The human mitochondrial 12 S rRNA A1555G mutation has been found to be associated with aminoglycoside-induced and non-syndromic deafness. However, putative nuclear modifier gene(s) have been proposed to regulate the phenotypic expression of this mutation. In yeast, the mutant alleles of MTO1, encoding a mitochondrial protein, manifest respiratory-deficient phenotype only when coupled with the mitochondrial 15 S rRNA Pr(454) mutation corresponding to human A1555G mutation. This suggests that the MTO1-like modifier gene may influence the phenotypic expression of human A1555G mutation. Here we report the identification of full-length cDNA and elucidation of genomic organization of the human MTO1 homolog. Human Mto1 is an evolutionarily conserved protein that implicates a role in the mitochondrial tRNA modification. Functional conservation of this protein is supported by the observation that isolated human MTO1 cDNA can complement the respiratory deficient phenotype of yeast mto1 cells carrying Pr(454) mutation. MTO1 is ubiquitously expressed in various tissues, but with a markedly elevated expression in tissues of high metabolic rates including cochlea. These observations suggest that human MTO1 is a structural and functional homolog of yeast MTO1. Thus, it may play an important role in the pathogenesis of deafness-associated A1555G mutation in 12 S rRNA gene or mutations in tRNA genes.

A variety of mitochondrial DNA (mtDNA) mutations have been found to be associated with many clinical abnormalities, including neuromuscular disorders, hearing loss, and diabetes (1–3). However, the nuclear background influences the phenotypic expression of pathogenic mtDNA mutations associated with human diseases. The first example is that the different nuclear backgrounds confer a marked advantage to either the A3242 mutation in the tRNA<sup>Cys(UCC)</sup> gene or the wild type mitochondrial genome (4, 5). The other example is that the nuclear background plays a determining role in biochemical phenotype of the deafness-associated A1555G mutation (6, 7). This mutation, which results from the A to G transition at position 1555 in the human mitochondrial 12 S rRNA gene, has been found to be associated with aminoglycoside-induced deafness and non-syndromic deafness in families of various ethnic backgrounds (8–10). The genetic and biochemical data strongly indicate that the A1555G mutation is a primary factor underlying the development of deafness (6, 7), but the expression of the deafness phenotype associated with this mutation requires contributions of nuclear modifier gene(s) or aminoglycoside antibiotics (6–12). The product of modifier nuclear gene(s), which may interact with the mutated 12 S rRNA, affects the phenotypic manifestation of the A1555G mutation by enhancing or suppressing the biochemical effect of the mutation (6). To date, the putative modifier nuclear gene(s) remain to be identified (13).

As shown in Fig. 1, the A1555G mutation is located in the region of small ribosomal RNA that is highly conserved from bacteria to mammals (14). The corresponding region in <i>Escherichia coli</i> forms an essential part of the decoding site of the ribosome (15) and is crucial for the subunit association either by RNA-protein or RNA-RNA interaction (16). The same region of the bacterial small ribosomal RNA is also known to bind aminoglycoside antibiotics (17), and mutations within this region conferring antibiotic resistance have been isolated in bacteria (18, 19) and yeast mitochondria (20, 21). In fact, the new G-C pair in the human mitochondrial 12 S rRNA created by the A1555G mutation facilitates the binding of aminoglycoside (22), which accounts for aminoglycosides induced hearing loss in the individuals carrying this mutation (8–10).

With the aim of identifying nuclear modifier genes, the yeast <i>Saccharomyces cerevisiae</i> has been used as a model organism to isolate the nuclear mutations that are involved in the phenotypic manifestation of the A1555G mutation. Interestingly, it was reported that the null mutations in MSS1 or MTO1, encoding highly conserved mitochondrial proteins, express their respiratory-deficient phenotypes only when their mtDNA carry the Pr<sup>454</sup> mutation corresponding to the deafness-associated A1555G mutation. In the Pr<sup>454</sup> background, <i>mss1</i> or <i>mto1</i> mutants fail to synthesize subunit 1 of cytochrome c oxidase (COX1), thereby leading to a respiratory-deficient phenotype.
Isolation of Human Homolog of Yeast MTO1 Gene

