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The Large Subunit of the Mammalian Mitochondrial Ribosome

ANALYSIS OF THE COMPLEMENT OF RIBOSOMAL PROTEINS PRESENT*

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Identification of all the protein components of the large subunit (39 S) of the mammalian mitochondrial ribosome has been achieved by carrying out proteolytic digestions of whole 39 S subunits followed by analysis of the resultant peptides by liquid chromatography and mass spectrometry. Peptide sequence information was used to search the human EST data bases and complete coding sequences were assembled. The human mitochondrial 39 S subunit has 48 distinct proteins. Twenty eight of these are homologs of the *Escherichia coli* 50 S ribosomal proteins L1, L2, L3, L4, L7/L12, L9, L10, L11, L13, L14, L15, L16, L17, L18, L19, L20, L21, L22, L23, L24, L27, L28, L30, L32, L33, L34, L35, and L36. Almost all of these proteins have homologs in *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Saccharomyces cerevisiae* mitochondrial ribosomes. No mitochondrial homologs to prokaryotic ribosomal proteins L5, L6, L25, L29, and L31 could be found either in the peptides obtained or by analysis of the available data bases. The remaining 20 proteins present in the 39 S subunits are specific to mitochondrial ribosomes. Proteins in this group have no apparent homologs in bacterial, chloroplast, archaebacterial, or cytosolic ribosomes. All but two of the proteins has a clear homolog in *D. melanogaster* while all can be found in the genome of *C. elegans*. Ten of the 20 mitochondrial specific 39 S proteins have homologs in *S. cerevisiae*. Homologs of 2 of these new classes of ribosomal proteins could be identified in the *Arabidopsis thaliana* genome.

Mammalian mitochondria are responsible for the synthesis of 13 proteins localized in the inner membrane. These proteins are components of the oligomeric complexes essential for oxidative phosphorylation and, hence, for the synthesis of about 90% of the ATP in eukaryotic organisms. The 55 S mammalian mitochondrial ribosomes consists of small (28 S) and large (39 S) subunits (1). In contrast to bacterial ribosomes which are about 65% RNA, mammalian mitochondrial ribosomes are only 33% RNA. The low percentage of RNA in these ribosomes reflects a reduction in the size of the rRNA and a compensating increase in the number of ribosomal proteins. For example, the small subunit of the mammalian mitochondrial ribosome contains a 12 S rRNA (about 950 nucleotides) and an estimated 29 proteins (2). In contrast, the *Escherichia coli* 30 S subunit has a 16 S rRNA (1542 nucleotides in length) and 21 proteins (3). The large subunit of the mammalian mitochondrial ribosome contains a 16 S rRNA (about 1560 nucleotides) and about 50 proteins (4, 5).

The identification of proteins in mammalian mitochondrial ribosomes has been challenging due to their low abundance. Recently 60 mammalian mitochondrial ribosomal proteins, 31 proteins from the large subunit and 29 proteins from the small subunit, have been characterized by different laboratories (2, 6–14). The identification of these proteins used two approaches. The traditional approach was to separate the proteins on two-dimensional gels or high performance liquid chromatography followed by sequence analysis using Edman chemistry or mass spectrometry (MS). More recently, proteins present in the 28 S subunit have been characterized by proteolytic digestion of whole subunits. Sequence information on the peptides present in this complex mixture was obtained by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS).[1] This strategy allowed the identification of 28 proteins of the small subunit including 14 proteins that had not previously been identified (2). In the present study, we have extended this approach to the 39 S subunit. In addition to direct analysis of 39 S digests by LC/MS, aliquots of the total digest were fractionated prior to reversed-phase LC/MS analysis to maximize the number of peptides sequenced. In the first approach, a portion of the total digest was fractionated by affinity selection of Cys-containing peptides on a monomeric avidin column following the modification of these residues with a biotin-conjugated reagent. A second fractionation approach involved on-line strong cation exchange (SCX)/reversed-phase LC/LC/MS/MS. The sequence information obtained was used in data base searches to allow the deduction of the complete amino acid sequences of these proteins. The present work has allowed us to identify what we believe is close to the full complement of ribosomal proteins present in this subunit including 17 new mammalian mitochondrial large subunit ribosomal proteins. Of these, 4 have homologs in prokaryotic ribosomes while 13 are members of new classes of large subunit ribosomal proteins.

MATERIALS AND METHODS

Preparation of Bovine Mitochondrial Ribosomal Subunits—Bovine mitochondrial ribosomes were prepared as described previously and the 55 S mitochondrial ribosomes were isolated on sucrose gradients (4). These ribosomes were diluted in buffer containing 20 mM Tris-HCl, pH 7.6, 2 mM $MgCl_2$, 40 mM KCl, and 2 mM dithiothreitol to dissociate the 55 S ribosomes into 28 and 39 S subunits. Two-ml samples were then

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[1] The abbreviations used are: LC/MS, liquid chromatography/mass spectrometry; SCX, strong cation exchange.

applied to 36-ml linear gradients (10–30% sucrose in the above buffer). Samples were subjected to centrifugation for 16 h at 24,000 rpm in a Beckman SW27 rotor. The gradients were fractionated and 39 S subunits were collected by centrifugation at 48,000 rpm for 6 h in a Beckman Type-50 rotor. The 39 S subunit pellet was resuspended in 0.1 ml of buffer containing 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 40 mM KCl, and 2 mM dithiothreitol and stored at −70 °C.

Peptide Sequencing by Mass Spectrometry—For each analysis, 5 pmol of purified 39 S subunits were digested with trypsin (Roche Molecular Biochemicals) or endoproteinase Lys-C (Wako Chemicals) as described previously (9, 12, 15). Nanoscale capillary LC/MS/MS analyzes of 39 S digests were done using an Ultimate capillary LC system (LC Packings) coupled to a quadrupole time-of-flight mass spectrometer (Micromass) fitted with a Z-spray ion source as described previously (2). In addition to the LC/MS/MS analysis, a multidimensional LC/LC/MS/MS approach similar to that described previously (16) was performed using SCX in the first LC dimension and reversed-phase in the second dimension. Peptides were sequentially step eluted from the 300 μm × 5-mm SCX column (LC Packings) packed with PolySulfoethyl Aspartamide material (PolyLC) onto a reversed-phase "trapping" cartridge using a 5 mM KH_2PO_4 buffer, pH 3.0, containing 0, 25, 35, 50, 60, 75, 100, or 200 mM KCl. Peptides from each salt fraction were then analyzed by sequential reversed phase LC/MS/MS analyses.

A second fractionation method involved the modification of Cys residues in an aliquot of 39 S subunits using the ICAT™ D0 reagent from Applied Biosystems. The biotin tag on this reagent allowed purification of Cys-containing peptides using avidin affinity chromatography as described previously (17, 18). For this analysis, 5 pmol of 39 S subunits was used for *in situ* biotinylation with the ICAT™ D0 reagent followed by digestion with trypsin as summarized in Fig. 1. Alkylation with the ICAT™ reagent was performed on the Agilent sequencing cartridge in the same manner as when using 4-vinylpyridine (19). Peptides were eluted with 80% acetonitrile or 40% acetonitrile, 40% isopropyl alcohol. Three different samples from this reaction were analyzed: 1) the total peptide mixture; 2) peptides not retained by the avidin column; and 3) bound peptides subsequently eluted from a 200-μl monomeric avidin column with 0.1% formic acid, 20% acetonitrile. Peptides in all three of these samples were analyzed by nanoscale LC/MS/MS. For all of the samples described above, uninterpreted peptide product ion spectra generated by LC/MS/MS were searched against the nonredundant protein data base and human and rat EST data bases for exact matches using the Mascot search program (20).

Computational Analysis—Peptide sequences obtained from Mascot searches of the protein and EST data bases were searched against the nonredundant protein data base using the FASTA algorithm (21). EST data base and genomic DNA searches of the peptide sequences were performed using the BLAST search program at the National Center for Biotechnology Information (22). Sequence analysis and homology comparisons were done using the GCG DNA analysis software package (Wisconsin Package Version 10, 1999, Genetics Computer Group (GCG), Vector NTI (Informax Inc.) and Biology WorkBench 3.2. The results were displayed using BOXSHADE (version 3.21, written by K. Hofmann and M. Baron). Prediction of the cleavage sites for the mitochondrial signal sequence was carried out using PSort and MitoProt II (23, 24).

