Abstracts of posters presented at the meeting

X-chromosome inactivation and sex determination

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The mammalian system of sex determination, in which the presence of a Y chromosome determines maleness, irrespective of the number of X chromosomes, appears fundamentally different from systems depending on X: autosome balance found in *Drosophila* and various other organisms. However, there are some similarities. In mammalian germ cells, the correct X-chromosome dosage (single in male and double in female) appears essential for cell survival. In the somatic cells, the number of active X chromosomes appears to depend on the number of autosomal sets, i.e. in triploids and tetraploids more than one X may remain active. The X chromosome is known to carry genes involved in sex determination and differentiation. The relationship between X-chromosome inactivation and sex determination is discussed.

Microdissection and microcloning of the centromeric and telomeric regions of the mouse Y chromosome

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We used a submetacentric marker Y chromosome to identify and microclone the putative sex-determining region and the region involved in pairing and recombination with the X. According to the G-band pattern, the marker Y has evolved from pericentric inversion. *In situ* hybridization with a ^{3}H-labelled *Bkm* probe confirmed that the *Bkm*-positive putative sex-determining region has moved to the short arm. We therefore microdissected and microcloned the short arm to provide recombinant DNA specific for this functionally important region. Electron microscopic analysis of X/Y pairing at meiosis confirmed that synapsis of the marker Y and the X still takes place at the distal end of both chromosomes. Hence, by microdissection of the distal region of the marker Y, we could generate DNA probes specific for the pairing/recombination region. Data on the molecular analysis of the microcloned probes are presented.

Deletion mapping of Yp and investigation of X–Y interchange in patients with aberrant sexual phenotypes

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A number of aberrant sexual phenotypes (eight XY females with pure gonadal dysgenesis, nineteen XX males and two XO males) have been investigated by Southern blot analysis with a series of nineteen Yp and four Yq DNA probes. By combining the patterns of Yp probes established in these patients, it has been possible to elaborate a model of Yp (based on the terminal transfer hypothesis) that gives the order of these sequences on the Y short arm and to suggest a location for the testis determinants.

The nineteen XX males reveal a striking variation in the amount of Yp evident in their genomes. Similarly, the two cases of XO males demonstrate differential portions of Yp in their genomic DNA. Fifteen of the nineteen XX males possess one or more of the Yp probes, with only four appearing to be devoid of Y sequences. The majority of XX males can be grouped to define a linear arrangement of Yp sequences. However, two of the XX males (KS and HM) and the XO male (RW) show anomalous patterns of transfer which can only be accommodated into this scheme by postulating inversion polymorphisms in the Y chromosomes of their fathers or more complex rearrangements generated during paternal meiosis.

Of the eight XY females examined, only one showed evidence of a deletion in Yp, the remaining seven apparently having intact Y chromosomes as judged with this battery of probes. This suggests that most XY females are not generated strictly as the reciprocals of X–Y interchanges which give rise to XX males; that is, being deleted for those probes which are found to be commonly transferred in XX males. This implies that other lesions predominate in creating pure gonadal dysgenesis. In the case of the deleted XY female, it has been shown that her Y chromosome carries her father's Xp telomere, suggesting that in this case an X–Y interchange may have been involved. By comparing sexual phenotypes in these various cases with the patterns of Yp sequences present, it is possible to suggest a distal location of TDF close to probe GMGY3.

Y-chromosome alphoid DNA

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In common with all other human chromosomes, the Y contains, at its centromere, alphoid satellite DNA. We have investigated the size of the region containing the alphoid DNA by digesting genomic DNA with the enzyme *Bam*HI (which does not have a site within the Y-chromosome consensus sequence), separating the fragments by field-inversion gel electrophoresis, blotting and probing with a Y-specific alphoid DNA probe. We have been able to show that there is extensive variation in the population for the size of the alphoid region, which varies from 400 kb to greater than 900 kb. We have examined DNA from several pairs of individuals that contain the same Y chromosome by common descent and one large family. No evidence for large-scale unequal crossing over was found in these cases.

Sex-specific hormonal regulation of yolk protein gene expression in *Drosophila melanogaster*

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The yolk protein genes of *Drosophila melanogaster* are encoded by single copy genes of the X chromosome. The genes are expressed in the fat body and ovarian follicle cells of the adult female. They thus provide an interesting system for studying sex-limited, tissue-specific and developmentally regulated gene expression.

Two regulatory systems have been described. The two insect hormones 20-hydroxyecdysone and juvenile hormone can affect the production of yolk proteins. 20-hydroxyecdysone affects fat body synthesis in males and females and juvenile hormone affects ovarian and fat body synthesis in females. These effects are mediated at the level of transcription, but there is no evidence for a direct interaction of the hormone/receptor complexes with *cis*-acting DNA sequences flanking the yolk protein genes.