EXPERIMENTAL PROCEDURES

Cell Lines, Culture Conditions, and RNA Extraction—Human hepatoblastoma cell line HepG2 was used for the extraction of RNA. Human osteosarcoma cell line 143BTK (27) was used for the subcellular location experiment. Both 143BTK and HepG2 cells were grown in regular Dulbecco’s modified Eagle’s medium (Invitrogen), supplemented with 10% fetal bovine serum. Total RNA was isolated from the human HepG2 cells using TRIzol reagent (Invitrogen) according to the manufacturer’s direction.

cDNA Cloning of Human MTO1—Peptide sequence of S. cerevisiae MTO1 was subjected to a BLAST search of GenBank nucleotide sequence data bases and two human EST clones (GenBank accession nos. A1074147 (np 914–1822) and BE735271 (np 308–1029)) were identified with a significant homology to yeast MTO1 open reading frame. For the amplification of 5′-terminal cDNA fragment, we used an antisense primer AGGGAGGCTTGCGACTGACCCGAAGACTG (np 1374–1402), whereas a sense primer CAGTCTCGGCTAGCTGCGCAG-GCTTCCTCT at the same position was used for the determination of 3′ end cDNA region. The 5′ and 3′ regions of cDNA were obtained by RT-PCR. First-strand cDNA for the use as a PCR template was generated from this RNA using SMART RACE cDNA amplification kit (CLONTECH). Touch PCR was performed with Advantage-2 GC 2 PCR (CLONTECH). To obtain the full-length coding region of MTO1 cDNA, RT-PCR was performed by using the high fidelity Pfu DNA polymerase (Promega) and total RNA isolated from HepG2 cells as template, with primers with HindIII site: 5′-CCCAAGTGTCGGCTTCAAAGTCAGATAG-3′ (np 55–76) and 5′-CCCAAGATGTTCAAGCTTCAAGATG-3′ (np 2431–2453). The predominant PCR product was purified by agarose gel electrophoresis and subsequently cloned into a pCR 2.1-TOPO vector (Invitrogen). Sequencing was done using a Dye Terminator cycle sequencing kit (PerkinElmer Life Sciences) and an ABI PRISM 3700 genetic analyzer. The resultant plasmid carrying the full-length coding region of human MTO1 cDNA was designed as pHMT01.

Gene Expression Analysis—The Blast homology searches were performed with web-based programs available from the National Center for Biotechnology Information (28). DNA and protein sequence alignments were carried out using the seqweb program GAP (CCG). The percentage of identical and similar amino acids was calculated. The working draft sequences from BACs AC025535 and AC068247 were assembled into a complete contiguous genomic sequence by using the Blast program to compare these sequences with each other and with the MTO1 cDNA and the genomic sequences we had compiled. We closed the remaining gaps in the sequence by making PCR primers that generated products across the gaps and sequencing these products. The splice donor and acceptor site sequence of human MTO1 genomic DNA was determined as described previously (29, 30).

To determine whether the MTO1 is expressed in the cochlea, PCR amplification of MTO1 cDNA was performed by using a human cochlear cDNA library (31) and HepG2 cDNA as template and using primers: 5′-TGTCAGCTTTCAAGCTTCAATTCGATAG-3′ (np 55–76) and 5′-GGATTCTC-TTACTCAGCGTCCTTCTAC-3′ (np 1814–1833). The resulting PCR products were then analyzed by gel electrophoresis and nucleotide sequence determination.

Complementation of a Yeast mto1 Mutant—The S. cerevisiae wild type strain used for this study was W301-1B (α, ade2-1, trp1-1, his 3-11,15, leu2-3,112, ural-3-1). The mto1 strain used in this study was E39/U1 (α, ural-3-1, mto1-1) (23). The W301-1B β′ strain was isolated by a margin of growth technique using ethidium bromide (31). All yeast strains were grown in YEP medium (0.5% yeast extract (Difco), Y, 1% Bacto-Peptone (Difco), P, and 2% glucose) and GlyYP medium (0.5% yeast extract (Difco), Y, 1% Bacto-Peptone (Difco), P, and 2% glycerol). Minimal medium contains 0.67% Difco yeast nitrogen base (YNB) without amino acids. This medium was supplemented with amino acids at 50 μg/ml or base at 25 μg/ml to complement auxotrophic requirements.

pDB20 was used for the expression of human MTO1 in S. cerevisiae. pDB20 is a URA3 and ADH1 promoter-based yeast expression vector.
**Isolation of Human Homolog of Yeast MTO1 Gene**

