SHORT COMMUNICATION

Chromosomal Localization of the Human Heme Oxygenase Genes: Heme Oxygenase-1 (HMOX1) Maps to Chromosome 22q12 and Heme Oxygenase-2 (HMOX2) Maps to Chromosome 16p13.3

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Heme oxygenase catalyzes the oxidation of heme to biliverdin, the precursor of the bile pigment bilirubin, and carbon monoxide, a putative neurotransmitter. We have employed polymerase chain reaction and fluorescence *in situ* hybridization to determine the chromosome localization of the genes coding for the two known heme oxygenase isozymes. Heme oxygenase-1 (HMOX1), the inducible form, was localized to human chromosome 22q12, while heme oxygenase-2 (HMOX2), the constitutive form, was localized to chromosome 16p13.3.

Heme oxygenase is the rate-limiting enzyme in the heme degradative pathway. It catalyzes the degradation of heme into two important products, biliverdin and carbon monoxide (3, 10). By the action of biliverdin reductase, biliverdin is readily converted to bilirubin, a potent antioxidant (2, 9, 11). The second product, carbon monoxide, recently has been reported to be a putative neurotransmitter (13). Heme oxygenase consists of two isozymes, an inducible heme oxygenase-1 (HMOX1) and a constitutive heme oxygenase-2 (HMOX2) (4, 12). They have been shown to be two different gene products by cDNA cloning (6, 14). We now report the chromosome localization of these two important genes in the human.

Mapping panel No. 2 (consisting of DNA isolated from 24 human/rodent somatic cell hybrids retaining one or two human chromosomes) obtained from NIGMS Human Genetic Mutant Cell Repository at the Coriell Institute for Medical Research (Camden, NJ) was screened by PCR (8). Several sets of primers were constructed for the amplification of HMOX1 using human genomic DNA as a template. However, only one set of primers, 5′-TGT GGC AGC TGT CTC AAA CCT CCA and 5′-TTG AGG CTG AGC CAG GAA CAG AGT, gave satisfactory results. These primers yielded a 175-bp PCR product whose sequence corresponded to 1234–1408 bp in the 3′ untranslated region of the published HMOX1 cDNA sequence (14). As shown in Fig. 1, an amplification product of appropriate size was observed only in the case of DNA preparation NA10888, which originated from a chromosome 22 monochromosomal somatic cell hybrid. A similarly sized product was observed with human genomic DNA but not with genomic DNA from mouse or hamster. This thus gives one line of evidence that HMOX1 is localized to human chromosome 22.

Since the genomic sequence of HMOX2 is not yet known, primers for PCR were designed from its published cDNA sequence (6). Primers 5′-GCT GAC CAA GGA CAT GGA GTA and 5′-GGT AGA GCT GCT TGA ACT GCT yielded a 284-bp product when human genomic DNA was used as a template. The authenticity of the PCR product, which corresponded to 324–607 bp in the translated region of HMOX2 cDNA, was verified by DNA sequencing. A PCR product identical to that obtained with human genomic DNA was observed for DNA preparations from two different monochromosomal somatic cell hybrids, putatively containing either chromosome 14 (NA10479) or chromosome 16 (NA10567). To clarify this result, we also analyzed DNA preparations from somatic cell hybrids outside of this panel. NA11100, a preparation from a hybrid cell line reportedly containing chromosome 16 as well as chromosome X, gave an amplification product similar to that given by NA10567 (Fig. 2). However, NA11535, a preparation from a hybrid cell reportedly containing only chromosome 14, failed to yield an amplification product, in contrast to the result obtained with NA10479 (Fig. 2). A possible explanation for this observation is that the somatic hybrid cell line from which the DNA preparation NA10479 is obtained may contain part of chromosome 16 as an impurity. Interestingly, this somatic hybrid cell line (although primarily containing chromosome 14) has been reported to contain chromosome 16 as an impurity (5). Thus, these data indicate that HMOX2 is localized to chromosome 16.

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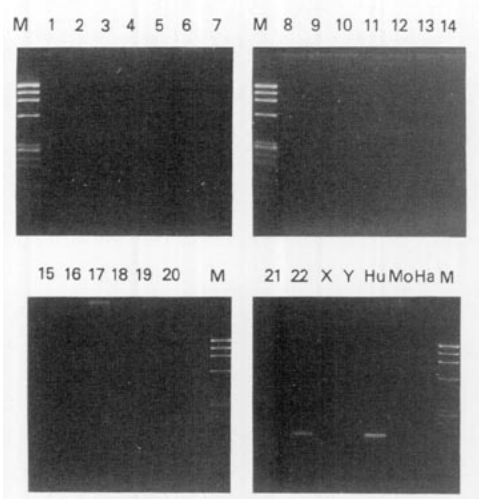


