Regional gene assignment of human porphobilinogen deaminase and esterase A₄ to chromosome 11q23 → 11qter

(gene mapping/somatic cell hybrids/Friend erythroleukemia cells/X chromosome-autosome translocation/acute intermittent porphyria)

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ABSTRACT The regional gene assignments for human porphobilinogen deaminase (PBGD; EC 4.3.1.8) and esterase A₄ (ESA4; EC 3.1.1.1) on chromosome 11 have been determined with somatic cell hybridization and immunologic, electrophoretic, and cytogenetic techniques. Dimethyl sulfoxide-induced erythroid differentiation of hybrid clones derived from the fusion of tetraploid Friend murine erythroleukemia (2S MEL) cells deficient in thymidine kinase and human Lesch-Nyhan fibroblasts (HLN) deficient in hypoxanthine phosphoribosyltransferase (HPRT⁻; EC 2.4.2.8) were examined for expression of human PBGD, ESA4, and lactate dehydrogenase A (LDHA; EC 1.1.1.27). Human PBGD was detected by rocket immunoelectrophoresis with rabbit anti-human PBGD IgG and by isoelectric focusing. The human chromosome complement of each clone was determined by cytogenetic and enzyme marker analyses. Of the five primary 2S MEL-HLN clones examined, three were positive for human PBGD. These were subcloned to yield a total of 10 secondary, tertiary, or quaternary clones. Analyses of these subclones permitted the regional assignment of human PBGD and ESA4 to the long arm of chromosome 11. Finer regional assignment of the loci for human PBGD and ESA4 was facilitated when two 2S MEL (HPRT⁻)-human fibroblast (HX/11) hybrids, each containing the X chromosome-autosome translocation (der11), t(X;11)(q25-26;q23) as the only human chromosome, were examined for expression of human PBGD, ESA4, and LDHA. One clone, HX/11-2, contained the intact X/11 translocated chromosome; in the other, HX/11-3, 11p was deleted, and the human X/11 derivative was translocated onto a mouse chromosome. HX/11-2 expressed human LDHA, but HX/11-3 did not, verifying that the latter human 11/X derivative did not include 11pter → 11p12; PBGD and ESA4 were not detected in either hybrid. These results confirm the location of the gene for human PBGD on chromosome 11 and establish the assignment of the loci for PBGD and ESA4 in the region 11q23 → 11qter.

Porphobilinogen deaminase (PBGD; EC 4.3.1.8) is the third enzyme in the mammalian heme biosynthetic pathway (1, 2). The enzyme sequentially deaminates 4 mol of the monopyrrole, porphobilinogen, to form the linear tetrapyrrole, hydroxymethylbilane, which is then nonenzymatically cyclized to uroporphyrinogen I (3). In the presence of uroporphyrinogen III co-synthase, hydroxymethylbilane is rapidly converted to the uroporphyrinogen III isomer (3–5). In 1980, we reported purification of homogeneous PBGD from human erythrocytes and the finding that the enzyme occurs in five forms, the free monomeric enzyme, and four stable enzyme-substrate intermediates (6). These intermediates represent the mono-, di-, tri- and tetrapyrrole-enzyme complexes in the stepwise conversion of the monopyrrole to the linear tetrapyrrole (6).

Half-normal activity of PBGD is the primary enzymatic defect in acute intermittent porphyria, a dominantly inherited porphyria (7–10). During acute attacks of this disease, hepatic δ -aminolevulinate synthase (ALAS; EC 2.3.1.37) the first enzyme in the heme biosynthetic pathway, is induced; δ -aminolevulinate and porphobilinogen accumulate because of the deficient PBGD activity; and inadequate amounts of heme are produced to retain the negative feedback control on ALAS (2).

