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Regulation of expression from the human beta-globin gene domain

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Using both erythroid cell tissue culture systems and transgenic mice, we have identified five DNA regions that regulate the transcription of the human beta-globin gene during development: (i) dominant locus-activating regions which flank the beta-globin locus, whose activation precedes the expression of any of the beta-like globin genes within the domain; (ii) positive and negative acting regions within the promoter; (iii) two transcriptional enhancer elements – one within the third exon and the other 600 bp downstream of the gene. These enhancers mediate the switch from foetal to adult globin gene expression.

Transient HPRT expression by injection of HPRT minigene DNA and its inhibition by antisense DNA

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Sensitive techniques are being developed to manipulate and monitor gene expression during pre-implantation development. The experiments involve injection of different constructs containing the mouse HPRT minigene into the male pronucleus and assay of transient expression of HPRT activity. The simultaneous assay of endogenous APRT activity provides an internal standard which enables comparisons between different injected eggs and different experimental procedures. The efficacy of different promoters in early cleavage stages may be studied using the expression of HPRT as a reporter function. Induction of the expression of the HPRT minigene 'driven' by the mouse metallothionein-I promoter is obtained by incubation of the embryos in the presence of cadmium. Negation of expression is obtained by simultaneous injection of HPRT antisense constructs together with the HPRT sense constructs.

Amplification and rearrangement in a germ line HSR of the mouse

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In various populations of wild mice of the species Mus musculus an additional chromosome segment in chromosome 1 is present, which may represent up to 1·8% of the haploid mouse genome. The segment has the characteristics of a homogeneously staining region (HSR) and is in contrast to the hitherto examined HRSs transmitted through the germ line.

Random DNA probes of this HSR were generated by microdissection and microcloning. Southern blot analyses of the cloned DNA probes and *in situ* hybridization experiments revealed amplification and rearrangement of wild mice DNA sequences in the HSR. However, the HSR DNA seemed not to be amplified

as a constant unit. Apparently the amplification process comprised DNA sequences of varying extent at different steps. Moreover, complicated rearrangements have taken place at a stage preceding amplification or during amplification.

Long-range physical mapping on the mouse *X* chromosome

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Molecular probes in the region of the *mdx* mutation on the mouse *X* chromosome are being used to construct physical maps utilising PFGE. These maps should facilitate precise localisation of the *mdx* mutation. Further, the average density of probes (random probes obtained by microdissection and known gene probes e.g. DMD, G6PD and CF8) in the proximal region of the mouse *X* chromosome is now one every 2 Mb, sufficient to contemplate the complete physical mapping of this region. Such a task will require the use of megabase separations on OFAGE gels and will be facilitated by the construction of a 'linking library' of the mouse *X* chromosome.

Molecular mapping around the *mdx* locus on the mouse X chromosome

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There is considerable interest regarding the status of the mouse *X*-linked muscular dystrophy mutation *mdx*. It has been suggested that the *mdx* locus is the homologue of the human *X*-linked locus Duchenne muscular dystrophy (DMD) or alternatively another human *X*-linked locus Emery–Dreifuss muscular dystrophy (EDMD). A *spretus/domesticus* interspecific cross, segregating for *mdx*, was established and serum pyruvate kinase levels and histological studies were used to determine expression of the *mdx* phenotype. Approximately 80 mice were analysed in this way and DNA samples recovered for analysis with DNA probes including human DMD exonic sequences. We have been able to construct a detailed molecular map in the region of the *mdx* locus which has implications for the relationship of *mdx* to both DMD and EDMD.

DNA fingerprinting – molecular genetics in business

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Since the publications of Professor Alec Jeffreys in 1985 on his discovery of the DNA fingerprinting probes, ICI Diagnostics have developed what is essentially a research technique into the first large scale genetic diagnostic service available to the public. ICI established Cellmark Diagnostics which opened for business in June this year and has already registered over 1200 cases. To date this technique has been used to establish family relationships in immigration and paternity disputes, and to identify samples in forensic cases. Animal pedigrees and cell line authentication are amongst many new areas of research and application for DNA fingerprinting.

