Abstracts of papers presented at the 13th Mammalian Molecular and Biochemical Genetics Workshop held at the Linnean Society Rooms, Piccadilly, London on 26 and 27 November 1986.

Molecular characterisation of the gene encoding ovine β-lactoglobulin

S. ALI AND A. J. CLARK
AFRC Institute of Physiology and Genetic Research, Edinburgh Research Station, West Mains Road, Edinburgh EH9 3JQ, U.K.

β-Lactoglobulin is the major whey protein in the milk of ruminants. It is an 18·3 kDa protein which appears to be encoded at a single locus. Phage lambda clones encoding β-lactoglobulins have been isolated from a sheep genomic library and characterized by restriction mapping and DNA sequencing. The gene is 4·9 kb long, is comprised of 7 exons and is specifically expressed in the lactating mammary gland. Comparison of the mRNA sequences of β-lactoglobulin with those encoding a mouse major urinary (MUP) protein suggests that the two genes are highly diverged members of a 'supergene' family. This view is supported by the finding that they share an almost identical intron/exon structure.

Molecular analysis of the halothane sensitivity locus in pigs

ALAN L. ARCHIBALD
AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, West Mains Road, Edinburgh EH9 3JQ, U.K.

Susceptibility to the related traits of porcine stress syndrome (sudden deaths) and pale soft exudative meat can be predicted from the response of individuals to a short exposure to the anaesthetic halothane. Sensitivity to halothane is controlled by a recessive gene at a single autosomal locus (*Hal*). The present halothane test fails to distinguish between the homozygous resistant animals and heterozygous carriers. The ability to detect restriction fragment length polymorphisms which are closely linked to the *Hal* locus should improve the genotyping for these traits. A cloned glucose-6-phosphate isomerase (*Gpi*) gene would be a useful initial probe for such RFLPs as the *Gpi* locus is tightly linked to the *Hal* locus at a distance of about 1 cM. Two oligonucleotides (a 36-mer and a 45-mer) were designed using data from the partial amino acid sequence of pig GPI and used to screen a pig gut cDNA library. The identity of positive colonies has been confirmed by sequence analysis.

A deletion mutation of mouse mtDNA associated with heteroplasmy

PIERRE BOURSOT, HIROMICHI YONEKAWA AND FRANÇOIS BONHOMME
Institut des Sciences de l'Evolution, U.S.T.L., 34060-Montpellier Cedex, France

During mtDNA RFLP survey of wild mice (*M. m. musculus*) we found two heteroplasmic animals. They contained a mixture of 20% of normal mtDNA (i.e. a molecule of usual length and restriction map) and 80% of a deletion mutant. The deletion was 5 kb long (31% of the mitochondrial genome) and encompassed six tRNA genes and seven proteins genes. Implications about the molecular mechanisms of the mtDNA evolution and the transmission genetics of this genome are discussed.

Mammalian kinetochore structure and function

JANE BOWER
MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K.

The kinetochores of mammalian chromosomes, the microtubule attachment sites which are visualized in the electron microscope as a pair of trilaminar plates, one on each side of the centromere of metaphase chromosomes, are composed of DNA, protein and possibly RNA. Using sera from patients suffering from the CREST syndrome (calcinosis, Raynaud's syndrome, oesophageal dismotility, sclerodactyly, telangiectasia), which contains a high titre of antikinetochore antibodies, the organization and function of kinetochores has been studied in several cell types by indirect immunofluorescence. Currently, little is known about the DNA and proteins involved in the mammalian kinetochore. A variety of approaches to purification and characterization is being used and preliminary results are described.

The molecular mapping of the mouse X chromosome

N. BROCKDORFF, E. M. C. FISHER, J. CAVANNA, M. F. LYON* AND S. D. M. BROWN
*Department of Biochemistry, St Mary's Hospital Medical School, Paddington, London W2 1PG, U.K., and * MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.*

A number of microclones isolated by microdissection of the mouse *X* chromosome (Fisher, Cavanna & Brown, 1985, *PNAS*, **82**, 5846–5849) have been mapped on the *X* chromosome with the use of interspecific *Mus spretus/Mus domesticus* crosses. Two microclones detect two distinct repeat 'islands' – complex clusters of related sequences. One microclone, mapped to a proximal region of the mouse *X* chromosome, detects a homologous sequence on the *Y* chomosome. The construction of these molecular maps, coupled with the use of techniques such as pulse-field gel electrophoresis, is allowing the detailed long-range sequence analysis of mammalian chromosomes.

