Proc. Nat. Acad. Sci. USA
Vol. 69, No. 1, pp. 69–73, January 1972

Cytological Mapping of Human X-Linked Genes by Use of Somatic Cell Hybrids Involving an X-Autosome Translocation

(mouse/hamster/human X-linked markers)

K. H. GRZESCHIK‡*, P. W. ALLDERDICE†, A. GRZESCHIK‡,
J. M. OPITZ§, O. J. MILLER†¶, AND M. SINISCALCO‡‖

‡ Department of Genetics, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York, 10461; † Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, 10032; § Department of Pediatrics and Medical Genetics, University of Wisconsin Medical School, Madison, Wis., 53706; ¶ Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, N.Y., 10032; and ‖ Department of Genetics, Faculty of Sciences, University of Naples, Naples, Italy.

Communicated by Harry Eagle, October 4, 1971

ABSTRACT **Man–mouse and man–Syrian hamster somatic hybrid cell lines were prepared by fusion of mouse A9 or hamster TG2 cells, which are deficient in hypoxanthine-guanine phosphoribosyl transferase, with cells of a diploid fibroblastic strain, KOP-1, derived from a woman heterozygous for an X-autosome translocation. 61 clones were derived in nonselective medium and 85 sublines of these were derived in selective media: 53 in hypoxanthine–aminopterine–thymidine and 32 in 8-azaguanine. All three human X-linked markers studied, i.e., hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and phosphoglycerate kinase (EC 2.7.2.3), were present together, or absent together, in most of these clones and sublines. However, loss or retention of only phosphoglycerate kinase was occasionally observed, even in the absence of selective growth, while no evidence of separation of hypoxanthine-guanine phosphoribosyl transferase from glucose-6-phosphate dehydrogenase occurred. Cytological examination of eight man–hamster clonal lines by the quinacrine fluorescent technique showed that human phosphoglycerate kinase was only present when the translocation chromosome carrying most of the long arm of the X chromosome was present. The presence of human glucose-6-phosphate dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase was not related to the presence or absence of this chromosome, but appeared to be correlated with the presence of the other translocation chromosome.**

The X chromosome is the only human chromosome for which a large number of markers are known. However, only a few of these are expressed in cultured cells, and information on their linkage relationship is scanty. In particular, nothing is known about their precise chromosomal locations. The somatic hybridization of mouse or hamster cell lines that are deficient in hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) with human cell strains derived from patients with structural aberrations of the X chromosome could be a useful experimental tool for the cytological mapping of those human genes whose X-linkage is already established from independent evidence (1). Man–mouse (2), man–Chinese hamster (3, 4), and man–Syrian hamster (Grzeschik, K.-H, Grzeschik, A., & Siniscalco, M., unpublished data) somatic hybrid cells undergo a progressive reduction in the number of human chromosomes, but selective retention or elimination of the human X-linked *HGPRT* locus can be induced in hybrids of this kind by growth in hypoxanthine-aminopterine-thymidine (HAT) or 8-azaguanine selective media (5), respectively.

By use of a human cell strain derived from a female heterozygous for an X-autosome translocation in which the structurally abnormal portions of the X are genetically active, it should be possible to obtain reduced hybrids containing only one of the two segments of the structurally abnormal X chromosome, and to map specific loci to one or the other segment of the X. We wish to report the assignment of the structural gene for phosphoglycerate kinase (PKG) to the long arm of the X chromosome by the use of this approach.

The human parental cell used in this study was a diploid fibroblastic strain (KOP-1) derived from a woman with a balanced translocation between an X chromosome and a D-group chromosome (Opitz, J. M. & Pallister, P. D., unpublished data). Studies (6) have established that the breakpoint is in the proximal part of the long arm of the X, with the major portion of the long arm attached to the long arm of chromosome 14. Furthermore, the structurally normal X has been shown to be genetically inert, since it is consistently the late-replicating X and the source of the sex chromatin body. Since there is no evidence that an inactive X chromosome can be reactivated by somatic cell hybridization, the only human X-linked genes expressed in hybrid cells in which KOP-1 is the human parent should be those carried on one or the other X-autosome translocation chromosome.