RESULTS

Nomenclature—Two-dimensional gel analysis of the proteins present in bovine liver mitochondrial 39 S subunits suggested the presence of as many as 50–52 ribosomal proteins. These proteins were designated as L1 through L52 in order of decreasing molecular weights (4). However, this nomenclature does not permit the easy comparison of the mammalian mitochondrial ribosomal proteins to those of other systems. Hence, we have adopted a system of nomenclature in which proteins with prokaryotic homologs are given the same number as the corresponding ribosomal protein in *E. coli* (Table I). For example, MRP-L7/L12 is the mammalian mitochondrial homolog of bacterial L7/L12. Proteins without bacterial homologs are given the next available number. Since bacterial ribosomes have proteins designated L1 through L36, we began designating the new mammalian mitochondrial ribosomal proteins beginning at MRP-L37. The 7 previously identified bovine mitochondrial 39 S proteins that do not have prokaryotic homologs are designated as MRP-L37 through MRP-L43 (6, 7, 13). The current article describes 4 homologs of bacterial ribosomal proteins (MRP-L18, MRP-L21, MRP-L28, and MRP-L35) and 13 large subunit ribosomal proteins representing new classes of ribosomal proteins (MRP-L44 to MRP-L56).

Characterization of Bovine Mitochondrial Ribosomal Proteins by Tandem Mass Spectrometry—To maximize the number of 39 S peptides sequenced in these experiments, several approaches were employed (Fig. 1). The basic approach involves analysis of proteolytic digests by nanoscale capillary LC/MS on a quadrupole time-of-flight mass spectrometer operated in a data-dependent MS to MS/MS switching mode. Using this approach, the mass spectrometer acquires MS scans until peptides are detected eluting from the LC column. At this point, the mass spectrometer switches to MS/MS mode, fragmenting up to 8 co-eluting peptides per MS/MS switch before resetting to MS mode to look for additional peptides. Digestion of intact 39 S subunits is expected to generate about 750 peptides when digested with endoproteinase Lys-C, or about 1500 peptides when digested with trypsin.

As a result of the extremely complex mixture of peptides present, each peak eluting from the LC column contained multiple peptides. Even though the mass spectrometer could rapidly acquire sequence data for up to 8 co-eluting peptides at a time, the number of peptides presented to the instrument for sequencing in a typical LC/MS/MS experiment overwhelmed the mass spectrometer's capacity for analysis. Thus, in a standard LC/MS/MS experiment, many peptides were never interrogated by MS/MS. By using the combination of approaches outlined in Fig. 1, the sequences of a total of 564 peptides were obtained. This number includes peptides observed in more than one run and peptides from co-purified contaminants. Of these, the sequences of 361 peptides were useful in allowing the identification of 47 large subunit ribosomal proteins.

The large number of potential peptides generated from digestion of intact 39 S subunits raised the question of whether adequate sample coverage could be obtained to allow identification of all of the proteins present. To improve the odds of obtaining some peptide sequence from all of the proteins comprising the 39 S subunit, three types of analyses were used: 1) replicate nanoscale capillary LC/MS/MS of both trypsin and Lys-C digests; 2) Cys-affinity labeling/fractionation (18); and 3) on-line multidimensional SCX-RP LC/LC/MS/MS (16). The most straightforward method for improving proteome coverage of 39 S proteins was found to be replicate analysis of identical digests of the subunit. For example, of 260 peptide matches obtained from triplicate LC/MS/MS analyses of a 39 S Lys-C digest, 141 peptides (55%) were sequenced in only one of the three replicates, 66 peptides (25%) were sequenced in only two of the three replicates, and 53 peptides (20%) were sequenced in all three replicate analyses. This data illustrates that the number of 39 S proteolytic peptides presented to the mass spectrometer for interrogation in an LC/MS/MS experiment exceeds the sequencing capacity of the instrument. Because chance (to some extent) plays a role in which peptide ions the instrument interrogates in each analysis (18), simply repeating the analysis results in a substantial number of new peptides interrogated in each experiment.

Another approach to improving proteome coverage is the use of multidimensional chromatographic separation of complex protein digests in conjunction with mass spectrometry. In this case, peptides are fractionated with an additional chromatographic step prior to the standard LC/MS/MS analysis. Thus, fewer peptides are presented to the mass spectrometer in each "fraction," allowing a higher number of peptides to be interrogated. One such approach uses an on-line SCX-RP LC/LC separation (16). When applied to 39 S subunit proteolytic digests,

TABLE I
Suggested names and some characteristics of the mammalian mitochondrial large subunit proteins

Protein	Family	Other name	p1[a]	Size[a]	Ref.
				kDa	
MRP-L1	L1	bMRP-16	8.2	34.5	This study, 13
MRP-L2	L2	MRP-L14	10.0	38.4	This study, 14
MRP-L3	L3	bMRP-15	9.5	38.6	This study, 13
MRP-L4	L4	bMRP-18	9.7	34.9	This study, 13
MRP-L7	L7/L12	MRPL31/34[b]	9.0	21.4	This study, 7
MRP-L9	L9	bMRP-28	10.1	30.2	This study, 13
MRP-L10	L10	MRP-L8	9.6	29.3	This study, 6
MRP-L11	L11	bMRP-32	9.9	20.7	This study, 13
MRP-L13	L13	bMRP-33	9.1	20.7	This study, 13
MRP-L14	L14	MRP-L32	10.3	15.9	This study, 6
MRP-L15	L15	MRP-L7	10.0	33.4	This study, 14
MRP-L16	L16	bMRP-25	10.1	28.5	This study, 13
MRP-L17	L17	MRP-L26	10.1	20.0	This study, 14
MRP-L18	L18		9.6	20.6	This study
MRP-L19	L19	MRP-L15[c]	9.5	33.5	This study, 7
MRP-L20	L20	bMRP-50	10.9	17.4	This study, 13
MRP-L21	L21		9.9	22.8	This study
MRP-L22	L22	MRP-L25	9.6	26.2	This study, 6
MRP-L23	L23	bMRP-38	9.6	17.8	This study, 13
MRP-L24	L24	MRP-L18	9.4	24.9	This study, 11
MRP-L27	L27	bMRP-55	10.4	16.1	This study, 13
MRP-L28	L28		8.3	30.2	This study
MRP-L30	L30	MRP-L28	10.0	18.6	This study, 6
MRP-L32	L32	bMRP-59b	9.8	21.4	This study, 13
MRP-L33	L33	bMRP-66	10.8	7.6	This study, 13
MRP-L34	L34	bMRP-68	12.3	10.2	This study, 13
MRP-L35	L35		11.3	21.5	This study
MRP-L36	L36	bMRP-69	11.3	11.8	13
MRP-L37	New	MRP-L2	8.5	47.8	This study, 7
MRP-L38	New	MRP-L3	7.2	44.6	This study, 7
MRP-L39	New	MRP-L5	7.6	38.7	This study, 14
MRP-L40	New	MRP-L22	9.6	24.5	This study, 6
MRP-L41	New	MRP-L27	9.6	15.4	This study, 6
MRP-L42	New	MRP-S31[d]	8.6	16.7	6, 2
MRP-L43	New	bMRP-36a	9.6	21.9	This study, 13
MRP-L44	New		8.7	37.5	This study
MRP-L45	New		9.1	35.2	This study
MRP-L46	New		6.6	31.7	This study
MRP-L47	New		10.3	29.6	This study
MRP-L48	New		9.0	23.9	This study
MRP-L49	New		9.5	19.2	This study
MRP-L50	New		7.7	18.3	This study
MRP-L51	New	MRP64	11.4	15.1	This study
MRP-L52	New		10.3	13.6	This study
MRP-L53	New		9.1	12.1	This study
MRP-L54	New		9.6	15.8	This study
MRP-L55	New		11.2	15.1	This study
MRP-L56	New		8.8	60.1	This study

[a] Molecular weights and pI values are calculated from full-length protein sequences.
[b] Also called MRP-L12.
[c] Also called RLX1.
[d] Found in small subunit in our study (2).

135 unique peptides were sequenced during SCX-RP LC/LC/MS/MS analysis. One peptide from MRP-L55 sequenced during this analysis (Fig. 2) was the only peptide from MRP-L55 observed in any of our analyses. Another LC/LC approach taken was the use of Cys affinity labeling with off-line avidin affinity fractionation of Cys-containing peptides (17, 18). In this approach, 39 S subunits were treated with a reagent (ICAT™ DO reagent) which alkylates Cys residues with a tag carrying a biotin moiety. The modified proteins were then cleaved with trypsin, and a portion of the resulting peptide mixture was used for peptide sequence analysis by LC/MS/MS. The remainder was applied to a monomeric avidin column to isolate the biotin-containing peptides. Nonalkylated peptides that were not retained by this column (avidin flow-through) were analyzed by LC/MS/MS as were the Cys-alkylated peptides retained by the column. A significant increase in the recovery of short, Cys-containing peptides was observed with the use of the ICAT™ D0 tag in the avidin bound fraction. None of these short peptides was observed in any of the non-ICAT™ experiments. Unique peptide information was also obtained in the avidin flow-through fraction as well as the direct unfractionated ICAT™-labeled aliquot. Combining the strategies outlined above allowed for the identification of 17 additional 39 S ribosomal subunit proteins including four prokaryotic ribosomal protein homologs in this subunit.

