GENOMICS **46,** 234–239 (1997)
ARTICLE NO. GE975038

Cloning, Sequencing, Gene Organization, and Localization of the Human Ribosomal Protein RPL23A Gene

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Received May 5, 1997; accepted September 18, 1997

The intron-containing gene for human ribosomal protein RPL23A has been cloned, sequenced, and localized. The gene is approximately 4.0 kb in length and contains five exons and four introns. All splice sites exactly match the AG/GT consensus rule. The transcript is about 0.6 kb and is detected in all tissues examined. In adult tissues, the RPL23A transcript is dramatically more abundant in pancreas, skeletal muscle, and heart, while much less abundant in kidney, brain, placenta, lung, and liver. A full-length cDNA clone of 576 nt was identified, and the nucleotide sequence was found to match the exon sequence precisely. The open reading frame encodes a polypeptide of 156 amino acids, which is absolutely conserved with the rat RPL23A protein. In the 5′ flanking region of the gene, a canonical TATA sequence and a defined CAAT box were found for the first time in a mammalian ribosomal protein gene. The intron-containing RPL23A gene was mapped to cytogenetic band 17q11 by fluorescence *in situ* hybridization.

INTRODUCTION

The ribosome is a cellular organelle responsible for synthesis of proteins in all cells. Its structural organization consists of about 80 ribosomal proteins (rp) and four RNA species (18S RNA in the 40 S r-subunit and 28S, 5S, and 5.8S RNA in the 60S r-subunit), which reflects a complex coordinating mechanism that has not yet been elucidated fully. Isolating all rp cDNAs and chromosomally mapping their intron-containing genes in higher eukaryotes are, therefore, the fundamental steps to elucidate the mechanism further.

Ribosomal genes, in general, seem to be present in families composed of multiple intronless pseudogenes and only one intron-containing functional gene (Mazuruk *et al.*, 1996). The pseudogene often lacks a functional promoter and is usually located around multiple repetitive elements (Dudov and Perry, 1984; Wagner and Perry, 1985). Because many pseudogene copies exist, it is relatively difficult to localize a functional rp gene in a complex genome. In mammals including humans, only a handful of intron-containing rp genes have been cloned and mapped until recently (Wiedemann *et al.*, 1987; Antoine and Fried, 1992; Foe *et al.*, 1992; Davies and Fried, 1993, 1995; Gallagher *et al.*, 1994; Annilo *et al.*, 1995; Nolte *et al.*, 1996; Mazuruk *et al.*, 1996).

The rp genes not only play a vital role in protein synthesis, but their mutations may also be implicated in some diseases. It has been speculated for a long time that haploinsufficiency of rp genes may play an important role by underlying the Turner phenotype in humans, which is predominantly a consequence of X-chromosome monosomy (Fisher *et al.*, 1990). In *Drosophila melanogaster*, it has been demonstrated that the rp gene L9 is located in *Minute* locus M(2)32D, and transferring a functional L9 gene into mutant flies can rescue the *Minute* phenotype completely (Schmidt *et al.*, 1996). Furthermore, analysis of the 5′ end of the human RPL7A gene has revealed that it is able to activate the *trk* proto-oncogene receptor kinase domain (Kozma *et al.*, 1988). Therefore, some rp genes may be related to oncogenesis through regulatory pathways.

In this report, we present the data about cloning, sequencing, gene organization, and chromosomal localization of the intron-containing human RPL23A gene. The potential promoter elements in the 5′ flanking region are also shown and discussed.

MATERIALS AND METHODS

BAC and cosmid clones. BAC clone 94912 was isolated from a total human BAC library (Research Genetics, Huntsville, AL) representing a 2.6× coverage of the haploid human genome. Cosmid clone 103C6 was identified from a chromosome 17-specific genomic library (17NC01, Los Alamos National Laboratory, Los Alamos, NM). A radioactively labeled human RPL23A full-length cDNA probe was used for screening the above genomic libraries.

