THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 275, No. 42, Issue of October 20, pp. 32585–32591, 2000

Printed in U.S.A.

A Proteomics Approach to the Identification of Mammalian Mitochondrial Small Subunit Ribosomal Proteins*

Received for publication, April 27, 2000, and in revised form, August 2, 2000
Published, JBC Papers in Press, August 9, 2000, DOI 10.1074/jbc.M003596200

Emine Cavdar Koc‡, William Burkhart§, Kevin Blackburn§, Arthur Moseley§, Hasan Koc¶, and Linda L. Spremulli‡‖

From the ‡Department of Chemistry and ¶School of Public Health, Environmental Science and Engineering, University of North Carolina, Chapel Hill, North Carolina 27599-3290 and the §Department of Structural Chemistry, Glaxo Wellcome Research and Development, Research Triangle Park, North Carolina 27709-3398

Mammalian mitochondrial small subunit ribosomal proteins were separated by two-dimensional polyacrylamide gel electrophoresis. The proteins in six individual spots were subjected to in-gel tryptic digestion. Peptides were separated by capillary liquid chromatography, and the sequences of selected peptides were obtained by electrospray tandem mass spectrometry. The peptide sequences obtained were used to screen human expressed sequence tag data bases, and complete consensus cDNAs were assembled. Mammalian mitochondrial small subunit ribosomal proteins from six different classes of ribosomal proteins were identified. Only two of these proteins have significant sequence similarities to ribosomal proteins from prokaryotes. These proteins correspond to *Escherichia coli* S10 and S14. Homologs of two human mitochondrial proteins not found in prokaryotes were observed in the genomes of *Drosophila melanogaster* and *Caenorhabditis elegans*. A homolog of one of these proteins was observed in *D. melanogaster* but not in *C. elegans*, while a homolog of the other was present in *C. elegans* but not in *D. melanogaster*. A homolog of one of the ribosomal proteins not found in prokaryotes was tentatively identified in the yeast genome. This latter protein is the first reported example of a ribosomal protein that is shared by mitochondrial ribosomes from lower and higher eukaryotes that does not have a homolog in prokaryotes.

Mammalian mitochondria carry out the synthesis of 13 polypeptides that are essential for oxidative phosphorylation and, hence, for the synthesis of the majority of the ATP used by eukaryotic organisms. The protein synthesizing system of mammalian mitochondria has a number of interesting features that are not observed in the corresponding systems in prokaryotes or the cell cytoplasm (1). The ribosomes present in mammalian mitochondria are 55–60 S particles and are composed of small (28 S) and a large (39 S) subunits (2). They are characterized by a low percentage of rRNA and a compensating increase in the number of ribosomal proteins (3).

Considerable progress has been made on the identification of the mitochondrial ribosomal proteins in yeast. About 50 different mitochondrial ribosomal proteins (MRPs)[1] have been identified in this organism (4). Additional protein components in yeast mitochondrial ribosomes remain to be determined. Surprisingly, less than half of the mitochondrial ribosomal proteins in yeast show significant sequence identities to the ribosomal proteins of other systems (4). Analysis of the protein composition of mammalian mitochondrial ribosomes indicates that they have more proteins than observed in bacterial ribosomes (5). Limited information is available on the identities of these proteins and on their relationships to bacterial ribosomal proteins. Recently, 18 proteins of the large subunit and 5 proteins of the small subunit of the mammalian mitochondrial ribosome have been characterized primarily by peptide sequencing coupled to the extensive use of the EST data bases to deduce the full-length cDNAs and the corresponding amino acid sequences (6–11). Of these proteins, 12 from the large subunit and 2 from the small subunit are homologs of bacterial ribosomal proteins. The remainder fall into new classes of ribosomal proteins. In the current work, peptide sequence information has been obtained for six new mitochondrial small subunit ribosomal proteins. The cDNAs and amino acid sequences have been assembled using EST data bases, and the genomes of *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans* have been searched for homologs. Only two out of the six new small subunit proteins found in mammalian mitochondrial ribosomes are similar to prokaryotic ribosomal proteins. The remaining four small subunit proteins fall into new classes of ribosomal proteins.

MATERIALS AND METHODS

Preparation of Bovine Mitochondrial Ribosomal Proteins for Two-dimensional Gel Electrophoresis—Bovine mitochondria and 28 S subunits were prepared as described previously by Matthews *et al.* (5), and the 28 S subunits were collected by centrifugation at 48,000 rpm for 6 h in a Beckman Type-50 rotor. The pellet containing about 5 A_{260} (approximately 420 pmol) (12) was resuspended in isoelectric focusing gel buffer containing 9.5 M urea, 2% Triton X-100, 2% ampholytes (consisting of 1.6% (v/v) pH 5–7 and 0.4% (v/v) pH 3–10), and 0.24 M 2-mercaptoethanol. The sample was prepared as described previously prior to loading on non-equilibrium pH gradient tube gels as described (9, 13). Following electrophoresis in the first dimension, gels were equilibrated in buffer (10% glycerol, 2% sodium dodecyl sulfate, 1% dithiothreitol, 62.5 mM Tris-HCl, pH 6.8) and subjected to electrophoresis in the second dimension on 12% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) (14). Gels were stained with Coomassie Brilliant Blue G-250.

* This work was supported by National Institutes of Health Grant GM32734. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) P82649, P82650, and P82663–P82670.

