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Localization of DNA Sequences in Region Xp21 of the Human X Chromosome: Search for Molecular Markers Close to the Duchenne Muscular Dystrophy Locus

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SUMMARY

Panels of somatic cell hybrid lines carrying various structural rearrangements of the human X chromosome short arm were analyzed with 21 X-chromosome-specific cloned DNA fragments. We mapped these molecular markers to five different regions of the short arm of the X chromosome. The results were confirmed by gene-dosage studies of human lymphoblasts with structurally abnormal X chromosomes. The ornithine transcarbamylase gene and four anonymous DNA sequences map within band Xp21, flanking the presumed locus for Duchenne muscular dystrophy.

INTRODUCTION

While more than 100 Mendelian traits are known to be X-linked, only about 20 of them have been assigned to a region of the X chromosome [1, 2]. For the

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majority of human X-linked traits, both regional localization and molecular nature are as yet unknown. With the development of methodologies to isolate chromosome-specific DNA sequences, an increasing number of X-chromosome-specific random DNA probes are becoming available [3–5]. It is important to know their precise chromosomal localizations for several reasons. First, this information will facilitate the establishment of linkage relationships of these new genetic markers with inherited diseases for which the molecular basis has not yet been elucidated. Closely linked markers can then be used for prenatal diagnosis and heterozygote detection in these disorders [6]. Second, it might help to identify new clusters of closely linked genes with precisely defined distances between them. So far, only two clusters have been established on the distal short arm and the distal long arm of the X chromosome [2]. A complete linkage map covering the entire human X chromosome will eventually result [7]. Third, sequences closely linked to any disease gene of unknown molecular nature can be used as tools for isolation of the gene itself.

Muscular dystrophy of Duchenne type (*DMD*) is an X-linked lethal recessive disorder of high incidence and high mutation rate whose primary biochemical defect is still unknown, reviewed in [8]. The localization of the *DMD* locus within band Xp21 is based on several sporadic cases of female patients with the features of the disease who also carried de novo balanced X-autosome translocations [9–15]. Although the rearrangements involved different autosomes, the breakpoints on the X chromosome were within band Xp21 in each case. Since the structurally normal X has been found to be inactive in the cells examined, it is assumed that small deletions and/or rearrangements of DNA sequences occurred within this region during the acquisition of the de novo translocations and were responsible for the DMD phenotype. Alternatively, a position effect could have inactivated the wild-type allele at the *DMD* locus. Two polymorphic DNA markers on Xp are flanking the *DMD* locus. Both *DXS7*, recognized by probe L1.28, and *DXS9*, recognized by probe RC8, are located outside of band Xp21 and are each about 17 cM away from the gene [16, 17]. Family linkage studies with these markers have provided independent evidence for the localization of *DMD* in the middle of Xp [17].

To find DNA sequences within band Xp21 and closer to the *DMD* locus, we produced somatic cell hybrids containing various rearrangements of the human X chromosome short arm. In these hybrid cell lines, different, well-characterized parts of the X chromosome have been retained on rodent backgrounds. These hybrids allow dissection of the short arm into five contiguous regions, two of them within band Xp21. We have screened the hybrid cell lines with 18 single-copy probes previously assigned to Xp. The results confirmed their X-chromosome location and assigned each of them to one of the five regions of the short arm.

MATERIALS AND METHODS

Somatic Cell Hybrid Lines

Eight types of somatic cell hybrids corresponding to 30 independent cell lines were analyzed. In all but two of them, the rodent background was hypoxanthine phos-

phoribosyltransferase (HPRT)-deficient Chinese hamster V79/380-6 lung fibroblasts. A mouse × human (IαA9 a gift from D. R. Cox) and a rat × human hybrid (XXII-18A) [18] contained structurally normal human X chromosomes and were used as controls.

Series XII and series XIII hybrids were derived from human donors with a balanced reciprocal translocation t(X;14)(p22.31;q21) transmitted from a mother (donor of fusion experiment XIII) to her son (donor of fusion experiment XII) [19]. Hybrid cell lines from fusion experiment XVIII contained an X chromosome with a small interstitial deletion del(X)(p21.1p21.3). This deletion was detected in a female patient (KC) with mental retardation but without the DMD phenotype and was associated with random inactivation of either the normal or the del(X) chromosome [20].

