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Pseudoautosomal genes in man

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Summary

MIC2, which encodes the 12E7 antigen, is the only well-defined pseudoautosomal gene in man. We have isolated cDNA and genomic sequences corresponding to _MIC2_ and have produced monoclonal antibodies reacting with the 12E7 antigen. These molecular tools have been used to investigate the genetics and biochemistry of the _MIC2_ system. Recent results suggest that _MIC2_ is the most proximal of the currently defined pseudoautosomal markers and that the escape of _MIC2_ from X-inactivation may be intrinsic to an associated HTF island found at the 5′ end of the gene. Investigation of the inter-relationship between _MIC2_ and the _XG_ locus has led us to postulate the existence of a second pseudoautosomal gene in man.

Key words: pseudoautosomal, *MIC2*, X-inactivation, human.

Introduction

Genes and sequences that exchange between the eutherian sex chromosomes during male meiosis fail to show complete sex linkage (Koller & Darlington, 1934; Haldane, 1936). The mode of inheritance of such genes and sequences has been termed pseudoautosomal (Burgoyne, 1982). For many years, evidence supporting the existence of the pseudoautosomal region was indirect and based on assumptions about chromsosomal behaviour during male meiosis. More recently, the existence of the pseudoautosomal region in man and mouse has been confirmed by a combination of molecular and classical genetic analysis.

In mouse, the *Sxr* mutation was shown to be caused by the pseudoautosomal inheritance of the male sex-determining gene (*Tdy*) (Evans, Burtenshaw & Cattanach, 1982; Singh & Jones, 1982). *Sts*, the gene encoding the microsomal enzyme steroid sulphatase, is present on both the X and Y chromosomes in mouse and its inheritance is almost certainly pseudoautosomal (Keitges, Rivest, Siniscalco & Gartler, 1985; Weissenbach *et al.*, this symposium; Bishop *et al.*, this symposium). A third indication of the pseudoautosomal region in the mouse is the inheritance of a retroviral sequence, *MOV*-15, newly integrated into the Y chromosome. Unlike *Sts* or *Sxr*, which show no sex linkage, *MOV*-15 exchanges between the sex chromosomes with a frequency of about 10%, implying the existence of a recombination gradient in the mouse pseudoautosomal region (Harbers, Soriano, Muller & Jaenisch, 1986).

In man, the first suggestion of the existence of a human pseudoautosomal region came from the chromosomal localization of the *MIC2* gene. A monoclonal antibody, 12E7, which defines a human cell surface antigen encoded by the *MIC2* gene was used to map the gene to both the X and Y chromosomes and the sublocalization on the X chromosome was well within the X–Y pairing region. Consistent with the X and Y localization, the *MIC2* gene was found to escape inactivation on the inactive X chromosome in female cells. Formal proof of the existence of the pseudoautosomal region, however, was obtained from studies of the inheritance of several DNA sequences isolated at random from the human Y chromosome (Cooke, Brown & Rappold, 1985; Simmler *et al.* 1985; Rouyer *et al.* 1986). These studies demonstrated that in male meiosis a single obligate recombination event occurs in the distal

Table 1. *Antibodies reacting with the 12E7 antigen*

Antibody	Immunoglobulin class	Immunogen	Reference
12E7	IgG1	T Cell ALL	Levy *et al* 1979
RFB1	IgG1	T Cell ALL	Bodger *et al* 1981
013	IgG1	Melanoma cell	Dracopoli *et al* 1985
F21	IgG1	Melanoma cell	Dracopoli *et al* 1983
BANR4A5 5	IgA	Red blood cells	Pym *et al.* unpublished
MSGB1	IgG2	Synthetic peptide	Darling *et al.* 1986*b*

