Opal Suppressor Phosphoserine tRNA Gene and Pseudogene Are Located On Human Chromosomes 19 and 22, Respectively*

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An opal suppressor phosphoserine tRNA gene and pseudogene have been isolated from a human DNA library and sequenced (O'Neill, V., Eden, F., Pratt, K., and Hatfield, D. (1985) *J. Biol. Chem.* 260, 2501–2508). Southern hybridization of human genomic DNA with an opal suppressor tRNA probe suggested that the gene and pseudogene are present in single copy. In this study, we have determined the chromosome location of the human gene and pseudogene by utilizing a 193-base pair fragment encoding the opal suppressor phosphoserine tRNA gene as probe to examine DNAs isolated from human-rodent somatic cell hybrids that have segregated human chromosomes. These studies show that the probe hybridized with two regions in the human genome; one is located on chromosome 19 and the second on chromosome 22. By comparing the restriction sites within these two regions to those previously determined for the human opal suppressor phosphoserine tRNA gene and pseudogene, we tentatively assigned the gene to chromosome 19 and the pseudogene to chromosome 22. These assignments were confirmed by utilizing a 350-base pair fragment which was isolated from the 5'-flanking region of the human gene as probe. This fragment hybridized only to chromosome 19, demonstrating unequivocally that the opal suppressor phosphoserine tRNA gene is located on chromosome 19. The flanking probe hybridized to a single homologous band in hamster and in mouse DNA to which the gene probe also hybridized, demonstrating that the 5'-flanking region of the opal suppressor tRNA gene is conserved in mammals. Restriction analysis of DNAs obtained from the white blood cells of 10 separate individuals demonstrates that the gene is polymorphic. This study provides two additional markers for the human genome and constitutes only the second set of two tRNA genes assigned to human chromosomes.

Two opal suppressor phosphoserine tRNAs have been characterized in mammalian, avian, and *Xenopus* tissues (Diamond et al., 1981; Hatfield et al., 1982). They are the only authentic, naturally occurring suppressor tRNAs identified in higher eukaryotes (Hatfield, 1985). They have several unique features which set them apart from all other eukaryotic tRNAs. For example, they 1) are transcribed beginning at the first nucleotide within the coding sequence of the gene; 2) maintain the 5'-triphosphate on the initial nucleotide of the mature tRNA during transport from the nucleus, and it remains intact on the tRNA in the cytoplasm; 3) are phosphorylated on their serine moiety to form phosphoseryl-tRNA (Hatfield et al., 1982; and Mizutani and Hashimoto, 1984); 4) contain 90 nucleotides making them the longest tRNAs sequenced to date; 5) contain two extra nucleotides between the universal U at position 8 and an A at position 14; 6) contain an extra unpaired nucleotide within the stem of loop IV; 7) are highly undermodified compared to other tRNAs (Diamond et al., 1881; Hatfield et al., 1982; and Kato et al., 1983); and 8) are aminoacylated by seryl-tRNA synthetase even though one of the isoacceptors has a tryptophan anticodon (Diamond et al., 1981). The genes encoding the opal suppressor phosphoserine tRNAs have been isolated and their sequences determined from human (O'Neill et al., 1985), rabbit (Pratt et al., 1985), chicken (Hatfield et al., 1983), and *Xenopus* genomes. The genes from these four organisms are identical in sequence with the exception that the human and rabbit genes have a U at position 11 while chicken and *Xenopus* have a C at this position. Humans and rabbits also contain an opal suppressor serine tRNA pseudogene in their genomes. Hybridization studies between genomic DNA and an opal suppressor tRNA probe demonstrated that the gene was present in single copy in these organisms and that the pseudogene was present in single copy in rabbits and humans. It seemed reasonable, therefore, that since the human genome contains only a single gene and pseudogene, we could map them on their respective chromosomes by analyzing human-rodent somatic cell hybrids (McBride et al., 1982a; McBride et al., 1982b; McBride et al., 1983; and McBride et al., 1986) with an opal suppressor tRNA gene probe. The results of these studies are presented herein.

MATERIALS AND METHODS

Preparation of Probes—A 193-bp AvaI–HindIII fragment encoding the human opal suppressor tRNA gene was prepared as described previously (O'Neill et al., 1985). A 350-bp HindIII–EcoRI fragment encoding that portion of the 5'-flanking region of the opal suppressor tRNA gene which is upstream of the HindIII site (O'Neill et al., 1985; and see Fig. 1) was prepared from the 1550-bp human DNA fragment which was cloned into pBR322 by previously described procedures (Hatfield et al., 1983; and O'Neill et al., 1985). The fragments were labeled with 32P utilizing an oligolabeling kit from Pharmacia P-L Biochemicals and deoxycytidine-5'-[a-32P]triphosphate from Amer- sham Corp. and used as probes.