Current interest has focused on the genetic and metabolic regulation of heme biosynthesis (2, 11–17). Because knowledge of the genetic organization of the structural genes for each of the heme biosynthetic enzymes might provide insight into the regulation of this pathway in man, we have directed our efforts to determine the chromosomal localization of the human gene loci for these enzymes. While the studies reported here were in progress, Meisler *et al.* assigned the gene for human PBGD to chromosome 11, using hybrids derived from human fibroblasts and mouse RAG, LM/TK, or A9 cells and using isoelectric focusing for detection of human PBGD (18). The genes for human lactate dehydrogenase A (LDHA; EC 1.1.1.27), esterase A₄ (ESA4; EC 3.1.1.1) and the β -globin gene complex also have been assigned to chromosome 11 (19–22). Because the specific activity of PBGD in human fibroblasts was about one-fifth that of human erythrocytes (23), we examined the expression of PBGD in tetraploid Friend murine erythroleukemia (2S MEL)-human Lesch-Nyhan fibroblast (HLN) hybrid lines, which previously were shown to selectively activate the synthesis of human β -globin but not δ -globin mRNA after dimethyl sulfoxide induction of hemoglobin production (24). Enzyme and cytogenetic analyses of homogeneous subclones derived from 2S MEL-HLN hybrids and 2S MEL-human fibroblast (HX/11) hybrids containing a single human chromosome 11 derivative (an X/11 translocation) (25), permitted the regional assignments of the human genes encoding PBGD, ESA4, peptidase S (PEPS) and phosphoglucomutase 2 (PGM2).

MATERIALS AND METHODS

Cells and Cell Culture. Two different sets of hybrid cells were used. The first was derived from the fusion of thymidine kinase-deficient 2S MEL cells with HLN, as described (24, 26). These hybrid lines were positive for human LDHA and produced human β -globin mRNA (24). Five primary 2S MEL-HLN hybrid clones were initially examined; three of these

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Abbreviations: ESA4, esterase A₄; G6PD, glucose-6-phosphate dehydrogenase; HLN, human Lesch-Nyhan fibroblasts; HPRT, hypoxanthine phosphoribosyltransferase; LDHA, lactate dehydrogenase A; 2S MEL, tetraploid Friend murine erythroleukemia; PEPS, peptidase S; PGM2, phosphoglucomutase 2; PBGD, porphobilinogen deaminase; ESD, esterase D; ALAS, δ -aminolevulinate synthase.

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strongly expressed human PBGD and were selected for subcloning (Table 1). Hybrids were subcloned by plating single cells into individual wells of a 96-well microtiter plate (Costar, Cambridge, MA). Of the four secondary clones generated, two derived from XX-8, [one positive (H21) and one negative (A31) for human PBGD] were chosen for further subcloning. Three cytogenetically homogeneous quaternary clones (H21-H7-2, -10, and -21) and two homogeneous tertiary clones (A31-18 and A31-23) were informative. The hybrid clones and the parental 2S MEL and HLN cells were cultured by standard techniques in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum, 2 mM L-glutamine, and 100 units of streptomycin and penicillin per ml. For subcloning, the medium was supplemented with 20% fetal calf serum.

The second set of hybrid clones (2S MEL-HX/11) was derived (L. Sanders-Haigh) from the fusion of hypoxanthine phosphoribosyltransferase-deficient (HPRT⁻) 2S MEL cell line with human fibroblasts (22, 25) carrying an X/11 translocation [46,X,t(X;11)(q25-26;q23)] (Genetic Cell Repository, GM 3552). Two hybrid clones (HX/11-2 and HX/11-3) from two independent fusions were grown in Iscove's minimal essential media (GIBCO) with 10% fetal calf serum/4 mM L-glutamine/0.8 mM glycine/0.1 mM hypoxanthine/0.1 μ M aminopterin/16 μ M thymidine.

Erythroid differentiation was induced in 2S MEL cells and 2S MEL-human fibroblast hybrids with 2% (vol/vol) dimethyl sulfoxide (24, 26). Induction conditions for the 2S MEL-HX/11 hybrid cells were identical except that Iscove's minimal essential medium without hypoxanthine, aminopterin, and thymidine was used. Prior to harvesting, aliquots were taken for determination of cell viability, number, and benzidine staining (26).

Cells with greater than 95% viability and 45–80% benzidine positivity were harvested by centrifugation at $700 \times g$ for 20 min at 4°C in a Sorvall GS-3 rotor. For enzyme studies, the cell pellet was washed three times in Dulbecco's phosphate-buffered saline (GIBCO). Cells (5×10^6) for PBGD analysis were resuspended in 1.0 ml of 1.0 mM potassium phosphate buffer/1 mM MgCl₂/1 mM dithiothreitol/0.05% Triton X-100. For PBGD activity assay and rocket immunoelectrophoresis, cells were lysed by three 10-sec pulses (output of 7) using a Branson Sonifier 200 (Branson Sonic Power, Danbury, CT). The lysate was then centrifuged at $35,000 \times g$ for 20 min in a Sorvall SS-34 rotor; the supernatant was collected and stored at -20°C. The mean PBGD specific activities (eight experiments) in induced and uninduced hybrid cells were 1.0 and 3.7 units/mg of protein, respectively. For PBGD isoelectric focusing, cell lysates were treated as above and then freeze-thawed twice in a dry ice/acetone slurry. The lysates were then heated at 60°C for 30 min and subsequently centrifuged at $35,000 \times g$ for 10 min in a Sorvall SS-34 rotor. The supernatant was collected and immediately subjected to isoelectric focusing as described below. For enzyme marker analyses, the washed cells were resuspended in distilled water (5×10^8 cells per ml), lysed by sonication, and centrifuged as described above.