Physical characterization of the human Xp11.3–11.4 region around the Norrie locus

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Norrie disease (ND) is an *X*-linked recessive disorder characterized by congenital blindness due to pseudotumorous proliferation of the retinal epithelium. In about one third of the cases mental retardation and hearing loss may occur. Close linkage has been shown between ND and the polymorphic locus DXS7 (probe L1.28, Xp11.3). In one of our Norrie families (male patient B.T.) with a complex clinical syndrome a submicroscopical deletion including L1.28 has been found. Here we present data on the physical characterization of the Xp11.3–11.4 region. Probe L1.28 has been mapped on a 450 kb *Sal*I FIGE fragment together with probe pX59R2 (DXS77, Xp11.3), which was not deleted in patient B.T. One of the breakpoints of the deletion has been localized on an about 45 kb *Apa*I fragment within the 450 kb *Sal*I fragment. The probe pX59R2 was found to be polymorphic for *Eco*RV and can be an additional marker for ND.

Use of a hypervariable region (HVR) probe alpha-globin 3′HVR to produce individual specific fingerprints

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This paper describes, giving details of methods, the production of DNA fingerprints using alpha-globin 3′HVR. Segregation analyses using large sibships are presented together with estimates of the individual specificity of the fingerprints. These studies demonstrate the probes' potential for use in forensic work, including paternity studies and also for linkage analysis.

Genetic mapping of microclones to mouse chromosome 7

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Over eighty progeny mice have been generated from an interspecific *spretus/domesticus* backcross segregating for two mouse chromosome 7 loci, pink-eye dilution (*p*) and chinchilla (c^{ch}). This resource has been used to map three anonymous DNA clones derived from a microdissection and microcloning of chromosome 7 and will be utilised to map further microclones and known mouse and human sequences in order to create a detailed map around the developmental locus, pudgy (*pu*). Such closely linked sequences will be analysed physically for their proximity or possible allelism to pudgy genic sequences.

Primary human cell cultures and established cell lines respond differently towards the action of tumour promoter okadaic acid

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Phosphorylation *in vivo* of primary human tumour cell cultures and established cell lines after treatment with the new tumour promoter okadaic acid led to quantitative and qualitative differences in the phosphoprotein pattern. A 60 kDa protein was predominantly phosphorylated. The protein was identified as a breakdown

product of nucleolin (M_r 110 kDa). Nucleolin has been shown earlier to be involved in the control of rDNA transcription. Phosphorylation of nucleolin is usually elevated in rapidly proliferating cells and tissues. The changes found in the phosphorylation pattern of cytoplasmic proteins found in the phosphorylation pattern of cytoplasmic proteins from primary cell cultures and established cell lines, especially those of nucleolin and its 60 kDa breakdown product suggest specific differences in the control of rDNA transcription.

Long-range mapping of human gene families

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The mammalian genome has become, due to technological advances, far easier to manipulate. One such advance is pulse field electrophoresis which enables DNA fragments as large as 2000 kb to move through an agarose gel. Using this technique two gene families have been studied in some detail. FIGE analysis of human DNA demonstrated a distance of 110 kb between the members of the alpha-1-antitrypsin gene family on the long arm of chromosome 14. The orientation of the alpha-1-antichymotrypsin relative to alpha-1-antitrypsin was also established.

A maximum distance of 160 kb was established for the distance between CA1 and CA3 on the long arm of chromosome 8 though further mapping may reduce this.

Five years into an introduction experiment: the Isle of May mice revisited

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In April 1982 77 mice from Eday, one of the Orkney islands, were introduced to the Isle of May. At this time the mice on the Isle of May were genetically invariable, and markedly different from the introduced mice, which were fixed for three Robertsonian translocations and alternative alleles at four biochemical loci, and were polymorphic at twelve other biochemical loci. The frequency of all introduced variants in the Isle of May population increased rapidly to a value around 50% of their frequency in the Eday population within only eighteen months of the introduction. Subsequently, changes have been much less dramatic, with a continued slow increase in the frequency of introduced fusions and alleles, slight seasonal changes, and a larger spasmodic increase in frequency of one introduced allele. An explanation for these changes will be offered and the wider implications of the results for population genetics theory discussed.

