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Are patterns of cell differentiation reflected in mammalian gene maps?

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There are two separate types of gene homologies. New genetic material can arise by gene duplication, either by regional duplication or by tetraploidization. Both these mechanisms of gene duplication give rise to *paralogous genes* through subsequent divergent evolution. Thus, paralogous genes exist within a species and have a common ancestral gene. *Orthologous* genes are found in different species and have diverged from their common ancestral gene as part of the process of speciation and separate evolution. Linkage conservation, as reflected by orthologous chromosomal regions, has been demonstrated for quite extensive parts of mammalian chromosomes. Three possible groups of paralogous chromosomal regions in mouse and man will be suggested. The phenomenon of *differential gene silencing* and its consequence for our ability to detect paralogies will be described and exemplified. It is suggested that there may be a temporal relationship between closely linked genes. This will be exemplified by genes likely to be active in cells derived from the neural crest. Thus, it seems that genes for inner ear defects in the mouse are often closely linked to coat colour genes. Some other genes clustering around pigment genes will be discussed.

Comparative aspects of the evolution of 'dispersed' and 'tandem' families of sequences in species of rodents

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One particular family of sequences (the MIF-1 family), currently receiving much attention, consists of approximately 20000 copies of a transcribed 5 kilobase sequence that are interspersed throughout several Mus, Apodemus and Rattus genomes, including an intimate juxtaposition with coding regions of the β-globin gene complex in *M. musculus* (see Brown & Dover, 1981, *J. mol. Biol.* **150**, 441 for references; C. Jahn, Colorado, pers. comm.). Comparative analysis of the repeat reveals high levels of sequence and structural homology and constancy of copy-

number between species. However, the majority of members within each species genome are uniquely identifiable by diagnostic sequence variants indicative of a possible continual process of 'gene conversion' by which family homogeneity is maintained. In addition the presence of subpopulations of variants might represent transient polymorphisms within this large and widely dispersed family during the conversion process. In contrast, a family of tandem repeats located at all the centromeres and common to *M. musculus* and *M. spretus* reveals a tenfold difference in copy-number whilst at the same time maintaining similar subpopulations in the same proportions in the genomes (Brown & Dover, 1980, *Nature* **285**, 47). Analysis of the proportions of the subpopulations in the DNA of an isolated *M. musculus* X-chromosome shows that individual subpopulations do not necessarily represent chromosome specific segments (Brown & Dover, 1980, *Nucl. Acid. Res.* **8**, 781), indicating only a partial independence of chromosome evolution. The possible molecular mechanisms responsible for the different evolutionary courses of dispersed and tandem families and for the possible fixation of sequence variants between individuals of a species will be discussed.

Screening and analysis of enzyme activity variants from wild-caught mice

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Populations of wild mice from Edinburgh, Leicestershire, Somerset, Greece and Denmark were screened for genetic variation in the activity of up to 20 enzymes. These variants have been analysed genetically and biochemically. In one enzyme system several different regulatory and structural loci are now known that affect the expression of the enzyme in different tissues.

Pigmentation and PTC tasting in the laboratory mouse

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Twenty-two inbred strains of mice were tested, over a ten-day period, for their reaction to phenylthiourea (PTC) in their drinking water. The mice could choose between drinking tap water and tap water containing 100 mg PTC per litre. Thirteen of the strains were pigmented (i.e. they contained the wild-type allele at the albino locus) but they had various combinations of alleles at the agouti, brown,

dilute, leaden and pink-eyed dilution loci. Eight other strains were albino (c/c) and one strain was homozygous for extreme dilution (c^e/c^e). The pigmented strains showed only a slight aversion to drinking the PTC solution. The albino and extreme dilution strains showed a much greater aversion to drinking PTC. Seven CXB Recombinant Inbred (RI) strains were then tested. The two albino RI strains showed a greater aversion to drinking PTC than did the five pigmented RI strains. However, two albino congenic strains, C3H-*c* and C57BL/10-*c*, showed no aversion to PTC. The evidence seems to indicate that a gene linked to the albino locus determines PTC aversion. However, this implies a degree of linkage disequilibrium in the general run of inbred strains which is difficult to explain.

Robertsonian translocations in British wild mice

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Since 1969 wild mice with Robertsonian translocations have been found in Switzerland, Italy, India, Marion Island, Yugoslavia, Spain and southern Germany. In our study Robertsonian-bearing mice have been found in Caithness (N.E. Scotland) and the Orkney islands of Eday and Westray. The karyotypes varied from $2n = 32$ to $2n = 36$ in Caithness and were $2n = 34$ on Eday and $2n = 36$ on Westray. Mice from Sutherland and the Orkney islands of Stronsay, South Ronaldsay and Mainland had the normal karyotype of $2n = 40$ as did mice from Fair Isle, Shetland, the Isle of May and six other sites in mainland Great Britain. G-band analysis showed Rb(9-12) and Rb(6-13) to occur in all mice in Caithness with Rb(4-10) and Rb(11-17) present in many. Eleven other associations were found either as homozygotes or heterozygotes. No variation was found within islands on Orkney. One translocation Rb(9-12) occurred in both Orkney and Caithness mice. A common ancestry for the island and Scottish mainland mice is suggested. The results of ethological and breeding experiments appear to show no evidence for an adaptive advantage of fused chromosomes. The large heterozygote disadvantage of 'standard karyotype' × 'metacentric' hybrids may provide an isolating mechanism according to the stasipatric theory of speciation. Recent evidence on gene flow within mouse populations seems to question this.