All these antibodies recognize molecules on the surface of somatic cell hybrids containing either a human X or Y chromosome as their sole karyotypic material in a rodent background. They also recognize molecules on the surface of 12E7-antigen-positive primary and secondary transfectants. All the antibodies work in immunoblot analysis and recognize a $32{\cdot}5\times10^3$ M_r molecule in whole cell lysates of human 'X only', 'Y only' and transfectant cell lines. All the antibodies detect elevated levels of expression of this molecule on the surface of amplified transfectants. None of the antibodies recognize molecules on the surface of mouse cells.

portion of the short arms of the X and Y chromosomes. The position of crossing over is variable and a gradient of recombination with respect to Y-specific sequences is observed. Cloning the *MIC2* gene provided DNA probes which were used to demonstrate that *MIC2* also exchanged during male meiosis, but X/Y recombination events which extend to include the *MIC2* gene occur with a much lower frequency than the other pseudoautosomal markers (Goodfellow, Darling, Thomas & Goodfellow, 1986).

The steroid sulphatase gene, *STS*, in man differs from its homologue in mouse and is not pseudoautosomal (Craig & Tolley, 1986; Fraser *et al.*, this symposium). *MIC2* is the only known human pseudoautosomal gene. We will consider here the biology and genetics of *MIC2* and the use of *MIC2* probes for studying X-inactivation. Finally, we will describe the relationship between *MIC2* and the X-linked locus *XG* which has led us to postulate the existence of a second pseudoautosomal gene in man.

Reagents for studying the *MIC2* gene

(A) *Antibodies*

The monoclonal antibody 12E7 was produced by Levy, Dilley, Fox & Warnke (1979). The antibody reacts with a cell surface antigen found on all human cells tested, with the exception of spermatozoa (Goodfellow, 1983). The 12E7 antigen is also found on the surface of gorilla and chimpanzee cells but is absent from the cells of orang-utan and other primates as well as all other animal species tested (Goodfellow, 1983). Table 1 describes additional monoclonal antibodies, all of which react with the 12E7 antigen. Although several of these antibodies are of different immunoglobulin class and recognize different antigenic epitopes, they share the unusual property of failing to immunoprecipitate the 12E7 antigen despite recognizing it by immunoblotting (Banting, Pym & Goodfellow, 1985).

(B) *cDNA clones*

The clone pSG1 was isolated by screening a bacteriophage λgt11 cDNA expression library with the 12E7 antibody (Darling *et al.* 1986*a*). Three lines of evidence support the conclusion that this is a cDNA clone corresponding to the *MIC2* gene. First, the chromosomal localization of the genomic sequences recognized by pSG1 is the same as the *MIC2* gene (see below). Second, 12E7 antigen-positive mouse cells have been constructed by DNA-mediated gene transfer using human DNA. Antigen-positive primary and secondary transfectants contain human sequences which react with pSG1 (B. Pym *et al.* unpublished observations). Furthermore, transfectants expressing increased amounts of 12E7 antigen have amplified the sequences reacting with pSG1. Third, a monoclonal antibody, MSGB1, has been produced by immunizing a mouse with a peptide, the sequence of which was derived from a conceptual translation of the nucleotide sequence of pSG1 (Darling *et al.* 1986*b*). The MSGB1 antibody reacts with the 12E7 antigen. Although sequence analysis suggests that pSG1 contains all of the coding sequence of the *MIC2* antigen, comparison with the message suggests that pSG1 does not contain the 5′ untranslated region of the *MIC2* transcript. A putative full-length clone, pNT23, has been isolated by screening a new cDNA library constructed with mRNA from the human teratocarcinoma cell line NTERA2 (M. V. Wiles and S. Darling, unpublished results).

(C) *Genomic sequences*

Cosmid and bacteriophage λ genomic libraries have been screened with the pSG1 clone and subclones derived from it. Over 50 kb of human genomic DNA has been isolated. This DNA contains the predicted 5′ end of the gene, but still lacks sequences at the 3′ end. Only the CpG-rich region (HTF island, see Bird,

1986) at the 5′ end of the gene has been analysed in detail.

