High-Resolution Comparative Mapping of the Proximal Region of the Mouse X Chromosome

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The murine homologues of the loci for McLeod syndrome (XK), Dent’s disease (CICN5), and synaptophysin (SYP) have been mapped to the proximal region of the mouse X chromosome and positioned with respect to other conserved loci in this region using a total of 948 progeny from two separate Mus musculus × Mus spretus backcrosses. In the mouse, the order of loci and evolutionary breakpoints (EB) has been established as centromere–(DXWas70, DXHXP34h)–EB–Clcn5–(Syp, DXMit55, DXMit28)–Tfe3–Gata1–EB–Xk–Cybb–telomere. In the proximal region of the human X chromosome short arm, the position of evolutionary breakpoints with respect to key loci has been established as DMD–EB–XR–PFC–EB–GATA1–C1CN5–EB–DXS1272E–ALAS2–EB–DXF34–centromere. These data have enabled us to construct a high-resolution genetic map for the ~3-cM interval between DXWas70 and Cybb on the mouse X chromosome, which encompasses 10 loci. This detailed map demonstrates the power of high-resolution genetic mapping in the mouse as a means of determining locus order in a small chromosomal region and of providing an accurate framework for the construction of physical maps. © 1995 Academic Press, Inc.

INTRODUCTION

The positioning of approximately 70 loci on the X chromosomes of both mouse and human has confirmed the prediction made by Ohno (1973) that X-linkage of genes is largely preserved in mammals (Herman et al., 1994; Willard et al., 1994). However, comparison of the relative positions of these loci in human and mouse has revealed that subchromosomal blocks of homologous loci have been rearranged with respect to each other during the 100 million years of evolutionary time that separate the two species (Amar et al., 1989; Blair et al., 1994a). A full understanding of the nature of these rearrangements is important for identifying mouse models for human genetic disease, and as an identical comparative map position is one criterion for inferring genetic homology between similar phenotypes.

The human–mouse comparative map of the X chromosome is composed of a minimum of eight chromosomal segments (Blair et al., 1994a). There are four blocks of homology in the ~20-Mb region of the proximal human X chromosome short arm; three lie in the proximal region of the mouse X chromosome, and the fourth lies in the distal region (Fig. 1). The largest block runs from Cybb to Pfc2 and, on the human X chromosome, is flanked on the CYBB boundary by DMD and on the PFC boundary by GATA1. In the mouse, the Cybb boundary is flanked by Gata1/Tfe3 and the Pfc boundary by Lamp2. The proximity of the small block of homology defined by Gata1/Tfe3 to the conserved segment that runs from Cybb to Pfc led to the suggestion that the current arrangement of loci has resulted from a partial inversion within a larger homologous block (Laval and Boyd, 1993a). GATA1 and TFE3 are known to lie approximately 150 kb apart in human (Derry et al., 1994) and have not been separated by recombination events in the mouse (Blair and Boyd, 1994a; Blair et al., 1994b; Merrell et al., 1995). In human, proximal to GATA1/TFE3 lies a homologous block that runs from DXS1272E (also known as XE169 and SMCX) to ALAS2 (Willard et al., 1994). The murine equivalent of this segment lies in the distal region of the mouse X chromosome (Chapman et al., 1994; Agulnik et al., 1994; Blair et al., 1994a). Finally, close to the centromere in both species lies a complex locus defined by DXF34 (Laval and Boyd, 1993a,b). While the construction of extensive YAC contigs has

