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Fine Structure Mapping of the Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT) Gene Region of the Human X Chromosome (Xq26)

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Summary

The Xq26-q27 region of the X chromosome is interesting, as an unusually large number of genes and anonymous RFLP probes have been mapped in this area. A number of studies have used classical linkage analysis in families to map this region. Here, we use mutant human T-lymphocyte clones known to be deleted for all or part of the hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene, to order anonymous probes known to map to Xq26. Fifty-seven T-cell clones were studied, including 44 derived from in vivo mutation and 13 from in vitro irradiated T-lymphocyte cultures. Twenty anonymous probes (DXS10, DXS11, DXS19, DXS37, DXS42, DXS51, DXS53, DXS59, DXS79, DXS86, DXS92, DXS99, DXS100d, DXS102, DXS107, DXS144, DXS172, DXS174, DXS177, and DNF1) were tested for codeletion with the *hprt* gene by Southern blotting methods. Five of these probes (DXS10, DXS53, DXS79, DXS86 and DXS177) showed codeletion with *hprt* in some mutants. The mutants established the following unambiguous ordering of the probes relative to the *hprt* gene: DXS53–DXS79–$^{5'}$*hprt*$^{3'}$–DXS86–DXS10–DXS177. The centromere appears to map proximal to DXS53. These mappings order several closely linked but previously unordered probes. In addition, these studies indicate that rather large deletions of the functionally haploid X chromosome can occur while still retaining T-cell viability.

Introduction

The q26-27 region of the human X chromosome contains a number of genes, including the hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene (Pai et al. 1980), X-linked lymphoproliferative syndrome (LYP) (Skare et al. 1989*a*, 1989*b*; Sylla et al. 1989), Lowe syndrome (OCRL) (Silver et al. 1987; Wadelius et al. 1989), X-linked idiopathic hypoparathyroidism (Thakker et al. 1989), Borjeson-Forssman-Lehmann syndrome (Turner et al. 1989), albinism-deafness syndrome (Shiloh et al. 1990), the coagulation factor IX (F9) (Camerino et al. 1984) gene, and the fragile X syndrome (FRAXA) (Giraud et al. 1976; Harvey et al. 1977), as well as a large number of anonymous RFLP probes (Mandel et al. 1989). A number of investigators have studied this region by using classical recombination studies of families with LYP, OCRL, F9 deficiency or FRAXA (Drayna and White 1985; Mandel et al. 1986; Murphy et al. 1987; Oberle et al. 1987; Arveiler et al. 1988; Brown et al. 1988; Mulley et al. 1988; Skare et al. 1989*a*, 1989*b*; Sylla et al. 1989; Wadelius et al. 1989; Reilly et al. 1990). The distance from LYP (Xq25-26) to F9 (q26.3-q27.1) appears to be of the order of 1.5–2.0 cM, with FRAXA (Xq27.3) about 2 cM telomeric to F9 (Davies et al. 1987). A number of anonymous probes have been placed in this region by the different investigators. Unfortunately, however, each investigator has used a different set of probes, and, in addition, most of these studies have used a small number of affected families, and thus the confidence limits on the recombination frequencies are large and the relative lods (when given) for alternate three-point orderings are not greatly different. Thus, it is difficult to work out a consistent, unambiguous map of the region.

Our laboratory has developed an *hprt* clonal assay

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to detect and measure mutations occurring in the *hprt* gene of human T-lymphocytes (Albertini et al. 1982; O'Neill et al. 1987, 1989). *hprt* mutant lymphocytes are selected by their ability to grow in the purine analogue 6-thioguanine, which kills the wild-type $hprt^+$ cells. Mutations occurring in vivo in lymphocytes either spontaneously or after exposure to mutagens can be detected (Morley et al. 1983; Albertini et al. 1988; Dempsey et al. 1985; Seifert et al. 1987; Cole et al. 1988; Hakoda et al. 1988; Messing et al. 1989; Tates et al. 1989; Nicklas et al. 1990). Background mutant frequencies in normal young adults are of the order of 5×10^{-6} (Morley et al. 1983; O'Neill et al. 1987, 1989; Messing et al. 1989; Tates et al. 1989) and rise up to 100-fold after mutagen exposures such as environmental exposures (Seifert et al. 1987; Cole et al. 1988; Hakoda et al. 1988) or cancer chemo- or radiotherapies (Dempsey et al. 1985; Messing et al. 1989; Nicklas et al. 1990). Newborns have an approximately 10-fold lower mutant frequency (Tatsumi et al. 1985; Henderson et al. 1986; Lippert et al. 1990; McGinniss et al. 1990). An in vitro *hprt* clonal assay in T-lymphocytes has also been developed (O'Neill et al. 1990*b*) to allow the determination of the mutagenicity of various compounds and spectra of the mutations induced.