Chromosomal localization of *MIC2*: physical studies

The *MIC2* gene was originally mapped to both the X and Y chromosome by exploiting the species specificity of the 12E7 antibody in somatic cell genetics (Goodfellow, 1983). Further localization of the X-located *MIC2* gene was achieved by studying hybrids containing different fragments of the X chromosome. Two hybrids are of particular interest. The hybrid UCLAB2 contains an X chromosome which has a short-arm terminal deletion of about 5×10^6 bp; this deletion removes the entire pseudoautosomal region as well as the genes *MIC2* and *STS* and several DNA sequences known to be restricted to the X chromosome. This result places an upper limit on the size of the pseudoautosomal region (Curry *et al.* 1984). The hybrid 817×175 contains an X–Y translocation chromosome which has resulted in the loss of *MIC2* but not *STS*. This makes *MIC2* the most distal of the known genes on the X chromosome (Mondello *et al.* 1987).

A similar conclusion has been reached by Geller, Shapiro & Mohandas (1986). The regional localization of the *MIC2* gene on the Y chromosome was achieved by *in situ* hybridization using the pSG1 probe (Buckle *et al.* 1985). In the human genome, only two sites of hybridization to pSG1 were found: the end of the X chromosome short arm and the end of the Y chromosome short arm. This localization is within the pairing region and is consistent with *MIC2* being part of the pseudoautosomal region.

Localization of *MIC2* within the pseudoautosomal region: family studies

DNA marker studies were undertaken to test for pseudoautosomal inheritance of *MIC2*. Numerous restriction fragment length polymorphisms were found to be detected with the probe pSG1. Unfortunately, the cDNA probe proved particularly difficult to use, as patterns of hybridization to DNA digested with most restriction enzymes tested produced multiple bands, many of which showed variation in the population. This complexity in the patterns of hybridization observed made it difficult to identify alleles. Simplification of the Southern blot patterns was obtained by using genomic probes. Like other pseudoautosomal DNA sequences, isolated at random from the Y chromosome, the genomic *MIC2* probes also recognized a large number of polymorphic sites. The *MIC2* polymorphisms appear to be due to simple base pair changes rather than more complex deletions or duplications.

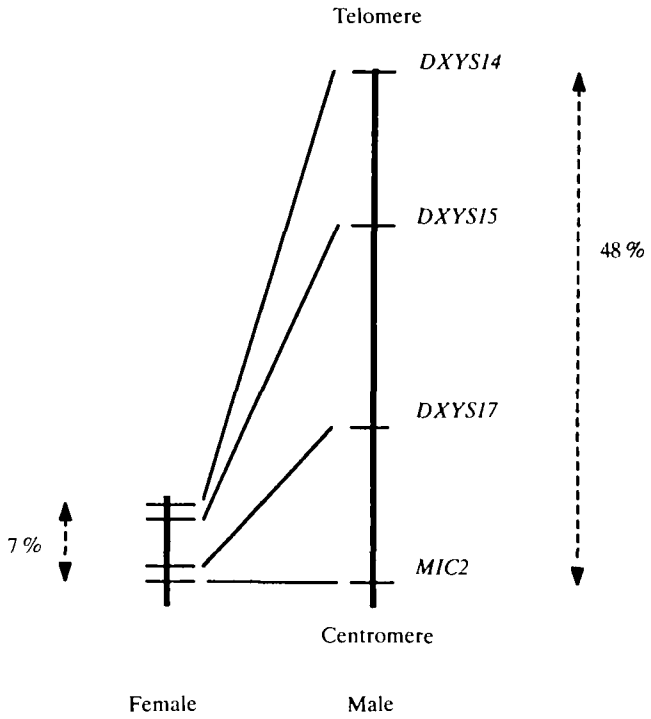

Fig. 1. Comparison of genetic distances separating pseudoautosomal loci in males and females.

