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Cloning and Sequence Analysis of the Human Mitochondrial Translational Initiation Factor 2 cDNA*

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Complete cDNAs encoding human mitochondrial translational initiation factor 2 (IF-2_{mt}) have been obtained from liver, heart, and fetal brain cDNA libraries. These cDNAs have a long open reading frame 2181 residues in length encoding a protein of 727 amino acids. Overall, human IF-2_{mt} has 30–40% identity to the corresponding prokaryotic factors. Surprisingly, it is no more homologous to yeast IF-2_{mt} than to the IF-2s from bacterial sources. The greatest region of conservation lies in the G-domain of this factor with less conservation in the COOH-terminal half of the protein and very little homology near the amino terminus. The 5′-untranslated leaders of the liver and heart cDNAs contain a number of short open reading frames. These sequences may play a role in the translational activity of the IF-2_{mt} mRNA. Northern analysis indicates that the IF-2_{mt} gene is expressed in all tissues but that the level of expression varies over a wide range.

The initiation of protein biosynthesis has been extensively studied in prokaryotes and in the cytoplasm of eukaryotic cells (1–5). During the initiation process, initiation factor 2 promotes the binding of the initiator tRNA to the small subunit of the ribosome in a GTP-dependent manner. Prokaryotic initiation factor 2 (IF-2)[1] is a single polypeptide chain having a molecular weight of 78,000–97,000. It promotes fMet-tRNA binding to 30 S ribosomal subunits in a mRNA-dependent reaction. Eukaryotic cytoplasmic initiation factor 2 (eIF-2) is a trimeric protein with a molecular weight of 145,000 (3). It is responsible for the binding of Met-tRNA to 40 S subunits. This reaction is believed to occur prior to the interaction of the subunit with mRNA (3).

The mechanism of translational initiation in animal mitochondria is not yet understood, but several studies suggest that it will have a number of unique features (6–9). To date, only one initiation factor has been identified in the animal mitochondrial system (10, 11). This factor, which was purified from bovine liver, is equivalent to prokaryotic IF-2 and has been designated IF-2_{mt} (11). It is a monomeric protein with a molecular weight of 85,000. IF-2_{mt} promotes the binding of fMet-tRNA to animal mitochondrial ribosomes in a GTP- and mRNA-dependent reaction.

Genes encoding IF-2 have been cloned from several prokaryotic microorganisms (12–15). The cDNA sequences encoding the α, β, and γ subunits of eIF-2 have also been recently reported (16–18). Although IF-2_{mt} in yeast has not been purified or characterized, its gene has been cloned (19). The sequence of this factor suggests that it has significant homology to bacterial IF-2 except near the amino terminus. In the present study, we have cloned and characterized the cDNAs for IF-2_{mt} from human liver, heart, and fetal brain.

EXPERIMENTAL PROCEDURES

Polymerase Chain Reaction—Highly conserved peptide sequences found in the G-domains of prokaryotic and mitochondrial IF-2s served as the basis for designing primers for cDNA synthesis and the polymerase chain reaction (PCR). The oligonucleotide primers used were as follows: P1R, C(T/C)TGIAC(G/A)TCNCCNCC based on the sequence GGDVQ(A/V); P2F, GCGGATCCIGTIGTNACNAT(C/A)ATGGG derived from the sequence PVVTIMG; P1F, GGAATTCCICAGGC(I/C)GA(T/C)CCIGCN(T/C)T based on the sequence PQADPAL; and P2R, GGAATTCCGTAGTCTTCGCA(I/C)AC(I/C)AC(G/A)TC(G/A)TA from the sequence YDVVCEDY. Residues added to the primers to facilitate restriction enzyme digestion during cloning are underlined. Residues indicated in parentheses designate degenerate positions.

For the production of cDNA clones, 2 μg of poly(A)$^+$ RNA from human liver was reverse transcribed with Moloney murine leukemia virus reverse transcriptase basically as described (20). 1 μl of the reaction mixture was used for the initial PCR, which was carried out using primers P1F and P1R. A portion (2 μl) of the first PCR reaction mixture was then amplified using the nested primers P2F and P2R. PCR reactions were analyzed on 4% NuSieve-agarose gels, and the products were purified from the gel using silicon powder (Sigma) (21). DNA fragments were digested with *Bam*HI and *Eco*RI and cloned into pTZ19R (Pharmacia Biotech Inc.) (22) The plasmid containing the cloned portion of the IF-2_{mt} cDNA is designated pTZIF2G, and the insert contained in this plasmid is designated IF2G when referred to below.