The *hprt* mutants generated in both in vivo and in vitro assays can be grown to sufficient numbers for molecular analysis including *hprt* Southern blotting and sequencing. About 15% of spontaneous *hprt* mutants in normal adults have gross changes (mostly deletions) in their *hprt* gene on Southern blots (Turner et al. 1985; Bradley et al. 1987; Nicklas et al. 1987, 1989; Hakoda et al. 1989). These frequencies are much higher in in vivo–irradiated (33%) or in vitro–irradiated (67%) T-lymphocytes, as would be expected (Nicklas et al. 1990; O'Neill et al. 1990*a*). Newborns also have higher percentages (66%–85%) of gross alterations, which predominantly involve exons 2 and 3 (McGinniss et al. 1989; Lippert et al. 1990). Here we use 57 *hprt* mutant T-lymphocyte clones containing partial or total deletions of their *hprt* gene and 20 anonymous X-linked probes to perform classical deletion mapping of the q26 region of the X chromosome.

Material and Methods

Mutant T-Lymphocyte Clones

The *hprt* mutant lymphocytes were isolated by use of an *hprt* clonal assay (Albertini et al. 1982; O'Neill et al. 1987, 1989). Forty-four of the mutants were isolated from fresh blood samples and were the result of in vivo mutations. In brief, peripheral blood or, in the case of newborns, umbilical cord blood was collected in heparin (sodium heparin; 143 units/10 ml whole blood) blood-collection tubes. The lymphocytes were then separated on Ficoll-Hypaque, and mitogenic stimulation was initiated with 1 μg phytohemagglutinin/ml in 15–20-ml cultures (1×10^6 cells/ml) in 25-cm^2 tissue culture flasks. All experiments used RPMI 1640 medium containing 20% nutrient medium HL-1, 5% prescreened defined supplemented bovine calf serum in a humidified atmosphere of 5% CO_2/95% air at 37°C, and T-cell growth factor (TCGF). Irradiated (8,000 rads) mycoplasma-free $hprt^-$ derivatives of WIL-2 lymphoblastoid cells designated TK6 were used as accessory feeder cells. After 36–40 h, the cells were centrifuged and washed, and the cell number was determined by use of a hemocytometer. The cells were then seeded in 96-well culture plates at 1–10 cells/well in nonselection medium and at 2×10^4 cells/well in 10 μM 6-thioguanine selection medium. Each well also contained $0.5–1 \times 10^4$ irradiated TK6 cells. After 7–14 d of culture, colony growth was determined by use of an inverted phase-contrast microscope. *hprt* mutant clones (those growing in 6-thioguanine) were isolated and propagated in vitro for molecular analysis.

Mutants were isolated from the blood of several groups of male individuals. Eight of the mutants were isolated from three normal young adults. Three mutants were isolated from two newborns (umbilical cord blood samples). Six mutants were derived from four elderly individuals. Twenty mutants were derived from seven individuals who received ^{131}I anti-ferritin radioimmunotherapy for hepatoma, and seven mutants were from three patients receiving cis-platinum therapy for testicular cancer.

Thirteen of the mutant lymphocyte clones were derived from in vitro γ-irradiated lymphocyte cultures plated for 6-thioguanine selection after 8 d phenotypic expression (O'Neill et al. 1990*b*). In brief, the mononuclear cell fraction isolated as above from fresh blood was suspended at 2×10^6 cells/ml in RPMI 1640 and was exposed to 300 rads irradiation from a ^{137}Cs source (Gammacell 1000 B; Nordion International). Then an equal volume of medium RPMI 1640 containing 40% HL-1 and 10% FBS was added, the cells were aliquoted at 20 ml/25-cm^2 flask, and phytohemagglutinin was added at 1 μg/ml. After 36–40 h incubation (designated as day 2), the cell number was de-

termined and the cells were plated in mass culture at 1×10^5 cells/ml in growth medium containing 2.5×10^5 irradiated feeder cells/cm^2 and optimal amounts of TCGF and were incubated for 3 d. On day 5, cells were subcultured as above. On day 8, the cells were plated for cloning efficiency (1 and 2 cells/well) and mutant selection (1×10^4 cells/well). Clones were isolated and propagated as above. Thirteen mutant clones from two individuals were studied.

The clones used in this study were chosen because they contained an *hprt* deletion extending 5′ and/or 3′ of the *hprt* gene as detected by Southern blotting with a 947-bp DNA probe containing the entire *hprt* coding sequence (Brennard et al. 1983). All the mutant clones came from male individuals and therefore had only 1 X chromosome. The list of mutant clones used in this study is shown in table 1.

