Abstracts of papers presented at the tenth Mammalian Genetics and Development Workshop (Incorporating the Promega Young Geneticists' Meeting) held at the Institute of Child Health, University College London on 17–19 November 1999

Edited by: ANDREW J. COPP[1] AND ELIZABETH M. C. FISHER[2]
[1]*Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK*
[2]*Neurogenetics Unit, Imperial College School of Medicine, St Mary's Hospital, Norfolk Place, London W2 1PG, UK*

Sponsored by: The Genetical Society, Promega (UK) Ltd and B&K Universal Group Ltd

Evidence for imprinting in type 2 diabetes: detection of parent-of-origin effects at the insulin gene

STEWART HUXTABLE, PHILIP SAKER, LEMA HADDAD, ANDREW HATTERSLEY, MARK WALKER and MARK McCARTHY
Section of Endocrinology, Imperial College School of Medicine, St Mary's Hospital, London, UK

Variation at the insulin gene (*INS*) VNTR is implicated in type 1 diabetes, polycystic ovarian syndrome and birthweight. Case-control studies inconsistently show class III VNTR association with type 2 diabetes, but may result from population stratification. We applied family-based association methods to 155 parent–offspring trios, ascertained via a Europid proband with type 2 diabetes. VNTR class was inferred from the –23*Hph*I SNP which is in tight linkage disequilibrium in Caucasians. Of 119 heterozygous parents, 65 transmitted class III and 54 class I ($P = 0{\cdot}16$). In 62 maternal transmissions, there was no deviation from expectation (26 vs 36, $P = 0{\cdot}21$). Fathers, in contrast, displayed excess class III transmission (39 vs 18, $P = 0{\cdot}003$). This parent-of-origin difference was clearly attributable to imprinting ($P = 0{\cdot}001$, parent-of-origin LRT). The *Fok*I SNP 3′ to INS distinguishes two class III haplotypes (VPH, PH) in type 1 diabetes. Only the VPH haplotype showed excess transmission to diabetic offspring ($P = 0{\cdot}02$), suggesting a complete reversal of type 1 diabetes susceptibility haplotypes in type 2 diabetes. Using family-based association methods, we have demonstrated that variation within the INS region influences type 2 diabetes susceptibility. This effect is mediated exclusively by paternally derived VNTR alleles, implicating imprinted genes (e.g. IGF2) in the pathogenesis of type 2 diabetes.

Identification of a locus for primary ciliary dyskinesia on chromosome 19

S. L. SPIDEN[1], M. MEEKS[1], A. J. WALNE[1], H. BLAU[2], H. MUSSAFFI-GEORGY[2], H. SIMPSON[3], M. EL FEHAID[4], M. CHEEBAB[4], M. AL-DABBAGH[4], H. D. HAMMUM[4], R. M. GARDINER[1], E. M. K. CHUNG[1] and H. M. MITCHISON[1]
[1]*Department of Paediatrics, Royal Free and University College Medical School, University College London, UK;* [2]*Schneider Children's Medical Center of Israel, Petech Tikva, Israel;* [3]*School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia;* [4]*Riyadh Al Kharj Hospital Programme, Saudi Arabia*

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder with an incidence of 1:20000 characterized by sinusitis, recurrent pulmonary infection, bronchiectasis and subfertility. Approximately 50% of patients also have laterality defects, most commonly situs inversus (Kartegener syndrome). Patients have reduced or absent cilia motility, and a wide range of abnormalities in the ultrastructure of the cilia are associated with this phenotype. Six families of Arabic origin were ascertained with a ciliary ultrastructural defect of absent outer dynein arms. The resource in total contains 37 individuals with 13 affected children, 4 of whom also have situs inversus. In five families the parents were first cousins, so a genome-wide linkage search was undertaken using homozygosity mapping. A region of excess homozygosity spanning 13 cM was identified on chromosome 19q13.4-qter, below marker *D19S418*. Haplotype analysis is consistent with linkage in four of the families. GENEHUNTER analysis gives a maximum multipoint lod score of 4·73 with an alpha of 0·7. This is the first definitive report of linkage for a PCD locus and work is under way to refine the critical region in order to identify potential candidate genes.

The molecular evolution of blood coagulation

COLIN DAVIDSON, EDWARD D. G. TUDDENHAM and JOHN H. McVEY
Haemostasis Research Group, MRC Clinical Science Centre, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

Vertebrate blood coagulation is a delicately balanced process whose central feature is the conversion of fibrinogen to fibrin by the protease thrombin. The plasma coagulation factors, factor VII (FVII), factor IX (FIX), factor X (FX), and prothrombin (PT) are zymogens of serine proteases. They are each activated by limited proteolysis of one or two peptide bonds. To be physiologically effective FXa and FIXa require assembly into complexes on phospholipid surfaces with non-enzymatic cofactors factor Va (FVa) and factor VIIIa (FVIIIa). The modular nature of the enzymes and cofactors indicates that the proteins evolved by domain duplication and exon shuffling and the organization and structure of the genes suggest that vertebrate haemostasis evolved as a result of gene duplication events. When did these gene duplication events occur? Do lower vertebrates have a subset of blood coagulation factors? To investigate these questions the FVII, FX and FIX gene duplication events were examined in chicken, the pufferfish *Fugu rubripes*, and lamprey *Lampetra fluviatilis* by database mining, degenerate PCR cloning and genomic library screening. Using this approach a genomic cosmid clone containing the chicken FVII and FX genes was isolated and characterized. The chicken FVII and FX genes were contained on one cosmid separated by $\sim 3{\cdot}0$ kb as found in mouse and man. The proximal promoters of the human FX and FVII have been characterized, including the 3 kb intergenic region. The tight linkage of FVII and FX genes was investigated for a functional role in coordinated gene regulation by comparative approach between the chicken and human intergenic regions in an attempt to identify novel transcription factor binding sites. Partial cDNA clones of FVII, FX and FIX have been isolated from chicken, pufferfish and lamprey liver RNA. The occurrence of coagulation factors VII, X and IX in Agnatha (jawless vertebrates) supports the hypothesis that extensive genome duplication took place early in vertebrate evolution (2R hypothesis). It appears the coagulation cascade evolved during the ~ 50 million years between the divergence of protochordates and the appearance of Agnatha and indicates a flurry of gene duplication and exon shuffling during this relatively brief evolutionary period.

Minisatellite mutation and meiotic crossing over are independent in mice

RUTH BARBER[1], YURI E. DUBROVA[1,2], MARK PLUMB[3] and ALEC J. JEFFREYS[1]
[1]*Department of Genetics, University of Leicester, Leicester LE1 7RH, UK;* [2]*N.I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow B-333, Russia;* [3]*Medical Research Council Radiation and Genome Stability Unit, Harwell, Oxon OX11 0RD, UK*

The ability of minisatellite loci to reflect radiation-induced structural damage in DNA makes them a useful marker for radiation exposure. However, the application of minisatellites as an indicator of environmental exposure is currently limited as the mechanisms of mutation induction at minisatellite loci remain unknown. A possible explanation for the elevated minisatellite mutation rate after exposure to ionizing radiation is that this is a reflection of a genome-wide increase in meiotic crossing over. To investigate whether this hypothesis is correct, we have simultaneously analysed the frequency of minisatellite mutation rate and crossover frequency in the offspring of male mice exposed either to 1 Gy of acute X-rays or to the anticancer drug cisplatin. The results of our study show that cisplatin has no effect on either minisatellite mutations or crossover frequencies during the most important stages of mouse spermatogenesis. In contrast, whilst crossover frequencies are unaffected, both pre-meiotic and meiotic exposure to ionizing radiation results in a dramatic increase in minisatellite mutation rates. The uncoupling of induced minisatellite instability and crossover frequency as demonstrated in the radiation study suggest that meiotic crossing over and minisatellite mutation in exposed mice are entirely independent processes. This may reflect the repair of double-strand DNA breaks prior to meiosis, as such damage would need to be present during meiotic division for an increase in legitimate crossover frequency to occur.

Genomic organization of the GART-SON-3SG locus; three genes in close proximity on chromosome 21

S. L. WYNN, R. A. FISHER, Q. LIU, D. WELLS, C. PAGEL, I. M. KHAN, P. ZAMMITT and L. BULUWELA
Gene Targeting Unit, Department of Neuromuscular Diseases, Imperial College School of Medicine, St Dunstan's Road, London W6 8RP, UK

The *SON* gene encodes a novel regulatory protein on human chromosome 21 within the Down syndrome

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

Critical Region. We have investigated the organization of the *SON* locus in both mouse and human genomes. The mouse *Son* gene spans a region of approximately 35 kb. The coding region is more than 8 kb in length and has been completely sequenced. The gene is organized into 11 coding exons and 1 non-coding 3′UTR exon, with over 70% of the coding region residing in one 5·7 kb exon. The gene also contains at least two alternative exons, N/C exon 1 and A/C exon 1, which can be used, by splicing, to generate truncated forms of protein. Further investigation of the mouse *Son* locus has identified the genes directly flanking *Son*. The glycinamide ribonucleotide formyltransferase (*Gart*) gene is encoded 5′ of *Son* in a head-to-head arrangement, with an intergenic region of 899 bp. Sequence comparison with the expressed sequence tagged (EST) database identified a novel gene, *3′Son* gene (3sg), within 65 bp of the 3′ end of *Son*. Gene expression and transgenic studies have been carried out in order to address the extent of co-regulation within this gene cluster.

