The cDNA for human elongation factor-1α was isolated from a cDNA library of human fibroblast cells. Using the cDNA as a probe, a number of chromosomal genes encoding the human elongation factor-1α were isolated. Characterization of the clones by restriction enzyme mapping and nucleotide sequence analysis has revealed that only one of them is an active gene, whereas all of the other genes are processed pseudogenes. The active gene consists of 8 exons and 7 introns spanning about 3.5 kilobases, and the sequence of its exons is completely identical to that of the human elongation factor-1α cDNA. The first non-coding exon of 33 base pairs is separated by a 943-base pair intron from the coding exons. The primer extension of human elongation factor-1α mRNA has indicated that the transcription of human elongation factor-1α gene starts from a C residue, and a "TATA" box was found 24 base pairs upstream of the initiation site. Three and five Sp1 binding sites are present on the 5'-flanking region and the 1st intron, respectively. Furthermore, one Ap-1 binding site is located in the 1st intron. By using nuclear extracts from HeLa cells, the promoter of human elongation factor-1α gene could stimulate in vitro transcription better than the adenovirus major late promoter.

Prokaryotic polypeptide chain elongation factor Tu (EF-Tu) and its eukaryotic counterpart EF-1α promote the GTP-dependent binding of an aminocacyl-tRNA to ribosomes (1). EF-Tu was purified from Escherichia coli (E. coli) as a protein of M, 43,000 (2), and the primary structure comprised of 393 amino acids residues was determined (3). Moreover, the tertiary structure has been analyzed by x-ray diffraction (4, 5).

In eukaryotic cells, two independent translational machineries are present, i.e. one in the cytosolic fraction and the other in the mitochondria. As for the elongation factors, EF-1α functions in the cytosolic fraction with 80 S ribosomes, while mitochondrial EF-Tu (mtEF-Tu) functions in mitochondria with 70 S ribosomes. Cytosolic EF-1α was purified from pig liver (6), Artemia salina (A. salina) (7), and yeast (8) and is shown to consist of a single polypeptide chain with M, 53,000. On the other hand, mtEF-Tu which is closer to prokaryotic EF-Tu than cytosolic EF-1α, was purified from yeast mitochondria as a protein of M, 48,000 (9). More recently, several groups (10, 11) have found thesaurin a, the major protein of Xenopus laevis previtellogenic oocytes, is homologous to EF-1α and suggested that it may be a stage-specific EF-1α.

To study the gene structure and regulation of its expression, the genes for EF-Tu and EF-1α were isolated from several species. In E. coli, EF-Tu is coded by two nearly identical but unlinked genes (tufA and tufB) (12). Saccharomyces cerevisiae (S. cerevisiae) cells have two genes for EF-1α (13-16) and one gene for mtEF-Tu (17). The two genes for yeast EF-1α code for a protein of 458 amino acids with an identical amino acid sequence (14). The amino acid sequence of mtEF-Tu coded by the nuclear gene tuM is more homologous to E. coli EF-Tu than yeast cytosolic EF-1α (17). More recently, EF-1α cDNA was isolated from higher organisms such as A. salina (18) and human (19), and the gene organizations were reported for A. salina (20) and Drosophila melanogaster (D. melanogaster) (21) EF-1α.

In this report, we describe isolation and characterization of the human chromosomal gene for EF-1α. Although there are many sequences related to EF-1α cDNA in the human genome, most of them are found to be processed pseudogenes. One expressed chromosomal gene for human EF-1α was isolated and the nucleotide sequence analysis revealed its intron/exon organization. Furthermore, the promoter of the EF-1α chromosomal gene could stimulate in vitro transcription better than the adenovirus major late promoter.

**EXPERIMENTAL PROCEDURES**

Materials—Restriction endonucleases were purchased from Takara Shuzo or Toyobo and were used essentially as recommended by the supplier except for the use of 200 μg/ml gelatin instead of bovine serum albumin. Klenow fragment of E. coli DNA polymerase I and T4 DNA ligase were the products of Takara Shuzo, and T4 polynucleotide kinase was obtained from Toyobo. Avian myeloblastosis virus reverse transcriptase and S1 nuclease were purchased from Seikagaku Kogyo and Pharmacia LKB Biotechnology Inc., respectively. [γ-32P]ATP was prepared from carrier-free H332P04 (Du Pont-New England Nuclear) as described by Walseth and Johnson (22). [α-32P]UTP (3000 Ci/mmole) and [α-32P]UTP (3000 Ci/mmole) were purchased from Amerham Corp. The 18- and 24-mer oligonucleotides used for hybridization and primer extension were synthesized using a DNA synthesizer (Applied Biosystems model 380A).