Table II summarizes the large amount of new peptide sequence information obtained here. For simplicity, peptide sequences obtained from the previously identified mitochondrial ribosomal proteins are not included in this table. Sequences of the peptides obtained were used to search the human EST data base using the tBLASTN program (National Center for Biotechnology Information). In most cases a number of EST clones gave positive hits for these sequences. Overlapping clones for these hits were obtained using the initial hits as virtual probes to rescreen the human EST data base. Consensus cDNAs were then assembled by repetitive searching and comparison of the EST sequences. Sequencing errors were corrected by comparing the sequences of overlapping clones. Where possible, EST

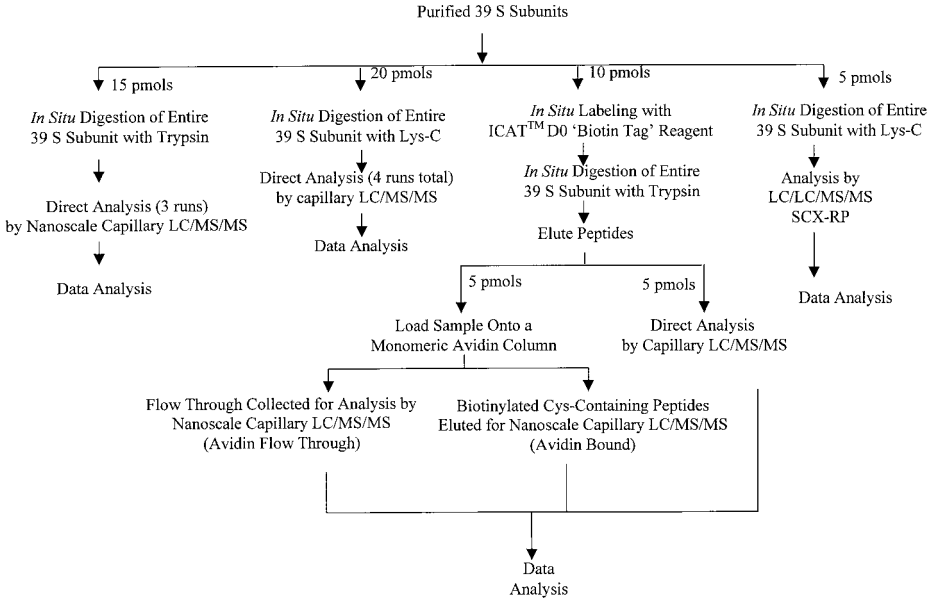

FIG. 1. **Strategies used to identify proteins present in the 39 S subunit of the mitochondrial ribosome.** Bovine mitochondrial 39 S ribosomal subunits were purified using two sequential sucrose gradients, and subjected to analysis by one of three approaches incorporating nanoscale capillary LC/MS/MS. Details are given under "Materials and Methods."

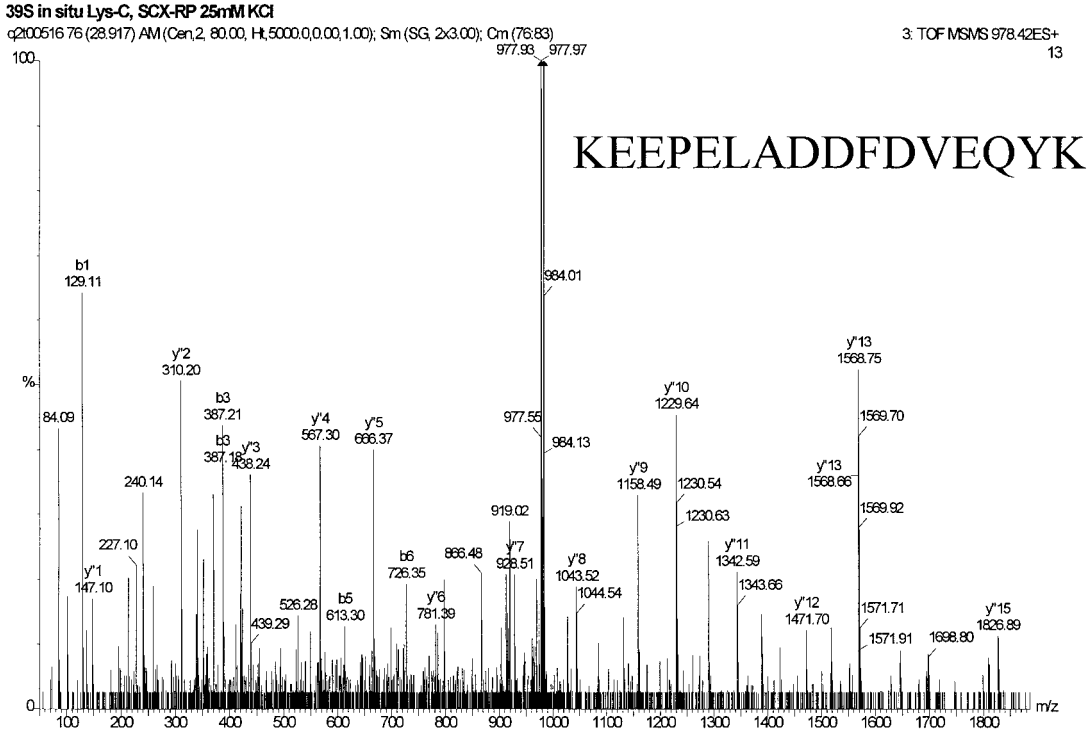

FIG. 2. **Product ion spectrum of a proteolytic peptide derived from MRP-L55 obtained by analysis of a Lys-C digest of the 39 S subunit by SCX-RP LC/LC/MS/MS.** This peptide was observed in the 25 mM KCl step elution from the SCX column. Product ions are labeled using the nomenclature of Roepstorff and Fohlman (47), with the peptide sequence shown on the spectrum.

assemblages from the TIGR data base were examined and used to facilitate the assembly of the full-length sequences and to help evaluate potential sequencing errors in the EST clones. The sequence of the longest possible cDNA was then assembled *in silico.* Generally, the fully assembled human sequence was used as a query against entries in the mouse ESTs and *Caenorhabditis elegans, Drosophila melanogaster,* and yeast genomic data bases.

The peptide sequence information obtained allowed the identification of 4 new homologs of prokaryotic ribosomal proteins. In addition, 13 novel large subunit ribosomal proteins were identified belonging to new classes. Peptides from 30 of the previously identified 31 large subunit proteins were also found in this analysis. The new sequence information provided here brings the total number of 39 S subunit ribosomal proteins to 48.

Some proteins from the small subunit were also detected in the analysis here. About 10–15% of the peptides detected in the 39 S subunit preparations were from known 28 S subunit proteins. For example, peptides from MRP-S30 were found in the analysis of both small and large subunit proteins (25, 26). Contamination of the 39 S subunit preparation by 28 S subunits arises primarily from transient interactions between the subunits at the 2 mM Mg^{2+} ion concentration used during their separation on sucrose gradients. Peptides arising from subunits of the pyruvate dehydrogenase and oxoglutarate dehydrogenase complexes were also observed in the 39 S preparation. These large oligomeric complexes sediment at 32 S (27)

TABLE II

Amino acid sequences of mature MRPs of Bos taurus 39 S subunits deduced from database searching of peptide product ion spectra

