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The human pyruvate dehydrogenase *Elα* genes: location, gene organization and expression

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The pyruvate dehydrogenase (PDH) complex catalyses the conversion of pyruvate to acetyl CoA in the mitochondrion and is a key regulatory enzyme in glucose metabolism. PDH deficiency is a relatively common inborn error of metabolism with a particularly broad spectrum of clinical presentation ranging from acute neonatal lactic acidosis to chronic neurodegeneration. Although the PDH complex is composed of multiple subunits which are the products of at least eight different gene loci, the mutation in almost all patients with PDH deficiency is in one particular subunit, the α subunit of the El component. This subunit is also of biochemical interest as it contains the regulatory sites which control the activity of the entire complex. We have isolated cDNA clones for the Elα subunit and mapped the corresponding genes by *in situ* hybridization and Southern blot analysis of somatic cell hybrids. There are two gene loci for the Elα subunit in the human genome. The major locus is on the short arm of the X chromosome in the region Xp22·1–22·2 and is subject to *X*-inactivation. The *X* chromosome location of these genes and varying patterns of *X*-inactivation in human females are a major factor in the clinical heterogeneity of PDH deficiency. The second PDH *Elα* locus is located on chromosome 4 in the region 4q22–23. This gene is only expressed in the testis after the onset of spermatogenesis and at this time the *X*-linked gene is inactivated. The product of the autosomal locus shares extensive homology with the *X*-linked form of PDH *Elα* but the gene organization is quite different. The coding information of the *X*-linked gene is arranged as eleven exons separated by introns ranging in size from 0·6 to 5·7 kb. By contrast, the autosomal PDH gene completely lacks introns and appears to be an active processed gene. The organization and expression of the PDH *Elα* genes is exactly comparable to that of another important enzyme of carbohydrate metabolism, phosphoglycerate kinase. There is an *X*-linked gene for this enzyme which is expressed in all somatic cells and a processed autosomal gene which is only expressed during spermatogenesis. This arrangement may be required to provide continuous expression of essential enzymes of energy metabolism during the prolonged maturation phase of haploid male germ cells.

Methylated DNA binding proteins in mammalian nuclei

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There is a strong correlation between DNA methylation and transcriptional inhibition in mammals. In bulk chromatin cytosine, but not 5-methylcytosine, is accessible to nucleases. This suggests the presence of nuclear factors which can differentiate between methylated DNA and non-methylated DNA. Using the electrophoretic mobility shift assay we have identified a protein, MeCP-1, which binds preferentially to methylated DNA. MeCP-1 is able to complex with a wide variety of unrelated DNA substrates only when they are methylated at CpG. Experiments using synthetic oligonucleotides show that MeCP-1 requires at least 15 symmetrically methylated CpGs for strong binding. We have recently identified another protein, MeCP-2, using the South-Western assay. The DNA binding properties of MeCP-2 show similarities to those of MeCP-1 and we are currently characterizing this protein further. The ability of MeCP-1 and MeCP-2 to recognise multiple methylated CpGs independent of sequence context implies they may play a role in methylation dependent transcriptional inhibition.

Transcription of an amplified sequence from a germline HSR in chromosome 1 of *Mus musculus*

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Homogeneously staining regions (HSRs) are extra segments on chromosomes, which are believed to be the cytological correlates of DNA amplification. Several populations of wild house mice (*Mus musculus*) are polymorphic for HSRs on chromosome 1. In contrast to all other HSRs described in literature (for example see Alitalo & Schwab, *Advances in Cancer Research* **47**, 235, 1986; Schimke, *Cancer* **10**, 1912, 1986) the HSRs of *Mus musculus* are transmitted throughout the germline (Traut *et al. Cytogenet. Cell Genet.* **38**, 290, 1984). Southern blots hybridized with HSR specific clones reveal that the HSRs of *Mus musculus* contain amplified mouse DNA. Furthermore one of these clones Lambda MmHSR5 (2·4 kb) detects a poly(A)$^+$RNA transcript from liver of mice. The transcript is overexpressed in the HSR mice, which is seen by the stronger hybridization signal in poly(A)$^+$RNA from HSR mice compared with that in poly(A)$^+$RNA from non-HSR mice. One clone of a c-DNA library of HSR mice was found to be complementary to a 1·3 kb poly(A)$^+$RNA. A stretch of 107 bp is identical in the c-DNA clone and in Lambda MmHSR5. This homology is also present in members of a low-copy 3·8 kb *Eco*RI family in non-HSR mice.

