BRIEF REPORTS

The Human Cytochrome b561 Gene (CYB561) Is Located at 17q11–qter

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Cytochrome b561 is a major transmembrane protein that is specific to catacholamine and neuropeptide secretory vesicles of the adrenal medulla, pituitary gland, and other neuroendocrine tissues. This 30-kDa cytochrome is present in both the small synaptic vesicles and the large dense core vesicles (chromaffin granules) of the tissues (1, 2). Its role is to supply reducing equivalents to two monooxygenases, dopamine β-hydroxylase in chromaffin granules and glycine peptidyl α-amidating monooxygenase in neurosecretory vesicles (3–6). The cytochrome fulfills this role by catalyzing the transfer of electrons from a cytoplasmic donor, ascorbate, across a phospholipid bilayer to the lumenal acceptor, semidehydroascorbate, in the interior of the vesicles. The continuously regenerated ascorbate within these vesicles is the immediate donor for the monooxygenases inside the neuroendocrine secretory vesicles (7). Cytochrome b561 thus appears to be a pure transmembrane electron "channel." The primary structure of bovine cytochrome b561 revealed that it has six transmembrane helices, with the N- and C-terminal ends of the protein both facing the cytoplasm (8). Subsequent structural analysis and biochemical evidence of bovine cytochrome b561 indicated that the N-terminal end is either buried in the membrane or facing the matrix (9). In this paper, we report that the human gene encoding cytochrome b561 (CYB561; GenBank Accession No. U06715) is localized to 17q11–qter.

A somatic cell mapping strategy was used to localize CYB561. The isolation and characterization of the hybrids has been described (10–12). Briefly, DNA samples from independent human–mouse and human–hamster somatic cell hybrids and subclones were digested with *Eco*RI, and the fragments were resolved on 0.7% agarose gels. Southern blots were prepared on nylon membranes and hybridized with a radiolabeled human CYB561 cDNA insert (GenBank Accession No. U06715). Blots were washed at high stringency (~0% divergence) in 0.1× SSC, 0.2% sodium dodecylsulfate at 65°C. After autoradiography, the presence of the hybridizing human sequences in the DNA samples was correlated with the specific human chromosomes retained in each of the somatic cell hybrids.

CYB561 was localized to human chromosome 17 by Southern analysis of DNA samples isolated from human–rodent somatic cell hybrids. The 21-kb and more weakly hybridizing 1.7- and 2.3-kb human sequences in *Eco*RI digests segregated concordantly with chromosome 17 and discordantly (≥27%) with all other human chromosomes (table reviewed but not included). The two discordancies with chromosome 17 may result from a break involving chromosome 17 in these hybrids.

The examination of several hybrids containing specific translocations and spontaneous breaks allowed regional localization of the cytochrome gene to 17q11–17qter. Several hybrids isolated after fusion of human fibroblast containing a 17;22 (p13;q11) reciprocal translocation with Chinese hamster cells (12) were examined. Two hybrids retaining the 17p13–qter translocation chromosome in the absence of a normal chromosome 17 also retained the cytochrome gene, whereas another hybrid retained only the reciprocal translocation chromosome, and the cytochrome gene was absent. One human–hamster and one human–mouse hybrid had spontaneous breaks involving chromosome 17 with retention of 17pter–q11, and the cytochrome gene was absent in both of these hybrids. The gene was present in another human–hamster hybrid retaining only 17q11–qter, and the gene could be regionally assigned to this segment.

An analysis of possible restriction fragment length polymorphism was performed. The DNAs isolated from peripheral leukocytes of 10 unrelated individuals were separately digested with 12 different restriction enzymes (*Eco*RI, *Bam*HI, *Hin*dIII, *Xba*I, *Sac*I, *Taq*I, *Msp*I, *Pvu*II, *Pst*I, *Bcl*II, *Eco*RV, and *Kpn*II). These blots were examined with the CYB561 cDNA probe, and no polymorphism was detected.

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