A second system is that of the sex-determination hierarchy of regulatory genes such as *transformer*, *transformer-2* and *doublesex* which act in *trans* to permit transcription of the yolk proteins only in females. It is not clear how these two systems are superimposed.

DNA including yolk protein genes 1 and 2 and the intergenic region give the correct sex specificity, tissue specificity and developmental profile when introduced into flies by P-element transformation. The sequence requirements for fat body and ovarian expression are separable and are located between YP1 and YP2. The fat body specificity and sex specificity have been further narrowed to a 125-base pair region from the intergenic region. As yet nothing is known about the sequence requirements for the correct expression of YP3 or about those required for 20-hydroxyecdysone and juvenile hormone to exert their effects on the genes.

If we are to understand the molecular basis of how transcription of this gene family is regulated, we must understand how the sex-gene and hormonal regulation systems interact and any other factors important in controlling their expression. We present experiments aimed at elucidating this problem.

Sexing the human conceptus by *in situ* hybridization

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We have used a DNA probe derived from the human Y chromosome to identify cells with a Y chromosome by *in situ* hybridization. The Y-probe was labelled with either biotin or tritium and detected, after *in situ* hybridization to cell preparations, by immunocytochemistry or autoradiography respectively. Control experiments with human lymphocytes showed that this approach distinguishes male and female cells in interphase or metaphase. We are now testing the system on fetal blood, amniotic fluid cells, chorionic villus cells and preimplantation embryos. This approach to prenatal diagnosis is likely to be particularly suitable for occasions where a rapid diagnosis is required (e.g. chorionic villus biopsy at 9–12 weeks) or when only a small number of cells (that are not necessarily in metaphase) are available. Preliminary results are presented.

A human Y-specific DNA sequence transcribed in testis

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During a screen for Y chromosomally transcribed DNA sequences, Northern blots of poly(A)$^+$ RNA of adult human testis tissue were hybridized with 18 different human Y-derived probes. Only probe pJA36B gave a distinct hybridization signal of 1·1 kb with testis RNA, but failed to hybridize with RNA of human lung, spleen, kidney and thymus. Therefore, we consider a limited transcription.

Clone pJA36B (DYS 14) was subsequently subcloned and the resulting subclones were tested for their presence in testis RNA. The positive subclone pJA36B2 was sequenced according to Sanger. Sequence analysis revealed an open reading frame of 522 bp, which was confirmed to be transcribed. From the nucleotide sequence the amino acid sequence was predicted and compared to the Dayhoff and EMBL Data libraries, revealing no homologies with known protein sequences.

Using a DNA panel of several patients with abnormal sex chromosomes, clone pJA36B was mapped to the neighbouring region of the *TDF* locus on Yp, being present in the DNA of two XX males. The corresponding cosmid clone of 40 kb length was mapped with several restriction enzymes and resulting subclones were tested for male-specific and male/female homologous hybridization patterns.

Y-chromosome conservation: identification and characterization of human Y-chromosomal sequences

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A variety of low-copy-number, Y-specific (and X/Y homologous) human sequences have been isolated by screening a library constructed from a somatic cell hybrid retaining the Y chromosome as the only human material. This exercise has produced a number of sequences of potential value. These include ten Y-only probes and three X/Y homologous probes, in addition to those previously reported (see *Human Gene Mapping* **8**).

Information on the chromosomal localization of these sequences has been obtained by probing DNA from a panel of Y-chromosome transfectants and from cell lines carrying an aberrant Y, or X, chromosome.

The sequence homology of several of the human Y-specific probes to a range of mammals has been examined by hybridization to restriction digests. Conservation of sequence, Y-localization and, in some cases, of restriction fragment length has been observed in primates under similar conditions of stringency (equivalent to 0·4 × SSC at 65 °C).

The destination of Y-specific sequences in X–Y interchange males

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It is now clear that the majority of so-called XX males owe their male differentiation to the presence of Y-specific sequences. However, in only a comparatively few cases has it been conclusively shown that the Y sequences have been transferred to Xp through an abnormal recombinational event termed X–Y interchange. Other explanations, including Y-autosomal translocation and mosaicism for Yp aberrations are possible.