Series 26 hybrids were constructed by fusion of V79/380-6 Chinese hamster cells with the fibroblasts of a female with DMD and a balanced translocation t(X;11)(p21.1;q13.5) [11]. The fibroblast strain (GM 1695) was obtained from the Human Genetic Mutant Cell Repository at the Institute of Medical Research, Camden, N.J. The subband localization of the breakpoints was determined in our laboratory. Chinese hamster cells and human fibroblasts were mixed in a 1 : 12 ratio and were cocultivated for 14–40 hrs as monolayer. Cell fusion was mediated by treatment with 50% (w/v) polyethyleneglycol solutions (PEG 1000 or PEG 6000, Baker, Phillipsburg, N.J.) as described [21]. Twenty-four hrs after fusion, the cells were plated at low density in RPMI 1640 medium (Gibco, Grand Island, N.Y.) enriched with 20% fetal bovine serum (Hyclone, Logan, Utah), penicillin-streptomycin (100 μg/ml, Gibco), and glutamine. Two days later, the medium was supplemented with HAT (10^{-4} M hypoxanthine, 4×10^{-7} M amethopterin, 1.6×10^{-5} M thymidine, 3.5×10^{-6} M glycine) [22]; ouabain (2×10^{-7} M) was added later to inhibit fibroblast growth. Within 3–4 weeks after fusion, 10 independent colonies of hybrid cells were isolated from 10 different plates and were further expanded in culture under HAT selective pressure. Counterselection against the active X chromosome was done by adding 2×10^{-5} M 8-azaguanine to regular medium.

In fusion experiment 28, the human parental cells were lymphoblastoid line GM 6007, which had been established from a female with DMD and a balanced translocation t(X;9)(p21;p22) [14]. The karyotype of the lymphoblasts was confirmed in our laboratory. Chinese hamster cells V79/380-6 were trypsinized, and 5×10^6 cells were mixed in suspension with an equal number of human lymphoblasts. The mixed cell pellet was washed with serum-free medium and resuspended in 1 ml of 45% PEG 6000 in medium for 1 min. Over 3 min, the PEG solution was diluted 1 to 10 with medium. The cells were then centrifuged for 10 min and resuspended in F12 medium with 20% fetal bovine serum. Plating at low density, selection, and counterselection of hybrid clones were performed as described above. Twelve independent hybrid clones were obtained.

In hybrid series 29, the human parental cells were lymphoblasts from a male (BB) who had a smaller Xp21 deletion than female KC. This patient and the characterization of series 29 hybrids are described in the accompanying paper [23].

Isozyme Characterization and Chromosome Analysis

All hybrid cell lines were screened for the presence of human glucose-6-phosphate dehydrogenase (G6PD), localized in region Xq26→q28 [24]. G6PD served as a marker for the derivative X chromosome in series 26 and 28 hybrids and for the active X chromosome in series XVIII hybrids. Series 26 hybrids were also analyzed for human steroid sulfatase (STS), which maps in region Xpter→p22 [25], and lactate dehydrogenase A (LDHA) in band 11p12 [26]. Expression of these two enzyme markers would confirm the presence of the der(11) t(X;11)(p21.1;q13.5) chromosome in the absence of the normal 11 and the normal X. Isozyme analyses were carried out by electrophoresis on cellulose acetate gels (Kalex, Manhassat, N.Y.) as published [27].

Informative hybrid cell lines chosen for DNA analyses were further expanded in culture. At the same passage, cells were harvested for chromosome preparation and quantitative karyotype analysis by trypsin-Giemsa banding [28], for preparation of cellu-

lar lysates and enzyme marker typing [19], and for DNA extraction. The presence or absence of an active X chromosome was enzymatically reassayed by G6PD electrophoresis at that time.

X-Chromosome Specific Probes

Sixteen of the 21 X-specific DNA sequences were isolated from two different genomic libraries constructed from sorted human X chromosomes. Probes RC8, RD6, RJ8, and M2C were derived from the λgtWes library of Davies et al. [3]. Probes p18-55, p99-6, pD2, pB24, pL1, and p75-42 [29] were isolated from a different X-enriched clone bank [4]. Probe p71-7A is the X-specific genomic equivalent of probe cDNA-71 isolated from a HeLa cDNA library [30]. Probes λ58HAI, λ48HAI, λ16BA, λ33HA, and p3SA have human inserts derived from a partial *Eco*RI digest of a Chinese hamster/human hybrid, containing only human chromosomes X and 20, cloned into Charon 4A [5]. Probe p51, a gift of P. Szabo, was isolated from a different rodent/human hybrid library in Charon 28 (P. Szabo, unpublished data, 1984). Plasmid pDP31, also referred to as probe A in the literature [31], was a gift of D. Page. It was derived from the Maniatis library of random human genomic DNA. It recognizes sequences at the *DXYS1* locus and contains a low-copy number repeat sequence. Probe C7 was isolated from a different partial genomic library (J. L. Mandel et al., unpublished data, 1984). The ornithine transcarbamylase probes pHI and 7-31 were isolated from a cDNA library [32].

