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Aneuploidy induction in mice: use of a new tester stock

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Irradiation of mouse germ-cells can lead to chromosome loss, but the extent to which it directly increases the frequency of non-disjunction is still uncertain. To investigate this problem, we have constructed a special TBH tester stock which carries the Robertsonian translocations Rb(4.15)4Rma, Rb(4.6)2Bnr and Rb(6.15)1Ald. This has tribrachial homology and thus gives a tricentromeric configuration, which leads to obligatory non-disjunction and 100% aneuploid gametes. Normal complementation of gametes nullisomic and disomic for chromosomes 4 or 15, and those with paternal disomy/maternal nullisomy for chromosome 6, allows the stock to be maintained by intercrossing. It is also homozygous for genetic markers B^{lt} on Chr 4 and *bt* on Chr 15, which help to determine which chromosome has been gained or lost in treated gametes in test-crosses. Since trisomics for chromosomes 4, 6 or 15 die in mid-gestation and monosomics much earlier, the only survivors from crosses of TBH to wild-type are the result of complementation between aneuploid gametes in the wild-type and TBH mice. Where there is chromosome loss in the wild-type stock the survivor will have two metacentric chromosomes, but only one after chromosome gain. Since Tease found a highly significant increase in pronuclear hyperhaploidy after irradiation of mouse oocytes in diakinesis this germ-cell stage was X-irradiated in our initial experiments. As expected, the frequency of survivors increased after irradiation (with 0·5–2·0 Gy). In twelve of these (11 in the irradiated series) the parental origin of the complementary chromosome gains and losses was determined: in each there was maternal loss (i.e. in wild-type gametes) combined with paternal (TBH) gain. So far, therefore, we have obtained no evidence for the induction of non-disjunction by irradiation.

Meiosis in a sterile male mouse with an isoYq marker chromosome

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A sterile male mouse with testis weights of $\pm 30\%$ of normal was found to possess a metacentric marker chromosome with a *Y*-characteristic staining pattern after C-banding (peripheral blood culture, bone marrow). Germ cells were used for whole mount spreading SC analysis and for 'air-dry' mitotic and meiotic observations. In many bone marrow and spermatogonial mitoses the '*Y*' showed a heterochromatic knob at the kinetochore position. Of the few pachytene spermatocytes available for SC analysis, most showed hairpin pairing of the *Y* chromosome. End to end pairing of both telomeres with the telomeric distal region of the *X* chromosome was also observed. Of 100 spermatogonial mitoses, 12 lacked the *Y* marker chromosome whereas one contained two. At diakinesis–metaphase I, it was absent in 17 of 100 cells. Of the remaining 83, 14·5% showed pairing between *X* and *Y*. Adjacent locations of univalent sex chromosomes were only infrequently seen. Air-dried pachytene cells were relatively few and almost always contained a sex vesicle with a heterochromatic body. Secondary spermatocytes were scanty and could contain the metacentric *Y* chromosome. No sperm were seen in the caput epididymis. The presumptive origin of isochromosomes in this case is at first glance conflicting with recent evidence that *Tdy* is on the short arm of the *Y* chromosome (*Proc. Natl. Acad. Sci. USA* 1988, **85**: 6442–6445; *Proc. Natl. Acad. Sci. USA* 1988, **85**: 6446–6449).

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Chromosome 2 rearrangement and interleukin-1 gene deregulation in radiation-induced murine acute myeloid leukaemia

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Rearrangement of chromosome (ch) 2 is a consistent feature of radiation-induced murine AML and there is circumstantial evidence that induction of such rearrangements in multipotential haemopoietic cells may initiate leukaemogenesis. In the search for the molecular consequences of ch2 rearrangement in these AMLs the interleukin *Il-1b* gene has been mapped by *in situ* hybridization to a frequent AML ch2 breakpoint region. Translocation of one *Il-1b* copy has been demonstrated in a t2;2 AML and in a study of four AMLs an association between *Il-1b* gene deregulation and ch2 rearrangement was apparent. A possible initiating role for *Il-1* deregulation in radiation–leukaemogenesis is discussed.