FIG. 1. Chromosome localization of HMOX1 by PCR analysis of somatic cell hybrids. NIGMS Mapping Panel No. 2 was employed for PCR screening using 5′-TGT GGC AGC TGT CTC AAA CCT CCA and 5′-TTG AGG CTG AGC CAG GAA CAG AGT as primers. PCR was performed on a Perkin–Elmer–Cetus GeneAmp PCR System 9600 using reagents from Perkin–Elmer (Norwalk, CT). Reaction mixture (50 μl) consisted of DNA preparation (88–175 ng), primers (0.5 μM), perfect-match DNA polymerase enhancer (0.5 μl; Stratagene, La Jolla, CA), deoxynucleoside triphosphates (200 μM), 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, and *Taq* DNA polymerase (2.5 units). Samples were kept at 95°C for 2 min, subjected to thermocycling (30 cycles of 30 s at 94°C, 30 s at 62°C, and 1 min at 72°C), and finally allowed to remain at 10 min at 72°C. The samples (25 μl) were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. **M**, size markers, ϕX174 RF DNA/*Hae*III fragments. Lanes **1–22**, **X**, and **Y**: PCR reaction performed with DNA preparations from somatic cell hybrids containing each respective chromosome. Hu, human genomic DNA. Mo, mouse genomic DNA. Ha, hamster genomic DNA.

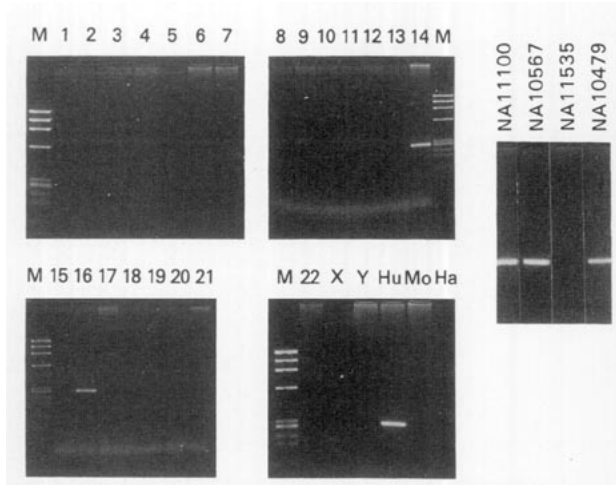


FIG. 2. Chromosome localization of HMOX2 by PCR analysis of somatic cell hybrids. PCR conditions were as described for Fig. 1 except that 5′-GCT GAC CAA GGA CAT GGA GTA and 5′-GGT AGA GCT GCT TGA ACT GCT were used as primers and 64°C was used as the annealing temperature. **M**, size markers, ϕX174 RF DNA/*Hae*III fragments. Lanes **1–22**, X, and Y: PCR reaction performed with DNA preparations from somatic cell hybrids containing each respective chromosome. Hu, human genomic DNA. Mo, mouse genomic DNA. Ha, hamster genomic DNA. NA11100, NA10567: PCR reactions performed with DNA preparations from respective somatic cell hybrids containing chromosome 16. NA11535, NA10479: PCR reactions performed with DNA preparations from somatic cell hybrids containing chromosome 14.