Cells of this KOP-1 strain were hybridized, by use of Sendai virus-mediated fusion (7), with mouse A9 cells (8) and with Syrian hamster TG2 cells (kindly provided by John W. Littlefield). The A9 line has a modal number of 57, with about 22 biarmed chromosomes. The TG2 line has a modal number of 46, with about 38 biarmed chromosomes. Both A9 and TG2 cells are deficient in HGPRT and are therefore resistant to

Abbreviations: HGPRT, hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8.); G6PD, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); PGK, phosphoglycerate kinase (EC 2.7.2.3.); HAT, hypoxanthine–aminopterine–thymidine selective growth medium; MEM, Eagle's minimal essential medium; MEM-TH, MEM enriched with hypoxanthine and thymidine.

* On leave of absence from the Institut für Humangenetik, der Universität Munster, West Germany.

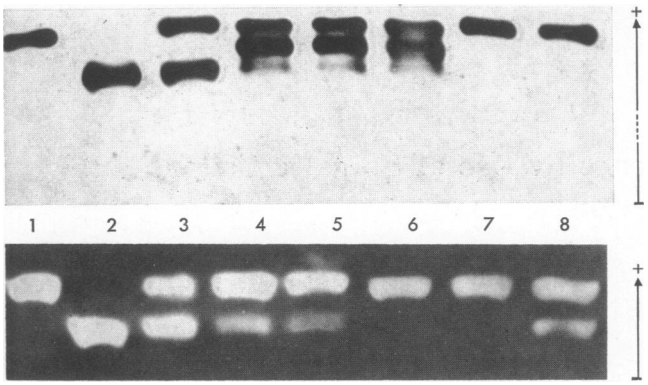

FIG. 1. G6PD (*upper panel*) and PGK (*lower panel*) isozyme patterns in some hybrid lines and controls. (*1*) Syrian hamster line, TG2. (*2*) Normal human fibroblastic strain. (*3*) Artificial mixture of equal amounts of cell lysates shown in channels *1* and *2*. (*4*) Hybrid KOP-1/TG2 clone 6 in MEM-TH soon after the appearance of the clone. (*5*) Same as the line shown in channel *4* after 4 weeks of further growth in MEM-TH. (6) Same as in channel *4* after 8 weeks of further growth in MEM-TH. (*7*) Subline in 8-azaguanine from hybrid clonal line shown in channel *6*. (*8*) Subline in 8-azaguanine from the hybrid clonal line shown in channel *4*.
Electrophoresis performed on cellogel (17).

8-azaguanine (9) and unable to grow in HAT medium (8). The fusion mixture was maintained in HAT medium for several weeks in order to get rid of the animal parental cells and to prevent the loss of the chromosome carrying the human *HGPRT* locus from any resultant hybrid cells. Two man–mouse (KOP-1/A9-1a and KOP-1/A9-2a) and two man–hamster (KOP-1/TG2-3 and KOP-1/TG2-3a) stock hybrid lines were first produced. Data from the man–hamster line have been pooled, since it is uncertain whether they arose from different fusion events.

The stock hybrid lines were frozen and stored within 7 weeks of the fusion. About 1 month later, they were thawed and cloned in regular medium supplemented with 17 μM thymidine and 100 μM hypoxanthine (MEM-TH medium) to prevent the massive death that usually follows the sudden transfer of HAT-cultured cells into regular medium. A total of 37 clones in MEM-TH were obtained from the man–mouse and 24 clones from the man–hamster stock hybrid cultures. Each clone was tested within 6 weeks for the presence of human X-chromosomal markers, HGPRT, glucose-6-phosphate dehydrogenase (G6PD), and PGK, and cytological studies were performed on four of the clones. The X-linkage of G6PD and HGPRT is well established. That of PGK has recently been proven by pedigree analysis (10, 11) and somatic-cell genetic studies (12). In addition, one of the pedigrees showed that PGK is not closely linked to G6PD (11).