Northern analysis. Northern analysis was performed by standard methods. The RNA blot was purchased from Clontech (Palo Alto, CA). The probe was labeled with [^{32}P]dCTP by *Taq* polymerase extension (Medori *et al.*, 1992).

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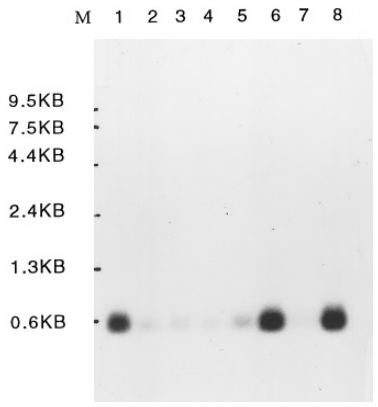

FIG. 1. Northern analysis of the human RPL23A gene. The blot contained poly(A)$^+$ mRNA at 2 μg per lane, hybridized to the radioactive RPL23A cDNA probe. The different tissues represented in the blot were: lane **1,** heart; lane **2,** brain; lane **3,** placenta; lane **4,** lung; lane **5,** liver; lane **6,** skeletal muscle; lane **7,** kidney; and lane **8,** pancreas. The positive signal is about 0.6 kb in length.

Full-length cDNA isolation and cDNA library. The GeneTrapper method was employed to isolate the full-length cDNA from a liver cDNA library. Both the GeneTrapper kit and the cDNA library were commercially purchased from Life Technologies (Gaithersburg, MD). For the GeneTrapper procedure, we exactly followed the manufacturer's instructions.

Primer designation for detecting the intron-containing gene. Several pairs of primers were designed based on the cDNA sequence (Fig. 2A). Primer set A consists of intraexon primers including Ribo-F1 (from 44 to 63 bp of the cDNA sequence) and Ribo-R1 (reverse primer from 197 to 216 bp of the cDNA sequence). Primer sets B and C consist of interexon primers. Both sets share Ribo-F1 as forward primer but use different reverse primers; i.e., set B uses Ribo-R2 (from 494 to 513 bp of the cDNA sequence) and set C uses Ribo-R3 (451 to 468 bp of the cDNA sequence). These three sets of the primers faithfully amplify 173-, 470-, and 425-bp DNA fragments, respectively, from the full-length cDNA clone. The PCR conditions were 94°C for 2.5 min (94°C for 30 s, 55°C for 30 s, 72°C for 60 s) × 30 cycles and 72°C holding for 5 min.

RESULTS

Northern Analysis

To isolate the disease-related gene from human chromosomes 19p13.1 and 6p23.1, we had previously performed several cDNA selections (Fan *et al.,* 1996; Fan *et al.,* 1997, in preparation) and repeatedly recovered cDNA fragment 1C4, which was 89% identical to rat RPL23A.

In an effort to estimate the transcript size and its expression pattern, we carried out a Northern analysis using multiple poly(A)$^+$ mRNA samples from different human tissues. The radioactively labeled cDNA fragment 1C4 was used as probe. As seen in Fig. 1, the result indicates that the transcript of the RPL23A gene is about 0.6 kb in length. It is present in all tissues as expected; however, its expression is dramatically abundant in pancreas, skeletal muscle, and heart while much lower in kidney, brain, placenta, lung, and liver. Since only one band was observed in all mRNA samples, we concluded that there was no alternative mRNA splicing for the human RPL23A gene.

Isolation of the Full-Length cDNA Clone

We used the GeneTrapper method to isolate the full-length cDNA clone from a liver cDNA library. It was found that the complete full-length cDNA clone is 576 nt. The open reading frame is 468 nt (from nucleotides 22 to 489) and codes for 156 amino acids (Fig. 2A). The 5′ untranslated region (UTR) is 21 nt and contains an oligopyrimidine tract, CTTTTC, which has been found in most of other mammalian rp genes (Mazuruk *et al.,* 1996). The translation start codon occurs in the context AAGATGG, which does not significantly depart from the optimum ACCATGG (Kozak, 1986). The 3′ UTR is 84 nt long and includes a 30-nt-long poly(A)$^+$ tail. The polyadenylation signal, AATATA instead of the usual consensus sequence AATAAA, is at positions 517 to 522 nt. It is 24 nucleotides upstream of the poly(A)$^+$ tail and is exactly the same as that of the rat RPL23A gene (Suzuki and Wool, 1993). Database searching indicates that the open reading frame is highly homologous to the rat RPL23A protein, with 89.6% nucleotide identity and 100% amino acid identity. Since 40% of the first 50 amino acids at the N-terminal consist of basic amino acids, this region may be an rRNA- or mRNA-binding domain.