Unusual molecular characteristics of a repeat sequence island within a Giemsa positive band on the mouse *X* chromosome

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The mouse genome contains fifty copies of a long complex repeat unit that are localized as a repeat sequence island at the A3 Giemsa positive (dark) band on the mouse *X* chromosome. The repeat units are not tandemly arranged but are juxtaposed and inserted by unrelated sequences of high repetition. The repeat sequence island possesses two notable features that have been suggested as diagnostic features of mammalian Giemsa positive bands. Firstly, the repeat sequence island encompasses a one megabase region devoid of CpG islands and, secondly, it features a high concentration of L1 long interspersed repeat sequences.

Establishment of the mouse chromosome 7 region with homology to the myotonic dystrophy region of human chromosome 19q

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A number of genetic markers, including ATP1A3, TGFB, CKMM and PRKCG define the genetic region on human chromosome 19 containing the myotonic dystrophy locus. These and a number of other DNA probes have been mapped to mouse chromosome 7 utilizing a mouse *Mus domesticus/Mus spretus* interspecific backcross segregating for the genetic markers pink-eye dilution (*p*) and chinchilla (c^{ch}). The establishment of a highly conserved synteny group between mouse chromosome 7 and human chromosome 19q indicates the likely position of the homologous gene locus to the human myotonic dystrophy gene on proximal mouse chromosome 7. In addition, we have mapped the muscle Ryanodine receptor gene (*Ryr*) to mouse chromosome 7 and

demonstrated its close linkage to the *Atpa-2*, *Tgfb1* and *Ckmm* cluster of genes. In humans, the malignant hyperthermia susceptibility locus (MHS) also maps close to this gene cluster and we propose *Ryr* as a candidate gene for MHS.

The molecular and genetic mapping of mouse chromosome 16

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Mouse chromosome 16 contains a number of genes and markers homologous to the long arm of human chromosome 21. This region of the genome has been the subject of extensive study in both man and mouse due to its involvement in Down's syndrome and Alzheimer's disease. We have begun the construction of genetic maps in this region of mouse chromosome 16, using an interspecific *Mus spretus/Mus domesticus* backcross. This will form the basis for the detailed physical mapping of the homologous mouse loci. In addition a number of mouse chromosome 16 probes outside the human chromosome 21 homologous region have been included in the analysis to develop a genetic map that spans the whole of mouse chromosome 16. We have constructed a genomic library from a somatic cell hybrid line which contains mouse chromosome 16 in a Chinese hamster background and isolated a number of mouse specific clones. We are now in the process of identifying unique DNA sequences from these clones to use as anonymous genomic probes on mouse chromosome 16. We are also constructing Not-1 linking clones that can readily be mapped and linked by the use of pulsed field gel electrophoresis.

