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Characterization of a panel of somatic cell hybrids for regional mapping of the mouse X chromosome

(X-chromosome–autosome translocations/X-chromosome breakpoints/X-chromosome deletions/subchromosomal localization)

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ABSTRACT **A panel of five hybrid cell lines containing mouse X chromosomes with various deletions has been obtained by fusing splenocytes from male mice carrying one of a series of reciprocal X–autosome translocations with the azaguanine-resistant Chinese hamster cell line CH3g. These hybrids have been extensively characterized by using the allozymes hypoxanthine/guanine phosphoribosyltransferase (encoded by the *Hprt* locus) and α-galactosidase (*Ags*) and a series of 11 X-chromosome-specific DNA probes whose localization had been previously established by linkage studies. Such studies have established the genetic breakpoints of the T(X;12)13Rl and T(X;2)14Rl X–autosome translocations on the X chromosome and provided additional information as to the X-chromosome genetic breakpoints of the T(X;16)16H, T(X;4)7Rl, and T(X;7)6Rl translocations. The data establish clearly that both the T(X;4)7Rl and T(X;12)13Rl X-chromosome breakpoints are proximal to *Hprt*, the breakpoint of the former being more centromeric, lying as it does in the 9-centimorgan interval between the ornithine transcarbamoylase (*Otc*) and *DXPas7* (M2C) loci. Similarly, it is now clear that the T(X;16)16H X–autosome translocation breakpoint lies distal to the *DXPas8* (St14-1) locus, narrowing the X-chromosome breakpoint down to a region flanked proximally by this marker and representing, as expected from previous data, the distal quarter of the *Hprt–Ta* subchromosomal span. These five hybrid cell lines provide, with the previously characterized EBS4 hybrid cell line, a nested series of seven mapping intervals distributed along the length of the mouse X chromosome. Their characterization not only allows further correlation of the genetic and cytological X-chromosome maps but also should permit the rapid identification of DNA probes specific for particular regions of the mouse X chromosome.**

While studies on the human genome have progressed rapidly due to our ability both to clone human DNA fragments and to assign such fragments to specific chromosomal regions, analogous studies on the mouse have until recently made less headway.

Isolation of chromosome-specific DNA fragments has been hampered by the problems encountered in constructing somatic cell hybrids containing single mouse chromosomes and the difficulty in obtaining high enrichment for specific mouse chromosomes by flow sorting (1). The problems associated with isolating chromosome-specific probes have been compounded by the limited opportunities for obtaining either chromosome or subchromosomal localization. This is due to the small number of hybrid panels available (2), the almost complete absence of murine cell lines containing duplications or deletions for particular chromosomal regions, and, last but not least, the limited amounts of allelic variation found between inbred mouse strains due to the small number of progenitor mice having contributed to their establishment (3).

Recent studies have shown that cloned mouse DNA fragments specific for a given chromosome can be generated relatively easily either by using chromosome flow sorting in conjunction with cell lines carrying Robertsonian translocations (4, 5) or by microcloning (6, 7). Moreover, the use of interspecific crosses between *Mus musculus domesticus* and *Mus spretus* mice has been shown to be a potent method for obtaining both chromosomal assignments for particular cloned genes or DNA fragments (8, 9) and high-resolution recombinational localization of genes or DNA fragments on particular chromosomes (5, 9, 10, 11).

To complement the recombinational mapping approach to the mouse X chromosome, we have isolated a panel of five somatic cell hybrids, each containing a different portion of the mouse X chromosome, by fusing splenocytes of male mice carrying reciprocal X–autosome translocations having different breakpoints with a Chinese hamster cell line.

Here we describe the isolation of this hybrid-cell panel and the use of a large array of X-chromosome-specific probes both to verify the panel and to allow the more precise localization of the X-chromosome breakpoints involved in the original translocations.

The use of this somatic cell hybrid panel has allowed confirmation of gene attributions to regions of the mouse X chromosome, such as the telomere, where possible high recombination frequencies might have been expected to complicate recombinational analysis. It is also proving useful for the rapid identification of probes specific for particular regions of the X chromosome.