One of the 37 man–mouse clones contained human PGK, but no human HGPRT or G6PD. Two of the 24 man–hamster clones showed the complementary pattern, with both human HGPRT and G6PD present and human PGK absent. In 36 of the 37 man–mouse clones, and 22 of the 24 man–hamster clones, all three human X-linked markers were still present, although in four man–mouse and five man–hamster clones there was a discrepancy, with different relative amounts of human G6PD and PGK, i.e., more human than mouse G6PD but more mouse than human PGK or vice versa (Table 1). We consider the variation in the relative amounts of enzyme products of X-linked genes to have the same significance as the complete loss of some of these products, since a clonal line showing this variation later underwent complete loss of human HGPRT and G6PD, with retention of human PGK (Fig. 1). Thus, the dissociation of the G6PD and PGK in the MEM-TH clones suggests the spontaneous loss of the *PGK* locus alone in a fraction of the cells, as if the loss occurred subsequent to the origin of the clone.

In order to test this idea, small inocula from each of the MEM-TH clonal lines were transferred into HAT selective media (at a 20-fold dilution of the cells). 15 of the 53 HAT sublines thus produced showed the same discrepancy in the retention of G6PD and PGK. Six of these came from the clonal lines that did not show such a discrepancy in the MEM-TH medium (Table 1). It thus appears that, under conditions in which the human structural locus for HGPRT is selectively

TABLE 1. *Mitotic separation of human X-linked markers in interspecific somatic cell hybrids involving a human strain with an X-autosome translocation*

	Clones in MEM-TH		Sublines in HAT			Sublines in 8-azaguanine	
Hybrid	No.	Phenotype*	+++	++(+)	++−	−−−	−−+
KOP-1/A9-1a	26	+++	23	1		None of 24 grew	
KOP-1/A9-2a	6	+++	4	2		3	3
	2	++(+)		2		1	1
	2	(++)+		1		1	
	1	−−+		1		1	
Total	11		4	6		6	4
KOP-1/TG2	17	+++	9	3		13	2
	3	++(+)		3		2	1
	2†	(++)+	1	1		2	
	2	++−		1	1	2	
Total	24		10	8	1	19	3

* Each + denotes the presence, and − the undetectability, of human HGPRT, G6PD, and PGK, respectively. Parentheses denote a diminished amount of product, relative to that expected.

† One of these clonal lines became −−+ after two months of continued propagation in the same MEM-TH medium (Fig. 1).

retained, the human *G6PD* locus is also retained but the human *PGK* locus is sometimes lost.

The use of 8-azaguanine selective medium for cells lacking HGPRT provides a more direct test of the hypothesis that the human *PGK* locus segregates independently from the human *HGPRT* locus in these hybrid cells. Small inocula from MEM-TH clonal lines containing all three human gene products were grown in the presence of 8-azaguanine. All of the 32 sublines produced in this way lacked human HGPRT and G6PD, but only 25 lacked human PGK, i.e., seven of the 32 still had human PGK (Table 1). From one of the man–mouse hybrid stock lines, KOP-1/A9-1a, on the other hand, it was impossible to obtain a viable subline in the 8-azaguanine selective medium (Table 1), a finding that could be explained by the assumption that the human *HGPRT* locus had been translocated to a mouse chromosome, whose loss could not be tolerated. The retention of human PGK in seven sublines despite the loss of both G6PD and HGPRT might reflect a high rate of separation of linked loci as a result of chromosome breakage, or may be the consequence of the localization of the *PGK* locus on a different translocation chromosome from that carrying *G6PD* and *HGPRT*. In either case, the locus for PGK must be rather distant from the other two loci.