In families ascertained at random, we have found a single X/Y recombination event including *MIC2* in 46 informative male meiosis. Testing the same families with other pseudoautosomal markers allowed us to construct a male meiotic map of the region. *MIC2* is the most proximal of the pseudoautosomal loci and shows no detectable linkage with the most distal pseudoautosomal marker *DXYS14* which is known to be close to the telomere (Cooke *et al.* 1985). These results are important for two reasons. First, the pseudoautosomal region is maximally 5×10^6 bp long and may be considerably smaller. 50 % recombination in 5×10^6 bp is some ten times higher than the expected average for that amount of DNA sequence in man (Renwick, 1969). It is also a considerably greater rate of recombination than is found over the same region in females (Fig. 1). Interestingly we, and others, have found no evidence for double recombinants in the pseudoautosomal region. Second, it has been argued on theoretical grounds (Bengtsson & Goodfellow, 1987) that the male sex-determining gene, *TDF*, will lie close to the pseudoautosomal region of man. If this is the case *MIC2* is a useful flanking marker for *TDF*.

The long-range restriction map constructed using pulsed-field gel electrophoresis techniques is consistent with the meiotic map of the pseudoautosomal region. Preliminary results suggest that *MIC2* lies less

a NDDPRPPNPPM

b DGENDDPRPPNPPM

c DGENDDPRP

Fig. 2. Conceptual translations of *MIC2*. (a) Synthetic peptide sequence used in production of monoclonal antibodies; (b) part of *MIC2* conceptual translation; (c) sequence containing epitopes recognized by the antibodies listed in Table 1.

than 5×10^5 bp away from sequences which are Y specific (C. Pritchard, personal communication).

Biochemistry of the *MIC2* gene product

Biochemical studies have been severely hampered because the available antibodies (see Table 1) fail to immunoprecipitate the 12E7 antigen reproducibly. The alternative approach of immunoblotting, though of limited value for immunological and biosynthetic analysis, demonstrated that the *MIC2* product is a cell surface molecule of $32{\cdot}5\times10^3$ M_r with a pI of about 5·0 (Banting *et al.* 1985).

Sequence analysis of the *MIC2* cDNA defined a long open reading frame. To check that the correct open reading frame had been identified, mice were immunized with a synthetic peptide corresponding to part of this sequence (Fig. 2). Monoclonal antibodies were produced that recognize both the immunizing peptide and the $32{\cdot}5\times10^3$ M_r *MIC2* cell surface product. MSGB1 is typical of these antibodies (Table 1). Whilst none of these antibodies immunoprecipitate specific polypeptides their ability to recognize the $32{\cdot}5\times10^3$ M_r cell surface molecule in immunoblot analysis demonstrates that the synthetic peptide sequence does correspond to part of the *MIC2* gene product, therefore, the correct open reading frame has been chosen for conceptual translation.

Recent epitope mapping studies have shown that all the antibodies listed in Table 1 recognize short, adjacent and/or overlapping continuous epitopes in the conceptual translation (G. S. Banting unpublished results). Since these antibodies bind to the surface of cells, this part of the sequence must be extracellular; definition of the transmembrane domain would allow predictions to be made regarding orientation of the molecule in the plasma membrane. Hydropathy profiles show two long hydrophobic stretches within the conceptual amino acid sequence, one at the extreme amino terminus. Such sequences could serve as transmembrane domains or signal sequences (Kyte & Doolittle, 1982).

Computer-assisted searches of available protein databases have provided no clue as to function since, with few exceptions, they have revealed no significant homology between the *MIC2* product and any previously published sequence. All the exceptions are matches found between the amino terminal region of the *MIC2* conceptual translation and the signal sequences of several precursor proteins. It is therefore likely that the amino terminus acts as a signal sequence directing the molecule to the cell surface.

The lack of homology between the *MIC2* product and previously described sequences suggests that the molecule may have a novel function. We have chosen to raise antibodies against synthetic peptides corresponding to potentially interesting regions of the conceptual translation in an attempt to investigate this function.

MIC2 as a tool to study X-inactivation

It would be predicted on gene-dosage grounds that *MIC2* would escape X-inactivation. Using somatic cell genetics it was found that *MIC2* does escape X-inactivation and cloning *MIC2* has provided a tool for studying the molecular basis of X-inactivation.