acd: a pleiotropic mutant with effects on the ploidy of differentiated tissues

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The *acd* (adrenocortical dysplasia) mutation was initially studied because it showed the signs of Addison's disease: hyperpigmentation, reduced growth and stress susceptibility. These are secondary to reduced adrenal weight (0·17 mg/g bodyweight *vs.* 0·29 mg/g for females), accompanied by elevated levels of ACTH. Microscopically the cortical cells are markedly enlarged (about 4-fold over normal littermates) and many contain very large nuclei (cross-sectional area 4-fold greater). The cytoplasm is packed with mitochondria typical of steroidogenic tissue and the cells produce both aldosterone and corticosterone *in vivo*. Ploidy levels were analysed, using a fluorescence-activated flow cytometer, in nuclei from adrenals of mutant and normal mice aged 1, 7, 14 and 28 days. The mutants had twice as many cells with a tetraploid DNA content as their normal littermates, at all ages. They also had twice as many cells with higher levels of ploidy. Significant differences were also found in liver cells. On day 1 25% of the mutant nuclei had a tetraploid or higher DNA content. This level of ploidy was not reached by normal littermates until 28 days, at which age over 40% of the mutant nuclei were polyploid. Small differences in ploidy were found in cardiac muscle. Differences in ploidy were also found in the spleens and thymuses of newborn mutant and normal littermates. Aneuploidy was detected in several tissue samples from *acd/acd* mice. Many of the greatly enlarged nuclei incorporated tritiated thymidine. This suggests that the increased ploidy arises from an inability of certain cells to proceed through mitosis following S-phase, rather than by the fusion of unreplicated diploid cells. Mitotic figures were much rarer in tissues from mutants. The mutation thus appears to affect the control of the cell-cycle in certain differentiating tissues as they proliferate in response to postnatal stimuli.

Death of mouse embryos homozygous for a null allele of glucose phosphate isomerase

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A null allele of the structural *Gpi-1s* gene, that encodes glucose phosphate isomerase enzyme (GPI-1; EC 5.3.1.9), arose in a mutation experiment at the MRC Radiobiology Unit and was designated *Gpi-1s*$^{a\text{-}m1H}$ (Peters & Ball, *Mouse News Letter* **74**, 92, 1986). The viability of homozygotes has been investigated. No offspring, homozygous for the null allele were produced by intercrossing two heterozygotes, so the homozygous condition was presumed to be embryonic lethal. Embryos were produced by crossing *Gpi-1s*^{a}/null heterozygous females and *Gpi-1s*^{b}/null heterozygous males. Homozygous null embryos were identified at different stages of development by electrophoresis and staining either for GPI-1 alone or GPI-1 plus phosphoglycerate kinase (PGK) activity. At 6·5 and 7·5 days post coitum homozygous null embryos were present at approximately the expected 25% frequency (37/165; 22·4% overall) although at 7·5 days the homozygous null embryos tended to be small. By 8·5 days most homozygous null embryos were dead, dying or developmentally retarded and by 9·5 days little remained except for some extraembryonic tissues.

Cell and tissue-specific expression of a human CMV major IEP promoter-*lacZ* fusion gene in transgenic mice

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Two independent lines of transgenic mice bearing a cytomegalovirus major immediate early promoter-*lacZ* fusion gene (CMZ) have been generated and a detailed *lacZ* expression profile obtained. There is a dual mode of *lacZ* activity during development of CMZ embryos. Expression is first observed at the 2-cell stage and is strain specific. At day 9 of development, there is a very specific pattern of expression that is limited to cells in the neural crest region. This pattern is transient and expression is subsequently detected in some of the derivatives of the neural crest, e.g. spinal ganglia of day 11 embryos. Neural crest outgrowth cultures show *lacZ* activity in migrating cells. These cells are being transplanted into newborn mouse brains. Initial results suggest that these cells have both a migratory and proliferative capacity *in vivo*.

The developmental potential of parthenogenetic cells in mouse chimeras

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We have analyzed the fate of parthenogenetic cells in fetal and adult parthenogenetic ↔ fertilized chimeras using both glucose-6-phosphate isomerase allozymes and a high copy number transgene as markers. Our results indicate that parthenogenetic cells are subject to a strong selection in all tissues, with the exception of the female germ line. However, the onset and the severity of selection are strikingly different in various tissues. So far we have no indication that strain background significantly changes the behaviour of parthenogenetic cells.