Cloning and Mapping the Intron-Containing Human RPL23A Gene

To obtain the intron-containing RPL23A gene, we screened a human genomic BAC library with the full-length cDNA probe. A total of 46 positive clones were identified from a 2.6× coverage of the human haploid genome, suggesting that about 30 to 40 copies of the RPL23A gene exist in the human genome. When all the positive BAC clones were tested by PCR using the three sets of primers, only one clone (94912) was found to contain introns. Southern analysis of restriction fragments showed that BAC clone 94912 contained a positive DNA fragment of 9 kb after *Bam*HI digestion, whereas most of the other positive clones contained a positive DNA fragment less than 3.0 kb.

To localize the intron-containing RPL23A gene, *in situ* hybridization was performed using BAC clone 94912 probe. Surprisingly, the results showed that this clone was mapped to two loci at 17q11 and 1p22.3–p31.1 (data not shown). Considering that the functional gene is no more than 10 kb and that the BAC clone houses a DNA insert of more than 120 kb, it is possible that one of the FISH signals was the result of some repetitive sequence or other gene family instead of the RPL23A gene. To get a shorter clone, we screened a chromosome 17-specific cosmid library (17NC01). The screening revealed numerous positive clones, and clone 103C6 was randomly chosen for further characterization. PCR analysis demonstrated that primer set B amplified a 3.1-kb DNA fragment and primer set C amplified a 2.85-kb DNA fragment using cosmid clone 103C6 as template (Fig. 3). It was noticed that though there

