammary glands from lines 7 and 45; the level of transgene protein in milk appeared to correlate with the proportion of secretory epithelial cells expressing BLG mRNA. Furthermore, *in situ* hybridization to metaphase chromosomes indicated that the transgene arrays in the variable lines are situated close to the centromere. We proposed that mosaicism of transgene expression is a consequence of the site and/or nature of the primary transgene integration event.

**Human minisatellite mutation rate after the Chernobyl accident**

YURI E. DUBROVA[1,2], ALEC J. JEFFREYS[2], VALERI N. NESTEROV[3], NIKOLAY G. KROUCHINSKY[3], VLADISLAV A. OSTAPENKO[3] and RITA NEUMANN[2]
[1] *N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow B-333, Russia*; [2] *Department of Genetics, University of Leicester, Leicester LE1 7RH, UK*; [3] *Research Institute for Radiation Medicine, Mogilev Branch, Mogilev, 212004, Belarus*

We are studying the radiation-induced increase in the mutation rate of minisatellite loci in mice and humans. It has recently been shown that low doses of acute ionizing $\gamma$-radiation cause a significant increase in minisatellite mutation rate in mice. Evidence for germline mutation induction was obtained at doses substantially lower than can be monitored by standard genetic techniques in mice. The frequency of minisatellite mutations among children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident was two times higher than in control families. In both experiments evidence for a radiation-induced increase in mutation rate was obtained from small population samples. The potential use of minisatellite loci in monitoring radiation-induced mutations in human populations will be discussed.

**Epigenetic changes in embryonic stem cells and their effects on fetal development and differentiation**

ROBERT FEIL, WENDY DEAN, LUCY BOWDEN and NICHOLAS D. ALLEN
*The Babraham Institute, Cambridge CB2 4AT, UK*

On prolonged culture, embryonic stem (ES) cells may lose their potential to go through the germ line. Furthermore, liveborn, completely ES-cell-derived mice have only been obtained using early passage stem cells. When derived from higher passage cells, the mice died at birth and were sometimes abnormally large (Nagy *et al. Proc. Natl. Acad. Sci. USA* 1993; **90**: 8424). We propose that on culture ES cells lose their full developmental potential because they progressively accumulate epigenetic changes in (imprinted) genes. We have analysed methylation changes in imprinted genes in relation to passage number of ES cells. Initially we studied allelic methylation patterns in the imprinted insulin-like growth factor 2 (*Igf2*) and *H19* genes (Feil *et al. Development* 1994; **120**: 2933; Feil *et al. Dev. Genet.* 1995; **3**), but have recently extended this to other imprinted genes with strong effects on growth. Interestingly, in the 'U2af binding protein related sequence' (*U2afbp-rs*) gene, allelic methylation was progressively lost on culture in about half the lines and methylation changes also occurred in other imprinted genes. We made completely ES-cell-derived embryos to determine whether the methylation changes persist during development, whether they affect imprinted gene expression in the embryo, and whether this leads to abnormal fetal growth. We find that the methylation changes do persist through fetal development and are likely to affect imprinted gene expression. This is because completely ES-cell-derived embryos obtained with higher passage stem cells were frequently abnormally large and displayed other distinct growth abnormalities.

**A locus control region can overcome heterochromatin-mediated position effect variegation in mice**

RICHARD FESTENSTEIN[1], MAURO TOLAINI[1], PAOLA CORBELLA[1], CLIO MAMALAKI[2], JENNY PARRINGTON[2], MARGARET FOX[2], ANTIGONI MILIOU[2], MARGARET JONES[3] and DIMITRIS KIOUSSIS[1]
[1] *Division of Molecular Immunology, NIMR, The Ridgeway, Mill Hill, London NW7 1AA, UK*; [2] *MRC Human Biochemical Genetics Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK*; [3] *Nuffield Department of Pathology, John Radcliffe Hospital, Oxford OX3 9SU, UK*

We have identified sequences within the human CD2 locus control region (LCR) which have no enhancer

activity but are necessary to enabling an open chromatin configuration regardless of the site of chromosomal integration. For this purpose we have taken advantage of the phenomenon of position effect variegation induced by the proximity of genes to centromeric heterochromatin. Transgenic mice carrying an hCD2 minigene attached only to the 3′ transcriptional enhancer exhibited variegated expression when the transgene integrated in the centromere, indicating that this enhancer was not able to overcome the negative effects of centromeric heterochromatin in all cells. In contrast, mice with a centromeric integration site of a transgene containing additional 3′ flanking sequences, with no enhancer activity, showed no variegation of expression. These data provide strong evidence that LCRs operate by ensuring an open chromatin configuration and that a short region with no enhancer activity plays an essential role in increasing the probability of establishing and/or maintaining an open chromatin domain.

