PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

MIC2: A Human Pseudoautosomal Gene [and Discussion]

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MIC2: a human pseudoautosomal gene

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MIC2 and *XGR* are the only known pseudoautosomal genes in man. *MIC2* encodes the 12E7 antigen, a human cell-surface molecule of unknown function. *XGR* regulates, in *cis*, the expression of the *XG* and *MIC2* genes.

DNA probes derived from the *MIC2* locus have been used in the construction of a meiotic map of the pseudoautosomal region and a long range restriction map into the X- and Y-specific chromosome domains. *MIC2* is the most proximal marker in the pseudoautosomal region and recombination between the sex chromosomes only rarely includes the *MIC2* locus. Our long-range restriction maps and chromosome walking experiments have localized the pseudoautosomal boundary within 40 kilobases adjacent to the 3′ end of the *MIC2* gene. The same maps have been used to predict the chromosomal location of *TDF*.

Introduction

The Y chromosome of man and other mammals is highly adapted for its role in male sex determination. It is composed of two distinct regions with antithetical genetic properties (Koller & Darlington 1934). One region is Y-chromosome-specific and encodes *TDF*, a gene required for male sex determination. Sequences derived from this region do not normally recombine with the X chromosome. Abnormal recombination, which occurs infrequently, leads to the generation of XX males or XY females and the breakdown of the chromosomal basis of sex determination (Ferguson-Smith 1966). The second region of the Y chromosome is shared by the X and Y chromosomes and is responsible for correct sex chromosome pairing and segregation in male meiosis. Sequences in this region are exchanged between the sex chromosomes by recombination and fail to show classical sex-linked inheritance. This behaviour has been described as 'pseudoautosomal', and the shared part of the sex chromosomes is known as the pseudoautosomal region (Burgoyne 1982).

Very few genes are encoded by the Y chromosome (Goodfellow *et al.* 1985). This is a direct consequence of the role of the Y chromosome in male sex determination and the evolution of dosage compensation by X-inactivation in mammals (Lyon 1974). To maintain gene dosage, genes shared by the sex chromosomes must either escape inactivation on the X chromosome or undergo inactivation on the Y chromosome. The X-located homologues of pseudoautosomal genes are known to escape X-inactivation (Goodfellow *et al.* 1984). There is no evidence for inactivation of Y-located genes. These considerations have led to the prediction that the genes encoded by the Y-specific region are either directly required for the male phenotype or are deleterious to the female phenotype. In contrast there is no obvious *a priori* restriction on the function of genes encoded by the pseudoautosomal region.

The paucity of genetic markers has in the past hindered the analysis of the human Y chromosome and most studies have been restricted to correlation of phenotype with gross chromosomal rearrangements (Davis 1981). The introduction of molecular cloning techniques has provided an unlimited supply of genetic markers for the Y chromosome and has led to construction of genetic maps of both the pseudoautosomal and the Y-specific regions (Davies *et al.* 1988). In addition, molecular cloning techniques have resulted in the isolation of the pseudoautosomal gene *MIC2* (Darling *et al.* 1986*a*, *b*) and a candidate sequence for *TDF* (Page *et al.* 1987).

In this review we consider the molecular cloning of *MIC2* and the use of *MIC2*-derived probes for investigating the genetics of the Y chromosome.

The 12E7 antigen

The monoclonal antibody 12E7 recognizes a human cell-surface molecule with a wide tissue distribution. The 12E7 antigen is found in man, gorilla and chimpanzee, but is not found in orangutan, gibbon or any other species tested (Goodfellow 1983). This species specificity was exploited to investigate the genetics of the 12E7 antigen. Human–rodent hybrids that retain the human X chromosome or the human Y chromosome express the 12E7 antigen; retention of no other human chromosome in the hybrids correlates with expression of the 12E7 antigen. These results define the X-located gene *MIC2X* and the Y-located gene *MIC2Y* (Goodfellow *et al.* 1980, 1983). Biochemical studies demonstrated that the 12E7 antigen is associated with a 32.5 kDa cell-surface molecule with a pI of 5.0. No differences were detected between the putative products of the *MIC2X* and *MIC2Y* loci (Banting *et al.* 1985).

