A Human Opal Suppressor tRNA Gene and Pseudogene*

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Veronica A. O’Neill, Francine C. Eden‡, Karen Pratt§, and Dolph L. Hatfield¶

From the Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

A human DNA library, cloned in bacteriophage λ, was screened with an opal suppressor tRNA probe. Two genes were isolated, subcloned into pBR322, and sequenced. One is a normal opal suppressor tRNA gene 87 nucleotides in length without intervening sequences. It has a TCA anticodon demonstrating that the mature tRNA reads the termination codon UGA. The 5' internal control region for transcription has two extra nucleotides compared to the consensus sequence for eucaryotic tRNA genes, while the 3' internal control region is normal. This gene differs from a previously sequenced chicken opal suppressor serine tRNA gene (Hatfield, D., Dudeck, B., and Eden, F. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4940-4944) only at position 11. The second human gene appears to be a pseudogene truncated near the 3' end, since in the cloverleaf form of the mature tRNA there are three noncomplementary bases in the acceptor stem. The two human genes have a high degree of homology and, excluding the truncated 3' terminus of the pseudogene, differ in only two positions. The flanking sequences of the pseudogene are about 90% homologous to the consensus sequence of the human Alu family of repeated sequences. This gene appears to have been inserted between two adjacent Alu family members.

Misreading of termination codons by major cytoplasmic tRNAs has been observed in higher eucaryotic cells. For example, the normal tryptophan tRNA in rabbit reticulocytes suppresses the UGA termination signal in rabbit β globin mRNA (11). Furthermore, a tyrosine tRNA from Drosophila (12) and two tyrosine tRNAs from tobacco plants (13, 14), each of which lacks the highly modified Q base in its anticodon, misread the UAG termination signal in tobacco mosaic virus.

Two opal suppressor serine tRNAs, which occur in mammalian, avian, and amphibian cells (15, 16), are examples of naturally occurring nonsense suppressor tRNAs in higher eucaryotes. Sequences of these tRNAs from bovine liver (15, 16) and the sequence of one of these tRNAs from human and mouse cells (17) have shown that they are 90 nucleotides in length and that they contain few modified bases. The opal suppressor tRNAs from bovine and chicken tissues specifically recognize the nonsense codon UGA in a ribosomal binding assay, suppress UGA in protein synthesis (15, 16, 18), and form phosphoseryl-tRNA (16). The kinase which phosphorylates the opal suppressor seryl-tRNAs has been purified from bovine liver and appears to specifically phosphorylate these seryl-tRNAs (19). The unique characteristic of the opal suppressor tRNAs to form phosphorylated-tRNA suggests that they have a specialized function in higher eucaryotes. The insertion of phosphoserine directly into protein in response to specific UGA codons (15, 16) or an involvement in the reverse metabolic pathway from serine to 3-phosphohydroxypyruvate (19) have been proposed as cellular functions.

The uniqueness of the opal suppressor serine tRNAs prompted us to isolate and sequence the gene or genes encoding them from the genomes of several higher eucaryotes. In an initial study, we sequenced an opal suppressor serine tRNA gene from the chicken genome (18). It was 87 nucleotides in length and contained a TCA anticodon. Although two opal suppressor tRNA species were observed in the tRNA population of chicken liver, the genome appeared to contain a single gene (18). To continue an evolutionary study of the opal suppressor tRNA genes in higher eucaryotes and to possibly resolve the disparity of two tRNA products and only a single gene, a human genome has been screened for the presence of the opal suppressor tRNA gene(s). We report here the isolation and sequence of two human tRNA genes which hybridize to an opal suppressor tRNA probe. One is the normal gene encoding this tRNA and the other appears to be a pseudogene.

MATERIALS AND METHODS

A DNA cleaved with HindIII, phiX DNA cleaved with HaeIII, a 123-bp DNA ladder, and a 1-kilobase pair DNA ladder were used as

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† Present address: Meloy Laboratories, Inc., Revlon Health Care Group, 6715 Electronic Drive, Springfield, VA 22151.

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§ To whom correspondence should be sent.

2501
gel markers and were purchased from Bethesda Research Laboratories; restriction endonucleases were purchased from Bethesda Research Laboratories, International Biotechnology, Inc., and New England BioLabs; human placental DNA (ultrapure) from Sigma; [γ-32P]ATP (specific activity 5000–6000 Ci/mmol), [α-32P]dATP, and [γ-32P]dCTP (specific activities approximately 400 Ci/mmol) and [3H]serine (specific activity 28 Ci/mmol) from Amersham Corp. The human DNA library subcloned into bacteriophage λ Charon 4a was obtained from Dr. M. Zasloff, NICHD, NIH, Bethesda, MD.