**Physical mapping of the murine casein locus**

SISILAMMA GEORGE, ALAN ARCHIBALD and JOHN CLARK
*BBSRC, Roslin Institute, Roslin, Edinburgh EH25 9PS, UK*

The caseins, which constitute the major protein components of milk, are encoded by at least five different genes ($\alpha$, $\beta$, $\gamma$, $\epsilon$ and $\kappa$) in mice. It is known that $\alpha$, $\beta$ and $\gamma$ genes map to mouse chromosome 5. by long-range restriction mapping and pulse field gel electrophoresis we have shown that all five genes are physically linked on a 340 kb *Xho* I fragment. This result also suggests that the murine casein locus may be larger than the bovine locus, which is contained in 200 kb. We have isolated four clones by screening a mouse 129 large fragment genomic library, in the Bacterial Artificial Chromosome (BAC) vector (pBeloBACII). Of the four clones, two contain the $\alpha$, $\beta$ genes, one contains the $\gamma$, $\epsilon$ genes and the other contains only the $\kappa$ gene. Screening the expanded BAC library we isolated four more clones, of which two contain three genes each ($\alpha$b, $\beta$, $\gamma$ and $\gamma$, $\epsilon$, $\kappa$ respectively). One clone contains $\beta$, $\gamma$ genes and the other contains the $\gamma$, $\epsilon$ genes. These clones should allow us to bridge the gaps between the earlier clones and develop a contig map of the locus. The order of the genes in the locus can be deduced as $\alpha$–$\beta$–$\gamma$–$\epsilon$–$\kappa$ from the new clones. Fine mapping of the clones containing $\alpha$- and $\beta$-genes showed that the $\alpha$-gene is ~ 14 kb in size and both genes together comprise ~ 34–35 kb with an inter-genic region of 7–8 kb. Moreover, the genes are in a tail-to-tail orientation ($\beta$ 5′–3′–3′–5 $\alpha$). Long-range PCR analysis showed that the $\kappa$ gene has a size of 10–13 kb.

**Inositol reduces the susceptibility of *curly tail* mutant mice to spinal neural tube defects**

NICHOLAS D. E. GREENE and ANDREW J. COPP
*Developmental Biology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

The *curly tail* (*ct*/*ct*) mouse provides a model system for the study of neural tube defects, which are among the commonest of congenital malformations in humans. Mice homozygous for the autosomal recessive *ct* mutation develop spinal NTD (tail flexion defects and/or spina bifida) in 40–60% of cases. This is due to a cell-type-specific proliferation defect in the gut endoderm and notochord which causes increased ventral curvature of the caudal region of the embryo leading to delayed closure of the posterior neuropore (PNP). In whole embryo culture abnormal *ct* embryos can be recognized on the basis of their enlarged PNP. We show that inositol supplementation of *curly tail* embryos in culture causes a significant reduction in PNP length and this appears to be a specific action in normalizing PNP closure, since no alterations in growth were observed. In addition, treatment with lithium, a known suppressor of inositol phosphate metabolism, counteracts the ameliorating effect of inositol. Labelling embryos with [$^3$H]inositol revealed that uptake is enhanced by supplementation but appears not to be limiting in affected embryos since there is no difference in incorporation into either the lipid- or water-soluble phases. In contrast to culture in hyperglycaemic conditions arachidonic acid, a downstream product of inositol metabolism, does not mimic the effect of inositol. However, another downstream effect, activation of protein kinase C (PKC), does appear to play a role since tissue plasminogen activator (TPA; an activator) mimics the protective effect. TPA and inositol treatment appear to upregulate expression of retinoic acid receptor $\beta$ (RAR$\beta$) in the caudal region (presumably due to PKC activation) and given that upregulation of RAR$\beta$ has already been implicated in the protective effect of retinoic acid treatment on *curly tail* embryos we propose that this pathway is involved in mediating the effect of inositol treatment.