Human–rodent somatic-cell hybrids that retain the inactive X chromosome also express the 12E7 antigen, implying that the *MIC2X* locus escapes X-inactivation (Goodfellow *et al.* 1984). These experiments suggested that *MIC2* is a pseudoautosomal gene, the first pseudoautosomal gene defined in man. Proof of this contention required cloning of the *MIC2* gene.

Molecular cloning and analysis of *MIC2*

φλgt11 is a cloning vector that allows expression of eukaryotic proteins as fusion products in bacterial cells (Young & Davis 1983). A φλgt11 library was constructed with complementary DNA (cDNA) sequences prepared from the human T cell line J6 and this library was screened with a mixture of two monoclonal antibodies that reacted with the 12E7 antigen (Darling *et al.* 1986*a*). A positive clone, designated SG1, was isolated and shown to be derived from the *MIC2* locus by three independent methods.

1. *In situ* hybridization of the cDNA clone to human chromosomes identified two sites of hybridization in the human genome: the end of the X chromosome short arm, Xp22.3-pter, and the Y chromosome short arm, Yp11-pter (Buckle *et al.* 1985).

2. Mouse cells were transfected with genomic human DNA and transfectants expressing the 12E7 antigen were isolated by using the fluorescence-activated cell sorter. DNA prepared from the antigen-positive transfectants reacted with the *MIC2* cDNA clone in Southern blot analysis (Darling *et al.* 1986*a*).

3. A monoclonal antibody, MSGB1, was raised against a peptide, the sequence of which was derived from a conceptual translation of the cDNA clone. The MSGB1 antibody recognizes the 12E7 antigen (Darling *et al.* 1986*b*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The cDNA clone has been used to isolate further cDNA clones and genomic sequences. The DNA sequence of one full length (or close to full length) cDNA clone is presented in figure 1, with its conceptual translation. A preliminary sequence of a partial cDNA clone has been published previously (Darling *et al.* 1986*b*). The published sequence contains an error that resulted in the premature termination of the coding region.

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CGTGGAGGCC GGGGCGGGGC GGGCGCAGCC GGCGCTGAGC TTGCAGGGCC GCTCCCCTCA CCCGCCCCCT TCGAGTCCCC GGGCTTCACC

CCACCCGGCC CGTGGGGGAG TATCTGTCCT GCCGCCTTCG CCCACGCCCT GCACTCCGGG ACCGTCCCTG CGCGCTCTGG GCGCACC

ATG GCC CGC GGG GCT GCG CTG GCG CTG CTG CTC TTC GGC CTG CTG GGT GTT CTG GTC GCC GCC CCG GAT GGT GGT
MET Ala Arg Gly Ala Ala Leu Ala Leu Leu Leu Phe Gly Leu Leu Gly Val Leu Val Ala Ala Pro Asp Gly Gly

TTC GAT TTA TCC GAT GCC CTT CCT GAC AAT GAA AAC AAG AAA CCC ACT GCA ATC CCC AAG AAA CCC AGT GCT GGG
Phe Asp Leu Ser Asp Ala Leu Pro Asp Asn Glu Asn Lys Lys Pro Thr Ala Ile Pro Lys Lys Pro Ser Ala Gly

GAT GAC TTT GAC TTA GGA GAT GCT GTT GTT GAT GGA GAA AAT GAC GAC CCA CGA CCA CCG AAC CCA CCC AAA CCG
Asp Asp Phe Asp Leu Gly Asp Ala Val Val Asp Gly Glu Asn Asp Asp Pro Arg Pro Pro Asn Pro Pro Lys Pro

ATG CCA AAT CCA AAC CCC AAC CAC CCT AGT TCC TCC GGT AGC TTT TCA GAT GCT GAC CTT GCG GAT GGC GTT TCA
Met Pro Asn Pro Asn Pro Asn His Pro Ser Ser Ser Gly Ser Phe Ser Asp Ala Asp Leu Ala Asp Gly Val Ser