Clonal growth of carcinogen-induced enzyme-deficient preneoplastic cell populations in mouse liver

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The cellular origin of preneoplastic subpopulations in liver during chemical carcinogenesis has not yet been clarified. An experimental approach to this problem became possible by using female phenotypic mosaics caused by X-chromosome inactivation ('lyonisation') occurring early in embryogenesis. Allozymes of the X-linked enzyme phosphoglycerate kinase (PGK-1) served as markers of the maternal (wildtype PGK-1B) and paternal (variant PGK-1A) X-chromosomes in crosses of near-congenic C3H mice. These PGK-allozymes are of similar activity and stability but can be separated electrophoretically and quantified by a fluorimetric method. Samples of adenosine triphosphatase-deficient preneoplastic populations punched out from cryostate serial sections of liver of 2-acetylaminofluorene-fed mice disclosed a PGK-allozyme pattern strikingly different from the norm. Thirty-nine of 40 preneoplastic nodules contained either a pure population of PGK-1A- or of PGK-1B-expressing cells. Mean contamination by the opposite PGK type was 1·1 %. In all cases the PGK expression in the preneoplastic nodules differed from the surrounding liver parenchyma. Determination in normal liver tissue of the patch size of coherent populations with a homogeneous expression of one PGK allozyme revealed an average of about eight hepatocytes per patch. Each microsample used for PGK determinations consisted of an average of 25 patches. It is evident from these results that 2-acetylaminofluorene-induced, adenosine triphosphatase-deficient preneoplastic subpopulations in the mouse liver are in all probability clones derived from a single cell.

Genetic basis of male sterility and segregation distortion due to *t*-haplotypes in the mouse

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The fertility and segregation distortion of male mice carrying different combinations of recombinant *t*-haplotypes derived from t^6, t^{w5} and t^{w32} suggests that the segregation distortion depends on at least three loci within the segment of chr. 17 occupied by *t*-haplotypes. There is a responder locus, *Tcr-1*, which responds by abnormal transmission to the action of two distorter loci, *Tcd-1* and *Tcd-2*, lying on either side of it. The first requirement for abnormal transmission is heterozygosity

for *t*-chromatin at the *Tcr-1* locus. If *t*-chromatin is also present, either in cis or trans, at both the *Tcd-1* and *Tcd-2* loci, then the homologue carrying the responder *t*-chromatin is transmitted at very high frequency. If only one or no distorter loci have *t*-chromatin then the responder may be transmitted at an abnormally low frequency. Male sterility depends on homozygosity for *t*-chromatin at one or both of two sterility loci, which have not yet been genetically separated from the distorter loci. This proposed system is similar to the genetic basis of segregation distortion in Drosophila, but more complex.

X-chromosome inactivation mosaicism in the three germ layers and the germ line of the mouse embryo

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Electrophoretic variant forms of the *X*-linked enzyme phosphoglycerate kinase (PGK-1) have been quantitated in 12½-day heterozygous female embryos as a measure of *X*-chromosome mosaicism in various tissues. Samples of yolk sac endoderm, yolk sac mesoderm, neural ectoderm, heart mesoderm, liver endoderm and primordial germ cells were analysed from each embryo. In all tissues except yolk sac endoderm, both PGK-1 isozymes were expressed. The covariance among tissues with respect to PGK-1 isozyme contribution suggests that all tissues examined are derived from the same pool of cells after *X*-inactivation. This pool is calculated to be approximately 47 cells minimum, which places the earliest time of *X*-chromosome inactivation in epiblast cells between 4½ and 5½ days *post coitus*. From the independent variance among tissues within an embryo, the primordial precursor pool sizes for each of the three germ layers and the germ line were estimated to be a minimum of 193 cells.