Hormonal and neuronal control of carbonic anhydrase isozymes

NICK CARTER, STEVE JEFFREY AND CHRISTIANE KELLY
Department of Child Health, St George's Hospital Medical School, London, U.K.

HARVEY ISENBERG, JULIE LLOYD AND YVONNE EDWARDS
MRC Human Biochemical Genetics Unit, Wolfson House, 4 Stephenson Way, London N.W.1, U.K.

There are at least five genetically independent isozymes of mammalian carbonic anhydrase, with differing specific activities and tissue distribution. There are striking hormonal and neuronal influences on specific isozymes; for example, carbonic anhydrase III protein is about 20-fold higher in male rat liver compared with female and this is specifically controlled by growth hormone patterns. Rat fast skeletal muscle shows an eight-fold induction of carbonic anhydrase III protein on denervation of the muscle and a similar induction can be obtained by thyroidectomy. The molecular basis for this hormonal and neuronal control is currently under investigation.

Electrophoretic variation in the Skomer vole

K. A. HARPER
Department of Genetics, University of Leicester, Leicester LE1 7RH U.K.

Seventy bank voles were trapped on Skomer Island during August 1986. They were found to be polymorphic for three systems already known in the mainland form. Their haemoglobin is of the southern variety described by Hall (1978). Data on the geographical variation on Skomer will be viewed in the light of preliminary data from the adjacent mainland.

Expression of human alpha-1-antitrypsin in transgenic mice

G. D. KELSEY,* S. POVEY,* A. BYGRAVE† AND R. LOVELL-BADGE†
** MRC Human Biochemical Genetics Unit and † MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London N.W.1, U.K.*

The serum protein alpha-1-antitryspin (AT) is a good model for liver-specific gene expression. To explore this, transgenic mice carrying copies of the human AT gene have been made following microinjection of a cosmid insert into fertilized mouse eggs. In three independent mouse lines, inheritance of human AT genes is accompanied by a high serum concentration of the human protein. These mice accumulate high levels of human AT RNA, preferentially in liver, and the developmental regulation of the introduced genes parallels that of the endogenous one. Whilst the mouse gene is almost entirely liver-specific in its expression, AT transcripts are present in a variety of human tissues other than liver, albeit at a lower level. In transgenic mice this pattern of expression of the human gene is largely maintained. Regulation of human AT gene expression is mediated in part by two transcription initiation sites with different tissue specificities.

$HPRT^-$ mice derived from $HPRT^-$ cultured embryonic stem cells

MICHAEL KUEHN, ALLAN BRADLEY, ELIZABETH ROBERTSON AND MARTIN EVANS
Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, U.K.

We have recently described a novel approach for producing transgenic mice utilizing cultured embryonic stem cell lines. Compared to other methods for manipulating the mouse genome, this approach can take advantage of somatic cell genetic techniques to select for specific variant cells prior to their use in the derivation of specific transgenic animals. Following multiple retroviral infection of stem cell cultures to induce insertional mutations we have selected for $HPRT^-$ clones by 6-thioguanine resistance and HAT sensitivity. DNA analysis shows that two such $HPRT^-$ clones have altered HPRT genes due to retrovirus insertion. These clones have contributed to the germ lines of ten chimaeric mice. F_1 mice heterozygous for the HPRT mutations are being used to establish $HPRT^-$ lines of mice which may provide an animal model for the Human Lesch–Nyhan syndrome.

Mapping a melanoma cDNA to the mouse B-locus

IAN J. JACKSON AND SHIGEKI SHIBAHARA*
*Molecular Development Group, MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, U.K., and * Frederick Miescher Institut, PO Box 2543, CH-4002, Basel, Switzerland*

The genetics of pigmentation of the mouse coat is one of the best understood mammalian genetic systems. There are several loci at which many alleles have been identified. We have been attempting to clone cDNA coding for tyrosinase, the putative product of the *albino* locus. We have independently isolated the same sequence, using different approaches. This clone does not map to the *albino* locus but rather we have localized it to the B-locus (which encodes the black/brown pigmentation). Experiments are under way to prove the identity of the clone with the B-locus. Other genetic aspects of the localization and the relationship of the cDNA to tyrosinase will be discussed.

I.J.J. is a Fellow of the Lister Institute of Preventative Medicine.