Methylation patterns around highly polymorphic loci on the human *X* chromosome; correlation with *X*-inactivation status

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Substantial evidence indicates that DNA methylation plays a role in the molecular mechanisms responsible for the maintenance of X-inactivation in mammals. The isolation of two probes, M27B and pMR24-1 (DXS255 and DXYS41; X-Y Committee Report, *Cytogen. Cell Genet.* **41**, 1987), which detect highly polymorphic loci on the human *X* chromosome has enabled us to examine methylation patterns at flanking CCGG restriction sites recognized by *Msp* 1 and its methylation sensitive isoschizomer *Hpa* II. Fifteen active, and seven inactive, *X* chromosomes have been examined in DNA prepared from males, cell hybrids and females with non-random patterns of *X* inactivation. Methylation at CCGG sites was observed on all fifteen active *X* chromosomes but not on any of the seven inactive *X* chromosomes when examined with the probe M27B which maps to a region subject to *X* inactivation, the proximal short arm. When the same samples were examined with pMR24-1 which lies in the pseudoautosomal region and therefore in a region which escapes from *X*-inactivation, CCGG sites were methylated irrespective of whether they lay on active or inactive *X* chromosomes or the *Y* chromosome. These results suggest that some CCGG sites may be consistently methylated on active *X* chromosomes and unmethylated when subjected to *X*-inactivation. This contrasts with the hypermethylation of GC rich regions at the 5′ end of housekeeping genes which occurs exclusively on inactive *X* chromosomes.

Identical sequences for a 3′ fragment of mouse Glucose-6-phosphate isomerase and Neuroleukin

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Neuroleukin is a 56 kDa neurotrophic factor found in skeletal muscle, brain, heart and kidneys that supports the survival of embryonic spinal neurones, skeletal motor neurones and sensory neurones. Neuroleukin is also a lymphokine product of lectin-stimulated T-cells and induces immunoglobulin secretion by cultured human peripheral blood mononuclear cells. Mouse neuroleukin has been cloned, the complete nucleotide sequence has

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been published and its cDNA has been transiently expressed in monkey COS-1 cells. The serum-free supernatant of the transfected, but not of control mock transfected, cells was shown to mimic the properties of neuroleukin isolated from mouse salivary glands. In our work on the molecular genetics of carbohydrate metabolism we have recently isolated a mouse glucose-6-phosphate isomerase (or phosphoglucose isomerase, PGI) cDNA clone using the yeast PGI gene (PGI 1) as a probe. We wish to report that there is complete sequence identity between the 1154 nucleotides at the 3′ end of this clone (coding and non-coding) and the sequence of mouse neuroleukin.

Developmental potential of parthenogenetic cells in mouse aggregation chimaeras

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We have aggregated parthenogenetic and normal embryos in order to analyse the fate of parthenogenetic cells in chimaeras. Results we have obtained indicate that parthenogenetic cells are at a severe disadvantage compared to fertilized cells. In the majority of chimaeras parthenogenetic cells were observed in a few tissues only where they constituted a minority of cells. Parthenogenetic cells, however, can contribute in the differentiation of all embryonic cell lineages and were found in all but one of the tissues we have assayed. Chimaeras with a relatively high contribution of parthogenetic cells in a majority of tissues were considerably smaller than their non-chimaeric litter-mates.

Homeobox gene networks: organization and possible involvement in development

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In all organisms so far analysed homeobox genes represent large gene families which are organized in clusters. Work in our laboratory focuses upon the *Hox-2* cluster which is located on mouse chromosome 11 and contains seven tightly linked members. From sequence analysis it would seem that the *Hox-2* cluster is related to at least one other murine cluster, *Hox-1*. The *Hox-2.6* gene displays extensive similarity in multiple regions of the protein to other vertebrate homeobox genes and to the *Drosophila Deformed* gene. Thus *Hox-2.6* is a member of a 'Dfd-like' family. Comparisons of other members of *Hox-2*, or *Hox-1*, show that each of these clusters is related to *ANT-C* and *BX-C*. Possibly the major mouse and *Drosophila* clusters arose from a common cluster that existed in the common progenitor that produced the arthropod and chordate lineages. The different but overlapping domains of homeobox gene expression suggests that these genes may play a role in the establishment of the body plan, possibly acting as a network of markers of positional information. They may also be involved in organogenesis. There appears to be a correlation between the anterior limit of expression and the position of a given gene within the cluster, be it in mouse or *Drosophila*. We would suggest that this correlation between anterior limit and position within the cluster must have existed prior to the divergence of the lineages that eventually produced arthropods and chordates.

Transgenic mice carrying the t-complex *Tcp-1* gene

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We are interested in the transmission ratio distortion (TRD) and sterility effects of the mouse *t*-complex. *Tcp-1*, a gene from the *t*-complex, has been cloned (Willison, Dudley & Potter, 1986) and been shown to be constitutively expressed in all cell types, but has a very high level of expression in testis. The protein exists in two

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forms, TCP-1A, the acidic form found in *t*-haplotype animals, and TCP-1B, the more basic form found in wild-type animals. TCP-1 is known to be a component of the cytoplasmic aspect of the trans Golgi network (TGN) in tissue culture cells and spermatids. We find TCP-1 associated with the developing acrosome in spermatids and thus the polypeptide may be involved in acrosome formation or function. To determine the effects of $Tcp\text{-}1^a$ upon sperm development and function, transgenic mice of the CBA/C57 strain, with the full length of $Tcp\text{-}1^a$ gene under the control of its own promoter, were produced. Five lines of mice have been generated. In two lines the transgene has been shown by PCR analysis to be expressed in all tissues with a high level of expression in testis, approximately four times the level of the endogenous gene. In these lines many of the males are sterile. Preliminary studies show this is due to a reduction in the numbers of mature spermatozoa. In one line no spermatids beyond the cap stage of spermiogenesis are observed.