DNA Probes

The list of 20 anonymous probes used in this study is shown in table 2. Probes were chosen that mapped to Xq25-q27 according to Human Gene Mapping 9 (Davies et al. 1987) and/or Human Gene Mapping 10 (Mandel et al. 1989).

Southern Blot Analysis

DNA was isolated from the mutant lymphocytes as described by Nicklas et al. (1987). In brief, $15–20 \times 10^6$ frozen cells were washed in 0.5 ml $T_{10}E_1$ (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and then were resus-

Table 1

Description of *hprt* Deletion T-Lymphocyte Clones Used

Source and Individual	Experiment	Clone	*hprt* Gene Change
In vivo normal young adults:			
1.....	M16	B10H11	Total gene deletion
1.....	MF52	A10H6	Exon 1 deletion
2.....	LS35	A5B4	Total gene deletion
2.....	LS1	A5H11	Total gene deletion
2.....	MF33	A4F9	exons 5–9 deleted
3.....	JB6	A9E8	Total gene deletion
3.....	JB6	A5G4	Total gene deletion
3.....	MF38A	A12G4	Exons 4–9 deleted
In vivo cis-platinum–treated adults:			
4.....	MF336B	B5A5	Total gene deletion
5.....	MF272	M20	Total gene deletion
5.....	MF272	M31	Total gene deletion
6.....	MF266	A5E2	Exons 1–3 deleted
6.....	MF274B	M31	Exons 1–3 deleted
6.....	MF274B	M32	Total gene deletion
6.....	MF274B	M1	Exons 1 and 2 deleted
In vitro–irradiated cells:			
2.....	LS148	G1	Total gene deletion
2.....	LS148	Y8	Exons 1–3 deleted
2.....	LS148	O3	Total gene deletion
2.....	LS148	V6	Total gene deletion
2.....	LS148	ID6	Total gene deletion
2.....	LS38	E5F9	Exons 4–9 deleted
2.....	LS155	E1	Total gene deletion
7.....	LS323	M24	Exon 1 deleted
7.....	LS323	M46	Total gene deletion
7.....	LS323	M158	Exons 1 and 2 deleted
7.....	LS345	M13	Total gene deletion
7.....	LS345	M23	Exon 1 deleted
7.....	LS345	M9	Total gene deletion
In vivo newborn:			
8.....	SS108	A15E5	Exons 4–9 deleted
8.....	SS108	A13H6	Exons 4–9 deleted
9.....	LS346	M16	Exons 1–3 deleted
In vivo elderly adults:			
10.....	MF241	A4D3	Total gene deletion
10.....	MF241	A8E2	Exons (5?)–9 deleted
10.....	MF241	A10C2	Exons 3–9 deleted
11.....	MF118	A4A10	Exons 4–9 deleted
12.....	MF130A	A7D2	Total gene deletion
13.....	MF130B	B6A2	Total gene deletion
In vivo–irradiated adults:			
14.....	MF268	A4A11	Total gene deletion
14.....	MF268	A4E4	Total gene deletion
14.....	MF268	A5A4	Total gene deletion
14.....	MF268	A5A9	Total gene deletion
14.....	MF268	A8H8	Total gene deletion
14.....	MF268	A9G3	Total gene deletion
14.....	MF268	A9G11	Total gene deletion
14.....	MF268	A11D4	Total gene deletion
14.....	MF268	A11E12	Total gene deletion
15.....	MF126	C6G10	Exons 5–9 deleted
16.....	MF123	A6B6	Total gene deletion
16.....	MF123	A6C11	Exons 1 and 2 deleted
16.....	MF123	A7F5	Total gene deletion
16.....	MF123	A6G4	Exons 5–9 deleted
16.....	MF123	A6G11	Total gene deletion
16.....	MF123	A7C9	Total gene deletion
17.....	LS215	A5C4	Total gene deletion
18.....	MF120	A5A11	Total gene deletion
19.....	MF121A	A3A10	Total gene deletion
20.....	MF312	M2	Exon 1 deleted

(*continued*)

Table 1 (continued)