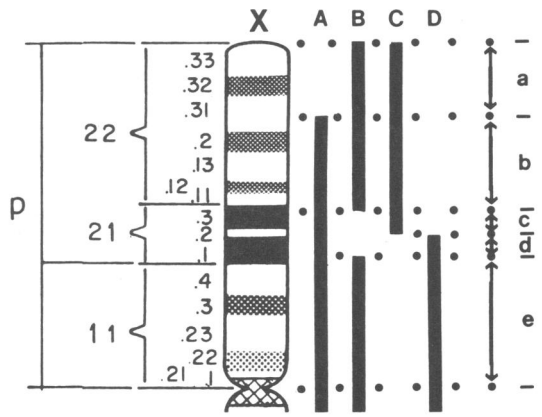
DNA Isolation, Digestion, Blotting, and Hybridization

Nuclear high molecular weight DNA was extracted from all hybrid and parental cells as described [33]. The DNA samples were cleaved with restriction enzymes *Eco*RI, *Hin*dIII, and *Taq*I, following the manufacturers' recommendations (Bethesda Research, Gaithersburg, Md.; New England Biolabs, Beverly, Mass.). Equal amounts of DNA digests as measured by the DAPI-fluorimetric assay [34] were electrophoresed and transferred to nitrocellulose filters (Schleicher & Schuell, Keene, N.H., BA 85). In most cases, the human inserts alone or the complete probes were ^{32}P-labeled by nick-translation [35] using *E. coli* polymerase I (New England Biolabs, Boehringer-Mannheim, Indianapolis, Ind.). In some instances, gel-purified inserts were labeled using T4 polymerase (Bethesda Research) according to the protocol of O'Farrell [36]. Hybridizations were carried out as reported [33] at 42°C or 68°C, depending on the presence or absence of 50% formamide. All filters were washed at high stringency with the final wash in 0.015 M sodium chloride, 0.0015 M trisodium citrate, 0.1% sodium dodecyl sulfate, at 68°C.

RESULTS

Construction of the Xp Mapping Panel

To search for single-copy DNA fragments localized to band Xp21, and to map regionally those which are outside of Xp21, we constructed a mapping panel that consists of two types of hybrids. First, to confirm that a DNA sequence or specific restriction fragment was indeed X-chromosomal, we used three completely independent hybrids with a structurally normal human X, but no intact autosomes, on a mouse, rat, or Chinese hamster background (hybrid cell lines IαA9, XXII-18A, and XIII-2A). Second, hybrids with four different parts of the X short arm allowed mapping of any DNA sequence to one of five regions of Xp (fig. 1). The rearranged X chromosomes were all present in the cells of the five human donors, one male and four females, used for the fusion experiments.



Names of Probes	No. of Probes	A	B	C	D	Xp Region
p18-55, p71-7A, λ3-SA, λRC8	4	-	+	+	-	a
λ33HA, pD2, p51 p99-6	4	+	+	+	-	b
pB24, L1, C7	3	+	-	+	-	c
OTC, M2C	2	+	-	-	+	d
p75-42, λ58HAI, λ48HAI λ16BA, λRD6, pL1.28	6	+	+	-	+	e
pDP31, λRJ8	2	+	+	-	+	Xq

FIG. 1.—Schematic representation of G-banding patterns on human X short arm based on band measurements of prometaphase chromosomes [39]. *Bars A, B, C,* and *D* indicate the Xp regions that are present in the different types of somatic cell hybrids; *a, b, c, d,* and *e* indicate the regions of Xp to which genes can be assigned using this somatic cell hybrid panel. The regions are expressed in ISCN 1981 nomenclature as follows: *a,* Xpter→p22.31; *b,* Xp22.31→p21.3; *c,* Xp21.3→p21.1 (DMD breakpoint); *d,* Xp21.1 (DMD breakpoint) → Xp21.1 (proximal deletion breakpoint that is very near Xp2100, i.e., the border between Xp11.4 and Xp21.1); *e,* Xp21.1→qter. For probes previously assigned to the X short arm, region *e* would end at the centromere. Results of the molecular hybridization with the different probes, and the regional localizations deduced, are summarized in *the lower part of the figure.*

Type A (experiments XII and XIII) represents the der(X),t(X;14)(p22.31; q21) chromosome that was selected for in six independently derived hybrid cell lines studied and had been selected against in four others [19].

The small interstitial deletion del(X)(p21.1p21.3) type B chromosome was present in the inactive state in three series XVIII hybrids in which the structurally normal genetically active X chromosome had been selected against in 8-azaguanine [20]. The del(X) chromosome was present in the active state in four other hybrids selected in HAT medium; in these cells, the normal inactive X was not present. One control hybrid contained both the normal and the deleted type B chromosome.

Type C is the der(11),t(X;11)(p21.1;q13.5) product of the de novo translocation in a female patient with DMD [11]. The chromosome was retained in one hybrid cell line from series 26 after counterselection. The control hybrid cell line in this series contained both reciprocal translocation products in addition to the structurally normal inactivated X.

Type D represents the der(X),t(X;9)(p21;p22) [14] product that was isolated in seven primary hybrid clones of series 28 by HAT selection. None of these hybrids had retained the inactive structurally normal X or the der(9) translocation product. A control hybrid line of this series carried the der(X) and the inactive normal X chromosome.

