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Human X-Linked Genes Regionally Mapped Utilizing X-Autosome Translocations and Somatic Cell Hybrids

(somatic cell genetics/X/9 and X/22 translocations/hypoxanthine phosphoribosyltransferase/glucose-6-phosphate dehydrogenase/phosphoglycerate kinase)

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ABSTRACT **Human genes coding for hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8; IMP:pyrophosphate phosphoribosyltransferase), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49; D-glucose-6-phosphate:NADP$^+$ 1-oxidoreductase), and phosphoglycerate kinase (PGK, EC 2.7.2.3; ATP:3-phospho-D-glycerate 1-phosphotransferase) have been assigned to specific regions on the long arm of the X chromosome by somatic cell genetic techniques. Gene assignment and linear order were determined by employing human somatic cells possessing an X/9 translocation or an X/22 translocation in man–mouse cell hybridization studies. The X/9 translocation involved the majority of the X long arm translocated to chromosome 9 and the X/22 translocation involved the distal half of the X long arm translocated to 22. In each case these rearrangements appeared to be reciprocal. Concordant segregation of X-linked enzymes and segments of the X chromosome generated by the translocations indicated assignment of the PGK gene to a proximal long arm region (q12-q22) and the HPRT and G6PD genes to the distal half (q22-qter) of the X long arm. Further evidence suggests a gene order on the X long arm of centromere-*PGK-HPRT-G6PD*.**

Man–rodent somatic cell hybrids have proved important for mapping the human genome (1, 2). Human genes can be assigned to specific chromosomes, since human chromosomes are preferentially lost in these cell hybrids. By employing cells with deleted or translocated human chromosomes in cell hybrid studies, one can determine gene order and assignment to a specific chromosomal region (3–6). The human X chromosome is of interest to map, since it is involved in sex determination; it is functionally inactivated in females, resulting in the same gene dosage as in males (7); and X-linked genes are involved in several inherited metabolic defects.

We employed two inherited X-autosome translocations and somatic cell hybrids to map genes coding for hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8; IMP:pyrophosphate phosphoribosyltransferase), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49; D-glucose-6-phosphate: NADP$^+$ 1-oxidoreductase), and phosphoglycerate kinase (PGK, EC 2.7.2.3; ATP:3-phospho-D-glycerate 1-phosphotransferase) to specific regions of the X long arm. These three genes have been shown to be X-linked by family and somatic cell genetic studies (3, 8–12), and enzyme deficiencies of HPRT, G6PD, and PGK in man have been associated, respectively, with Lesch–Nyhan disease (8), drug-induced hemolytic anemia (13), and chronic hemolytic anemia (14).

Abbreviations: HPRT, hypoxanthine phosphoribosyl transferase; G6PD, glucose-6-phosphate dehydrogenase; PGK, phosphoglycerate kinase; HAT, hypoxanthine/aminopterin/thymidine; DMEM, Dulbecco's modified Eagle's medium.

Mapping the X chromosome in cell hybrids is aided by a system to select for or against hybrid cells possessing human HRPT (15). This procedure, in concert with an X/9 and an X/22 translocation involving different segments of the X long arm translocated to different autosomes, was employed to locate *HPRT*, *G6PD*, and *PGK* genes on specific regions of the X. In cells with the X/9 translocation, the breakpoint on the X long arm was close to the centromere. The X breakpoint in X/22 translocation cells was near the center of the X long arm. By comparison of the concordant segregation of the three genes and the different X chromosome segments in cell hybrids, the gene coding for PGK was assigned to the proximal long arm region between the two X breakpoints and the genes for HPRT and G6PD were assigned to the terminal half of the X long arm.

MATERIALS AND METHODS

Parental Cells. Human cells were AnLy fibroblasts [46,X,t(Xq−;9p+)(q12;p24)] with the X long arm region q12-qter translocated to the 9 short arm (16), and CaVa leukocytes [46,X,t(Xq−;22q+)(q22;q13)] with the q22-qter region translocated to the long arm of 22 (Figs. 1 and 2). We are grateful to Dr. Catherine Palmer for CaVa cells. AnLy fibroblasts were propagated on Eagle's Basal Medium (Diploid) in 10% fetal calf serum with penicillin, streptomycin, and kanamycin (GIBCO) (17). CaVa leukocytes were isolated from blood with citric acid/sodium citrate/dextrose anticoagulant by sedimentation in Plasmagel (Laboratoire Roger Bellon) for 2 hr. Mouse parental (HPRT$^-$) cells, RAG (18) and A9 (19), were propagated on Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO-high glucose formula) supplemented with 10% fetal calf serum and antibiotics.