Mapping of Loa, a mouse motor deficit mutation, to distal chromosome 12

A. S. WITHERDEN[1], S. J. NICHOLSON[1], M. HAFEZPARAST[1], N. A. BERMINGHAM[1,5], J. PETERS[2], S. T. BALL[2], D. C. ROGERS[3], J. E. MARTIN[4] and E. M. C. FISHER[1]

[1]*Department of Neurogenetics, Imperial College School of Medicine at St Mary's, Norfolk Place, London W2 1PG, UK;* [2]*Mammalian Genetics Unit, Medical Research Council, Harwell, Didcot, Oxon OX11 0RD, UK;* [3]*Smithkline Beecham, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK;* [4]*Department of Morbid Anatomy, The Royal London Hospital, Whitechapel, London E1 1BB, UK;* [5]*Howard Hughes Medical Institute, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA*

Motor neuron disease is a progressive genetic disorder with 5 sufferers per 100000 people in Europe and the UK. Approximately 20% of these cases are familial with predominantly autosomal dominant inheritance but, with the exception of SOD1, the causal genes remain unknown. Our mouse model of motor neuron degeneration will provide access to new genes involved in the pathology of these cells. The mutant mouse called legs at odd angles (Loa) has a dominantly inherited motor neuron dysfunction. Heterozygotes have an early/mid-onset phenotype characterized by progressive loss of motor function in the hind limbs, with vacuolation of the lower motor neurons. There is no evidence of muscle pathology and heterozygotes have a normal life span. However, Loa is lethal in homozygous mice within 24 hours of birth. To map and subsequently clone the Loa gene, a large intraspecific backcross between the mutant mouse and the inbred strain C57BL/6 was set up. Affected N1 mice were backcrossed to C57BL/6 to generate over 1000 affected N2 animals. These were used to genetically map the Loa mutation to a 1·6 cM region of distal MMU12 flanked by *D12Mit17* and *D12Mit181*. We are constructing a contig by screening the WI/MIT820 mouse YAC library with existing markers from this region and STSs that we generate from the YACs. These YACs are also being used to isolate polymorphic microsatellites which can be genetically mapped and thereby used to narrow down the critical region. Comparative mapping between mouse and human genomes demonstrates a syntenic region at HSA14q32. Online databases are monitored for mouse and human genes near or within the critical region. Exon trapping and cDNA selection methods will be employed to isolate candidate genes. Any genes that map to the critical region will be further analysed and sequenced in affected and wild-type mice and the Loa gene identified.

Characterization of a mouse model of neuromuscular disease generated by transgenic insertional mutagenesis

DEREK PAISLEY and ANDREW WARD

School of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK

In a screen of 78 lines of transgenic mice for insertional mutant phenotypes, amongst a number of lines identified, one displayed a striking neuromuscular disorder. Homozygotes are smaller than their siblings, exhibit progressive ataxia and tremor, have cerebellums of reduced size and die between P19 and P25. A combination of fluorescence *in situ* hybridization (FISH) and genetic mapping with microsatellite markers has located the multiple-copy transgene array at the mid-region of chromosome 4 – extremely close to the classical mouse mutant scrambler. The scrambler mutant results from aberrant splicing of the mouse disabled homologue gene, *mdab1*, and displays a phenotype which closely resembles that of our insertional mutant. Northern analysis of *mdab1* expression, coupled with mapping and phenotypic data, suggests that we have identified a new allele of the *mdab1* gene.

A mutation in the basolateral secretory Na^+-K^+-2Cl^-cotransporter gene *slc12a2* causes deafness and vestibular dysfunction in the mouse

JAMES GAZZARD[1], SHAZIA S. CHAUDHRY[2], NATALIE SIMPSON[2], BRADLEY A. SCHULTE[3], MICHAEL J. DIXON[2] and KAREN P. STEEL[1]
[1]*MRC Institute of Hearing Research, University Park, Nottingham NG7 2RD, UK;* [2]*School of Biological Sciences and Department of Dental Medicine and Surgery, 3.239 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK;* [3]*Department of Pathology and Laboratory Medicine and Department of Otolaryngology and Communicative Sciences, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA*

The mouse mutant shaker-with-syndactylism (sy) is profoundly deaf and displays systemic skeletal abnormalities. We genetically mapped fused phalanges (syfp), a hearing allele of sy. The markers *D18Mit124*, -52, and the gene *s1c12a2* are non-recombinant with syfp. The original sy allele contains a $\sim 0{\cdot}7$ cM deletion. Analysis of PCR amplification products indicated that primers designed to *s1c12a2* were deleted from the genomic DNA of sy. *s1c2a2* encodes a Na^+-K^+-2Cl^- cotransporter important in auditory and vestibular K^+ recycling, no syndactylism (syns), an allele of sy that is deaf but does not have a skeletal phenotype, was found to contain the mutation nt2955 ins(A) in exon 21 of *s1c12a2*. This mutation causes a frameshift with the introduction of a termination codon after 13 amino acid residues. Histological analysis of 1-month-old syns homozygous mutant inner ears revealed a phenotype consistent with abnormal endolymph production. Reissner's membrane and the walls of the vestibular compartments had collapsed. Severe abnormalities were observed in the cells of the organ of Corti and the stria vascularis, syns mutants also displayed reduced lumen diameter of the semicircular canals. Mutation of *s1c12a2* results in deafness in the mouse; therefore the human orthologue of *s1c12a2*, when mutated, may cause deafness in the human population.

Supported by the MRC.

Different roles for *Pax6* in optic vesicle and facial epithelia mediate early morphogenetic events during murine eye development

J. MARTIN COLLINSON[1], ROBERT E. HILL[2] and JOHN D. WEST[1]
[1]*Edinburgh University, Department of Reproductive and Developmental Sciences, Anatomy Building, Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland, UK;* [1]*MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, UK*

Chimaeric mice were made by aggregating *Pax6*-null and wild-type 8-cell mouse embryos, in order to study early stages of development. Histological analysis of the distribution of $-/-$ cells at E9.5, during the interaction between the optic vesicle and the prospective lens epithelium, showed: (1) During lens development *Pax6* is required prior to placode formation; most $-/-$ cells are eliminated from an area of facial epithelium which is wider than that which will go on to form the lens. (2) There is segregation of $-/-$ and $+/+$ cells in the optic vesicle at E9.5, most likely a result of different adhesive properties of wild-type and mutant cells. (3) there is a role for *Pax6* during proximo-distal patterning of the optic vesicle. (4) *Pax6* is required in the optic vesicle to maintain contact with the overlying lens epithelium and thus $-/-$ optic vesicles are inefficient at inducing a lens placode. Together, these results demonstrate requirements for functional *Pax6* in both the optic vesicle and facial epithelia in order to mediate the interactions between the two tissues during the earliest stages of eye development.

***HIRA*, a putative transcriptional regulator, is critical for mammalian development**

HANNAH FARMER, ROBIN BUCKLE, CATHERINE ROBERTS and PETER SCAMBLER
Institute of Child Health, 30 Guilford Street, London WC1 1EH, UK

HIRA was isolated during positional cloning of the region of chromosome 22q11 commonly deleted in DiGeorge Syndrome (DGS). DGS is a complex developmental anomaly, thought to arise from the failure of neural crest cells to migrate or interact properly during early development. *Hira* expression is particularly high in the developing neuroepithelium and rostral neural crest, and plays a critical role during development, since *Hira* knockout mice are embryonic lethal by E10.5 with a wide range of malformation. *HIRA* encodes a WD40 repeat protein with strong similarity to two yeast transcriptional co-repressors HIR1/HIR2. Predicted to function as part

of a protein complex, *HIRA* was used in a yeast two-hybrid screen of an early mouse embryonic library. Interactors include homeodomain-containing transcription factors *Pax3* and *Pax7*, as well as core histones H3 and H2B. *Pax3* was of particular interest because of its known role in development where it is expressed in the neural crest and derived lineages. *Pax3* mutations in mice give rise to the Splotch phenotype, where homozygotes show features of DGS. We have mapped the *Pax3* interaction domain, and have shown that *HIRA* can stimulate *Pax3*-driven expression from the MITF promoter, a target of *Pax3*, in cell transfection experiments. Our data suggest that *HIRA* regulates transcription, possibly by modulating chromatin structure at target promoters.