Scurfy mutant mice show haematological abnormalities resembling those in Wiskott–Aldrich syndrome

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The detailed knowledge of the five homologous segments of mouse and human *X*-chromosomes aids in the search for mouse homologues of human disease. The locus of the mutant scurfy, *sf*, which causes scaly skin, diarrhoea and early death, lies in an *X*-chromosome segment homologous with that in which the human Wiskott–Aldrich syndrome (WAS) lies. WAS causes eczema, diarrhoea and early death, due to haematological problems including platelet deficiency and immunodeficiency. Studies of affected scurfy animals have shown that this mutant also results in platelet deficiency, accompanied by a reduced red cell count and raised white cell count. There is gastrointestinal bleeding and most animals appear to die of severe anaemia. It is not yet clear if there is immunodeficiency. Thus, scurfy resembles WAS, and may be homologous with it. Differences are that the genetic positions of the two loci within the homologous segments may be different, and that scurfy males show hypogonadism, which is not seen in WAS.

A possible involvement of the *Zfx* gene in sex determination of the wood lemming

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The importance of the *X* chromosome in mammalian sex determination has been well implicated in the wood lemming, *Myopus schisticolor*. A rearranged *X* chromosome designated *X**, harbours a mutated gene which represses the male determining effect of the *Y* chromosome, such that *X* Y* embryos develop normally into females. Recently, a phylogenetically conserved zinc finger protein (*ZFY*) gene identified on the sex determining region of the human and mouse *Y* chromosomes was postulated to be the putative testis-determining factor (*TDF*) gene. A highly homologous gene, designated *ZFX*, was also mapped on the *X* chromosome. Southern analysis of DNA samples derived from wood lemmings of *XX*, *X* O*, *X* Y* females and *XY* male localized the *ZFY*-related sequence (*Zfy* and *Zfx*) on both the *Y* and *X* chromosomes. The *X* and *X** chromosomes each contain a single *Zfx* gene. Molecular differences between the two alleles are readily detected with different DNA probes from the human *ZFY/ZFX* genes. These two *Zfx* alleles on the *X* and *X** are designated as *Zfx* and *Zfx** gene respectively. Chromosome *in situ* hybridization localized the *Zfx* sequences at bands p11–12 on the short arms of both the *X* and the *X** chromosomes, at or proximal to a postulated breakpoint involved in the generation of *X** from *X*. At least 15 copies of the *Zfy* sequences are present on the *Y* chromosome. Multiple copies of *Zfy* sequences on the *Y* chromosome are highly unusual. Most eutherians, except the mouse and the South American rodent, *Akodon azarae*, have only a single copy of *Zfy* and *Zfx* genes on their *Y* and *X* chromosomes respectively. Southern blot analysis of *Akodon azarae* DNA indicated that its *Y* chromosome contains about 7–8 copies of the *Zfy* sequences. Interestingly, *XY* sex reversals are either present naturally as in *Akodon* or *Myopus*, or experimentally inducible in the mouse. It is still unknown if all 15 copies of the *Zfy* sequences are expressed in the wood lemming. Although the incompatibility of the *Tdy* gene on the *Y* chromosome to other testis determining genes on either the autosomes or the *X* chromosomes is still considered to be important in the sex reversal in these species, their multiple *Zfy* sequences may constitute a common factor in potentiating this sex reversal process. If the *Zfy* gene(s) is indeed the *Tdy* in the wood lemming, the genetic rearrangement in the *Zfx** gene may have resulted in an incompatible gene with the *Zfy* gene(s) in determining sex of the *X* Y* embryos. These observations argue for a possible involvement of both the *Zfy* and *Zfx* genes in testis determination of the wood lemming.

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Pairing failure at meiosis: cause or result of gametogenic incompetence?