**Isolation of Human EF-1α cDNA—Plasmid pNK1 containing yeast**
Fig. 1. Nucleotide sequences of human EF-1α cDNA and one of pseudogenes (λEF8). The dashed line indicates identity between the two sequences. The amino acid sequence of human EF-1α was deduced from the nucleotide sequence of cDNA. The 18-mer oligonucleotide used for the identification of the expressed chromosomal gene is boxed. The 15-nucleotide direct repeats framing the XEFS gene are underlined. The dashed line indicates identity between the two sequences. The amino acid sequence of human EF-1α was deduced from the nucleotide sequence of cDNA. The 18-mer oligonucleotide used for the identification of the expressed chromosomal gene is boxed. The 15-nucleotide direct repeats framing the XEFS gene are underlined.
EF-1α chromosomal gene (14) was digested with Clal and HindIII, and the 1-kb Clal-HindIII fragment was isolated by agarose gel electrophoresis. The Clal-HindIII fragment was labeled with 32P by nick translation using [α-32P]dCTP (23).

A human cDNA library constructed with mRNA from human fibroblast GM 637 cells using pCD vector system (24), was kindly provided to us by Dr. H. Okayama (National Institutes of Health, Bethesda, MD). About 40,000 colonies were screened by colony hybridization under low stringency (17) using the 32P-labeled Clal-HindIII fragment of pNK1. Plasmid DNAs were prepared from positive clones and characterized by restriction enzyme mapping and Southern hybridization analysis. One of the full-length cDNA clones (1.8 kb long) was designated as pAN7, and the nucleotide sequence was determined as described (25).

Southern Hybridization Analysis—Human chromosomal DNA was extracted from human leukocyte according to the method of Gross-Bellard et al. (26). Each 10 μg of chromosomal DNA was digested with various restriction enzymes, and subjected to 0.8% agarose gel electrophoresis. DNA fragments were then transferred to a nitrocellulose filter (Schleicher & Schuell) as described (23) and hybridized with a 32P-labeled BamHI fragment of pAN7 containing human EF-1α cDNA.

Cloning of Human EF-1α Chromosomal Gene—Human gene libraries constructed with human fetal liver DNA (27) and human placenta DNA (28) were provided by Dr. T. Maniatis (Harvard University) and Dr. M. Shibuya (Institute of Medical Science, University of Tokyo), respectively. The libraries were first screened by plaque hybridization (23) using the 32P-labeled 2-kb BamHI fragment containing human EF-1α cDNA. In some cases, the positive clones were screened using the 18-mer oligonucleotide which was labeled at their 5' end using T4 polynucleotide kinase and [γ-32P]ATP. DNAs were prepared as described (23) and characterized by restriction enzyme mapping and Southern hybridization analysis using 32P-labeled pAN7 cDNA. DNA fragments hybridizing with pAN7 cDNA was subcloned into the appropriate site of pBR322 or pUC119. The nucleotide sequence of the EF-1α gene region was determined by the dideoxynucleotide chain termination procedure after subcloning into M13 mp8 or mp9 (25).

Primer Extension—Total cellular RNA was extracted from human HL-60 cells by the guanidine thiocyanate/cesium chloride method as described (29). For the primer extension, the 32P-labeled 24-mer synthetic oligonucleotide (5 pmol) and 5 μg of poly(A) mRNA were denatured together at 90 °C for 3 min and gradually chilled (14). The mixture was then adjusted to contain, in 50 μl, 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 8 mM MgCl₂, 0.5 mM each of dNTPs, and 40 units of RNase inhibitor (Takara Shuzo). The cDNA was synthesized by incubation at 42 °C for 90 min with 25 units of avian myeloblastosis virus reverse transcriptase, and analyzed by electrophoresis through 8% polyacrylamide gel containing 7 M urea. As a size marker, 32P-labeled HaeIII-digested pBR322 was electrophoresed in parallel.