Stat3b supports embryonic stem cell self-renewal

CRAIG STRACEY, TOM BURDON and AUSTIN SMITH
Centre for Genome Research, University of Edinburgh, UK

The propagation of undifferentiated embryonic stem (ES) cells in culture is dependent on the presence of LIF or related cytokines. Such factors act through a common transmembrane signal transducer, gp130. The signalling pathways downstream of gp130 lead to the activation of several signalling intermediates. Of these, activation of the latent transcription factor Stat3 has been shown to be essential for signalling ES cell self-renewal. The Stat3 gene encodes two proteins, Stat3a and Stat3b, generated via an alternative splicing event. The two molecules share the first 715 amino acids, with the C-terminal 55 amino acids of Stat3a being replaced by 7 alternative amino acids in Stat3b. In this study the role of Stat3 variants in signalling self-renewal has been investigated by overexpression in ES cells. It has previously been demonstrated that Stat3b displays prolonged activation and has distinct activity from Stat3a. We have shown that overexpression of Stat3b is sufficient to sustain self-renewal in the absence of added LIF, whilst Stat3a overexpression has no effect. This activity is unique to Stat3B as expression of a truncation of Stat3a to amino acid 715 drives differentiation of ES cells. This is the first demonstration of a biological role for Stat3b.

Re-replication in fission yeast arises from uncontrolled origin firing

STEPHANIE K. YANOW, M. A. NASSER HAJIBAGHERI and PAUL NURSE
Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, UK

In fission yeast, S phase can be uncoupled from mitosis resulting in multiple rounds of DNA replication. The gene *Cdc18+* has been isolated in our laboratory and when overexpressed results in cells persisting in S phase with increasing DNA content. This gene has been identified as a key component of the complex that regulates the onset of DNA synthesis in yeast and other eukaryotes. However, its specific function in the initiation of replication remains unclear. We have directed our studies toward understanding how *Cdc18* is capable of disrupting the normal cellular controls ensuring that each segment of DNA is replicated once and only once per cell cycle. To establish the effects of high levels of *Cdc18* on genomic replication we have examined these re-replicating cells by electron microscopy. As *Cdc18* protein accumulates we detect a dramatic increase in the frequency of initiation events on chromosomal DNA. Most notably, we have observed multiple initiations within enlarging bubbles suggesting that the increased ploidy in these cells arises from sequential re-initiation of origins. Also striking is the high frequency of clusters of replication bubbles simultaneously elongating along short stretches of DNA. To ascertain whether these new initiation events are specific to origins of replication, we have used neutral/neutral 2D gel electrophoresis to examine the firing efficiency at a specific origin when *Cdc18* is overexpressed. Using this technique we have found that in conjunction with the abnormal initiation events observed by electron microscopy we detect a novel replication intermediate present at this locus which persists as ploidy increases. This structure migrates in the gel with increased mass and complexity, suggesting it could correspond to a bubble-within-bubble or complex branch structure. We have also shown that this structure is specific to *Cdc18*-induced re-replication and is not present in cells undergoing multiple complete rounds of S phase.

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

Quantification of hypoxanthine phosphoribosyl transferase mRNA in individual human oocytes and embryos using competitive RT-PCR

DEBORAH M. TAYLOR, ASANGLA AO, ROBERT M. L. WINSTON and ALAN H. HANDYSIDE
Department of Reproductive Sciences and Medicine, Division of Paediatrics, Obstetrics and Gynaecology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

Preimplantation genetic diagnosis (PGD) of inherited disease allows the selective transfer of unaffected embryos therefore avoiding the possibility of termination of an affected pregnancy following conventional prenatal diagnosis. Diagnosis based on PCR of gDNA from single cells is susceptible to amplification failure, contamination and allele drop-out. RT-PCR offers an attractive alternative with the availability of multiple templates. However, it is crucial that embryonic rather than maternal transcripts are analysed. We have quantitatively characterized *HPRT* gene expression in individual oocytes and embryos throughout human preimplantation development. The amount of *HPRT* transcripts decreased significantly ($P = 0{\cdot}0006$) from 7 fg of cDNA in the unfertilized oocyte ($n = 7$) and pronucleate zygote stage ($n = 8$) to 1·2 fg at the 4-cell stage ($n = 7$) and did not increase at the 8-cell stage ($n = 7$) or the blastocyst stage ($n = 5$). This drop presumably reflects the degradation of the oocyte-derived maternal message and coincides with the time at which global embryonic gene activation occurs in the human. No loss of oocyte-derived transcripts occurs over the same period *in vitro* culture in the absence of fertilization and cell cleavage.

Chromosome analysis of human day 5 embryos

P. RUANGVUTILERT, J. D. A. DELHANTY, P. SERHAL, M. SIMOPOULOU and J. C. HARPER
Department of Obstetrics and Gynaecology, University College London, 86–96 Chenies Mews, London WC1E 6HX, UK

From studies using fluorescence *in situ* hybridization (FISH) analysis on human cleavage embryos, four groups of chromosome patterns have been classified: uniformly normal, uniformly abnormal, mosaic and chaotic. Analysis of blastocyst chromosomes with conventional karyotyping has shown that all four groups of chromosome patterns are also observed, even though only up to 6 nuclei were analysed per blastocyst. In the present study, human embryos were cultured from day 3 to 5 in extended cultured medium and were analysed by a two-round multicolour FISH procedure to ensure that almost every nucleus was examined. Probes for chromosomes X, Y and 18 were used in the first round and images captured on a cooled CCD camera. In the second round, probes for chromosomes 13 and 21 were used and the images recaptured to ensure accurate analysis of each nucleus. Nineteen arrested embryos and 12 embryos that had reached the blastocyst stage with more than 30 nuclei were analysed. Only 3 arrested embryos were uniformly diploid and 1 blastocyst considered borderline normal. The remaining 27 embryos were mosaic. For preimplantation genetic diagnosis, this high prevalence of mosaicism that persists to the blastocyst stage may pose similar problems to the mosaicism seen in cleavage-stage embryos.

Studies of 'sex body' proteins in male and female meiosis suggest functions unrelated to meiotic sex chromosome inactivation (MSCI)

JAMES TURNER[1], SHANTHA MAHADEVAIAH[1], HENRI-JEAN GARCHON[2], RICARDO BENAVENTE[3], HILDO OFFENBERG[4], CHRISTA HEYTING[4] and PAUL BURGOYNE[1]
[1]*Laboratory of Developmental Genetics, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK;* [2]*INSERM Unit 25, Hôpital Necker, Paris Cedex 15, France;* [3]*Department of Cell and Developmental Biology, Theodor Boveri-Institute (Biocenter), University of Würzburg, Am Hubland, D-97074 Würzburg, Germany;* [4]*Department of Genetics, Agricultural University, Dreijenlaan 2, NL-6703 HA Wageningen, The Netherlands*

During male meiosis the asynapsed regions of the X and Y chromosomes are inactivated (MSCI) and the 'XY' or 'sex body' has been viewed as the morphological manifestation of MSCI. An increasing number of proteins have been found to locate to the sex body during pachytene and diplotene of male meiosis, which is well after MSCI is initiated; this suggests they may have roles unrelated to MSCI. We have compared the expression of three of these proteins during male meiosis with that in XY female meiosis. One of these proteins, XY77, proved to be restricted to the sex body in males. However, a protein detected by an antibody against Xlr, together with a protein detected by a recently identified antibody (2E3), were found associated with the asynapsed X (but not the Y) of XY pachytene oocytes in the absence of MSCI. These proteins also associated with asynapsed autosomal axes in mice carrying an autosome–autosome translocation. We conclude that

these latter two proteins locate to the sex body during pachytene simply because of the presence of asynpased axes. Possible independent roles for MSCI and these sex body proteins will be discussed.