Ribosomal Binding of Human Reticulocyte Seryl-tRNA—Human reticulocyte tRNA (obtained from Dr. R. Forget, Yale University School of Medicine, New Haven, CT) was aminocylated with [3H]serine in the presence of rabbit reticulocyte aminocyl-tRNA synthetases (20), the resulting [3H]seryl-tRNA fractionated over a RPC-5 column (20), the late eluting minor isoacceptors isolated (21), and ribosomal binding studies carried out (21) as previously described.

Gene Isolation and Sequencing—Screening of the human library, phage and plasmid DNA preparation and isolation, gel electrophoresis, restriction analyses of DNA, subcloning of human genes into the plasmid pBR322, and Southern blotting were as previously described (18, 22). Agarose gels contained 1% agarose unless otherwise identified in figure legends. Figures with photographs show the separated fragments as light bands in a dark background; and figures with autoradiograms show fragments which hybridized to the probe as dark bands in a light background. The sizes of DNA fragments were estimated on developed gels by the migration of marker DNAs.

**TABLE I**

Binding of human reticulocyte [3H]seryl-tRNA to ribosomes in response to UGA

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<th>Fractions</th>
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<th>cpm added</th>
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<td>Fractions 65–68</td>
<td>+UGA 1261, -UGA 6814, Δ 5883</td>
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<td>Fractions 70–74</td>
<td>+UGA 1669, -UGA 5932, Δ 1261</td>
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*Total [3H]seryl-tRNA from human reticulocytes was fractionated on a RPC-5 column, and the appropriate fractions were pooled as shown in the table and prepared for codon recognition studies as given under “Materials and Methods.”

*Amount of [3H]seryl-tRNA bound to ribosomes in the presence of UGA minus the amount bound in the absence of UGA.

*Amount of [3H]seryl-tRNA added to each assay.

ATP (specific activity 5000–6000 Ci/mmol), [α-32P]dATP, and [γ-32P]dCTP (specific activities approximately 400 Ci/mmol) and [3H]serine (specific activity 28 Ci/mmol) from Amersham Corp. The human DNA library subcloned into bacteriophage λ Charon 4a was obtained from Dr. M. Zasloff, NICHD, NIH, Bethesda, MD.

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Fig. 2. Localization of HG-2 within the recombinant DNA in λ and in pBR322. 1.75 and 1.0 μg of recombinant DNA in λ and in pBR322, respectively, were digested with various restriction endonucleases. Gels were developed, stained, and photographed, and hybridization assays were carried out with 2 × 10⁶ cpm of [³²P]tRNA probe. Autoradiograms were exposed for 24 h. In A each lane represents digestion of DNA from recombinant phages with: a) AvaI; b) BamHI; c) EcoRI; d) HindIII; e) PstI; f) PvuII; and g) SstI. In B each lane represents digestion of DNA from recombinant phages (lanes a, c, and e) and from recombinant plasmid (lanes b, d, and f). A 360-bp DNA fragment which hybridized to the probe was generated during AvaI digests of the recombinant phage encoding HG-2 (lane a). This fragment was subcloned into the AvaI site of pBR322 and was released during digests of the recombinant plasmid with AvaI (lane b). Other lanes show digests with AvaI and in addition: c and d) HaeIII; and e and f) HindIII.

Sequencing of DNA was carried out by the Maxam and Gilbert technique (23) except that the G + A reaction was in 60% formic acid.

Probes—Opal suppressor tRNA was purified from chicken liver and labeled with ³²P as described previously (18). Total tRNA, prepared from rabbit liver (21), was dephosphorylated and 5' end labeled using phage T4 polynucleotide kinase and γ-³²P]ATP. A 193-bp HindIII-AvaI fragment encoding the normal human gene was labeled with ³²P by Lohstrand Labs Limited of Bethesda, MD. A 170-bp MspI-AvaI fragment was isolated from the 3' flanking sequence of the human pseudogene (designated as HG-2), dephosphorylated, and end labeled with ³²P as described above. Five separate fragments were prepared from the 19,000-bp chicken DNA insert in λ (see Fig. 8 for the location and size of each fragment) and subcloned into pBR322. Each recombinant plasmid was nick translated (24) with [γ-³²P]dCTP and [α-³²P]dATP and used as probe.