Sequence	*m/z*	M_r	Protein match	Swiss-Prot No.	
				Bovine	Human
RLQIGMIEGGVLQEPRRIYE	615.04,4+	2456.16	MRP-L18	P83017	Q9H0U6
TSLSSPPWPEVVLPDPAEEAR	1138.73,2+	2275.45	MRP-L21	P83013	P83031
TSLSSPPWPEVVLPDPAEEARHHAEVVEK	804.24,4+	3205.60	MRP-L21		
VLLVGADDFTLLGRPLLGK	998.88,2+	1996.17	MRP-L21		
DLVRVEATVIEK	686.25,2+	1370.78	MRP-L21		
IIVNPQTVLRINTIEIAPR	720.58,3+	2158.70	MRP-L21		
RIIVNPQTVLRINTIEIAPRLC	660.00,4+	2636.53	MRP-L21		
NGQRERVEDVPIPVHYPPESQLGLWGG-EGWLK	911.27,4+	3641.84	MRP-L28	P83014	Q13084
AFVIPEAEAEWVGLTILDEAVEK	1208.35,2+	2415.22	MRP-L28		
VYVEELVEQLQQQALSEPAVVQK	1314.17,2+	2626.34	MRP-L28		
EFVFCNKTQSK	886.74,2+	1771.46	MRP-L35	P83033	Q9NZE8
LREFVFCNK	799.25,2+	1596.48	MRP-L35		
LLDKMTTSFWK	685.19,2+	1368.37	MRP-L35		
RRNWYADDPYQMYHDRTNLK	661.17,4+	2640.68	MRP-L35		
LQETFSLDLLK	653.70,2+	1305.39	MRP-L44	P83015	Q9H9J2
EAVLLNLK	450.71,2+	899.42	MRP-L44		
DNQELSEQGTSFSQTCLTQFFEDAFPDL-PTEGVK	1304.96,3+	3912.77	MRP-L44		
LIAEGPGETVLVAEEEAAR	977.55,2+	1953.01	MRP-L44		
KLIAEGPGETVLVAEEEAARVALRK	663.12,4+	2648.48	MRP-L44		
LYGFTENRRPWDYSKPK	719.66,3+	2155.98	MRP-L44		
DVLEYVVFEK	620.88,2+	1239.75	MRP-L45	P83016	Q9BRJ2
WSFVESLEPPQVVQVR	950.54,2+	1899.06	MRP-L45		
TVMIPGPQLKPGEEYEELQR	772.11,3+	2313.29	MRP-L45		
LMYGQEDVPRDVLEYVVFEK	810.45,3+	2428.32	MRP-L45		
HLANPYGSWRMHGK	551.84,3+	1652.52	MRP-L45		
QLTPMQEEMAALLQQMEIER	796.85,3+	2388.15	MRP-L46	P83023	Q9H2W6
FLQFKPGARITDADVK	903.45,2+	1804.90	MRP-L46		
NILLVQDLEDMWEQK	937.26,2+	1872.93	MRP-L46		
LDRNLILLVK	598.77,2+	1195.54	MRP-L46		
FLGNAPCGHYK	656.29,2+	1310.58	MRP-L46		
EELGDYLQPK	596.31,2+	1190.62	MRP-L46		
SGASWTCQQLR + 1 PB-ICAT-D0(C)[a]	839.69,2+	1677.36	MRP-L47	P83018	Q9HD33
LWYVLLK	467.79,2+	933.58	MRP-L47		
ERNMLLTLEQEAK	787.89,2+	1573.98	MRP-L47		
QWPIPWYLNK	672.72,2+	1343.70	MRP-L47		
FFAMPYVDRFIR	521.15,3+	1560.44	MRP-L47		
HLVKPEEPK	538.81,2+	1075.62	MRP-L48	P83019	Q9Y5J0
TMEVLQLQDQGNK	752.37,3+	2254.11	MRP-L48		
MLLDSVLTTHERVVQISSLNATFAEIF-LEIIQQSNLPEGVK	1114.54,4+	4454.16	MRP-L48		
ARPELEELLAK	634.76,2+	1267.52	MRP-L48		
VEGDIWALQKDVEDFLSPLLGK	824.73,3+	2471.19	MRP-L49	P83020	Q13405
DVEDFLSPLLGK	666.83,2+	1331.66	MRP-L49		
TPITQVNEVTGTLRIK	590.56,3+	1768.68	MRP-L49		
QPVVAETVEEVK	664.36,2+	1326.72	MRP-L50	P83021	Q9NX15
EPILVCPPLR + 1 PB-ICAT-D0(C)[a]	789.78,2+	1577.55	MRP-L50		
EVFGSSVPTSWQEISLEDVHMK	835.54,3+	2501.19	MRP-L50		
DLQSRLESHVK	437.91,3+	1310.73	MRP-L50		
DLQSRLESHVKEVFGSSVPTSWQEISLED-VHMK	950.2,4+	3796.80	MRP-L50		
LHQMCR + 1 PB-ICAT-D0(C)[a]	615.18,2+	1228.34	MRP-L50		
RAMFGVYDNIGILGNFEK	1022.28,2+	2043.02	MRP-L51	P83022	Q9P0N7
AMFGVYDNIGILGNFEKHPK	1125.26,2+	2248.50	MRP-L51		
RMVGNRMFIDDLHNLNK	691.53,3+	2072.04	MRP-L51		
LRQGLAANPSGYGPLTELPDWSYADGRP-APPMK	881.95,4+	3523.77	MRP-L52	P83028	P83029
QGLAANPSGYGPLTELPDWSYADGRAPP-MKGQLR	928.20,4+	3708.77	MRP-L52		
FARRVVLLSQEMDAGLQAWQLRQQEK	775.75,4+	3099.64	MRP-L52		
VQFCPFEK + 1 PB-ICAT-D0(C)[a]	720.21,2+	1438.41	MRP-L53	P83024	P83030
VQFCPFEKNVESTR	709.20,3+	2124.58	MRP-L53		
NVESTRTFLQAVSSK	898.27,2+	1794.91	MRP-L53		
GAAVGETLKDPEVCTDPVQLTTHAMGV-NIYK + PE-Cys[b]	841.24,4+	3361.67	MRP-L54	P83025	P83032
EGQDVVLKPDSEYPEWLFQMNVGPPK	1501.36,2+	3001.45	MRP-L54		
KEEPELADDFDVEQYK	977.23,2+	1952.46	MRP-L55	P83026	P83027
NANENLLPGYLKPETMLMIWTPVPNTEM-SWDK	1244.65,3+	3731.80	MRP-L56	P83095	P83096
WAGGGFLSTVGDLLK	760.77,2+	1519.80	MRP-L56		
YAMAWGVVEK	577.18,2+	1152.56	MRP-L56		

[a] PB-ICAT-D0(C), cysteine residues alkylated with ICAT™ D0 reagent (monoisotopic mass = 545.23 Da).

[b] PE-Cys, cysteine residues alkylated with pyridylethyl (monoisotopic mass = 208.07 Da).

around the same buoyant density as ribosomal subunits. Peptides from two proteins expected to be associated with ribosomes were also detected in the 39 S preparations. These were from chaperone Hsp70 and from an unknown protein with similarity to both the release factor family of proteins and to the peptidyl-tRNA hydrolase family of proteins.

The molecular masses of the 48 proteins identified in the 39 S subunit, following removal of the predicted signal sequence, range from 5.4 to 54.6 kDa (Fig. 3). This range of sizes is in good agreement with the previous spectrum observed in bovine mitochondrial large subunits (4, 28). The total calculated molecular mass of the large subunit proteins following removal of the proposed N-terminal signal sequences is about 1030 kDa. This value combined with the mass of the 16 S rRNA present in the