‖ To whom correspondence should be addressed: Dept. of Chemistry, University of North Carolina, Campus Box 3290, Chapel Hill, NC 27599-3290. Tel.: 919-966-1567; Fax: 919-966-3675; E-mail: linda_spremulli@unc.edu.

[1] The abbreviations used are: MRP, mitochondrial ribosomal protein; LC/MS/MS, liquid chromatography-tandem mass spectrometric analysis; MS, mass spectrometry; EST, expressed sequence tag; PAGE, polyacrylamide gel electrophoresis.

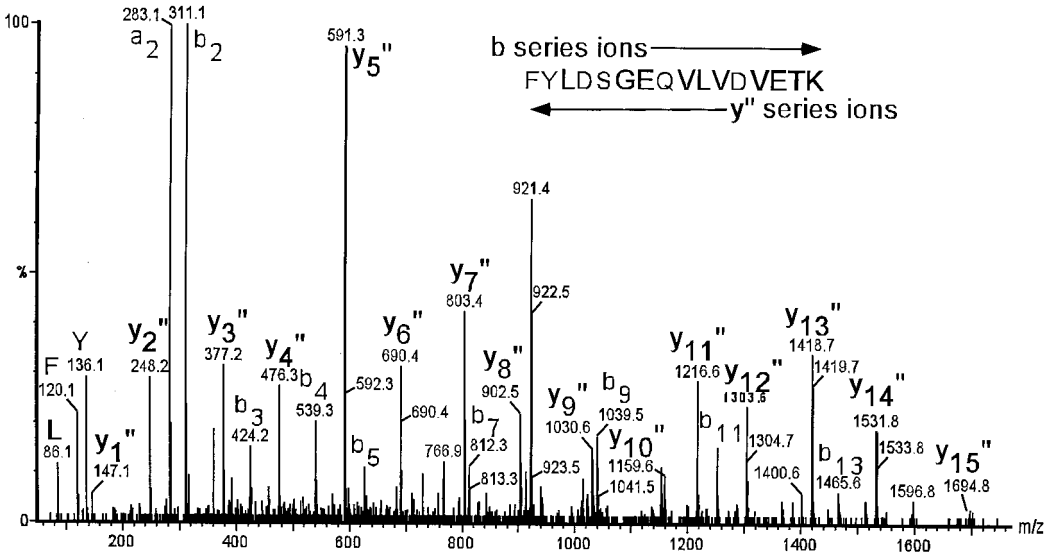

FIG. 1. **Product ion spectrum of the tryptic peptide at *m/z* 921.4 from MRP-S25.** The y″ and b series ions are labeled according to the nomenclature of Roepstorff and Fohlman (21).

Peptide Sequencing by Mass Spectrometry—Seven randomly picked spots from the two-dimensional PAGE of the mitochondrial 28 S subunit were excised and digested in-gel with trypsin (Roche Molecular Biochemicals) in 10 mM Tris-HCl, pH 8.0, according to the procedure of Shevchenko *et al.* (15) except that alkylation of sulfhydryls was accomplished with 4-vinylpyridine.

Liquid chromatography-tandem mass spectrometric (LC/MS/MS) analyses of in-gel digests were done using an Ultimate capillary liquid chromatography system (LC Packings, San Francisco, CA) coupled to a quadrupole time-of-flight mass spectrometer (Micromass, Manchester, United Kingdom) fitted with a Z-spray ion source as described previously (9). Uninterpreted peptide product ion spectra generated by LC/MS/MS were searched against the nonredundant protein data base and a human EST data base for exact matches using the Mascot search program (16). High quality spectra that had no exact matches in either the protein or EST data bases were sequenced *de novo* either manually or with the aid of the PepSeq program (Micromass).

Computational Analysis—Peptide sequences obtained from Mascot searches of the protein and EST data bases and those obtained by *de novo* sequencing from peptide product ion spectra were searched against the nonredundant protein data base using the FASTA algorithm (17). For peptides with no exact matches in the data bases, sequences obtained by *de novo* sequencing were used for FASTA searches. Because mass spectrometry cannot distinguish between the isobaric (same nominal residue molecular weight) amino acids Leu and Ile, initial data base searches were carried out using Leu in the peptide sequences. Hits with an Ile at these positions were considered exact matches. If no hits were obtained when Leu was present in the search sequence, the search was redone with Ile. The isobaric amino acids Phe and oxidized Met (a common artifact of PAGE) were distinguished by diagnostic loss of methanesulfenic acid (64 Da) from oxidized Met (18). Because the protease trypsin that cleaves on the C-terminal side of Arg and Lys residues was used for in-gel digestion, Lys residues could be distinguished with a fairly high certainty from isobaric Gln residues. EST data base and genomic DNA searches of the peptide sequences were performed using the BLAST search program (19). Sequence analysis and homology comparisons were done using the GCG DNA analysis software package (Wisconsin Package version 10 (1999); Genetics Computer Group, Madison, WI) and 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 (20).