MATERIAL AND METHODS

X–Autosome Translocation-Bearing Mice. The T(X;7)6Rl, T(X;4)7Rl, T(X;12)13Rl, and T(X;2)14Rl stocks (12, 13) are maintained by one of us (L.B.R.) at Oak Ridge National Laboratory. Mice carrying Searle's T(X;16)16H translocation were raised in the Institut Pasteur. Male translocation carriers were provisionally identified by coat-color markers and their genotype was confirmed by both histological examination and weighing of the testes, which establishes the absence of spermatogenesis. In the mouse nomenclature system for translocations, T, standing for translocation, is followed by a bracketed reference to the chromosomes involved—e.g., T(X;2). This is followed by a number and letter(s) that identify the particular translocation. The letter(s) identify the person or laboratory having isolated the

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Abbreviations: HGPRT, hypoxanthine/guanine phosphoribosyltransferase; cM, centimorgan.

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translocation—e.g., T(X;2)14Rl. For convenience the translocations are sometimes referred to in the text without the bracketed information—e.g., T14Rl.

Hybrid Cell Isolation. Mixtures, 1:1 and 3:1, of the azaguanine-resistant Chinese hamster fibroblast cell line CH3g and splenocytes from translocation-carrying mice were fused by using polyethylene glycol 4000 and standard procedures. The resulting suspension was plated on peritoneal exudate feeder cells in Dulbecco's modified Eagle's medium (DMEM) + 20% fetal calf serum. Twenty-four hours later the cultures were passed onto medium containing hypoxanthine, aminopterin, and thymidine (HAT). The primary hybrid colonies appearing were isolated, individually cloned, and recloned by limiting dilution.

Some subclones were back-selected in medium containing 6-thioguanine at 10 μg/ml.

Primary hybrids are denoted by a letter corresponding to the fusion in question and a clone number. Subclones have additional letters and numbers following the primary code.

Cytogenetic Analysis. Hybrid cells were analyzed as described by Seabright (14) and Schnedl (15). The trypsin Giemsa-banding method (16) was used for studies on the N15 line karyotypes.

Allozyme Analysis. *Hypoxanthine/guanine phosphoribosyltransferase (HGPRT).* HGPRT was tested by the technique described by Chasin and Urlaub (17) and Chapman *et al.* (18) with modification. The isoelectrofocusing gradient extended from pH 4.0 to 8.0. Gels were developed with either [^{3}H]hypoxanthine or [^{3}H]guanine.

α-Galactosidase. After successive washings with phosphate-buffered saline (composition, in g/liter; NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 1.15; KH_2PO_4, 0.2), cells were resuspended in an equal volume of cold water containing 10 mM phenylmethylsulfonyl fluoride, frozen at −20°C, and subjected to three successive cycles of freezing and thawing. Resulting lysates were centrifuged at 2000 rpm for 5 min in a Beckman TJ-6 centrifuge, then treated with neuraminidase (from *Clostridium perfringens*) as described by Lusis and Paigen (19). The treated cell lysates were electrophoresed on 250-μm-thick cellulose acetate sheets (Cellogel; Chemetron, Milan, Italy) and stained as described by Fox *et al.* (20).

DNA Preparation from Cell Culture, Digestion with Restriction Enzymes, Gel Electrophoresis, and DNA Transfer. These procedures were as described in refs. 5 and 21.

Probe Preparation and Blot Hybridization. Conditions for the use of probes 66, 87, 100, 52, and 45 are described in ref. 5; for probe P-23 (*Plp*) in ref. 11; for p19 (A-raf) in ref. 22; for pHPT5 (*Hprt*), G28B (GdX-*G6pd*), and St14-1 in ref. 21; and for 80Y/B in ref. 23. Probes M2C (24) and pMN 152 (*Otc*) were nick-translated as in ref. 5, hybridized in 40% (vol/vol) formamide at 42°C, and washed, respectively, with 300 mM NaCl/30 mM sodium citrate or 60 mM NaCl/6 mM sodium citrate at 65°C.