Table 2

***hprt* Linked Probes Used**

DX No.	Probe	Vector	Insert Size (enzyme)(kb)	Fragment Size(s) (kb) *PstI*	Fragment Size(s) (kb) *Hin*dIII	Donor	Reference
DXS10	36B-2	puc9	2.0 (*Hin*dIII)	13.0	2.18	ATCC	Silver et al. 1987
DXS11	p22-33	pBR322	1.8 (*Hin*dIII)	11.0, 3.7	1.55	B. Sylla	Nussbaum et al. 1985
DXS19	pX46d	puc8	1.8 (*Hin*dIII)	9.0	1.7	B. N. White	Holden et al. 1984
DXS37	30RIb	pAT153	6.28 (*Eco*RI)	17.0	9.8, 3.45	B. N. White	Holden et al. 1984
DXS42	p7F1	puc19	1.8 (*Eco*RI)	6.2, 5.15	4.3	B. Sylla	Reilly et al. 1988
DXS51	p52a	pBR322	5.3 (*Eco*RI/*Hin*dIII)	13.0	3.45	ATCC	Drayna and White 1985
DXS53	St16	pBR329	5.9 (*Eco*RI)	2.5, 1.5, 1.23, 1.0	5.13	B. Sylla	Oberle et al. 1986
DXS59	L2.9	pBR322	1.99 (*Eco*RI)	4.8	7.0[a], 1.3	E. Bakker	Wieacker et al. 1984
DXS79	07-03	EMBL-3	2.3[b] (*Eco*RI)	5.0	6.2	ATCC	Murphy et al. 1985
DXS86	ST1	pBR329	6.0 (*Eco*RI)	5.0, 1.35	7.8, 5.4[a], 3.5[a]	J.-L. Mandel	Oberle et al. 1986, 1987
DXS92	pXG-16	pAT153	3.3 (*Hin*dIII)	6.0	5.5 or 9.5	ATCC	Davatelis et al. 1985
DXS99	pX58dIIIc	puc8	2.4 (*Hin*dIII)	5.6	2.2	B. N. White	Mulligan et al. 1987
DXS100d	pX45d	puc8	2.5 (*Hin*dIII)	6.5 or 3.7	2.6	B. N. White	Holden et al. 1984
DXS102	cX38.1	pAT153	2.6 (*Eco*RI)	5.15	9.5, 2.4	ATCC	Hofker et al. 1987
DXS107	cpX234	puc12	0.15 (*Bam*HI)	.9	2.9	ATCC	Hofker et al. 1986
DXS144	C11	pEMBL8	1.0 (*PstI*)	2.65, 1.2	7.5[a], 5.6, 3.3, 1.48, 1.35	J.-L. Mandel	Oberle et al. 1987
DXS172	pX71c	pAT153?	1.0 (*Eco*RI)	11	5.4	B. N. White	Holden et al. 1984
DXS174	pX82d	pAT153?	1.45 (*Eco*RI)	1.7	5.7	B. N. White	Holden et al. 1984
DXS177	plambda2.7	λgtwes	7 (*Eco*RI)	8.5[a], 6.35, 3.7, 2.4, 1.75, 1.5, 7.0[c], 2.1[c], 1.38[c]	13.5, 9.4, 5.3, 4.65[a], 2.95[a], 8.2[c]	ATCC	Cooke et al. 1983, Davies et al. 1987
DNF1	pAX-6	pAT153	1.44 (*PstI*)	6.3, 3.4, 2.75, 2.1	6.8, 4.3[a], 1.2	ATCC	Balazs et al. 1984

[a] Faint fragment.
[b] Total insert size is 18 kb; 2.3-kb *Eco*RI subfragment was used for the hybridization probe.
[c] Cross-hybridizing fragment (presumably not in Xq26, as they were not codeleted with *hprt*).

pended in 2.5 ml TENS (25 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA, 0.6% SDS). The cell suspension was then heated to 65°C for 15 min, followed by an overnight incubation at 37°C with proteinase K (1.5 mg). The next day, 1.0 mg proteinase K was added to each sample, which then was incubated for 2 h at 37°C. The solution was then phenol extracted twice and chloroform/isoamyl alcohol extracted three times. After ethanol precipitation, the DNA pellet was resuspended in $T_{10}E_1$ and digested with restriction enzymes (*Hin*dIII or *Pst*I). After fractionation of the DNA fragments on a 0.7% agarose gel, the DNA was transferred to nitrocellulose paper (Schleicher & Schuell) as described previously (Nicklas et al. 1987).

Prehybridization was in 50% formamide, 5 × SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0), 10 × Denhardt's, 50 mM Tris-HCl, pH 7.5, and 500 mg sheared and denatured herring sperm DNA/ml for 4–6 h at 42°C. Hybridization was performed overnight at 42°C in a solution of 50% formamide, 5 × SSC, 1 × Denhardt's, 20 mM Tris-HCl, pH 7.5, 10% dextran sulfate, 250 mg sheared and denatured herring sperm DNA/ml, and 1–1.5 × 10^6 cpm of oligonucleotide-labeled probe/ml (Feinberg and Vogelstein 1984). Autoradiography of the nitrocellulose was performed at −80°C for 1–3 d by using Kodak XAR-5 film and Dupont Lightning Plus screens (Nicklas et al. 1990). Blots were washed free of probe (two washes, each at 70°C for 15 min in distilled water) and were placed in prehybridization solution for reprobing. The same four nitrocellulose filters were used for all 20 probings.