In order to discriminate between these two possibilities, direct cytological examination was performed in the different types of clones by use of the quinacrine fluorescence technique (13). A typical partial fluorescent karyotype of the X-autosome translocation chromosomes and the normal X from a cell of the human parental line are shown in Fig. 2. The long t(14q,Xq) chromosome is easily distinguishable from the TG2 Syrian hamster chromosomes, and less easily distinguishable from the A9 mouse chromosomes, because of its distinctive quinacrine fluorescent banding pattern and the presence of satellites (Figs. 2 and 3). The short Xq- translocation chromosome is even less easily distinguishable from the one or two morphologically similar mouse or hamster chromosomes with similar fluorescent patterns that are sometimes present. Cytological analysis of 94 metaphase cells from three of the clones derived in the nonselective MEM-TH medium provides some evidence that the human *PGK* locus is carried by the long X-autosome translocation chromosome, and the *G6PD* and *HGPRT* loci by the other. Thus, the long translocation chromosome is clearly present in only one, and absent from at least 14 of 17 man–hamster hybrid cells from clone 8, which had lost human PGK but retained human G6PD and HGPRT. The presence or absence of the other translocation chromosome, Xq-, could not be determined in the 17 cells from this clone, but in two other clones, 6 and 10, both translocation chromosomes appeared to be present along with all three human X-linked gene products. Unfortunately, chromosome studies for the series of man–mouse hybrids were unsatisfactory because of difficulty in obtaining preparations of sufficient quality to permit distinguishing either X-autosome translocation chromosome from similar A9 chromosomes.

In view of the uncertainty of identification of the Xq- chromosome, and the much greater ease and accuracy of recognition of the longer t(14q,Xq) chromosome, 335 cells from five man–hamster hybrid sublines derived in selective media were scored under a microscope for the presence or absence of the t(14q,Xq) chromosome. This chromosome was present in a large proportion (80–90%) of the cells from two sublines in which human PGK was present, and virtually absent in cells

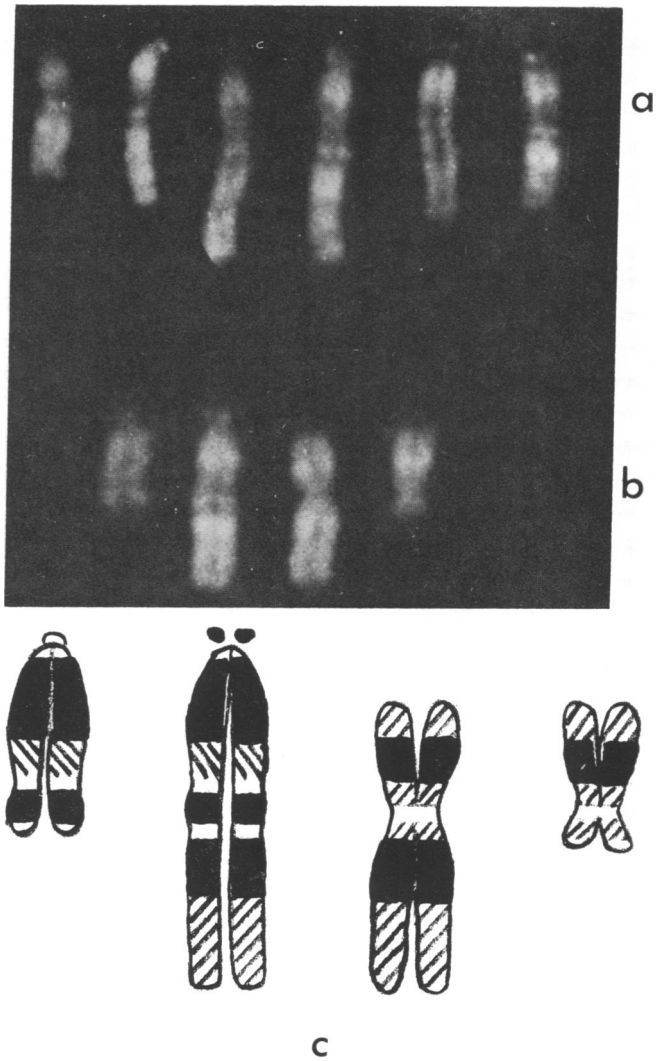

FIG. 2. (*a*) Translocation chromosome t(14q,Xq) from six man–Syrian hamster hybrid cells. (*b*) Partial karyotype of chromosomes 14, t(14q,Xq), X, and Xq- from the human parent. (*c*) Sketches of chromosomes in *b* and in the same order.

