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ASSIGNMENT OF THE MYELOPEROXIDASE GENE *MPO* TO HUMAN CHROMOSOME 17 USING SOMATIC CELL HYBRIDS AND FLOW-SORTED CHROMOSOMES

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Summary A cDNA coding for human myeloperoxidase (MPO) was used as a probe to study MPO gene structure and to determine the chromosomal location of the gene in the human genome. Southern blot hybridization of restriction endonuclease digests of human DNA with the MPO cDNA probe showed that a single gene for human MPO was present in the human genome. Southern blot hybridization experiments with human-mouse cell hybrid DNAs containing various subsets of human chromosomes revealed that the human MPO gene is located on chromosome 17. This conclusion was supported by DNA spot-blot hybridization using flow-sorted human chromosomes.

INTRODUCTION

Myeloperoxidase (MPO) is a main hemoprotein of human polymorphonuclear leukocytes (Schultz and Kaminker, 1962) and is involved in the chloride ion-mediated bactericidal action of these cells (Klebanoff, 1975). Multiple forms of MPO were isolated from human leukocytes and leukemia HL-60 cells (Yamada *et al.*, 1981; Pember *et al.*, 1983; Morita *et al.*, 1986; Miyasaki *et al.*, 1986). The MPO production in HL-60 cells is suppressed when these cells are induced for differentiation by several agents such as a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate

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TPA, retinoic acid and dimethyl sulfoxide (Yamada *et al.*, 1987; Johnson *et al.*, 1987; Weil *et al.*, 1987) and therefore the presence or absence of MPO is considered as a marker for determining malignancy of leukemic cells. The MPO consists of two large subunits of 59,000 daltons and two small subunits of 15,000 daltons (Yamada *et al.*, 1981; Andrews and Krinsky, 1981). The two large subunits are linked by a disulfide bond (Andrews and Krinsky, 1981; Morita *et al.*, 1986).

We recently isolated cDNA coding for the carboxyl terminal portion of human MPO from a cDNA library of HL-60 cells (Yamada *et al.*, 1987). The other groups have also isolated and characterized cDNAs coding for human MPO (Chang *et al.*, 1986; Johnson *et al.*, 1987; Weil *et al.*, 1987; Morishita *et al.*, 1987). In this communication we have used the cloned human MPO cDNA as a hybridization probe to examine the segregation of the MPO gene in a panel of human-mouse cell hybrids by Southern blot analysis of cell hybrid DNA. The human MPO gene was assigned to chromosome 17. DNA spot-blot analysis of flow-sorted human metaphase chromosomes also supported this conclusion.

MATERIALS AND METHODS

DNA probes. The cDNA clone, pMP1, encoding carboxy-terminus of human MPO was isolated from a cDNA library constructed from HL-60 cell poly(A)$^+$ RNA in pBR322 as described previously (Yamada *et al.*, 1987). The cDNA contained 1,278 base pairs (bp) and was composed of three *Pst*I fragments of 430, 654 and 194 bp (Yamada *et al.*, 1987). The *Pst*I fragment of 654 bp shown in Fig. 1 was isolated from a *Pst*I digest of pMP1 DNA by agarose gel electrophoresis. A 440-bp *Kpn*I-*Xba*I fragment specific for human c-*erb*B-2 (Semba *et al.*, 1985) was isolated from pKX044 obtained from Japanese Cancer Research Resources Bank (JCRB). DNA probes were labeled with [α-^{32}P]dCTP (Amersham) by random primer extension (Feinberg and Vogelstein, 1983) and used for blot hybridization.

Human-mouse cell hybrid DNAs. A panel of 14 human-mouse cell hybrids containing various subsets of human chromosome was constructed as described previously (Shimizu *et al.*, 1980). High molecular weight DNAs from the cell hybrids, mouse A9 cells and human placenta were isolated as described previously (Hunts *et al.*, 1985).