GGT GGA GAA GGA AAA GGA GGC AGT GAT GGT GGA GGC AGC CAC AGG AAA GAA GGG GAA GAG GCC GAC GCC CCA GGC
Gly Gly Glu Gly Lys Gly Gly Ser Asp Gly Gly Gly Ser His Arg Lys Glu Gly Glu Glu Ala Asp Ala Pro Gly

GTG ATC CCC GGG ATT GTG GGG GCT GTC GTG GTC GCC GTG GCT GGA GCC ATC TCT AGC TTC ATT GCT TAC CAG AAA
Val Ile Pro Gly Ile Val Gly Ala Val Val Val Ala Val Ala Gly Ala Ile Ser Ser Phe Ile Ala Tyr Gln Lys

AAG AAG CTA TGC TTC AAA GAA AAT GCA GAA CAA GGG GAG GTG GAC ATG GAG AGC CAC CGG AAT GCC AAC GCA GAG
Lys Lys Leu Cys Phe Lys Glu Asn Ala Glu Gln Gly Glu Val Asp Met Glu Ser His Arg Asn Ala Asn Ala Glu

CCA GCT GTT CAG CGT ACT CTT TTA GAG AAA TAGAAGATTG TCGGCAGAAA CAGCCCAGGC GTTGGCAGCA GGGTTAGAAC
Pro Ala Val Gln Arg Thr Leu Leu Glu Lys

AGCTGCCTG AGGCTCCTCCC TGAAGGACAC CTGCCTGAGA GCAGAGATGG AGGCCTTCTG TTCACGGCGG ATTCTTTGTT TTAATCTTGC

GATGTGCTTT GCTTGTTGCT GGGCGGATGA TGTTTACTAA CGATGAATTT TACATCCAAA GGGGGATAGG CACTTGGACC CCCATTCTCC

AAGGCCCGGG GGGGCGGTTT CCCATGGGAT GTGAAAGGCT GGCCATTATT AAGTCCCTGT AACTCAAATG TCAACCCCAC CGAGGCACCC

CCCCGTCCCC CAGAATCTTG GCTGTTTACA AATCACGTGT CCATCGAGCA CGTCTGAAAC CCCTGGTAGC CCCGACTTCT TTTTAATTAA

AATAAGGTAA GCCCTTCAAT TTGTTTCTTC AATATTTCTT TCATTTGTAG GGATATTTGT TTTTCATATC AGACTAATAA AAAGAAATTA

GAAACCAAAA
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FIGURE 1. Nucleotide sequence of the *MIC2* cDNA clone, NT23. The conceptual translation of the cDNA is shown. NT23 was isolated from a cDNA library constructed with mRNA from a human testicular teratocarcinoma cell line by screening with a previously described cDNA, SG1 (Darling *et al.* 1986*a*, *b*). DNA sequence analysis was done on both strands by using the dideoxy chain termination procedure (Sanger *et al.* 1977) after cloning into M13 vectors (Messing 1983). Synthetic oligonucleotide primers were used to confirm the sequence in areas of ambiguity.

The *MIC2* product contains two hydrophobic regions corresponding to an N-terminal signal sequence and a putative transmembrane domain adjacent to the C terminus. Unusual features of the protein sequence include a high concentration of proline residues in the middle region of the molecule and many charged pairs of amino acids. These features of the sequence present no obvious clue as to the function of the *MIC2* gene. A search of available protein and nucleic acid sequence data bases has failed to find related sequences (Banting *et al.* 1988).