**ME2: a novel receptor expressed in the mouse embryo**

KATERINA HADJANTONAKIS and PETER LITTLE
*Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AZ, UK*

We have identified ME2, a novel protein with homology to several distinct receptor families. ME2

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contains seven putative transmembrane domains, suggesting that it is a G-protein coupled receptor related to the receptors for a variety of regulatory peptides including PACAP, VIP, GHRH, calcitonin and parathyroid hormone. The extracellular region of ME2 contains multiple EGF-like repeats with homology to the Notch/Delta/Serrate family of cell surface proteins. To date only two other seven-pass transmembrane G-protein coupled receptors have been identified with large extracellular domains containing EGF-like repeats, these being EMR-1 and CD97. Unlike EMR-1 and CD97, ME2 also contains a number of cadherin-like repeats, similar to those found in the classic cadherins, protocadherins, desmocollins and the *Drosophila Fat* gene – though only *Drosophila Fat* and ME2 contain both EGF-like and cadherin-like repeats in their extracellular regions. We have shown that *ME2* is a member of a gene family that is conserved in organisms as divergent as humans and *Drosophila*, and that it is the first seven-pass transmembrane receptor shown to be expressed during early vertebrate development. In the mouse embryo, transcripts are first detected before the onset of gastrulation. As gastrulation proceeds, expression of the gene is maintained in the primitive streak region of the embryo. Subsequently transcripts are confined to ectodermal lineages, with the predominant sites of expression being within the developing central nervous system. Interesting features of this very dynamic expression pattern include the delineation of segments in the developing hindbrain and neural tube, whilst in the adult *ME2* transcripts are localized to the brain and eye. We have mapped the *ME2* gene in mouse and man; in both these genomes the *ME2* locus is contained in a region associated with gastrulation and neurulation mutants/disorders.

**Chromosomal mosaicism in human preimplantation embryos**

JOYCE HARPER[1,2], CLARE CONN[1], ALAN HANDYSIDE[2], ROBERT WINSTON[2] and JOY DELHANTY[1]

[1] *Department of Genetics and Biometry, Galton Laboratory, Wolfson House, University College London, 4 Stephenson Way, London NW1 2HE, UK*; [2] *Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK*

Preimplantation diagnosis of inherited disorders can be an alternative to prenatal diagnosis and recurrent abortions for certain couples. Embryos are generated by standard *in vitro* fertilization (IVF) procedures. On day 3 post-insemination, when the embryos have reached the 4- to 10-cell stage, one or two cells can be biopsied and tested to determine the status of the embryo. This procedure is currently used for the detection of the ΔF508 mutation in cystic fibrosis, the sex of embryos for X-linked disease and unbalanced translocations. Since it is difficult to obtain bandable metaphases from these cells, we initially used dual fluorescent *in situ* hybridization (FISH) with probes for chromosomes X and Y to sex embryos for couples at risk of transferring X-linked diseases, where only normal female embryos are transferred to the patient. During this programme we detected a high level of mosaicism in human preimplantation embryos. We extended this work to embryos donated from patients undergoing routine IVF and used autosomal probes as well as probes for the sex chromosomes. More recently we have used multi-colour FISH with probes for chromosomes X, Y and 1 for the preimplantation diagnosis of sex, the additional autosomal probe helping to ensure the most normal embryos are replaced. Collectively this work has shown that four groups of chromosomal arrangements exist in human preimplantation embryos: (a) embryos that are normal diploid in all cells examined, (b) embryos showing uniform aneuploidy in all cells, such as XO, XXX or XXY, (c) mosaic embryos which are mainly diploid with a few cells being aneuploid, and (d) embryos where every cell in the embryo shows a different chromosome complement, which we have termed 'chaotic'. Even though an XX cell has always been representative of a female embryo and so a misdiagnosis of sex would be unlikely, these findings have important consequences for the diagnosis of chromosome abnormalities such as unbalanced translocations. In our first series of preimplantation diagnosis of abnormal karyotypes (parents t(13;14), t(6;21) and gonadal mosaic for chromosome 21) we detected the same four groups of chromosome arrangements, with obviously a higher proportion of embryos being uniformly abnormal, e.g. trisomy 21. These findings have important consequences for preimplantation diagnosis and early human development.