Hybrid Cells. AnLy cells were fused with RAG and A9 while in monolayer (17) and CaVa leukocytes were fused with RAG in suspension (20). Hybrid clones were selected after fusion in DMEM supplemented with hypoxanthine/aminopterin/thymidine (HAT) (15). In DMEM–HAT, mouse (HPRT$^-$) cells die, human leukocytes in suspension are removed, and AnLy fibroblasts grow slowly. Independently derived hybrid colonies were cloned (primary clones) (20).

Selection and Counterselection. HPRT$^-$ cells die in HAT but survive in 8-azaguanine-supplemented medium (15). Primary clones growing in HAT require the functioning human *HPRT* locus on the Xq$^-$ or X-autosome to survive (Figs. 1 and 2). Three X/9 and four X/22 translocation hybrid clones were chosen for counterselection experiments (Table 1) (3, 12, 22). Primary clones were withdrawn from HAT medium by growing for two passages on medium containing hypoxanthine and

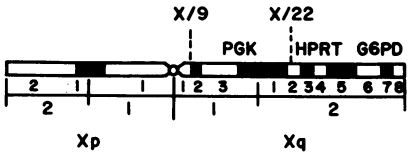

FIG. 1. Diagram of the human X chromosome and band locations, based on the 1971 Paris Conference (25). The short arm, p, is separated into two regions composed of numbered bands. The end of the short arm is designated pter. The long arm, q, is separated into two regions composed of numbered bands. The long arm terminus is qter. Breakpoints for the X/9 and X/22 markers are at q12 and q22, respectively. The gene coding for PGK was assigned to the q12-q22 segment. *HPRT* and *G6PD* genes were assigned to the q22-qter segment (see Fig. 2).

thymidine. After additional passages, each hybrid population was plated in 8-azaguanine (20 μg/ml) or HAT-supplemented DMEM. Subclones that lose human HPRT survive on 8-azaguanine-DMEM; clones that retain human HPRT survive on HAT–DMEM.

Chromosome Analyses: Human chromosomes were identified by G-banding (23) and C-banding (24) techniques. Terminology follows recommendations of the 1971 Paris Conference on nomenclature (25) (Fig. 1). AnLy and CaVa marker chromosomes are demonstrated in Fig. 2.

Enzyme Analyses: Cell hybrids were harvested (17) and enzymes were identified by starch gel electrophoresis. Procedures for HPRT, PGK, and G6PD were described (11).

RESULTS

Linkage of *HPRT*, *G6PD*, and *PGK* genes was examined in 204 man–rodent hybrids involving human cells with cytologically normal X chromosomes. Hybrid clones grown on HAT medium were obtained from 10 fusion experiments using human cells from six individuals, three HPRT$^-$ mouse lines, and two HPRT$^-$ Chinese hamster lines. The three human enzymes segregated together in 183 (90%) of the cell hybrids tested, confirming the X-linkage of these genes. One hundred-

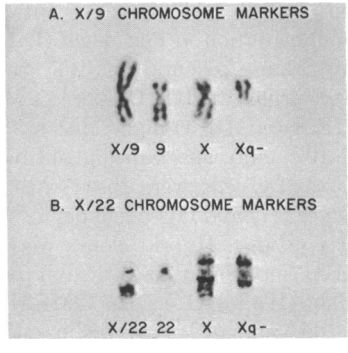

FIG. 2. (A) Partial karyotype of AnLy fibroblasts [46,X,t(Xq−;9p+)(q12;p24)] demonstrating the X/9 (Buffalo) and Xq− translocations by Giemsa-9 banding. The X/9 marker involves the X long arm segment q12-qter translocated to the 9 short arm at p24. The Xq− marker extends from the X short arm terminus to the breakpoint at q12 which is designated pter-q12. See Fig. 1. (B) Partial karyotype of CaVa lymphocytes [46,X,t(Xq−,22q+)(q22;q13)] demonstrating the X/22 (Indy) and Xq− translocations by Giemsa–trypsin banding. The X/22 marker is composed of the X long arm segment q22-qter translocated to the 22 long arm at q13. The Xq− marker extends from the pter to the q22 breakpoint (pter-q22). See Fig. 1.