RESULTS

Isolation of Human EF-1α cDNA and Southern Hybridization Analysis of Human Chromosomal DNA—By using yeast EF-1α cDNA as a probe, we isolated human EF-1α cDNA from the cDNA library constructed with mRNA from human fibroblast GM 637 cells. One of the full length cDNA was designated as pAN7, and the complete nucleotide sequence was determined (Fig. 1). The coding sequence of pAN7 cDNA

FIG. 2. Southern hybridization analysis of human EF-1α gene. Genomic DNA from human leukocytes was digested with BglII (lane 1), EcoRI (lane 2), and HindIII (lane 3) and electrophoresed on a 0.8% agarose gel. DNA was transferred to a nitrocellulose filter and hybridized as described under "Experimental Procedures." A 32P-labeled BamHI fragment of human EF-1α cDNA was used as the probe. The DNA size marker was electrophoresed in parallel and is given indicated in kilobases. ori, origin of electrophoresis.

FIG. 3. The organization of human EF-1α chromosomal gene. Boxes and lines between them represent 8 exons and 7 introns, respectively. The size scale is indicated by a bar of 500 bp length on the upper right side of the figure. Coding sequences are indicated by solid boxes, while non-coding regions are represented by open boxes. The location of the major recognition sites for restriction enzymes are given above the gene. The sequencing strategy is shown under the restriction map, and arrows represent the direction and length of sequence determined by each independent experiment.
**Fig. 4.** Nucleotide sequence of human EF-la chromosomal gene.

The coding sequence of exons is translated and numbered from the ATG initiation codon. The TATA box on the 5' flanking region and "ATTAAA" polyadenylation signal are underlined. The initiation site for transcription and the polyadenylation site are marked by *. The putative Sp1 binding sites are boxed in the 5' flanking region and the first intron.
was 1386 bp long and was identical to the coding sequence of human EF-1α cDNA published by Brands et al. (19).

When purified DNA from human leukocytes was cleaved with EcoRI, BglII, or HindIII and analyzed by Southern hybridization using the human EF-la cDNA, more than 10 distinct bands were observed in each of the restriction enzyme-digested DNA (Fig. 2). Since subsequent analyses showed that some of the human EF-la genes contain HindIII, BglII, or EcoRI sites within the gene, the number of different DNA segments hybridizing to EF-la cDNA could not be determined.

Cloning of Human EF-1α Chromosomal Gene—In order to characterize the chromosomal gene for EF-1α, human gene libraries derived from human fetal liver DNA and human placenta DNA were screened using the human EF-la cDNA as a probe. From about 1.5 million plaques, 218 positive clones were obtained. Five of them were plaque-purified, and recombinant λDNAs were characterized by the restriction enzyme mapping, Southern hybridization, and the partial nucleotide sequence analysis. Although DNA fragments from these five clones hybridized very strongly with human EF-1α cDNA they did not contain any introns, but several point mutations, deletions, and insertions indicating that they are pseudogenes of human EF-1α. The complete nucleotide sequence of one of the clones (λEF8) is shown in Fig. 1, together with that of the human EF-1α cDNA. The overall homology between the nucleotide sequences of the λEF-8 pseudogene and human EF-1α cDNA was 97%. The pseudogene is terminated by a sequence of perfect 15-nucleotide direct repeats at both the 5’ and 3’ ends.

To isolate the active chromosomal gene for human EF-1α, the 70 clones identified by hybridization with the cDNA were rescreened using the oligonucleotide probe specific for the cDNA sequence. The 18-mer oligonucleotide probe contained the sequence of the nucleotide positions of 1562-1579 of the cDNA sequence. The 18-mer oligonucleotide probe contained the sequence of perfect 15-nucleotide direct repeats at both the 5’ and 3’ ends.
3'-non-coding region of the cDNA. This sequence was chosen because the 3'-non-coding region is known to mutate more rapidly than the coding sequence (30), and frequent mutations were found in this region of the pseudogenes which had been sequenced (Fig. 1). By screening with the oligonucleotide probe five clones gave positive results, and one of them (XEFg 58) was plaque-purified. Southern hybridization analysis of λEFg 58 DNA indicated that the 7-kb EcoRI fragment contained the DNA fragment hybridizing with the EF-1α cDNA. The 7-kb EcoRI fragment was subcloned at the EcoRI site of pUC 119 to yield plasmid pEFg1.

