Genet. Res., Camb. (1984), **43**, *pp.* 213–220
Printed in Great Britain

Abstracts of papers presented at the 10th Mammalian Biochemical Genetics Workshop meeting held at the Linnean Society Rooms, Piccadilly, London on 23 and 24 November 1983.

The isolation of MUP gene variants for the study of hormonal and tissue regulated gene control

By RAYA AL-SHAWI, A. J. CLARK and J. O. BISHOP

Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh

The major urinary proteins of the mouse are the products of a large recently diverged gene family. Certain tissues express different groups of MUP genes. The genes are under multihormonal control and variation in hormonal responsiveness is detected between members that are expressed in the same tissue as well as between members that are expressed in different tissues. Differences in MUP gene expression are found in inbred mouse strains. These features make the system ideal for identifying DNA sequences that determine tissue specific expression and differences in hormonal responsiveness.

New source of variation in high sulphur keratins from hair; differences between strains of mice

By J. A. BIRD STEWART and L. K. BARNETT

Department of Genetics, University of Leeds, Leeds LS2 9JT

The highly insoluble high sulphur keratins of mammalian hair have not been successfully characterized by physico-chemical methods. They are known to vary in sheep when the animal's diet is altered in sulphur content. We report a method of extraction and separation of these proteins based on SDS acrylamide gels, and the finding of strain differences in gel pattern and amino acid composition of protein extracted from the hair of three lines of mice.

Recombinant DNA probes in retinitis pigmentosa

By S. S. BHATTACHARYA, A. F. WRIGHT, H. COOKE, E. M. SOUTHERN and H. J. EVANS

MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh

EcoRI generated fragments of flow sorted human *X*-chromosome DNA were cloned in phage λgt Wes. A library of some 7×10^4 recombinants was obtained.

Nearly 95% of the clones in this library contained inserts which were highly repeated in the human genome and were eliminated by a two stage screening procedure using radioactively labelled total human DNA. So far ten *X*-chromosome specific single copy sequences have been isolated of which four have been mapped to specific subregions. Three X-probes identify restriction fragment length polymorphism (RFLP) with TaqI, HindIII and EcoRI respectively. Thirty obligate heterozygote for X-linked retinitis pigmentosa (XLRP) have been screened using 3 X-probes showing RFLPs with TaqI. The segregation of RFLP alleles in informative XLRP families is being analysed.

Sequences homologous to mouse mitochondrial DNA found in mouse genomic DNA

By D. JANE BOWER and L. H. ERRINGTON

Department of Genetics, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JN

A cosmid library of mouse DNA was screened with cDNA made on a template of mouse neural retina RNA. The colonies which gave the strongest signal included two containing 12 kb of the 16·3 kb mouse mitochondrial chromosome, as judged by restriction pattern, variable copy number in different tissues, abundance of up to 10% of total RNA in liver, kidney, heart and brain, and RNA size classes. Nineteen other clones which were picked contained genomic segments of up to 39 kb, which all contained sequences which cross-hybridized with high specificity to three of the larger mitochondrial RNA species.

Expression of cloned sequences of the mammalian HPRT gene

By JOHN BRENNAND, MARGARET FOX and C. THOMAS CASKEY*

*Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, *Baylor College of Medicine, Houston, TX, USA*

cDNA sequences of the human and Chinese hamster hypoxanthine guanine phosphorybosyltransferase (HPRT) genes have been isolated. These sequences have been utilized in the construction of chimaeric plasmids containing gene control elements of viral or mammalian origin. These plasmids are capable of transferring the $HPRT^+$ phenotype into HPRT deficient hamster, mouse and human cultured fibroblasts, following calcium phosphate mediated DNA transfection. The molecular mechanisms of this effect are currently under study. In addition, the ability of the plasmids to function *in vivo* is being investigated following their microinjection into the male pronucleus of fertilized mouse ovum and reimplantation into pseudopregnant females.

The cloning of the mammalian fatty acid synthetase gene

By I. R. CAMERON, R. DILS, C. J. SKIDMORE and F. A. WEIR

Department of Physiology & Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ

Fatty acid synthetase (FAS) is a polypeptide of 250000 molecular weight whose expression is hormonally controlled in mammary gland. Poly(A)+RNA has been isolated from membrane-bound polyribosomes prepared from lactating rabbit mammary gland. A high molecular weight fraction of this RNA has been cloned into pAT153 both by homopolymer tailing and by the method of Okayama & Berg (*Mol. Cell. Biol.* **2**, 161–170, 1982). A comparison of these methods will be presented.

Chromosomal organization of the mouse major urinary protein gene complex

By A. J. CLARK and J. O. BISHOP

Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh

The multigene family which codes for the mouse major urinary proteins (MUPs) consists of about 35 genes. Most of these are members of two different groups, Group 1 and Group 2, which can be distinguished by nucleic acid hybridization. The mouse genome contains approximately equal numbers (c. 15) of Group 1 and Group 2 genes and most, if not all, of these are located on chromosome 4. Here we describe the chromosomal organization of the MUP gene complex. We show by 'chromosome walking' that Group 1 and Group 2 genes are linked to each other in a head to head fashion, with 13 Kb of DNA between the two 5′ ends of the genes. We also show that MUP genes are linked to each other in a tail to tail fashion, and that there is approximately 24 Kb separating the 3′ ends of two genes. Overall, the MUP gene cluster(s) accounts for at least 650 Kb of mouse chromosomal DNA.