Results

A total of 57 *hprt* mutant T-cell clones were used in this study (table 1). Mutant clones arising in vivo in normal young adults, elderly adults, and newborns and in irradiation-exposed or cis-platinum-exposed

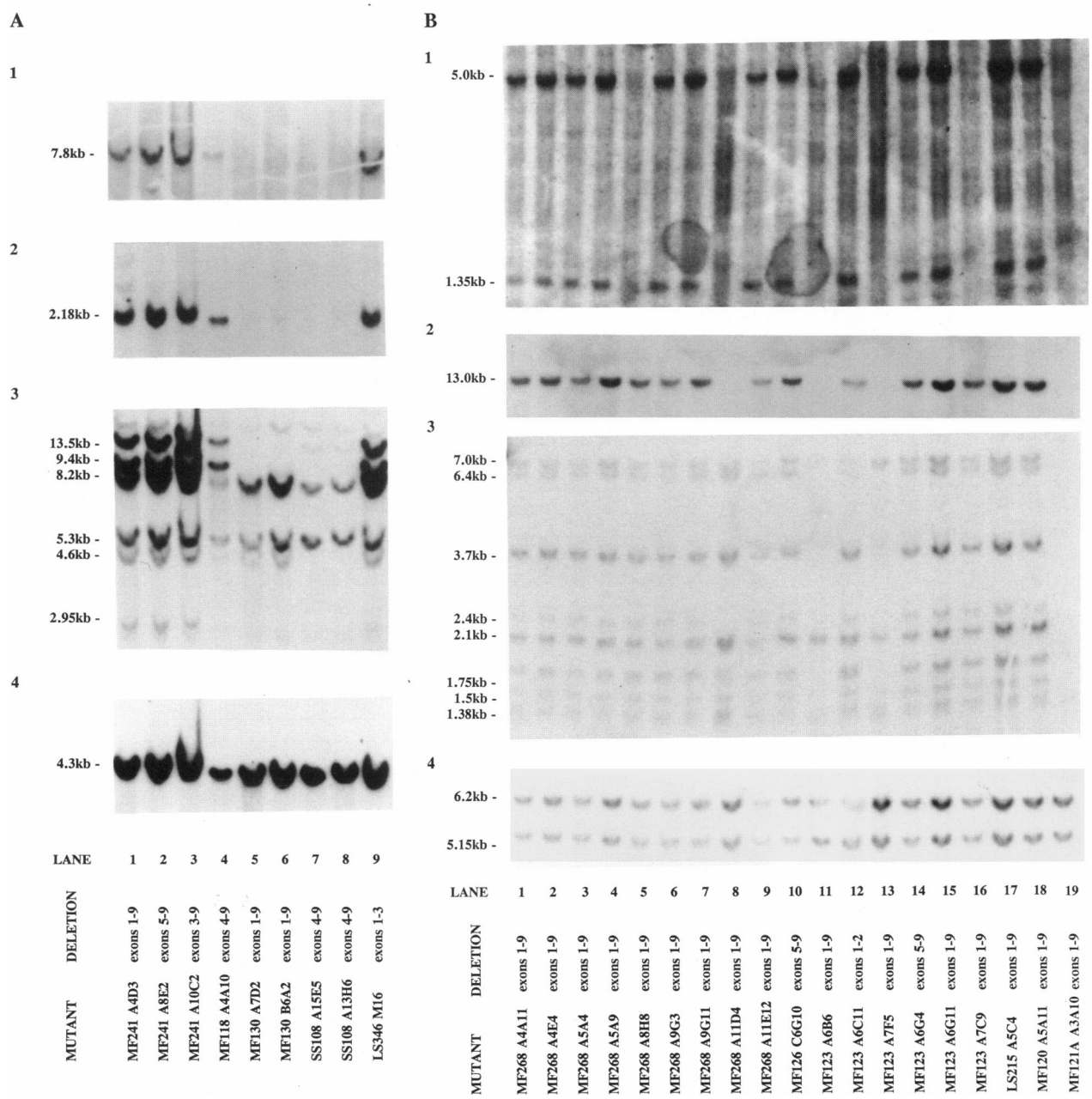

Figure 1 *A, Hin*dIII Southern blots of DNA from nine *hprt* mutant clones from elderly and newborns. The same filter of *Hin*dIII-digested DNAs is shown probed with DXS86, (panel 1), DXS10 (panel 2), DXS177 (panel 3), and DXS42 (panel 4). The two exon 4–9 deletion mutants in lanes 7 and 8 place DXS86, DXS10, and DXS177 off the 3′ end of the *hprt* gene, as both mutants have deletion of all three markers. In panel 4, no deletions lost hybridization with DXS42, indicating that it maps too far away for codeletion with *hprt*. This blot also demonstrates that all lanes of the blot have DNA. *B, Pst*I Southern blots of DNA from 19 *hprt* mutant clones from in vivo irradiated adults. The same filter of *Pst*I-digested DNAs is shown probed with DXS86 (panel 1), DXS10 (panel 2), DXS177 (panel 3), and DXS42 (panel 4). In panel 1, six of the total deletions have lost DXS86 (lanes 5, 8, 11, 13, 16, and 19); however, in panel 2, only four of these have lost DXS10 (lanes 5, 8, 11, 13, 16, and 19). This creates the order DXS10–DXS86–*hprt*. In panel 3, only three of those total deletions have lost hybridization with DXS177 (lanes 11, 13, and 19), which creates the final order of DXS177–DXS10–DXS86–*hprt*. In panel 4, no deletions lost hybridization with DXS42, indicating that it maps too far away for codeletion with *hprt*. This blot also demonstrates that all lanes of the blot have DNA.

Table 3

Extent of Deletion in 57 *hprt* Deletion Mutants

Clone (type[a])	DXS53	DXS79	5′hprt	3′hprt	DXS86	DXS10	DXS177
MF130 B6A2 (E)	—	—	—	—	—	—	—
LS148 G1 (V)	—	—	—	—	—	—	
MF123 A6G11 (R)	—	—	—	—			
MF268 A11E12 (R)	—	—	—	—			
LS155 E1 (V)	—	—	—	—			
LS148 V6 (V)		—	—	—	—	—	—
MF268 A11D4 (R)		—	—	—	—	—	
LS345 M9 (V)		—	—	—	—	—	
MF121A A3A10 (R)		—	—	—	—	—	—
MF123 A7C9 (R)		—	—	—	—		
MF268 A8H8 (R)		—	—	—	—		
MF268 A9G3 (R)		—	—	—			
MF268 A4E4 (R)		—	—	—			
MF268 A5A4 (R)		—	—	—			
LS323 M46 (V)		—	—	—			