TABLE 1. *Distribution of PGK, HPRT, and G6PD in X/9 and X/22 translocation hybrids*

	Total clones	Medium[a]	Enzymes[b] PGK	HPRT	G6PD
A. X/9 translocation hybrids					
Hybrid Series	Primary				
ALA	7	HAT	+	+	+
ALR	6	HAT	+	+	+
Subclone selection experiments					
Primary clones	Subclones				
ALA-1		HAT	+	+	+
	6	HAT	+	+	+
	6	8-aza	−	−	−
ALA-8		HAT	+	+	+
	7	HAT	+	+	+
	7	8-aza	−	−	−
ALR-1		HAT	+	+	+
	6	HAT	+	+	+
	11	8-aza	−	−	−
B. X/22 translocation hybrids					
Hybrid Series	Primary				
REX	26	HAT	+	+	+
	8	HAT	−	+	+
Subclone selection experiments					
Primary clones	Subclones				
REX-11		HAT	+	+	+
	7	HAT	−	+	+
	6	8-aza	−	−	−
	1[c]	8-aza	−	−	+
REX-57		HAT	+	+	+
	1	HAT	+	+	+
	3	HAT	−	+	+
	3[c]	HAT	−	+	−
	7	8-aza	−	−	−
REX-12		HAT	−	+	+
	4	8-aza	−	−	−
REX-60		HAT	−	+	+
	4	8-aza	−	−	−

[a] Supplemented with HAT and 8-azaguanine (8-aza).
[b] Enzyme activity represented by (+); no activity (−).
[c] Subclones considered variants. See *text*.

ten of the 183 hybrids were primary clones, the others were subclones. Twenty-one hybrids (10%) deviated from this pattern. Nine were PGK$^-$, HPRT$^+$, G6PD$^+$, two were PGK$^+$, HPRT$^+$, G6PD$^-$, and 10 were PGK$^-$, HPRT$^+$, G6PD$^-$. Eleven variants were primary clones; 10 were subclones. The 10% discordancy is attributed to spontaneous X chromosome breakage and retention of the human *HPRT* gene under selection pressure (11, 28). For nonselected linkage groups, 4–6% discordancy was observed in these hybrids (11, 26).

Human cells possessing either an X/9 or an X/22 translocation (Figs. 1 and 2) were employed for regional mapping (29, 30). Human AnLy fibroblasts, which possess the translocation involving X and 9, were fused with RAG (ALR hybrids) and A9 (ALA hybrids). Seven ALA and six ALR hybrids retained HPRT, G6PD, and PGK (Fig. 3, Table 1). Three independent hybrids (ALA-1, ALA-8, ALR-1), withdrawn from HAT medium, were subcloned in 8-

azaguanine-supplemented media to counterselect for clones that had lost human HPRT, and in HAT medium to backselect for clones that had retained human HPRT. All 19 HAT subclones retained human HPRT, G6PD, and PGK (Table 1). Chromosome studies demonstrated that two ALR-1 HAT subclones possessed the X/9 translocation and the three enzymes (Fig. 4, Table 2). Twenty-four subclones counterselected on 8-azaguanine did not possess the three enzymes (Table 1). Chromosome analyses on two such subclones did not demonstrate the X/9 translocation (Table 2). The Xq^- chromosome was absent in HAT and 8-azaguanine subclones (Table 2). Concordant segregation of the X/9 translocation, PGK, HPRT, and G6PD, supports assignment of the three genes to the long arm of the X chromosome distal to the q12 band (Fig. 1). The unaltered X was considered inactive in cell hybrids, as in lymphocytes (ref. 16; C. Palmer, personal communication), since ALR-1 BSAg-9 and ALR-1 BSAg-10 grown on 8-azaguanine did not express human HPRT, G6PD, and PGK, although the unaltered X was observed in 30% and 25% of the cells, respectively (Table 2).