Cell Hybrids—The human and rodent parental cells, the cell fusion


2 B. J. Lee, K. H. You, and D. Hatfield, unpublished data.

3 The abbreviations used are: bp, base pairs; SDS, sodium dodecyl sulfate; kb, kilobase.

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procedure, and the isolation and characterization of the hybrids and have been described (McBride et al., 1982a; McBride et al., 1982b; McBride et al., 1983; and McBride et al., 1986). In general, hybrid cells were analyzed for the presence of all human chromosomes (except Y) by standard isoenzyme analyses, as well as by Southern analysis with probes from previously localized genes, and frequently by cytogenetic analyses.

**DNA Isolation and Filter Hybridization** — DNA was isolated from hybrid cell lines, digested with EcoRI, size-fractionated by (0.7%) agarose gel electrophoresis, and transferred to positively charged nylon membranes as described (McBride et al., 1982a; McBride et al., 1982b; McBride et al., 1983; McBride et al., 1986). Membranes were hybridized for 24–48 h at 42 °C with 32P-labeled probes in 50% formamide containing 5 × SSPE (1 × SSPE is 0.15 M NaCl, 0.01 M sodium phosphate, 0.001 M EDTA, pH 7.4), 5 × Denhardt’s solution (1 × is 0.2% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 10% dextran sulfate, 0.2% SDS, and sheared denatured herring sperm DNA at 150 µg/ml. Membranes were washed twice at 0.015 M NaCl, 0.01 M sodium citrate, pH 7.0 containing 0.2% SDS and 4 × at 55 °C in 0.1 × NaCl/Cit containing 0.2% SDS. After autoradiography with intensifying screens, the probe was removed from membranes in 0.4 M NaOH at 42 °C. The membranes were neutralized, prehybridized with carrier DNA, and hybridized with a second probe.

**RESULTS**

Two probes were used to map the chromosome location of the opal suppressor tRNA gene and pseudogene (O’Neill et al., 1985) in the human genome. One probe was a 193-bp fragment encoding the human gene. The second probe was a 350-bp fragment that occurs upstream of the gene and is separated from the gene by 45 bp (see Fig. 1).

The human opal suppressor tRNA gene was chromosomally mapped by Southern analysis of EcoRI-digested DNAs isolated from human-rodent somatic cell hybrids segregating human chromosmes. Using the 193-bp probe containing the opal suppressor tRNA gene, two hybridizing (5.5- and 8.5-kb) bands were detected in EcoRI digests of human placental DNA which were readily distinguished from 1.6-, 1.7-, and 7.4-kb Chinese hamster (Fig. 2A) or 2.1-kb mouse (Fig. 3A) homologous sequences. These two hybridizing human bands are located on different human chromosomes since they segregate independently in the different somatic cell hybrids (Figs. 2A and 3A). A series of 31 human-hamster and 52 human-mouse hybrids was examined, and the results indicate that the 5.5-kb band is located on human chromosome 19 while the 8.5-kb band is found on chromosome 22 (Table I).

Based upon previously described sizes of EcoRI fragments containing the opal suppressor tRNA gene and pseudogene (O’Neill et al., 1985), the gene can be assigned to chromosome 19 and the pseudogene to chromosome 22.

After autoradiography, the probe was then stripped from the Southern blots, and we hybridized these same blots with a 350-bp probe isolated from the 5’-flanking region of the gene. This probe detected only the 5.5-kb human EcoRI fragment (Figs. 2B and 3B) and confirmed the assignment of the active gene to chromosome 19. Hybridization of the flanking probe, although of reduced intensity, was observed with a single homologous band in hamster and mouse DNA, and it indicates good evolutionary conservation of a portion of the 5’-flanking sequence that is separated from the gene by 45 bp. Assignment of the opal suppressor tRNA gene (5.5-kb EcoRI fragment) to human chromosome 19 is based upon discordant segregation (≥12%) of the gene with all human chromosomes in the somatic cell hybrids except chromosome 19 (Table I). Concordant segregation of the gene with one human chromosome is expected and the infrequent discordancy (5% = 3%) of the gene with chromosome 19 requires explanation. The gene was not detected in hybrids which did not contain chromosome 19. The four discordancies all involved human-hamster hybrids in which the gene and human peptidase D expression were not detected but human glucose phosphate isomerase expression was observed. The glucose phosphate isomerase and human peptidase D loci have been assigned previously (Brook et al., 1985; Hulsebos et al., 1985; and Lusis et al., 1985) to the chromosome 19 long arm (19q) and short arm (19p), respectively, and a fragile site has been reported on this chromosome at 19q13 (Sutherland et al., 1985). Our results are explicable if the opal suppressor tRNA gene is located on the short arm of chromosome 19 (19p) and all four hybrids contain a chromosome 19 break with retention of only 19q. An equally plausible explanation for these results.
The opal suppressor tRNA gene and pseudogene were detected as 5.5- and 8.5-kb bands, respectively, in EcoRI digests of somatic cell hybrid DNAs after Southern hybridization with the 193-bp AvaI-HindIII probe. Detection of the human gene and pseudogene is correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy indicates presence of the chromosome when the gene is absent or presence of the chromosome despite the presence of the gene, and the sum of these numbers divided by total hybrids examined ($\times 100$) represents percent discordancy. The human-hamster hybrids consisted of 17 primary clones and 14 subclones (13 positive for 5.5-kb and 17 positive for 8.5-kb bands of 52 total) and the human-mouse hybrids consisted of 13 primary hybrids and 39 subclones (13 positive for 5.5-kb and 8 positive for 8.5-kb bands of 52 total). The isolation and characterization of the hybrid cell lines have been described (McBride et al., 1982a, 1982b, 1983, and 1986).