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enabled the definition of gene order and approximate distances between genes in the proximal human X chromosome short arm (Willard et al., 1994), many of these loci have not been positioned, or not been positioned with sufficient detail, on the mouse X chromosome, and therefore the locations of evolutionary breakpoints in this region are poorly defined. This paper describes the mapping of the murine homologues of three such loci (XK, CICN5, and SYP; see Fig. 1). The gene encoding XK is a novel membrane transport protein that has been recently cloned but has not yet been mapped in the mouse (Ho et al., 1994). XK is mutated in patients suffering from McLeod syndrome, a complex disorder characterized by abnormalities in the neuromuscular and hemopoietic systems. XK lies ~200 kb distal to CYBB on the human X chromosome and therefore could lie either proximal to CYBB or close to Dmd in the central region of the mouse X chromosome. The isolation of CICN5 (previously CIC-K2), a candidate gene for the X-linked nephropathiai Dent's disease, has also been reported recently (Fisher et al., 1994). This gene, CICN5, is a new member of the voltage-gated chloride channel family and lies close to DXS255, between GATA1 and DXS1272E on the human X chromosome, and therefore could lie in any one of several sites on the mouse X chromosome (Fig. 1). The murine homologue of syntaphynin (SYP) has been mapped previously to the proximal region of the mouse X chromosome by somatic cell hybrid analysis, but has not been ordered with respect to other loci in the region (Özçelik et al., 1980).

We report here the mapping of these three loci to the proximal region of the mouse X chromosome and their positioning with respect to other conserved loci in the region using a total of 948 progeny from two separate Mus musculus × Mus spretus backcrosses. These data and others obtained from mapping murine-specific probes and microsatellite-based loci on the same backcrosses have enabled us to construct a high-resolution genetic map for the ~3-cM interval between DXWas70 and Cybb, which encompasses 10 loci and two evolutionary breakpoints. As a result, the human–mouse comparative map of the region has been significantly improved.

**MATERIALS AND METHODS**

**Interspecific backcrosses.** Loci were first positioned using an interspecific backcross bred at the MRC Radiobiology Unit and then, to achieve further separation of markers, on the European Collaborative Backcross. The backcross bred at the RBU (backcross A) comprises two sections: (3H1 or C3H ? × Mus spretus δ) F1 females were mated to 3H1 or C3H δ; 3H1 is on F1 hybrid produced by mating C3H/Hef ? to 101/H δ. No significant difference has been found between any of the genetic distances calculated for X chromosomal loci, and therefore the results from both sections have been pooled (Blair et al., 1990). A total of 235 animals have been typed for four loci in the proximal region of the mouse X chromosome, DXWas70, DXHx874, Tfe3, and Pfc (Laval et al., 1991; Blair et al., 1994a and unpublished data). The second backcross (B), the European Collaborative Interspecific Backcross (EUCIB), was produced by mating (C57BL/6 ? × M. spretus δ) F1 females to either C57BL/6 δ or M. spretus δ (Breen et al., 1994). Approximately 900 backcross animals had been previously typed for DXWas70 and four other markers on the mouse X chromosome (DXMx8, Xist, Pip, and Grpr).

**Probes and filter hybridizations.** Hybridization probes were used to detect most of the conserved loci, and the murine repeat sequence, DXWas70, used in this study (see Table 1 and Results). Partial human cDNAs were used to detect the murine homologues of the McLeod syndrome gene, XK (Ho et al., 1994), the voltage-gated chloride channel gene, CICN5, implicated in Dent's disease (Fisher et al., 1994), and the GATA binding protein 1, GATA1 (Laval and Boyd, 1993a). The hybridization probe used to detect the murine homologue of the syntaphyn in gene, SYP, was generated using primers designed from exon 4 of the human cDNA. The forward (GCAACAGATT-GTACTTGAAGC) and reverse (GCCGCTGTTACTACTC) primers represent positions 1570–1591 and 1735–1752, respectively, of the sequence given in Özçelik and co-workers (1990). Primers were used to amplify and label an approximately 180-bp product from mouse genomic DNA using standard polymerase chain reaction (PCR) conditions described previously (Blair et al., 1985). Reactions were carried out in the presence of 1 mM MgCl2 using an annealing temperature of 55°C. Probes were labeled with [32P]dCTP by nick-translation or multiprimer using commercially available kits (Amersham International plc). Southern blotting and filter hybridi-
TABLE 1
X-Linked RFLVs or APVs Detected at Each Locus