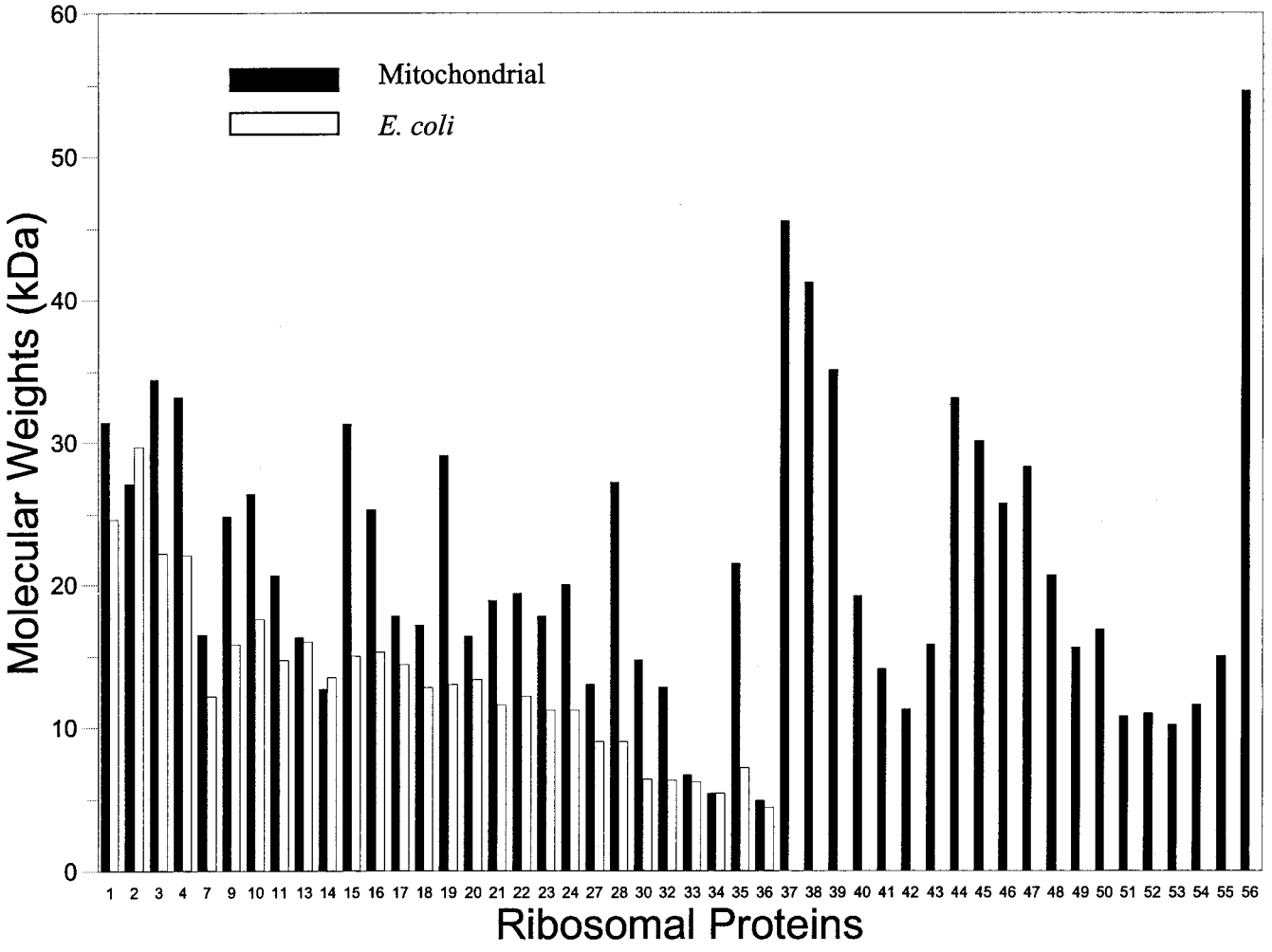

FIG. 3. **Molecular weights of mammalian mitochondrial 39 S proteins and comparison to *E. coli* 50 S ribosomal proteins (L1-L36).** The molecular weights of the MRPs are calculated after the removal of putative N-terminal import signals as predicted by MitoProt II and/or PSORT.

large subunit (about 550 kDa) brings the total estimated molecular mass of the large subunit to 1580 kDa. This value is in good agreement with the protein content of the ribosome calculated from the buoyant density of this subunit and the estimated sizes of each individual protein from the two-dimensional gels (4, 28). The 28 S subunits has been calculated to be around 1000 kDa based on the molecular masses of individual protein components and 12 S rRNA (2). Thus, the total calculated mass of mammalian mitochondrial ribosomes would be about 2.6×10^6 Da. This number also agrees with previous estimates (4, 28). Recently, the molecular mass of the rat mitochondrial ribosome was calculated to be 3.57×10^6 Da using static light scattering experiments (29). Analysis of the ribosomal proteins in the preparations used in these experiments indicated the presence of 86 ribosomal proteins, a number of which had high molecular masses (up to 86 kDa). We and several other groups (4, 5, 13, 28) have failed to observe such large ribosomal proteins in preparations of bovine mitochondrial ribosomes (data not shown). The apparent discrepancy between the size of the mitochondrial ribosome determined by biophysical methods and that calculated on the basis of the sizes of the known proteins and rRNAs remains to be resolved.

The Mitochondrial Large Subunit Proteins with Prokaryotic Homologs—Previously, prokaryotic homologs of 24 large subunit ribosomal proteins in mammalian mitochondrial ribosomes were identified. The present work adds 4 new homologs to this group (MRP-L18, MRP-L21, MRP-L28, and MRP-L35). Thus, a total of 28 large subunit proteins in mammalian mitochondria have bacterial homologs. MRP-L36, previously shown to be present in mitochondrial 39 S subunits, was not detected in our preparations due to its small size and its high arginine and lysine content which gives rise to small peptides upon digestion with trypsin.

MRP-L18—Only one peptide was obtained for MRP-L18 (Table II). This peptide was sufficient to identify mitochondrial L18 which was described as "ribosomal L18p/L5e family" in GenBank™. The full-length MRP-L18 protein is 180 residues in length. Homologs are present in other mammals, *C. elegans,* and *D. melanogaster*, however, there is no clear homolog of L18 in yeast mitochondria (Table III and IV). MRP-L18 is the only one of the three proteins known to interact with 5 S rRNA that is found in mammalian mitochondrial ribosomes. Even though the overall identity of MRP-L18 to prokaryotic L18s is low, the consensus residues describing L18p/L5e families are highly conserved except several arginine residues generally found at the N-terminal end of the prokaryotic, archaeal, and eukaryotic L18p/L5e proteins (30) (Fig. 4).

MRP-L21—Six peptides were obtained for MRP-L21 (Table II). Initial screening of the EST data bases with these peptides provided a hit, but it was not identified as an L21 homolog. The full-length protein is 205 residues in length. After the removal of the predicted import signal, the size of MRP-L21 remains significantly larger than *E. coli* L21 (Fig. 3). All but two of the mammalian mitochondrial large subunit proteins are larger

TABLE III
Percentage identity of human mitochondrial ribosomal proteins that are also found in other species

	% Identity to					
Human	Mouse	Drosophila	*C. elegans*	Yeast	*E. coli*	Arath[a]
MRP-L18	83.3	39	38.9	ND[b]	33.9	23.7
MRP-L21	80	38.5	32.7	24.5	23.3	28
MRP-L28	82.8	44	41.3	25.4	21.3	<20
MRP-L35	76.5	42.5	25.5	ND	26.6	28.5
MRP-L44	87	36.5	24.4	20.4	ND	ND
MRP-L45	79.4	40.8	35.9	ND	ND	ND
MRP-L46	81.2	37.7	26.1	29.5	ND	29
MRP-L47	85.7	43.3	33.2	21.8	ND	ND
MRP-L48	81	27.6	23.1	ND	ND	ND
MRP-L49	82.5	36.2	31.2	27.8	ND	ND
MRP-L50	77.9	31.9	24	ND	ND	ND
MRP-L51	81.3	37.3	30.2	29.4 (S10)	ND	ND
MRP-L52	85.1	35.9	28.6	ND	ND	ND
MRP-L53	85.6	ND	24.8	ND	ND	ND
MRP-L54	70.2	41.9	37.5	30.5	ND	28.6
MRP-L55	71.7	37.4	31.2	ND	ND	ND
MRP-L56	89	ND	39.2	ND	21.3	ND

[a] Arath, *Arabidopsis thaliana*.
[b] ND, Not detected.

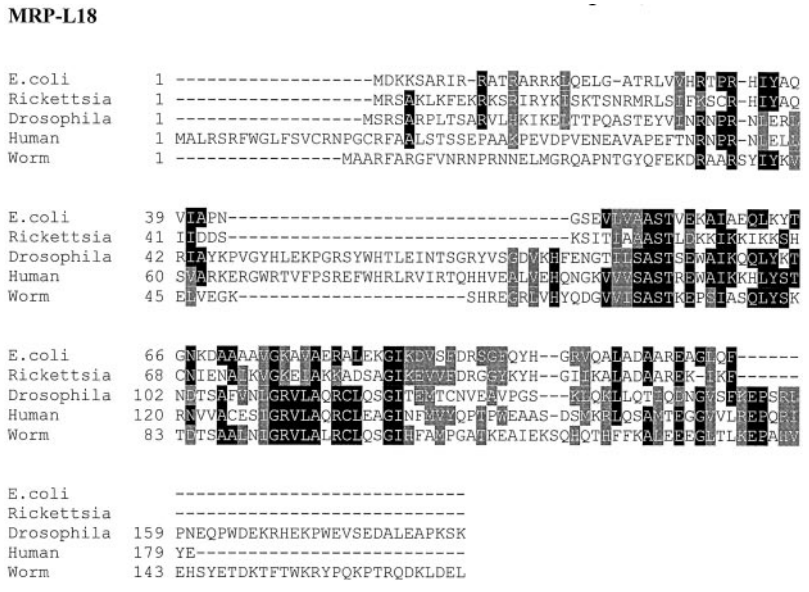

FIG. 4. **Amino acid sequence of human MRP-L18 and alignment with homologs from other species.** *Human*, *Drosophila,* and *worm* represent the corresponding mitochondrial species. The alignment was done with the CLUSTALW program in Biology Workbench and the results are displayed in BOXSHADE. Accession numbers of all the homologs, except *Rickettsia prowazekii* (Q9ZCS1) are given in Table IV.

than their prokaryotic counterparts (Fig. 3). Homologs of MRP-L21 are present in other mammals, *C. elegans,* and *D. melanogaster* (Table III and IV). Of these, the *Drosophila* MRP-L21 is more closely related to the mammalian protein than is *C. elegans* MRP-L21. This theme is shared by most of the large subunit proteins (Table III). Mammalian MRP-L21 is more closely related to its homolog from *S. cerevisiae* mitochondria than to its bacterial homolog. This pattern is not particularly typical since, in many cases, mammalian mitochondrial ribosomal proteins are more closely related to their bacterial counterparts than to the yeast mitochondrial homologs.