Marker Enzyme and Cytogenetic Analyses. Marker enzymes for specific human chromosomes were determined by established electrophoretic methods (27–31). For cytogenetic analyses, metaphase spreads were prepared by standard techniques (32), and the chromosomes were banded to distinguish mouse from human chromosomes with the Giemsa 11 technique (33) and then destained and banded with quinacrine hydrochloride (34) or trypsin/Giemsa (32) techniques. Hybrid clones were subcloned until judged homogeneous by the examination of at least 30 metaphase spreads. It was notable that a high frequency of chromosomal breakage and rearrangement was ob-

served in 2S MEL-HLN hybrids. Human PBGD assays, marker-enzyme electrophoresis, and cytogenetic analyses were performed on cell hybrids harvested from the same passage.

Detection of Human PBGD. PBGD activity was determined in cell lysates by the methods of Anderson and Desnick (6). One unit of enzymatic activity represents 1 nmol of uroporphyrin formed per hr at 37°C. Immunoreactive human PBGD was detected with a monospecific rabbit anti-human PBGD IgG raised against homogeneous PBGD-A as described (23).

Rocket immunoelectrophoresis for human PBGD was carried out as described (23), except that the 1% agarose gel solution contained 7.5 μ l of rabbit anti-human PBGD IgG. After immunoelectrophoresis of lysates containing 3–5 units/ml of PBGD activity from human erythrocytes, murine erythrocytes, 2S MEL cells, 2S MEL-HLN hybrid cells, or 2S MEL-HX/11 hybrid cells, rocket immunoprecipitates were only observed for PBGD from the human sources. This method was sensitive enough to detect human PBGD in mixtures of human and 2S MEL PBGD when less than 5% of the total activity was human.

Isoelectric focusing of PBGD was carried out as described (23) by using polyacrylamide slab gels with a pH gradient of 3.5–9.5 prepared with LKB Ampholines as described by the manufacturer (35). Gels were prefocused lengthwise for 1 hr at 800 V (constant voltage) at 4°C. The anode contained 1 M H₃PO₄ and the cathode contained 1 M NaOH. Aliquots of hybrid lysates (200–300 μ l) were pipetted onto Whatman no. 17 paper strips which were placed 1 cm from the cathode. Gels were focused for 6 hr at 800 V (constant voltage). Then gels were stained for PBGD activity, and the pH gradient was determined as described (23). Isoelectric focusing of mixtures, each containing varying proportions of mouse and human PBGD, revealed that this method was sensitive enough to detect human PBGD when less than 5% of the total activity was human.

RESULTS

Phenotypic Expression of Human PBGD, ESA4, and LDHA in Parental and Hybrid Lines. Expression of human PBGD in parental and hybrid lines was detected by rocket immunoelectrophoresis by using monospecific rabbit anti-human PBGD IgG and by isoelectric focusing. In Fig. 1, a representative rocket immunoelectrophoretic gel shows rockets for a human erythrocyte lysate and hybrid subclones XII-4-20 and XX-8-H21-H7-10, the latter of which contained only the human chromosomal rearrangement t(4;11). Note that rockets were not observed for lysates of 2S MEL cells or hybrid clone HX/11-2. Human PBGD expression in hybrid cells was also shown by isoelectric focusing (Fig. 2). The five PBGD intermediates, designated A–E (6), from human erythrocytes focused at pI values

FIG. 1. Rocket immunoelectrophoresis of human PBGD in cell lysates. Lanes: 1 and 6, human erythrocytes; 2, 2S MEL cells; 3 and 4, 2S MEL-HLN subclones XX8-H21-H7-H10 and XII-4-20; 5, 2S MEL-H11/X-2 hybrid cells.