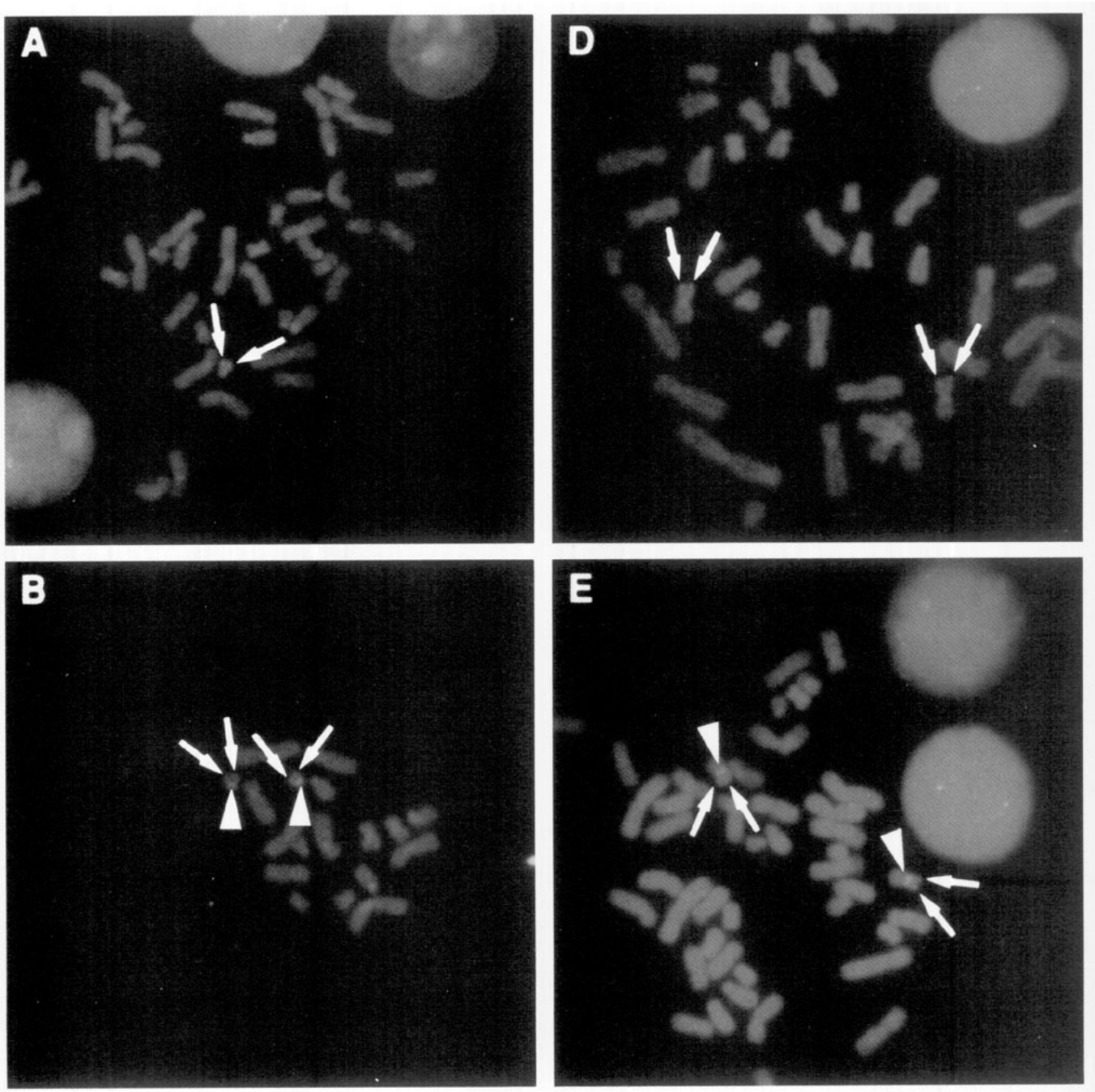
Independent confirmation of the chromosomal localization was obtained using fluorescence *in situ* hybridization. HMOX1 and HMOX2 genes were cloned by PCR screening of a human genomic P1 library using primers and conditions described above for chromosome localizations (Genome Systems, Inc., St. Louis, MO; unpublished results). The cloned DNA preparations were labeled by nick–translation with digoxigenin-dUTP, combined with sheared human DNA, and hybridized to normal human metaphase chromosomes in a solution containing 50% formamide, 10% dextran sulfate, and 2× SSC. Signal was detected with antidigoxigenin–FITC. Chromosomes were then counterstained and analyzed (Bios Laboratories, New Haven, CT).

As expected, the HMOX1 probe specifically localized to chromosome 22 (Fig. 3A). Localization of this gene was further confirmed by the cohybridization of a probe specific for centromeres of chromosomes 22 and 14 (D14Z1/D22Z1) to this chromosome (Fig. 3B). A total of 80 metaphase cells were analyzed with 71 showing specific signal. Measurements of 20 hybridized chromosomes showed that HMOX1 is located 62% of the distance from the p terminus to the q terminus of the chromosome; this is an area corresponding to band 22q12 (Fig. 3C).

HMOX2 specifically hybridized to chromosome 16 (Fig. 3D). The location was confirmed by the cohybridization of a probe that is specific for the centromere of chromosome 16 (D16Z2) to the same chromosome (Fig. 3E). A total of 85 metaphase cells were analyzed, with 81 showing specific signal. Giemsa banding pattern showed that the signal was located at the terminus of the short arm of the chromosome. This indicates that HMOX2 is localized to chromosome band 16p13.3 (Fig. 3F).

Work over the last few years has shed new light on the possible biological importance of the heme oxygenase enzymes. They are primarily thought to play key roles in protection against oxidative stress (3) in their synthesis of bilirubin, a "chain-breaking antioxidant" (9). They also produce carbon monoxide, a neurotransmitter that appears to be a physiological regulator of cGMP (13). Moreover, heme oxygenase-1 is a heat-shock protein

FIG. 3. Chromosome localization of HMOX1 and HMOX2 by fluorescence *in situ* hybridization. (**A**) Hybridization of HMOX1 probe (indicated by arrows) to chromosome 22. (**B**) Cohybridization of HMOX1 probe (indicated by arrows) and centromere-specific probe D14Z1/D22Z1 (indicated by arrowheads) to chromosome 22. (**C**) Idiogram of chromosome 22 showing the subchromosomal location of heme oxygenase-1 to 22q12. (**D**) Hybridization of HMOX2 probe (indicated by arrows) to chromosome 16. (**E**) Cohybridization of HMOX2 probe (indicated by arrows) and centromere-specific probe D16Z2 (indicated by arrows) and centromere-specific probe D16Z2 (indicated by arrowheads) to chromosome 16. (**F**) Idiogram of chromosome 16 showing the subchromosomal location of heme oxygenase-2 to 16p13.3.