Regulation of mouse homeobox genes in embryos and cultured embryonic stem cells

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In our laboratory cloning and analysis of the structure and expression of several murine homeobox containing genes from the Hox 2 cluster, has revealed a complex pattern of expression. Northern and *in situ* hybridization experiments show that the Hox 2.1 and 2.6 genes are tissue-specifically, spatially and temporally restricted during mouse development. To approach the molecular basis of these patterns we have also examined gene expression in teratocarcinoma and embryonic stem cells. Our results on the endogenous and transfected versions of these genes indicate that multiple 5′ ends are used and they show variation in different tissues. Multiple elements appear to control expression via transcription and mRNA stability.

The genetics of bitterness and sweetness tasting in mice

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There seems to be a large number of genes which determine the ability of mice to taste bitter substances. The five which have been studied are *Soa* (sucrose acetate), *Rua* (raffinose acetate), *Qui* (quinine), *Glb* (glycine) and *Cyx* (cycloheximide). The last four are linked in the order *Cyx–Qui–Rua–Glb*, probably on chromosome 6. It is not yet known if *Soa* is also in this cluster of genes. In contrast to bitterness, only one sweetness tasting gene has been identified, *Sac* (saccharin). On evidence from BXD RI strains and from a wide survey of inbred strains, the gene(s) which determine saccharin tasting also determines the ability to taste other sweet substances, e.g. acesulfam, dulcin and sugars. It is suggested that there are many different receptors for bitterness but only one receptor for sweetness.

A novel repeat sequence island on the mouse *X* chromosome

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A microclone (141) isolated from a microdissection of the mouse *X* chromosome identifies a cluster of related sequences. This repeat island maps to a euchromatic area of the *X* chromosome. The island consists of a cluster of approximately 50 copies of an *X*-specific sequence, that is genetically and physically limited to a small region of the *X* chromosome. Pulse field gel analysis indicates that the island sequences are confined to a region less than 1 Mb in size and are bordered by a number of rare cutter restriction sites. The island sequences are themselves part of a longer complex repeat unit that is dispersed amongst a variety of other unrelated sequences within the island. The absence of unmethylated rare cutter restriction sites within the island suggests this may be a relatively large, genetically inert region of the mouse *X* chromosome.

Use of a mouse embryonic stem cell line deleted for part of the HPRT gene to demonstrate homologous recombination

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We have found that the spontaneous mutation in the embryonic stem cell line used by Hooper *et al.* to produce HPRT in the deficient mice, is a deletion of the first two exons of the HPRT structural gene. The ability to make targetted genetic modifications *in vitro* and then reintroduce them into the mammalian germ line, via the stem cell route, would be of great value. Because of the strong selection available for HPRT enzyme activity and the clearly defined nature of the mutation, this cell line was a good target for correcting the HPRT deficiency by homologous recombination. A plasmid, containing the promoter and exon 1 of human HPRT, plus mouse exons 2 and 3, providing about 4 kb of sequence in common with the target locus, was introduced into the cell line. A frequency of correction greater than 4×10^{-5} was observed.

Embryonic expression of proto-oncogene *int-1* in the mouse neural tube

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The proto-oncogene *int-1* is implicated in mouse mammary tumour formation and encodes a protein that is probably secreted and is of unknown function. *Int-1* has a highly restricted pattern of normal expression, apparently limited to the mature testis and a transient expression during mouse development. To gain clues as to the normal function of *int-1*, we have examined the embryonic sites of *int-1* RNA accumulation by *in situ* hybridization. *Int-1* expression is restricted to a subset of cells in the developing central nervous system. Computer-aided reconstruction has been used to visualise the spatial and temporal pattern of *int-1* expression. We will discuss possible functions of *int-1* in the mouse neural tube in the light of the recent finding that the *Drosophila* homologue of *int-1* is *wingless*, a gene involved in pattern formation.

Expression of fibroblast growth factor related proto-oncogene *int-2* in gastrulating mouse embryos

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Several cellular genes have been implicated in MMTV-elicited mouse mammary tumorigenesis. Although there are no apparent sequence homologies among these genes, the insertion of MMTV either 5′ or 3′ is a common feature. One such gene, *int-2*, has recently been identified as homologous with members of the FGF family. As no adult sites of *int-2* expression have been observed, it seemed likely that *int-2* may be an embryonic/foetal expressed gene. We have used *in situ* hybridization to localize *int-2* expression to newly formed embryonic mesoderm during its early migratory period through the primitive streak and to extraembryonic sites. The potential role of *int-2* in these early events will be discussed.

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