Structure of the Human EF-1α Chromosomal Gene—A fine restriction enzyme map of the human EF-1α chromosomal gene was constructed using pEFg1, and the nucleotide sequence was determined according to the strategy shown in Fig. 3. Fig. 4 presents about 5 kb of the nucleotide sequence containing the human EF-1α chromosomal gene. When the human EF-1α cDNA sequence was aligned with the exons of the chromosomal gene sequence, both sequences matched completely and revealed the structural organization of the human EF-1α gene (Fig. 3). The gene is split by 7 introns, and all of the splice donor and acceptor sites conform to the GT...AG rule (31) for the nucleotides immediately flanking exon borders. The first exon, consisting of 33 nucleotides, is located 943 bp upstream from the second exon which contains the ATG initiation codon. Other introns are relatively short and consist of 83–376 bp.

The Initiation Site for Translation—The transcription start point of human EF-1α gene was determined by the primer extension method. Poly(A) mRNA was prepared from human HL-60 cells and hybridized with the excess of 5'-labeled 24-mer primer, 5'TGGTGTCTGGCGGCAAAACC-GTTTG-3', which is complementary to nucleotide positions 584–607 (Fig. 4). After incubation with avian myeloblastosis virus reverse transcriptase, the synthesized cDNA was separated by polyacrylamide gel electrophoresis. As shown in Fig. 5, two bands of 32 and 33 nucleotides were obtained. Since avian myeloblastosis virus reverse transcriptase can erroneously transcribe the "cap" structure giving one nucleotide longer cDNA (32), we assigned the major initiation site of transcription of human EF-1α gene to the C residue at position 576 (Fig. 4).

Transcription of EF-1α Gene in a Cell-free System—We transcribed the human EF-1α gene in a cell-free system derived from HeLa cells. pEFg1 DNA was truncated at the PsI site and Apal sites, which are located at 128 and 428 nucleotides, respectively, downstream of the start site determined by the primer extension method. The DNA templates were incubated with the HeLa cell nuclear extract (29) in the presence of [α-32P]UTP, and the run-off RNA products were analyzed by polyacrylamide gel electrophoresis. As shown in Fig. 6, the Apal-digested pEFg1 DNA gave a transcript of 428 nucleotides long, while an RNA of 124 nucleotides long was produced by using the PsI-digested pEFg1 as a template. These results suggest that the transcription start site determined by primer extension was used also as the initiation site in the cell-free transcription system. To examine the relative strength of the human EF-1α gene promoter, pAdSmaF containing the adenovirus major late promoter was digested with SmaI and transcribed in parallel under the same conditions. The analysis of the RNA products (Fig. 6) indicates that the human EF-1α promoter is stronger than the adenovirus major late promoter. Both templates, Apal-digested pEFg1 and PsI-digested pEFg1, directed the labeling of additional products...
In this paper, we described the isolation and structural analysis of the human EF-1α gene. By screening 1.5 × 10⁶ plaques of human gene library with human EF-1α cDNA, we have obtained more than 200 positive clones. Assuming an average size of 15 kb for the inserts in the human gene library and a genome size of 3 × 10⁹ bp, we can expect one in 2 × 10⁶ plaques carrying a particular single-copy sequence. This suggests that there may be more than 20 DNA segments homologous to the EF-1α gene per human haploid genome, which agrees with the result obtained by the Southern hybridization (Fig. 6). For EF-1α, the complete nucleotide sequence analysis of each of the resultant transcript into the genome of germline cells (34).

DISCUSSION

In this paper, we described the isolation and structural analysis of the human EF-1α gene. By screening 1.5 × 10⁶ plaques of human gene library with human EF-1α cDNA, we have obtained more than 200 positive clones. Assuming an average size of 15 kb for the inserts in the human gene library and a genome size of 3 × 10⁹ bp, we can expect one in 2 × 10⁶ plaques carrying a particular single-copy sequence. This suggests that there may be more than 20 DNA segments homologous to the EF-1α gene per human haploid genome, which agrees with the result obtained by the Southern hybridization analysis of human genomic DNA with the EF-1α cDNA (Fig. 2).