Control of gene expression in mammalian cells is still poorly understood. Sequences rich in the CpG dinucleotide pair at the 5′ ends of genes have been implicated as playing an important role in their expression (Bird, 1986). Because of the large number of *Hpa*II sites present in the G+C-rich regions, the descriptive term 'HTF-island' (*Hpa*II tiny fragment island) has been applied to such regions. A general feature of HTF-islands is a lack of 5-methylcytosine residues and hypomethylation within HTF-islands is positively correlated with gene activity. Analysis of the HTF-island of two X-linked genes (*PGK* and *HPRT*) has shown methylation of the HTF-islands on the transcriptionally silent inactive X chromosome, whereas the same islands on the active X are unmethylated (Yen *et al.* 1984; Keith, Singer-Sam & Riggs, 1986).

There is an HTF-island at the 5′ end of the *MIC2* gene. Preliminary sequence analysis of 1·2 kb around the presumed first exon indicates that it is very GC rich (67%) with abundant CpG dinucleotide pairs. Southern blot analysis of genomic DNAs doubly digested with *Hin*dIII and rare cutting methylation-sensitive restriction enzymes reveal a cluster of unmethylated sites including *BssH*II, *Sac*II, *Nar*I and *Nae*I (Fig. 3). Comparison of hybridization patterns to DNAs prepared from peripheral blood leukocytes of males (XY), females (XX) and Turners syndrome female (XO) proved that there were no differences in methylation of sites within the HTF-island on the active X, inactive X or Y chromosome. DNAs from a

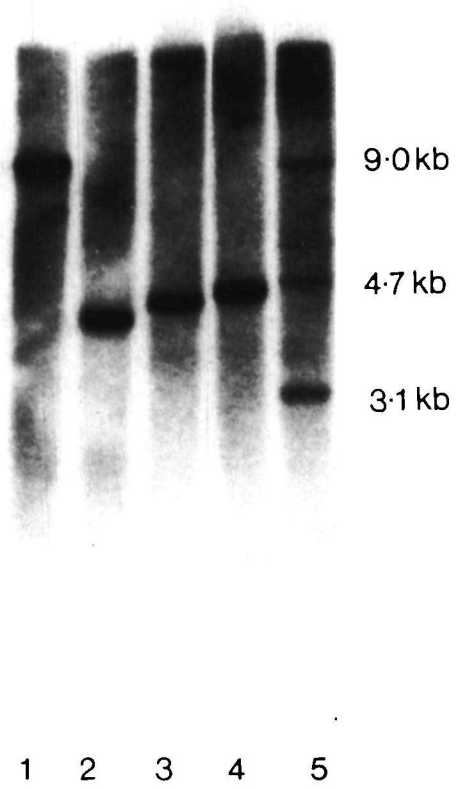

Fig. 3. Southern blot analysis demonstrating a lack of methylation in the 5′ region of *MIC2*. PGF (46XY male lymphoblastoid cell line) DNA digested with *Hin*dIII alone and in combination with methylation-sensitive restriction endonucleases. Hybridization was with p2B (a 1·2 kb *Eco*RI subclone which flanks the HTF-island). 1. *Hin*dIII, 2. *Hin*dIII/*Bss*HII, 3. *Hin*dIII/*Sac*II, 4. *Hin*dIII/*Nar*I, 5. *Hin*dIII/*Nae*I.