(32). pDB20-HMT01 was constructed to by inserting full-length coding region of human MTO1 cDNA (pHMTO1) at HindIII site of pDB20. To determine whether the N-terminal targeting sequence is essential for the function, a human cDNA lacking the N-terminal sequence was obtained by PCR amplification using the pHMTO1 as the template. For this purpose, the antisense primer 5'-CCCAAGCTCTCAATGCTGTTATGTTTGTCGTTGTCG-3' (np 309–329) and sense primer 5'-CCCAAGATTCAGTTTTAGGTTCGCTGTTGTCG-3' (np 2453–2463) were designed for PCR amplification. The PCR product (2114 bp) was cloned into a PCR 2.1-TOPO vector (Invitrogen). After sequence determination, the insert was subsequently subcloned into pDB20 to generate pDB20-HMT01-2. These constructs were transformed into the mt01 strain of S. cerevisiae E39U1 by the method of Sheeh and Gietz (33). The Ura3+ transformants were selected at 30 °C on minimal glucose medium. The transformants were then replica-plated on GYP and GlyYP plates, which were incubated at 30 °C for 4 days. Colonies growing on GlyYP medium were subject to further analysis.

Subcellular Localization of Human MTO1—A human MTO1 cDNA lacking its natural stop codon was obtained by PCR using the pHMTO1 as template. The antisense primer 5'-CCCAAGCTCTCAATGCTGTTATGCTGTTGTCG-3' (np 55–76) with a HindIII site and sense primer 5'-GGATTCTCTTCTCAGAGCTCGTCTCT-3' (np 1814–1833) with an engineered HindIII site immediately upstream of the natural stop code were used for the PCR amplification. The PCR products were firstly cloned into PCR 2.1-TOPO vector (Invitrogen). After sequence determination, the insert was subsequently subcloned into the expression vector pEGP-N1 (CLONTECH).

The resultant constructs were transfected into 143B cells using SuperFect™ transfection reagent (Qiagen) according to the manufacturer’s protocol. Transfected cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum for 30 h. The chamber slides were then washed with PBS, fixed in 4% paraformaldehyde plus PBS for 10 min, and permeabilized in 0.1% Triton-X-100 plus PBS for 10 min. The cells were then incubated with mouse anti-human COX1, Alexa Fluor 594 conjugate (10 μg/ml, Molecular Probes) and rabbit anti-GFP, IgG, Alexa Fluor 488 conjugate (4 μg/ml, Molecular Probes) in 5% normal goat serum plus PBS at 37 °C for 2 h. The chamber slides were then viewed under a Zeiss confocal fluorescence microscope.

**RESULTS**

Identification of cDNA Encoding Human MTO1 Homolog—S. cerevisiae Mto1p sequence was subjected to a BLAST search of the NCBI/GenBank™ human EST data base. Two ESTs derived from human kidney (GenBank™ accession no. A074147) and placenta (GenBank™ accession no. BE735271) were identified with significant homology to the N and C terminal of S. cerevisiae MTO1 open reading frame (Fig. 2A), respectively.

Based on the sequence of the cDNA identified by a search of the human EST data base, we designed primers to isolate and characterize the full-length coding region of human MTO1 and also examine the noncoding 5’ and 3’ region of the mRNA. Using total RNA isolated from HepG2 cells as the template, a cDNA that containing an open reading frame of 1,856 bp has been identified. This cDNA encodes a protein of 692 amino acids with a predicted molecular mass of 77,293 daltons. To investigate the 5’ sequence of the human MTO1 transcript, we performed 5'-RACE amplification of cDNA ends using total RNA isolated from HepG2 cells. Through 5'-RACE amplification of cDNA ends, four clones with identical sequences were obtained. The cDNA fragment contains seven ATG codons. Assuming the first ATG to be the initiation codon, the 5'-untranslated region consists of 90 bp (Fig. 2B). However, through 3'-RACE amplification of cDNA ends, five different clones with various lengths were obtained. Four of these clones share identical 3' untranslated region, whereas the 3'-untranslated region of clone 5 extends additional 356 bp. Using the primers homologous to the 5' and 3' ends of the cDNA, a full-length MTO1 cDNA with 2,537 bp has been isolated and deposited in GenBank™ (accession no. AF319422). As shown in Fig. 2B, four other transcripts, which are likely splice variants with 2,612 bp (isoform II), 2,627 bp (isoform III), 2,246 bp (isoform IV), and 2,961 bp (isoform V), have been obtained, and their cDNA sequences have been deposited in GenBank™ (accession nos. AY078985, AF469111, AF469110, and AY078986, respectively).