<table>
<thead>
<tr>
<th>Human locus symbol</th>
<th>Mouse locus symbol</th>
<th>Probe name</th>
<th>Gene name/details</th>
<th>Reference*</th>
<th>Restriction enzyme for RFLV</th>
<th>3H1 X-linked hands (kb) or product (bp)</th>
<th>M. spretus X-linked bands (kb) or product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>DXWas70</td>
<td></td>
<td>X-linked DNA segment</td>
<td>1</td>
<td>TaqI</td>
<td>3.5, 1.8</td>
<td>4.0, 3.0</td>
</tr>
<tr>
<td>DFX34</td>
<td>DFX34</td>
<td></td>
<td>X-linked conserved sequence</td>
<td>2</td>
<td>EcoRI</td>
<td>4.0, 1.3</td>
<td>3.0</td>
</tr>
<tr>
<td>CLCN5</td>
<td>Clcn5</td>
<td>RL-L6²</td>
<td>Voltage-gated chloride channel N5</td>
<td>3</td>
<td>PvuII</td>
<td>9.4</td>
<td>3.5</td>
</tr>
<tr>
<td>SYP</td>
<td>Syp</td>
<td>mSYPE4⁴</td>
<td>Synaptophysin</td>
<td>4</td>
<td>TaqI</td>
<td>5.0</td>
<td>3.0</td>
</tr>
<tr>
<td>GATA1</td>
<td>Gata1</td>
<td>K14⁴</td>
<td>GATA binding protein 1</td>
<td>5</td>
<td>EcoRI</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>XK</td>
<td>Xk</td>
<td>XX²</td>
<td>McLeod syndrome gene</td>
<td>6</td>
<td>TaqI</td>
<td>0.8</td>
<td>2.2</td>
</tr>
<tr>
<td>CYBB</td>
<td>Cybb</td>
<td>CyBB⁴</td>
<td>Cytochrome h245 β chain</td>
<td>7</td>
<td>TaqI</td>
<td>4.0</td>
<td>2.3, 1.8</td>
</tr>
<tr>
<td>PFC</td>
<td>Pfc</td>
<td>mP-830²</td>
<td>Properdin factor complement</td>
<td>8</td>
<td>TaqI</td>
<td>0.1</td>
<td>5.0</td>
</tr>
<tr>
<td>TFE3</td>
<td>Tfe3</td>
<td>APV</td>
<td>Transcription factor enhancer 3</td>
<td>9, 10</td>
<td>HhaI</td>
<td>655</td>
<td>267, 188</td>
</tr>
<tr>
<td>—</td>
<td>DXMit526</td>
<td>APV</td>
<td>X-linked minisatellite</td>
<td>11, 12</td>
<td>HhaI</td>
<td>220</td>
<td>206</td>
</tr>
<tr>
<td>—</td>
<td>DXMit55</td>
<td>APV</td>
<td>X-linked minisatellite</td>
<td>11, 12</td>
<td>HhaI</td>
<td>116</td>
<td>100</td>
</tr>
<tr>
<td>—</td>
<td>DXMit54</td>
<td>APV</td>
<td>X-linked minisatellite</td>
<td>11, 12</td>
<td>HhaI</td>
<td>144</td>
<td>212</td>
</tr>
</tbody>
</table>

* References: (1) Diesteche et al., 1987; (2) Laval and Boyd, 1993b; (3) Fisher et al., 1994; (4) Öztürk et al., 1990; (5) Zon et al., 1990; (6) Ho et al., 1994; (7) Royer-Pokora et al., 1986; (8) Goundis and Reid, 1988; (9) Roman et al., 1992; (10) Blair and Boyd, 1994b; (11) Dietrich et al., 1992; (12) Research Genetics, Huntsville, AL.

¹ Mouse genomic DNA.
² Human genomic DNA.
³ Human cDNA.
⁴ Mouse cDNA.
⁵ Requires digestion with HhaI to observe the APV.

RESULTS

Amplification product variants (APVs) or restriction fragment length variants (RFLVs) between M. musculus strains and M. spretus were identified for all loci, and X-linkage confirmed using their F1 progeny (see Table 1; data not shown). Prior to the high-resolution mapping described below, loci were localized to the proximal region of the mouse X chromosome using a panel of backcross animals with single recombination events that divided the X chromosome into 10 intervals (Laval and Boyd, 1993b). In this way, we were able to position Xk, Clcn5, and Syp proximal to Pfc (data not shown). These three loci, and the others described in Table 1, were mapped against two M. musculus x M. spretus backcross panels (see Materials and Methods).