RESULTS

To obtain a panel of somatic cell hybrids carrying partially deleted murine X chromosomes, splenocytes of mice carrying X–autosome translocations were fused with the azaguanine-resistant Chinese hamster cell line CH3g as described for the T(X;16)16H translocation in Fig. 1. Selection on HAT medium for a functional HGPRT gene (*Hprt*) led to hybrid cells retaining the part of the mouse X chromosome that includes the *Hprt* gene. The reciprocal translocation chromosome may or may not also be retained. Successive subcloning steps proved sufficient to ensure the loss, in the majority of cases, of the unselected part of the X chromosome.

Thioguanine selection of early-passage clones that still contain both the reciprocal X–autosome and autosome–X chromosome fragments could lead to the loss of the X-

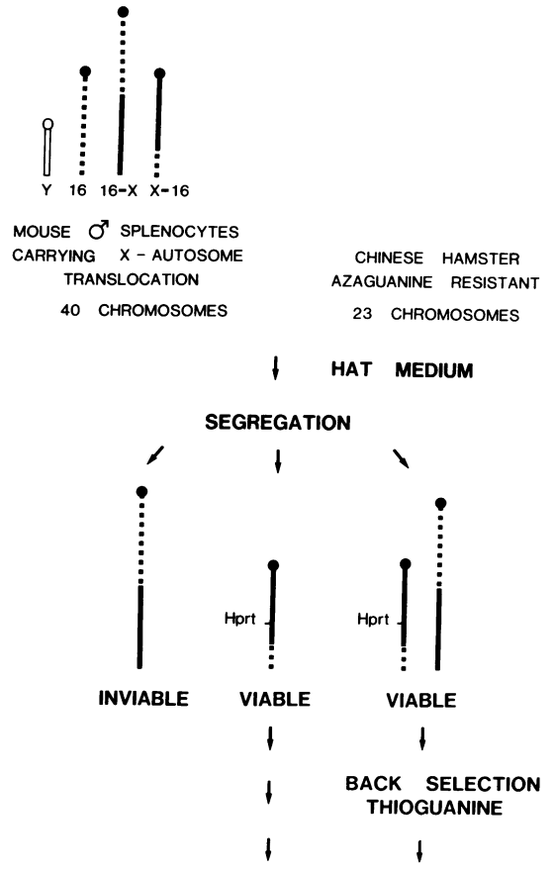

FIG. 1. Fusion and selection procedures used to obtain hybrid cells containing deleted mouse X chromosomes from male translocation carrier mice.

chromosome fragments linked to the *Hprt* gene with occasional fortuitous retention of the unselected part of the X chromosome (see Fig. 1). In practice, few of the thioguanine-resistant clones analyzed had kept the unselected portion of the X chromosome, and all the results reported here are based on clones in which the X-chromosome fragment is retained in the hybrid cell population by HAT selective pressure.

The hybrids constructed here have used the T(X;7)6Rl, T(X;2)14Rl, T(X;16)16H, T(X;4)7Rl, and T(X;12)13Rl X–autosome translocations. Genetic and cytological data show the first two of these X–autosome translocations to have distal X-chromosome breakpoints, the latter two to have proximal breakpoints, and the T(X;16)16H breakpoint to be centrally located (Table 1). The code of the fusion series corresponding to each translocation is indicated in the table.

Screening of the hybrid clones was carried out by using cDNA probe for *Otc*, which marks the proximal part of the X chromosome, the probe G28B, which marks the central part of the X chromosome, and, as distal marker, probe 45, which localizes cytologically to distal band XF3 or XF4 (M. G. Mattei and P.A., unpublished data) (see also Fig. 2). Hybrids such as those derived from the T(X;4)7Rl and T(X;12)13Rl that carried X chromosomes with deletions would be expected to give profiles of the type Otc^-, $G28B^+$, 45^+ for these DNA markers. Hybrids derived from T(X;2)14Rl, T(X;13)16H, and T(X;7)6Rl should, on the other hand, have a profile of the type Otc^+, $G28B^+$, 45^-.