The cDNA clone has been used to isolate genomic sequences derived from the *MIC2* locus. These sequences have been used for an investigation of the promoter region at the 5′ end of the gene and as a source of polymorphic probes for family studies. The *MIC2* gene is unusually large for the size of the transcript. The mRNA is about 1.3 kilobases (the cDNA clone in figure 1 is 1242 base pairs (b.p.) long) and this derives from over 50 kilobases of genomic DNA. The

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

5′ end of the gene contains a region that is high in G+C content (67%) and shows no suppression of the occurrence of CpG dinucleotide pairs (Goodfellow *et al.* 1988). These features are characteristic of the 5′ regions of many mammalian genes and may have functional importance for gene expression (Bird 1986). Another feature of these CpG-rich regions is the lack of methylation of the cytosine in the CpG pair. The absence of 5-methyl cytosine residues allows cleavage by methylation-sensitive restriction enzymes such as *Hpa*II, generating multiple small *Hpa*II fragments (hence the name HTF island or *Hpa*II tiny fragment island). These regions are also cleaved by methylation-sensitive enzymes used in long-range restriction mapping (Brown & Bird 1986). Several X-located genes have CpG-rich 5′ regions; these regions are unmethylated on the active X and methylated on the inactive X (Yen *et al.* 1984; Keith *et al.* 1986). This correlation suggests that methylation of CpG-rich regions may play a role in X-inactivation. Consistent with this view the *MIC2* CpG-rich region is unmethylated on the inactive X as well as on the active X and Y chromosome (Goodfellow *et al.* 1988). In this case escape from inactivation is correlated with lack of methylation at the CpG-rich region. Methylation at CpG sites within the body of the gene is variable and shows a poor correlation with X-inactivation (Mondello *et al.* 1988).

The *MIC2* cDNA clones recognize many restriction-fragment polymorphisms in genomic DNA; however, the patterns generated with most restriction enzymes are difficult to analyse. Single-copy probes derived from genomic *MIC2* sequences have proved to be more convenient tools for use in family studies and the construction of meiotic maps of the pseudoautosomal region (Goodfellow *et al.* 1986*a*). *MIC2* probes have also been used in the construction of deletion maps and long-range restriction maps of the Y chromosome.

Mapping the Y chromosome with *MIC2* probes

DNA probes, derived from sequences isolated at random from the human Y chromosome, were used to demonstrate recombination between the X and Y chromosomes and to construct a meiotic map of the pseudoautosomal region (Simmler *et al.* 1985; Cooke *et al.* 1985; Rouyer *et al.* 1986; Goodfellow *et al.* 1986*a*). The most distal sequence, *DXYS14*, exchanges in 50% of cases, implying that an obligate recombination occurs in the pseudoautosomal region in each male meiotic event. In an initial search for recombination events that included the *MIC2* locus we studied 46 informative male meioses and found one exchange between the X and Y chromosomes. This constitutes formal proof that *MIC2* is a pseudoautosomal gene (Goodfellow *et al.* 1986*a*).

Figure 2 summarizes the meiotic maps of the pseudoautosomal region for both male and female meioses. There are dramatic differences in the rates of recombination between the pseudoautosomal marker loci in male versus female meioses. The most proximal marker, *MIC2*, shows no detectable linkage with the most distal marker, *DXYS14*, in male meioses. In female meioses, however, *MIC2* and *DXYS14* recombine at a rate of only 2.4% ($\theta = 0.024$, $Z_1 = 20.0$, one lod unit† confidence interval 0.00–0.05) (combined data of Weissenbach *et al.* (1987) and our unpublished data). Whereas *MIC2* and *DXYS17* recombine at a rate of 15% in male meioses, no recombination between the pair of markers is observed in female meioses. The male map is more than ten times larger than the female map and emphasizes the high levels of recombination in this region in males. Both indirect evidence (Mondello *et al.* 1987;

† A lod score represents the $\log_{10}$ of the likelihood (L) ratio: L (recombination fraction)/L (0.5).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