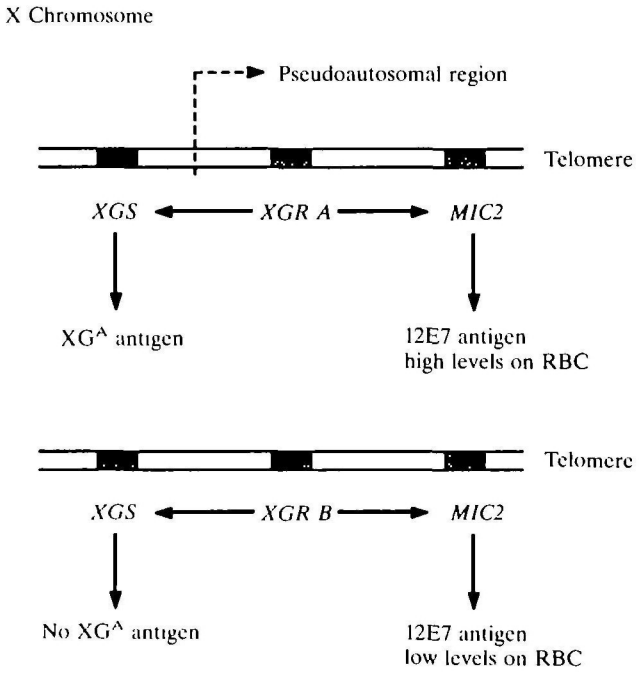

Fig. 4. Relationships between the proposed regulatory gene *XGR* and XG^a and 12E7 antigen expression.

number of lymphoblastoid B cell lines show a similar lack of methylation. The HTF-islands associated with *PGK* or *HPRT* are methylated on the inactive X chromosome in XX individuals (Yen *et al.* 1984; Keith *et al.* 1986). Lack of methylation of the *MIC2* HTF-island on the inactive X chromosome correlates with escape from inactivation. It is similarly unmethylated on the Y chromosome carrying an active gene. Methylation within the body of *MIC2* is, however, variable as measured by comparison of hybridization to *Msp*I- and *Hpa*II-digested DNAs. In DNA prepared from blood cells from Turners syndrome females (X^{active} O) two *Hpa*II sites we have examined are always cleaved whereas DNAs prepared from blood of normal females (X^{active} $X^{inactive}$) show that those sites are either not cleaved or are only partially cleaved on the inactive X chromosome (C. Mondello, unpublished observation).

One plausible interpretation of these results is that escape from methylation and perhaps X-inactivation generally is an intrinsic property of the HTF-island of *MIC2*. Alternatively, the HTF-islands of genes which are X-inactivated may be differentially susceptible to methylation on the inactive X chromosome.

The relationship between *XG* and *MIC2*: a theory postulating a second pseudoautosomal gene

The expression of the 12E7 antigen on red blood cells is polymorphic. Two phenotypes can be recognized: high level 12E7-antigen expression and low level 12E7-antigen expression. There is a complex interaction between the *XG* blood group locus and the 12E7-antigen polymorphism. All individuals that are XG^a-antigen positive are high level expressors of the 12E7 antigen. XG^a-antigen-negative males can be either high or low level expressors of 12E7 antigen. These results suggested the existence of a Y-located gene, *YG*, which was polymorphic and regulated the level of 12E7-antigen expression on red blood cells (Goodfellow & Tippett, 1981). Formal proof of a Y-located genetic element was obtained from sib-analysis. It was predicted that XG^a-antigen-negative brothers would all share the same 12E7-antigen phenotype. Of about 200 brothers tested, 194 shared the same 12E7-antigen phenotype. This result can only be explained by the influence of a Y-located gene (Tippett, Shaw, Green & Daniels, 1986). The six exceptional cases were originally thought to be artefactual due to technical problems, however, at least in one case, the

exception is real. When we investigated the one exceptional brother and his family by DNA analysis it was discovered that he was recombinant at *MIC2*. This result has led us to postulate a new model in which a *cis*-acting regulator controls both XG^a-antigen expression and 12E7-antigen expression on red blood cells (Fig. 4). The regulator, *XGR*, is polymorphic with two alleles. The A allele induces the structural *XG* locus to produce the XG^a antigen and induces the *MIC2* locus to produce high levels of the 12E7 antigen in red blood cells. The B allele induces only low levels of the 12E7 antigen and fails to induce the expression of the XG^a antigen. The regulator is postulated to be pseudoautosomal in order to explain the exceptional case described above. This theory is explained in more detail in Goodfellow, Pritchard, Tippett & Goodfellow (1987).