**Genomic Structure of the Human MTO1 Gene**—We used the human MTO1 cDNA sequence as a query to search available EMBO/GenBank™ High Throughput Genome data base. This search has led to the identification of working draft sequences of two bacterial artificial chromosome (BAC) clones (AC025535 and AC068247), each containing partial genomic MTO1 sequence (Fig. 3B). To define the actual intron-exon genomic organization of the human MTO1 gene, we used single site PCR to obtain and sequence genomic fragments spanning the predicted exon-intron junctions, used long range genomic PCR to estimate the size of each intron, and subsequently sequenced the PCR products. We have used these BAC sequence data in conjunction with our sequence data to assemble a 41-kb genomic sequence from the MTO1 region of chromosome 6. Fig. 3A revealed that the MTO1 gene is composed of 12 exons, which range from 118 to 522 bp in size, and 11 introns varying from 92 bp (intron 5) to 9,614 bp (intron 9). Additionally, a putative polyadenylation signal (AATTAA; np 2510–2516) and a poly(A) were observed. The intron-exon junctions and polyadenylation signals are shown in Table I. The complete nucleotide sequence of MTO1 gene has been deposited in GenBank™ (accession no. AF442963). Furthermore, we have assembled the sequence of the other four transcripts. As shown in Fig. 3C, these transcripts are caused by the alternative splicing. The insertion exon 6A (75 bp) of isoforms II and V produces a protein product of 717 amino acids, whereas isoform V contains exon 12A. The isoform IV skipping the exon 4 resulted in a protein of 595 amino acids, whereas the retention of intron 5 led to a shorting protein with 312 amino acids in isoform III. The genomic organization of these MTO1 transcripts derived from these data is summarized in Fig. 3C. Isoforms II, IV, and V may be of special interest, as they comprise in-frame insertion or deletion.

**Comparison of Amino Acid Sequences of Human Mto1 with Those of Homologs from Other Species**—The protein alignment or deletion.

Mus musculus, Caenorhabditis elegans, Schizosaccharomyces pombe, S. cerevisiae (23), and E. coli (34), revealed an extensive conservation of amino acid sequences (Fig. 4). In particular, the identity of the overall predicted amino acid sequences of human Mto1 with homologs of S. cerevisiae, M.
The similarity of human to other species of *musculus, C. elegans, S. pombe*, and *E. coli* is 49, 87, 53, 46, and 49%, respectively, whereas the similarity is 59, 90, 61, 55, and 58%, respectively. 

Like the yeast homolog, the human MTO1 protein contains the typical features of mitochondrial target sequence with regularly spaced basic, hydroxylated, and hydrophobic residues (35). The Mitoprot program (www.mips.biochem.mpg.de/proj/medgen/mitop) predicts with a high probability that human MTO1 protein localizes to mitochondrion. This protein belongs to the GIDA family, which has been shown to be involved in tRNA modification (25, 26). From these data, the high sequence conservation between the human and *S. cerevisiae* suggests that they appear to be structural homologs and leads us to test in yeast whether the human protein can complement the characterized function of yeast *mto1* mutation.

**Functional Complementation of the Yeast mto1 Mutant by Human MTO1 cDNA**—To examine the functional conservation of the human *MTO1*, we tested its ability to complement the respiratory defect of the yeast *mto1* mutant carrying the PR454 allele of mitochondrial 15 S rRNA gene. First, the entire coding region of human MTO1 cDNA was cloned into an expression vector pDB20, designed as pDB20-HMTO1. To test whether the putative mitochondrial targeting sequence is essential for the function of MTO1, we also constructed a plasmid pDB20-HMTO1–2 containing a 2.1-kb human MTO1 cDNA lacking N-terminal sequence. These constructs were then transformed into a yeast *mto1* strain E39/U1 carrying the mitochondrial PR454 allele, and resultant transformants were tested for their growth on glycerol-rich medium. As illustrated in Fig. 5, the growth defect of the *mto1* mutant on glycerol medium was restored by the entire coding region of human MTO1 cDNA (pDB20-HMTO1) but not by the cDNA lacking N-terminal sequence (pDB20-HMTO1–2). These data suggest that human MTO1 is able to functionally complement the yeast *mto1* mutation and N-terminal targeting sequence is essential for the function of MTO1. These results strongly indicated that human MTO1 is the functional homolog of yeast MTO1.