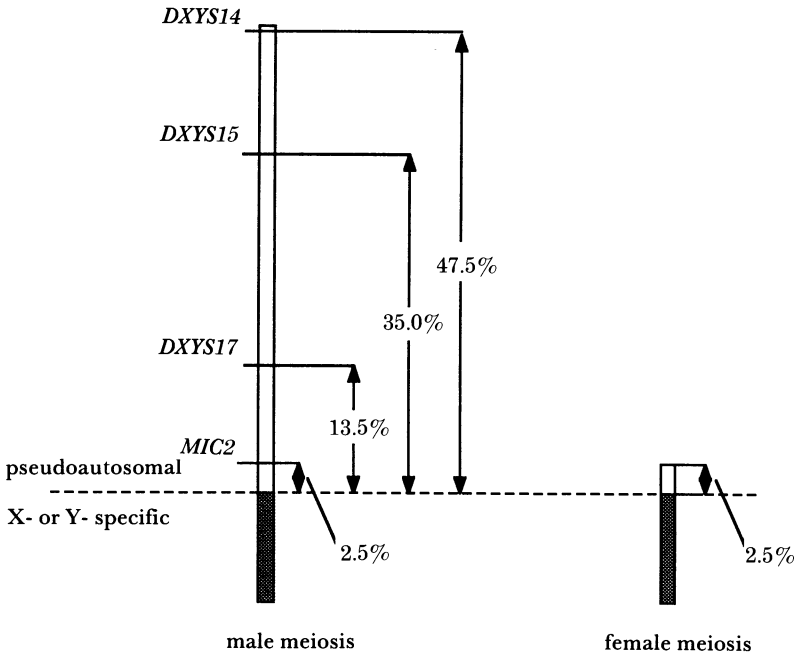

FIGURE 2. Meiotic maps of the pseudoautosomal region for male and female meiosis. Recombination rates with respect to sex-specific sequences and marker loci *DXYS14*, *DXYS15*, *DXYS17* and *MIC2* are given for male meioses. The distances between pairs of markers in male meioses are additive and consistent with values relative to sex-specific sequences. Few recombinants are observed in female meioses and only the estimated genetic size of the region in females is shown. Data are a further compilation of our unpublished results with those compiled by Weissenbach *et al.* (1987).

Rouyer *et al.* 1986) and direct measurement using pulsed field gel electrophoresis (PFGE) (J. Weissenbach, this symposium) suggest that the pseudoautosomal region is about 3.0×10^6 b.p. in size. In man, one unit of recombination is on average equated with approximately 1×10^6 b.p. (Renwick 1969). The genetic size of the pseudoautosomal region when measured in female meiosis is consistent with the physical size of the region. In male meiosis, one unit of recombination in the region is equivalent to 1×10^5 b.p. (100 kilobases) or less. Recombination in the pseudoautosomal region in male meiosis therefore occurs at a frequency greater than ten times the genome average.

In summary, *MIC2* only rarely exchanges between the sex chromosomes and is the most proximal of the pseudoautosomal markers in man.

A DELETION MAP OF THE Y CHROMOSOME

The genomes of the majority of XX males contain Y chromosome sequences (Affara *et al.* 1986*a*, *b*; Guellaen *et al.* 1984; Petit *et al.* 1987). These sequences derive from an abnormal exchange between the X and Y chromosomes resulting in the transfer of Y sequences, including *TDF*, to the tip of the X chromosome (Andersson *et al.* 1986; Buckle *et al.* 1987). The amount of Y-derived sequences transferred is variable but, by definition, must always include *TDF*. Analysis of the variable amounts of Y sequences present in XX males has allowed construction of deletion maps of the Y chromosome (Affara *et al.* 1986*a*; Vergnaud *et al.* 1986). From these maps it was concluded that *TDF* is located on the distal part of the short arm of Y chromosome

adjacent to the pseudoautosomal region. These studies defined Y-specific proximal flanking markers and implied that *MIC2* was the closest known distal flanking marker for *TDF*.

The regional localization of *MIC2* has been exploited to demonstrate that not all XX males are generated by a terminal exchange between the X and Y chromosomes. It was argued that exchange XX males will inherit the Y chromosome allele of *MIC2* on the paternal X chromosome (except in 2% of cases in which normal recombination at *MIC2* might also occur). Petit *et al.* (1987) constructed somatic cell hybrids to define the *MIC2* allele present on the Y chromosome in the fathers of XX males and were able to demonstrate that in three XX males the Y chromosome *MIC2* allele had not been inherited. A similar case was reported by Goodfellow & Goodfellow (1988). However, it should be stressed that the majority of XX males are due to aberrant X–Y exchange.