from the three sublines in which human PGK was absent (Table 2). There was no correlation between the presence or absence of the t(14q,Xq) chromosome and the presence or absence of either human G6PD or HGPRT. However, in the relatively small proportion of the 335 cells from the five sublines that were photographed, a good correlation was found between the presence of the presumptive Xq- chromosome and the presence of human G6PD and HGPRT. Thus, cells derived from clone 6 in 8-azaguanine contained no presumptive Xq- chromosome and no human G6PD or HGPRT, whereas many of the cells derived from clone 8 and clone 10 in HAT did show such a chromosome and both human G6PD and HGPRT.

Chromosome studies revealed an additional interesting finding. The man–mouse hybrids appeared to have been derived from fusions of a single human with a single mouse cell, whereas the man–hamster hybrids showed a higher multiplicity of hamster chromosomes, probably indicating fusion of two hamster cells with a single human cell. The relative amounts of human and animal enzymes present in the two classes of hybrids are consistent with this interpretation.

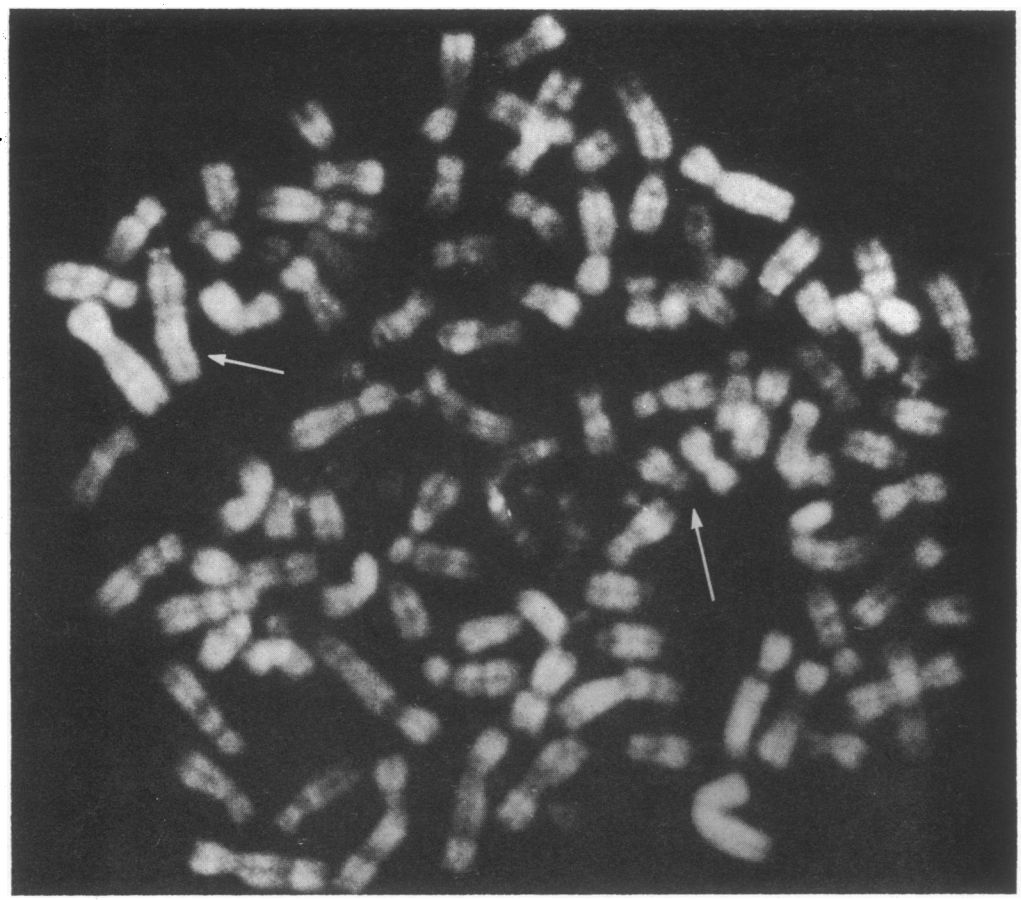

FIG. 3. A man–Syrian hamster hybrid cell. *Arrows* point to the human t(14q,Xq) and presumptive Xq- chromosomes. Note the large number of chromosomes, almost all of hamster origin.

