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Existence of Glucose-6-Phosphate Dehydrogenase-Like Locus on Chromosome 17

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SUMMARY

Hybridization of DNA samples prepared from flow-sorted human chromosomes with a cDNA probe for the X-linked glucose-6-phosphate dehydrogenase (G6PD) suggested the existence of the G6PD-like locus on chromosome 17. Southern hybridization analysis of endonuclease-digested DNA samples from the human-mouse hybrid cell line with human chromosome 17, and from control human and mouse cells, proved that not only X chromosomes, but also chromosome 17, contain DNA sequences that are hybridizable with the G6PD cDNA probe. The G6PD-like locus on chromosome 17 could be a putative pseudogene or a functional gene for the fetal brain-specific G6PD isozyme or other protein.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, E.C.1.1.1 49, abbreviation G6PD) plays a key role in the generation of NADPH, particularly in mature red blood cells; and the genetic deficiency of the enzyme is associated with chronic and drug- or food-induced hemolytic anemia in humans. The gene for G6PD is located at the q28 position of the human X chromosome [1]. In addition to the X-linked G6PD, an autosomal isozyme, commonly designated as glucose dehydrogenase or hexose-6-phosphate dehydrogenase (β-D-glucose: NAD(P) oxidoreductase, E.C.1.1.1 47, abbreviation GDH), exists in the microsomes of human and other mammals [2, 3]. This isozyme oxidizes glucose and galactose-6-phosphate, as well as

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glucose-6-phosphate, and uses NAD as well as NADP as a coenzyme. The locus for GDH was assigned to chromosome 1 [4]. The existence of still another G6PD isozyme in human fetal brain was previously suggested [5]. This paper reports the existence of a G6PD-like locus on chromosome 17.

MATERIALS AND METHODS

G6PD cDNA Probe

An insertion of 2.0-kilobase pairs (kbp) of G6PD cDNA clone λG6PD-19 was labeled with [α^{32}P]dATP (5,000 Ci/m mol, from New England Nuclear, Boston, Mass.) by nick-translation [6] and used as a hybridization probe. The 2.0-kbp insertion encoded 204 amino acid residues that were completely compatible with the COOH-terminal part of the X-linked human G6PD and had a 3′ noncoding region of 1.36 kpb. Details of cloning and characterization of this cDNA clone was reported [7].

Southern Hybridization

DNA samples prepared from flow-sorted human chromosomes were spotted on nitrocellulose filters and hybridized with the [^{32}P]-labeled G6PD cDNA probe (about 1.5×10^6 cpm/ml, about 1 mCi/μg DNA). DNA samples were prepared from human cells and mouse cells and from fibroblasts of a human-mouse hybrid cell line that contains human chromosome 17 only (provided by Dr. T. Mohandas, Harbor UCLA Medical Center, Torrance, Calif.). These samples (about 10 μg each) were completely digested by *Pst*I (100 U), overnight, under the conditions recommended by the supplier. Other restriction enzymes, *Hin*dIII, *Eco*RI, and *Xba*I were also used for the digestion. However, these restriction enzymes were found to be less satisfactory for distinguishing between human DNA bands and mouse DNA bands. The DNA fragments were separated by agarose gel electrophoresis and transferred onto nitrocellulose filters, as described by Southern [8]. Prehybridization was carried out in a solution containing 50% formamide, 3 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 1 × Denhardt's solution, and salmon sperm DNA (100 μg/ml) for 4 hrs at 42°C; and hy-

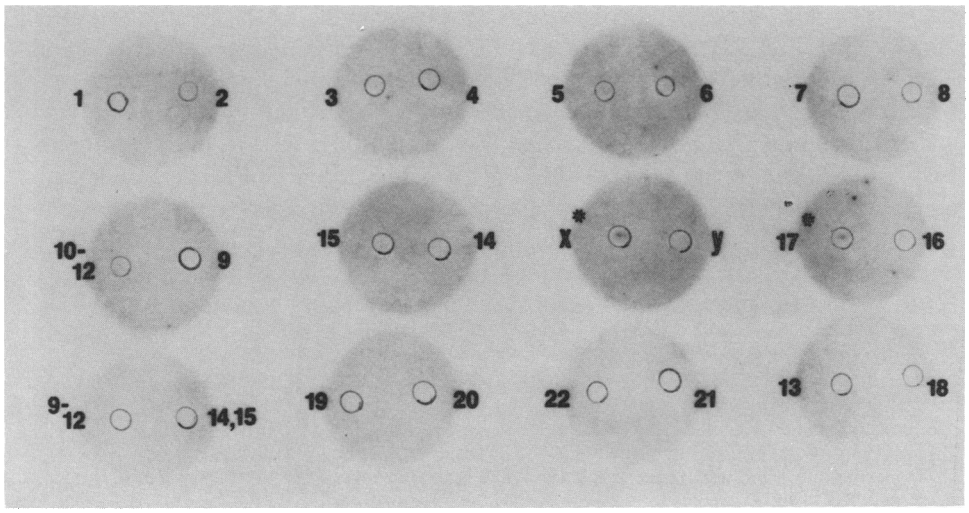

FIG. 1.—Hybridization of DNA from flow-sorted chromosomes by G6PD cDNA probe. DNA dots are encircled. *Asterisks* indicate positive hybridization on chromosomes X and 17.