Construction of long-range restriction maps

PFGE extends the size separation range for DNA molecules in agarose gels up to several million base pairs. Combined with restriction enzymes that cut infrequently in the genome, PFGE can be used to construct long-range restriction maps of complex genomes. Most 'rare cutting' restriction enzymes recognize sequences containing CpG dinucleotide pairs and are sensitive to cytosine methylation. In consequence, 'rare cutting' enzymes frequently cleave the CpG-rich regions found at the 5′ end of many genes (Brown & Bird 1986).

From a series of experiments designed to facilitate 'reverse genetic' approaches to cloning *TDF*, a Y-specific sequence, *DYS104*, was isolated from the region adjacent to *TDF* (Pritchard *et al.* 1987*a*). Another sequence, *DYS13*, with similar properties, was isolated by Affara *et al.* (1986*a*,*b*). Using probes for *MIC2*, *DYS104* and *DYS13*, a long-range restriction map was constructed (Pritchard *et al.* 1987*b*). The starting point for this map was the CpG-rich region at the 5′ end of *MIC2*. This region has been sequenced (Goodfellow *et al.* 1988) and several sites for 'rare cutting' restriction enzymes were demonstrated to be unmethylated in genomic DNA, providing an 'anchor point' upon which a long-range restriction map was based. The map we obtained contained several features of interest (figure 3).

1. For several enzymes all three sequences are located on the same restriction fragments. As *MIC2* is pseudoautosomal and *DYS104* and *DYS13* are Y-specific these fragments must span the boundary of the pseudoautosomal region. This conclusion was confirmed by demonstrating that *MIC2* probes recognize different restriction fragments on the X and the Y chromosomes. The smallest fragment that differs between the X and Y chromosomes is 150 kilobases. This defines the maximum distance between the *MIC2* CpG-rich region and the boundary. The position of the boundary has been further localized, by chromosome-walking experiments, to a region within 40 kilobases of the 3′ end of the *MIC2* gene.

The genetic distance between *MIC2* and the boundary is approximately 2.5 cM†. This recombination occurs in a physical distance of only 100 kilobases. Thus there is no evidence for suppression of recombination in the immediate vicinity of the boundary.

2. On the Y chromosome there are two clusters of 'rare cutting' cleavage sites detected by the probes. The first cluster is at the 5′ end of *MIC2*, the second cluster is approximately 260 kilobases proximal to *MIC2*. We suggested that this cluster was defining a gene and, on

† The morgan is the unit of relative distance between genes on a chromosome. One centimorgan (cM) represents a crossover value of 1%.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