Haplotype transmission distortion and evidence for linkage of the *CHRNA7* gene region to schizophrenia in Southern African Bantu families

BRIEN P. RILEY[1], MPHALA MOGUDI-CARTER[2], TREFOR JENKINS[3], ROBERT WILLIAMSON[4], DAVID COLLIER[1] and ROBIN MURRAY[1]
[1]*Department of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK;* [2]*Department of Psychiatry, Baragwanath Hospital, Soweto, South Africa,* [3]*Department of Human Genetics, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa,* [4]*The Murdoch Institute, Royal Children's Hospital, Parkville, Melbourne, Australia*

Recent reports have strongly linked markers near the a-7 nicotinic cholinergic receptor subunit gene on human chromosome 15q13–q14 to a sensory gating deficit common in schizophrenics, and have shown positive though non-significant results linking this region to the primary phenotype of schizophrenia in a sample of North American families. We therefore tested for linkage between markers in this region of chromosome 15q and schizophrenia in a sample of 16 families multiply affected with schizophrenia drawn from the Bantu-speaking black population of South Africa. An initial replication using markers from the original study gave an affected-only LOD score maximum of 1·08 under a recessive model at $Q = 0{\cdot}00$ for D15S1360, a dinucleotide polymorphism found on the same YAC as the a-7 receptor gene. Non-parametric affected-only multipoint analysis gave a Z-score of 1·29, $P = 0{\cdot}098$, for D15S1360, and $Z = 1{\cdot}45$, $P = 0{\cdot}075$ for D15S118. We then increased the resolution of the map with an extended set of 20 markers. Again, two peaks were observed, with NPL scores of 1·81, $P = 0{\cdot}037$, at D15S1043 and 1·79 at D15S1360 and 1·80 at D15S1010, both $P = 0{\cdot}037$. Transmission disequilibrium testing of data from D15S1360 gave an allele-wise and genotype-wise c2 of 6·59, 2 d.f. $P = 0{\cdot}037$. Haplotype transmission disequilibrium testing using a restricted allele and haplotype set from D15S1043 and D15S1360 gave a global c2 of 10·647, 4 d.f., $P = 0{\cdot}007$, and a maximum c2 of 6·567, 1 d.f., $P = 0{\cdot}004$ for excess transmission of the 1·2 haplotype into affected offspring.

Variation in alphoid DNA and trisomy 21; a cause of nondisjunction?

KLIO MARATOU, YASMEEN SIDDIQUE, ANNA M. KESSLING and GAIL E. DAVIES
Academic Unit of Medical and Community Genetics, Imperial College of Science, Technology and Medicine, London, UK

The cause of nondisjunction of chromosome 21 remains largely unknown. The α-satellite (alphoid) DNA family is a class of tandemly arranged repetitive DNAs, which appears to be important for centromere function or formation. We hypothesize that variation in alphoid DNA structure has a role in trisomy formation. The chromosome 21 and 13 α-satellite subfamilies share 99·77% sequence homology. We have developed novel methodology to identify specifically the chromosome 21 subfamily and determine whether any specific patterns of variation are associated with the nondisjoining chromosomes 21. The combined alphoid size of both chromosome 21 homologues was examined in 23 maternal meiosis I families with an affected child with trisomy 21 and 38 controls. Data were divided into nondisjoining mothers, disjoining father, female controls and male controls. Analysis based on parametric (Games–Howel method) and nonparametric (Wilcoxon two-sample test) techniques showed statistically significant differences in combined alphoid size between nondisjoining mothers and female controls, and nondisjoining mothers and all controls. This preliminary evidence indicates a potential role for alphoid size in predisposition to nondisjunction and further supports the role of alphoid DNA in correct chromosome segregation.

Mutations in a novel gene in Ellis van Creveld syndrome and Weyers acrofacial dysostosis

VICTOR L. RUIZ-PEREZ[1], SUSAN E. IDE[3], TIM STROM[2], BETTINA LORENZ[2], DAVID WILSON[3], MICHAEL WRIGHT[3], CLAIR FRANCOMANO[4], MIHAEL H. POLYMEROPOULOS[3], THOMAS MEITINGER[2] and JUDITH GOODSHIP
[1]*School of Biochemistry and Genetics, Newcastle University, UK;* [2]*Department of Medical Genetics, Ludwig-Maximilians-Universität, Munich, Germany;* [3]*Pharmacogenetics Division, Novartis, USA;* [4]*National Human Genome Research Institute, USA*

Ellis van Creveld (EvC) syndrome [MIM225500] is an autosomal recessive skeletal dysplasia characterized by short limbs, short ribs and postaxial polydactyly.

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

Congenital cardiac defects occur in 60% of affected individuals, with a defect of primum atrial septation often leading to a common atrium. The disorder was mapped to chromosome 4p16 in Amish, Mexican, Ecuadorean and Brazilian families. We narrowed the critical region by homozygosity mapping and now report mutations in a novel gene, encoding a 992 amino acid protein. We have observed expression around fetal vertebrae and upper limb bones and also in the heart and kidney. The mutations we have seen include five truncating mutations, four of which are homozygous. A homozygous amino acid deletion DK302 was found a child of European gypsy origin. There has been debate about clinical manifestations in heterozygous carriers and it has also been noted that Weyers acrofacial dysostosis [193530], an autosomal dominant disorder with similar but milder phenotype, maps to 4p16. It was therefore particularly interesting to find mutations in a child with EvC syndrome whose father has Weyers dysostosis. This child is a compound heterozygote with a frameshift on the maternal allele and an amino acid change S307P inherited from the father.

A possible new method for detecting congenital heart defects prenatally?

MARIA KOLATSI-JOANNOU, DEBORAH J. HENDERSON and ANDREW J. COPP
Neural Development Unit, *Institute of Child Health*, *University College London*, *30 Guilford Street*, *London WC1N 1EH*, *UK*

Conotruncal heart defects account for up to 20% of congenital heart malformations and require surgery within the first few months of birth. These heart defects can only be detected by specialized ultrasound quite late in pregnancy. To date there are no biochemical tests (comparable to alpha-fetoprotein for neural tube defects) that can detect congenital heart defects. We showed previously (Henderson *et al.* (1997), *Mech. Dev.* **69**, 39–51), that homozygous splotch (Sp2H) embryos overexpress a proteoglycan of the extracellular matrix called versican. These homozygous splotch embryos not only have open neural tube defects, but also develop conotruncal heart defects as a result of failure of cardiac neural crest cells to colonize the developing outflow tract. We hypothesized that excess versican may be detectable in the amniotic fluid of splotch homozygotes, perhaps providing a means of identifying early embryos developing conotruncal heart defects. To test this idea, we have produced antibodies to a region that is highly specific to the versican molecule. We are currently devising an enzyme-linked immunosorbent assay (ELISA) that may provide a novel diagnostic tool to be used in future to detect congenital heart anomalies in humans.

IGF-II ameliorates the dystrophic phenotype

JANET SMITH[1], ANDREW WARD[2], CRAIG GOLDSMITH[1], FLORINE DE HAAS VAN DORSSER[1] and RIFCA LE DIEU[1]
[1]*School of Biosciences*, *University of Birmingham*, *Edgbaston*, *Birmingham B15 2TT*, *UK*; [1]*School of Biology and Biochemistry*, *University of Bath*, *Bath BA2 7AY*, *UK*

Duchenne muscular dystrophy (DMD) is a congenital disease with an early onset of severe crippling phenotype and fatality in the late teenage years [1]. The classic histopathological features are reproduced in the animal model for this disease, the mdx mouse [2]. A key feature of these muscles is up-regulated cell and muscle fibre turnover, which includes high levels of programmed cell death (PCD). Previously we have shown that PCD is down-regulated by the peptide growth factor IGF-II both *in vivo* and *in vitro* [3]. We now report results of breeding an IGF-II transgene [4] onto the mdx mouse (Igf-2-mdx) which results in high levels of ectopic IGF-II expression throughout the animal's lifespan. We show that skeletal muscle PCD is directly correlated with the loss of the dystrophin gene and with the dystrophic phenotype. Furthermore we find that the IGF-II transgene can rescue all the histopathological features of the dystrophic phenotype which are associated with regeneration. We observe also that expression of the dystrophin-related protein, utrophin, is more widespread in Igf-2-mdx animals compared with their transgene-negative siblings, suggesting that the IGF-II effect is via this protein. These data clearly raise an exciting new possibility for treatment of DMD using gene therapy.

1. Moser, H. (1984). *Hum. Genet.* **66**, 17–40.
2. Bulfield, G., *et al.* (1984). *Proc. Natl. Acad. Sci. USA* **81**, 1189–1192.
3. Smith, J., *et al.* (1995). *Cell Death* **2**, 243–251.
4. Ward, *et al.* (1994). *Proc. Natl. Acad. Sci. USA* **91**, 10365–10369.