The presence of the different fragments of the X chromosome was determined by complete karyotyping of hybrid metaphase cells after trypsin-Giemsa banding and by isozyme characterizations. In addition, most cell lines contained different subsets of human autosomes. To confirm the presence of the specific X-chromosomal region and the absence of the other X chromosome, we searched for restriction fragment length polymorphisms (RFLPs) in the human female donors. DNA extracted from all human parental cell lines carrying abnormal X chromosomes was cleaved with restriction enzyme *Taq*I, blotted, and hybridized with three different probes. Probe pDP31 detects the allelic X- and Y-specific fragments of the *DXYS1* locus on the long arm of the X chromosome [31]; probes RC8 [16] and L1.28 [17] detect X-specific alleles at *DXS9* in region b and at *DXS7* in region e, respectively (fig. 1). The only RFLPs detected were with probes RC8 and pDP31 in the parental line of series XVIII hybrids (chromosome type B in fig. 1). Figure 2 illustrates the results obtained by Southern-blot analyses of the series XVIII hybrid cell lines and parental cell lines with pDP31. The del(X) chromosome carried an 11.8-kilobase pair (kbp)

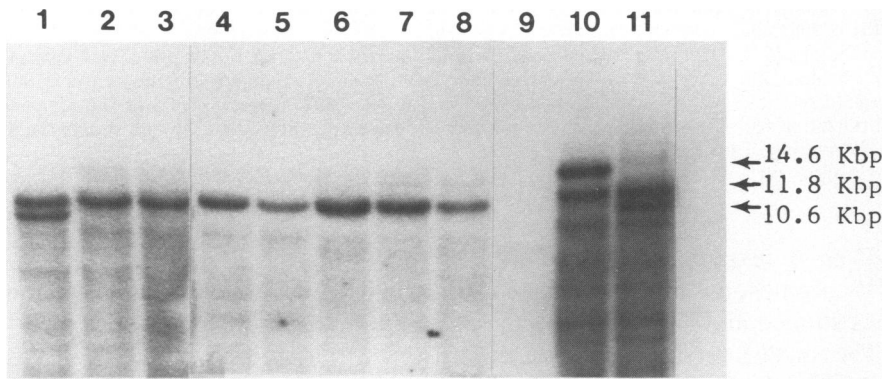


FIG. 2.—Hybridization of probe pDP31 to 5 μg each of *Taq*I-cleaved DNA of series XVIII hybrids (*lanes 1–8*) derived from human female donor KC with del(X)(p21.1p21.3) (*lane 11*) and from Chinese hamster 380-6 cells (*lane 9*). The hybrid in *lane 1* has the normal and the deleted X chromosome. The hybrids in *lanes 2–8* contain the deleted X chromosome in the active or the inactive state. The DNA in *lane 10* is from the human male BB with a smaller Xp21 deletion [23]. The 11.8-kbp and 10.6-kbp fragments are X-specific alleles, and the 14.6-kbp, Y-chromosome specific.

fragment (lanes 1–8), while the normal X chromosome carried a 10.6-kbp fragment (lane 1). The results were consistent with the chromosome and isozyme analyses [20].

Concomitant analysis of these hybrid cell lines allows dissection of the short arm of the X chromosome into regions a–e (fig. 1), with the *DMD* locus near the border between regions c and d. This "border" may represent a small region in itself, since type C and type D chromosomes are not the reciprocal products of a single translocation but are derived from two different breakage events, both of which resulted in inactivation of the wild-type allele at the *DMD* locus. Any DNA sequences that would map to both type C and type D chromosomes, if they are overlapping, or to neither, if there is a gap between them, could be postulated to derive from within or very near the *DMD* locus.

Screening of the Xp Mapping Panel with Single-Copy DNA Sequences

We analyzed 21 different X-specific DNA sequences that had been isolated in seven different laboratories. Table 1 summarizes the names, locus symbols, insert sizes, and published localizations of these probes, while figure 1 illustrates the regional assignments derived from our studies. The localization of each probe to one of the five regions a–e was based on the presence of homologous sequences in the rearranged chromosome types A–D.