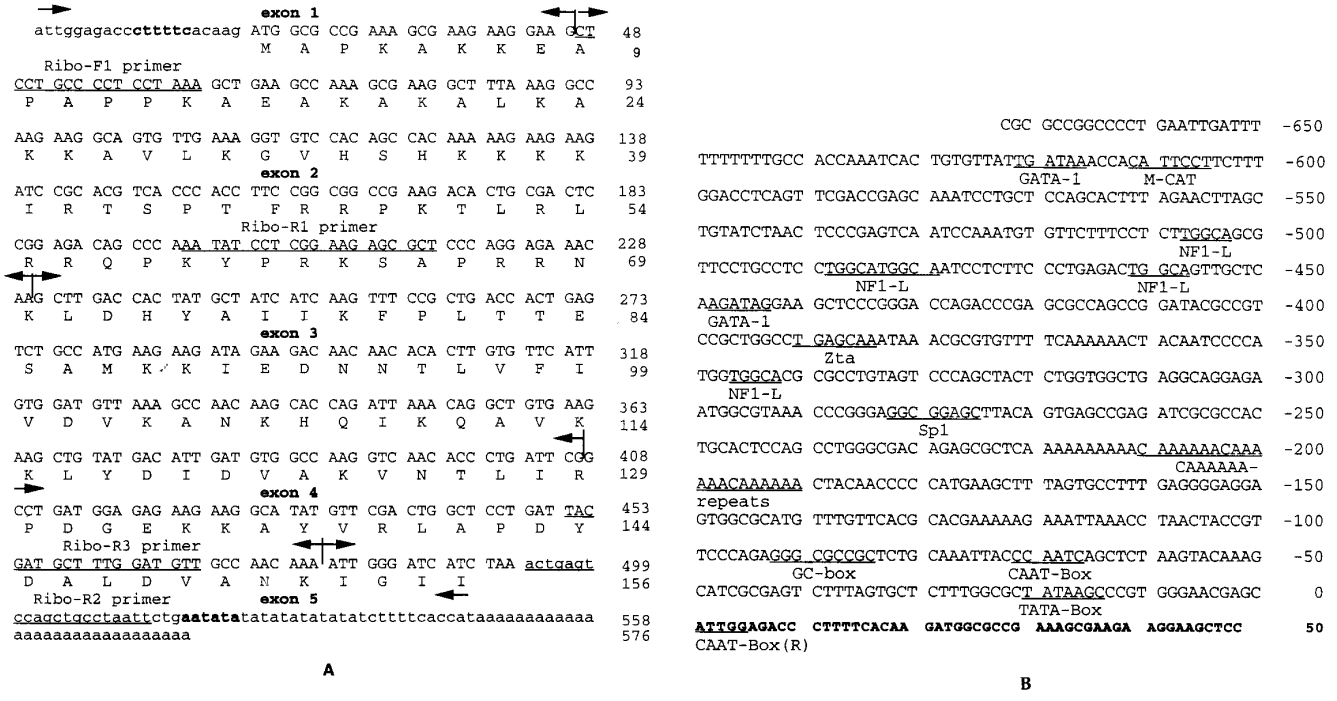

FIG. 2. (**A**) DNA sequence of the full-length cDNA of the human RPL23A gene. The coding region is in capital letters, and the noncoding region is in lowercase letters. The deduced amino acids are shown in single-letter code below the second base of the codon. The exons are divided by vertical lines at the boundary and marked by arrows. The oligopyrimidine, CTTTTC, and the 3′ poly(A) signal, AATATA, are shown in boldface letters. The primers used for searching the intron-containing gene are indicated as underlined sequence. (**B**) DNA sequence of the 5′ flanking region of the RPL23A gene. The potential promoter elements or transcription factor-binding motifs are underlined, and the corresponding names are under each line. In addition to the potential promoter elements, a CpG island is present from −543 to −287 bp and a $(CAAAAAA)_3$ repeat is present from −272 to −241 bp. The minus number is for flanking sequence, and the boldface letters are for the transcribed sequence.

was only a 45-bp difference between primer sets B and C in the cDNA sequence (Fig. 2A), the DNA fragments amplified from the genomic clone demonstrated a 250-bp difference (Fig. 3), indicating that an intron exists between the two primers. This prediction was proved later by DNA sequencing. When genomic clone 103C6 was used for the FISH experiment, the results clearly showed that the intron-containing human RPL23A gene is localized to chromosome 17 at 17q11 only (data not shown).

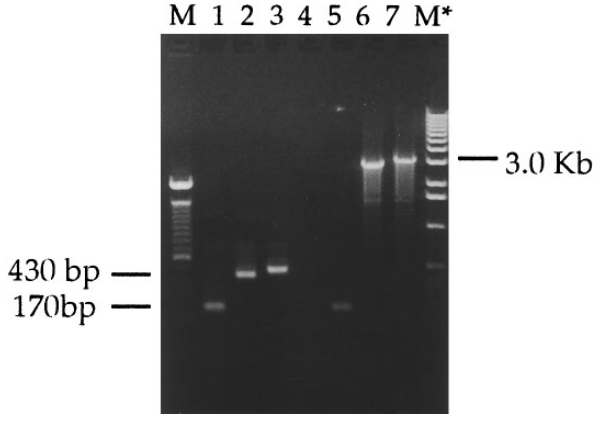

FIG. 3. PCR analysis of the human RPL23A cDNA and the intron-containing gene (cosmid clone 103C6) using three different sets of primers. Lane **M,** the 100-bp DNA marker; lane **M*,** the 1-kb DNA marker. Lanes **1** to **3** show the PCR product from the full-length RPL23A cDNA clone, lanes **5** to **7** show the PCR product from chromosome 17-specific cosmid clone 103C6, and lane **4** is the PCR control without DNA template. Primer set A was used for lanes **1** and **5;** primer set C was used for lanes **2** and **6;** and primer set B was used for lanes **3** and **7.** The numbers to the left and right of the figure indicate the approximate DNA size. For primer details, see Materials and Methods.