When 225 animals from the first interspecific backcross were typed for DXWas70 and Pfc, 18 single recombination events were identified in this interval, and thus the estimated genetic distance between these two loci is 8.0 ± 1.8 cM. These 18 recombination events were typed for all of the loci given in Table 1, including Xk, Clcn5, and Syp (Figs. 1 and 2). Pedigree analysis established the order of loci as (DXWas70, DXHxf34h)–(2.2 ± 0.9)–(Clcn5, Syp, DXMit26, DXMit55)–(0.5 ± 0.5)–(Gata1, Tfe3, Xk)–(0.5 ± 0.5)–Cybb–(2.6 ± 1.1)–DXMit54–(2.2 ± 0.9)–Pfc (Fig. 3a). Cosegregating loci are shown in brackets, and the figures represent the calculated genetic distance (in centi- morgans) between adjacent loci. These results demonstrate that the conserved segment known previously to contain Gata1 and Tfe3 can be extended to contain Clcn5 and Syp. In addition, as Xk was found to lie proximal to Cybb and not close to Dmd in the central region of the mouse X chromosome, the conserved segment previously defined by Cybb–Pfc can be redefined as the region delineated by Xk and Pfc.

As we could not determine the order of several loci in the first backcross, we identified a further set of recombination events in this region from the high-resolution European Collaborative Interspecific Backcross (Breen et al., 1994). We had previously scored this backcross for DXWas70, and, as the results described above indicated that the DXWas70–DXMit54 interval would be relevant to our studies, we scored the entire backcross for DXMit54 to identify all of the single recombination events between these two markers. Of 723 backcross animals scored for both loci, 38 were found to contain recombination events in this interval, and the genetic distance between the two loci was calculated to be 5.3 ± 0.8 cM, which was not significantly different from that measured in the first backcross (5.7 ± 1.5 cM; 13 recombination events were detected in
225 backcross progeny scored for both \( DXWas70 \) and \( DXMit54 \). Variants at the loci described in Table 1 were scored in 29 backcross animals that carried recombination events between \( DXWas70 \) and \( DXMit54 \). Pedigree analysis enabled us to define the order of loci and estimate the genetic distances between loci to be \( \text{Clen5} \sim (0.7 \pm 0.4) \sim \text{Syp} \sim (1.0 \pm 0.5) \sim \text{DXMit55} \sim (0.5 \pm 0.3) \sim \text{Tfe3} \sim (0.2 \pm 0.2) \sim \text{Gata1} \sim (0.7 \pm 0.4) \sim \text{Xk} \sim (2.2 \pm 0.6) \sim \text{DXMit54} \) (Fig. 3b). As not all of the recombinants in any defined interval could be scored for variants at all loci, genetic distances were estimated by calculating the expected number of total recombinants from the proportion actually scored (values are in centimorgans). The results obtained from the EUCIB backcross established an order for the conserved loci in the region as \( DXHXF34 \sim \text{Clen5} \sim \text{Syp} \sim \text{Tfe3} \sim \text{Gata1} \sim \text{Xk} \sim \text{Cybb} \). Furthermore, \( \text{Xk} \) has been placed distal to \( \text{Gata1} \), and there appears to be no hidden rearrangements in the boundary between these blocks.