MF274 M32 (C)		—	—	—			
LS148 O3 (V)		—	—	—			
MF120 A5A11 (R)		—	—	—			
LS148 ID6 (V)		—	—	—			
LS345 M13 (V)		—	—	—			
MF241 A4D3 (E)		—	—	—			
MF272 M31 (C)			—	—	—	—	—
JB6 A9E8 (A)			—	—	—	—	—
MF130 A7D2 (E)			—	—	—	—	—
MF123 A6B6 (R)			—	—	—	—	—
MF123 A7F5 (R)			—	—	—	—	—
LS35 A5B4 (A)			—	—	—	—	
LS1 A5H11 (A)			—	—			
MF336 B5A5 (C)			—	—			
MF272 M20 (C)			—	—			

(*continued*)

individuals were studied, as were mutants from in vitro–irradiated T-lymphocyte cultures. These clones arose in 20 different male individuals. (One individual [individual 2] provided cells for both in vivo and in vitro studies.) These particular mutant clones were chosen because they had *hprt* deletions which extended either 5′ (e.g., an exon 1 deletion; 11 mutants), 3′ (e.g., an exon 4–9 deletion; 10 mutants) or both 5′ and 3′ (i.e., total *hprt* gene deletions; 36 mutants) of the *hprt* gene. Thus, combined, these mutants contained 47 breakpoints occurring 5′ of the *hprt* gene and 46 breakpoints occurring 3′ of the *hprt* gene.

DNA from each of the 57 clones was digested with *Pst*I or *Hin*dIII and was transferred to nitrocellulose for probing with the 20 anonymous probes listed in table 2. Presence of the expected fragment(s) on the Southern blot indicated that that probe was not codeleted with *hprt,* while absence of the fragment(s) indicated codeletion (fig. 1). All of these clones (with the exception of SS108 A15E5 and SS108 A13H6) represent independent mutations either because they are from different individuals, had different deletions on Southern blots, had different TCR gene rearrangements, or were shown here to have different deletions of the flanking probes. Mutants SS108 A15E5 and SS108 A13H6 were from the same newborn, and, although they had different T-cell receptor rearrangements (not a definitive test of independence for mutant clones from newborns because of a high rate of prethymic mutation), they showed identical Southern blot changes, including an identical new fragment. Here, they also showed identical deletion of the flanking probes and are thus assumed to be sibling mutants (progeny of one original mutant cell).