Conclusions

Molecular and genetic analysis of the *MIC2* gene of man has revealed important clues as to the structure of the pseudoautosomal region of man. We anticipate that further analysis of this system will provide insights into the mechanism of X-inactivation and the evolution of the pseudoautosomal region. *MIC2* is a flanking marker for the pseudoautosomal and X- and Y-chromosome-specific regions. That location makes *MIC2* a convenient starting point for experiments designed to clone *TDF*.

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References

Banting, G. S., Pym, B. & Goodfellow, P. N. (1985). Biochemical analysis of an antigen produced by both human sex chromosomes. *EMBO J.* **4**, 1967–1972.

Bengtsson, B O. & Goodfellow, P. N. (1987). The effect of recombination between the X and Y chromosomes of mammals. *Ann. Hum Genet.* **51**, 57–64.

Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature, Lond.* **321**, 209–213.

Bodger, M. P., Francis, G. E., Delia, D., Granger, S. M. & Janossy, G. (1981). A monoclonal antibody specific for immature human haematopoietic cells and T lineage cells. *J. Immunol.* **127**, 2269–2274.

Buckle, V., Mondello, C., Darling, S., Craig, I. W. & Goodfellow, P. N. (1985). Homologous expressed genes in the human sex chromosome pairing region. *Nature, Lond.* **317**, 739–741.

Burgoyne, P. S. (1982). Genetic homology and crossing over in the X and Y chromosomes of mammals. *Hum. Genet.* **61**, 85–90.

Cooke, H. J., Brown, W. R. A. & Rappold, G. A. (1985). Hypervariable telomeric sequences from the human sex chromosomes are pseudoautosomal. *Nature, Lond.* **317**, 687–692.

Craig, I. W. & Tolley, E. (1986). Steroid sulphatase and the conservation of mammalian X chromosomes. *Trends in Genetics* **22**, 201–204.

Curry, C. J. R., Magenis, R. E., Brown, M., Lauman, J. T., Tsa, J. T., O'Langue, P., Goodfellow, P. N., Mohandas, T., Bergner, E. A. & Shapiro, L. M. (1984). Inherited chondrodysplasia punctata due to a deletion of the terminal short arm of an X chromosome. *N. Engl J. Med.* **311**, 1010–1015.

Darling, S. M., Banting, G. S., Pym, B., Wolfe, J. & Goodfellow, P. N. (1986*a*). Cloning an expressed gene shared by the human sex chromosomes. *Proc. natn. Acad. Sci. U.S.A.* **83**, 135–139.

Darling, S. M., Goodfellow, P. J., Pym, B., Banting, G. S., Pritchard, C. & Goodfellow, P N. (1986*b*). Molecular genetics of *MIC2*: a gene shared by the human X and Y chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **51**, 205–212.

Dracopoli, N. C., Rettig, W. J., Albino, A. P., Esposito, D , Archidiacono, N., Rocchi, M., Siniscalco, M. & Old, L. J. (1985). Genes controlling gp25/30 cell surface molecules map to chromosomes X and Y and escape X-inactivation. *Am. J. Hum. Genet* **37**, 199–207.

Dracopoli, N C., Rettig, W. J., Spengler, B. A., Oettgen, H. F., Biedler, J. L. & Old, L. J. (1983). Assignment of genes determining human cell surface antigens defined by monoclonal antibodies to chromosomes 12,X and Y. *Cytogenet. Cell Genet.* **40**, 456.

Evans, E. P., Burtenshaw, M. & Cattanach, B. M. (1982). Meiotic crossing over between X and Y chromosomes of male mice carrying the sex reversing (*Sxr*) factor. *Nature, Lond.* **300**, 443–445.

Geller, R. L., Shapiro, L J. & Mohandas, T. K. (1986). Fine mapping of the distal short arm of the human X chromosome using X/Y translocations. *Am J. Hum. Genet.* **38**, 884–890.