The X-chromosome constitution of the hybrids identified on this basis was further characterized by using the allozymes HGPRT and α-galactosidase (encoded by the *Ags* locus) and an extended series of cloned X-chromosome-specific DNA fragments. Fig. 2 shows the position of the relevant loci,

Table 1. Characteristics of X–autosome translocations

Translocation name	Cytological breakpoints		Recombination breakpoints*		Code of derived hybrid cell lines
	Chromosome X	Autosome	Chromosome X	Autosome	
T(X;7)6Rl	XF1	7B3	*Ta*–R6 = 7	R6–*p* = 2	N
T(X;4)7Rl	XA2	4D1	R7–*Ta* = 4–23	*b*–R7 = 19	G
T(X;12)13Rl	XA3	12A	—	—	I
T(X;2)14Rl	XF	2C	—	—	E
T(X;16)16H	XD–distal	16B5	*Ta*–T16 = 4	*md*–T16 = 40	B

Data are from refs. 12 and 13.
*Numbers are recombination distance in centimorgans (cM).

including those defined by the DNA probes, as previously established by using an interspecific mouse cross (5, 11, 21–23).

When HGPRT activity of the hybrid cell lines, including at least one from each fusion series, was compared to that of the Chinese hamster parent, all the lines were found to contain HGPRT, although this activity appears to vary in extent from one hybrid cell line to another (data not shown). Unlike previous authors (27), we were unable to separate the Chinese hamster and mouse forms of HGPRT. Controls run on the frequency of reversion of the CH3g fusion parent make it, however, unlikely that the hybrids are revertants, a conclusion subsequently confirmed by the extended probe analysis.

α-Galactosidase testing showed that hybrid clones such as B20c12 derived from the T(X;16)16H translocation and E11 derived from T(X;2)14Rl were both α-galactosidase negative, suggesting that the breakpoints of both T16H and T14Rl are proximal to this locus (Fig. 3). The N15 clone was α-galactosidase positive, indicating on the other hand that the T6Rl breakpoint is distal to *Ags*. α-Galactosidase analysis of the other hybrids whose breakpoint is proximal to the *Hprt* locus (series I and G) are not informative. Examples of probe analysis are shown in Fig. 4 for the P-23 (*Plp*) probe, which localizes distally to XF1 (11), and the 80 Y/B probe, which detects at least three blocks of sequences, one locus [named (X-Y)A or 80c] being centromerically located, the other two [(X-Y)B and (X-Y)C] localizing to the region mapping between probes 52 and 45 (23). Hybridization with P-23 showed that hybrid E11 lacks the mouse-specific *Plp* band which is, however, present in hybrid N15 (Fig. 4*B*). Similarly, the I series hybrids I5d2 and I5d3 clearly lack the centromerically located (X-Y)A locus, which is present in the G series hybrids G13n23 and G13n28 (Fig. 4*A*). The results for all the hybrid clones and markers examined are summarized in Fig. 5, and the genetic breakpoints on the X chromosome of the five X–autosome translocations adduced from these data are summarized on the left of Fig. 2.

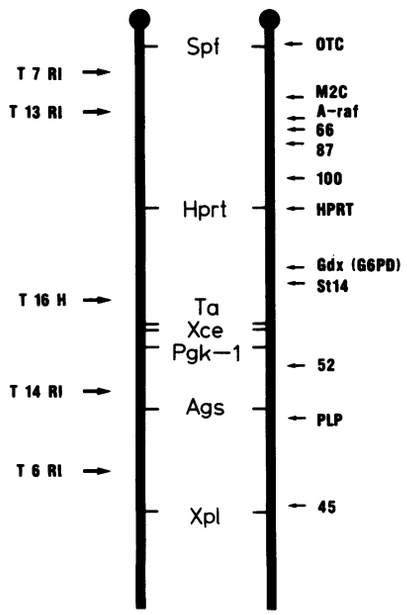

FIG. 2. Map positions of the X-chromosome sequences identified by the various DNA probes used in this study (right). Other key X-chromosome-specific loci are shown in the center, and the positions of the X–autosome translocation breakpoints on the X chromosome resulting from our analysis are shown on the left.

DISCUSSION

We now have available to us three interlocking characterized panels for examining mouse X-chromosome structure. The first consists of over 250 backcross progeny from an interspecific mouse cross (*Mus spretus* and *Mus musculus domesticus*); the second, a group of hybrid cell lines containing various partially deleted mouse X chromosomes; and the third, a panel of specific probes mapping over the entire length of the mouse X chromosome.