Five of the 20 probes were deleted in some of the mutants. The use of these mutant clones, which contained deletions of differing lengths, allowed the unambiguous ordering of these five probes relative to

Table 3 (continued)

Clone (type[a])	DXS53	DXS79	5'hprt	3'hprt	DXS86	DXS10	DXS177
MF268 A9G11 (R)			—				
LS215 A5C4 (R)			—				
MF268 A5A9 (R)			—				
M16 B10H11 (A)			—				
JB6 A5G4 (A)			—				
MF268 A4A11 (R)			—				
LS323 M24 (V)	—	—					
MF123 A6C11 (R)		—					
MF52 A10H6 (A)			—				
MF274 M31 (C)			—				
LS345 M23 (V)			—				
MF312 M2 (R)			—				
MF274 M1 (C)			—				
LS323 M158 (V)			—				
MF266 A5E2 (C)			—				
LS148 Y8 (V)			—				
LS346 M16 (N)			—				
SS108 A15E5 (N)				—	—	—	—
SS108 A13H6 (N)				—	—	—	—
LS38 E5F9 (V)				—	—	—	
MF126 C6G10 (R)				—			
MF38A A12G4 (A)				—			
MF33 A4F9 (A)				—			
MF118 A4A10 (E)				—			
MF123 A6G4 (R)				—			
MF241 A8E2 (E)				—			
MF241 A10C2 (E)				—			

[a] Mutant origin is as follows: E = in vivo elderly adult; V = in vitro–irradiated cells; R = in vivo–irradiated adult; C = in vivo cis-platinum–treated adult; A = in vivo normal young adult; N = in vivo newborn.

hprt and to each other (table 3). Both the resulting map of the *hprt* gene region and the number of breakpoints lying in each part of the region are shown in figure 2.

Discussion

The Xq26-q27 region has been intensely studied. In addition to the mapping studies of OCRL, LYP, F9, and FRAXA families (Drayna and White 1985; Mandel et al. 1986; Murphy et al. 1987; Oberle et al. 1987; Arveiler et al. 1988; Brown et al. 1988; Mulley et al. 1988; Skare et al. 1989*a*, 1989*b*; Sylla et al. 1989; Wadelius et al. 1989; Reilly et al. 1990), individuals with breakpoints in the region have been studied (Suthers et al. 1989; Reilly et al. 1990). Physical mapping studies using somatic cell (pushme-pullyu) hybrids are also underway (Brown et al. 1989). A number of investigators have begun pulsed-field maps and/or chromosome-walking or chromosome-jumping studies (Anson et al. 1988; Nguyen et al. 1988, 1989; Patterson et al. 1988; Reilly et al. 1990). In addition, several groups have begun using yeast artificial chromosomes (YACS) to clone the Xq24-q28 (Little et al. 1989; Wada et al. 1990), Xq27-qter (Poustka et al. 1989), or Xq28 region (Kenwrick and Gitschier 1989; Feil et al. 1990). Also, one research group is

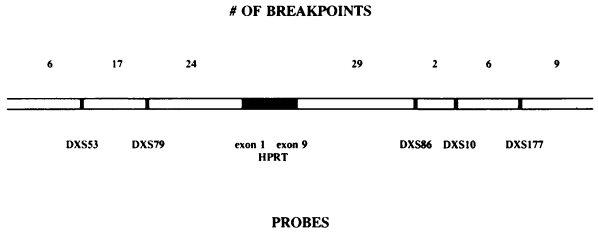

Figure 2 Map of *hprt* gene region. The number of breakpoints mapping between each of the loci is shown above the map.

creating band-specific libraries of Xq27 by dissection, PCR, and cloning (Lüdecke et al. 1990). However, a definitive ordered mapping of the probes near *hprt* has not been determined.

Our studies have allowed the unambiguous ordering of five *hprt*-linked probes relative to the *hprt* gene. These results confirm most other data in the literature but conflict with some studies. The initial study of DXS10 placed it very near *hprt* (Boggs and Nussbaum 1984), although later studies have placed it as much as 12 cM away (Brown et al. 1988). Our studies confirm its close linkage to *hprt*. DXS10 and DXS79 have previously been very closely linked (Murphy et al. 1983), although F9 was also very closely linked in the same study. Reilly et al. (1990) studied a woman with Lowe syndrome caused by a breakpoint in the gene. DXS10, DXS86, DXS177, and *hprt* were all distal to the breakpoint. They also report that DXS10 and DXS86 share a 460-kb *Bss*HII fragment and that these two markers show no recombination. This close linkage is in very good agreement with our results, as we obtained only two breakpoints mapping between these probes.

The main conflicts with our results occur in the work of Brown et al. (1988), who reported a large mapping study of 405 individuals from FRAXA families. Their map places DXS100 just distal to *hprt* and DXS10 12 cM more distal. This placement of DXS100 is in conflict with a number of other groups' results, which place it proximal to *hprt* (Oberle et al. 1987; Skare et al. 1989*b;* Reilly et al. 1990), although another group has placed it distal (Mulligan et al. 1985). The evidence of Reilly et al. (1990) is most compelling, as they mapped DXS100 proximal to a breakpoint in a translocation carrier while *hprt* mapped distal. Brown et al.'s placements of DXS100 and DXS10 are clearly in error, as we have DXS10 codeleted and thus closely linked to *hprt* while DXS100 was never codeleted with *hprt* and thus is not as closely linked.