Five Exons and Four Introns Exist within the Human RPL23A Gene

We completely sequenced the intron-containing RPL23A gene after cloning a 9-kb *Bam*HI fragment from cosmid clone 103C6 into a plasmid vector (Blue-Script). As seen in Fig. 4, the human RPL23A gene contains five exons and four introns. It spans 3947 bp at full length, and all exon–intron junctions (splice sites) precisely follow the GT/AG consensus rule. The full-length cDNA sequence and the genomic sequence can be obtained from GenBank under Accession No. U37230 (cDNA sequence) and AF001689 (genomic sequence). Based on the presence of introns and the precise match between the exons and the cDNA sequence, we believe that cosmid clone 103C6 derived from human chromosome 17 represents the functional gene. The other RPL23A genes identified at 6p21.3 and 19p13.1 are pseudogenes since they lack introns and contain many nonsense mutations (data not shown).

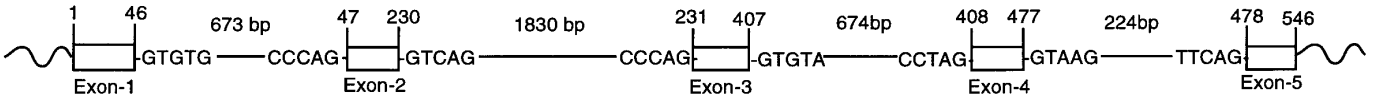

FIG. 4. The organization of the human RPL23A gene. The gene is located at chromosome 17q11 and spans 3947 bp, containing five exons and four introns. Rectangles represent exons while lines represent introns. The numbers over both ends of the boxes mark the beginning and ending basepair of the exons, and the number above each line indicates intron length. The letters represent five nucleotide sequences at the 5′ and 3′ ends of each intron.

The Potential Promoter Elements in the 5′ Flanking Region

From 673 bp of available 5′ flanking region sequence (Fig. 2B), numerous putative promoter elements were identified. Surprisingly, a TATA-box, TATAAGC, instead of the typical consensus sequence, TATAAAA, is present from −15 to −21 bp. The box is surrounded by a G + C-rich sequence as GGCGCTATAAGCCCG. In addition, two CCAAT-boxes were found. One is a canonical CCAAT box from −67 to −72 bp, and one is an inverted CCAAT-box, ATTGG, downstream of the transcriptional startpoint from +1 to +5 bp. Since promoter elements in rp genes are often located both upstream and downstream of the cap site (Hariharan and Perry, 1991), this is no surprise.

Other potential promoter elements include a GC-box from −85 to −93 bp, a Sp1 element from −276 to −283 bp, five NF1-like elements (TGGCA) scattered from −343 to −508 bp (i.e., from −343 to −347 bp, from −458 to −462 bp, from −480 to −484 bp, from −485 to −489 bp, and from −504 to −508 bp), a Zta element from −335 to −341 bp, two GATA-boxes from −444 to −449 bp and from −617 to −622 bp, respectively, and a M-CAT element from −606 to −612 bp. There is a CpG island from −287 to −543 bp. Interestingly, a $(CAAAAAA)_3$ repeat is present from −222 to −191 (Fig. 2B).