**Expression of Human MTO1**—We investigated the tissue-specific expression of *MTO1* in a number of human tissues. The 32P-labeled *MTO1* probe detected an mRNA of /H110112.6 kb, in good agreement with the length of the cDNA (2537 bp). *MTO1* is ubiquitously expressed, but there are significant variations in steady-state *MTO1* mRNA levels among tissues (Fig. 6). In particular, the expression in the brain and lung appears low compared with other tissues with high metabolic rates such as liver and skeletal muscle, kidney, and heart. In addition, an mRNA with /H110114.0 kb appears in the liver and skeletal muscle, indicating that this RNA may be additional isoform of *MTO1* in these tissues. To examine if the human MTO1 gene is expressed in the cochlea, we performed the PCR amplification of *MTO1* cDNA using the human cochlear cDNA library (36) as template. As shown in Fig. 6C, the size of PCR fragment derived from cochlear cDNA library is identical to that amplified from HepG2 RNA. The nucleotide sequence analysis of these fragments confirmed that this PCR product is indeed *MTO1* cDNA, suggesting that the MTO1 is also expressed in the cochlea.

**Subcellular Location of the Human MTO1**—To determine the subcellular localization of the product of human MTO1, the cDNA lacking its natural stop codon was obtained by PCR using the pHMTO1 as the template. After sequence determination, the insert was subsequently subcloned into pEGFP-N1...
Isolation of Human Homolog of Yeast MTO1 Gene

TABLE I

| Exon | Splice donor sequence | Intron size (bp) | Splice acceptor sequence | Exon |
|------|-----------------------|-----------------|--------------------------|------|
| 1    | ACGATCGGTAAGGAG       | 4140            | TAAAGCAGATGCC            | 1    |
| 2    | CATCGAGTTAAGAAT       | 81              | TATCTTAGGTCAGA           | 2    |
| 3    | GTTTGGGCTACGTAT       | 6760            | TTTTGACAGAAAGAA          | 3    |
| 4    | GATTAAGGTTAAGATA      | 6078            | GTTGTATAGCAGAA           | 4    |
| 5    | GACCTCGGTAAGGGC       | 92              | TATTCTAGATACGT           | 5    |
| 6    | CAGCAGGTTAAGAAA       | 549             | TTGGTCAGGTACT            | 6    |
| 7    | AGCTCAAGTTAAGAGA      | 1235            | CTCTTGTAGGTTG            | 7    |
| 8    | CTGCGAGGTATACCTC      | 205             | CTTCCTAGGGTATA           | 8    |
| 9    | CTGTCAGGTTATGCT      | 9614            | ATTTGCAAGGCTCT          | 9    |
| 10   | ATAGAAGGTTAGAAA       | 5382            | GGTGACAGGCTCT           | 10   |
| 11   | ACAGACGGTAAAGAAA      | 2677            | GATTTTATAGTCC            | 11   |

Poly(A) site: AAGTCTACCAATTTAAAGTGCTATCATTC

(CLONTECH). This construct was then transiently transfected into the human 143B cell line for the transient expression. Fig. 7 showed that the immunofluorescence pattern of transfected 143B cells doubled-labeled with a monoclonal antibody specific for the GFP and mouse monoclonal antibody to COX1, a subunit of cytochrome c oxidase complex in the mitochondrial inner membrane. A typical mitochondrial staining pattern was observed with both antibodies (Fig. 7, A and B) and superimposition of two panels showed complete overlap of the two patterns (Fig. 7C). No other subcellular structure exhibited immunoreactivity with the GFP antibody, demonstrating that human Mto1 localizes exclusively to mitochondria, even when overexpressed.

DISCUSSION

In this study, we have identified the full-length cDNA sequence for the human MTO1 gene. The general genomic structure of this gene has been elucidated. Alignment of the product of human MTO1 with yeast Mto1p as well as other homologs, including mouse, S. pombe, E. coli, and C. elegans, displays a high amino acid sequence conservation as well as similarity in size. In particular, the amino acid sequence identity between human Mto1 and S. cerevisiae Mto1p (23) is 49%, whereas the identity between human Mto1 and E. coli GidA (34) is 49%. The N-terminal sequence of Mto1p, like that of its homologs, is a characteristic mitochondrial targeting sequence (35). Immunofluorescence analysis of 143B cells expressing MTO1-GFP fusion protein demonstrated the co-localization of the MTO1 protein with the mitochondrial protein COX1. This observation strongly indicated that this protein functions in the mitochondria. This evolutionary conservation between distantly related species suggests that human MTO1 and its homologs possess a fundamental cellular role.