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

An *in vivo Drosophila* assay for wild-type and mutant human *HOX* gene function

FRANCES R. GOODMAN[1], PETER J. SCAMBLER[1] and R. S. MANN[2]
[1]*Molecular Medicine Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK;* [2]*Department of Biochemistry & Molecular Biophysics, Columbia University College of Physicians & Surgeons, 701 West 168th Street, HHSC 1108, New York, NY 10032, USA*

While studying human malformations caused by *HOX* gene mutations, we have shown (1) that the penetrance and severity of synpolydactyly increase with increasing expansions of an N-terminal polyalanine tract in HOXD13; (2) that a novel phenotype, similar to but distinct from synpolydactyly, segregates with two different deletions in *HOXD13*; and (3) that expansion of a polyalanine tract in *HOXA13* causes hand–foot–genital syndrome. These findings raise interesting questions about the functional effects of the mutations: the deletions, which we predict would result in null alleles, produce a different phenotype from that seen in the corresponding 'knock-out' mice, while the expansion could confer either gain or loss of function. We are now using a *Drosophila* assay system to study the effects of these mutations. Whereas wild-type human *HOXD13* and *HOXA13* act as hypomorphs of their *Drosophila* homologue *Abdominal-B*, a mutant form of *HOXD13* carrying one of our human deletion mutations is completely inactive. Moreover, mutant forms of *HOXD13* carrying increasingly long polyalanine tract expansions exert an increasingly strong dominant negative effect. These results shed important light on the phenotypes produced by *HOX* gene mutations in humans and mice, as well as demonstrating the value of *Drosophila* for investigating human *HOX* gene function.

Functional characterization of two novel thyroid hormone receptor (TR) beta proteins

ICSM Molecular Endocrinology Group, Division of Medicine and MRC Clinical Sciences Centre, Hammersmith Hospital, London W12 0NN, UK

Thyroid hormone (T_3) receptors are ligand-dependent transcription factors. We have identified two novel rat TRbeta cDNAs: TRbeta3 and TRdeltabeta3. The TRbeta3 protein has a unique N-terminal domain, whereas TRdeltabeta3 uses the next in frame ATG and lacks a DNA binding domain. Tissue-specific expression of these isoforms is regulated by thyroid status, suggesting that the proteins possess different functional activities. *In vitro* transcription/translation and site-directed mutagenesis studies confirmed that TRdeltabeta3 is produced from the next in frame ATG. Fusion proteins were tested in gel shift assays using a DR+4 T_3 response element (TRE). Like TRbeta1, TRbeta3 bound DNA as a heterodimer with RXR, whereas TRdeltabeta3 failed to bind DNA. To test their T_3 responsiveness, the receptors were co-transfected with reporter genes driven by two TREs. The TREs were induced 5-fold or 9-fold by TRbeta1 plus T_3 relative to activity in the absence of ligand. TRbeta3 mediated T_3 responses of a similar potency. In contrast, TRdeltabeta3 failed to mediate a T_3 response with either TRE. Preliminary data from competition experiments suggest that TRdeltabeta3 acts as a dominant negative antagonist to modulate the activity of other TR proteins in a tissue-specific manner.

Analysis of the imprinting status of GRB10, a prime candidate for Silver–Russell syndrome, in human fetal development

MEGAN P. HITCHINS[1], DAVID MONK[1,2], GILLIAN BELL[1], PHILIP STANIER[1], MICHAEL A. PREECE[1,2] and GUDRUN E. MOORE[1]
[1]*Fetal and Maternal Medicine Unit, Institute of Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, London, UK;* [2]*Biochemistry, Endocrinology and Metabolism Unit, Institute of Child Health, London, UK*

Silver–Russell syndrome (SRS) is characterized by intrauterine and postnatal growth retardation, lateral asymmetry and small triangular facies. Maternal uniparental disomy for chromosome 7 (mUPD7) occurs in ~7% of patients. The demonstration of consistent heterodisomy in a subset of mUPD7 subjects indicates SRS is due to disruption of one or more imprinted genes. A *de novo* duplication of the maternal 7p11·2–13, which included the growth factor receptor bound protein 10 (GRB10) gene, was recently identified in a SRS patient. GRB10 binds to the insulin and insulin-like growth factor receptors, acting as an inhibitor of the respective post-receptor cascades. Murine Grb10 maps to the homologous region of proximal chromosome 11, which displays an imprinted growth phenotype. Grb10 was identified as a maternally expressed gene through subtractive hybridization using normal and androgenetic mouse embryos. To investigate a potential role for GRB10 in SRS we are analysing its imprinting status during human development using intragenic polymorphisms. In contrast to its mouse homologue, GRB10 is expressed preferentially from the paternal allele in fetal brain, but is biallelic in a multitude of other fetal tissues. We have also found evidence for multiple

splice-forms of GRB10, which are being analysed individually for isoform-specific imprinting.

Analysis of expression and imprinting of *p57Kip2* from a modified BAC transgene

ROSALIND M. JOHN, SHEILA C. BARTON and M. AZIM SURANI
Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge CB2 1QR, UK

p57Kip2 is a maternally expressed, imprinted gene which encodes a transcript of approximately 1·4 kb and spans a 3 kb genomic region. It lies within the established imprinted domain on distal chromosome 7 where a number of imprinted genes are clustered. This region is syntenic to human chromosome 11p15 where the gene order and imprinting are conserved. In humans, mutations of this region are associated with Beckwith–Weidermann syndrome (BWS). We have isolated and characterized bacterial artificial chromosomes (BACs) which span approximately 320 kb around the *p57Kip2* locus. To identify the control *cis*-elements involved in expression and imprinting of *p57Kip2* we are testing the ability of these BACs to imprint in the mouse. Using a recently described technique which allows the modification of *E. coli*-based genomic clones, we have homologously recombined a two-colour reporter gene into the 3′ UTR of *p57Kip2* and generated transgenic mouse lines by pronuclear injection. One transgene directs a strikingly restricted expression profile while a second, overlapping transgene appears to contain all the enhancers required for appropriate expression. Preliminary analysis of transgenic lines suggests that key regulatory elements for the imprinted expression of *p57Kip2* lie at a distance from the gene. Implications for BWS will be discussed.

Identification of three biallelically expressed genes in the centre of the imprinting cluster on distal chromosome 7 in the mouse

MARTINA PAULSEN[1,2], OLIVIA FRANCK[1], OSMAN EL-MAARRI[1], SABINE ENGERMANN[1], MARTIN STOERICKE[1], KAREN R. DAVIES[3], WOLF REIK[3] and JOERN WALTER[1]
[1]*Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 73, D-14195 Berlin, Germany;* [2]*present address; University of Cambridge, Department of Anatomy, Downing Street, Cambridge CB2 3DY, UK;* [3]*The Babraham Institute, Laboratory of Developmental Genetics and Imprinting, Cambridge CB2 4AT, UK*

In human and mouse most imprinted genes are arranged in chromosomal clusters. Their linked organization suggests coordinated mechanisms controlling imprinting and gene expression. We have established a complete contig of clones along the murine imprinting cluster on distal chromosome 7 which is syntenic with the human imprinting region at 11p15.5 associated with the Beckwith–Wiedemann syndrome. The cluster comprises about 1 megabase of DNA, contains at least eight imprinted genes, and is demarcated by the two maternally expressed genes *Tssc3* and *H19*. We have sequenced a 250 kb long region in the centre of the cluster. Sequence analysis by gene prediction programmes, comparison with the human genomic sequence, and database searches for homologous expressed sequence tags (EST) revealed the existence of three new genes (*Tssc4*, *Tssc6* and *Trpc8*) between the imprinted *Kcnq1* and *Mash2* genes. *Tssc4*, *Tssc6* and *Trpc8* are biallelically expressed in embryonic and newborn tissues. This indicates that the imprinting cluster contains two imprinted subdomains that are separated by a set of genes that escape imprinting.

Placental defects in mice associated with imprinting on chromosome 12: implications for human placentopathy

PANTELIS GEORGIADES, MARIE WATKINS and ANNE FERGUSON-SMITH
Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

The genetic control of mouse placental development during late gestation is largely unknown when compared with that of the fetus. Understanding this is important because correct placental development/function is necessary for correct fetal development/viability. This is also important for humans because several placentopathies of unknown genetic basis are associated with adverse clinical outcomes for the fetus and/or mother. An example is the defined placento-

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

pathy associated with pre-eclampsia in the mother and increased death incidence of the fetus. Some of the known imprinted genes are required for normal murine placental development. Even though mouse chromosome 12 (ch12) contains imprinted genes, none of them have been isolated and their putative involvement in placental development is not known. To examine the function of imprinted genes on ch12, we generated mouse conceptuses which inherited both copies of ch12 from the father (paternal uniparental disomy 12; pUPD12) or mother (maternal uniparental disomy 12; mUPD12) and thus had ch12-linked imprinted gene(s) abnormally expressed. The pUPD12 embryos die during late gestation and their placentae show a variety of defects. These include abnormal fetal blood vessel vascularization and blood distribution within the placental labyrinth, abnormal behaviour of glycogen cells (the murine equivalent of human extravillous trophoblast) and abnormal morphology of the central maternal artery supplying the placenta. Thus, the correct expression of ch12-linked imprinted gene(s) is necessary for late placental cell behaviours whose genetic basis was, up to now, unknown. Intriguingly, all these defects taken together have been reported in the analogous structures of the human pre-eclamptic placenta.