**A *cdc* (cell division cycle)-like gene located on human chromosome 2q21**

CLARE B. HARVEY[1], YANGXI WANG[1], NED MANTEI[2] and DALLAS M. SWALLOW[1]

[1] *MRC Human Biochemical Genetics Unit, The Galton Laboratory (UCL), 4 Stephenson Way, London NW1 2HE, UK*; [2] *Department of Neurobiology, ETH-Hönggerberg, CH-8093 Zurich, Switzerland*

In proliferating cells various factors are required to ensure that the genome is replicated once and with fidelity before the cell divides. It has been suggested that 'licensing factors' are required. Candidates for such a role are some of the so-called cell division cycle (*cdc*) and mini-chromosomal maintenance (*mcm*) genes in yeast, in particular, *mcm2/3/5*, *cdc46* and

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*cdc21*. These appear to belong to a family of evolutionarily related genes and occur in a wide range of species. These genes contain the motif IDEFDKM in a 200 amino acid domain which has been suggested to have ATP-dependent DNA binding activity. We have identified three exons of what may be new human member of this gene family. These sequences reside on the same genomic clone as the first exon of the lactase gene, which maps to human chromosome 2q21. The exons were identified by their similarity to expressed sequence tagged sites (ESTs) in the databases and to a recently reported rat cDNA isolated from intestinal crypts. Two of the human exons show more than 90% identity at both the protein and nucleotide level to part of the 3′ end of the rat sequence. The 5′ ends of the rat sequence and of one of the overlapping ESTs extend into the conserved domain of the *cdc46/21/mcm2/3/5* gene family. Semi-quantitative RNA-PCR was used to study the tissue distribution. Like lactase, the transcript is expressed at high levels in human intestine, but unlike lactase it is expressed in all the fetal tissues so far tested and in some adult tissues, including muscle and testis. So far this pattern of expression does not give clear indications of the likely role in the cell division cycle, but further work in progress will provide more information on the pattern of expression and the existence of other members of the gene family.

**A methylation-dependent DNA-binding activity specific for the gametic methylation imprint in the promoter region of the mouse *Xist* gene**

JOHN HUNTRISS, ROBERTO LORENZI, MAURIZIO ZUCCOTTI and MARILYN MONK
*Molecular Embryology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK*

Gametic imprinting of the regulatory mouse *Xist* gene involves two adjacent CpG sites in the promoter region which are methylated in the egg but not in the sperm. Differential methylation at these sites in sperm and egg correlates with exclusive expression of the paternal allele of the *Xist* gene in preimplantation development and paternal X-inactivation in extra-embryonic tissues of the female mouse embryo. Using an oligonucleotide containing these two CpG sites as a probe in band shift assays, we have demonstrated the presence of a sequence-specific methylation-dependent binding activity to the methylated *Xist* promoter. This activity, termed the *Xist* imprint–methylated DNA binding protein (XI–MDBP), was observed in nuclear extracts from embryonic stem cells but was not present in male or female somatic tissues. In agreement with bandshift data, Southwestern blotting identifies a protein in embryonic stem cells with an approximate molecular weight of 102 kDa that binds strongly to the methylated *Xist* promoter oligonucleotide but only weakly to the unmethylated oligonucleotide. Competition experiments with methylated or ummethylated heterologous oligonucleotides that comprise the consensus binding site sequences of the transcription factors SP1 and AP1 demonstrate that the XI–MDBP is sequence specific and methylation specific. The binding of XI–MDBP to the methylated maternal *Xist* promoter region suggests a role for this protein in the repression of transcription from the maternal allele or in the prevention of erasure of preimplantation methylation from these critical imprinted CpG sites.

**The developmental potential of mouse cells that are deficient in glycolysis**

ANNEMARIE KELLY and JOHN D. WEST
*Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK*

Glucose phosphate isomerase (GPI) is an important glycolytic enzyme which catalyses the interconversion of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P). Therefore, its activity, coupled with other factors, determines the amount of G6P that enters the glycolytic pathway. Mouse embryos, homozygous for a null allele of GPI, failed to complete gastrulation and were resorbed by 11·5 days *post coitum* (d.p.c.). Aggregation chimaeras were produced between homozygous GPI null embryos and wild-type mouse embryos to examine whether homozygous GPI null cells could survive for longer when combined with normal cells. Homozygous GPI null cells were identified by *in situ* hybridization to a transgenic lineage marker and fetal homozygous GPI null chimaeras were identified at 12·5 d.p.c. A series of chimaeras was allowed to develop to term and 11 were produced. One female was identified as a homozygous GPI null chimaera, indicating that homozygous GPI null cells can be rescued and survive in adult chimaeras. *In situ* hybridization also revealed the presence of GPI null oocytes in the chimaeric ovary. Breeding data from this female confirmed that she was a null chimaera and showed that homozygous GPI null oocytes survive and are functional in aggregation chimaeras.

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