Evolutionary studies of mouse 16S mitochondrial RNA

By PHILIPPE FORT and FRANÇOIS BONHOMME*

*Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford OX3 0SQ, *Institut des Sciences de L'Evolution, U.S.T.L. Place E. Bataillon 4060 Montpellier, France*

The 5′ terminus of mitochondrial 16S rRNA has been examined for its variability among and within species of European and South-Asian mice. The observed variations are mainly base substitutions, the number of which being up to 20%

in the case of interspecific variations, and 4% in the case of intraspecific ones. A further computation of phylogenetic trees, using the maximum of parsimony and maximum of likelihood methods, shows a discrepancy between the mitochondrial (16S rRNA) and nuclear (Protein analysis) data. Though the explanation of this discrepancy is unclear, it could rely on the absence of recombination and on the maternal mode of inheritance of mitochondria.

Evolution of pseudogenes

By STEPHEN HARRIS and ALEC J. JEFFREYS

Genetics Department, University of Leicester, Leicester

Pseudogenes are commonly found associated with functional gene families within the higher eukaryotes. It is generally accepted that they are functionless sequences which are 'neutral' in terms of natural selection and therefore soon lose their identity in the genome. Phylogenetic analysis in man and the primates in fact suggests that some pseudogenes are ancient and highly stable entities within gene clusters. In addition they can be involved in major rearrangements, which affect a whole cluster, and may themselves be progenitors of other pseudogenes.

Molecular and genetic analysis of two serum protease inhibitors in the mouse

By ROBERT E. HILL, PHILIP H. SHAW, RICHARD K. BARTH, RICHARD R. MEEHAN and NICHOLAS D. HASTIE

MRC CAPCU, Western General Hospital, Edinburgh EH4 2XU

We have isolated cDNA clones for mouse α_1-antitrypsin and α_1-antichymotrypsin, serum protease inhibitors important in the inflammatory response. We show using recombinant inbred strains and mouse-chinese hamster hybrid cell lines that these two genes map to chromosome 12, approximately 1 map unit apart. Sequence analyses show these two cDNA clones to be from related genes having approximately 55% homology at the DNA level. The two proteases inhibitors, however, are expressed in a different spectrum of tissues and are regulated differently during the inflammatory response. The expression of α_1-antichymotrypsin mRNA is polymorphic between inbred mouse strains; BALB/c and DBA/2 mice, for example, show multiple sized RNA species for α_1-antichymotrypsin, whereas C57BL/6 and C3H/He mice express only a single sized species. This difference in RNA expression appears to be due to a regulatory locus which maps close to the structural genes.

A reversible biotinylating agent: use in Western blots

By M. J. HOBART, B. A. FERNIE and P. J. LACHMANN

MITI, MRC Centre, Hills Road, Cambridge

N-biotinyl penicillamine can be used to reversibly biotinylate proteins, especially antibodies, for probing 'Western' blots. It promises to be useful for repeated probing of the same blot. However, its use reveals two 'genuine artefacts': (*a*) Surprisingly, many 'normal' individuals have monoclonal antibodies to foreign serum proteins. Since these band patterns are probably heritable, they may represent the expression of germ-line coded genes. (*b*) There appears to be a biotin-binding protein in human serum. It is polymorphic and seems to be controlled by two loci.

Sequence homology in the 3′ untranslated region of various actin genes

By JULIE LLOYD, GERRY GILLESPIE and YVONNE EDWARDS

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

At least six different mammalian actin isoforms have been identified and in general their protein sequences and hence their nucleotide sequences in the coding region are highly conserved. Recombinants containing DNA homologous to human skeletal actin and non muscle βactin RNAs have been isolated from a fetal muscle cDNA library. Sequence analysis shows that both clones contain the 3′ untranslated region and most of the coding sequence. The noncoding sequence is relatively diverged and is gene specific. Sequence comparisons between human and rat genes (Shani *et al.* 1980) reveal greater homology in this region between the same genes across species than amongst different genes of the same species. Gillespie, G., Lloyd, J., Isenberg, H., Hopkinson, D. A., Edwards, Y. (1983). *Eur. J. Biochem.* Manuscript submitted.

A structural locus for mouse kidney histidine decarboxylase: proximal and distant regulatory loci

By S. A. M. MARTIN, R. J. MIDDLETON* and GRAHAME BULFIELD*

*Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN, *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

Mouse kidney histidine decarboxylase (HDC; E.C.4 . 1 . 1 . 22) levels are modulated by at least three hormones (oestrogen, testosterone and thyroxine), and have

a defined temporal profile. Allelic variation at the HDC structural locus, *Hdc-s*, determines (1) heat stability of the enzyme and (2) its K_m for the cofactor pyridoxal-5′-phosphate. *Hdc-s* has been mapped to chromosome 2 using both recombinant inbred strains and conventional crosses. The spatial and operational relationships with the structural gene of four loci which regulate different aspects of HDC expression will be described.