DISCUSSION

The use of man–mouse or man–hamster somatic cell hybrids for the determination of human linkage groups and cytological maps is based on the assumption that a single human gene locus, and the chromosomes carrying it, can be selectively retained by use of the appropriate selective media, while the other human chromosomes will be lost, except for an occasional randomly retained chromosome. In the present study, *both* portions of the X chromosome appear to have been retained in the majority of hybrid clones maintained in HAT or MEM-TH media, and both lost in medium with 8-azaguanine. However, separation of the X-linked markers was observed in 11 exceptional clones, four of which arose in the absence of any selective procedure. When some of these, together with suitable controls, were submitted to cytological study, a close correlation between the presence of the t(14q,Xq) chromo-

TABLE 2. *Correlation between the presence of human X-linked markers and the long t(14q,Xq) translocation chromosome in clones of the KOP-1/TG2 hybrid line and six sublines derived in selective media*

Clone no.	Culture medium	Human X-linked markers			No. of metaphases with t(14q,Xq)	
		HGPRT	G6PD	PGK	Present	Absent
6	MEM-TH	+	+	+	Not studied	
	8-azaguanine	−	−	+	56	14
8	MEM-TH	+	+	−	1	14
	HAT	+	+	−	5	85
	8-azaguanine	−	−	−	0	75
10	MEM-TH	+	+	(+)	20	5
	HAT	+	+	(+)	39	13
12	MEM-TH	+	+	(+)	Not studied	
	HAT	+	+	(+)	45	5
	8-azaguanine	−	−	trace	4	46

some and human PKG became apparent, thus indicating that the *PGK* locus is on the long arm of the X chromosome. The absence of correlation between the t(14q,Xq) chromosome and human G6PD or HGPRT probably indicates that neither locus is carried by the portion of the long arm of the X carried by the t(14q,Xq) chromosome, i.e., that *G6PD* or *HGPRT* loci are carried by the Xq- chromosome: either on the short arm or the very most proximal portion of the long arm, or one on either side of the centromere.

The mitotic separation of two human X-linked markers in man–mouse somatic cell hybrids (HGPRT-deficient mouse x normal human cells) was reported earlier by our group and related to the occurrence of *in vitro* X-chromosome breakage (14). However, this event has thus far been observed only after long propagation (over 8 months) of the hybrid cells in the HAT selective medium, probably because the rare exceptional cells, with their reduced number of human chromosomes, slowly overgrow all the others.

Recently, we also (12) showed that separation of X-linked loci did not occur among 175 clones derived from somatic cell hybrids of the same type that had been transferred into regular (nonselective) medium soon after the isolation of hybrid colonies in HAT after the fusion experiment. The same set of data disclosed a regular correlation between the amounts of human G6PD and PGK, relative to the amounts of the corresponding enzymes of mouse origin.

Thus, the high incidence of exceptions (12 out of 61) among the clones grown in MEM-TH, in spite of the absence of long propagation in the HAT-selective medium, suggests that the mitotic separation of human PGK from human HGPRT and G6PD is in this case due to the preexisting cytological aberration present in the human parent, namely the localization of PGK and of HGPRT and G6PD at different portions of the X chromosome involved in the translocation; that is, PGK is carried by the t(14q,Xq) chromosome and the other two loci by the other translocation chromosome at an appreciable distance from one another, as suggested by independent evidence (14, 15). This interpretation is further strengthened by the occurrence of 11 additional and independent exceptions in the short-term cultures of MEM-TH clones, in HAT or 8-azaguanine selective media (Table 1).