NMR structure and mutagenesis of the methyl-DNA binding domain of MeCP2

ANDREW FREE[1], ROBERT I. D. WAKEFIELD[2], BRIAN O. SMITH[2], XINSHENG NAN[1,3], ALICE SOTERIOU[2], DUSAN UHRIN[2], PAUL N. BARLOW[2] and ADRIAN P. BIRD[1]

[1]*Institute of Cell and Molecular Biology*, *University of Edinburgh*, *King's Buildings*, *Mayfield Road*, *Edinburgh EH9 3JR*, *UK;* [2]*Edinburgh Centre for Protein Technology*, *University of Edinburgh*, *King's Buildings*, *West Mains Road*, *Edinburgh EH9 3JJ*, *UK;* [3]*Present address: MRC Human Genetics Unit*, *Western General Hospital*, *Crewe Road*, *Edinburgh EH4 2XU*, *UK*

Methylation of vertebrate genomic DNA at cytosine residues located in the dinucleotide sequence CpG has a negative effect upon the transcription of nearby genes and is implicated in X-chromosome inactivation and imprinting mechanisms. Current evidence suggests that the majority of the repressive effects of methylation upon transcription are mediated via general methyl-CpG binding proteins, a family of which has been identified on the basis of a conserved methyl-DNA binding domain (MBD). One of the best characterized is MeCP2, an abundant chromosomal protein which binds with high affinity to individual methyl-CpG pairs and is essential for embryonic development. The MBD of MeCP2 confers its ability to recognize methyl-CpG specifically. We have determined a solution NMR structure for this domain, which is shown to form a novel wedge-shaped structure including a positively charged DNA-binding surface. We have carried out site-directed mutagenesis of residues within this surface and in other parts of the MBD, and analysed the DNA binding characteristics of the mutant proteins by fluorescence anisotropy and mobility-shift assay. This identifies residues D121, Y123 and A131 as implicated in DNA recognition, and F157 and possibly K135 as necessary for the structural integrity of the domain. The flexibility of a large mobile loop is thought to be critical for DNA binding, as judged by replacement of a glycine residue at its tip (G114) with proline. We have also been able to rationalize the effects of point mutations of R106, R133 and F155 of MeCP2, which are found in spontaneous cases of Rett syndrome. A model for methyl-CpG recognition is proposed.

Functional analysis of a *cis*-acting imprinting control element at the *H19* locus

ROBERT A. DREWELL[1], JAMES D. BRENTON[1], JUSTIN F.-X. AINSCOUGH[1], SHEILA C. BARTON[1], KATHY J. HILTON[1], KATHARINE L. ARNEY[1], LUISA DANDOLO[2] and M. AZIM SURANI[1]

[1]*Wellcome Trust/Cancer Research Campaign Institute of Cancer and Developmental Biology and Physiological Laboratory*, *Tennis Court Road*, *Cambridge CB2 1QR*, *UK;* [2]*1QR*, *1 ICGM*, *INSERM*, *Paris*, *France*

Genomic imprinting involves the selective expression of an imprinted gene from a single parental allele and the silencing of the other allele. The molecular mechanism responsible for the inactivation of one allele is unknown, but it is thought to involve *cis*-acting control elements which may regulate chromatin modifications and DNA methylation. We have examined the function in imprinting of a 1·2 kb element from the region upstream of the *H19* gene by targeted deletion at the endogenous mouse locus. This element had previously been shown to function as a *cis*-acting silencer of transgenic reporter genes in *Drosophila*. The deletion revealed that this element functions as a silencer on the paternally inherited *H19* allele. Its deletion resulted in a cell-type-specific loss of silencing but had no detectable effect on the neigbouring *Igf2* gene. Expression of the maternal *H19* allele was not disrupted. Despite derepression of the paternal *H19* allele, the deletion surprisingly had no effect on differential methylation at the *H19* locus. The ability of the 1·2 kb element to function as a *cis*-

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

acting silencer in *Drosophila* and in regulating imprinting of the *H19* gene in mice, may be indicative of an evolutionary conservation of some aspects of epigenetic mechanisms.

Mapping and novel gene identification within the Ids to Dmd region of the mouse X chromosome

R. BATE[1], A.-M. MALLON[1], P. DENNY[1], M. A. STRIVENS[1], M. R. M. BOTCHERBY[2], R. STRAW[2], D. CLARKE[2], P. WESTON[2], J. GREYSTRONG[2], G. HUNTER[2], C. KIMBERLEY[2], A. DANGEL[3], D. CUNNINGHAM[3], G. E. HERMAN[3], W. MILLER[4], M. PLATZER[5], T. WIEHE[5], G. NORDSIEK[5], A. ROSENTHAL[5], M. RHODES[2] and S. D. M. BROWN[1]
[1]*MRC UK Mouse Genome Centre and Mammalian Genetics Unit, Harwell, Oxon, UK;* [2]*MRC Human Genome Mapping Project Resource Centre, Hinxton, Cambridge, UK;* [3]*Department of Pediatrics and Children's Hospital Research Foundation, Ohio State University, Columbus, OH 43205, USA;* [4]*Department of Computer Science, The Pennsylvania State University, University Park, PA 16802, USA;* [5]*Institut für Molekulare Biotechnologie, Beutenbergstasse 11, D-07745 Jena, Germany*

Comparison of mouse and homologous human sequences allows us to use evolutionary sequence conservation, alongside gene and exon prediction packages, to identify genes. The first region of interest encompasses two murine X-linked mutations: bare patches (Bpa) and striated (Str). We have completed the sequencing of approximately 0·5 Mb from this region. This sequence has been compared with the homologous human sequence on Xq28 by pairwise alignment. A number of other mouse genes were identified by the use of homology searches and gene prediction packages. Analysis of the mouse sequence using NIX identified a new gene, based on homology to ESTs and overlapping predicted exons. RT-PCR experiments were carried out which confirmed the predicted exons and identified expression in testis. Additional analysis of this gene has identified weak homology to the *Xlr* gene family. The second region of interest is between F8 and Dmd, which is relatively gene-poor, and spans two evolutionary breakpoints. The genetic map of the region has been refined and a clone map is currently being generated for sequencing. The evolutionary breakpoints may be identified by sequence analysis, as well as any potential genes. Future work will include expression studies of any novel genes, and investigation of the breakpoint regions.

Genetic analysis of deafness: screening for new mouse deafness mutations and their modifiers

R. E. HARDISTY[1], A. KIERNAN[2], A. ERVEN[2], D. SAW[1], P. MBURU[1], A. VARELA[1], J. PETERS[1], P. NOLAN[1], S. VOEGELING[3], J. L. GUENET[3], J. HUNTER[4], and S. D. M. BROWN[1]
[1]*MRC Mammalian Genetics Unit and UK Mouse Genome Centre, Harwell, Oxon OX11 0RD, UK;* [2]*MRC Institute of Hearing Research, Nottingham NG7 2RD, UK;* [3]*Institut Pasteur, Paris, France;* [4]*SmithKline Beecham Pharmaceuticals, Harlow CM19 5AW, UK*

The bulk of genetic deafness in the human population is non-syndromic and caused by sensorineural defects in the inner ear neuroepithelium, the organ of Corti. A large number of mouse deaf mutants are available, many carrying neuroepithelial defects. However, despite this apparently large catalogue, mouse models for many human deafness loci are not available. We have embarked upon a major mouse mutagenesis programme to recover a large number of novel mutant phenotypes, including new mouse deaf mutants. As part of our genome-wide screen for dominant mutations, we have incorporated tests for auditory and vestibular function, including a click-box emitting a high frequency soundburst to test for deafness as well as balance tests to assess vestibular function. From 10000 mice screened to date 29 phenotypes with vestibular and/or hearing anomalies have been detected, and a number subjected to inheritance testing. Of the proven mutants there are 9 with vestibular dysfunction, 1 mouse with vestibular and hearing deficits, and 2 mice with severe hearing impairment alone. Of the 9 vestibular mutations, 5 map to proximal chromosome 4 in the vicinity of the Wheels locus, a dominant mutation demonstrating circling behaviour and a circadian rhythm phenotype. Morphological studies indicate that these mutations have a truncation of the lateral semi-circular canal. All five mutants were derived from different mutagenized males and appear to represent new alleles at the Wheels locus. We have also employed mutagenesis in sensitized screens to identify modifiers involved in known auditory transduction pathways. Shaker1 (sh1), a recessive deafness mutation in the mouse, encodes myosin VIIA which appears to function in stereocilia positioning at the apical hair cell surface in the neuroepithelia. To identify modifiers of the sh1 locus, mutagenized males are mated to sh1 heterozygote females and F1 offspring screened for auditory and vestibular anomalies. Progeny testing of affected F1s distinguishes between new dominant

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

deafness mutations, new sh1 alleles and dominant enhancers of the sh1 mutation. Currently, 1000 F1 progeny have been analysed and several potential mutants are undergoing progeny testing. Both direct and modifier screens will enhance our knowledge of the panoply of genes involved in auditory system function.