Southern blot analysis. DNA was completely digested with a restriction endonuclease and fractionated by electrophoresis through a 0.7% agarose gel. DNA fragments were transferred to nitrocellulose filter (Schleicher & Schuell, BA85) by the method of Southern (1975). The filters were prehybridized for 6–14 hr at 42°C in 50% formamide, 5 × SSC (1 × SSC consisted of 0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0), 5 × Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 0.1% sodium dodecyl sulfate (SDS) and 100 μg/ml denatured salmon sperm DNA. The hybridization buffer contains 4 parts of 50% formamide, 5 × SSC, 1 × Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 0.1% SDS, 100 μg/ml denatured salmon

sperm DNA and 1 part of 50% dextran sulfate. The filters were hybridized with ^{32}P-labeled probe for 20–24 hr at 42°C. After hybridization, the filters were washed twice for 15 min in 2 × SSC–0.1% SDS at room temperature, twice for 30 min in 0.5 × SSC–0.1% SDS at 50°C and twice for 30 min in 0.1 × SSC–0.1% SDS at room temperature, and autoradiographed with intensifier screen on Fuji RX X-ray film at −70°C.

Spot-blot analysis of flow-sorted chromosomes. Spontaneously transformed human B-lymphoblast line, GM130B, was obtained from the Human Genetic Mutant Cell Repository (Camden, N.J., USA) and used for sorting human chromosomes. The methods for metaphase chromosome preparation, chromosome sorting and spot-blot hybridization were carried out according to Lebo *et al.* (1985) with some modifications. Briefly, metaphase chromosomes were stained with Hoechst 33258 and sorted into 16 fractions by a FACS440 cell sorter (Becton-Dickinson). Fifty thousand chromosomes of each type were sorted directly onto nitrocellulose filter discs (25-mm diameter; Schleicher & Schuell, BA85) where the chromosomal DNA was denatured and used for hybridization as described above except that washings were twice for 30 min in 0.1 × SSC–0.1% SDS at 60°C for MPO cDNA probe or twice for 30 min in 0.5 × SSC–0.1% SDS at 60°C for c-*erb*B-2 probe. These washing conditions were chosen to assure that the primary gene sequence gives a considerably intenser signal than homologous gene sequences.

RESULTS

DNAs from human placenta and mouse A9 cells were digested with either restriction endonuclease *Eco*RI or *Hin*dIII. A Southern blot of the digests was hybridized with a pMP1 *Pst*I fragment of 654 bp encoding carboxy-terminus of human MPO (Fig. 1). As shown in Fig. 2, MPO cDNA strongly hybridized to the 20-kb *Eco*RI fragment (lane 1) and the 15-kb *Hin*dIII fragment (lane 3) in human DNA. These results suggest that a single MPO gene is present in the human genome. Several minor bands were also observed in both the *Eco*RI and *Hin*dIII digests under the hybridization conditions used, suggesting that there may exist several MPO-related sequences in the human genome. In the digest of the mouse cell line A9 DNA, a single 8-kb *Eco*RI fragment (lane 2) and a single 8.4-kb *Hin*dIII fragment (lane 4) were detected. This represents cross-hybridization of the human MPO probe with a homologous sequence in the mouse genome.

For chromosome assignment of the human MPO gene, a panel of 14 human-mouse cell hybrids were used. DNA from the hybrids was digested with *Eco*RI and blot hybridized with the human MPO probe. The 20-kb *Eco*RI fragment (arrows in Fig. 3) specific for human MPO gene was detected in 10 hybrids (TA4, TA5, B4A1-17a, C3B4, D5B1, B5B2, B4A4, B2B4, B4B4 and B2B1) out of 14. Four hybrids (TA1, TA2, TA3 and C2B5) were negative. The chromosomal profile of the hybrid cells was compared to the results of the Southern blot analysis, as shown