DNA Sequencing—All DNA sequencing was carried out using double-stranded DNA plasmids as templates (23). Both strands of cDNA clones were sequenced using the sequenase system (U. S. Biochemical Corp.) or with *Taq* DNA polymerase in the Automatic DNA Sequencing Facility at the University of North Carolina. Amino acid and nucleotide sequences were analyzed using the GCG software package from the University of Wisconsin Genetics Computer Group running on a VAX computer.

Screening cDNA Libraries—The insert in pTZIF2G was excised, amplified using PCR, and radioactively labeled using random hexamer primers and the Klenow fragment of DNA polymerase I (24). Screening cDNA libraries and the purification and sequencing of positive clones were performed according to previously described methods (22). Three human cDNA libraries from Stratagene were used: a $(N)_6$/oligo(dT)-primed fetal brain library, a heart $(N)_6$/oligo(dT)-primed ZAPII cDNA library, and an oligo(dT)-primed liver λUni-ZAP XR cDNA library. Approximately 1×10^6 plaques from each library were screened with ^{32}P-labeled IF2G DNA as a probe (22). Inserts from positive plaques were excised *in vivo* from the λZAP vectors according to the manufacturer's suggested protocol. Cells carrying pBluescript SK$^-$ plasmids containing IF-2_{mt} cDNAs were identified by colony hybridization with the IF2G probe.

Northern and Southern Analysis—A nylon membrane containing poly(A)$^+$ RNA from various tissues (Clontech) was used for the North-

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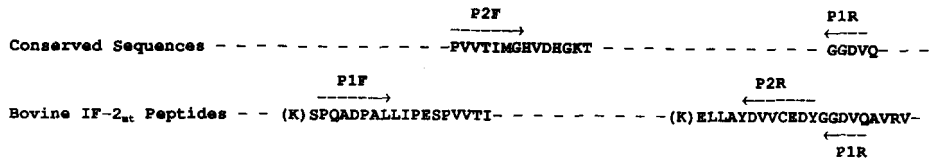
The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L34600.

[1] The abbreviations used are: IF-2, initiation factor 2; IF-2_{mt}, mitochondrial translational initiation factor 2; PCR, polymerase chain reaction; kb, kilobase(s); bp, base pair(s); UTL, untranslated leader; eIF-2, eukaryotic cytoplasmic initiation factor 2; uORF, upstream open reading frame.



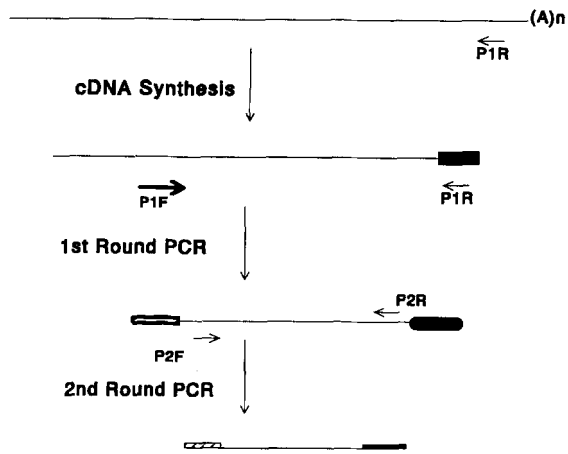


FIG. 1. **Conserved sequences in IF-2 and cloning strategy.** The primer P2F was designed based on a sequence conserved in prokaryotic IF-2s. Primer P1R was based on a sequence found in yeast IF-2_{mt} and in a peptide obtained from bovine IF-2_{mt}. Primers P1F and P2R were based on sequence information obtained with bovine IF-2_{mt}. Primer P1R was used to prepare a specific cDNA using human liver poly(A)$^+$ RNA and reverse transcriptase. Two rounds of PCR were then carried out using the indicated primers.

ern analysis. The blot was hybridized using standard methods (22). A nylon membrane containing *Eco*RI-digested genomic DNA from various sources (Clontech) was used for the Southern analysis (22).