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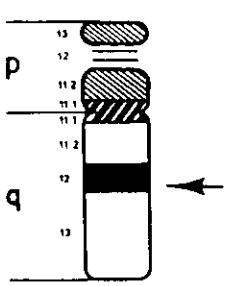

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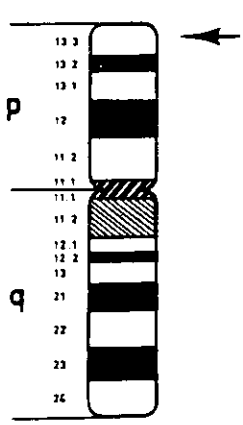

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that is now thought to be capable of protecting neurons in specific areas of the brain (1). With these functions, it seems clear that gene mutations could lead to severe consequences, i.e., hereditary diseases. The present chromosomal localization of the two genes should allow for them to be considered as potential "candidate genes" in a number of diseases mapped to chromosomes 16 and 22. On chromosome 16, in particular, a number of anemias, thalassemias, etc. would be of interest to investigate as well as familial Mediterranean fever, recently mapped to 16p (7).

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REFERENCES

1. Ewing, J. F., and Maines, M. D. (1991). Rapid induction of heme oxygenase 1 mRNA and protein by hyperthermia in rat brain: Heme oxygenase 2 is not a heat shock protein. *Proc. Natl. Acad. Sci. USA* **88:** 5364–5368.
2. Kutty, R. K., and Maines, M. D. (1981). Purification and characterization of biliverdin reductase. *J. Biol. Chem.* **256:** 3956–3962.
3. Maines, M. D. (1992). "Heme Oxygenase: Clinical Applications and Functions," CRC Press, Boca Raton, FL.
4. Maines, M. D., Trakshel, G. M., and Kutty, R. K. (1986). Characterization of two constitutive forms of rat liver microsomal heme oxygenase: Only one molecular species of the enzyme is inducible. *J. Biol. Chem.* **261:** 411–419.
5. Mares, A., Jr., Ledbetter, S. A., Ledbetter, D. H., Roberts, R., and Hejtmanick, J. F. (1991). Isolation of human chromosome 14-only somatic cell hybrid: Analysis using *Alu*- and line-based PCR. *Genomics* **11:** 215–218.
6. McCoubrey, W. K., Jr., Ewing, J. F., and Maines, M. D. (1992). Human heme oxygenase-2: Characterization and expression of a full-length cDNA and evidence suggesting that the two HO-2 transcripts may differ by choice of polyadenylation signal. *Arch. Biochem. Biophys.* **295:** 13–20.
7. Pras, E., Aksentijevich, I., Gruberg, L., Balow, J., Prosen, L., Dean, M., Steinberg, A., Pras, M., and Kastner, D. (1992). Mapping of a gene causing familial Mediterranean fever to the short arm of chromosome 16. *N. Engl. J. Med.* **326:** 1509–1513.
8. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:** 487–491.
9. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., and Ames, B. N. (1987). Bilirubin is an antioxidant of possible physiological importance. *Science* **235:** 1043–1046.
10. Tenhunen, R., Marver, H. S., and Schmid, R. (1969). Microsomal heme oxygenase: Characterization of the enzyme. *J. Biol. Chem.* **244:** 6388–6394.
11. Tenhunen, R., Ross, M. E., Marver, H. S., and Schmid, R. (1970). Reduced nicotinamide-adenine dinucleotide phosphate dependent biliverdin reductase: Partial purification and characterization. *Biochemistry* **9:** 298–303.
12. Trakshel, G. M., Kutty, R. K., and Maines, M. D. (1986). Purification and characterization of the major constitutive form of testicular heme oxygenase: The noninducible form. *J. Biol. Chem.* **261:** 11131–11137.
13. Verma, A., Hirsch, D. J., Glatt, C. E., Ronnett, G. V., and Snyder, S. H. (1993). Carbon monoxide: A putative neural messenger. *Science* **259:** 381–384.
14. Yoshida, T., Biro, P., Cohen, T., Muller, R. M., and Shibahara, S. (1988). Human heme oxygenase cDNA and induction of its mRNA by hemin. *Eur. J. Biochem.* **171:** 457–461.