Isolation of a mammalian cell variant altered in DNA ligase and poly ADP-ribosyl transferase activity

By BARBARA MURRAY, DEBBIE CREISSEN, MANOOCHEHR TAVASSOLI, JUDY IRWIN and SYDNEY SHALL

Cell and Molecular Biology Laboratory, University of Sussex, Brighton BN1 9QG, Sussex

Nuclear ADP-ribosyl transferase, which catalyses the formation of poly(ADP-ribose) – modified chromatin proteins from NAD+, is required for efficient DNA excision repair.

We have isolated a variant from mouse leukaemia L1210 cells which is altered in its response to the poly ADP-ribosyl transferase inhibitor 3-aminobenzamide. The variant does not show potentiation of cytotoxicity to dimethyl sulphate in the presence of 3-aminobenzamide, as occurs in the parental L1210 cells. The basal DNA ligase level is increased 2-fold over the wild type level and is not activated by dimethyl sulphate, as is the wild type. The apparent V_{max} of the variant poly ADP-ribosyl transferase is slightly higher than the wild type enzyme.

Genetic determination of pyruvate kinase and triosephosphate isomerase in the mouse

By J. PETERS and G. BULFIELD*

*MRC Radiobiology Unit, Harwell; *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

Four isozymes of pyruvate kinase (EC 2.7.1.40) have been described in mammals. We report the results of genetic studies which indicate that a single structural gene, *Pk-3*, determines two of these isozymes, M_1 (the heart form) and M_2 (the kidney form), even though it had been shown that they are encoded by different mRNAs. This parallels findings for the L (liver) and L′ (red cell) isozymes for both are known to be determined by the *Pk-1* structural gene but like M_1 and M_2, are encoded by different mRNAs. We also report a mutant of triosephosphate isomerase (EC 5.3.1.1) and the location of *Tpi-1* on chromosome 6.

Rearrangements in the *H-2* complex of mouse *t* haplotypes

By J. H. ROGERS* and K. R. WILLISON†

**MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH,*
†Chester Beatty Laboratories, Fulham Road, London SW3 6JB

The *t* haplotypes are variants of mouse chromosome 17 which differ from the standard chromosome 17 over a larger region, including both the *H-2* complex and several genes of developmental interest. We have investigated the *H-2* complex of *t* haplotypes by probing Southern blots with DNA clones (kindly given by M. Steinmetz). The restriction patterns seen in *t* differ from those of standard mouse strains mainly by deletions and duplications of certain regions. One major rearrangement that is specific to all *t* haplotypes is in the *Qa* region, where certain *H-2*-related genes are missing due to the loss of several large homology units. We are now cloning this region from *t* to characterize the rearrangements in more detail.

Aldehyde dehydrogenases in human-rat hybrids

By I. SANTISTEBAN[1], S. POVEY[1], M. SMITH[2], and D. A. HOPKINSON[1]

[1]*MRC Human Biochemical Genetics Unit, University College London,*
[2]*Department of Pediatrics, University of California, Irvine*

A number of different aldehyde dehydrogenases in human and rat tissues have been characterized with respect to their substrate specificity, tissue distribution and electrophoretic mobility. One of these components is specially active against benzaldehyde, can utilize NAD^+ and $NADP^+$, is strong in lung and stomach and weakly expressed in liver. An apparently homologous enzyme has been identified in rat tissues. The human BENZ isozyme was found to be expressed in somatic cell hybrids between a rat hepatoma cell line and human fibroblasts, and in hybrids between a related rat hepatoma line and cells from human fetal liver. Attempts to correlate the presence of human BENZ with a human chromosome are still in progress. The expression of rodent BENZ in these hybrids was extremely variable. Its presence or absence was not correlated with the presence or absence of the human enzyme. In hybrids expressing both forms no heteromeric isozymes were observed.

Further studies on vole esterases

By ROBERT SEMEONOFF

Department of Genetics, University of Leicester, Leicester

The field vole, *Microtus agrestis*, is polymorphic for the presence of a serum esterase of distinctive properties. An analogous polymorphism is found in the bank

vole, *Clethrionomys glareolus*. I shall report on progress towards the purification of the enzymes in both species, and on the search for its expression in other tissues.

Mammalian myoglobin genes

By P. WELLER, A. BLANCHETOT and A. JEFFREYS

Department of Genetics, University of Leicester, Leicester LE1 7RH

The myoglobin gene is estimated from amino acid sequence comparisons to have arisen from duplication of a primitive globin-like gene about 600–800 million years ago. Sequence analysis of the grey seal myoglobin gene, isolated from a cDNA library, showed it to be 9·2 kb, 5–10 times the length of haemoglobin genes, but with equivalent arrangement of exons. Hybridization of human DNA with the seal myoglobin gene detected a single functional human myoglobin gene, plus an extensive family of sequences related to the central exon of the myoglobin gene. Sequence analysis of the functional gene showed that its organization is similar to that of the seal gene, but with some interesting promoter differences.