Since the original interspecific backcross segregated for the X-chromosome loci tabby (*Ta*), jimpy (*jp*), and *Hprt*, we have been able to position the panel of cloned DNA fragments relative to these known markers (5, 10, 11, 21–23, 26), thus identifying a series of loci that have, in their turn, become important tools in, for example, characterizing hybrid cell panels such as that described here having various partially deleted X chromosomes (Fig. 5).

This extensive characterization of the hybrid panel has allowed us to divide the mouse X chromosome into six nested sections: that extending from the centromere to *DXPas7* (M2C), from *DXPas7* (probe M2C) to *A-Raf* (probe p19-1) or *DXPas3* (probe 66), from *A-Raf* (p19-1) or *DXPas3* (probe 66)

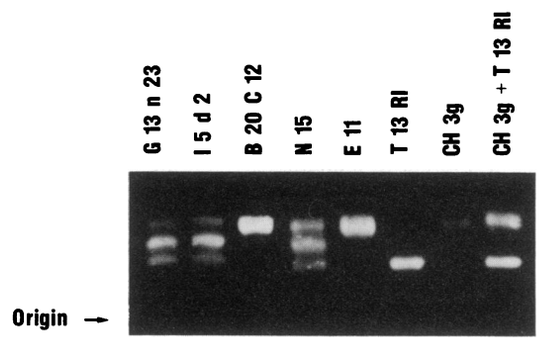

FIG. 3. α-Galactosidase activity of the various hybrid cell lines. Control lysates were the unfused Chinese hamster cell line (lane CH3g), as well as a mixture of this cell line with fibroblasts from a T13Rl-carrying mouse. The Chinese hamster enzyme migrates more rapidly than that of the mouse (lanes CH3g vs. lane Ch3g + T13Rl vs. T13Rl). In all hybrid cell lines containing both the mouse and hamster forms of the enzyme (lanes G13n23, I5d2, and N15) an additional heterodimeric band can be seen.

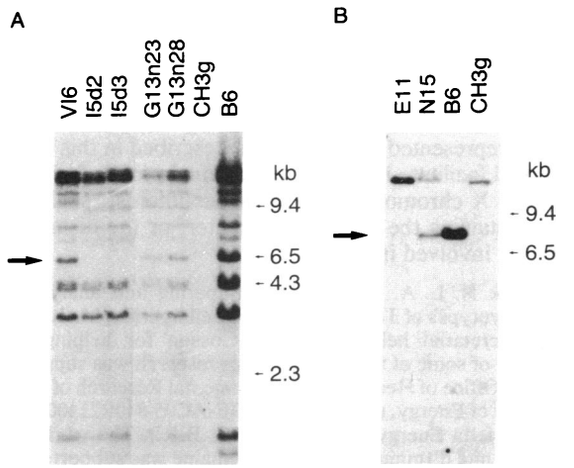

FIG. 4. Hybridization pattern of various hybrid cell lines. (*A*) Probe 80Y/B. The cell lines tested were as follows: VI-6, Chinese hamster–mouse hybrid cell line containing mouse chromosomes X and 16; I5d2 and I5d3, Chinese hamster–mouse hybrid cell lines derived from the T13RI translocation; and G13n23 and G13n28, Chinese hamster–mouse hybrid cell lines derived from the T7RI translocation. The control B6 lane contains female C57BL/6 DNA and the CH3g lane, Chinese hamster DNA. The arrowed band indicates the centromerically localized mouse X-chromosome-specific 80c band [also known as (X-Y)A] lost in the I series but retained in the G series hybrids. kb, Kilobases. (*B*) Probe P-23 (*Plp*). The cell lines tested were as follows: E11, Chinese hamster–mouse hybrid derived from translocation T14RI; and N15, Chinese hamster–mouse hybrid derived from translocation T6RI. Control lanes: B6 refers to female C57BL/6 DNA and CH3g, to Chinese hamster DNA. The arrow indicates the mouse-specific *Plp* band lost in hybrid E11 but retained in hybrid N15.

to *DXPas8* (St14), from *DXPas8* (St14) to *DXPas2* (probe 52), from *DXPas2* (probe 52) to *Plp* (P-23), and, last, from *Plp* (P-23) to the telomere and therefore including the X–Y pairing region. The largest of these regions is some 22 centimorgans (cM), the smallest, some 5–6 cM. To these six regions can be added that defined by the EBS4 somatic cell hybrid isolated by Peter Lalley (25), which we have recharacterized as being deleted proximally to the *Hprt* locus (5).