New mouse models for deafness and vestibular defects

ALEXANDRA ERVEN[1], AMY E. KIERNAN[1], MARTIN HRAB de ANGELIS[2], HELMUT FUCHS[2], RUDI BALLING[2], JEAN-LOUIS GUENET[3], KAREN B. AVRAHAM[4], ORIT BEN-DAVID[4], SARAH VREUGDE[4], PAT NOLAN[5], JO PETERS[5], BRUCE CATTANACH[5], MICHAEL SKYNNER[6], NICK ALLEN[6], STEVE D. M. BROWN[5] and KAREN P. STEEL[1]
[1]*MRC Institute of Hearing Research*, *University Park*, *Nottingham NG7 2RD*, *UK*; [2]*GSF-Forschungszentrum für Umwelt und Gesundheit*, *Institut für Saugetiergenetik*, *Neuherberg*, *Postfach 1129*, *Oberschleiheim 85758*, *Bavaria*, *Germany*; [3]*Unité de Génétique des Mammifères de l'Institut Pasteur*, *Rue du Docteur Roux 25*, *75724 Paris*, *Cedex 15*, *France*; [4]*Department of Human Genetics and Molecular Medicine*, *Sackler School of Medicine*, *Tel Aviv University*, *Tel Aviv 69978*, *Israel*; [5]*Mammalian Genetics Unit*, *MRC*, *Harwell*, *Didcot OX11 0RD*, *UK*; [6]*Department of Neurobiology*, *Babraham Institute*, *Babraham*, *Cambridge CB2 4AT*, *UK*

The detection of genes involved in deafness and/or vestibular defects is ongoing (Holme and Steel (1999) *Curr. Opin. Genet. Dev.* **9**, 309–314). The localized genes represent only a fraction of the loci involved in hearing and balance, and many more remain to be detected. Mutations can be induced by treatment of germ cells with ionizing radiation, transgenic methods or *N*-ethyl-*N*-nitrosourea (ENU) injection. Such methods are, however, random events and this approach will identify more genes not yet known to cause deafness or balance defects. After mutagenesis offspring are screened for potential new deafness mutations using a calibrated tone burst of 20 kHz at 90 dB SPL and the mice are monitored for the lack of a Preyer reflex (ear flick). Mice are also monitored for waltzing or shaking behaviour indicating vestibular defects. In this study, different mutagenesis methods have given rise to a variety of dominant and recessive phenotypes, which we have characterized. The mutant Head-shaking circler (Hsc), induced by X-ray mutagenesis, shows thinning of all semicircular canals. A presumed allelic series of mutants including Eddy (Edy) and Dizzy (Dz), arising from ENU mutagenesis, show a truncation of the lateral semicircular canal. The recessive mutant, Tasmanian Devil (tde), resulting from insertional mutagenesis, displays thin, disorganized stereocilia of the hair cells in the organ of Corti. The Beethoven (Bth) mutant, dominantly inherited and arising from ENU mutagenesis, lacks inner hair cells. ENU mutagenesis has also given rise to a mutant with abnormal middle ear ossicles, Doarad (Dor). These mutants will contribute to the unravelling of the genetics of deafness and the understanding of the development of the mammalian inner ear.

This project is supported by the MRC and the EU (contract number: CT97-2715).

Differential gene expression in mouse endocardial cushions

VICTORIA A. REED, DEBORAH J. HENDERSON and ANDREW J. COPP
Neural Development Unit, *Institute of Child Health*, *University College London*, *30 Guilford Street*, *London WC1N 1EH*, *UK*

Congenital heart defects are among the commonest birth defects. The majority result from the defective formation and fusion of endocardial cushions, leading to malformation of the septa and valves. It is therefore of importance to understand the mechanisms underlying normal endocardial cushion development. A review of the literature has revealed differential gene expression between the atrio-ventricular and outflow tract cushions. However, these studies have been conducted on a variety of animal models, and without comparison between the two types of cushions. Our aim was to confirm and extend the comparison of atrio-ventricular and outflow tract endocardial cushion gene expression in a single animal model. Expression patterns for genes including the transcription factors Msx-1 and Msx-2, an extracellular matrix molecule tenascin, and BMP-2, a signalling molecule, confirms that genes are differentially expressed between outflow tract and atrio-ventricular cushions. The most prominent developmental difference between atrio-ventricular and outflow tract cushions is the population of the outflow tract by neural crest cells. Therefore, differential gene expression could be a result of an influence of neural crest cells in the outflow tract. However, preliminary data utilizing the mutant mouse splotch, which has neural crest migration defects, suggests that crest cells may not be influencing gene expression in the outflow tract. Two alternative explanations are that the outflow tract may be derived from outside the heart-forming field, or that the heart-forming region and heart tube may be subject to anterior–posterior positional patterning.

Expression and localization of the genes for arylamine N-acetyl transferases in mice

SOTIRIA BOUKOUVALA[1], GIANNOULIS FAKIS[1], KATALIN PINTER[1], VERONIKA BUCKLE[2], HELEN PRIDDLE[3] and EDITH SIM[1]
[1]*Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK;* [1]*Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK;* [2]*Centre for Genome Research, University of Edinburgh, Edinburgh EH9 3JQ, UK*

Arylamine N-acetyltransferases (NATs) are known as enzymes metabolizing arylamines and hydrazines. In mice there are three genes, *Nat1*, *Nat2* and *Nat3*, encoding for three NAT isoenzymes. NAT1 and NAT2 have well-characterized substrates, while the substrate specificity of NAT3 remains unclear. In addition to its xenobiotic substrates, NAT2 was recently shown to metabolize the endogenous folate catabolite *p*-aminobenzoic glutamate (pABGlu), suggesting a potential role of the enzyme in development. This was further supported by the fact that *Nat2* is expressed as early as the blastocyst stage. We now provide evidence that, unlike *Nat2*, *Nat1* and *Nat3* are not expressed very early in development and also demonstrate, for the first time, expression of *Nat3 in vivo*, in the spleen. Interest has now focused on studying the regulation of expression of *Nat2*, as sequence analysis of the region upstream of the gene indicates an unusual splicing pattern for the *Nat2* transcript. We also report progress towards the generation of *Nat2* knockout mice, which will allow further investigation of the role of NAT2 in development. Finally, fluorescence *in situ* hybridization has allowed localization of the three *Nat* genes in the same region of murine chromosome 8, confirming previous studies based on linkage analysis.

We would like to thank Action Research (SPARKS), the Wellcome Trust and the Bodossaki Foundation (Greece) for funding.

The molecular mechanisms of neurulation

JENNIFER N. MURDOCH and ANDREW J. COPP
Neural Development Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK

Primary neurulation is the process that creates the brain and the spinal cord rostral to the mid-sacral level of the body axis. The morphological processes of primary neurulation are well documented, but the molecular mechanisms that are involved are not fully understood. A review of the literature reveals that over 30 genes are involved in neurulation, since disruption of these genes, either by null mutation or by overexpression, gives rise to embryos with neural tube defects. These genes encode proteins with a wide variety of functions, including transcription factors (for example, *Pax3*, AP-2, Hes-1), tyrosine kinases (Abl, Arg), signalling molecules (Shh, Notch3), gap junction proteins (Cx43) and even a lipoprotein (Apob). We propose that these genes can be grouped into classes that affect five different morphological or cellular processes of neurulation: (a) mesenchymal proliferation/expansion; (b) neural crest cell proliferation and emigration; (c) dorsolateral hinge point formation; (d) apoptosis and remodelling in the dorsal midline; and (e) neuroepithelial proliferation and differentiation. In addition, several genes belong to distinct signalling pathways. For example, Shh, Ptc and Gli3 are all part of the Shh signalling pathway, and Notch3, RBP-Jk and Hes-1 function in the Notch signalling pathway. Therefore, the molecular mechanisms involved in neural tube closure are beginning to become apparent.