RESULTS

Characterization of Bovine Mitochondrial Ribosomal Proteins by Tandem Mass Spectrometry—As a first step toward understanding the protein components of mammalian mitochondrial ribosomes, small subunit proteins were separated by two-dimensional PAGE. The protein spots from the two-dimensional PAGE were excised and subjected to in-gel digestion using trypsin. After digestion, the resulting peptide mixtures were analyzed by nanoscale capillary LC/MS/MS using a quadrupole time-of-flight mass spectrometer. The instrument was operated in a data-dependent MS to MS/MS switching mode where peptide ions detected in an MS survey scan trigger a switch to MS/MS for obtaining peptide product ion (fragmentation) spectra. Product ion spectra of peptides contain primarily ions originating from either the peptide C terminus (y″ ion series) or N terminus (b ion series), which are formed by cleavage of amide bonds along the peptide backbone (21). Adjacent y″ or b ions differ by the corresponding amino acid residue mass, enabling assignment of peptide amino acid sequence. A representative spectrum for one of the peptides analyzed is shown in Fig. 1. Initially, uninterpreted peptide product ion spectra were searched against the nonredundant protein and human EST data bases using the Mascot program. This program searches all entries in the data base for peptide sequences that would yield product ion spectra with a fragmentation pattern identical to that observed for peptide product ion spectra obtained by LC/MS/MS. In cases where no exact matches were retrieved, spectra were subjected to manual interpretation/sequence assignment (*de novo* sequencing). Peptide sequence matches obtained from Mascot data base searches and sequences derived from *de novo* sequencing are shown in Table I.

Nomenclature—Two-dimensional patterns and molecular weights of bovine mitochondrial ribosomal small subunit proteins were reported previously and the proteins thought to be present in these ribosomes were designated S1 to S33, in order of decreasing molecular weight (5). However, it is now clear that this system for designating mammalian mitochondrial proteins does not provide a consistent way to define them. For example, using this system, bovine MRP-S18 is the same protein as rat MRP-S13. To simplify the nomenclature and to facilitate a comparison between proteins in mammalian mitochondrial ribosomes and in bacteria, we have adopted a nomenclature in which proteins are designated by their prokaryotic homolog (for example S12 in bacteria is designated MRP-S12 in mammalian mitochondria). Proteins without bacterial homologs are given the next available number. Since there are 21 proteins in the bacterial ribosome, we have begun designating the new mammalian mitochondrial ribosomal proteins beginning at MRP-S22. This approach to the nomenclature of organellar ribosomal proteins has recently been adopted for chlo-

TABLE I

Amino acid sequences of mature MRPs of Bos taurus derived from data base searching or de novo sequencing of peptide product ion spectra of two-dimensional gel spots

(I/L) and (Q/K), amino acid residues leucine/isoleucine and glutamine/lysine can not be distinguished by mass spectrometry. *X*, unidentified amino acid residue C-terminal to tryptic cleavage site.

Protein name	Sequence	THC no.[a]	Swiss-Prot no.[b]	Family
MRP-S22		359730	P82650 (H)	New
Peptide 1	LMTQAQLEEATR		P82649 (B)	
Peptide 2	LKMPPVLEER			
Peptide 3	VPINDVLAEDK			
Peptide 4	KIDGLLIDQIQR			
Peptide 5	VFAKTEAQK			
MRP-S26 identified before as MRP-S13		385286	S78421 (R)	New
Peptide 1	*XX*MAWN(Q/K)AENR([c])			
MRP-S23		298895[d]	Q9Y3D9 (H)	New
Peptide 1	ALLAEGVLIR		P82666 (B)	
Peptide 2	AFDLFNPNFK			
MRP-S10		371177	P82664 (H)	S10
Peptide 1	NLPEGVAMEVTK		P82670 (B)	
Peptide 2	AVLDSYEYFAVLAAK			
MRP-S25		330112[d]	P82663 (H)	New
Peptide 1	VMTVNYNTHGELGEGAR		P82669 (B)	
Peptide 2	FYLDSGEQVLVDVETK			
MRP-S24		260485[d]	P82668 (H)	New
Peptide 1	GDKPVTYEEAHAPHYIAHR		P82667 (B)	
Peptide 2	TVEDVFLR			
Peptide 3	LHLQTVPSK			
MRP-S14		328681	O60783 (H)	S14
Peptide 1	H(I/L)EEVADEE(I/L)AA(I/L)PR([c])		P82665 (B)	

[a] Numbers are from the human gene index of the Institute for Genomic Research.

[b] H, Swiss-Prot number for the complete human sequence; B, Swiss-Prot number for the bovine peptides; R, MIPS Data Base accession number for rat N-terminal peptide (6).

[c] Obtained from *de novo* sequencing.

[d] Consensus cDNA sequences in the THC files must be corrected according to EST sequences obtained from TBLASTN searches to obtain completely correct protein sequences. Note that the reverse complement of some THC entries must be used to obtain the protein sequence.

roplast ribosomal proteins (22, 23).

Overall Approach to the Assembly of Mammalian Mitochondrial Ribosomal Protein Sequences from cDNA Clones—Sequences of the peptides obtained from bovine mitochondrial ribosomes (Table I) were used to search the human EST data base using the tBLASTN program (National Center for Biotechnology Information). A number of hits were obtained for some but not all of the peptides used as virtual screening probes. Overlapping clones for these hits were obtained using the initial hit as a virtual probe to rescreen the human EST data base. For all the peptide sequences obtained, consensus cDNAs were then assembled by repetitive searching and comparison of EST sequences. The sequence of the longest possible cDNA was assembled *in silico.* Sequencing errors were corrected by comparison of overlapping clones. The fully assembled human sequence was then used as a query against entries in other data bases.