Molecular cloning in the region of the mouse *T* locus

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The *T* locus of the mouse is involved in the axis formation of the mouse embryo. *T/T* homozygotes lack a notochord and the posterior part of the embryo and die at approx. 10 days of gestation.

We have taken a genetic and molecular approach towards the cloning of this interesting developmental gene. Two DNA markers located close to the *T* gene has been identified amongst a pool of random DNA fragments derived from microdissected metaphase chromosomes 17. Genetic fine structure analyses enabled us to orient these markers relative to the *T* gene. The closer marker has been used as a start point for chromosome walking and jumping covering a total of 400 kb of genomic DNA close to T. Several genes have been identified on the genomic regions cloned. Two genes have been cloned and further investigated. The molecular analysis of these genes and other recent results on the attempted cloning of the *T* gene will be presented.

Genetic mapping of the mouse X-inactivation centre

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Evidence from studies of mice carrying X-autosome translocations indicates that there is a single X-inactivation centre on the mouse *X*-chromosome that lies distal to the T(X;16)16H breakpoint and proximal to the *X* chromosome breakpoint found in the HD3 Embryonic Stem cell line. Genetic mapping in mouse interspecific crosses of a number of genic and random DNA probes has defined the genetic position of the mouse X-inactivation centre on the mouse *X* chromosome. The X-inactivation centre region is localized between the mouse *Dmd* locus that lies proximal to the T16H breakpoint and the random probe *DXSmh44* that lies distal to the HD3 breakpoint. The X-inactivation centre maps close to the *Ta* locus and the *Xce* locus. In addition, the mapping demonstrates that the *Zfx* locus (the mouse X homologue to the putative testis-determining gene) and the androgen receptor gene lie within the *Dmd* to *DXSmh44* interval and therefore close to the inactivation centre. It follows that the genetic limits of the X-inactivation centre on the mouse *X* chromosome are, proximally, the *Dmd* locus and, distally, the *DXSmh44* locus – a genetic region of some 9 cM. One probe, *DXSmh120*, lies between the T16H and HD3 breakpoints and within the mouse X-inactivation centre region.

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Phenotypic effects of a presumed deletion of t-complex distorter-1 resemble the *t*-complex allele rather than wild type

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Transmission ratio distortion and male sterility caused by the mouse *t*-complex have been shown to be due to the time action of three or more distorter genes on a responder gene. For better understanding of the action of these genes, and for their cloning, it would be valuable to know whether they act as hypomorphs, or neomorphs, antimorphs, etc. The radiation-induced brachyury allele T^{22H} was shown to involve a small deletion covering the DNA marker D17Leh48, which lies close to the locus of *t*-complex distorter-1, *Tcd-1*. In double heterozygotes with various complete or partial *t*-haplotypes T^{22H} showed phenotypic effects on both male sterility and transmission ratio distortion which resembled those of *Tcd-1* itself rather than its wild-type allele. Four out of seven tested T^{22H}/t^{w32} males were sterile, and the remaining 3 poorly fertile, whereas control males were normally fertile. The transmission ratio of $5T^{22H}/t^{6}$ males was 99% (137/139) whereas that of control $+/t^{6}$ males was 63% (42/67). It is concluded that the locus of *Tcd-1* is deleted in T^{22H}, and that the *t*-form of *Tcd-1* is either a hypomorph or an amorph. Thus, the harmful effect of the distorter loci on the responder is due to the lack of some factor, and the *t*-form of the responder is in some way resistant to this lack.

Sex differentiation and tempo of growth: probabilities versus switches

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On the basis of anatomy and histology, the sexual development of mammals is generally subdivided into four stages: (1) pregonadal; (2) the stage of the indifferent gonad; (3) the sexual differentiation of the gonad; and (4) the consequent development of other sexual characters. Stage 3 which is often referred to by the term 'sex determination', is thought to be separated from stage 2 by a switch brought about by the presence or absence of a testis-determining gene located on the *Y* chromosome. However, quantitative data indicate that the rates of growth of XX and XY gonads differ from the beginning of their development, the rate of growth being faster in XY than in XX gonads. These findings contradict the idea of an indifferent gonad. Additional data have shown that the developmental rate of XY embryos exceeds that of XX embryos from the earliest stages of development, thus making the idea of a switch inappropriate. Testicular development in stage 3 requires the formation of Sertoli cells at a time before ovarian differentiation would have been initiated. A model is proposed which illustrates the conclusion that fast developmental rates enhance the probability of testis formation, while, conversely, slow developmental rates increase the probability of the gonad becoming an ovary.