Further localization of the genes was obtained in X/22 translocation (REX) hybrids. Twenty-six REX hybrids retained all three enzymes. However, eight hybrids retained only HPRT and G6PD, indicating loss of the chromosome region coding for PGK (Table 1). Two X/22 hybrids (REX-11 and REX-57) possessing all three enzymes and two hybrids (REX-12 and REX-60) possessing HPRT and G6PD were subcloned on 8-azaguanine and HAT media. From the REX-11 and REX-57 subclone series on HAT medium, one subclone retained all three enzymes, and 10 lost PGK but retained HPRT and G6PD (Table 1). Chromosome studies on REX-11 and REX-57 HAT subclones that expressed HPRT and G6PD demonstrated the X/22 translocated chromosome but not the X or Xq^- chromosomes (Fig. 5, Table 2). Thirteen 8-azaguanine subclones from REX-11 and REX-57 lost all three enzymes. Chromosome analyses on REX-11 and REX-57 8-azaguanine subclones did not demonstrate the X, X/22, or Xq^- chromosomes (Table 2). REX-12 and REX-60 primary clones expressed HPRT and G6PD. Chromosome studies on REX-12 demonstrated the X/22 translocation but not the X or Xq^- chromosomes (Table 2). Eight subclones were isolated from REX-12 and REX-60 on 8-azaguanine DMEM and all were HPRT and G6PD negative (Table 1). Chromosome analyses on two subclones could not identify an X/22 translocation (Table 2). Segregation of PGK, HPRT, and G6PD in X/22 translocation hybrids demonstrated the concordant distribution of HPRT, G6PD, and the X/22 translocation. PGK segregated independently. These results support the assignment of *HPRT* and *G6PD* genes to the terminal segment of the X long arm (Fig. 1).

Four variant subclones were observed in REX hybrids (Tables 1 and 2). Three REX-57 HAT subclones expressed only HPRT and one REX-11 8-azaguanine subclone expressed only G6PD. Chromosome analysis on REX-57 BSH-C did not demonstrate a recognizable X, X/22, Xq^-, or a rearranged X/22 (Table 2). Spontaneous X chromosome breakage or rearrangement was most likely the reason for these exceptional subclones.

If an enzyme coded by an autosomal locus segregated with HPRT in X/9 and X/22 translocation hybrids, a gene assignment could be made to either chromosome 9 or 22, respectively. Twenty-two additional human enzymes (11, 17, 26, 27) coded by autosomal genes were tested for segregation with HPRT. None segregated with the X-linked markers.

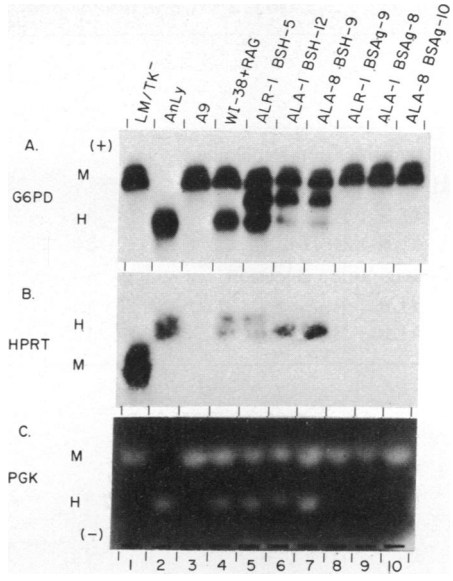

FIG. 3. Electrophoretic patterns of G6PD (A), HPRT (B), and PGK (C). (*1*) mouse LM/TK^- $HPRT^+$ (TK is thymidine kinase) (32). (*2*) human fibroblasts. (*3*) mouse ($HPRT^-$). (*4*) mixture of human (H) and mouse (M) ($HPRT^-$) homogenates. (*5–7*) X/9 translocation hybrid subclones on HAT medium. (*8–10*) X/9 translocation hybrid subclones counterselected on 8-azaguanine medium. Mouse and human enzymes in X/22 translocation hybrids migrated the same as in X/9 hybrids.

DISCUSSION

In the absence of classical genetic recombination in somatic cell hybrids, a series of hybrids segregating different segments of a chromosome are particularly useful for determining linear order and gene assignment to chromosomal regions. Utilizing