RESULTS AND DISCUSSION

Molecular Cloning of Human IF-2_{mt} cDNA—The overall strategy used to obtain a cDNA clone for human liver IF-2_{mt} was based on two pieces of information: preliminary partial peptide sequence data that had been obtained on bovine IF-2_{mt}[2] and the conservation of certain sequences in IF-2, particularly in the G-domain. A cDNA specific for IF-2_{mt} was prepared using human liver mRNA and the reverse primer P1R (Fig. 1). The sequence of the degenerate oligonucleotide primer P1R was based on the peptide GGDVQ(A/V), which is located at the end of the G-domain. This peptide sequence was obtained from partial sequence analysis of the bovine liver IF-2_{mt}.[2] It is also found in yeast IF-2_{mt}, and, thus, there was a strong possibility that it would be present in human IF-2_{mt}. Following first strand cDNA synthesis, PCR was used to specifically amplify potential IF-2_{mt} sequences present. A first round of PCR was carried out using degenerate oligonucleotide primers (P1F and P1R) both derived from peptide sequences obtained from the bovine liver IF-2_{mt} (Fig. 1). A product of the expected size (460 bp) was present among the products after the first round of PCR amplification (data not shown).

To ensure that this band was indeed derived from the IF-2_{mt} cDNA, a second round of PCR was carried out. This step was based on the observed sequence conservation of the IF-2s from various organisms (Fig. 1). The peptide sequences of the factors from *Escherichia coli* (12), *Bacillus stearothermophilus* (14), *Streptococcus faecium* (15), and *Bacillus subtilis* (13), along with *Saccharomyces cerevisiae* IF-2_{mt} (19), were analyzed using the PILEUP program in the GCG software package. The sense primer P2F was designed from one of these sequences, PVVTIMG, which marks the amino-terminal boundary of the G-domain of prokaryotic IF-2 (Fig. 1). A second round of PCR was carried out using a nested set of primers lying inside the first pair of primers (the P2F primer derived from sequences conserved in IF-2s and P2R derived from peptide sequence information obtained from bovine IF-2_{mt}). Gel analysis indicated that the major product corresponded well to the size predicted to span the region between the selected primers. The product was cloned and sequenced. The amino acid sequence deduced from the cDNA clone shares 59% identity to the yeast IF-2_{mt} and 69% identity to *E. coli* IF-2. It exhibits over 90% identity to the bovine IF-2_{mt} peptide sequences shown in Fig. 1. It has 27% sequence identity to eIF-2γ but no significant homology to either the α or β subunits of eIF-2. The cDNA obtained, therefore, most likely encodes a portion of the G-domain of human liver IF-2_{mt}.

The liver IF-2_{mt} cDNA was used to screen a human liver oligo(dT)-primed λUni-ZAP cDNA library. 16 positive clones with inserts ranging in size from 500 to 2500 bp were purified, and the plasmids containing the inserts were excised *in vivo*. The largest clone characterized carries a 2541-bp-long insert and was sequenced in both directions. This clone is derived from a mRNA containing a 270-base pair 5′-untranslated leader (UTL), a 2181-base pair open reading frame encoding IF-2_{mt}, a 26-base pair 3′-untranslated leader, and a poly(A) tail of over 60 residues (Fig. 2). Recently, cloning of human infant brain-expressed sequence tags has yielded two clones containing sequences encompassing about 165 amino acids that are homologous to bacterial IF-2s (25). These expressed sequence tag clones are 98–99% identical to regions in the G-domain and near the COOH terminus of the human IF-2_{mt} cDNA identified here. Therefore, we believe that these expressed sequenced tags are derived from human IF-2_{mt} cDNAs.

Human heart and human fetal brain cDNA libraries were also screened, and IF-2_{mt} cDNA clones were isolated from them. The complete nucleotide sequences of two clones with 2.5-kb inserts were obtained from the heart and fetal brain libraries. The IF-2_{mt} cDNAs from heart and fetal brain share essentially the same coding sequence and 3′-untranslated regions. The heart and liver cDNA share the same 5′-untranslated leader except that the heart IF-2_{mt} cDNA has a slightly different sequence at the very 5′-end. The 5′-UTL of the fetal

[2] M. Farwell, J. Ma, W. Burkhart, and L. Spremulli, unpublished observations.