MTO1 homologs of other species have been shown to be involved in the tRNA modification, thus playing an important role in the translation process. In particular, the E. coli gidA mutants (homolog of MTO1) are defective in the biosynthesis of the hypermodified nucleoside mnm<sup>5</sup>s<sup>2</sup>U34 (25). This modified nucleotide, found in the wobble position of several bacterial tRNAs specific for glutamate, lysine, arginine, leucine, and glutamine, has a pivotal role in the function of tRNAs, including both tRNA identity and the codon recognition specificity (26). It has been shown that the defect in modification of this nucleotide caused the deficient aminoclastic of these tRNAs (37), as well as an alternation in codon-anticodon interaction (38), consequently affecting the efficiency and accuracy of translation (39, 40). The synthesis of mnm<sup>5</sup>s<sup>2</sup>U is complex and has been proposed to occur in the following step (25): U34 mnm<sup>5</sup>s<sup>2</sup>U X gidA cmnm5s2U34 mnmC1 nm5s2U34 mnmC2 nm5s2U34.

The E. coli mnmE (homolog of MSS1) product was shown to be responsible for the first step of the modification in the 5-position of the uracil (39), and it is very likely that the MnmE activity precedes that of product of gidA (homolog of MTO1) (25). The evidence that the Miss1p and Mto1p of S. cerevisiae form a complex strongly supports this idea that they are members of the same pathway (23).

tRNA modification may influence the decoding process in the A site of small ribosomal RNA. In S. cerevisiae, mutants of mtol and mss1 displayed a respiratory deficiency phenotype only under the coexistence of the P<sup>R</sup><sub>454</sub> mutation in mitochondrial 15 S rRNA gene. The Mto1p and Miss1p both interact with the decoding region of 15 S rRNA, particularly at the site of P<sup>R</sup><sub>454</sub> (23, 24). This P<sup>R</sup><sub>454</sub> mutation, corresponding to the deafness-linked A1555G mutation, is a part of the A site of ribosomal essential for the decoding process (15, 18, 20). It was shown that those ribosomes carrying this mutated 15 S rRNA have a decreased level of natural frame shifting, suggesting a more stringent proofreading (21). Recently, it was proposed that Miss1p and Mto1p in yeast are responsible for the tRNA modification and that these hypermodified tRNAs are less efficient for the decoding of the codon ending in G than A (25). Mutations in MSS1 or MTO1 are proposed to cause the defect in the synthesis of mnm<sup>5</sup>s<sup>2</sup>U34 and consequently reduce the tRNA modification. This deficiency could be stronger with the more stringent P<sup>R</sup><sub>454</sub> ribosome, thereby causing the respiratory deficient phenotype. These observations strongly indicated that the products of MSS1 and MTO1 in yeast affect the phenotypic expression of the mitochondrial P<sup>R</sup><sub>454</sub> mutation.

The human MTO1, similar to other mitochondrial proteins encoded by nuclear genes, such as frataxin (41), SURF1 (42), and SCO2 (43), is predominately expressed in the tissues with...
**Sequence alignment of human Mto1p with its homologs.** The alignment was generated using the GCG program. The organisms and corresponding accession numbers used for this analysis are as follows: *M. musculus* Mto1, AAK63070; *S. cerevisiae* Mto1p, P53070; *E. coli* GIDA, P17112; *S. pombe* Mto1p, 013670; and *C. elegans*, Q20680. Numbers give the position of residues in proteins in relation to the first methionine of the human Mto1. Amino acid residues shaded dark are identical; those shaded dark gray and light gray are similar in at least five residues and four residues of seven homologs, respectively.
high metabolic rate, including skeletal muscle, liver, and heart, as well as cochlea. In particular, the MTO1 transcripts, ranging from 2.2 to 3.0 kb, are expressed at higher level in liver than other tissues. Using RT-PCR, molecular cloning, and cDNA sequencing, five different cDNAs have been identified in the HepG2 cells. Among these, a cDNA with 2,537 bp is consistent with the 2.6-kb transcript identified in various tissues by Northern blot analysis, whereas four other cDNAs are in good agreement with various transcripts ranging from 2.2 to 2.9 kb in the liver. These isoforms, particularly with in-frame insertion and deletion, result from alternative splicing. Isoforms II and V, resulting from the retention of exon 6A, produce a protein with 717 amino acids, whereas a truncated protein of 598 residues that is translated from isoform IV is the result of the deletion of exons 4. In addition, a 4.0-kb transcript, detected in the liver and skeletal muscle, is probably an additional isoform with special function in these tissues. It has been shown that different protein isoforms may regulate or interact with other proteins or participate in different cellular processes.