TABLE 1

CLONED HUMAN X CHROMOSOME SEQUENCES STUDIED

Probe	Locus	Insert (kbp)	RFLP	Published localization	Reference	Our localization
p18-55 ...	DXS70	1.9	−	pter→p21	[29]	a (pter→p22.31)
p71-7A...	DXS69	3.5	−	pter→p21	[29]	a "
λ3-SA ...	DXS22	5.2	−	p22.3→p22	[2]	a "
λRC8	DXS9	6.2	+	p22.3→p21	[37]	a "
λ33HA...	DXS2	2.0	−	p22→p21	[2]	b (p22.31→p21.3)
p99-6	DXS41	1.8	+	pter→p21	[29]	b "
pD2	DXS43	0.4	+	pter→p21	[29]	b "
p51.....	NA	1.2		none		b "
pB24	DXS67	0.9	+	pter→p21	[29]	c (p21.3→p21.1#)
L1.....	DXS68	1.7	−	pter→p21	[2]	c "
C7.....	DXS28	2.7	+	p22→p11	[38]	c "
M2C.....	DXS32	4.2	−	p22→p11	[38]	d (p21.1#→p21.1*)
pHI	OTC	1.2	+	pter→qter	[32]	d "
p75-42 ...	NA	1.5	−	p21→cen	[29]	e (p21.1*→qter)
λ58HAI..	DXS14	3.5	+	p21→cen	[29]	e "
λ48HAI..	NA	2.4	−	pter→q1	[5]	e "
λ16BA...	DXS71	5.8	−	p21→cen	[2]	e "
λRD6....	DXS34	?	−	p11.3→p11	[37]	e "
pL1.28...	DXS7	1.25	+	p11.3→p11	[37]	e "
pDP31 ...	DXYS1	4.5	+	q13→q22	[2]	e "
λRJ8.....	DXS35	10	−	q24→q28	[37]	e "

NOTE: NA = none assigned. p99-6 has only been studied by dosage and has been excluded from Xp21.3→p21.1. p21.1# denotes breakpoint in t(X;11) and t(X;9) translocations. p21.1* denotes proximal breakpoint in KC's del(X) (p21.1p21.3) chromosome.

Preliminary localizations were obtained by hybridizing each probe against a screening panel of hybrids with the different types of X-chromosome fragments and of one hybrid carrying only a normal human X chromosome. For further confirmation, other pertinent independently derived hybrid cell lines were analyzed with a different restriction endonuclease. In addition, concordant results were obtained when filters were hybridized several times with different probes or hybridized simultaneously with probes localized to different regions of the X short arm.

Regional assignments are summarized in table 1. Four sequences were localized within region Xp22.31→pter (region a). One of them, probe 71-7A, initially isolated from a cDNA library [30], recognized also Y-specific sequences of weaker homology; this Y-specific signal (data not shown) was obtained in hybrids from series XII that retained a Y chromosome in the presence or absence of the translocated X chromosome. Four probes mapped to region Xp22.31→p21.3 (region b). For two of them (RD6 and 99-6), we have determined only that they are localized outside of the deletion in Xp21, while further regional mapping has been reported [2, 37]. Eight probes were mapped to Xp21.1→Xqter (region e), and two of those (pDP31 and RJ8) were known to be on the long arm of the X chromosome [2]. Since the other six had previously been reported to be on the short arm [2, 29, 37], we conclude that they must be in region Xp21→cen.

Assignments of X-specific Probes to Region Xp21

Four anonymous probes and cOTC were found within region Xp21.1→p21.3 defined by the deletion present in the type B chromosome, as illustrated in figure 3. This filter contained DNA samples from eight series XVIII hybrids carrying the chromosome type B. Probe D2 detected a 1.3-kbp *Hin*dIII restriction fragment in all hybrids regardless of the presence of the Xp21 deletion. In contrast, probe B24, when hybridized simultaneously to the same filter, detected a 1.8-kbp *Hin*dIII fragment only in the first lane that contained the control hybrid with the normal X chromosome in addition to the deleted type B chromosome. This indicated that the sequence homologous to probe B24 is localized within the Xp21 deletion while that recognized by probe D2 resides outside.

The autoradiogram in figure 4 has been obtained after cohybridization of probes L1.28 and M2C against DNA of series XVIII hybrids. It again demonstrates localization of M2C within the Xp21 deletion based on absence of a detectable signal in the hybrids carrying the type B chromosome (lanes 2–6). Similar results were obtained with the OTC probe.

Furthermore, localization of probes within the Xp21 deletion was confirmed by direct gene-dosage studies. In figure 5, the probes B24 and D2 were hybridized to DNA samples from human cells containing one, two, or four copies of the X chromosome. The signal produced by B24 was of the same intensity in DNA from the female heterozygous for the Xp21 deletion (46,X,del[X]) as in DNA from a normal male (46,XY), while with probe D2, the intensity was similar to 46,XX DNA and substantially greater than with male