MRP-S22—The sequences of five tryptic peptides were obtained from this protein using mass spectrometry (Table I) and its full sequence (360 amino acids) was deduced (Fig. 2). MitoProt II assigns a 71% probability that MRP-S22 is localized in mitochondria and predicts cleavage following residue 30 (20). Examination of the sequence of the MRP-S22 using MotifFinder (software available via the World Wide Web) indicates that this protein does not contain any of the motifs found in the PROSITE data base including any known RNA binding motifs. The Swiss-Prot and MitoProt II protein data bases were searched using the full-length sequence of human MRP-S22 to find homologs of this mitochondrial ribosomal protein. No significant similarities were found to any known prokaryotic or eukaryotic protein in the data bases. Therefore, MRP-S22 is categorized as a member of a "new" class of ribosomal proteins.

A complete mouse homolog for MRP-S22 is present in the mouse EST data base. Human and mouse MRP-S22 align well except in the region corresponding to the mitochondrial import signal peptide. Overall, these two proteins are 78.8% identical (Table II). Homologs were also observed in *C. elegans* and *D. melanogaster* (Fig. 2 and Table II). Alignment of the *C. elegans* and *D. melanogaster* proteins with the human MRP-S22 shows that the conserved regions are all located in the N-terminal and middle sections of the protein (Fig. 2). Both the *C. elegans* and *D. melanogaster* proteins have C-terminal extensions not observed in the mammalian proteins. These extensions do not share significant homology, suggesting that they may not play an essential role in the biological function of this protein. No sequence corresponding to the MRP-S22 mitochondrial ribosomal protein could be detected in the yeast genome when human, *C. elegans*, or *D. melanogaster* sequences were used as virtual probes. This observation suggests that prokaryotic and fungal mitochondrial ribosomes do not have this particular ribosomal protein in common.

MRP-S26—The sequence of only one peptide was obtained from the tryptic digest of this protein (Table I). This peptide was derived from a previously identified protein designated MRP-S13 in rat and MRP-S18 in bovine mitochondrial ribosomes (6). Therefore, no further analysis was done for this protein.

MRP-S23—The sequences of two peptides were obtained from MRP-S23 by mass spectrometry (Table I). The protein encoded by this cDNA is 190 amino acids in length (Fig. 3). Neither PSort nor MitoProt II predicts a mitochondrial localization for the MRP-S23 protein. To help ensure that this protein was actually a ribosomal protein present in the small subunit, the intensity of this spot was examined in two-dimensional gels of subunits prepared directly from crude ribosomes on a single sucrose gradient and in 28 S subunit preparations obtained in two sequential sucrose gradients. The first gradient was used to prepare 55 S ribosomes, which were dissociated into subunits that were purified in the second sucrose gradient. The intensity of the MRP-S23 was comparable in both types of