bridization was carried out in the prehybridization solution supplemented with 5% dextran sulfate and the [^{32}P]-labeled G6PD cDNA probe (about 10^6 cpm/ml, about 1 mCi/μg DNA) for 18 hrs at 42°C. After the hybridization, the filters were washed with 0.1 × SSC containing 0.1% sodium dodecylsulfate twice at room temperature and twice at 52°C for 30 min. Densitometry of autoradiograms for the hybridization patterns was carried out by a recording densitometer (Joyce, Loebl & Co., England). The restriction enzymes were obtained from Bethesda Research, Gaithersburg, Md., and the nitrocellulose filters (BA 85) were obtained from Schleicher & Schuell, Keene, N.H.

RESULTS AND DISCUSSION

Dot-blot hybridization of DNA samples from flow-sorted chromosomes with the G6PD cDNA probe indicated that chromosome 17, as well as the X chromosome, contained DNA sequences that are homologous to the G6PD locus (fig. 1). DNA samples prepared from all other chromosomes, including chromosome 1, did not hybridize with the probe. An existence of the G6PD-like locus on chromosome 17 was further confirmed by the Southern hybridization analysis of DNA from the mouse-human hybrid cell line. This hybrid cell line contains human chromosome 17, but not other human chromosomes. Hybridization patterns of *PstI* fragments are shown in figure 2. The control human DNA indicated four positive bands, that is, 5 kbp (weak), 4 kbp (strong), 2 kbp (strongest), and 0.6 kbp (weak); and mouse DNA indicated two positive bands, that is, 3.5 kbp and 0.6 kbp. DNA obtained from the human-mouse hybrid cells

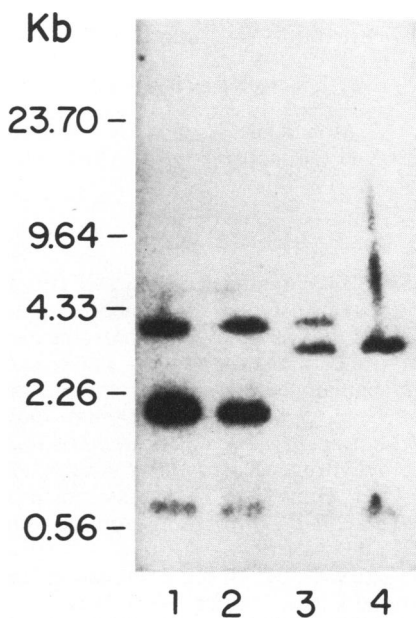

FIG. 2.—Southern blot hybridization of DNA samples from human, mouse, and mouse-human hybrid cells. DNA samples were digested by *PstI* and subjected to hybridization with G6PD cDNA. *Lane 1:* Human female DNA; *2:* human male DNA; *3:* DNA from a mouse-human hybrid cell line with human chromosome 17, but with no other human chromosomes; *4:* mouse DNA.

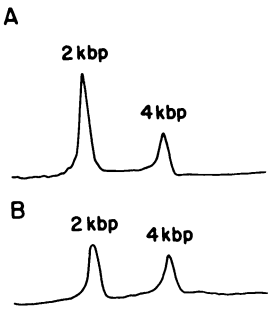

FIG. 3.—Densitometry of the hybridization bands shown in figure 3. *A*, Human female DNA pattern shown in *lane 1*, figure 2. B, Human male DNA pattern shown in *lane 2*, figure 2.

contained the 4-kbp band in addition to the two bands existing in mouse DNA, indicating that the 4-kbp band belongs to human chromosome 17. Furthermore, densitometric comparison of the relative intensities of the 4-kbp band and the 2-kbp band of DNA samples from human male and female clearly indicated the autosomal origin of the 4-kbp band (fig. 3). It can be concluded that chromosome 17 contains a DNA sequence that is homologous to the X-linked G6PD.

The G6PD-like locus on chromosome 17 could be related to a putative pseudogene for G6PD. Alternatively, it could be for the G6PD isozyme that was reported to exist in fetal human brain [5]. This isozyme has not yet been well characterized. The possibility that the locus on chromosome 17 is for another protein has not been totally excluded.

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