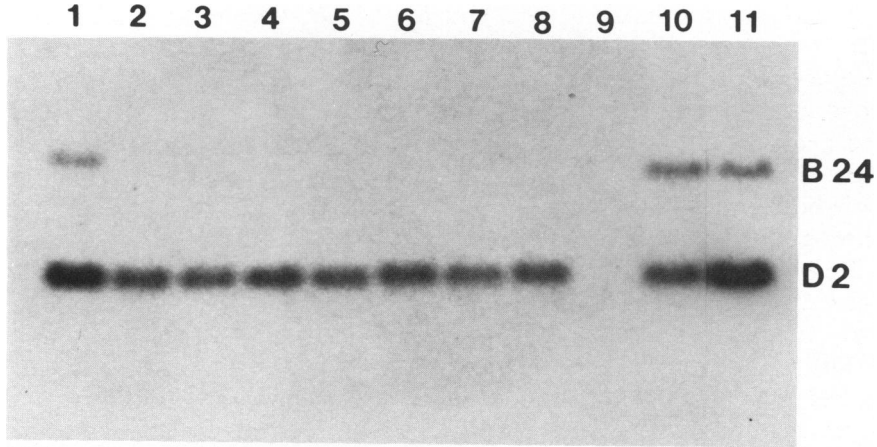


FIG. 3.—Cohybridization of probes D2 and B24 to 7.5 μg of *Hin*dIII-cleaved DNA of series XVIII hybrids and parental cell controls. The samples are the same as in figure 2. Note that the sequence homologous to probe B24 is deleted from the del(X) chromosome of female KC (hybrids in *lanes 2–8*) but is present in total DNA of male del(X) carrier BB [23] (*lane 10*).

DNA. Likewise, within each lane, the B24- and D2-derived bands were of similar intensity except for the sample in lane XX^d, which appeared to contain a single copy of B24 sequences and two copies hybridizing with D2. Dosage analysis with the OTC probe (fig. 6, lanes 1–7) revealed that the female cell line heterozygous for the Xp21 deletion (fig. 6, lane 4) produced a signal of the same intensity as the male lines (fig. 6, lanes 2 and 3).

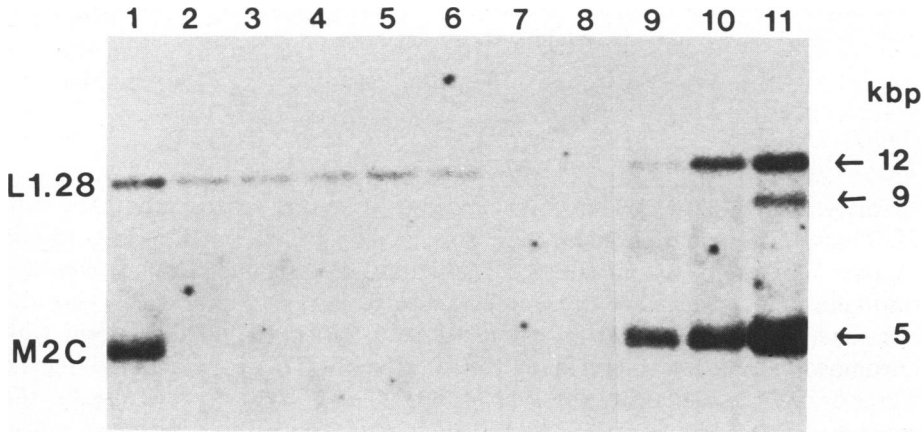


FIG. 4.—Cohybridization of probes M2C and L1.28 to 4–5 μg of *Taq*I-cleaved DNA of series XVIII hybrids and controls. The hybrid in *lane 1* has both the del(X) and the normal X, while the hybrids in *lanes 2–6* have only the del(X) in 80%–100% of cells. *Lane 7*, counterselected derivative of hybrid in *lane 6* that has a reduced del(X) frequency of 10%. *Lane 8*, Chinese hamster control. *Lane 9*, human male BB with del(Xp21) [23]. *Lane 10*, female KC with del(Xp21) [20], human parent of hybrids in *lanes 1–7*. *Lane 11*, lymphoblastoid cell line with 48,XXXX karyotype, heterozygous for the RFLP at the *DXS7* locus.

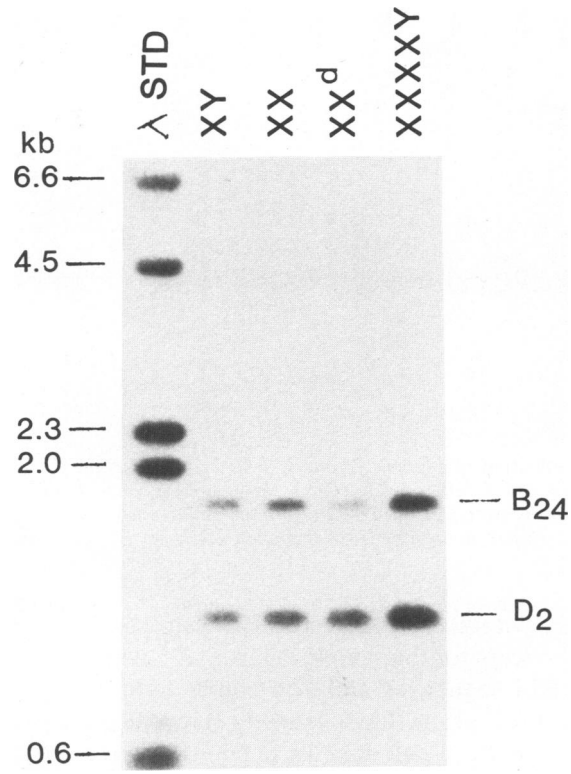


FIG. 5.—Cohybridization of probes pB24 and pD2 to various genomic DNA samples: *Hin*dIII-cleaved (1 μg) DNA of the following cell lines was loaded to a 0.8% agarose horizontal gel slab; 46,XY lymphoblast DNA; 46,XX lymphoblast DNA; 46,X del(X) (p21.1p21.3) lymphoblast DNA; 49,XXXXY lymphoblast DNA. The X-chromosome constitution is indicated *above each lane*. Radiolabeled *Hin*dIII λ phage DNA served as a molecular weight marker.