RESULTS

Occurrence of Opal Suppressor tRNAs in Human Cells—[³²H]Seryl-tRNA from human reticulocytes was fractionated on a RPC-5 column, the late eluting minor isoacceptors isolated, and their response to UGA in a ribosomal binding assay determined. The results are shown in Table I. Clearly, there are two seryl-tRNA isoacceptors which occur in minor levels in human reticulocytes (20) that recognize the nonsense codon UGA.
plaque were purified, amplified, and used as a source of DNA.

Eight positive plaques were detected. Phages from each bacteriophage pattern, and one phage had a different pattern. The restriction RNA Probe-8.8 two restriction patterns were observed. Seven phages had one pattern, and sequence determination.

**Fig. 3.** Restriction endonuclease mapping and sequence strategy of DNA fragments encoding HG-1 and HG-2. In A the restriction endonuclease cleavage sites within the 1550-bp AvoI fragment encoding HG-1 (upper graph) and the 318-bp HindIII-AvoI fragment encoding HG-1 (lower graph) are shown. In B the restriction endonuclease cleavage sites within the 360-bp AvoI fragment encoding HG-2 are shown. Location of HG-1 and HG-2 is indicated by the heavy dark lines. The arrows show the direction and extent of nucleotide sequences derived from end labeling of these sites and sequence determination.

Isolation of Recombinant Phages which Hybridize to the RNA Probe—8.8 \times 10^5 plaques from a human DNA library in bacteriophage λ were screened with an opal suppressor tRNA probe. Eight positive plaques were detected. Phages from each plaque were purified, amplified, and used as a source of DNA. The DNA was mapped with restriction endonucleases, and two restriction patterns were observed. Seven phages had one pattern, and one phage had a different pattern. The restriction patterns of the two different phages are shown in Fig. 1 (designated HG-1) and in Fig. 2 (designated HG-2), respectively. Also shown in each figure are the restriction fragments that hybridized to the tRNA probe.

The sizes of the recombinant DNAs encoding HG-1 and HG-2 in bacteriophage λ Charon 4a were estimated from restriction digests with EcoRI endonuclease (lane a in Figs. 1A and 2A). The sizes of the DNA inserts were estimated to be 16,200 bp for HG-1 and 19,000 bp for HG-2.

The tRNA probe hybridized to a single restriction fragment encoding either HG-1 or HG-2 with each restriction endonuclease (Figs. 1A and 2A, respectively). A 1550-bp AvoI fragment encoding HG-1 (lane a of Fig. 1B) and a 360-bp AvoI fragment encoding HG-2 (lane a of Fig. 2B) were ligated separately into the AvoI site of pBR322 for further restriction analysis and sequencing. Comparisons of HG-1 in λ and in pBR322 digested with AvoI, with HindIII, and with AvoI in combination with other restriction endonucleases are shown in Fig. 1B. Similar experiments with HG-2 are shown in Fig. 2B. In the case of HG-1, the gene was further localized to a 193-bp HindIII-AvoI fragment.

**Sequence Analysis**—The sequences of a 318-bp region of the fragment encoding HG-1 and of the 360-bp (351 nucleotides sequenced) AvoI fragment encoding HG-2 were determined. Restriction endonuclease cleavage sites in the two fragments and the strategy used in deriving the sequences are shown in Fig. 3, A (HG-1) and B (HG-2). The arrows show the direction and extent of sequencing.

The nucleotide sequences of the anticoding strands of HG-1 and HG-2, along with their 5' and 3' flanking sequences, are shown in Fig. 4. For comparison, the nucleotide sequence of the chicken opal suppressor tRNA gene (18) is also shown. The regions encoding the genes are underlined. Each gene contains a TCA anticodon (designated by the asterisks in the figure) specifying a tRNA product which would read UGA. HG-1 is 87 nucleotides in length. It does not have an intervening sequence, and the CCA terminus of the mature tRNA is not encoded. HG-1 differs from the chicken opal suppressor tRNA gene only at position 11 where the human gene has a T and the chicken gene a C. The 5' and 3' flanking sequences of these two species show little homology with the exception of several common stretches of nucleotides in the -6 and -40 region. The termination signal for transcription, which is present as a T cluster in the anticydinding strand of the 3' flanking sequence of tRNA genes (25), is designated by the + symbol under HG-1 and under the chicken gene.

