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The use of PCR in mapping human genes

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The polymerase chain reaction (PCR) can be used to amplify specifically human genes on a background of rodent DNA. We have exploited this specificity to allow us to identify which human chromosomes are present in somatic cell hybrids as an alternative to protein markers. This analysis has now been extended to the mapping of a human gene for which genomic sequence was available.

Linkage analysis of the halothane sensitivity locus in pigs

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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-induced malignant hyperthermia is controlled by a recessive gene at a single autosomal locus (*Hal*). As halothane sensitivity is a recessive trait, the present halothane test fails to distinguish between the homozygous resistant animals and heterozygous carriers. The porcine stress syndromes resemble some of the inherited disorders of Man, such as cystic fibrosis and Huntington's disease, which are monogenic diseases where the underlying lesion remains unknown. A molecular genetic approach has been used successfully, in the case of Duchenne muscular dystrophy, to isolate linked polymorphic markers (restriction fragment length polymorphisms, RFLPs) for the prediction of genotypes at the disease locus and for the isolation and characterisation of the disease gene itself. This approach is being used to look for predictive genetic markers for porcine stress and, in the longer term, for the *Hal* gene itself. Linkage between the *Hal* locus and a number of blood group and biochemical marker loci is well documented. The closest of the known markers is the glucose-6-phosphate isomerase gene (*Gpi*). cDNA clones for Gpi have been isolated by two different strategies using oligonucleotide probes in two laboratories. These cDNA clones and the corresponding genomic clones have been characterized. RFLPs detected using these Gpi clones have been shown to be linked to the *Hal* locus. The polymorphic information content of the Gpi DNA polymorphisms is greater than that for the Gpi protein polymorphism. Another of the known members of the *Hal* linkage group is the 6-phosphogluconate dehydrogenase gene (*Pgd*). A new Pgd protein variant has been identified, thus enhancing the value of this locus in linkage analysis. The usefulness of these, and other, polymorphic markers for the prediction of *Hal* genotypes will be discussed.

Characterization of human fibroblast ecto-5′ nucleotidase and assignment to chromosome 6q

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We have used a whole-cell assay in which [^{14}C]AMP was converted to adenosine to demonstrate that human MRC-5 fibroblasts express on their surface a 5′ nucleotidase (5NT) whose properties are similar to those shown

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by ecto-5NT purified from placenta and lymphocytes. The activity had a broad optimum from pH 6·5–8·0, was stimulated by Mg^{2+} but was not dependent on it and was released from the plasma membrane by phospholipase C. The K_{app} and V_{max} for AMP were $38 \pm 3{\cdot}4$ nmol per 10^6 cells and $173{\cdot}9 \pm 19{\cdot}2$ nmol per min per 10^6 cells respectively ($n = 8$). The enzyme recognised 5′-, but not 2′- or 3′-purines, nor pNPP or β-glycerophosphate. Strong inhibition was observed with ADP and the analogue α,β-methylene ADP. Deoxy purine nucleotides were weak inhibitors as were most pyrimidine nucleotides except dTMP. In contrast, all Chinese hamster cell lines that we tested lacked 5NT. This difference enabled us to demonstrate 100% concordance between the inheritance of human chromosome 6 and the expression of ecto-5NT in a panel of 26 human × Chinese hamster hybrids. This observation was confirmed by demonstrating the co-segregation of 5NT and HLA Class I. Regional localisation was obtained by measuring expression of 5NT in a panel of human × mouse hybrids which contained translocation fragments of 6 in the absence of normal 6 or the reciprocal translocation. The shortest region of overlap corresponded to 6q14–q21.

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CpG-rich island libraries from the mouse *X* chromosome

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The vector NotλEMBL3A has been used to construct libraries from a hybrid cell line (C18) containing the mouse *X* chromosome on a human background. Mouse *X* chromosome clones were obtained by screening with total mouse DNA. The libraries contain clones that encompass sites for the rare cutter restriction enzymes *Not* I and *Eag* I. The recognition sites for these enzymes are CpG rich and methylation sensitive and it is therefore expected that a large proportion of these clones will contain CpG rich islands. It has been estimated that *Not* I sites occur in 1 in 5 CpG rich islands, whilst *Eag* I sites should occur in most, if not all, islands. Because CpG rich islands are found in close association with a variety of genes, it is hoped that the genetic mapping of these clones will provide candidate sequences for a number of genetic loci on the mouse *X* chromosome. In addition, the occurrence of rare cutter sites in the islands will be useful in the physical mapping of the chromosome by pulsed field gel electrophoresis. Preliminary data on the characterisation and localization of some of these clones will be presented.