Craniofacial development: analysis of *Hox* gene expression plasticity in mouse cranial neural crest

PAUL A. TRAINOR and ROBB KRUMLAUF
Developmental Neurobiology, National Institute for Medical Research, Mill Hill, UK

The current model for craniofacial patterning is one in which the neural crest cell population carries positional information acquired in the neuroepithelium to the peripheral nervous system and branchial arches thereby ensuring registration and maintenance of rostro-caudal identity between different tissues. The molecular basis for this model stems from *Hox* gene expression patterns in the cranial neural crest, cranial ganglia and branchial arches emulating the expression patterns characteristic of their rhombomeric origins. Recently in disagreement with this model, grafting studies and molecular analyses have suggested that rhombomere, neural crest and branchial arch patterns of *Hox* gene expression should be considered as independently regulated events and that rhombomere patterns of *Hox* gene expression are not always cell autonomous. In this series of experiments we have directly challenged the *Hox* gene autonomy or plasticity of rhombomere and cranial neural crest cells in ectopic environments in mouse embryos. In posterior-to-anterior hindbrain grafts we observe autonomy of *Hox* gene expression in the rhombomeric tissue in accordance with the posterior dominance model;

however, *Hox* gene expression in the neural crest is entirely plastic. In anterior-to-posterior hindbrain grafts we do not observe induction of *Hox* gene expression in either the grafted rhombomeric or neural crest tissue appropriate to its new location. Our experiments provide clear evidence at the cellular level for the independent regulation of *Hox* gene expression in rhombomeres and neural crest cells and suggests that there may be an environmental signal in the cranial mesenchyme which is essential for correct *Hox* gene expression patterns in the neural crest. The pre-patterning model is therefore inappropriate and insufficient to describe craniofacial patterning and a much more complex set of molecular and cellular interactions are taking place during vertebrate head development.

A transgenic approach to studying the establishment of topographic organization in the mouse optic tract

TILL MARQUARDT, ANASTASSIA STOYKOVA, NICOLE ANDREJEWSKI, RUTH ASHERY and PETER GRUSS
Max-Planck-Institut für biophysikalische Chemie, Abt. Molekulare Zellbiologie, Am Fassberg 11, D-37077 Goettingen, Germany

We generated a transgenic mouse line in which a highly conserved retina-specific regulatory element of *Pax6*, referred to as the alpha-element, drives expression of the axonal marker tau-lacZ. We found that the expression of the transcription factor *Pax6* in the retina can be separated into complementary territories that apparently are directed by distinct *cis*-regulatory systems. The expression directed by the alpha-element could be mapped to two separate domains in the temporal and nasal retina. The element was further dissected to reveal *cis*-regulatory components required for regional expression. In the later postnatal period, expression is confined to *Pax6*-positive retinal ganglion cells (RGCs) in the nasal and temporal retina that send their axonal projections to two stripes in the rostral- and caudalmost superior colliculus, as well as the dorsal- and ventralmost lateral geniculate nucleus. The restricted and stereotypic innervation pattern by the labelled RGC axons of the transgenic mouse line provides us with an ideal tool to study the establishment of topographic organization in the mammalian visual system. Work is in progress to examine the development of topographic specificity in the axonal trajectories of two achiasmatic mouse mutants generated in our laboratory. Furthermore conditional gene targeting is utilized to elucidate the role of *Pax6* in the differentiation of the respective retinal territories.

Isolation and characterization of WH2.3, a new member of the 'Winged Helix' family, expressed during eye development

ANGELA GOUGE[1], ADRAIN HARDY[1], JANE SOWDEN[2] and HAZEL SMITH[1]
[1]*The Galton Laboratory, Biology, University College London, London, UK;* [2]*The Institute of Child Health, University College London, London, UK*

Eye development is a highly complex process. The development of the retina involves the production of seven different cell types, found in three separate layers. Specification and differentiation of these different cell types is regulated by a combination of extrinsic and intrinsic factors [1]. Evidence is accumulating that transcription factors play a major role in the intrinsic control of retinal cell fate. We report here the cloning and characterization of a putative new member of the 'Winged Helix' family of transcription factors called WH2.3. This new cDNA appears to be the mouse homologue of the *Fugu Whn* gene [2,3], and has been mapped to mouse chromosome 5 by fluorescence *in situ* hybridisation. RNA transcripts of WH2.3 are visible in the brain, spinal cord and retina. It seems likely that this new gene plays a role in the proliferation of neuronal cell types before subsequent determination and differentiation.

1. Cepko *et al.* (1996). *Proc. Natl. Acad. Sci. USA* **93**, 589–595.
2. Schuddekopf *et al.* (1996). *Proc. Natl. Acad. Sci. USA* **93**, 9661–9664.
3. Schlake *et al.* (1997). *Proc. Natl. Acad. Sci. USA* **94**, 3842–3847.

***Trans*-regulatory mechanisms directing *Pax6* activity in the retina**

NICOLE ANDREJEWSKI, TILL MARQUARDT, MARC HALLONET, MARTIN SCHWARZ and PETER GRUSS
Max-Planck-Institut für Biophysikalische Chemie, Abt. Molekulare Zellbiologie, Am Fassberg 11, D-37077 Goettingen, Germany

Pax6 has an essential role in eye development since the *Pax6* null mutant mice totally lack eyes. *Pax6* expression in the retina is complementary to *Pax2* and *Vax1* expression in the optic stalk. Both *Pax2* and *Vax1 null mutant mice show expansion of Pax6* expression into the optic stalk. Taken together these facts suggest reciprocal regulation of *Pax6* and *Pax2/Vax1*. In a detailed analysis of *Pax6* upstream regulation a highly conserved regulatory element

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press

driving *Pax6* expression in the retina, the alpha-element, has been isolated. Two functional assays have been used to examine the interactions between the probable upstream factors and the alpha-element. Using bandshift assays we showed a direct binding of *Pax6*, *Pax2*, *Vax1* and *Six3* to distinct as well as overlapping sequences of this element. Cotransfection experiments as a strong indicator for a possible *in vivo* function confirmed the presumption that *Pax6* up-regulates and *Pax2* downregulates the alpha-element. In summary, these findings imply that *Pax2*, and probably *Vax1* and *Six3*, define the correct pattern of *Pax6* activity in the optic cup (including an auto-regulatory loop) and might direct the specification of the optic cup/optic stalk boundary. The *in vivo* functions of these protein–DNA interactions are currently under further investigation.

Characterization of a new human t-box gene, *TBX20*, and its mouse homologue, *Tbx20*

MORITZ MEINS[1,2], S. S. BHATTACHARYA[2] and JANE SOWDEN[1]
[1]*Institute of Child Health*, *University College London*, *30 Guilford Street*, *London WC1N 1EH*, *UK;* [2]*Institute of Ophthalmology*, *University College*, *London*, *London*, *UK*

T-box transcription factors contain a novel type of DNA binding domain, the T-box, and are encoded by an ancient gene family. Four T-box genes, *omb*, *Trg*, *org1* and *H15*, have been identified in *Drosophila*, whereas in mammals the T-box gene family has expanded, and 12 human T-box genes have been identified. We report the identification and expression analysis of a novel human T-box gene, *TBX20*, and its mouse homologue, *Tbx20*, which are closely related to the *Drosophila H15* gene. *H15* expression in leg imaginal discs correlates with commitment to a ventral fate, implicating this gene in early patterning events in the fly. We identified a human homologue of *H15* by genome databases searches. The human *TBX20* gene maps to chromosome 7p14–15. Its T-box domain is encoded by 5 exons and shares 71% amino acid identity with the *H15* T-box domain. *TBX20* is expressed in the developing human eye from 9 weeks, and in the fetal heart and limb at 11–12 weeks post-conception. In parallel we characterized the mouse *Tbx20* gene, which is highly similar to *TBX20* in both its sequence and expression pattern. Our expression data indicate a likely role of *TBX20*/*Tbx20* in regulating differentiation of ocular and heart tissues.

Cell-specific knockout of *Pax6* in the developing eye

RUTH ASHERY-PADAN, TILL MARQUARDT and PETER GRUSS
Max-Planck Institute of Biophysical Chemistry, *Department of Molecular Cell Biology*, *D37077*, *Gottingen*, *Germany*

Mice homozygous for null *Pax6* allele die soon after birth with various abnormalities: they are devoid of eyes and reveal abnormal brain and pancreas development. In order to study the role of *Pax6* in specific cell types, during selected time and in the adult we are currently using the Cre/loxP1 approach. We established a mouse line in which the paired box of *Pax6* was flanked by two loxP1 sequences. The floxed *Pax6* allele is hypomorphic due to aberrant splicing products between *Pax6* and the transcripts of the neomycin-resistance gene. Indeed the phenotype was completely reversed when the selection cassette was removed, allowing the use of the floxed *Pax6* mice for conditional knockout experiments. In order to study the role of *Pax6* in the developing eye, we established transgenic mouse lines that express the Cre under the regulation of *Pax6* regulatory elements. In one line, Cre activity is observed in the developing lens while in the other, recombination is detected exclusively in the developing retina. These lines are being employed to study the role of *Pax6* in specific cells within the developing eye and for lineage tracing of the cells in which these regulatory elements are active.

https://doi.org/10.1017/S0016672300004675 Published online by Cambridge University Press