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Characterization of autoantigens in an inherited autoimmune disease

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The CREST syndrome is a form of progressive systemic sclerosis in which characteristic anti-kinetochore antibodies occur with very high frequency. Serum from one CREST patient described here reacts not only with the kinetochores of chromosomes, but also with an ubiquitous cytoplasmic antigen. Indirect immunofluorescent assay of human or hamster cells with the serum gives a pattern of speckling throughout the cytoplasm, followimg the actin stress fibre lines in addition to the nuclear immunofluorescence of the kinetochores. The cytoplasmic antigen, associated with a phosphorylated polypeptide of $\simeq$ 70 kD present in all mammalian cell types examined, is mainly found on coated vesicles.

Y chromosome polymorphism and diversity in wild mice

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A cloned *Y* specific sequence belonging to a moderately repeated family was used as a probe to detect species specific polymorphisms on the *Y* chromosome of wild mice. RFLP analysis reveals diagnostic patterns which were used to study the evolutionary history of this family of sequences in the genus as a whole and *Y* chromosome gene flow along the hybridization zone between the semispecies *M.m. domesticus* and *M.m. musculus*. The putative origin of laboratory mice strains will be discussed in the light of these results.

The structure and expression of the mouse *Tcp-1* gene

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We have previously reported the identification of a cDNA clone corresponding to the mRNA of the mouse *Tcp-1* gene. TCP-1 is a polypeptide encoded in the mouse *t*-complex and was originally described by Silver *et al.* (1979). We have shown that the mRNA for TCP-1 is expressed at high levels in post meiotic male germ-cells, but is also present in all cell types so far tested but at lower levels. We have now isolated phage and cosmid clones encompassing the entire *Tcp-1* gene cluster and have determined a detailed map of the intron and exon structure. The entire nucleotide sequence of the cDNA has revealed several amino acid changes between the wild-type form of the polypeptide TCP-1B and the *t*-mutant form TCP-1A. Progress towards raising anti-sera to the polypeptide by using synthetic oligopeptides and bacterial fusion proteins as antigens will be discussed.

Problems of polymorphism

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Polymorphism, as defined by Ford, involves serious problems, in that it is present to a degree not easily accommodated within the fabric of Mendelism acting on likely numbers over likely times. Models involving the imprinting of parts of chromosomes by their previous environment could explain both the genesis and the maintenance of this variability. A simple model will be explored in which heterozygosity at one meiotic event predisposes to conversion at the next. Chromosomal imprinting is now demonstrable in mammals, both for the *X* chromosome and autosomes, and the model may be operational using techniques available.

Genes affecting the concentration and the activity of erythrocyte and liver pyruvate kinase in the mouse

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Both the erythrocyte and liver isoenzymes of pyruvate kinase are coded for by the same structual gene, *Pk-1*. We have several mutants affecting the measurable activity of PK in liver or RBC or both. Using a specific antiserum to liver PK we have titrated the concentration of PK in both tissues of these mutants and genetically determined the relationship of each mutation to the structural gene. It is concluded that some mutations affect the activity and some the concentration of PK in both tissues; more surprisingly we have tissue specific mutations: one affecting enzyme concentration and the other enzyme activity.

Enhancer dependence of the mouse major urinary protein genes in tissue culture cells

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The mouse major urinary proteins (MUPs) are a closely related group of proteins which are synthesized in the liver, secreted into the blood and rapidly excreted in the urine. MUPs are the most abundant male mouse liver product and are also expressed to a lesser extent in some other secretory tissues, the lachrymal, submaxillary, parotid and mammary glands. The MUP genes that are expressed in the liver are under multihormonal control and different genes display different patterns of hormonal regulation as well as tissue-specific expression. MUPs are, therefore, a good model system for investigating the control of gene expression. The homologous rat gene, the $\alpha_{2\mu}$-globulin gene, was the first tissue-specific gene demonstrated to be glucocorticoid inducible in a heterologous cell system (mouse L cells) (Kurtz, D. (1981). *Nature* **291**, 629).

Data will be presented on the transient expression in BHK tk^- cells of constructs containing various mup promoter sequences linked to the HSV-tk gene in either the presence or absence of the SV40 enhancer region. These data firmly demonstrate the enhancer dependence of the mup promoter in fibroblasts. Finally, it is suggested that this enhancer dependence involves a *deregulatory* effect on the mup promoter by the enhancer while the mup promoter and associated sequences has a *down* regulatory effect on the SV40 early promoter.

Characterization of the human jejunal brush border hydrolases, sucrase, aminopeptidase and lactase

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The restricted tissue distribution of the membrane-bound glycoprotein hydrolases of the jejunal brush border has limited both the biochemical and somatic-cell genetic analysis of these enzymes in man. None of the genes

have been mapped to human chromosomes and the relationship of the enzymes one to another and to well characterized enzymes with similar biological activity present in other tissues is not clear. We have been producing and making use of monoclonal antibodies to assist in the characterization of the brush border hydrolases and ultimately the isolation of their genes. I shall describe some of our preliminary findings on the biochemical genetics of sucrase, aminopeptidase and lactase.

The isolation of cDNA clones from the mouse tyrosinase gene

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The *C* locus (*albino* locus) of mouse is an excellent candidate for applying molecular biological techniques to a gene extensively studied by classical genetics. The locus is thought to encode tyrosinase, the enzyme in common with both phaeomelanin and eumelanin synthesis.