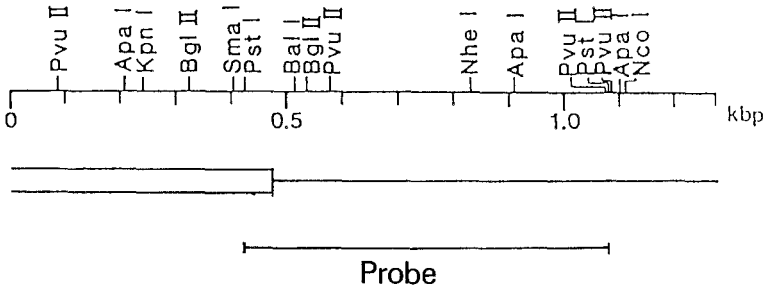

Fig. 1. Restriction enzyme map of MPO cDNA. The cDNA clone, pMP1, encoding the carboxy-terminus of human myeloperoxidase (MPO) was isolated from a cDNA library constructed from HL-60 cell poly(A)$^+$ RNA in pBR322 as described previously (Yamada *et al.*, 1987). The upper line indicates a detailed restriction map of the cDNA. The cDNA consists of 1,278 bp containing an open reading frame of 474 bp and a noncoding region of 804 bp. The middle line indicates the position of the open reading frame (the boxed area). The *Pst*I fragment of 654 bp, shown in the lower line, was labeled with ^{32}P by random primer extension and used as a probe for blot hybridization.

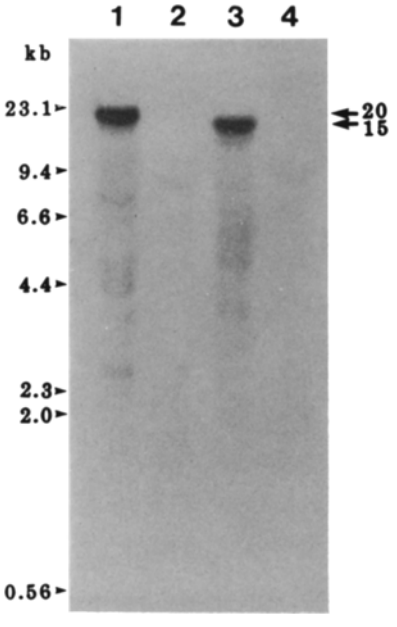

Fig. 2. Southern blot analysis of the myeloperoxidase (MPO) gene. DNAs (5 μg each) from human placenta (lanes 2 and 3) and mouse A9 cells (lanes 2 and 4) were digested with either *Eco*RI (lanes 1 and 2) or *Hin*dIII (lanes 3 and 4) and analyzed by Southern hybridization using MPO cDNA as a probe (see Fig. 1). *Hin*dIII-digested lambda phage DNA was used as a size marker and the sizes are shown in kilobases (kb).

Table 1. Correlation of MPO sequence with human chromosome 17 in human-mouse somatic cell hybrids.

Human chromosome		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	7p+Xq−[a]	
Concordant	+/+[b]	6	3	7	6	0	2	2	2	2	5	6	5	4	3	3	4	10	9	6	3	8	3	1	0	8	2
	−/−	2	1	3	1	3	4	2	2	4	3	3	2	1	3	3	1	4	2	3	2	3	3	0	4	0	1
Discordant	+/−	4	7	3	4	10	8	0	8	8	5	4	5	6	7	7	6	0	1	4	7	2	7	1	10	0	6
	−/+	2	3	1	3	1	0	1	2	0	1	1	2	3	1	1	3	0	2	1	2	1	1	3	0	1	0
Discordant clones		6	10	4	7	11	8	1	10	8	6	5	7	9	8	8	9	0	3	5	9	3	8	4	10	1	6
Percent discordant		43	71	29	50	79	57	20	71	57	43	36	50	64	57	57	64	0	21	36	64	21	57	80	71	11	67

[a] 7p+ = t(7;X)(7qter → 7p22::Xq21 → Xqter), Xq− = t(7;X)(Xpter → Xq21::7p22 → 7pter). [b] Presence of 20-kb MPO sequence/chromosome (number of clones).