The sequence of HG-2 appears to be an incomplete structure for a tRNA. It has a C at position 87 instead of a G which is present in the other two tRNA genes. Bases at position 86 (G), 82 (T), and 83 (T) do not complement bases at positions 81 (G), 84 (C), and 5 (G) in the cloverleaf form of the mature tRNA. Therefore, HG-2 apparently is a pseudogene which is truncated near the 3' terminus. The two human genes show greater than 90% homology in their sequences, differing only in positions 5, 56, 82, 83, 84, 86, and 87. A termination signal for transcription was not detected in 170 bp sequenced in the 3' flanking DNA of HG-2. The 5' and 3' flanking sequences of HG-2 have about 90% homology in their sequences, differing only in positions 5, 56, 82, 83, 84, 86, and 87. A termination signal for transcription was not detected in 170 bp sequenced in the 3' flanking DNA of HG-2. The 5' and 3' flanking sequences of HG-2 have about 90% homology to the consensus sequence of the human Alu family of repeated sequences (26) see “Discussion”.

**Other Alu Family Members within the Flanking Sequences of HG-1 and HG-2**—A 170-bp MspI-AvoI fragment of the 3' flanking sequence of HG-2 (see Fig. 3B) was used as a probe to investigate the possibility that Alu family members also occur in the recombinant DNA encoding HG-1 and in other regions of recombinant DNA encoding the Alu-HG-2 sequence. Fig. 5 shows that Alu family members occur in restriction fragments of both recombinant DNAs. This is to be expected since Alu family members are present 500,000 times in the human genome and occur on average every 5000 base pairs (27).

Hybridization of HG-1, HG-2, and Their Flanking Sequences
with the Corresponding Sequences from Chicken DNA—To determine if the sequences surrounding HG-1 and HG-2 have been conserved in the flanking sequences of the chicken opal suppressor gene, probes were constructed from chicken DNA up to 3350 bp on the 5' side and 1800 bp on the 3' side of the human genes (see Fig. 6). The only chicken fragment which hybridized to the human DNA contained the opal suppressor gene found in the chicken genome (18). Opal suppressor tRNA genes are underlined and the asterisks show the position of the anticodon.

The bases at the 5' end of HG-2 which do not complement those at the 5' end are indicated by x. The termination signal in the 3' flanking sequences of HG-1 and the chicken gene are indicated by +.

**Fig. 4. Sequences of a 318-bp region encoding HG-1 and a 360-bp region encoding HG-2.** The human sequences are compared to the sequence determined for the opal suppressor tRNA gene and flanking regions of chicken DNA (18). Opal suppressor tRNA genes are underlined and the asterisks show the position of the anticodon. The bases at the 3' end of HG-2 which do not complement those at the 3' end are indicated by x. The termination signal in the 3' flanking sequences of HG-1 and the chicken gene are indicated by +.

**TABLE**

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<tr>
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**DISCUSSION**

The sequence of a human opal suppressor tRNA gene determined in the present study is very similar to that of an opal suppressor tRNA gene found in the chicken genome (18). They differ from each other at only a single position. The expected tRNA products of both genes are shown in their cloverleaf form in Fig. 8. The expected product of a second human gene (HG-2) is also shown in this figure. Some bases at the 5' and 3' ends of HG-2 are not complementary, and, therefore, this gene appears to be a pseudogene which has been truncated near the 3' terminus.
Transcription of tRNA genes involves two internal control regions designated as the 5' and the 3' internal promoter regions or as the A box and B box (28, 29). The consensus sequence of the A and B boxes for eucaryotic tRNAs and tRNA genes sequenced to date are RRYNNARYGG and GTTCAATTC, respectively (R = purine, Y = pyrimidine, and N = any base). The sequence for the region corresponding to the A box in the opal suppressor tRNA genes, including the human pseudogene, is GATCCTCAGTGG (nucleotides 9–20 in Fig. 4). Compared to the consensus sequence this region contains two extra nucleotides at positions 14 and 15 (underlined in above sequence). Whether the extra nucleotides in the A box result in a “down” promoter and effect a low level of transcription of these genes in vivo remains to be established. The tRNA products are indeed present in minor levels in cells (15, 16, 18, 20). The nucleotides in the B box of the chicken and human opal suppressor tRNA genes and the human pseudogene are GTTCAATTC (nucleotides 66–74 in above sequence). Whether the extra nucleotides upstream of these genes may exert on levels of expression.

The possibility that the 5' flanking sequences may have a role in controlling the expression of the opal suppressor tRNA genes should also be considered. Several studies have demonstrated a positive or a negative role of the 5' flanking sequences of eucaryotic tRNA genes on the level of transcription (30–35). Comparison of the 5' flanking sequences of the human and chicken opal suppressor tRNA genes shows several short stretches of homology in the −5 to −40 nucleotide region which may be involved in regulating expression. Homologies in this region and even further upstream are quite extensive when comparing human and rabbit opal suppressor tRNA genes; and a more complete analysis of the homologies in the 5' flanking sequences will be presented elsewhere. We are engaged in mutagenesis and transcription studies which should determine the effect that sequences upstream of these genes may exert on levels of expression.