FIG. 5. Overexpression of human MTO1 suppresses the defective growth of the mto1 mutant on glycerol medium. The yeast strains were spread on glucose-rich medium and glycerol-rich medium and incubated at 30 °C for 4 days. Sector 1, wild type haploid strain W303–1B pY; sector 2, wild type haploid strain W303–1B pET; sector 3, the yeast mto1 mutant (E39/11); sector 4, the yeast mto1 mutant transformed with vector pDB20; sector 5, the yeast mto1 mutant transformed with pDB20-HMTO1–2 (lacking N-terminal sequence); sector 6, the yeast mto1 mutant transformed with pDB20-HMTO1.

FIG. 6. Analysis of MTO1 expression. A, a human 8-lane multiple-tissue blot (CLONTECH) containing 2 μg of poly (A)+ RNA/lane was hybridized with a 32P-labeled fragment containing human 2398-bp MTO1 cDNA probe according to the manufacturer’s protocol. B, the blot was then stripped and re-hybridized with 32P-labeled human β-actin probe as a control. C, PCR analysis of MTO1 cDNAs in human cochlear cDNA library and HepG2 cDNA.

FIG. 7. Human Mto1 is a mitochondrial protein. 143B cells were transiently transfected with pEGFP-N1-MTO1. Intracellular distribution of MTO1-GFP fusion protein was visualized using the GFP antibody conjugated with Alexa Fluor (A). The expression of the COX1 subunit in the same cell was assessed by the successive application of monoclonal antibody against human COX1 conjugated with Alexa Fluor 594 (B). Superimposition of A and B shows the complete overlap of the two staining patterns (C).
with distinct sets of target (44). One example is that the splicing variants of the Wilms tumor 1 gene encode proteins that have opposite effects on tumorigenicity (45). It is possible that different isoforms of MTO1 have different functions in various tissues. Further characterization of these isoforms is now being carried out in this laboratory.

Mutations in mitochondrial tRNA and rRNA genes are often associated with a wide spectrum of human diseases caused by mitochondrial disorders (1). Among these, the A1555G mutation in the 12 S rRNA gene is one of the most common causes of non-syndromic deafness and aminoglycoside-induced deafness (2). In the absence of aminoglycosides, the A1555G mutation produces a clinical phenotype that ranges from severe congenital deafness to normal hearing (8, 9). This mutation locates at a highly conserved decoding region of 12 S rRNA that has been shown to be crucial for RNA-protein or RNA-RNA interaction (16). An overall decrease in the rate of mitochondrial protein labeling was observed in the mutant lymphoblastoid cell lines derived from an Arab-Israeli family, compared with that in control cell lines (6). However, more severe mitochondrial dysfunction was observed in the lymphoblastoid cell lines derived from asymptomatic individuals than those from asymptomatic individuals carrying the A1555G mutation (6). By contrast, under a constant nuclear background, there were very similar biochemical defects including reduction in the rate of mitochondrial protein synthesis between transmitochondrial cell lines derived from symptomatic and asymptomatic individuals (7). These genetic and biochemical data firmly suggest that the A1555G mutation is a primary factor underlying the development of deafness (6, 7), but the nuclear background determines the penetrance of the A1555G mutation in the Arab-Israeli family (6, 7). It is possible that the biochemical defect associated with this A1555G mutation primarily results from the fact that the hypermodified tRNAs, synthesized by the participation of MTO1, are less efficient for the decoding of the codon ending in G than A, as proposed in the yeast mitochondrion (10). A wobble modification of the A1555G mutation by enhancing or suppressing the variability of A1555G mutation could play an important role in the phenotypic expression of pathogenic mutations in the mitochondrial tRNA genes such as the A3243G MELAS mutation and the A8344G MERRF mutation.

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