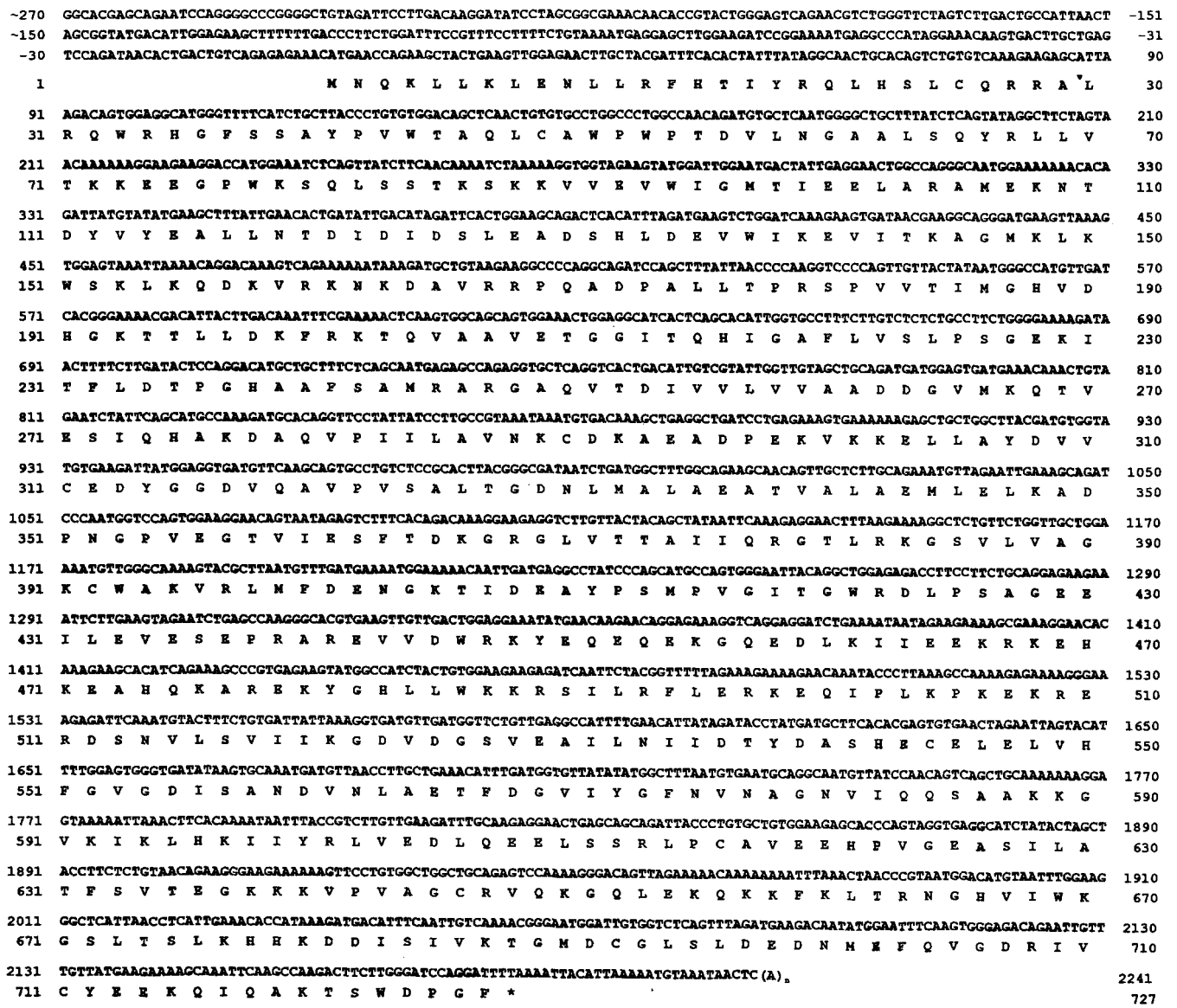


FIG. 2. **Nucleotide and deduced amino acid sequences of human liver IF-2_{mt}.** Sequences in the 5′-untranslated leader are designated as *negative numbers*. The nucleotide numbered +1 corresponds to the first base of the initiation codon ATG at the beginning of the longest open reading frame. The putative polyadenylation signals are *underlined*, and $(A)_n$ denotes the poly(A) tail. The proposed processing site for the mitochondrial processing endopeptidase is indicated by a *solid inverted triangle*.