FIG. 2. Isoelectric focusing of human and murine PBGD in cell lysates. Lanes: 1, human erythrocytes; 2 and 3, 2S MEL-HLN XX-8 hybrid subclones H21-H7-10 and A31-18, respectively; 4, 2S MEL-HX/11-3 hybrid; 5, 2S MEL cells. Note that human PBGD was detected only in clone H21-H7-10.

of about 6.6, 6.3, 6.1, 5.9, and 5.8, respectively. The PBGD intermediates from 2S MEL cells focused at pI values of 5.8, 5.7, 5.6, 5.5, and 5.4. The pI values varied within ± 0.1 pH from run to run. Typically, two major murine activity bands were observed at pI values 5.8 and 5.3 in all hybrid clones. Hybrids were scored positive for human PBGD when at least one human activity band, typically at pI 6.6, was observed in addition to those corresponding to the murine PBGD intermediates.

Segregation of PBGD, ESA4, and LDHA in Hybrid Cells. Table 1 shows the human chromosomal constitution of the 2S MEL-HLN and 2S MEL-HX/11 hybrid clones used for these analyses. Table 2 indicates the expression of human PBGD, ESA4, LDHA, PEPS, PGM2, esterase D (ESD), and glucose-6-phosphate dehydrogenase (G6PD). From the original 2S MEL-HLN hybrid line, XX-8, two cytogenetically homogeneous tertiary clones (A31-18 and A31-23) and three homogeneous quaternary clones (H21-H7-2, -10, and -21) provided pertinent information. Note that the tertiary subclone H21-H7 contained only two chromosomes that were classified as human by G-11 staining. These chromosomes were identified as 9q and t(4;11) by quinacrine banding. The quaternary clone H21-H7-10, which expressed human PBGD (Table 2), contained only the single human chromosome derivative t(4;11)(4pter $\rightarrow$ 4q21;11q13 $\rightarrow$ 11qter), shown in Figs. 3 and 4. The human PBGD gene was localized to the region 11q because the primary clone XIX-25 contained an intact chromosome 4 but was negative for PBGD. Furthermore, the primary clone XII-4 and its subclone XII-4-20 contained a mouse/human translocation (Fig. 3), in which the human derivative was 11q11 $\rightarrow$ 11qter, and were positive for human PBGD. Assignment of PBGD to human chromosome 11p was further excluded by findings in the secondary and tertiary subclones of XX-8, A31 and A31-23, which were positive for LDHA and for the short arm of 11 (Fig. 3) but were negative for PBGD. In addition, the primary clone XIX-10 contained a human-human translocation t(11;13)(11p12 $\rightarrow$ 11qter;13q14 $\rightarrow$ 13qter) (Fig. 3). This clone was positive for human PBGD, ESA4, and ESD, the latter gene having been localized on 13q (36).

Examination of the 2S MEL-HX/11 hybrids provided finer localization of the gene for human PBGD. Hybrid 2S MEL-

Table 1. Human chromosomal constitution of 2S MEL-human fibroblast hybrids

Hybrid clones	Human chromosomes	Human-human translocations
2S MEL-HLN:		
Primary:		
XIX-25	3,4,6,8,12,15,19	—
XVIII-5	10,14	—
XIX-10	4,6,14,19	11;13q*
XII-4	10,11,14	11q/M†
XX-8	4,6,8,9,11,12,13,14,15,18,21,22	4p11q‡,11p4q§
Secondary:		
XIX-10-A1	4,14,9	—
XII-4-20	14	11q/M†
XX-8-H21	4,9,13,15,21	4p11q‡
XX-8-A31	4,9q,15,21	11p4q§
Tertiary:		
XX-8-A31-18	15,21	11p4q§
XX-8-A31-23	4,9q,15,21	11p4q§
XX-8-H21-H7	9q	4p11q‡
Quaternary:		
XX-8-H21-H7-2	9q	—
XX-8-H21-H7-10	—	4p11q‡
XX-8-H21-H7-21	9q	—
2S MEL-HX/11:		
Primary:		
HX/11-2		X/11¶
HX/11-3		X/11/M

The human chromosomal constitution of 2S MEL-HLN primary hybrids was heterogeneous (50 metaphase spreads were examined). Human-human and human-mouse rearrangements were also present but were difficult to identify cytogenetically. Therefore, in addition to cytogenetic analyses, the presence of the human chromosomes in all hybrid lines was based on marker-enzyme data (27–31). Identification of the human chromosomes present in all secondary, tertiary, and quaternary clones was based on enzyme-marker and cytogenetic analyses, which corresponded in every case.

* 11;13q = t(11;13)(11p12 $\rightarrow$ 11qter;13q14 $\rightarrow$ 13qter).