MRP-L28—Two peptide fragments encoded by the same bovine cDNA were identified as fragments from a protein similar to the prokaryotic L28 ribosomal protein family in Swiss-Prot searches (Table II). MRP-L28 is 256 residues in length and has a putative signal peptide cleavage site at residue 25 with a 91% probability to localize in mitochondria. Human MRP-L28 is about 3 times longer than its *E. coli* counterpart (Fig. 3). The region showing homology to the L28 family of proteins is located in the middle section of MRP-L28. This region corresponds to the C-terminal end of prokaryotic and chloroplast L28s. The C-terminal 128 residues of human MRP-L28 was previously identified as a "human melanoma-associated antigen" (Swiss-Prot O13084) that is recognized by certain melanoma-specific tumor-infiltrating lymphocytes (31). Extensive EST data base searches indicate that this data base entry does not represent the complete open reading frame for this protein. The observation that a mitochondrial ribosomal protein is related to a tumor antigen is reminiscent of the observation that a small subunit ribosomal protein has been identified as an antigen occurring in diabetic patients (2).

MRP-L35—The full-length human protein was assembled from cDNA clones by using any of the three peptides from MRP-L35 (Table II). Comparison of the sequence of this protein with the proteins in the Swiss-Prot data base revealed high homology (27.3%) to *Spinacia oleracea* chloroplast L35 (P23326). Human MRP-L35 is 26.6% identical to *E. coli* L35 and is reasonably well conserved among the various mitochondrial sequences available (Table III). The sequences of MRP-L35 homologs are well conserved in their C-terminal halves. Mitochondrial L35 homologs have additional sequences in their N-terminal halves although the sequences of these extensions are not particularly conserved. Regions of high homology between the mitochondrial proteins start where the alignment with *E. coli* L35 begins. A clear *Saccharomyces cerevisiae* mitochondrial L35 homolog cannot be found in the data base searches performed using either *E. coli* L35 or the human MRP-L35 sequence as a query.

TABLE IV
Swiss-Prot and GenBank™ accession numbers of human mitochondrial large subunit ribosomal proteins and their homologs found in various species

Protein	Human	*Drosophila*	*C. elegans*	Yeast	*E. coli*	*Arabidopsis*[a]
MRP-L18	Q9H0U6 (XP_004239)	AAF58460	AAA27999		P02419	BAB01882
MRP-L21	P83031 (AW632056)	AAF49183	AAF49183	MRPL49/YJL096W	P02422	AAF79387
MRP-L28	Q13084[b]	AAF52188	AAC26291	P36525 (Mrpl24p)	P02428	O22795
MRP-L35	Q9NZE8 (XP_002554)	AAF55944	CAA86518	YCR018C	P07085	AAC63677
MRP-L44	Q9H9J2 (BAB14234)	AAF52025	P34384	P36516 (YmL3/Mrpl3p)		
MRP-L45	Q9BRJ2 (BAB27440)[c]	AAF56032	AAK29715[b]			
MRP-L46	Q9H2W6 (AF210056)	AAF47568	AAF60808	P36528 (Mrpl17p/YNL252c)		AAF63174
MRP-L47	Q9HD33 (AF285120_1)	AAF54390	AAB52351	P36517 (MRPL4/YLR439W)		
MRP-L48	Q9Y5J0 (AF151876_1)	AAF51347	AAB88341			
MRP-L49	QI3405 (NP_004918)	AAF48212	CAA99906	NP_009996 (Img2)		
MRP-L50	Q9NX15 (BAA91207)	AAF09579	CAA92691			
MRP-L51	Q9P0N7 (AAF36161)	AAF52690	CAA92984	RSM10 (small subunit, S10)		
MRP-L52	P83029 (BAB23216)[c]	AAF59209	CAA90335			
MRP-L53	P83030 (BAB22849)[c]		CAA94201			
MRP-L54	P83032 (BAB22654)	AAF46662	CAB02987	P36532 (Mrpl37p/YBR268w)		AAF01557
MRP-L55	P83026 (BAB28535)[c]	AAF55632	AAD12855			
MRP-L56	AAG37911[c]		CAA16386			

[a] Some are putative chloroplast ribosomal proteins.
[b] Partial protein sequence.
[c] Mouse sequence.

New Classes of Ribosomal Proteins in Mammalian Mitochondrial Ribosomes—Thirteen proteins that did not have homology to any known prokaryotic ribosomal protein were identified based on the peptides present in whole 39 S subunit digests (Table II). These proteins are designated as "new" classes of mammalian mitochondrial large subunit proteins to reflect their lack of homology to other known ribosomal proteins. Seven of these mitochondrial specific ribosomal proteins have no obvious homologs in yeast. All but two of these proteins can be identified in the *D. melanogaster* genome. With few exceptions (see below) none of the new classes of ribosomal proteins identified here has significant similarities to other known proteins nor do they contain recognizable common protein motifs. Almost all of these ribosomal proteins have mitochondrial import signals. The proteins newly identified here bring the number of large subunit mammalian mitochondrial ribosomal proteins without bacterial homologs to 20 (6, 7, 11, 13, 14, 32).

The New Classes of Ribosomal Proteins with Homologs in Yeast and Arabidopsis—Five of the new classes of ribosomal proteins identified in this study have homologs in yeast (MRP-L44, MRP-L46, MRP-L47, MRP-L49, and MRP-L54). The yeast homologs of human MRP-L44, MRP-L46, MRP-L47, and MRP-L54 were previously identified as yeast mitochondrial ribosomal large subunit proteins (Table IV) (33). MRP-L51 shows considerable homology (29.4%) to a small subunit ribosomal protein in yeast (S10). However, no peptides from this protein were detected in small subunit preparations from bovine mitochondria. Hence, we believe that it is a *bona fide* large subunit ribosomal protein in animals. It is possible that this protein lies on the interface of the two subunits and fractionates differently between the two subunits in yeast and mammals. This study, along with the previous efforts of Mitochondrial Ribosome Consortium and Professor Watanabe's group (University of Tokyo), increases the number of mitochondrial specific ribosomal proteins shared by yeast and mammalian mitochondrial ribosomes to 10.

BLAST analysis suggests that human MRP-L44 and its yeast homolog (MRPL3) have some similarities to RNase III from prokaryotes. However, important conserved regions in RNase III are not conserved in the mitochondrial ribosomal protein and it is unlikely to have RNase activity. The yeast homolog of MRP-L49 is described as Img2, a protein which is required for mitochondrial genome integrity. Previous work has shown that there is a connection between the stability of the mitochondrial genome and transcription and translation in this organelle (34, 35). MRP-L49 (Img2) could potentially play a role in the coordination of mitochondrial gene expression and maintenance of mitochondrial DNA that is observed in yeast and postulated to occur in higher eukaryotes (34).

Homologs of the new family of ribosomal proteins MRP-L46 and MRP-L54 are found in both yeast and *Arabidopsis thaliana* (Table IV). The *A. thaliana* homologs of MRP-L46 and MRP-L54 are about 29% identical to the human proteins. MRP-L46 in *C. elegans* is considerably longer than the corresponding proteins from other mitochondria (Fig. 5). As seen in these alignments, highly conserved regions in human, *Drosophila,* and yeast homologs are also conserved in *A. thaliana*. Several of these regions have not been conserved in *C. elegans* (Fig. 5). The MRP-L54 homologs found in the mitochondria of various organisms are all about the same size and show two regions of particularly high conservation.

Other New Ribosomal Proteins—Seven of the ribosomal proteins without prokaryotic homologs also lack homologs in yeast (Table IV). The locations of these new proteins in the 39 S subunit and their roles in translation remain to be determined. One of the new classes of ribosomal proteins, MRP-L56, is about 21% identical to prokaryotic serine β-lactamases. However, most of the active center residues of prokaryotic serine β-lactamases are not conserved, suggesting that this protein is not a mammalian mitochondrial serine β-lactamase homolog. One of the new proteins identified here (MRP-L51) has recently been observed by Suzuki *et al*. (36) in 55 S ribosomes and was designated MRP64 in that study. Suzuki *et al.* (36) also identified a protein (MRP63) from 55 S preparations. We have not observed any peptides from this small protein in 28 S or 39 S subunit preparations. This group has further reported that a subunit (CI-B14) of NADH-ubiquinone oxidoreductase (Complex I) is present in preparations of 55 S ribosomes (36). However, we have not observed peptides from this protein in either 28 S or 39 S subunit preparations.

The current analysis of peptide digests of whole large subunits leads to the confirmation of the presence of 30 large subunit ribosomal proteins reported previously including proteins that represent new classes of ribosomal proteins and proteins having prokaryotic homologs (6, 7, 11, 13, 14, 32).