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Many translocations cause gametogenic arrest, preceded by asynapsis in multivalents. An analysis has been made of synaptonemal complexes of translocation configurations in five autosomal rearrangements in the mouse. In both oocytes and spermatocytes of each translocation, some pachytene configurations were fully paired while others exhibited varying degrees of asynapsis. The data were further classified into (1) cells in which all homologues other than those involved in the translocation were fully paired, and (2) cells containing generalized pairing anomalies. Most pairing errors in spermatocytes consisted of univalent *X* and *Y* chromosomes. The results show that multivalents with asynapsis are significantly more frequent in oocytes and spermatocytes containing other pairing anomalies, whereas fully synapsed configurations are preferentially present in cells with normal pairing. This suggests that pairing failure in translocation multivalents is more likely to occur in abnormal than in normal gametocytes. We propose that chromosome rearrangements can exert deleterious effects on gametogenesis – perhaps because of delayed progression through meiotic prophase – and that cells thus affected experience particular difficulties in achieving full synapsis of translocation configurations. The resulting asynapsis could cause further problems to the cell, thus promoting gametogenic breakdown.

Extensive non-homologous pairing during late zygotene in male mice heterozygous for reciprocal translocations

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The pairing behaviour of two reciprocal translocations T(5;15)4Ad and T(16;17)43H was studied in male mice by analyzing synaptonemal complexes (SCs). In about 70% of pachytene cells of T4Ad/+ males a translocation quadrivalent was present, whereas in the remaining cells only normal shaped bivalent SCs were recognizable indicating non-homologous pairing of segments proximal to the translocation breakpoints. In case of trans position of T4Ad to Rb(5.15)3 Bnr the incidence of cells with non-homologous pairing increased to 100% by forming a regular trivalent. In the latter case the translocated chromosomes start pairing homologous distally to the breakpoints and continue to synapse towards the centromeres by changing directly from homologous to non-homologous pairing. The transition from homologous to non-homologous pairing most probably takes place, when synapsis reaches the breakpoints of the reciprocal translocation chromosomes. The Rb3Bnr/T4Ad males are fertile and the non-disjunction rate of trivalent chromosomes in these males is similar to single Rb3Bnr/+ males. In compound heterozygous males Rb(16.17)8Lub/T(16;17)43H again non-homologous pairing takes place proximal to the breakpoints. In that case the proximal regions of chromosomes 17 synapse from opposite directions. As a consequence the centromeres of both acrocentric chromosomes and the Rb chromosome are situated apart from each other within the trivalent formed. This special arrangement of centromeres during pachytene does not seem to increase the non-disjunction rate in comparison to single Rb8Lub/+ males.

Chromosome damage and non-disjunction in normal and chromosomally mutant mice irradiated at the diakinesis stage of female meiosis

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We have irradiated diakinesis primary oocytes 4 h after the hCG step of superovulation with 0·6 Gy of X-rays. Three karyotypes were used: normal mice (+/+), homozyotes for the translocation T(1;11.13S)70H that

combines T(1;13)70H and Rb(11.13)4Bnr (T/T) and a derivate of this translocation with a small 1^{13} heteromorphic marker bivalent (T/T*). After fertilization, pronuclear chromosomes were scored for the number of centromeric regions, marker chromosomes, dicentrics and fragments using preferential staining of centric heterochromatin. In the order +/+, T/T, T/T*, female pronuclei had hyperploid chromosome counts of 11·7% ($n = 221$), 12·0% ($n = 242$) and 16·6% ($n = 126$). The frequencies of dicentrics/female pronucleus in this order were 0·07, 0·16 and 0·11 and the frequencies of fragments were 0·13, 0·18 and 0·31. In about half of the hyperploid chromosome spreads, a dicentric chromosome was included. Also when nuclei contained two $11{\cdot}13^{1}$ long translocation chromosomes, in about 50% of cases (5 out of 12) one was structurally changed. Both observations point into the direction of chromatid exchange, effectuated before metaphase, anaphase I as the mechanism for the irradiation induced non-disjunction here observed.

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