† 11q/M = (der11)t(11;M)(11q11 $\rightarrow$ 11qter;murine).

‡ 4p11q = t(4;11)(4pter $\rightarrow$ 4q21;11q13 $\rightarrow$ 11qter).

§ 11p4q = t(11;14)(11pter $\rightarrow$ 11p12;4q21 $\rightarrow$ 4qter).

¶ X/11 = (der11),t(X;11)(q25 or 26;q23).

|| X/11/M = (der11),t(X;11)(q25 or 26;q23;murine).

HX/11-2 contained the human-human X/11 translocation (der11),t(X;11)(q25-26;q23) as the only human chromosome. In the 2S MEL-HX/11-3 hybrid, the human 11p was deleted, and the human X/11 derivative was translocated onto a large murine chromosome (Figs. 3 and 4). Because both of the X/11 translocations in these hybrids were deficient for the region 11q23 $\rightarrow$ qter and because neither hybrid expressed human PBGD, the gene for this enzyme was localized to this region.

Analogously, the structural gene for human ESA4 was found to segregate concordantly with the human PBGD gene in all of the hybrid clones (Table 2). Thus, the gene for human ESA4 also can be assigned to the region 11q23 $\rightarrow$ 11qter.

These hybrid clones also provided information for the finer regional localization of the genes for human PEPS and PGM2 on chromosome 4. H21-H7 quaternary subclones that contained the human-human translocation t(4;11) expressed human PEPS, thus tentatively localizing the structural gene to the region 4pter $\rightarrow$ 4q21. Subclones of A31 and H21-H7 segregated for human PGM2 (Table 2). All tertiary and quaternary clones derived from H21-H7 were negative for human PGM2, suggesting that this locus was excluded from the region 4pter $\rightarrow$ 4q21 (proximal portion). However, all A31 subclones contained 4q21 (distal portion) $\rightarrow$ 4qter and expressed human PGM2. The

Table 2. Segregation of human PBGD and enzyme markers for human chromosomes 4, 11, 13, and X in 2S MEL-human hybrids

Clone	Human PBGD		Human chromosome						
	RIE*	IEF*	11	4	13	X			
			LDHA	ESA4	PEPS	PGM2	ESD	G6PD	
2S MEL-HLN:									
Primary:									
XIX-25	-	-	-	-	+	+	-	-	
XVIII-5	-	-	-	-	-	-	-	-	
XIX-10	+	+	+	+	+	+	+	-	
XII-4	+	+	-	+	-	-	-	-	
XX-8	+	+	+	+	+	+	+	-	
Secondary:									
XIX-10-A1	-	-	-	-	+	+	-	-	
XII-4-20	+	+	-	+	-	-	-	-	
XX-8-H21	+	+	-	+	+	-	+	-	
XX-8-A31	-	-	+	-	-	+	-	-	
Tertiary:									
XX-8-A31-18	-	-	+	-	-	+	-	-	
XX-8-A31-23	-	-	+	-	+	+	-	-	
XX-8-H21-H7	+	+	-	+	+	-	-	-	
Quaternary:									
XX-8-H21-H7-2	-	-	-	-	-	-	-	-	
XX-8-H21-H7-10	+	+	-	+	+	-	-	-	
XX-8-H21-H7-21	-	-	-	-	-	-	-	-	
2S MEL-HX/11:									
Primary:									
HX/11-2	-	-	+	-	-	-	-	+	
HX/11-3	-	-	-	-	-	-	-	+	

* Human PBGD was specifically detected by rocket immunoelectrophoresis (RIE) and isoelectric focusing (IEF). See text for experimental details.

† 2S MEL-HX/11 hybrids were cultured in hypoxanthine/aminopterin/thymidine media to ensure that the X autosomal translocation was retained.

regional gene assignments for PBGD and ESA4 and the tentative assignments for PEPS and PGM2 are diagrammatically represented in Fig. 4.