When 5 clones were picked up randomly from these positive plaques, none of them contained introns and their sequences did not match completely with that of the cDNA. It seems, therefore, that most of the DNA segments related to the EF-1α cDNA in the human genome are pseudogenes for EF-1α. The complete nucleotide sequence analysis of one of them indicated that it is a full length copy of the spliced resultant transcript into the genome of germline cells (34).

The presence of the 15-nucleotide perfect repeats framing the junctions of exon and introns are indicated by (Fig. 7) (32). The amino acid sequences indicate the consensus sequences of the phosphoryl residues in the amino acid sequences of EF-la and EF-Tu from human (hEF-1α) (30), D. melanogaster (dEF-1α) (31), S. cerevisiae (yEF-1α) (33-36), chloroplast of Euglena gracilis (tufC) (37), mitochondria of S. cerevisiae (tufM) (17), and E. coli (tufA) (42) are aligned to give maximal homology by introducing several gaps (-).

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possible that there may exist more than one actively expressed gene in the human genome. Furthermore, the human genome would contain the genes for mitochondrial EF-Tu and the sauran which was found in Xenopus previtellogenetic oocytes as a protein homologous to EF-1α (10, 11).

So far, eukaryotic EF-1α genes have been isolated from S. cerevisiae (13-16), A. salina (20), chloroplast of Euglena gracilis (37), D. melanogaster (21), and human (this report). All EF-1α genes, except the yeast EF-1α gene, contain introns: 4 in A. salina EF-1α gene; 1 in D. melanogaster EF-1α gene; and 7 in the human EF-1α gene. Some of these introns are within 5′- or 3′-non-coding regions. When the amino acid sequences predicted by the EF-1α genes were aligned, it was found that the four splice junctions are conserved between the A. salina and human EF-1α genes (Fig. 7). Furthermore, the position of the 2nd intron of the chloroplast EF-Tu gene is close to the last intron of the human and A. salina EF-1α genes. Examination of the correlation of the exons with the structural domains of E. coli EF-Tu molecules (4, 5) revealed the following properties. The 2nd exon of human EF-1α gene corresponds to the N-terminal domain of E. coli EF-Tu, which can be cleaved off by a limited trypsin digestion. In this domain, the conserved phosphoryl binding sequence (Gly-X-X-Gly-Lys) (38) is located. The amino acid sequence of the 3rd exon comprises two β-sheets and one α-helix, and contains the second phosphoryl binding site of Asp-X-Gly in which the Asp residue can be linked with GDP via Mg²⁺.

The 4th exon contains the typical mononucleotide fold of three β-strands in which the guanine-specific binding site of Asn-Lys-X-Asp is present. Furthermore, the x-ray analysis of E. coli EF-Tu indicates that there are additional domains consisting of amino acids 200-296 and 297-393 at the C terminus of the molecule (4). These domains may correspond to the 6th and 7th exon of the human EF-1α gene, respectively.

EF-Tu in E. coli and EF-1α in yeast are one of the most abundant proteins in these organisms, and 4-6% of total soluble proteins in E. coli and yeast are EF-Tu or EF-1α, respectively (2, 8). In mammalian cells, EF-1α is also present abundantly in almost all kinds of tissues, and the EF-1α gene can be regarded as one of the so-called "housekeeping" genes. Using the nuclear extracts from HeLa cells, the promoter of the EF-1α gene is located at a cytosine residue which is embedded in a sequence similar to the AP1 binding site (C/GTGACTC/AA) which indicates that the promoter of the EF-la gene is located at a cytosine residue which is embedded in a sequence similar to the AP1 binding site (C/GTGACTC/AA) (40) is located. The amino acid sequence of the 3rd exon comprises two @-sheets and one a-helix, and contains the second phosphoryl binding sequence (Gly-X-X-Gly-Lys) (38) is located. The amino acid sequence of the 3rd exon comprises two β-sheets and one α-helix, and contains the second phosphoryl binding site of Asp-X-Gly in which the Asp residue can be linked with GDP via Mg²⁺.

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