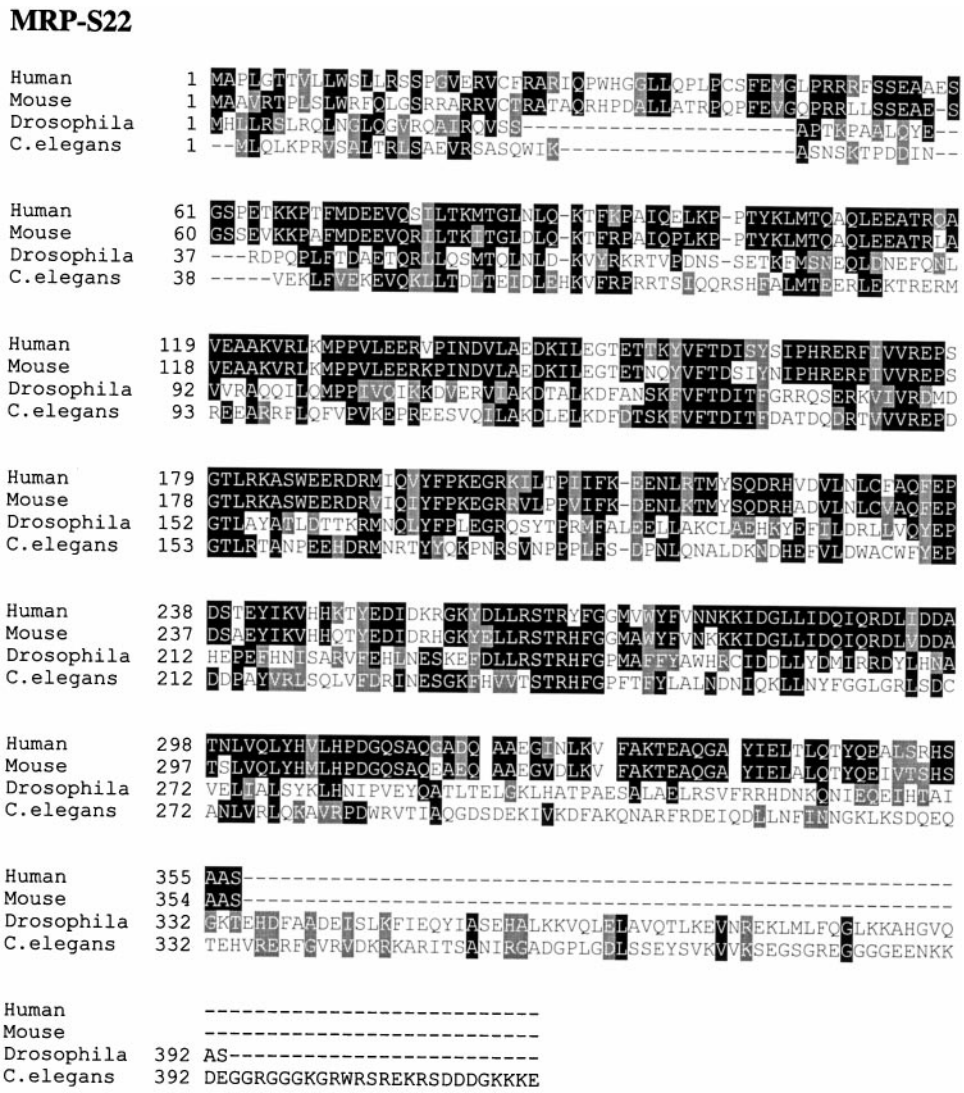

FIG. 2. **Sequence of MRP-S22 and alignment with homologs in other organisms.** The sequence of the human cDNA was assembled using ESTs AA313665, AA463594, W74092, and AI004657. The mouse sequence was assembled from EST clones. The sequences of the *D. melanogaster* (AAF56757) and *C. elegans* homologs (CE24801) were obtained from the data bases.

TABLE II
Percentage identity of human mitochondrial ribosomal proteins to homologs found in other species

ND, homologs of human ribosomal proteins are not detected in data base searches.

Human protein	Identity to				
	Mouse	*Drosophila*	*C. elegans*	Yeast	*E. coli*
	%	%	%	%	%
MRP-S10	80.2	44.8	36.4	21.1	30.4
MRP-S14	86.7	67.6	40.7	30.4	35
MRP-S22	78.3	31.4	32.2	ND	ND
MRP-S23	76	ND	40.6	ND	ND
MRP-S24	81.3	37.4	ND	ND	ND
MRP-S25	89.5	56	39.3	24.4	ND

preparations. The observation that MRP-S23 protein is present in 55 S monosomes and in 28 S subunits prepared from them is strongly suggestive that it is a *bona fide* ribosomal protein.

A complete mouse cDNA was deduced from ESTs (Table I and Fig. 3). Alignment of the human and mouse sequences gives 76% identity. A homolog to human MRP-S23 is also present in the *C. elegans* genome (Fig. 3). Searches of the *D. melanogaster* genome detects a number of hits covering small stretches with similarity to MRP-S23. However, most of these hits are not in predicted reading frames. No sequence corresponding to this human mitochondrial ribosomal protein could be detected in searches of the yeast genome using either the human or *C. elegans* MRP-S23 protein as a probe.

Analysis of the amino acid sequence of the MRP-S23 using PROSITE does not indicate the presence of any common motifs including RNA binding or ribosomal protein motifs. A BLOCKS analysis provides several possible poorly aligning blocks. One of these is to a region of the S8 family of ribosomal proteins. However, alignment of the S8 sequences from several sources with the sequence of the human MRP-S23 polypeptide gives identities of less than 18%.

MRP-S10—The sequences of two tryptic peptides obtained from one spot allowed it to be identified as the bovine mitochondrial homolog of bacterial S10. Mouse, *C. elegans*, and *D. melanogaster* MRP-S10 are also readily identified in the data bases (Table II). Alignment of the human MRP-S10 with the corresponding proteins from several prokaryotes indicates that the alignment begins around residue 70 in the mammalian mitochondrial protein (Fig. 4). MRP-S10 is 24–36% identical to various bacterial S10 proteins examined. Interestingly, the regions most highly conserved between the prokaryotic S10 proteins do not correspond to the regions that are most conserved between the corresponding mitochondrial proteins. The

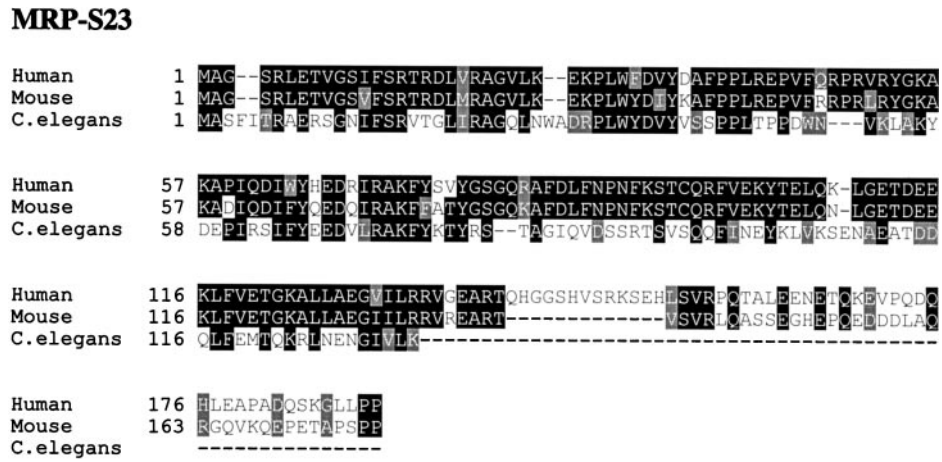

FIG. 3. **Sequence of human MRP-S23 and alignment with homologous proteins.** The sequence of human MRP-S23 was assembled using ESTs AW407459 and AW157159. The mouse protein was obtained from assembled ESTs. *C. elegans* MRP-S23 homolog is Swiss-Prot accession number P34748. Examination of the Swiss-Prot protein data base reveals a postulated human protein (Q9Y3D9) that matches the N-terminal 137 residues of human MRP-S23 (Table I). The sequence of this protein was predicted based on an open reading frame (CGI-138) observed in *C. elegans*. The sequence of this predicted protein appears to be correct for the first 137 residues and then to shift into an alternative frame due to an error in the cDNA sequence used.