Analysis of Hybrid Cell Lines Constructed from Two Different Females with DMD

Analysis of hybrid cell lines constructed from two different females with DMD and X-autosome translocations assigned probes mapped to Xp21 to one of two subregions within the Xp21 deletion. For example, sequences hybridizing with OTC were present in figure 6, lane 11 (hybrid carrying the chromosome type D), and absent in figure 6, lanes 13 and 15 (hybrid with chromosome type C), thus refining the location of *OTC* within region d (fig. 1). Three anonymous sequences (B24, L1, and C7) mapped to region c distal to the presumed *DMD* locus, and one (M2C) mapped proximal to region d that also contains the *OTC* locus.

The hybrids in figure 6, lanes 9 and 10 (series 29 hybrids), were derived from a male lymphoblastoid cell line (BB) (figure 6, lane 3) that contains a very subtle interstitial deletion of Xp21 [23]. All three samples shown here hybridized normally with the OTC probe.

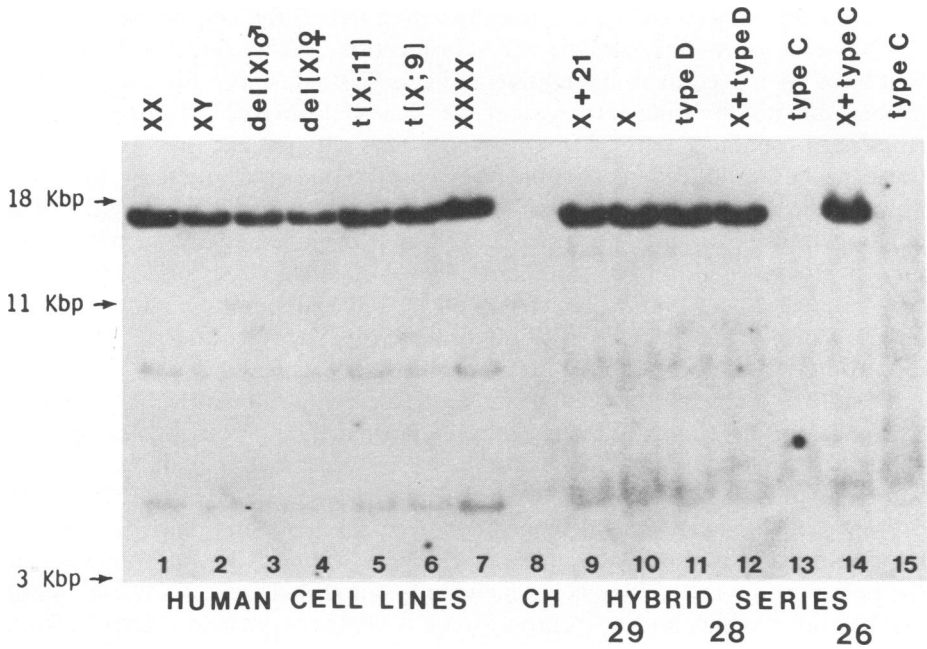


FIG. 6.—*Hin*dIII digests of human lymphoblast cell lines and Chinese hamster cells (*lane 8*) (5 μg each) and of series 26, 28, and 29 hybrids (10 μg each) hybridized with probe pHI (OTC). For each DNA sample, the content of X-chromosomal material is noted. The intensity of the major fragment in *lane 4*, female KC with del(Xp21), is similar to that in *lane 2* (46,XY male) and distinctly weaker than that in *lane 1* (46,XX female). *Lane 3* contains DNA from male patient BB with a smaller Xp21 deletion [23]. *Lanes 5* and *6*, fibroblasts and lymphoblasts, respectively, of female patients with DMD and X/autosome translocations. *Lane 7*, lymphoblastoid cell line GM 1415 with 48,XXXX karyotype. *Lanes 9* and *10*, Chinese hamster/human hybrids (series 29) with patient BB's del(Xp21) chromosome, alone (*lane 10*) or together with a chromosome 21 (*lane 9*). *Lanes 11* and *12*, series 28 hybrids. *Lanes 13–15*, series 26 hybrids. *Lane 15* contains 20 μg of the hybrid DNA in *lane 13;* cross-hybridizing Chinese hamster fragments are evident. This blot provides evidence that all three OTC-specific bands of hybridization are derived from the X chromosome and that all three of them map proximal to the DMD breakpoint (type D positive and type C negative). Furthermore, it provides dosage confirmation that female KC is hemizygous for the sequence, and, lastly, that the sequence is present and apparently unrearranged in the male del(Xp21) patient BB. The hybrid sample in *lane 10* shows that the sequence is present on his X chromosome and not translocated to an autosome.