Goodfellow, P. (1983). Expression of the 12E7 antigen is controlled independently by genes on the human X and Y chromosomes. *Different.* **23**, 535–539.

Goodfellow, P. J., Darling, S. M., Thomas, N. S. & Goodfellow, P. N. (1986). A pseudoautosomal gene in man. *Science* **234**, 740–743.

Goodfellow, P. J., Pritchard, C., Tippett, P. & Goodfellow, P. N. (1987). Recombination between the X and Y chromosomes at the *YG* locus: implications for the relationship between *MIC2*, *XG* and *YG*. *Ann. Hum. Genet.* **51**, 161–167.

Goodfellow, P. N. & Tippett, P. (1981). A human polymorphism related to Xg blood groups. *Nature, Lond.* **289**, 404–405.

Haldane, J. B. S. (1936). A search for incomplete sex-linkage in man. *Ann. Eugenic.* **7**, 28–57.

Harbers, K., Soriano, P., Muller, U. & Jaenisch, R. (1986). High frequency of unequal recombination in the pseudoautosomal region shown by proviral

insertion in transgenic mouse. *Nature, Lond.* **324**, 682–685.

KEITGES, E., RIVEST, M., SINISCALCO, M. & GARTLER, S. M. (1985). X-linkage of steroid sulphatase in the mouse is evidence for a functional Y-linked allele. *Nature, Lond.* **315**, 226–227.

KEITH, D. H., SINGER-SAM, J. & RIGGS, A. D. (1986). Active X chromosome DNA is unmethylated at eight CCGG sites clustered in a guanine-plus-cytosine rich island at the 5′ end of the gene for phosphoglycerate kinase. *Mol. cell. Biol.* **6**, 4122–4125.

KOLLER, P. C. & DARLINGTON, C. D. (1934). The genetical and mechanical properties of the sex chromosomes. 1. *Rattus norvegicus*. *J. Genet.* **29**, 159–173.

KYTE, J. & DOOLITTLE, R. F. (1982). A simple method of displaying the hydropathic character of a protein. *J. Molec. Biol.* **157**, 105–132.

LEVY, R., DILLEY, J., FOX, R. I. & WARNKE, R. (1979). A human thymus leukaemia antigen defined by hybridoma monoclonal antibodies. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6552–6556.

MONDELLO, C., ROPERS, H-H., CRAIG, I. W., TOLLEY, E. & GOODFELLOW, P. N. (1987). Physical mapping of genes and sequences at the end of the human X chromosome short arm. *Ann. Hum. Genet.* **51**, 137–143.

RENWICK, J. H. (1969). Progress in mapping human autosomes. *Brit. Med. Bull.* **25**, 65–73.

ROUYER, F., SIMMLER, M-C., JOHNSSON, C., VERGNAUD, G., COOKE, H. J. & WEISSENBACH, J. (1986). A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Nature, Lond.* **319**, 291–295.

SIMMLER, M-C., ROUYER, F., VERGNAUD, G., NYSTROM-LAHTI, M., NGO, K. Y., DE LA CHAPELLE, A. & WEISSENBACH, J. (1985). Pseudoautosomal DNA sequences in the pairing region of the human sex chromosomes. *Nature, Lond.* **317**, 692–697.

SINGH, L. & JONES, K. W. (1982). Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent non-reciprocal crossover involving the X and an aberrant Y chromosome. *Cell* **28**, 205–216.

TIPPETT, P., SHAW, M. A., GREEN, C. A. & DANIELS, G. L. (1986). The 12E7 red cell quantitative polymorphism: control by the Y-borne locus, Yg. *Ann. Hum. Genet.* **50**, 339–347.

YEN, P. H., PATEL, P., CHINAULT, A. C., MOHANDAS, T. & SHAPIRO, L. J. (1984). Differential methylation of hypoxanthine phosphoribosyltransferase genes on active and inactive human X chromosomes. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1759–1763.