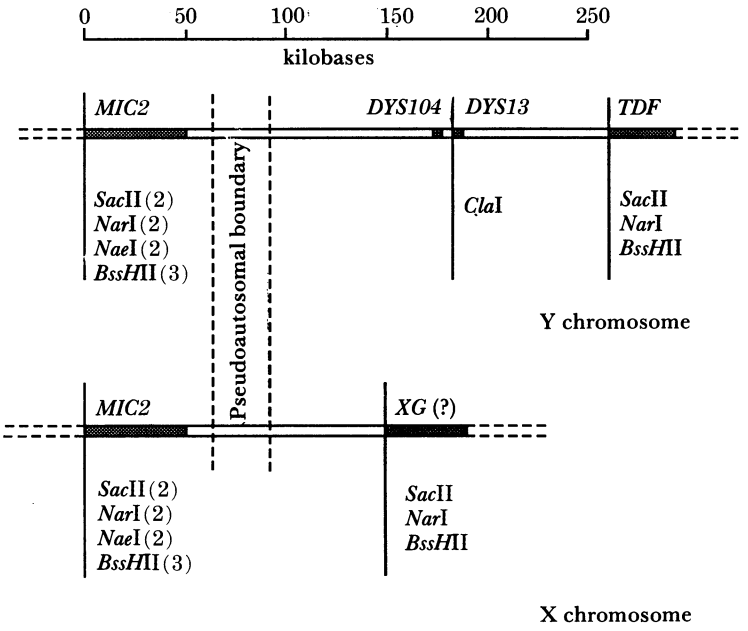

FIGURE 3. Long-range restriction map across the pseudoautosomal boundary. Rare-cutting restriction-enzyme recognition sites in the *MIC2* HTF-island and the X- and Y-specific cluster of sites are shown. The number of sites at the *MIC2* island are given in parentheses. The *Cla*I site that separates *DYS104* and *DYS13* and their positions relative to it are shown. The positions of *DYS104*, *DYS13* and the pseudoautosomal boundaries were refined by chromosomal walking experiments (our unpublished observations).

the basis of chromosomal location, we predicted that this gene was *TDF*. This prediction appears to have been confirmed by the elegant studies of Page *et al.* (1987).

3. On the X chromosome there are also two clusters of 'rare cutting' cleavage sites detected by the *MIC2* probe. The second cluster is specific to the X chromosome and we have suggested that this may represent the structural gene for the Xg blood-group antigen. This prediction is based on the finding of genetic interaction between *MIC2*, *XG* and a third locus *XGR* (Goodfellow *et al.* 1986*b*). On red blood cells, expression of the *XG* antigen and the 12E7 antigen is coordinately regulated in *cis* by *XGR*, a pseudoautosomal locus. This locus was previously believed to be Y-specific and was named *YG* (Goodfellow& Tippett 1981; Tippett *et al.* 1986). Recombination occurs between *XGR* and *XG*, but *XGR* has not been separated from *MIC2* and it is possible that *XGR* is a polymorphism at the *MIC2* locus. Nevertheless, the *cis* interaction between *XGR*, *MIC2* and *XG* is easier to understand if *XG* and *MIC2* are in close proximity.

CONCLUSIONS

Two pseudoautosomal genes have been defined in mammals: *Sts* in mouse and *MIC2* in man. The murine *Sts* locus encodes the enzyme steroid sulphatase and this locus exchanges with a high frequency between the sex chromosomes in male meiosis (Keitges *et al.* 1985). The equivalent locus in man, *STS*, is not pseudoautosomal, but is located on the X chromosome adjacent to the pseudoautosomal region (Geller *et al.* 1986; Mondello *et al.* 1987). *STS* is subject to only partial X-inactivation (Migeon *et al.* 1982) and this might imply that *STS* was originally pseudoautosomal and has been lost recently from the Y chromosome during

evolution. The presence of an incomplete copy of the *STS* gene on the Y chromosome is consistent with this suggestion (Frazer *et al.* 1987; Yen *et al.* 1987).

MIC2, and the related *XGR* locus, are the only known pseudoautosomal genes in man. The position of *MIC2* close to the pseudoautosomal boundary has made it a valuable genetic marker for analysing the structure of the human Y chromosome. In particular, it was possible to construct long-range restriction maps, based on *MIC2*, which precisely defined the chromosomal location of *TDF* (Pritchard *et al.* 1987*b*; Page *et al.* 1987). The same restriction maps have located the pseudoautosomal boundary immediately adjacent to the 3′ end of the *MIC2* gene. It should be possible to clone the boundary region by chromosome-walking methods starting with cloned *MIC2* sequences.

We thank our many friends who have helped to study *MIC2* and the human Y chromosome. This manuscript was prepared with editorial help provided by Clare Middlemiss.

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Discussion

H. Sharma (71 *Barrack Road, Hounslow, U.K.*) Is there homology to any known protein sequence with *MIC* genes? Is it conserved in species?

P. N. Goodfellow. Outside the putative signal sequence we have not detected sequence homology between *MIC2* and any other described sequence; this is true both at the protein and nucleotide sequence level.

MIC2-related sequences are present in primates, but have not been detected by high-stringency hybridization in other animals.

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