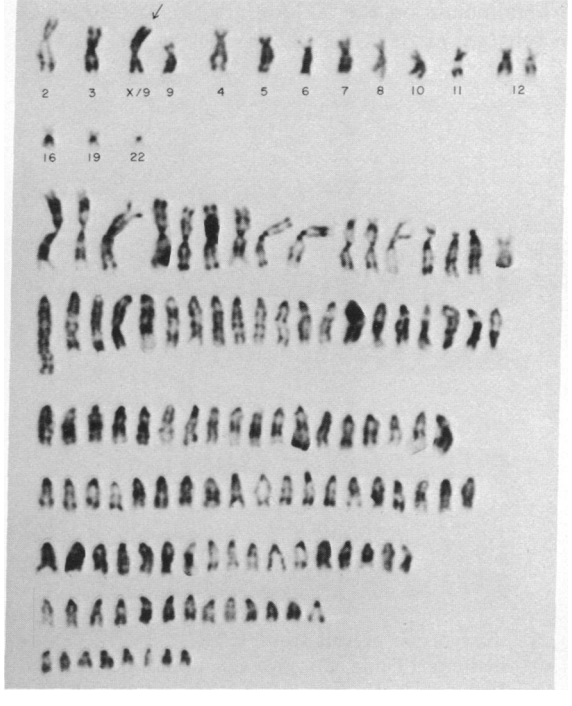

FIG. 4. Karyotype of cell from ALR-1 BSH-6 subcloned on HAT medium (Table 2). Human chromosomes are observed in the top two rows; mouse (RAG) chromosomes are in the last seven rows. Note retention of the X/9 marker. The Xq− chromosome was not detected by G or C banding.

TABLE 2. *Chromosome and enzyme distribution in X/9 and X/22 hybrid subclones*

	Cells[a]	Chromosomes[b]				Enzymes[c]		
		X/9	9	X	Xq−	PGK	HPRT	G6PD
A. X/9 translocation subclones								
HAT selection								
ALR-1 BSH-5	30	22	8	0	0	+	+	+
ALR-1 BSH-6	24	22	4	2	0	+	+	+
8-azaguanine selection								
ALR-1 BSAg-9	13	0	8	4	0	−	−	−
ALR-1 BSAg-10	12	0	6	3	0	−	−	−
		X/22	22	X	Xq−			
B. X/22 translocation subclones								
HAT selection								
REX-11 BSH-C	16	15	1	0	0	−	+	+
REX-11 BSH-F	26	24	2	0	0	−	+	+
REX-57 BSH-B	14	13	0	0	0	−	+	+
REX-57 BSH-C	15[d]	0	0	0	0	−	+	−
REX-12 (primary)	33	26	6	0	0	−	+	+
8-azaguanine selection								
REX-11 BSAg-B	18	0	0	0	0	−	−	−
REX-57 BSAg-E	8	0	0	0	0	−	−	−
REX-12 BSAg-D	27	0	0	0	0	−	−	−
REX-60 BSAg-C	31	0	0	0	0	−	−	−

[a] Total cells counted for chromosomes.
[b] Cells in which the specific chromosome was observed.
[c] (+) represents activity; (−) represents no activity.
[d] Absence of the X/22 marker and G6PD but presence of HPRT indicates chromosome breakage.

two human X-autosome translocations, we employed this strategy to determine the regional location of human genes coding for the X-linked enzymes HPRT, G6PD, and PGK.

The breakpoints on the X long arm in AnLy and CaVa human cells are at q12 and q22, respectively (Fig. 1). This separates the X chromosome in AnLy cells into two segments, pter-q12 and q12-qter (Fig. 1). In CaVa cells the X is separated into two segments, pter-q22 and q22-qter (Fig. 1). By comparing the segregation of HPRT, G6PD, PGK, and the marker chromosomes in AnLy and CaVa × mouse cell hybrids, the enzyme genes can be assigned to one of three X chromosome regions, pter-q12, q12-q22, or q22-qter (Fig. 1). Since HPRT is required for growth in the HAT selection system, one of these regions must be retained in the ALR, ALA, and REX cell hybrids. It follows that other genes coding for X-linked enzymes can be mapped by segregation with the marker chromosomes. For this strategy to be effective, the unaltered X must be nonfunctional or absent in hybrids. The unaltered X was inactive in lymphocytes of both translocation carriers (ref. 16; C. Palmer, personal communication). In hybrids, the unaltered X was observed in clones growing on 8-azaguanine DMEM although X-linked enzymes were not expressed (Table 2). This demonstrated that the unaltered human X remained inactive in hybrid cells.