Effect of chronic electrical stimulation on muscle gene expression

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Chronic electrical stimulation of fast skeletal muscle induces a switch in contractile protein isoforms to those normally found in slow muscle, an increase in the enzymes of anaerobic metabolism and a decrease in those of glycolysis. We studied the levels of specific mRNAs isolated from rabbit fast muscle which had been stimulated continuously at 10 Hz for up to 21 days. cDNA probes which encode part of the myosin heavy chain (MHC) and myosin light chain (MLC), actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and carbonic anhydrase (CAIII) genes were used for the quantitative assessment of the corresponding mRNAs. Levels of fast muscle MHC and MLC mRNAs fell to about 10 and 50% respectively of control values within 10 days of stimulation. On the other hand, the level of an MHC mRNA which could be either an intermediate or slow fibre type increased during this time. Actin mRNA declined initially and then returned to control levels after 21 days. Slow muscle specific CAIII mRNA exhibited a rapid increase on stimulation and GAPDH mRNA declined in accordance with changes in muscle leading to a more oxidative metabolism. These alterations in specific mRNA levels are in broad agreement with changes in the corresponding protein.

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Cloning of cDNAs for a bovine immunoglobulin to testosterone

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Steroid antibodies are of considerable value for elucidating the actions of steroid hormones and can be used to immunomodulate physiological processes dependent on hormone response mechanisms. Of particular interest is the role of steroid hormones in regulating the reproductive cycle. To obtain large quantities of specific allogenic anti-testosterone antibody for passive immunization studies a mouse–bovine heterohybridoma has been produced which secretes a bovine monoclonal antibody to testosterone. From this heterohybridoma we have cloned cDNAs for the heavy and light chains of the anti-testosterone antibody. The heavy-chain is of the subclass IgG_1. CDR3 is unusually large, 14 amino acids long. Both FR3 and FR4 have invariant amino acids at the expected positions consistent with immunoglobulin heavy-chains from other species. The light-chain is of the lambda type. This work will lead to a molecular examination of the interactions between antibody molecules and steriod antigens relevant to the use of antibodies for physiological immunomodulation.

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A highly unstable mouse minisatellite locus

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Human DNA fingerprinting probes 33·6 and 33·15 have been used to detect sets of highly variable minisatellites in the mouse genome. These DNA fingerprints are less complex in inbred strains of mice than in wild mice and are largely strain specific. Analysis of recombinant inbred strains has shown that these DNA fingerprints are composed of loci which are dispersed through the mouse genome and are of heterogeneous stability. One locus in particular, *Ms6-hm*, was found to be very unstable. This locus has been cloned to investigate the rate and mechanism of mutation at minisatellites. A 7 kb allele from a C57BL/6J mouse collapsed on cloning to a stable 300 bp insert in pUC13. This contained the residual minisatellite embedded in a repetitive element of the MT family. We have used the BXD RIs to map *Ms6-hm* close to *Ifa* on chromosome 4. Through analysis of a large pedigree we estimate the germline mutation rate per gamete for *Ms6-hm* to be 3–4%. A significant rate of somatic mutation is also seen at this locus; mice with three alleles in both somatic and germline tissue have been found. At low stringency the 300 bp probe detects other highly variable minisatellites in the mouse genome. Together these loci generate a virtually inividual specific DNA fingerprint for mice even from within an inbred strain.

Study of the PGD Locus on Chromosome 1p

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A partial cDNA for human 6-phosphogluconate dehydrogenase (*PGD*) was isolated from a lung library using a rat cDNA probe. Somatic cell hybrid analysis shows that the cDNA hybridizes to three independently segregating loci suggesting the possibility of PGD pseudogenes. It also shows that one of the three loci lies within 1p36.13-1pter, the chromosomal region to which PGD has previously been assigned. The cDNA detects a *Bam*HI RFLP at the 1p locus. Linkage between this RFLP and the PGD protein polymorphism has been studied to confirm the authenticity of the cDNA. Results so far show strong but incomplete allele association. Linkage of the RFLP to other 1p markers is being examined to refine the genetic map of this chromosomal region. Relative frequencies of the RFLP and the PGD protein polymorphism are such that the RFLP increases the informativeness of the PGD locus as a marker by a factor of about 10.