**DISCUSSION**

Previous mapping studies of \( M. \text{musculus} \times M. \text{spretus} \) interspecific backcrosses established in our laboratory had established that the order of loci and evolutionary breakpoints (EBs) in the most proximal region of the mouse X chromosome was centromere–(\( DXWas70, DXHXF34 \))–EB–(\( DXMit26, \text{Gata1}, \text{Tfe3} \))–EB–Cybb–telomere (Laval and Boyd, 1993b; Blair and Boyd, 1994a,b; see Fig. 1). In this paper we have positioned several additional markers into this interval and produced a high-resolution genetic map of the region based on 948 meioses with an order of loci and evolutionary breakpoints as centromere–(\( DXWas70, DXHXF34 \))–EB–\( \text{Clen5} \)–(Syp, \( DXMit55, DXMit26 \))–Tfe3–Gata1–EB–Xk–Cybb–telomere (Fig. 4). This genetic map of ordered loci will provide an invaluable starting point for the construction of a YAC contig across the region and the subsequent production of a physical map. However, even at this resolution, we have been unable to separate the three loci Syp, \( DXMit55 \), and \( DXMit26 \) by recombination, and therefore we suggest that they must lie within a few hundred kilobases of each other. We have also been unable to detect any recombination between \( DXWas70 \) and \( DXHXF34 \) in 225 mice in backcross A or in any of the 29 recombination events scored for these two loci in the \( DXWas70 \) to \( DXMit54 \) interval from backcross B. These observations indicate that the RFLVs detected at \( DXWas70 \) and \( DXHXF34 \) must lie extremely close on the mouse X chromosome and/or that it is possible that \( DXHXF34 \) is the more proximal of the two loci.

The data reported here provide a greatly enhanced definition of the conserved segment previously represented by Gata1 and Tfe3, which lie approximately 150 kb apart on the human X chromosome (Derry et al., 1994). This segment now extends from \( \text{Clen5} \) to \( \text{Gata1} \).
that the relationship between the genetic and the physical distances is constant for the whole region. It is of interest to note that the evolutionary breakpoints that define this conserved segment on the human X chromosome lie in the vicinity of a cluster of repeated sequences associated with pseudogenes of the ornithine aminotransferase locus. The proximal boundary lies between ClCN5 and DXS1272E in the region of OATL2, and the distal boundary lies above GATA1 close to OATL1 (Willard et al., 1994). There is as yet no evidence that any OAT pseudogenes or related sequences lie in this region of the mouse X chromosome, and it remains to be determined whether these sequences have played a role in the subchromosomal rearrangements that have led to the current order of loci on the human X chromosome.

Mapping of human disease loci in the mouse indicates the position of loci potentially associated with homologous mutant phenotypes. Thus, any existing mouse mutants that are homologues for McLeod syndrome or Dent's disease should map to the proximal region of the mouse X chromosome. Loci responsible for two murine mutations (tattered, Td, and scurfy, sf) have been positioned in the proximal region of the mouse X chromosome (Lyon and Searle, 1989; Lyon et al., 1990). Both loci have been shown to cosegregate in separate backcrosses of ~200–300 progeny with Gata1, Tfe3, and DXMit26 (Blair et al., 1994b; Merrell et al., 1995). However, neither of the phenotypes associated with these mutations shows any obvious similarities with the features of McLeod syndrome or Dent's disease. Scurfy, which is characterized by a tight, scaly skin and hematological abnormalities, has been proposed as a murine homologue for the human immunodeficiency disease, Wiskott–Aldrich syndrome (WAS) (Lyon et al., 1990). Its cosegregation with Gata1 and Tfe3 supports this hypothesis, as the WAS gene has been recently cloned and shown to lie between GATA1 and TFE3 (Derry et al., 1994). Tattered, which is characterized by patches or streaks of scarred skin first visible at 5–6 days, has been proposed to be homologous to incontinentia pigmenti type 1 (IP1), a skin disorder associated with a series of scattered X-autosome translocation breakpoints lying between DXS255 and the centromere (Gorski et al., 1991; Hatchwell, 1994).

The mapping data of Merrell et al. (1995) place Td between DXWas70 and Cybb on the mouse X chromosome, which does not exclude it as a candidate for the murine homologue of IP1. The data presented here should enable the position of Td to be determined with respect to the evolutionary breakpoints in the region and thereby provide evidence for or against the proposed homology.

In conclusion, we have constructed an integrated comparative and high-resolution genetic map of the DXHXF34–Cybb region of the mouse X chromosome, which spans approximately 3 cM and contains 10 loci, including those that encode the human disease genes XK and ClCN5. This detailed map demonstrates the
power of high-resolution genetic mapping in the mouse as a means of determining locus order in a small chromosomal region and of providing an accurate framework for the construction of physical maps.

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REFERENCES