FIG. 4. **Amino acid sequence of human MRP-S10 and alignment with homologous proteins from other species.** The human sequence was from NM_018141. The mouse protein was obtained from assembled EST sequences. The *C. elegans* sequence is from accession number CE20222; the *D. melanogaster* homolog is CG4247, and the *E. coli* homolog is P02364.

plant mitochondrial homologs known are 22–25% identical to the human sequence. No homolog for S10 can be detected in searches of the yeast genome when human MRP-S10 is used as a query in a BLAST search. However, when the *Escherichia coli* S10 sequence is used in a BLAST search, a mitochondrial homolog (CAA90780) is detected with 29% identity to the *E. coli* sequence. Alignment of this yeast mitochondrial S10 homolog and the human mitochondrial S10 using the GCG program GAP did not give a convincing alignment (21% identity scattered throughout the sequence with no more than three contiguous amino acids). This observation indicates that the sequences of human and yeast mitochondrial S10 homologs are more closely related to the prokaryotic S10 proteins than they are to each other.

MRP-S25—Two peptides were generated from this protein (Table I) and allowed the deduction of the sequence of the full-length protein from both human and mouse (Fig. 5). Homologs are also present in *C. elegans* and *D. melanogaster*. No homolog to this protein could be detected in the yeast genome using the human sequence as the query in an advanced Blast or Psi-Blast search. However, when the *D. melanogaster* sequence was used as a virtual screening probe, a yeast homolog with 31% sequence identity to the *D. melanogaster* sequence was found. This yeast homolog is 24% identical to the human MRP-S25 (Table II). No known ribosomal proteins from other sources show significant similarities to MRP-S25. Therefore, this protein represents a new class of ribosomal protein. No common protein sequence motifs can be detected.

MRP-S24—Three peptides were obtained from MRP-S24 (Table I), and the complete sequences of the corresponding proteins from both human and mouse were assembled from different ESTs (Fig. 6). A full-length genomic clone (AC004985–2) was obtained and indicated the presence of three introns and four exons.

The sequences of the MRP-S24 proteins from human, bovine, mouse, and rat are strongly conserved (Fig. 6 and Table II). The sequence of the mammalian MRP-S24 protein does not appear to be homologous to any of the known prokaryotic ribosomal proteins. Searches of the *D. melanogaster* genome using the human MRP-S24 protein as a cyberprobe indicates the presence of a homolog to the human protein. However, no homolog can be detected in the *C. elegans* genome. Thus, it appears that this ribosomal protein is not found in a recognizable form in all animals. Alternatively, it is possible that sequencing errors or a complex genomic organization might make it difficult to locate the worm homolog. Finally, probing the yeast genome with either the human or *D. melanogaster* MRP-S24 sequences fails to locate a homolog (Table II).

MRP-S14—One peptide was obtained allowing identification of the human and mouse mitochondrial homolog of bacterial S14 (Fig. 7 and Table I). The chromosomal gene for mitochondrial MRP-S14 has been located. It covers about 9 kilobase

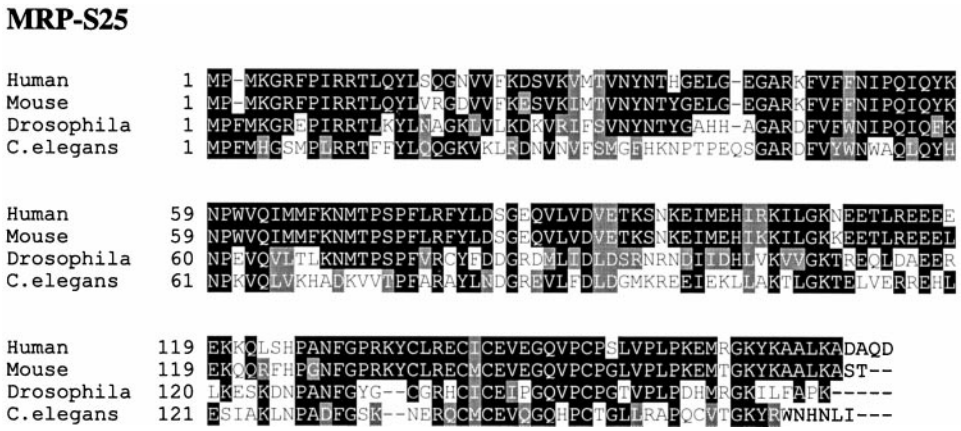

FIG. 5. **Sequence of human MRP-S25 and alignment with its homologs.** The human sequence was assembled using EST data base entries AW249185 and AI243774. The mouse protein was also obtained from assembled EST sequences. The *C. elegans* sequence is CE22547; the *D. melanogaster* homolog is CG14413, and the *S. cerevisiae* homolog is data base entry YKL167c.

FIG. 6. **Alignment of human MRP-S24 protein with its homologs.** The human sequence was assembled using EST data base entries AI870515, AI086409, W46469, and AW022634. The mouse protein was also obtained from assembled EST sequences. The *D. melanogaster* homolog is CG13608.