In an ongoing study of 15 patients, we have used high-resolution chromosome analysis, DNA measurement by flow cytometry, *in situ* hybridization and Southern analysis of flow-sorted chromosomes to obtain evidence for the regional chromosomal localization of Y-specific sequences. So far, chromosome analysis has shown evidence of transfer of Yp11.3 to the distal end of one X chromosome in 11 out of 15 cases. Flow cytometry has shown that one X chromosome is 3·8% larger than the other in 9 out of 12 cases. *In situ* hybridization using the Y-specific repeat probes GMGY7 and GMGY10 has revealed the presence of these sequences at the distal end of one X chromosome in all 4 cases tested. Southern analysis of flow-sorted chromosomes in 2 cases demonstrates that the Y sequences are carried by the fraction containing the X. To date, we have strong evidence that the Y-specific sequences have been transferred to one of the X chromosomes in 12 of the 15 cases, and in 11 of these the sequences map to distal Xp. In no case have we positive evidence of a mechanism other than X–Y interchange.

Natural variants of functional genes on the mouse Y chromosome

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The comparison of congenic lines of mice, carrying Y chromosomes originating from different inbred strains crossed onto common genetic backgrounds, has provided evidence for natural variants of functional genes affecting a number of characters. This poster summarizes the results obtained in Leeds over the past few years.

We have examined two pairs of Y chromosomes, which were chosen because Y variation had previously been described. These were from CBA/FaCam, which was compared with C57BL/Fa, and from PHH as against PHL. We have observed differences between the congenic lines which we ascribe to Y-chromosomal variation for the following characters: testis weight; proportion of morphologically abnormal spermatozoa; serum testosterone levels; sensitivity of certain target organs to exogenous testosterone; expression of serologically defined male-specific antigen; expression of H-Y antigen; aggressive behaviour of previously isolated males; and sexual behaviour with oestrous females. In addition, we have preliminary evidence for effects on skeletal growth.

At least four distinct Y-chromosomal loci must be invoked to explain the distribution of the effects between strains. The possible relationship between these loci and other known loci on the mouse Y chromosome is discussed.

Characterization of the regions of close homology on Yp and Xq13-q24

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The region Xq13-q24 has been proposed to be around 98% homologous with Yp, to be the result of an Xq to Yp transposition event during the evolution of apes to man and to constitute up to a quarter of the Y euchromatin.

Eight XY-homologous probes, characterized in this department, were mapped using somatic cell hybrids and individuals with deletions of the Y chromosome, to Xq13-q24 and Yp. Six of these XY-homologous probes were isolated from the same library. This library has now generated 60 Y-probes none of which map into the heterochromatin. On this basis, the region is one tenth of the Y-euchromatin or approximately 1 Mbp. All eight probes were used to screen panels of normal males and females digested with 24 different restriction enzymes. It was estimated that more than 2 kbp of DNA were studied directly at the restriction sites or approximately 0·2% of the XY-homologous region.

As might be expected, the variation between the X and the Y is greater than between X chromosomes. Each probe shows distinct X- and Y-specific bands with three to five restriction enzymes but only three probes reveal X polymorphisms. Two of these probes revealed single polymorphisms with frequencies of less than 5%. The third probe reveals three different polymorphisms with frequencies of around 40% with *Rsa*I (GTAC), *Mbo*I (GATC), and *Hin*fI (GANTC). These three polymorphisms behave as if closely linked and, from the pattern of alleles generated by any one of the polymorphic enzymes, one can predict exactly the pattern for the other two enzymes in the same individual. As these three restriction sites cannot overlap it would appear that these enzymes are cutting in a region rich in GATC-related sequences which is in one form on around 40% of X chromosomes and another on the remaining 60%. Furthermore, no Y polymorphisms were observed despite there being X/Y differences. This perhaps indicates some selective pressure for the differences observed between the X and the Y.

Identification of an 11 kb, Y-specific, low copy sequence mapping to distal Yp

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A cosmid, designated Y104, was isolated from a library constructed from a somatic cell hybrid retaining the Y chromosome as the only human material; this cosmid has been found to contain a human 11 kb *Eco*RI low copy number restriction fragment. Confirmation of its Y-chromosomal origin and a provisional assignment to Yp was obtained by hybridization of either the purified fragment or the total cosmid DNA (preannealed with human genomic DNA to remove signals from repetitive sequences) to a filter with DNA samples from various cell lines and an independent 'Y-only' somatic cell hybrid. No equivalent signals were observed in restriction digests of DNA from females or an X-only hybrid. *In situ* hybridization indicated that the purified 11 kb fragment could be assigned to distal Yp (19% of all grains scored to distal Yp – about 1% genome). When preannealed total cosmid DNA was employed, hybridization was observed at the same site and a second site was also detected on Yq.

The observation of a long, low copy 'Y-only' sequence mapping to distal Yp is of interest in that it is presumably distally located to the block(s) of Xq/Yp homologous sequence and proximal to the pseudoautosomal region. Lack of significant homology to sequences in mice and rabbits suggest that this sequence is not located in a region directly involved in sex determination.