Absence of 5 S rRNA in 39 S Subunits—Mammalian mitochondrial DNA does not possess a gene encoding a 5 S rRNA. However, recent data on the presence of imported 5 S rRNA in mitochondria (37) and the presence of MRP-L18, a homolog of a prokaryotic 5 S rRNA-binding protein L18, in mitochondrial

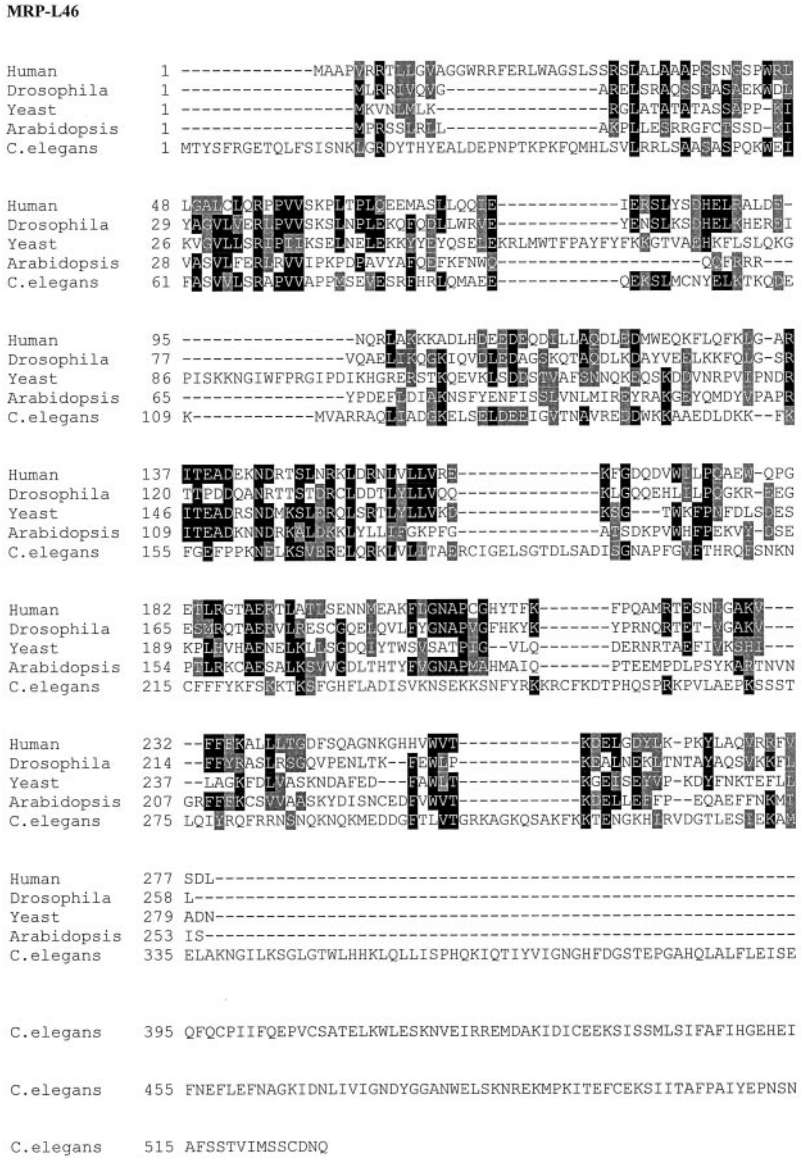

FIG. 5. **Amino acid sequence of human MRP-L46 and alignment with homologous proteins from other species.** Accession numbers of all the homologs are given in Table IV. The alignment was done using the CLUSTALW program in Biology Workbench version 3.2 and the results are displayed with BOXSHADE.

39 S subunits, forced us to question the possible incorporation of cytoplasmic 5 S rRNA into 39 S subunits. To demonstrate the absence of rRNA species similar to 5 S rRNA, ribosomal RNAs from bovine mitochondrial 39 S subunits, cytoplasmic 80 S ribosomes, and *E. coli* 50 S subunits were prepared. Denaturing gel analysis of rRNA extracted from bovine mitochondrial 39 S subunits indicated that no 5 S rRNA species corresponding to imported cytoplasmic 5 S rRNA was present (data not shown). In contrast, the 5 S rRNA was readily observed in cytoplasmic 80 S ribosomes and in *E. coli* 50 S subunits. Therefore, although the cytoplasmic 5 S rRNA appears to be imported into mammalian mitochondria, it is not incorporated into mitochondrial ribosomes.

DISCUSSION

The 17 ribosomal proteins described in this paper and the 31 previously characterized proteins increase the total number of proteins identified in mammalian mitochondrial 39 S subunits to 48. Twenty eight of these proteins show significant sequence similarities to bacterial ribosomal proteins. The remaining 20 proteins identified in this and previous studies belong to new classes of ribosomal proteins (6, 7, 11, 13, 14, 32).

The mammalian mitochondrial 39 S subunit contains a 16 S rRNA which is about 1500 nucleotides in length. It is highly truncated compared with the 23 S large subunit rRNA of prokaryotic 50 S subunits. In general, the 16 S rRNA is not randomly shortened throughout its structure. Rather, it shows striking deletions of a number of features of the secondary structure (Fig. 6). For example, almost all of domain I and most of domain III of 23 S rRNA have been deleted (*red* in Fig. 6) while most of the crucial helices involved in peptidyl transferase activity in domains IV, V, and VI have been preserved in the predicted structure of human mitochondrial 16 S rRNA.

Recently, crystal structures of the large ribosomal subunit from an archaea, *Haloarcula marismortiu,* and of the 70 S ribosome from the prokaryote, *Thermus thermophilus*, were determined at 2.4- and 5.5-Å resolution, respectively (38, 39). The regions of 23 S rRNA present in the mitochondrial 16 S rRNA were examined using the reconstructed three-dimensional model of the *T. thermophilus* 50 S subunit at 5.5-Å resolution to supply the coordinates (39). A front (small subunit interaction side) or crown view is shown in Fig. 7*A* with the regions retained in the 16 S rRNA shown in *blue* and the regions missing shown in *red*. A back view is shown in Fig. 7*B* again showing regions that are present in *blue* and regions that are absent in *red*. These views illustrate that most of the regions of the large subunit rRNA missing in the mammalian

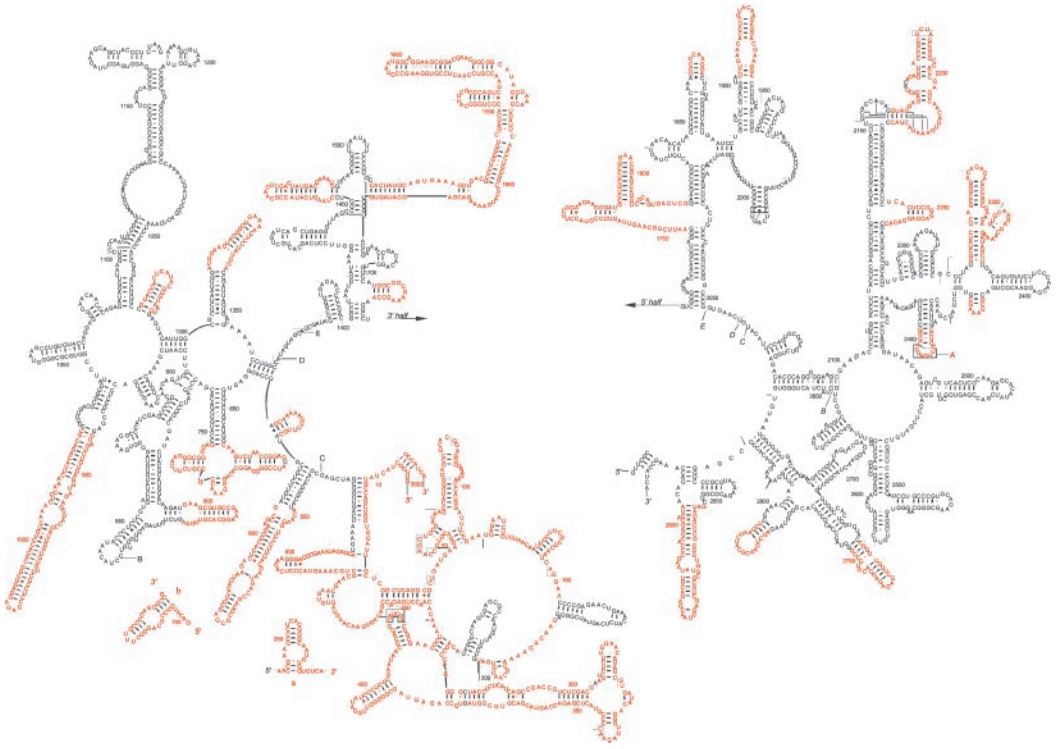

FIG. 6. **Secondary structure comparison of large subunit rRNA from *H. marismortiu* 50 S subunits (23 S) and human mitochondrial 39 S subunits (16 S).** After superimposing the secondary structure of human mitochondrial 16 S rRNA on the *H. marismortiu* 23 S rRNA, the missing secondary structures in mitochondrial rRNA were colored in *red*. (R. R. Gutell, S. Subashchandran, M. Schnare, Y. Du, N. Lin, L. Madabusi, K. Muller, N. Pande, N. Yu, Z. Shang, S. Date, D. Konings, V. Schweiker, B. Weiser, and J. J. Cannone, manuscript in preparation (data can be found at www.rna.icmb.utexas.edu/). The remaining secondary structure is shown in *black*.