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Widespread expression of human alpha-1-antitrypsin in transgenic mice revealed by *in situ* hybridization

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In situ hybridization was used to examine the spatial and developmental control of human α_1-antitrypsin (α_1AT) mRNA expression in transgenic mice. In addition to expression in yolk sac and liver, human α_1AT mRNA was detected in gut, stomach, pancreas, nasal epithelium, pharynx, bronchi, spinal ganglia and ossifying cartilage of transgenic fetuses at 14·5 days post coitum. Expression in transgenic adults was no longer found in the pancreas but was found in the kidney and salivary gland. In each tissue, expression was confined to a specific cell population. This pattern of α_1AT expression was found to correlate with that seen in several human fetal and adult tissues. These results suggest a wider role of α_1AT in human physiology and development than previously suspected, and may help to explain the various pathological conditions which have been tenuously associated with α_1AT deficiency. Expression of the endogenous α_1AT gene in mice was confined to a subset of these tissues, suggesting that the *cis*-acting human sequences which regulate the expression of the transgene and the *cis*-acting sequences associated with the mouse gene interact differently with transcription factors present in mouse cells.

Approaches to the role of homeobox genes in vertebrate development

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A number of murine genes that potentially play an important role in mammalian development have been isolated by virtue of their sequence similarity to known *Drosophila* morphogenetic loci. In most, but not all cases, sequence identity is restricted to a 183 bp motif termed the homeobox. This work focuses on the murine homeobox gene *Hox 2.1* that forms part of a complex of homeobox genes on chromosome 11. This gene is transiently activated in F9 teratocarcinoma cells induced to differentiate with the potential morphogen retinoic acid. The kinetics of expression differ, depending upon whether the stem cells differentiate into visceral or parietal endoderm-like cells. Data on the regulation of *Hox 2.1* suggests that post-transcriptional events are involved in the activation. Deletion-transfection data has begun to identify sequences in the 3′ end of the RNA involved in mRNA instability. Consistent with the idea of homeobox proteins being developmentally regulated transcription factors is the nuclear localisation of *Hox 2.1*, shown by antibody tagging. None of the *Hox 2* has so far been found to be allelic with known mouse mutants, therefore efforts are being made to address the function of these proteins by gene transfer or reverse genetics. The strong evolutionary conservation of homeobox proteins predicted by sequence data permits the use of heterologous systems for these experiments. Preliminary results suggest that the inappropriate expression of RNA from the mouse *Hox 2.1* gene in developing *Xenopus* embryos is associated with specific phenotypic defects. These experiments provide the opportunity to examine different regions of the mouse homeobox protein to define functional domains and correlate them with biological roles.

Cloning and expression of mouse carbonic anhydrase III (CAIII)

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The carbonic anhydrase (*CA*) gene family includes at least eight related gene sequences which encode several kinetically distinct isozymes. In mice, cDNA clones have been reported which determine two of the cytoplasmic

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isozymes, the erythrocyte specific CAI and the more ubiquitously expressed CAII. Using an isozyme-specific antibody we have isolated a cDNA clone for the mouse CAIII enzyme. This cDNA has been fully sequenced and used as probe to examine CAIII expression in mouse tissues. In man and other large mammals CAIII expression is confined to skeletal muscle; however, in the mouse and rat, CAIII is also expressed in the liver. The pattern of CA expression in rat liver is sexually dimorphic; however, examination of mouse liver CAIII RNA and protein shows that this phenomenon does not occur in mice and is peculiar to rats.

Characterisation of a novel gene at the human Pi locus

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During a study of the alpha-1-antitrypsin protein and its locus (*Pi*) by isoelectric focusing and RFLP analysis of 130 individuals of the PiZ (deficiency) phenotype, we have identified a novel allele that resembles the *PiZ* gene in several of its properties. Compound heterozygotes of the novel allele (PiZ_{Tun}) and *PiZ* are clinically indistinguishable from the usual *PiZZ* homozygotes and its protein product is also deficient in the plasma. Combined genetic and direct analysis indicates that the new allele is due to a mutation in exon V that is identical in all respects to the *Z* mutation but on a different genetic background.

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