I have made a cDNA library from mRNA of pigmented B16 melanoma cells, by cloning into the expression vector λgtll. I screened the library with antiserum specific for tyrosinase and obtained several positive clones. These clones are now being used to examine their structure and their transcription in melanoma cells. They are being mapped in the mouse genome with respect to the *C* locus.

Isolation and analysis of a cDNA clone for the human muscle specific carbonic anhydrase, CAIII

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Human CAIII recombinants were isolated from an adult skeletal muscle cDNA library prepared in the bacteriophage expression of vector λgtll. A full length cDNA was characterized by nucleotide sequence analysis. The CAIII probe has been used to investigate the developmental pattern of expression of human CAIII and to assign the gene for human CAIII to chromosome 8.

Linkage and possible homology of tasting genes and salivary protein genes in mouse and man

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There are three closely-linked tasting genes in the mouse. *Qui* (quinine) *Rua* (raffinose acetate) and *Cyx* (cycloheximide). The SDP (strain distribution pattern) of *Qui* and *Rua* in the fourteen tested BXD RI strains is identical to the SDP of a gene which is detected by a cDNA probe for 'salivary proline-rich protein' genes. The salivary protein genes are on chromosome 8 (Azen, (1984), *Science* **226**, 967). Therefore the tasting genes are probably on chromosome 8. In Man an homologous group of salivary protein genes is on Chr. 12. Could there be some homology between tasting genes and salivary protein genes? Or could there be a physiological relationship between tasting and saliva?

Genetic analysis of altered sex ratio phenotypes for the enzyme histidine decarboxylase in the mouse kidney

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Histidine decarboxylase (HDC) activity is normally repressed by testosterone and induced by oestrogen in the mouse kidney. Genetic variation exists amongst strains of mice in their response to sex hormones which leads to an altered sex ratio for HDC activity. Genetic analysis of the phenotype for high HDC activity in males in a feral strain has indicated the difference to be due to a single gene. This gene confers insensitivity to testosterone and the effective factor responsible maps at or near the structural locus for HDC. In addition, analysis of a further abnormal sex ratio phenotype will be presented. In this strain (NZB), the female/male activity ratio is greatly increased. Evidence exists for this difference also being a single gene effect.

Tissue specific *X*-chromosome expression and methylation in development

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Previous work has shown that the timing and specificity of *X*-chromosome inactivation is coupled to early differentiation events in the female mouse embryo and that the inactive *X* is reactivated in the female germ-line around the time of meiosis. Since there are differences in methylation patterns on the active and inactive *X* chromosomes we asked whether changes in overall DNA methylation in different embryonic lineages might accompany changes in *X* chromosome activity. A specific demethylation event in the germ-line is proposed.

Characterization of heat shock protein (hsp70) during mouse embryogenesis

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Although a large body of information on hsp70 is available only little is known about the physiological role of this protein during embryogenesis where proliferation is especially enhanced at certain stages of the foetal development. We isolated mouse embryos as early as day 6 after fertilization. At 2 days intervals foetuses were isolated and analysed for the presence of hsp70 and heat shock mRNA by PAGE and Northern blotting, respectively. At day 14 a burst of heat shock mRNA and hsp70 is observed, which declines continuously till birth. Freshly born mice hardly contain any hsp70. These observations suggest that the hsp70 is also involved in certain steps during foetal development, apart from its traditional well-known role when stress conditions prevail. Since hsp70 appears late during embryogenesis its function could be involved in the massive growth process which takes place post organogeny, rather than being involved in differentiation. With the aid of a 2·3 kb genomic probe we have tentatively assigned the hsp70 gene locus to chr. 1,2,5, from human metaphases.

Organization of actin and myosin genes in mouse

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Actin and myosin genes are members of a multigene family and are co-ordinately expressed during differentiation. We have investigated gene expression at the mRNA level and found two modes of expression: actin and alkali

myosin light-chain genes are co-expressed whereas myosin heavy-chain genes are sequentially expressed. We have characterized five myosin heavy-chain cDNA probes specific for the embryonic, perinatal, adult skeletal and α-(V1) cardiac myosin heavy-chain genes, three cDNA probes specific for the adult skeletal, cardiac ventricular, and embryonic atrial alkali myosin light-chain genes, and three cDNA probes specific for the skeletal, α-cardiac and non-muscular actin genes. We used these probes on Southern blots of genomic DNA to detect restriction fragment-length polymorphisms defining alleles in two mouse species. In this way we followed the segregation of these genes in 42 offspring. We found that the genes are not linked except for the myosin heavy-chain genes which are sequentially expressed. Sequential expression may require linkage as in the case of the globin genes.

Isolation of a genomic clone with the coding sequence of carcino-embryonic antigen (CEA)

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Two mixed oligonucleotides corresponding to part of the known amino acid sequence of carcino embryonic antigen were employed to screen a human genomic DNA cosmid library. Two cosmids were isolated which contained sequences detected by both oligonucleotide probes. 1·0 kb region from one of the cosmids was sequenced and the 5′ end of a CEA (or CEA-like) gene identified. The gene has a hydrophobic leader sequence. 5′ coding sequences contained features which are presumed to be important in regulating expression.