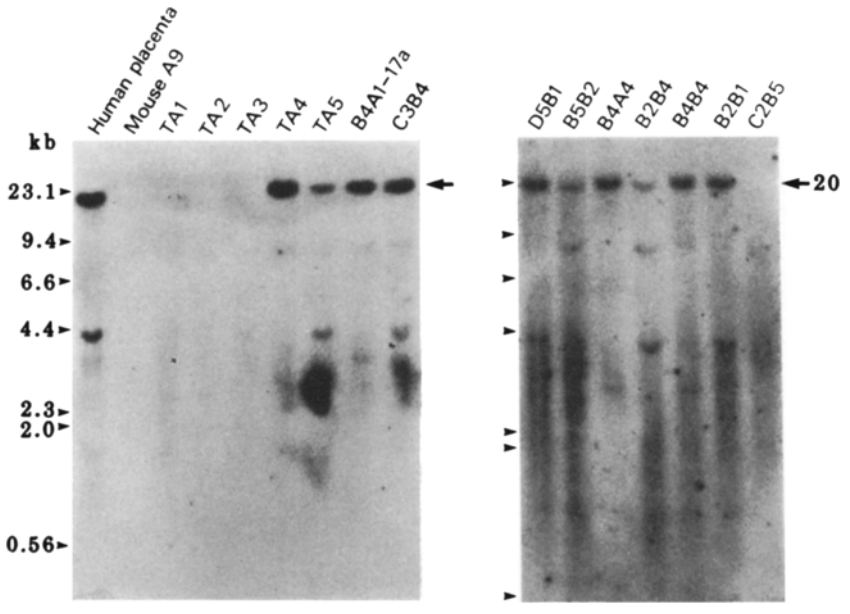

Fig. 3. Southern blot analysis of *Eco*RI-digested DNAs from human-mouse cell hybrids using the human MPO cDNA probe. DNAs (5 μg each) from human placenta, the mouse cell line A9 and 14 clones of human-mouse cell hybrids were digested with *Eco*RI and analyzed by Southern hybridization using ^{32}P-labeled human MPO cDNA probe (see Fig. 1).

in Table 1. We found that the presence of human chromosome 17 corresponds to the presence of the 20-kb MPO sequence. The absence of human chromosome 17 correlated with the absence of the 20-kb MPO sequence. The other chromosomes could be eliminated with discordances of greater than 11%. These results indicate that the MPO gene is located on human chromosome 17.

To confirm the localization of the MPO gene on chromosome 17, we carried out DNA spot-blot analysis of flow-sorted human metaphase chromosomes. Metaphase chromosomes were prepared from human B-lymphoblast line GM130B, stained with Hoechst 33258, then analyzed and sorted by a FACS440 cell sorter. As shown in Fig. 4(A), these chromosomes were fractionated into 16 groups, A through O, containing one to four types of chromosomes. Fifty thousand chromosomes of each type in each group were sorted directly onto nitrocellulose filter discs. Chromosomal DNA immobilized on filter discs were hybridized with the human MPO probe. As shown in Fig. 4(B), the most intense signal was obtained with DNA from the group L containing chromosome 17 and Y. The hybridization experiment using a 440-bp *Kpn*I-*Xba*I fragment of human c-*erb*B-2 gene (Semba *et al.*, 1985) assigned to chromosome 17 (Schechter *et al.*, 1985; Coussens *et al.*, 1985; Fukushige *et al.*, 1986) as a probe confirmed the presence of chromosome 17 in the group L as shown in