DISCUSSION

Structurally abnormal X chromosomes with breaks in the short arm, that had been identified in human donors, were isolated away from normal X homologs and reciprocal translocation products in five independent series of hybrids. Hybrid cell DNA samples were screened, by Southern blot hybridization, with 21 single-copy X-specific probes, and the loci identified by these probes could be assigned to one of five distinct regions of the human X short arm. In line with our interest in developing DNA markers for X-linked muscular dystrophy, five probes (20%) mapped within the Xp21 interstitial deletion of patient KC. Three of them mapped distal and two proximal to the *DMD* locus as defined by the breakpoints in X/autosome translocation carriers with DMD.

As illustrated here and in the accompanying paper, the approaches of high-resolution cytogenetics, somatic cell hybridization, and nucleic acid hybridization have been used in an interactive and synergistic fashion. Five single-copy probes did not hybridize to patient KC's del(X)(p21.1p21.3) chromosome, isolated in somatic cell hybrids. On dosage blots with patient KC's total DNA, these probes produced restriction fragments of the same intensity as those obtained with male DNA. These studies have confirmed the presence of a deletion in this patient and have ruled out a balanced rearrangement. The presence of a deletion had also been suggested clinically, because patient KC was heterozygous for OTC deficiency and for chronic granulomatous disease (CGD) [20]. Furthermore, in in situ hybridization experiments, the OTC probe did not hybridize to her del(X) chromosome [40].

To utilize this patient's deletion for the screening of human DNA probes, it was necessary to eliminate the structurally normal X by producing interspecies somatic cell hybrids. Since her X chromosomes were randomly inactivated, we were able to isolate the del(X) in both the active and the inactive state [20]. The presence of the del(X) and absence of the normal X were initially determined by chromosome and isozyme analyses. Studies of the restriction fragments at the polymorphic *DXYS1* locus independently confirmed these results in all but one hybrid in which an Xp− chromosome was present that was derived from the normal X (data not shown). Since human chromosomes in rodent × human somatic cell hybrids often undergo spontaneous structural rearrangements, the results obtained with DNA probes that mapped on Xp, but outside the deletion (table 1), are relevant for the potential detection of such events. Thus, the molecular hybridization data served to validate the X-chromosome constitution in the somatic cell hybrids, in addition to providing localization of probes to one of five distinct regions. Given the relative size of the Xp21 deletion (approximately 10% of Xp at metaphase and even less at prophase [20]), it appears surprising that five of the 21 probes screened fell into the deleted segment. However, the probes were not truly random, since two of them (C7 and M2C) had previously been localized to the middle of the X short arm [38].

While large numbers of X-chromosome-specific sequences can be isolated from X-enriched libraries with relative ease, the search for polymorphisms that are useful in linkage studies is more laborious. Therefore, it may be desirable to know the precise intrachromosomal location of random probes before choosing candidates for RFLP searches. Our series XVIII hybrids serve to identify probes within Xp21 that are potentially clinically useful as markers for DMD. Sets of RFLPs flanking the disease locus are much more powerful in the detection of recombination than a single marker [17]. The closest available approximation to the *DMD* locus is represented by the breakpoints in the DMD females with de novo X/autosome translocations [9–15]. We have isolated the products of two such translocations in somatic cell hybrids, as have others [37, 41]. These hybrids allowed the mapping of probes distally or proximally to the presumed *DMD* locus. Of the five probes identified within band p21, three mapped distal and two proximal. At least one in each category detects an *Msp*I

polymorphism: OTC proximal [42] and B24 and C7 distal to the DMD breakpoint [29].

To further dissect this small region, cells from the male patient BB, described in the accompanying paper [23], will be useful. As demonstrated by molecular analyses, his deletion, which is much smaller, appears to fall entirely within the larger deletion present in the female patient KC. All five of the sequences that map within KC's deletion are present in BB. So far, only a single sequence has been mapped to BB's smaller deletion: probe 754, which detects a high frequency polymorphism and maps to the centromere side of *DMD* but distal to *OTC* [23]. In addition, there is clinical evidence that loci for chronic granulomatous disease and for a form of retinitis pigmentosa map within this small region [20, 23]. Linkage between *XLRP* and *DXS7,* detected by L1.28, has also been reported [43]. The precise linear order and linkage relationships of the genes in this region can be established utilizing RFLP markers that are developed with sequences mapped to one of the subregions of band Xp21. Furthermore, these probes will provide starting material for the isolation of the sequences at these loci and for the elucidation of the molecular defects.

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