If this interpretation is correct, we must also conclude that, for unknown reasons, both portions of the X chromosomes tend to be retained in interspecific hybrids under the selective conditions used in our experiments. The observations of Ruddle *et al.* (16) are compatible with this interpretation. They observed retention of all these three X-linked markers in a series of 15 primary hybrid lines, six derived from the fusion of KOP-1, and nine from the fusion of KOP-2 (a strain derived from the son of the source of KOP-1, who also carries the translocation chromosomes) with HGPRT-deficient mouse RAG cells. None of the hybrid lines studied by Ruddle and his collaborators were cloned or subcultured in different selective media, i.e., conditions in which separation of markers are more easily observed. Nevertheless, their results fit well with our own finding that 80% of the lines retained all three markers. This is in accord with other evidence (our unpublished data) that chromosome loss in interspecific somatic hybrids may not be entirely random. Selective retention of both parts of a chromosome involved in a translocation could be a barrier to the detection of new linkage groups by this method, unless extensive cloning in regular media, followed by growth in selective media, is applied. Under these conditions, however, the use of hybrids of the type that we have described provides a powerful tool to determine the locations of genes along a specific chromosome.

We thank Dr. P. D. Pallister for providing access to his patient, and Drs. B. Horecker and Salome G. Waelsch for making available facilities in the Division of Biological Sciences at the Albert Einstein College of Medicine, where M.S. was a Visiting Professor, 1970–71. This work was supported in part by the USPHS under Grants GM 11301, 13415, 18153, and CA 12504, and by a grant from the National Foundation-March of Dimes. Request for reprints should be addressed to M. S., Department of Genetics, Albert Einstein College of Medicine, 1300 Eastchester Road and Morris Park Avenue, Bronx, N.Y. 10461. GKH is a recipient of a fellowship of the Deutsche Forschungsgemeinschaft.

1. Siniscalco, M. (1970) "Control Mechanisms" in *Expression of Cellular Phenotype*, ed. Padykula, M. A. (Academic Press, N.Y.), p. 205–221.
2. Weiss, M. C. & Green, H. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 1104–1111.
3. Kao, F.-T. & Puck, T. T. (1970) *Nature* **228**, 329–332.
4. Westerveld, A., Meera Khan, P., Visser, R. P. L. S. & Bootsma, D. (1971) *Nature New Biol.* **234**, 20–24.
5. Nabholz, M., Miggiano, V. & Bodmer, W. (1969) *Nature* **223**, 358–363.
6. Allderdice, P. W., Miller, O. J., Klinger, H. P., Opitz, J. M. & Pallister, P. D. (1971) *Proc. Int. Congr. Hum. Genet., 4th* 14–15.
7. Harris, H. & Watkins, J. F. (1965) *Nature* **205**, 640–646.
8. Littlefield, J. W. (1966) *Exp. Cell Res.* **41**, 190–196.
9. Szybalska, E. H. & Szybalski, W. (1962) *Proc. Nat. Acad. Sci. USA* **48**, 2026–2034.
10. Valentine, W. N., Hsieh, H.-S., Paglia, D. E., Anderson, H. M., Baughan, M. A., Jaffe, E. R. & Garson, O. M. (1969) *N. Engl. J. Med.* **280**, 528–534.
11. Chen, F. H., Malcolm, L. A., Yoshida, A. & Giblett, E. R. (1971) *Amer. J. Hum. Genet.* **23**, 87–91.
12. Meera Khan, P., Westerveld, A., Grzeschik, K. H., Deys, B. F., Garson, O. M. & Siniscalco, M., *Amer. J. Hum. Genet.*, in press.
13. Caspersson, T., Zech, L., Johansson, C. & Modest, E. J. (1970) *Chromosoma* **30**, 215–227.
14. Miller, O. J., Cook, P. R., Meera Khan, P., Shin, S. & Siniscalco, M. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 116–120.
15. Nyhan, W. L., Bakay, B., Connor, J. D., Marks, J. F. & Keele, D. K. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 214–218.
16. Ruddle, F. H., Chapman, V. M., Ricciuti, F., Klebe, R., Murnane, M. & Meera Khan, P. (1971) *Nature New Biol.* **232**, 69–73.
17. Meera Khan, P. (1971) *Arch. Bioch. Biophys.* **145**, 470–483.