mitochondrial 16 S rRNA are located on the periphery of the subunit and on the back side away from the decoding region. RNA components creating the core structure of the peptidyl transferase site and much of the body have been retained in the 16 S rRNA. The RNA in the central protuberance formed by 5 S rRNA, a portion of the L1 stalk and portions of the bottom of the subunit are missing in the mammalian mitochondrial 16 S rRNA (shown in *red* in Fig. 7). It has been shown previously that 5 S rRNA is not needed for peptidyl transferase activity (40). The conservation of critical regions of the rRNA suggests that the basic functioning of the mitochondrial ribosome will be analogous to that of the bacterial and archaeal ribosomes.

The putative locations of the mitochondrial ribosomal proteins that have prokaryotic homologs have been examined using the model of the 50 S subunit (Fig. 7, *C* and *D*). Unfortunately, only some of the prokaryotic large subunit proteins can be localized due to the lack of complete structural information on the bacterial ribosome. The mitochondrial proteins that can be localized are L1, L2, L3, L4, L7/L12, L9, L13, L14, L15, L16, L18, L19, L20, L21, L22, L23, L24, L28, L30, L32, L33, and L34 (Fig. 7, *C* and *D*). The other prokaryotic homologs present in mammalian mitochondrial ribosomes, L10, L17, L20, L35, and L36, cannot be localized (39). Two of the missing prokaryotic homologs, L5 and L25, are located in the central protuberance of the 50 S structure (shown in yellow in Fig. 7, *C* and *D*) where the protein-protein interaction (L5 and S13) occurs at the subunit interface (39).

In prokaryotes L28 is categorized as a secondary rRNA-binding protein that can be cross-linked to domain I of 23 S rRNA (41, 42) where the mitochondrial 16 S rRNA is truncated. Therefore, the long human mitochondrial MRP-L28 might compensate for the truncated portion of the mammalian mitochondrial 16 S rRNA. This analysis indicates that truncation of the rRNA in the large subunit may account in part for the larger size and additional number of ribosomal proteins in mitochondria. However, a more complete understanding of structure/function relationships in mitochondrial ribosomes will be required before a clear interpretation of the data is possible.

No proteins corresponding to bacterial ribosomal proteins L5, L6, L25, L26, L29, and L31 could be found. L5 and L25 are 5 S rRNA-binding proteins and are, therefore, not expected to be present in the 39 S subunit (43). However, a clear mammalian mitochondrial homolog of the 5 S rRNA-binding protein of the L18p/L5e family is observed in mitochondrial ribosomes. Ribosomal L18p/L5e family proteins are one of the three 5 S rRNA-binding proteins in prokaryotic and cytoplasmic ribosomes (41). L18 is also one of the eight essential ribosomal proteins required to form active subribosomal particles of *Thermus aquaticus* ribosomes containing both 23 and 5 S rRNAs (31). Recently, the arginine-rich N-terminal portion of *Pyrococcus furiosus*, L18 was shown to be involved in 5 S rRNA binding (30). This region is missing in animal mitochondrial MRP-L18s as would be expected based on the lack of this rRNA in mitochondrial ribosomes.

The large subunit protein L26 sometimes fractionates with the small subunit and is identical to S20. No homologs to the prokaryotic L26/S20 could be identified in either 28 S or 39 S subunits. This observation is not surprising since the region of the rRNA to which this protein binds is not present in the mammalian mitochondrial rRNA (2). The ribosomal protein designated L8 is actually a complex between the L7/L12 dimer and L10 in prokaryotic ribosomes and, thus, does not represent a separate ribosomal protein. Hence, the mitochondrial 39 S subunit has 28 of the distinct 33 *bona fide E. coli* 50 S subunit proteins.

About half of the proteins in the large subunit of the mammalian mitochondrial ribosome do not have homologs in prokaryotic ribosomes. The roles and locations of all these riboso-

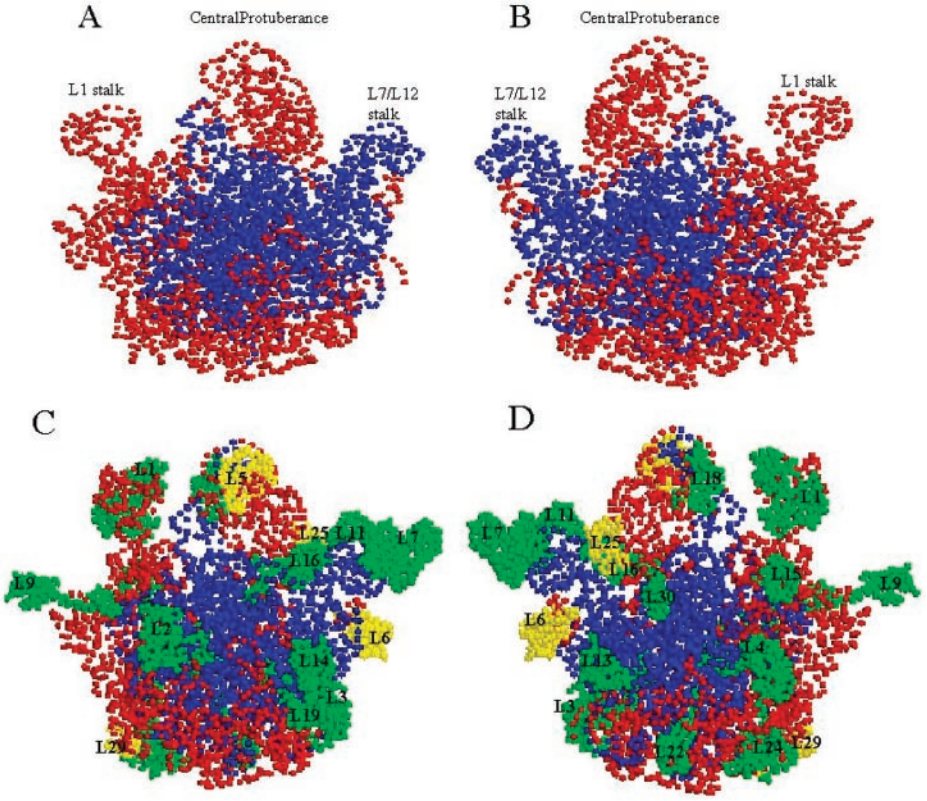

FIG. 7. **Models of the mitochondrial large subunit based on the crystal structure of the *T. thermophilus* 50 S subunit.** The coordinates of the *T. thermophilus* 50 S subunit were obtained from the Protein Data Bank (accession number 1GIY) (39). In all the panels, the large subunit rRNA is represented by *blue* and *red dots*. Each model is shown in two different views (the front view representing the side that interacts with the small subunit and the back view). *A* and *B,* three-dimensional models of the *T. thermophilus* 50 S subunit showing the front view (*A*) and the back view (*B*) of the rRNA in the subunit, respectively. The *blue regions* are the regions conserved in both prokaryotic and mitochondrial rRNAs and the *red regions* are the portions missing in the mitochondrial large subunit rRNA. *C* and *D,* the prokaryotic ribosomal proteins that have homologs in mammalian mitochondrial ribosomes were located on the three-dimensional model of the 50 S subunit (*green*). The prokaryotic ribosomal proteins that do not have homologs in mammalian mitochondrial ribosomes are shown in *yellow*. *Panel C* is the front view (from the 30 S subunit side) while *D* is the back view of the large subunit. Again, regions of the 23 S rRNA present in the 16 S rRNA are shown in *blue* and regions missing are shown in *red*.

mal proteins remain to be determined. Some of these new ribosomal proteins may have evolved additional roles for the cell such as promoting the interaction of ribosomes with the mitochondrial inner membrane and coordinating translation with the maintenance and expression of mitochondrial DNA (44, 45). Components of the mitochondrial protein biosynthetic system may also play pivotal roles in apoptosis and in mitochondrial diseases (2, 25, 46).

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