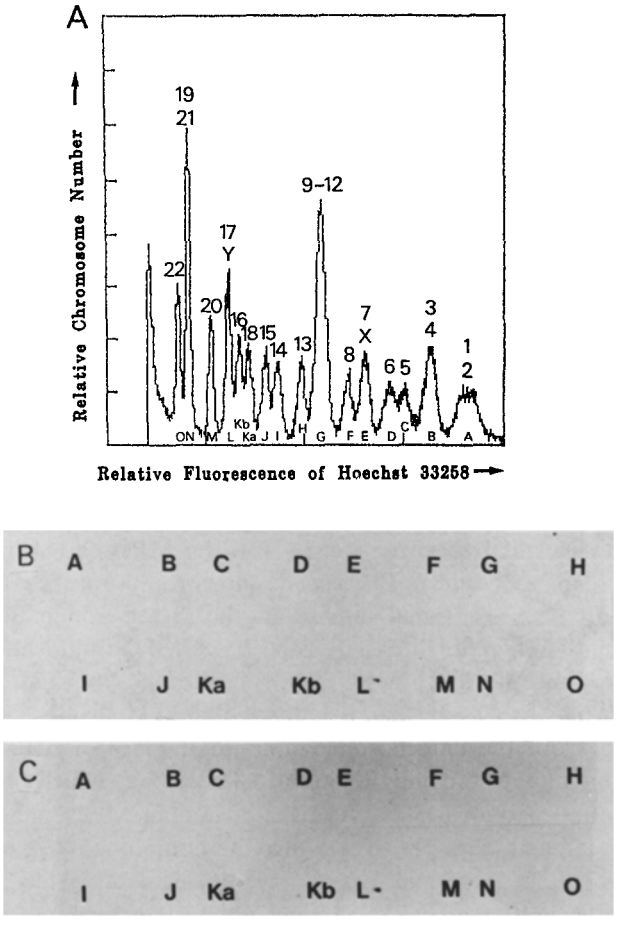

Fig. 4. Spot-blot analysis of flow-sorted human chromosomes. (A) Flow karyotype of human B-lymphoblast line GM130B. Numbers in the figure represent the positions of human chromosomes. (B, C) Fifty thousand chromosomes of each type in each group shown in (A) were directly sorted onto nitrocellulose filter discs. Hybridization was carried out with either the MPO cDNA probe (B) or the c-*erb*B-2 probe (C).

Fig. 4(C). Weak signals in the group Kb, detected by the MPO and c-*erb*B-2 probes, may represent contamination from the neighboring group L. These results together with cell hybrid analysis indicate that the MPO gene is located on human chromosome 17.

DISCUSSION

MPO consists of two large subunits and two small subunits (Yamada *et al.*, 1981; Andrews and Krinsky, 1981). Studies on the biosynthesis of MPO have shown

that these large and small subunits are generated from a single polypeptide chain precursor (Yamada, 1982; Olsson *et al.*, 1984; Koeffler *et al.*, 1985; Akin and Kinkade, 1986). Our present study provided evidence that MPO is synthesized from a gene located on chromosome 17. The chromosome assignment based on the segregation of the MPO gene in a panel of human-mouse cell hybrids was confirmed because the same segregation pattern was obtained when a marker for chromosome 17 (Schechter *et al.*, 1985; Coussens *et al.*, 1985; Fukushige *et al.*, 1986), a 440-bp *Kpn*I-*Xba*I fragment of human c-*erb*B-2 gene (Semba *et al.*, 1985), was used as a probe (data not shown). Also, spot-blot hybridization using flow-sorted human chromosomes provided a definitive evidence for the assignment.

Southern blot analysis of human leukemia HL-60 cell DNA with the MPO cDNA probe indicated that the fragments detected in the *Eco*RI digests are identical in number, size and signal intensities to those in the placenta DNA (data not shown). Thus, the MPO gene was not rearranged and not amplified in leukemia HL-60 cells as reported by others (Chang *et al.*, 1986; Morishita *et al.*, 1987).

Several minor bands hybridized weakly with an MPO cDNA probe as shown in Fig. 3. Although we could not determine the chromosome localization of these bands because of their low signal intensities, the segregation pattern of some of these was apparently different from that of the 20-kb MPO fragment. For example, a hybrid cell TA4 had the 20-kb MPO band, but not 4.4-kb band. In contrast, a hybrid cell TA3 had the 4.4-kb band, but not the 20-kb band. Thus the 4.4-kb must be derived from the chromosome other than 17. An MPO cDNA probe used in this study contains a coding region of 48 base pairs which corresponds to 16 amino acid residues of the carboxyl terminal of human MPO and a 3′ noncoding region of 610 base pairs (Fig. 1). Therefore, the homologous regions between the MPO cDNA probe and these minor bands are not necessarily localized within the coding region of MPO. Molecular cloning and further characterization of these bands are needed to reveal the significance of these sequence homology.

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