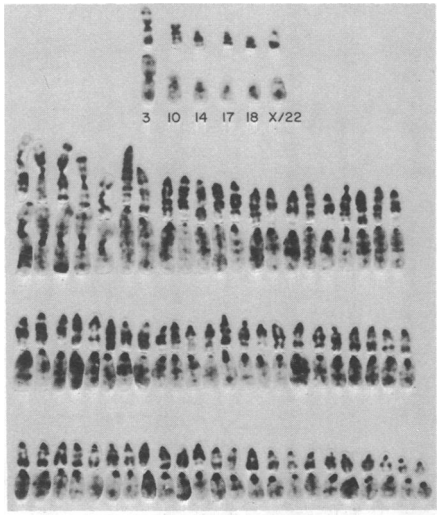

FIG. 5. Karyotype of cell from REX-11 BSH-C subcloned on HAT medium (Table 2). G-banded chromosomes are in top row of each of three sets of chromosomes. C- banded chromosomes are in second row of each set. Human chromosomes are in first set; others are mouse (RAG). The X/22 translocation was retained. The Xq− marker was not detected.

In X/9 translocation hybrids, HPRT, PGK, and G6PD were never separated (Table 1). This result predicted that the three enzymes would segregate with either the Xq^- or the X/9 translocation. Chromosome and enzyme results demonstrated concordant segregation of the three enzymes with the X/9 translocated chromosome (Tables 1 and 2). It was concluded that *PGK*, *HPRT*, and *G6PD* genes are located in the X long arm region distal to the q12 band (Fig. 1).

Since nearly the entire X long arm is involved in the X/9 translocation, it is predicted that more distal breakpoints on the X long arm would localize the three genes to smaller

regions. In X/22 translocation hybrids, HPRT and G6PD segregated independently of PGK (Tables 1 and 2). It was predicted that HPRT and G6PD would segregate with the Xq^- or X/22 translocation, while PGK would segregate with the opposite segment. Segregation data demonstrated that HPRT, G6PD, and the X/22 translocation were concordant. The two enzyme genes were assigned to the X long arm q22-qter region (Fig. 1). This confirmed the X long arm assignment of *HPRT* and *G6PD* genes obtained from AnLy (X/9) hybrids. Since *PGK*, *HPRT*, and *G6PD* genes were assigned to the X long arm distal to the q12 breakpoint in X/9 hybrids and *HPRT* and *G6PD* genes were assigned to the X long arm distal to the q22 breakpoint in X/22 hybrids, it is concluded that the *PGK* gene is located in the q12-q22 region of the X chromosome (Fig. 1).

Ricciuti and Ruddle have employed an X/14 (KOP) translocation in cell hybrids for mapping HPRT, G6PD, and PGK. They demonstrated the concordant segregation of the X/14 translocation, PGK, HPRT, and G6PD, suggesting assignment of the three genes to the long arm of the X in the q13-qter region (3, 22). This evidence is in agreement with the X/9 translocation data presented here. However, in another cell hybrid study by Grzeschik *et al.* (12), using the same X/14 translocation cells as Ricciuti and Ruddle (3, 22), it was concluded that the *PGK* gene was located on the long arm and the genes for HPRT and G6PD were on the short arm of the X. These conflicting reports demonstrated a need to use different X-autosome translocations to determine the X chromosome map. P. S. Gerald and G. Bruns (personal communication), employing cells with an X/19 translocation, have cell hybrid segregation data to support the assignment of *HPRT* and *G6PD* to the distal half (q24-qter) of the X long arm (28). Pearson *et al.* (31), in a preliminary report, have utilized cells with an X/22 (Breda) translocation, which also supports the assignment of *HPRT* and *G6PD* to the distal half (q23-qter) of the X long arm. These two findings are in agreement with the results of the X/22 (Indy) translocation hybrids presented here. Pearson *et al.* (31) have presented preliminary and unconfirmed evidence to suggest that *G6PD* is the most terminal marker of the three. In human X/3 translocation hybrids, *G6PD* was assigned to the terminal q26-qter region of the X long arm.

The somatic cell genetic data presented here support the assignment of the *PGK* gene to the q12-q22 region and the *HPRT* and *G6PD* genes to the q22-qter region of the X long arm (Fig. 1). These regional assignments are in good agreement with evidence from other laboratories. The combined observations lead to the conclusion that the gene order on the X long arm is centromere-*PGK-HPRT-G6PD*. It is conceivable that this somatic cell approach to determining gene order and regional mapping could be extended to the precise localization of a gene by making extensive use of an array of induced or inherited deletions and translocations along a specific chromosome.