First meiotic pairing of the X and Y in the human male

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An EM investigation of surface-spread XY bivalents was performed on three normal subjects of proven fertility having a 46,XY karyotype. The X and Y demonstrated an Xp/Yp synaptonemal complex in all the 218 spermatocytes investigated. A preliminary more detailed computer-digitizing analysis of this segment indicates that during an intermediate phase there is a looser alignment of a much larger segment. We conclude that Xp–Yp exchange pairing is restricted to the very tips of Xp/Yp, forming the primary pairing segment. An additional association between Xp and Xq was seen in about 50 % of cells. Measurements by the computer digitizer localized this association to the segment shown by molecular technology (by others) to be homologous with the Y, using the probes DXS69 and DXYS1. We interpret this association as an attempt to homologous synapsis between Xp, Xq and Yq. A further association, of the very tips of the long arms of X and Y, was seen in about 50 % of cells (range 48–52). One possible explanation for this is some Xq/Yq homology within this segment. We may speculate that this represents a secondary pairing segment, where (in analogy with the situation of the primary pairing segment at Xp/Yp) gene activity may be retained in somatic XX cells; and with recombination there is a potential for partial sex-linkage.

Mechanical constraints on the synapsis between the homologous sequences of X and Y may lead to selective breakage and reunion. Thus a reshuffling of the partial XY homology will take place, which could be of relevance for the origin of syndromes such as the fragile X mental retardation. It may added that the so-called 'dense body' of the Y, corresponding to the heterochromatic block Yq12, shows characteristics of protein composition, such as heavy silver staining and association to nucleoli of acrocentrics. The latter might be the mechanism behind documented cases of 15q;Yq and 22;Yq translocation, similar to the origin of Robertsonians.

Structure and localization of *Hpa*II tiny fragment islands on the human Y chromosome

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As a strategy for finding genes on the Y chromosome, we have been screening a Y-specific cosmid library for *Hpa*II tiny fragment (HTF) islands. At present, three candidate clones (CY019, CY022 and CY24) have been isolated, restriction maps of which reveal a clustering of *Hpa*II sites. These clones have been confirmed to be homologous to sequences on the Y chromosome by Southern blot analysis of male and female DNA and of DNA from somatic cell hybrids. The localization of CY019 has been further studied by *in situ* hybridization. In addition to their Y chromosome location, sequences in CY022 and CY24 have also been shown to be present on the X chromosome.

We have gone on to discover the distribution of *Hpa*II tiny fragment islands in the vicinity of CY019 using field-inversion gel electrophoresis.

A deletion map of the long arm of the human Y chromosome (Yq) and the detection of the possible paracentric inversions

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30 DNA probes isolated from two DNA libraries enriched for the Y chromosome have been localized to Yq.

By studying the blot hybridization pattern of these probes on the DNA of seventeen patients, fifteen with iso(Yp) chromosome but all having various deletions of Yq, a consensus order of these sequences along this part of the Y chromosome has been obtained.

The probes have defined 16 different breakpoints among these individuals as compared to 2 by chromosome banding techniques. This demonstrates the importance of combining both molecular and cytogenetic methods for the detailed analysis of chromosome rearrangements.

In general, the probe sequences follow a linear order on the long arm of these patients, but four out of the eighteen cases displayed alternative patterns, which could best be explained by the occurrence of paracentric inversions. Although it is possible that these anomalous cases could be the result of more complex rearrangements in the formation of the altered chromosome, these findings raise the question of whether a common order of sequences on the Y exists in the general population, or whether there are structural variants.

Cloning and characterization of bovine Y-derived sequences

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We have constructed a bovine plasmid library enriched for Y-specific DNA sequences by the deletion enrichment method. The resulting clones were analysed by hybridization to Southern blots of male and female *Eco*RI digests of bovine genomic DNA. From 200 clones tested, two (B.C.1.2 and 1.34) were entirely male specific, six gave a male–female differential hybridization pattern and the remaining reacted similarly with male and female DNA. Interspecific somatic cell hybrid studies confirmed that the B.C.1.2 male-specific probe was derived from the Y chromosome. Sequencing results revealed that the B.C.1.2 insert was 49 nucleotides long. This fragment is present at about 2000–2500 copies in the male bovine genome. When we tested the species specificity on DNA from different mammals this Y probe remained bovine specific. The B.C.1.2 probe revealed one monomorphic band with each restriction enzyme used. No transcription has been observed on RNA blots of different bovine tissues tested. The male specificity and repeated nature of the B.C.1.2 sequence has enabled us to use it as a molecular probe for sex determination on small numbers of embryo cells by identifying the Y chromosome.