Structure and evolution of the spectrin gene family

A. P. McMAHON,* D. H. GIEBELHAUS,† J. BAILES,* J. CHAMPION* AND R. T. MOON†
** Laboratory of Developmental Biochemistry, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K., and † Department of Pharmacology SJ-30, School of Medicine, University of Washington, Seattle, WA, 98195, U.S.A.*

Spectrin, the principal cytoskeletal component of the red blood cell, mediates the attachment of microfilaments to the cell membrane. The resulting network confers on the cell specific biophysical properties affecting cell shape,

elasticity and lateral movement of membrane components. Recently, spectrin analogues have been found in non-erythroid vertebrate cells. In order to understand the role of these non-erythroid forms of spectrin, and to investigate the evolution of the spectrin gene family, we have isolated cDNA clones encoding human and other vertebrate non-erythroid spectrin. The structure and evolution of this family of proteins will be discussed.

Data from mouse *X*-autosome translocations on *X*-chromosome inactivation and reactivation

K. A. WAREHAM,* E. D. WILLIAMS,* P. H. GLENISTER,† J. ZENTHON,† M. F. LYON† AND E. P. EVANS†

** Department of Pathology, Welsh National School of Medicine, Cardiff, U.K., and † MRC Radiobiology Unit, Chilton, Didcot, Oxon, U.K.*

The gene sparse-fur, *spf*, causes deficiency of the liver enzyme ornithine carbamoyl transferase (OCT). In T(X;4)37H the break is distal and in T(X;11)38H proximal to the *spf* locus. The livers of T38H + / + *spf* females showed the typical mosaic pattern of OCT staining, but in similar T37H females all liver cells were OCT positive. The interpretation was that in T37H physical continuity between the OCT locus and the inactivation centre was broken and hence the normal allele on the translocated chromosome remained active in all cells. Translocation T(X;16)16H causes non-random inactivation with the normal X inactive in all cells. Hence T16H*spf*/ + + females should be OCT negative. In fact, small numbers of OCT positive liver cells were seen. The numbers of positive cells increased as the animals aged, but the size of patches did not. This suggested reactivation of the inactivated normal allele of OCT, and is believed to be the first evidence of reactivation of an *X*-linked gene *in vivo* in a eutherian mammal.

CKBE – A new human gene characterized by ectopic expression of creatine kinase B

T. F. WIENKER,* B. WIERINGO,† E. C. M. MARIMAN,† C. R. MULLER,‡ G. SCHEUERBRANDT,§ K. BENDER* AND H. H. ROPERS†

** Institute of Human Genetics, University of Freiburg, F.R.G., † Institute of Anthropogenetics, University of Nijmegen, The Netherlands, ‡ Institute of Human Genetics, University of Wurzburg, F.R.G., § CK-Test-Laboratory, Breitnau, F.R.G.*

In the course of a large-scale screening programme directed to the early diagnosis of Duchenne muscular dystrophy in newborn males, a rare familial trait characterized by high levels of creatine kinase type BB (EC 2.7.3.2.) either in thrombocytes or in erythrocytes has been discovered. The underlying mutation (CKBE) segregates in a regular autosomal dominant fashion, and has been mapped by linkage analysis in information pedigrees to the distal long arm of chromosome 14. Results of linkage studies involving conventional markers (Gm, Pi) and DNA-probe defined RFLPs (D14 S1, IGHG4) will be presented. Potentially, the CKBE mutation will contribute to the understanding of mechanisms governing tissue specificity of gene expression.

Gene expression during early mouse development

DAVID G. WILKINSON, JANET CHAMPION, JULIET A. BAILES AND ANDREW P. McMAHON

Laboratory of Developmental Biochemistry, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The mouse embryo is undergoing dramatic morphological changes between 8 and 10 days of development, and our objective is to study this at the level of specific gene regulation. Two-dimensional gel electrophoretic analyses detect only one embryonic protein whose relative rate of synthesis is substantially increased during this period. We have set out to isolate cDNA clones corresponding to this protein by the differential screening of an embryonic cDNA library. Four classes of cDNA clones were isolated, each of which specifies a 600 b mRNA that drastically increases in amount between 9 and 10 days of development. *In situ* hybridization showed that this mRNA is localized in embryonic erythrocytes, and also in the liver of the 12½-day embryo. These data suggest that these cDNA clones encode embryonic globins, and this has been confirmed by DNA sequence analysis. Further experiments to isolate additional developmentally regulated transcripts will be discussed.

Studies on an homologous enzyme in two species of vole

P. WHITLEY, K. A. HARPER AND R. SEMEONOFF
Department of Genetics, University of Leicester, Leicester LE1 7RH, U.K.

Both field voles and bank voles are polymorphic for a heat-resistant inhibitor-resistant esterase. We shall present data on the relative activity levels of each enzyme against closely related substrates, and introduce an investigation of the tissue distribution of this enzyme.

https://doi.org/10.1017/S0016672300027166 Published online by Cambridge University Press