DISCUSSION

Several seminal studies have indicated that the heme biosynthetic pathway is under tight regulatory control. The demonstration that hepatic ALAS, the first and rate-limiting enzyme in the pathway, undergoes negative feedback repression [at the transcriptional level (14-16)] by heme (37-40) and the finding that the heme biosynthetic enzymes are sequentially induced during erythroid differentiation of diploid MEL cells (17) have stimulated investigations to elucidate the mechanisms respon-

FIG. 3. Partial karyotypes of the human chromosomes in 2S MEL-human fibroblast hybrids. (A, B, and C) The translocation is shown between normal human chromosomes 4 (left) and 11 (right) as observed by quinacrine staining. The rearrangement in hybrid XX-8-H21-H7-10 was $t(4;11)$, $(4pter \rightarrow 4q21; 11q13 \rightarrow 11qter)$ (A), in hybrid XX-8-A31-18 was $t(4;11)$, $(4q21 \rightarrow 4qter; 11pter \rightarrow 11q13)$ (B), and in hybrid HX/11-2 was $t(11;X)$, $(11pter \rightarrow 11p23; Xq25 \text{ or } 26 \rightarrow Xter)$ (C). (D) In HX/11-3, a mouse-human translocation occurred at 11q11 as shown by Giemsa 11 staining. (E) In XII-4-20, a mouse-human translocation occurred at 11q11, as shown by Giemsa 11 (left) and trypsin/Giemsa (center) staining compared to an intact human chromosome 11 (right). (F) In XIX-10, the rearrangement $t(11;13)$, $(11p12 \rightarrow 11qter; 13q14 \rightarrow 13qter)$ was observed, by Giemsa 11 (left) and trypsin/Giemsa (right center) staining. Trypsin/Giemsa-stained intact human chromosome 11 (left center) and 13 (right) are shown for comparison.

sible for the control of heme biosynthesis. Because insights into the organization of the genes controlling human heme biosynthesis can be obtained from somatic cell hybridization studies, we employed these techniques to elucidate the chromosomal organization of the human heme biosynthetic enzymes. Tetraploid MEL-human fibroblast hybrids were chosen for these studies because the efficiency of hemoglobin inducibility was greatly increased in 2S MEL-human hybrids compared to that in 1S hybrids (26). Furthermore, the induced 2S MEL-human fibroblast hybrids expressed significantly greater amounts of PBGD than did uninduced 2S MEL cells or human fibroblasts.

During the course of these studies, Meisler *et al.* reported that the gene for human PBGD was localized to chromosome 11 (20). The results described here confirm the assignment of the structural gene for PBGD to chromosome 11 and further localize the gene to the region $q23 \rightarrow qter$.

It was notable that human ESA4 cosegregated with human

FIG. 4. Diagrammatic representation of the regional gene assignments for human PBGD, ESA4, PEPS, and PGM2 on chromosomes 4 and 11. Chromosomes 4, 11, and X and the pertinent translocations in the 2S MEL-human fibroblast hybrid clones are shown, as observed with the Giemsa staining technique. PBGD and ESA4 were localized to the region $11q23 \rightarrow 11qter$ and $11q22 \rightarrow 11q23$, respectively. PEPS and PGM2 were assigned to $4pter \rightarrow 4q12$ and $4q21$, respectively.

PBGD in all hybrid clones (Table 2). Previously, ESA4 was assigned to the region 11cen → 11q22 (21) based on three independent studies (41–43). Our finding that HX/11 subclones were negative for ESA4 implies that the locus had been deleted from this translocation. Although the breakpoint of the X/11 translocation has been interpreted to be 11q23 (22, 25), it is not possible to exclude the distal portion of band q22 as the breakpoint. Thus, our findings and those of previous reports (41–43) imply the localization of ESA4 to the region 11q22 → 11q23.

The 2S MEL–HLN XX-8 subclones also provided data for the finer regional assignments of the genes for PEPS and PGM2 to the regions 4pter → 4q21 and 4q21 → 4qter, respectively (Fig. 4). Consistent with our findings (43), the smallest region of overlap for human PEPS, based on previous studies (44–46), was 4pter → 4q12. Based on several studies (44–47), the smallest region of overlap for human PGM2 was 4p14 → 4q21 (48). Bocian *et al.* (46) have assigned the gene to the region 4pter → 4q12. Our data are discrepant with those of Bocian *et al.* because homogeneous subclone A31-18 was positive for human PGM2 and did not contain the region of their assignment. Based on our findings and those of the other studies, it is likely that human PGM2 is in the region 4q21; further studies of families with 4q aberrations are required to confirm this assignment.

Preliminary results with these hybrids to map the structural genes for other enzymes in the heme biosynthetic pathway [e.g., δ -aminolevulinic dehydratase (ALAD; EC 4.2.1.24), and uroporphyrinogen III cosynthase] indicate that the gene for human ALAD is not linked to the gene for PBGD. It is notable that in *Escherichia coli* the gene for PBGD is tightly linked to the uroporphyrinogen III cosynthase gene, whereas the ALAD gene is distant from these two genes (49).

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