Comparison of the 5' and 3' flanking sequences of the human pseudogene to those of the consensus sequence of the human Alu family genes (26) shows about 90% homology between these regions (see Fig. 9). This degree of homology...
Fig. 7. Hybridization of total human DNA with the opal suppressor tRNA gene. In A each lane represents the digestion of 40 µg of human genomic DNA with a different restriction endonuclease, electrophoresis of the resulting digests on an agarose gel, and transfer of the DNA to nitrocellulose filter paper and Southern hybridizations with 1.4 × 10^6 cpm of a 193-bp HindIII-AuAI fragment. Autoradiograms, which were exposed for 21 days, are shown from digestions with: a) AuAI; b) BamHI; c) EcoRI; d) HindIII; e) PstI; and f) SstI. In B each lane represents the digestion of 1 µg of DNA from recombinant phages encoding HG-2 (lanes a, c, e, g, i, and k) or HG-1 (lanes b, d, f, h, j, and l) with a different restriction endonuclease, electrophoresis of the resulting digests on a 1.8% agarose gel, and Southern hybridization with 5 × 10^6 cpm of tRNA probe. Autoradiograms, which were exposed for 30 h, are shown from digestions with: a) and b) AuAI; c and d) BamHI; e and f) EcoRI; g and h) HindIII; i and j) PstI; and k and l) SstI. λ DNA cleaved with HindIII and phiX DNA cleaved with HaeIII were used as markers on both agarose gels to determine the sizes of DNA fragments.

Fig. 8. Comparison of the expected tRNA products from HG-1, HG-2, and the chicken opal suppressor tRNA gene. The base difference between the human gene (HG-1) and the chicken gene is shown at position 11 as indicated by the arrow on the left-hand cloverleaf. Arrows on the right-hand cloverleaf show the base differences between the human pseudogene (HG-2) and the human normal gene (HG-1).

is an expected level between any random Alu sequence in the same genome. The 5' flanking sequence of the human pseudogene shows close homology to the 3' end of the Alu element; and its 3' flanking sequence is very similar to the 5' end of the Alu element. A segment of the 3' end of the upstream Alu element and a portion of the 5' end of the downstream Alu element (designated by the bases within the brackets in the figure) appear to have been deleted. We conclude, therefore, that the pseudogene has been inserted between two Alu family members, resulting in the loss of sequences from both partners during the recombination event.

Human reticulocytes contain two minor seryl-tRNAs which recognize the nonsense codon UGA in a ribosomal binding assay (see Table I). However, we have thus far found only a single gene in the chicken genome (18) and a single normal gene in the human genome. It is not clear if a second undetected gene is present which codes for the second isoacceptor (see Ref. 16 for the tRNA structures) or if both isoacceptors are encoded in a single gene and the differences in structures occur by post-transcriptional modification. Other possibilities which may account for the presence of a single gene and two very similar tRNA isoacceptors have been considered elsewhere (18).

Digestion of human genomic DNA with various restriction endonucleases and hybridization of the resulting fractionated DNA to a probe encoding the opal suppressor tRNA gene demonstrated the presence of three positive fragments in some digests. Two of these bands correspond to the normal opal suppressor tRNA gene and to the pseudogene. It is not known if the third region encodes a gene for the second isoacceptor discussed above, another pseudogene, an additional copy of the normal gene, or possibly is the result of incomplete genomic DNA methylation. In addition, it should be noted that it is possible that more than one or two copies of the opal suppressor tRNA gene may occur in the human genome. For example, multiple copies of the same gene having extensive flanking regions which have been highly conserved may occur. Digestion of such regions with different restriction...
endonucleases would yield only one or a few fragments which would hybridize with the probe.

The present study provides further evidence that the sequences of the opal suppressor serine tRNAs, present in higher vertebrates, are highly conserved. The fact that these serine tRNAs form phosphoseryl-tRNA makes them targets for examination of the functions and regulatory mechanisms of these opal suppressor tRNAs.

Acknowledgment—We express our sincere appreciation to Eileen Kessler for her assistance in screening the human library for the presence of the opal suppressor tRNA genes.

REFERENCES

### Human Opal Suppressor tRNA Gene and Pseudogene

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