brain IF-2_{mt} cDNA, however, is very different from those found in the heart and liver clones. It is possible that this difference may have arisen from the transcription of separate genes or may be the result of the cloning process.

Analysis of the IF-2_{mt} cDNA Sequence—The 5′-UTL of the human liver IF-2_{mt} cDNA is 270 bp in length and contains 3 ATG triplets, all of which are out of frame with respect to the initiation codon of IF-2_{mt} (Fig. 2). Each of the three ATGs in the 5′-untranslated leader marks the beginning of a short upstream open reading frame (uORF). Between uORF1 and uORF2, there is a T-rich segment consisting of 50% T's. The reading frames of uORF2 and uORF3 overlap with uORF3 contained within uORF2. None of the three uORFs overlaps the sequence coding for IF-2_{mt}, and uORF2 terminates 31 bases upstream from the initiation codon. According to the widely accepted scanning model for the initiation of translation in the eukaryotic cytoplasm, the 40 S ribosomal subunit and initiation factors bind to the 5′-end of the mRNA and migrate along the 5′-UTL until the first AUG codon in a good context is reached. This AUG codon is usually the first AUG from the 5′-end of the mRNA. Initiation generally occurs at this position (26). Experimental evidence indicates that uORFs strongly inhibit the translation of the downstream protein-coding sequence, presumably due to a low efficiency of ribosomal reinitiation at the downstream open reading frame (27–29). The translation of the IF-2_{mt} open reading frame may be down-regulated by the presence of these uORFs.

The long open reading frame is 2181 residues long and encodes a 727-amino acid protein. The translational initiation codon for IF-2_{mt} has been tentatively identified as the first in-frame AUG of this long open reading frame. The 3′-untranslated region of the cDNA is unusually short, and this 26-bp-long sequence is 85% A/T. As shown in Fig. 2, two sequences, ATTAAA and AATAAC, upstream from the poly(A) tail resemble the consensus sequence AATAAA for polyadenylation (30). Therefore, one or both of them is probably used as a polyadenylation signal for the human IF-2_{mt} gene. It has been reported that the eIF-2β gene may also use ATTAAA as the signal for polyadenylation (17).

Most mitochondrial proteins are synthesized as precursors

Bstea
Bsubt
Sfaec
Ecoli
Mthum
Mtyst

TABLE I
Homologies between IF-2 polypeptide sequences

Species compared	Identity to human IF-2_{mt}[a]			
	Amino terminus[b]	Center[c]	COOH terminus[d]	Overall[e]
	%			
B. stearothermophilus	8	66	37	37
B. subtilis	11	70	35	38
S. faecium	10	63	35	35
E. coli	12	69	34	36
Mitochondria, *S. cerevisiae*	18	59	32	32

[a] Calculated as the sum of identical residues shared at the aligned positions between the two sequences divided by the total number of overlapping residues (Fig. 3).

[b,c,d] The amino terminus is defined as the amino acid sequence of human IF-2_{mt} between Met-1 and Ser-180. The Center is defined as the residues from Pro-181 to Asn-330 and encompasses the G-domain. The COOH-terminus is the sequence from Leu-331 to Phe-727.

[e] Calculated as the length-weighted average of the amino terminus, Center, and COOH terminus values.