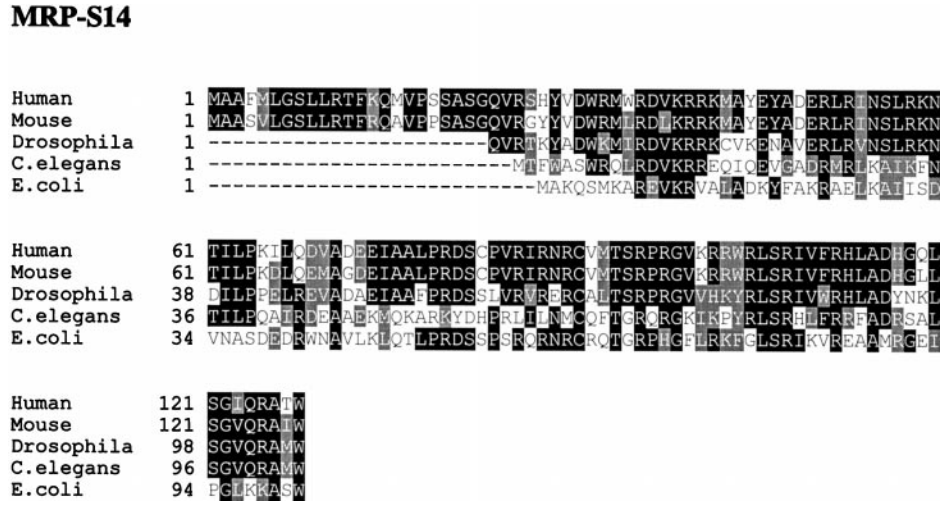

FIG. 7. **Amino acid sequence of human MRP-S14 and alignment with homologous proteins.** The human sequence was from CAB16601. The mouse protein was obtained from assembled EST sequences. The *C. elegans* sequence is from accession number P49391 (CE02309), the *D. melanogaster* homolog is CGI12211, and the *S. cerevisiae* homolog is data base entry P10663.

pairs of DNA and consists of three exons and two introns. Cyberprobing the *C. elegans* genome with the human MRP-S14 results in the identification of the worm homolog (Swiss-Prot P49391). This protein is 40.7% identical to the human protein when the full sequences are aligned (Table II). The *D. melanogaster* genome also has a putative MRP-S14 homolog (AE003512). However, due to a probable sequencing error, the *D. melanogaster* homolog could not be obtained from the given coding sequence. Instead, the partial putative S14 sequence is embedded in a long open reading frame of unknown function (CG12211). Alignment of the animal and yeast S14 homologs (Fig. 7) clearly shows that the animal proteins are more closely related to each other than they are to yeast mitochondrial S14 as expected. The animal proteins have clusters of conserved sequences in blocks throughout the length of the protein. These highly conserved regions are only partially conserved in yeast S14. The human mitochondrial MRP-S14 is also well conserved when compared the prokaryotic S14 proteins (Fig. 7, Table II). Once again the human S14 is more closely related to the prokaryotic proteins than to the yeast MRP-S14. The prokaryotic

S14 proteins are most highly conserved between each other, and with human mitochondrial S14, in the C-terminal half of the protein.

DISCUSSION

The identification and characterization of the proteins present in mitochondrial ribosomes has been difficult due to their low abundance. During the past several years, significant progress has been made in identifying some of the proteins present using a combination of protein sequencing and data base searching. In the present report, high sensitivity peptide sequencing by mass spectrometry has been used to identify seven different classes of mammalian mitochondrial ribosomal proteins from the small subunit, only one of which had been characterized previously (6). This work increases the number of proteins identified in the small subunit to 11.

The small subunit of the mammalian mitochondrial ribosome is thought to have about 33 different proteins. This number is considerably higher than the 21 proteins found in the 30 S subunit of prokaryotic ribosomes (24). Therefore, a number of the mammalian mitochondrial ribosomal proteins are not expected to be homologs of prokaryotic ribosomal proteins. Two of the six ribosomal proteins described in this paper (MRP-S10 and MRP-S14) and two of the previously characterized ribosomal proteins (MRP-S12 and MRP-S7) show significant sequence similarities to bacterial ribosomal proteins (9, 10).

S10 is located in the head of the prokaryotic 30 S subunit (25, 26). It is classified as a tertiary rRNA-binding protein since its assembly into the small subunit is strongly dependent on the presence of other proteins rather than arising from a tight direct interaction with the small subunit rRNA. Foot-printing and UV cross-linking experiments indicate that S10 is close to, or interacts with, helix 39 in the 3′-domain of 16 S rRNA (27, 28). A comparison of the secondary structures of the *E. coli* 16 S rRNA and bovine mitochondrial 12 S rRNA indicates that the region corresponding to helix 39 is not conserved in mitochondrial 12 S rRNA. This observation strongly suggests that S10 interacts primarily with other ribosomal proteins in the mitochondrial 28 S subunit. *E. coli* S10 can be cross-linked to residue A9 in tRNAs bound to the A-site of the ribosome (29). A similar proximity to the A-site is likely in the mammalian mitochondrial ribosome.

The present work has also identified a human mitochondrial protein in the S14 family of ribosomal proteins. Like S10, S14 is a tertiary rRNA-binding protein located in the head of the small subunit. S14 gives weak rRNA footprints located in the 3′ domain of the 16 S rRNA (27). A zinc-finger motif found in several members of the S14 family has been postulated to contribute to an interaction with rRNA (30, 31). However, no zinc finger motif is present in mammalian mitochondrial S14. Photoaffinity labeling of ribosomal proteins with puromycin derivatives labels S14 in *E. coli* ribosomes suggesting that this protein, although in the small subunit, is located in the proximity of the peptidyltransferase center in the 50 S subunit.