The murine *Pgk-1* multigene family

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Besides the functional mouse *Pgk-1* and *Pgk-2* genes at least eight pgk homologous regions exist in the mouse genome. Seven *Pgk-1* homologous regions have been cloned and sequenced completely. They have to be classified as processed pseudogenes, some of them showing the typical characteristics of retroposons. While most of these genes are mutated extensively as usually observed with pseudogenes, two genes represent the actual mouse *Pgk-1* cDNA sequence in the coding part except for substitutions in the third position of three codons. These genes can code for a functional PGK protein but, lacking as they do classical promoter structures are probably not expressed. They must have originated recently. Puzzling is the observed extensive difference relative

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to the 3′ untranslated region of the published mouse *Pgk-1* cDNA. Three genes are truncated at the 5′ end at nearly identical positions, corresponding to the middle of *Pgk-1* cDNA. The known four rare human variants of PGK-1 show single amino-acid exchanges in the same area of genomic DNA. We have recently cloned and sequenced this region from the mouse pgk allele $Pgk\text{-}1^a$ and localized a base exchange in the coding region relative to $Pgk\text{-}1^b$, resulting in an amino-acid replacement of Lys with Thr in PGK-1A in position 156.

The genetics of insulin dependent diabetes mellitus (type I, IDDM) in the non-obese diabetic (NOD) mouse strain

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Inbred NOD mice spontaneously develop diabetes from the fourth month of life onwards. The disease is characterised by selective immune destruction of the beta (insulin producing) cells of the pancreatic islets, and is a close model for IDDM in man. Genetic analysis of NOD mice has shown that the disease is controlled by at least three autosomal recessive genes. One maps to the major histocompatibility complex and it is likely, although as yet unproven, that this is an MHC class II gene. A recessive mode of inheritance for such a gene is unusual. A second recessive gene maps to chromosome 9, near the Thy 1 locus and the third is not yet mapped. We will describe experiments establishing the role of two types of T lymphocyte in the induction of the disease following cell transfer, and plans to explore the role of MHC class II genes using transgenic NOD mice.

Experimentally-induced giant chromosomes in the mouse:

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Giant chromosomes have been induced from the highly polyploid nuclei of mouse trophoblast giant cells. Two methods for condensation have been used; fusion of giant cells with mitotically blocked HELA cells and incubation of giant nuclei in *Xenopus laevis* egg extracts. Such giant chromosomes may be useful for the mapping and cloning of mouse genes.

The spermatogenic block in H–Y negative XOSxr^b Mice

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The original Y-derived Sxr fragment (Sxr^a) contains genetic information involved in testis determination (*Tdy*), spermatogenesis (*Spy*) and expression H–Y antigen (*Hya*); it may be that *Spy* and *Hya* are the same gene. The deleted Sxr fragment, Sxr^b (≡ Sxr′) retains *Tdy* but has lost *Spy* and *Hya*. Quantitative analysis of germ cells in male mice constructed with a univalent *X* chromosome and the Sxr^b fragment i.e. XOSxr^b, demonstrates that a major block in germ cell proliferation occurs 4–5 days after birth which leads to a progressive deficiency of differentiating spermatogonia. This implies that *Spy* is expressed in normal control mice during this period.

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Sex chromosome pairing in male mice carrying the sex-reversal (*Sxr*) factor

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The presence of the sex-reversal factor (*Sxr*) on the *Y* chromosome of X/Y*Sxr* mice prevents sex chromosome pairing in a substantial proportion of pachytene spermatocytes. Under exceptional circumstances, it is possible to generate males with *Sxr* on the *X* rather than the *Y* chromosome, or on both *X* and *Y* chromosomes, or on a *Y* chromosome of different origin to that on which the factor arose. Males of such novel *Sxr* genotypes were examined here to determine whether chromosomal location of or homozygosity for the factor influenced its effect on *XY* pairing. It was found that the rate of *X–Y* separation at pachytene was elevated in all *Sxr* genotypes. Moreover, the univalent sex chromosomes were observed to self-pair in a proportion of spermatocytes from all males. Our observations did not support a recent suggestion that *X–Y* separation in X/Y*Sxr* males was the result of self-pairing of the *Y* competing with normal synapsis of the *X* and *Y* chromosomes. Rather, they indicate that either heterozygosity of *Sxr* in the *XY* pairing region is sufficient to disrupt synapsis, or alternatively, that *Sxr* is an ineffficient initiator of sex-chromosome pairing.

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