Whilst the clones varied markedly in the number of mouse chromosomes they contained, when detailed banding studies on the N15 and B48c hybrids were carried out, the expected translocation chromosome was in each case identified, rendering it unlikely that large-scale fragmentation or interstitial deletion of the translocated X chromosomes had occurred in the hybrid cell lines.

This conclusion is supported by the results obtained with the marker panel, since no inconsistencies in the marker profiles of the hybrid cells examined were found, except in one case. This was the M5 clone from the T6RI series (data not shown) which was $Hprt^+$ in the apparent absence of other mouse X-chromosome markers and therefore either is a Chinese hamster revertant or has acquired a small fragment of the mouse X chromosome including the *Hprt* locus.

The introduction of interspecific mouse crosses has radically changed the possibilities available for genetic localization of cloned DNA fragments by allowing increased restriction fragment length variation to be detected and the somewhat limited polymorphism among inbred mouse strains to be overcome (5, 8–11, 21–23). The classical genetic approaches to probe localization in the mouse thus opened up possess an intrinsically greater potential precision than that available through the use of somatic cell hybrids. The nonrandom distribution of X–autosome translocation breakpoints along the X chromosome in the mouse would in any case preclude high-resolution mapping of some chromosomal regions (13). For example, while several translocation breakpoints are known in the region between the centromere and *Hprt*, there are few, in addition to T14RI described here, currently thought to map to the region between *Ta* and *jp*, a distance of some 20 cM. Somatic cell hybrid panels nevertheless retain a central position in strategies involving both gross regional

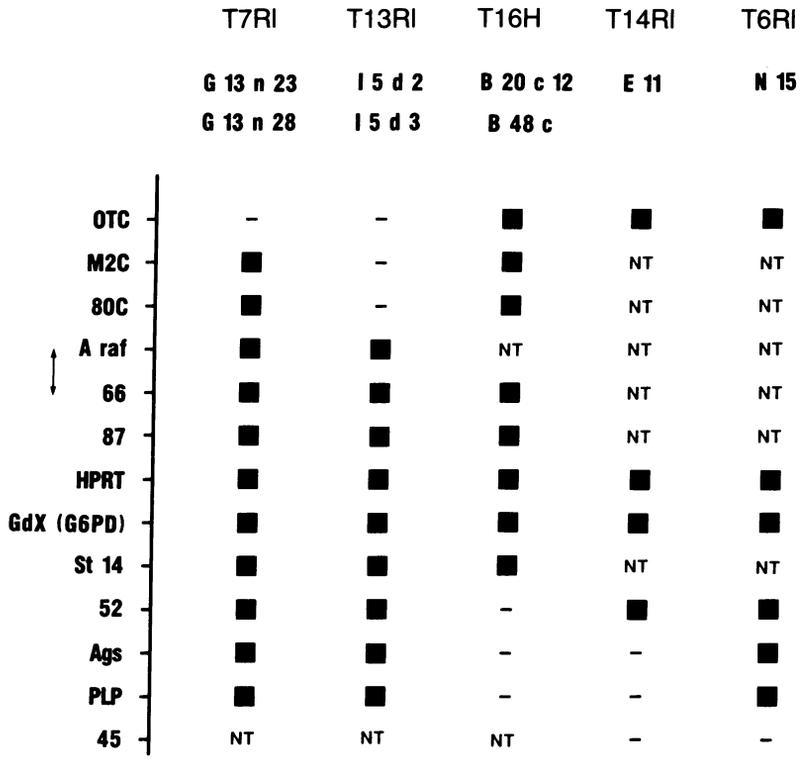

FIG. 5. Summary of the results obtained with the various probes on the panel of translocation-derived hybrid cell lines. ■, Hybridization; –, no hybridization; NT, not tested.

mapping and enrichment and identification of probes for particular subchromosomal regions.