DISCUSSION

The ribosome is among the most important complexes in all cells; therefore, its components, the rp proteins, are well conserved evolutionarily (Jones *et al.*, 1991; Rhoads and Roufa, 1991; Suzuki and Wool, 1993). The deduced human RPL23A protein is 100% identical to the rat RPL23A protein at the amino acid level, indicating that RPL23A is such an extremely vital component that it has not incurred a single amino acid change since the divergence of human and murine (about 75 million years). The highly conserved sequences are present not only in the coding region, but also in the noncoding area. The first one is the oligopyrimidine tract, CTTTTC, located in the 5′ UTR, which has been found in 17 human rp genes and 8 murine rp genes to date (Levy *et al.*, 1991; Mazuruk *et al.*, 1996). The second is the polyadenylation signal located in the 3′ UTR. In both the human and the rat RPL23A genes, the signal sequence is AATATA instead of AATAAA. Furthermore, there is a $(TA)_8$ dinucleotide repeat immediately downstream of the AATATA signal in the human RPL23A gene. In yeast, the $(TA)_n$ repeat is an efficiency element for polyadenylation in the 3′ UTR of the mRNA (Guo *et al.*, 1995; Egli *et al.*, 1995). Since the rp proteins are vital components in all cells, the $(TA)_n$ repeat may be a conserved element employed to "guarantee" efficient polyadenylation, or it may play another unknown role in mRNA translation.

The 5′ flanking region of the human RPL23A gene contains a canonical TATA-box. Although common in polymerase II-transcribed promoters, this has not been found in any other known mammalian rp genes (Hariharan and Perry, 1990; Safrany and Perry, 1993). There are three features of this element in the human RPL23A gene. First, the TATA-box and the oligopyrimidine tract, CTTTTC, coexist in the same flanking region. It is said that the rp genes are regulated by TATA-less promoters (Hariharan and Perry, 1990) and the oligopyrimidine element actually plays a TATA-box-like function and defines the transcriptional start position, but this is clearly not the case for this gene. Second, the TATA-box is closer to the transcription start point (from −15 to −21 bp) compared to other genes (from −19 to −29 bp). Third, its consensus sequence is not TATAAAA, but TATAAGC. Although the sequence TATAAGC is rare, it does occur in some genes (Lewin, 1994). Since TATAAGC is surrounded by a G + C-rich sequence, we believe it is the functional TATA-box for the human RPL23A gene.

Compared to other rp genes, the human RPL23A gene contains three unusual promoter elements (Fig. 2B). The first one is the M-CAT element, which supposedly mediates both muscle-specific and non-muscle-specific transcription (Larkin *et al.*, 1996). The second is a Zta element, which is supposedly bound by the Epstein–Barr virus Zta transactivator (Chang *et al.*, 1990). Third, there are five copies of the NF1-like or TGGCA elements. It is said that the NF1-like proteins play a role in transcription of liver-specific genes (Paonessa *et al.*, 1988). The human RPL23A gene, however, is not a liver-specific gene at all. Instead, it is a housekeeping gene. The presence of a CpG island in the flanking region from −287 to −543 bp is another piece of evidence. Since NF1-like proteins are a group of heterogeneous *trans*-acting factors, some may be ubiquitously expressed in a variety of tissues (Lichtsteiner *et al.*, 1987; Lijam and Sussman, 1995) and serve as both transcription activator and repressor through different combinations of a common set of regulatory molecules. If this is the case, then the presence of TGGCA elements indicates that either expression of

the human RPL23A gene is regulated by a complex mechanism or the same gene plays an unknown second function. It is known that many rp proteins have a second function during cell growth and differentiation (Nolte *et al.*, 1996), so it is possible that the RPL23A gene has functions other than serving in ribosome. Interestingly, there is a $(CAAAAAA)_3$ repeat located from −222 to −191 bp. It may be a polymorphic marker in the 5′ of the gene, or it may play some regulatory role in gene expression.

In this report, we presented the data about cloning the human RPL23A gene. The study was first initiated from chromosome 19 and the HLA complex (6p21.3) by cDNA selection, and it was subsequently demonstrated that these chromosomal substrates actually encoded intronless pseudogenes. This fact indicates that cDNA selection cannot distinguish intronless (or pseudogene) genes from intron-containing (or functional) genes. Since the selection is a DNA hybridization process, as long as the cDNA and genomic DNA are homologous enough for cross-hybridization, the cDNA will be selected. We concluded that gene mapping based on cDNA selection is not reliable for certain genes.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-Eng-48. We express our thanks to the LLNL Sequence Core for sequencing support and to Dr. Wu-bo Li and Joe Jeffrey from BRL for the initial assistance with the GeneTrapper method.

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