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* See text for explanation of the four discordancies (5%) of the gene with chromosome 19.

**DISCUSSION**

The human genome contains a single opal suppressor phosphoserine tRNA gene and a single opal suppressor tRNA pseudogene (O’Neill et al., 1985). By utilizing a 193-bp fragment encoding the opal suppressor tRNA gene as probe and hybridizing this probe to DNAs isolated from human-rodent somatic cell hybrids which have segregated human chromosomes (McBride et al., 1982a; McBride et al., 1982b; McBride...
Fig. 4. Southern hybridization of SacI-digested human DNAs isolated from the peripheral leukocytes of 10 normal individuals (lanes 1–10) with the gene probe (left panel) or the flanking probe (right panel). A restriction fragment length polymorphism was detected on chromosome 19 due to the presence of a SacI site resulting in a 8.6-kb hybridizing sequence. Lane 3 is homozygous for the 8.6-kb band and lanes 6 and 10 are heterozygous; lanes 1, 2, 4, 5, 7, 8, and 9 are homozygous for the 7.0-kb band, but a faint 8.6-kb band is observed in these lanes due to incomplete digestion. The invariant 3.1-kb hybridizing band detected only with the 193-kb probe represents the pseudogene on chromosome 22. A smaller amount of DNA is present in lane 6 than in the other lanes. DNA was isolated from leukocytes by techniques given under "Materials and Methods."

et al., 1983; McBride et al., 1986), the gene and pseudogene were mapped to human chromosomes 19 and 22, respectively. A 350-bp fragment which was isolated from the 5'-flanking region of the gene (see Fig. 1) was also used as probe to hybridize to human-rodent somatic cell hybrid DNAs. The latter probe recognized only a region on chromosome 19. The gene, therefore, occurs on chromosome 19. We tentatively localized the gene and pseudogene in this study to chromosomes 19 and 22, respectively, based on restriction analyses of the two regions (O'Neill et al., 1985). The use of the latter probe confirmed these assignments.

Our understanding of the occurrence of the opal suppressor phosphoserine tRNA gene in the mammalian population is further enhanced by examining the hybridization of the gene and flanking probes to mouse and hamster DNAs. For example, the gene probe hybridized to only a single homologous band in mouse DNA, indicating that the gene occurs in single copy. The mouse genome does not contain a detectable pseudogene since a single hybridizing band was observed in mouse DNAs digested with eight different restriction endonucleases, whereas two or three bands were found in corresponding human and hamster DNA digests. The flanking probe hybridized to the same band in mouse DNA as the gene probe. The latter observation provides further evidence that the flanking region is conserved in mammals. We had previously shown that the 5'-flanking region of the human and rabbit genes has a high degree of homology, and the possible significance of this observation has been discussed (Pratt et al., 1985). Hamster DNA, on the other hand, contains three bands, which hybridized to the gene probe, comprising at least one pseudogene in addition to a functional gene. The flanking probe appeared to hybridize to only one of these three bands. As noted above, the 5'-flanking region seems to be conserved in mammals and, most certainly, the DNA fragment hybridizing to probes covering the sequences of the flanking regions and structural gene represents a region including the functional phosphoserine tRNA gene and not the pseudogene. The faintly hybridizing 7.4-kb band from hamster DNA may represent a second pseudogene, but it could also result from polymorphism of the hamster pseudogene or an EcoRI site within the sequence hybridizing with the 193-bp probe.

It is of interest to note that the structural genes for seven aminocyl-tRNA synthetases have been mapped to chromosomes in the human genome (see Gerken et al., 1986; and Wasmuth and Carlock, 1986; and references therein). Four of these genes occur on chromosome 5 demonstrating substantial clustering for some members of this class of genes. However, to our knowledge the assignments of the opal suppressor phosphoserine gene and pseudogene to chromosomes 19 and 22, respectively, are only the second set of tRNA genes mapped to human chromosomes. Naylor et al. (1983) have determined that two tRNA<sup>Met</sup> genes, which are nonallelic, are located on human chromosome 6. Such studies as these provide additional markers for the human genome and contribute to our understanding of the genomic organization of the tRNA multigene family in man. These results indicate that tRNA genes are dispersed throughout the genome rather than all being clustered on a single chromosome. The opal suppressor phosphoserine tRNA gene also provides another useful polymorphic marker for mapping genes on chromosome 19 by genetic linkage analysis.

REFERENCES