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1. Ruddle, F. H. (1973) *Nature* **242**, 165–169.
2. New Haven Conference (1973) *First International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, X:3, 1974* (The National Foundation, New York); and (1974) *Cytogenet. Cell Genet.* **13**, 1–216.
3. Ricciuti, F. C. & Ruddle, F. H. (1973) *Nature* **241**, 180–182.
4. Creagen, R. P., Tan, Y. H., Chen, S., Tischfield, J. & Ruddle, F. H. (1974) *Cytogenet. Cell Genet.* **13**, 83–85.
5. McDougall, J. K., Kucherlapati, R. & Ruddle, F. H. (1973) *Nature New Biol.* **245**, 172–175.
6. Burgerhout, W. G., Someren, H. van & Bootsma, D. (1973) *Humangenetik* **20**, 159–162.
7. Lyon, M. F. (1972) *Biol. Rev.* **47**, 1–35.
8. Seegmiller, J. E., Rosenbloom, F. M. & Kelley, W. N. (1967) *Science* **155**, 1682–1684.
9. Davidson, R. G., Nitowsky, H. M. & Childs, B. (1963) *Proc. Nat. Acad. Sci. USA* **50**, 481–485.
10. Chen, S. H., Malcolm, L. A., Yoshida, A. & Giblett, E. R. (1971) *Am. J. Hum. Genet.* **23**, 87–91.
11. Shows, T. B. (1974) in *Somatic Cell Hybridization*, eds. Davidson, R. L. & de la Cruz, F. (Raven Press, New York), pp. 15–25.
12. Grzeschik, K. A., Allderdice, P. W., Grzeschik, A., Optiz, J. M., Miller, O. J. & Siniscalco, M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 69–73.
13. Beutler, E. (1972) in *The Metabolic Basis of Inherited Disease*, eds. Stanburg, J., Wyngaarden, J. & Frederickson, D. (McGraw-Hill, New York), pp. 1358–1388.
14. Valentine, W., Hsieh, H., Paglia, D., Anderson, H., Baughan, M., Jaffe, E. & Carson, O. M. (1969) *N. Engl. J. Med.* **280**, 528–534.
15. Littlefield, J. W. (1964) *Science* **145**, 709–710.
16. Cohen, M. M., Lin, C.-C., Sybert, V. & Orecchio, E. J. (1972) *Am. J. Hum. Genet.* **24**, 583–597.
17. Shows, T. B. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 348–352.
18. Klebe, R. J., Chen, T. R. & Ruddle, F. H. (1970) *J. Cell Biol.* **45**, 74–82.
19. Littlefield, J. W. (1966) *Exp. Cell Res.* **41**, 190–196.
20. Miggiano, V., Nabholtz, M. & Bodmer, W. (1969) "Heterospecific genome interaction," *Wistar Inst. Symp. Monogr.* **9**, 61–76.
21. Puck, T. T., Marcus, P. T. & Cieciura, S. J. (1956) *J. Exp. Med.* **103**, 273–281.
22. Ricciuti, F. C. & Ruddle, F. H. (1973) *Genetics* **74**, 661–678.
23. Patil, S. R., Marrick, S. & Lubs, H. A. (1971) *Science* **173**, 821–822.
24. Dev, V. G., Miller, D. A., Allderdice, P. W. & Miller, O. J. (1972) *Exp. Cell Res.* **73**, 259–262.
25. Paris Conference (1971) *Standardization in Human Cytogenetics. Birth Defects: Original Article Series VIII:7, 1972* (The National Foundation, New York).
26. Shows, T. B. (1972) *Biochem. Genet.* **7**, 193–204.
27. Lalley, P. A., Rattazzi, M. C. & Shows, T. B. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1569–1573.
28. Gerald, P. S. & Brown, J. A. (1974) *Cytogenet. Cell Genet.* **13**, 29–34.
29. Shows, T. B. & Brown, J. A. (1974) *Cytogenet. Cell Genet.* **13**, 146–149.
30. Shows, T. B. & Brown, J. A. (1975) *Cytogenet. Cell Genet.*, in press.
31. Pearson, P. L., van der Linden, A. G. J. M. & Hagemeijer, A. (1974) *Cytogenet. Cell Genet.* **13**, 136–142.
32. Kit, S., Dubbs, D. R., Piekarski, L. J. & Hau, T. C. (1973) *Exp. Cell Res.* **31**, 297–312.