The panel, moreover, allows verification of data obtained from genetic recombination studies, particularly for special chromosomal regions such as telomeres, for which it is desirable to have independent evidence. In this respect the N15 hybrid clone derived from the T6Rl translocation, the most distally broken of those we have analyzed, may prove useful in studies involving the X–Y pairing region.

Prior to this work, no genetic localization of the X-chromosome breakpoints in the T(X;12)13Rl or T(X;2)14Rl translocations had been reported (12, 13) to our knowledge, and the recombination distances for the T(X;4)7Rl translocation breakpoint had relatively wide confidence limits (12). The simultaneous availability of defined probes and the hybrid cell panel has enabled us to demonstrate that the T(X;12)13Rl translocation breakpoint must be proximal to *Hprt* in the region defined by the loci *DXPas3* (probe 66) and (X-Y)A or 80c (Fig. 2).

Marker-signal loss from the T(X;4)7Rl hybrid clones accompanied by marker retention in T(X;12)13Rl-derived hybrid clones moreover implies that the breakpoint in the former is more centromeric than that of the latter, lying as it does between *Otc* and *DXPas7* (M2C). The relative ordering of the T(X;4)7Rl and T(X;12)13Rl breakpoints is consistent with that observed cytologically for these two translocations, which have been attributed, respectively, to bands XA2 and XA3 (12).

The T(X;2)14Rl translocation, on the other hand, apparently lies between the *DXPas2* (probe 52) and the *Ags* loci, a region of some 19 cM. This localization can be compared with previous cytological observations, suggesting that T(X;2)14Rl is in XF and the recent *in situ* localization to XF1 of the *Plp* locus mapping some 2 cM distal to *Ags* (11).

Our results for the T(X;7)6Rl translocation suggest that the breakpoint lies in the 11-cM region defined by *Plp* and *DXPas1* (probe 45) loci (11). This is slightly more distal than the genetic localization observed by Russell (12) but is probably consistent with the cytologically observed breakpoint in XF1 (12), *Plp*, as already mentioned, lying in XF1. It should be noted that the genetic distance of 7 cM observed by Russell for *Ta*–T6Rl would place the breakpoint very close to locus *DXPas2* (probe 52), which cytologically appears to map to XD (M. G. Mattei and P.A., unpublished data). A possible explanation for the discrepancy between Russell's original genetic data and that reported here could be recombination suppression by T6Rl, though the combined evidence from crosses involving several markers gave no indications of this (12). *In situ* hybridization analysis of T(X;7)6Rl metaphases with probes 52, 45, and P-23 (*Plp*) should provide independent evidence for this breakpoint location.

Somatic cell hybrids carrying deleted X chromosomes derived from the T(X;16)16H translocation have previously been isolated by Francke and Taggart (27) but have not as yet apparently been extensively typed for loci defined by molecular probes. The data we have obtained from our T(X;16)16H-derived hybrids B48c and B20c12 suggest that the X-chromosome breakpoint in this translocation must be distal to GdX-(*G6pd*) and *DXPas8* (St14). Published genetic data indicate a breakpoint three-fourths of the map distance from *Bn* to *Ta*, and the *Ta*–T16 recombination distance has been given as 4 cM (13). Our data, which are free from the complication of the marked cross-over suppression found with this translocation (13), agree with these findings and show that the breakpoint must lie in the 8-cM interval defined by the *DXPas8* (St14-1) and *Ta* loci.

Since the X-chromosome breakpoints of all the translocations used here have been cytologically localized (12, 13), the improved genetic data concerning these breakpoints allow better correlation between the genetic and physical X chromosome maps (Fig. 2). This in turn has major implications for further molecular studies of particular chromosomal regions, where recourse is made to techniques such as microcloning (6, 7), and for the use of X–autosome translocations for probe ordering by *in situ* hybridization, when groups of clustered probes are being examined. Availability of the six nested sub-X-chromosomal segments represented by the hybrids described in this publication should facilitate isolation of additional probes for parts of the mouse X chromosome of particular interest, such as the region containing the inactivation center, or the distal region apparently involved in X–Y pairing.

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