Three of the four currently identified mammalian mitochondrial small subunit ribosomal proteins that have prokaryotic homologs (S7, S10, and S14) are located in the head of the small subunit. Of these, S7 is a primary rRNA-binding protein (9). The assembly of both S10 and S14 into the small subunit is dependent on the presence of S7. The location of small subunit ribosomal proteins that are not homologs of known prokaryotic ribosomal proteins will require an extensive investigation of the structure of the mammalian mitochondrial ribosome.

Acknowledgment—We thank Mary Moyer (Glaxo-Wellcome) for outstanding technical contributions to this work.

REFERENCES

1. Pel, H., and Grivell, L. (1994) *Mol. Biol. Rep.* **19,** 183–194
2. O'Brien, T. W., and Matthews, D. E. (1976) in *Handbook of Genetics* (King, R. C., ed) pp. 535–580, Plenum, NY
3. De Vries, H., and van der Koogh-Schuuring, R. (1973) *Biochem. Biophys. Res. Commun.* **54,** 308–314
4. Graack, H.-R., and Wittmann-Liebold, B. (1998) *Biochem. J.* **329,** 433–448
5. Matthews, D. E., Hessler, R. A., Denslow, N. D., Edwards, J. S., and O'Brien, T. W. (1982) *J. Biol. Chem.* **257,** 8788–8794
6. Goldschmidt-Reisin, S., Kitakawa, M., Herfurth, E., Wittmann-Liebold, B., Grohmann, L., and Graack, H.-R. (1998) *J. Biol. Chem.* **273,** 34828–34836
7. Graack, H.-R., Bryant, M., and O'Brien, T. W. (1999) *Biochemistry* **38,** 16569–16577
8. O'Brien, T. W., Fiesler, S., Denslow, N. D., Thiede, B., Wittmann-Liebold, B., Mouge, E., Sylvester, J. E., and Graack, H.-R. (1999) *J. Biol. Chem.* **274,** 36043–36051
9. Koc, E. C., Blackburn, K., Burkhart, W., and Spremulli, L. L. (1999) *Biochem. Biophys. Res. Commun.* **266,** 141–146
10. Mariottini, P., Shah, Z. H., Toivonen, J., Bagni, C., Spelbrink, J., Amaldi, F., and Jacobs, H. (1999) *J. Biol. Chem.* **274,** 31853–31862
11. O'Brien, T. W., Liu, J., Sylvester, J., Mourgey, E. B., Fischel-Ghodsian, N., Thiede, B., Wittmann-Liebold, B., and Graack, H.-R. (2000) *J. Biol. Chem.* **275,** 18153–18159
12. Hamilton, M. G., and O'Brien, T. W. (1974) *Biochemistry* **13,** 5400–5403
13. Cahill, A., Baio, D., and Cunningham, C. (1995) *Anal. Biochem.* **232,** 47–55
14. Laemmli, U. K. (1970) *Nature* **227,** 680–685
15. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) *Anal. Chem.* **68,** 850–858
16. Perkins, D. N., Pappin, D. J., Creasy, D. M., and Cotrell, J. S. (1999) *Electrophoresis* **20,** 3551–3567
17. Pearson, W. R., and Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 2444–2448
18. Jiang, X., Smith, J. B., and Abraham, E. C. (1996) *J. Mass Spectrom.* **31,** 1309–1310
19. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402
20. Claros, M. G., and Vincens, P. (1996) *Eur. J. Biochem.* **241,** 770–786
21. Roepstorff, P., and Fohlman, J. (1984) *Biomed. Mass Spectrom.* **11,** 601
22. Yamaguchi, K., and Subramanian, A. R. (2000) *J. Biol. Chem.* **275,** 28466–28482
23. Yamaguchi, K., von Knoblauch, K., and Subramanian, A. R. (2000) *J. Biol. Chem.* **275,** 28455–28465
24. Wittmann, H. (1986) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., and Kramer, G., eds) pp. 1–27, Springer-Verlag, New York
25. Noller, H., Moazed, D., Stern, S., Powers, T., Allen, P., Robertson, J., Weiser, B., and Triman, K. (1990) in *The Ribosome: Structure, Function and Evolution* (Hill, W., Dahlberg, A., Garrett, R., Moore, P., Schlessinger, D., and Warner, J., eds) pp. 73–92, ASM Press, Washington, D. C.
26. Wittmann-Liebold, B. (1985) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., and Kramer, G., eds) pp. 326–361, Springer-Verlag, New York
27. Mueller, F., and Brimacombe, M. (1997) *J. Mol. Biol.* **271,** 545–565
28. Samaha, R. R., O'Brien, B., O'Brien, T. W., and Noller, H. F. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7884–7888
29. Abdurashidova, G. G., Tsvetkova, E. A., and Budowsky, E. I. (1991) *Nucleic Acids Res.* **19,** 1909–1915
30. Chan, Y. L., Suzuki, K., Olvera, J., and Wool, I. G. (1993) *Nucleic Acids Res.* **21,** 649–655
31. Tsiboli, P., Triantafillidou, D., Franceschi, F., and Choli-Papadopoulou, T. (1998) *Eur. J. Biochem.* **256,** 136–141