Location of *Xce* in the mouse *X* chromosome and effects on PGK-1 expression

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Heterozygotes for alleles at the *Xce* locus, which is located on the *X*, typically express non-random *X*-inactivation. Random *X*-inactivation in *Xce* homozygotes is assumed but not yet demonstrated. The precise location of *Xce* in the *X* is also

not yet established but the available data indicate that it probably lies closely proximal to *Ta*. Further evidence on the location of *Xce* has now been obtained by screening Mo^{blo} and *Ta* recombinants from $Mo^{blo}Pgk\text{-}1^{b}Ta\ Xce^{a}/+Pgk\text{-}1^{a}+Xce^{c}\times+Pgk\text{-}1^{b}+Xce^{a}/Y$ crosses. The *Xce* genotypes of both Mo^{blo} and *Ta* progeny were determined in test-matings using *Ta* as the marker for assessing *X* chromosome expression. No. $Mo^{blo}-Pgk\text{-}1$ recombinants or *Ta-Xce* recombinants were found, so confirming that the *Pgk-1* locus lies closer to Mo^{blo} than to *Ta* and the Xce locus lies closer to *Ta* than to Mo^{blo}. In the *Ta* test the effects of the *Xce* alleles in the recombinant chromosomes were similar to those previously found. However, quantitative assessment of PGK-1 expression in Xce^{a} and Xce^{c} homozygotes and in heterozygotes for recombinant Xce^{a} and Xce^{b} chromosomes has shown that although *Xce* allele substitutions always shift PGK-1 expression in the direction expected, the degree of shift is short of expectation. The basis for the quantitatively discordant results is not known but may be attributable to the presence of the Mo^{blo} and *Ta* markers on only some of the *X* chromosomes used.

Intersexuality in the horse

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Of three intersexual horses karyotyped, two were found to be $2n = 64$, *XX* intersexes with no evidence of a *Y* chromosome, while the third was a mosaic of 63, *XO*/64, *XY* cells. The degree of disruption to normal testicular development differed between the two types of intersexes, and there was also a disparity in the time at which spermatogenesis was arrested. In the *XX* intersex Leydig cell hyperplasia was apparent, but the seminiferous tubules were structurally normal although lined only with Sertoli cells. By comparison the *XO*/*XY* intersex which also had Leydig cell hyperplasia, showed greater disruption to normal testicular development with a breakdown of the membranic boundaries surrounding the seminiferous tubules. Spermatogenesis in the *XO*/*XY* intersex had progressed to a later stage than in the *XX* intersex, as primary spermatocytes were present. A fourth animal which was a phenotypic female displaying stallion behaviour was found to have a $2n = 64$, *XY* karyotype. It was classified as a case of equine testicular feminization (*Tfm*), although it differed from human and mice *Tfm* individuals in that it had a male psychosexual orientation.

The actions of genetic factors on the *Y*-chromosome of the mouse *Mus musculus*

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Recent studies on allelic variants of the *Y*-chromosome in mice have shown that there are several effects of the *Y*-linked locus (or loci) involved. Congenic strains were used in the present study to define the effects of the *Y*-chromosome. Reciprocal CBA/FaCam × C57BL/Fa F_1 males were repeatedly backcrossed to inbred females to give two lines of male progeny on each genetic background. Similarly PHH and PHH. YL males were crossed on to the CBA/FaCam background. In these strains the only difference between a pair of lines is in the origin of the *Y*-chromosome. Hence a consistent difference between two congenic lines provides definitive evidence of a *Y*-chromosome effect. Comparing the *Y*-chromosomes of C57BL/Fa and CBA/FaCam, there are statistically significant allelic effects on the H-Y antigen (as determined by time of skin graft rejection), serum testosterone levels (by radioimmunoassay), spermatozoan abnormality, testis weight and aggressive behaviour. Comparison of PHH and PHH . YL *Y*-chromosome shows effects on testosterone levels, sexual and aggressive behaviours, spermatozoan abnormality and testis weight but not on the speed of graft rejection (H-Y antigen). Many of these effects show an interaction with genetic background (i.e. they are not expressed in all strains). Taking these interactions into account, our evidence so far suggests that a minimum of two loci must be postulated to explain the various *Y*-linked effects described above.

Induction of congenital anomalies in offspring of female mice exposed to varying doses of X-rays prior to conception

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The possibility of combining a test for the induction of congenital malformations with a dominant lethal test has been investigated. Female mice exposed to various doses of X-rays (108–504 rad) were mated at four different weekly intervals after exposure. Uterine contents were examined at late pregnancy in order to detect early foetal death (dominant lethals) and malformations in the live embryos. It was found that two trends were apparent from the data on dominant lethals and abnormal embryos, in that for both the incidences tended to rise with increase in dose at each weekly interval, and at any given dose the incidences tended to

increase with time after irradiation. For both dominant lethals and abnormal embryos, the greatest incidences were reached when treatment was given three weeks before mating, when after 504 rad the values were $59{\cdot}1 \pm 4{\cdot}7\,\%$ and $12{\cdot}5 \pm 3{\cdot}1\,\%$ respectively. Dwarfism and exencephaly were the two most common malformations found. The results obtained parallel those shown by known genetic effects reported by other workers and suggest that testing treated animals for incidence of congenital malformations may provide a useful means of assessment of genetic hazards of radiation or chemicals.