Adding our data to that of the literature allows the placement of some of the other RFLP probes and disease genes proximal or distal to *hprt*. DXS37 and DXS19 map proximally to *hprt* (Brown et al. 1988; Skare et al. 1989*b*), with DXS19 probably more proximal (Brown et al. 1988; Skare et al. 1989*b*), although previously their order had been thought to be the reverse (Mulligan et al. 1985). DXS11 also maps proximal to *hprt* (Drayna and White 1985; Sylla et al. 1989; Wadelius et al. 1989), as does DXS42 (Lesko and Nussbaum 1986), although Drayna and White (1985) originally had the latter distal to *hprt*. Skare et al. (1989*a,* 1989*b*) obtain the order proximal–DXS37–DXS42–LYP–distal in mapping studies of LYP families. OCRL has also been mapped very close to DXS42 and to DXS86 (Wadelius et al. 1989). Reilly et al. (1990) have shown that DXS10 and DXS42 flank OCRL. DXS144 appears to map distal to DXS86, as DXS86 was mapped between DXS100 and DXS144 (Oberle et al. 1987). DXS144 was also mapped between DXS100 and DXS51 (Arveiler et al. 1988). DXS102 was mapped distal to DXS51 but proximal to F9 (Arveiler et al. 1988) and apparently maps just 300 kb from F9 (Nguyen et al. 1989). DXS99 has been placed very close to F9 (Mulligan et al. 1987) but proximal to DXS51 (Brown et al. 1988).

The question arises as to the relative orientation of DXS177–DXS10–DXS86–*hprt*–DXS79–DXS53 with regard to the centromere. There is evidence for both orientations. Supporting a mapping of the centromere proximal to DXS177, Sylla et al. (1989) place DXS86 closer to LYP (and DXS37) than to *hprt,* on the basis of recombination frequencies, while Murphy and Ruddle (1985) reported the order centromere–*hprt*–DXS79, on the basis of studies in chromosome-mediated gene-transfer lines, and Turner et al. (1989) state that DXS10 is proximal to DXS86. (The latter placement, however, has been retracted; J. C. Mulley, personal communication). Supporting a mapping of the centromere proximal to DXS53, Brown et al. (1988) placed DXS10 distal to *hprt,* Wadelius et al. (1989) placed DXS86 closer to OCRL (and DXS42) than to DXS10, Oberle et al. (1986) mapped DXS53 proximal to DXS86 by using a panel of somatic cell hybrids, and Reilly et al. (1990) mapped *hprt* proximal to DXS10, DXS86 by recombination frequencies. This order is only 15 times more likely than that which maps *hprt* distal, but it is supported by a recombinant in which OCRL crossed over with DXS86 but not with *hprt* (R. L. Nussbaum, personal communication).

Thus, although there are conflicting data, the preponderance of the information available gives the order centromere–DXS53–DXS79–$^{5'}$*hprt*$^{3'}$–DXS86–DXS10–DXS177–telomere. The major pieces of evidence supporting this orientation are the somatic cell hybrids of Oberle et al. (1986) and the recombinant individual of Nussbaum.

Although the exact amount of DNA deleted in the mutants cannot be determined as yet, many mutants must have lost more than 460 kb (the size of the DXS10–DXS86 *Bss*HII fragment [Reilly et al. 1990]). This 460 kb is 10 times the size of the *hprt* gene itself. Since the DXS10-DXS86 distance appears to be only

a small part of the map in figure 2, it may be that some of these deletions are much larger. One mutant (MF130 B6A2) has deleted *hprt* and all five linked markers. The question of what is the largest possible deletion is of interest because viable X chromosome deletions are expected, in general, to be smaller than viable autosomal deletions, since, for X chromosome deletions, there is no remaining functional allele on the other homologous chromosome. Thus, the limitation on the deletion size of the *hprt* mutants used here is provided by the presence of flanking T-cell vital genes. Further study of the breakpoints of the largest deletions should map these linked vital genes.

These studies map five anonymous probes around the *hprt* gene. Some of these results contradict those of published genetic linkage maps based on family studies; however, other reports of physical maps contradicting linkage maps exist (Higgins et al. 1990). Studies are in progress to map additional new probes and to use additional new *hprt* deletion mutants. Pulsed-field gel analysis with the DXS10, DXS86, DXS177, DXS53, DXS79, and *hprt* probes is underway to determine the exact distances between these loci. This information will be used to determine exact deletion size in the *hprt* mutants.

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