FIG. 3. **Comparison of primary sequences of IF-2 from *B. stearothermophilus* (*Bstea*), *B. subtilis* (*Bsubt*), *S. faecium* (*Sfaec*), *E. coli* (*Ecoli*), human liver mitochondria (*Mthum*), and *S. cerevisiae* mitochondria (*Mtyst*).** The sequence alignment was made using PILEUP from the GCG computer software package, and the conserved residues *shaded* in *black* were made using BOXSHADE (netserv@EMBL-Heidelberg.de).

on cytosolic polysomes and are subsequently imported into mitochondria. The vast majority of these precursors carry amino-terminal presequences, which contain the information required for their targeting to mitochondria. However, no significant sequence homology has been found in the mitochondrial targeting sequences. In general, presequences of mitochondrial proteins are rich in positively charged residues, lack acidic residues, and most have a high content of hydroxylated residues (31). In addition, many of these sequences appear to be able to form amphiphilic α helices. The first 30 amino acids of human liver IF-2_{mt} from the amino terminus contain a number of basic residues, including 2 lysines, 4 arginines, and 2 hydroxylated amino acids. The mitochondrial targeting sequence at the amino terminus of human IF-2_{mt} is predicted to form an amphiphilic α helix between residues 1 and 15. A putative cleavage site is predicted between Ala-29 and Leu-30 based on the analysis of cleavage patterns in other mitochondrial import sequences.

The predicted mature form of IF-2_{mt} consists of 698 amino acid residues and has a molecular weight of 77,500. Its isoelectric point is calculated to be 6.6. Previous work has indicated that bovine IF-2_{mt} has an apparent molecular weight of 85,000 on SDS-polyacrylamide gel electrophoresis. Thus, human and bovine IF-2_{mt} appear to be similar in size. Human liver IF-2_{mt} is similar to prokaryotic IF-2 (except *E. coli* IF-2α) in size. It is about 50 residues longer than yeast IF-2_{mt}, assuming that the transit sequences are about the same length (19).

As indicated in Fig. 3 and Table I, the peptide sequence of the mature form of human IF-2_{mt} displays extensive homology to the COOH-terminal two thirds of prokaryotic IF-2s. However, it shows no significant similarity to the amino-terminal peptide sequences of these factors. The amino-terminal peptide sequence (Leu-30 to Ser-180) of the mature human IF-2_{mt} has 29% charged residues. The corresponding regions in the IF-2 of *E. coli* or yeast mitochondria contain approximately 26% charged residues. Using Chou-Fasman and Garnier-Osguthorpe-Robson methods, we predict that both the human mitochondrial factor and *E. coli* IF-2 have similar secondary structures at this region (32, 33).

There is striking primary sequence homology in the G-domain of IF-2, the central domain corresponding to residues 392–540 of *E. coli* IF-2α and residues 181–330 of IF-2_{mt}. The central domain of human IF-2_{mt} exhibits an average 67% identity to prokaryotic IF-2s (Table I). However, it has only 59% identity, the lowest among those compared, to its yeast mitochondrial counterpart. The alternative β sheet/α helix secondary structure predicted to be present in the G-domain of IF-2 is retained in the central domain of human IF-2_{mt} (33). The

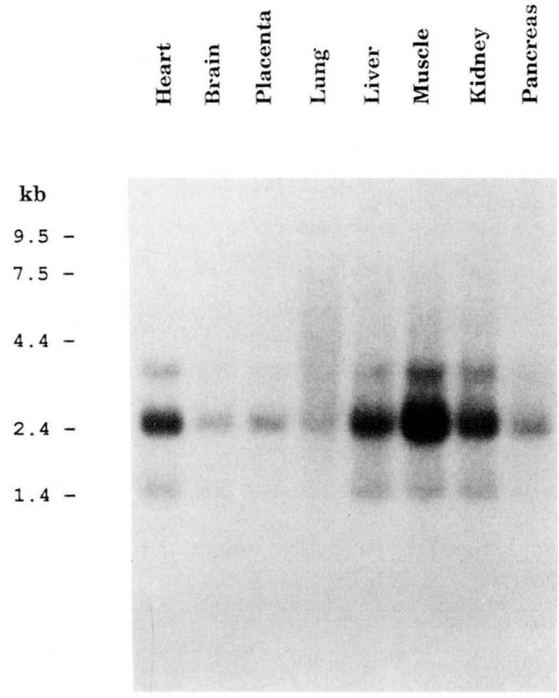


FIG. 4. **Northern analysis of the expression of IF-2_{mt} mRNA in different human tissues.**

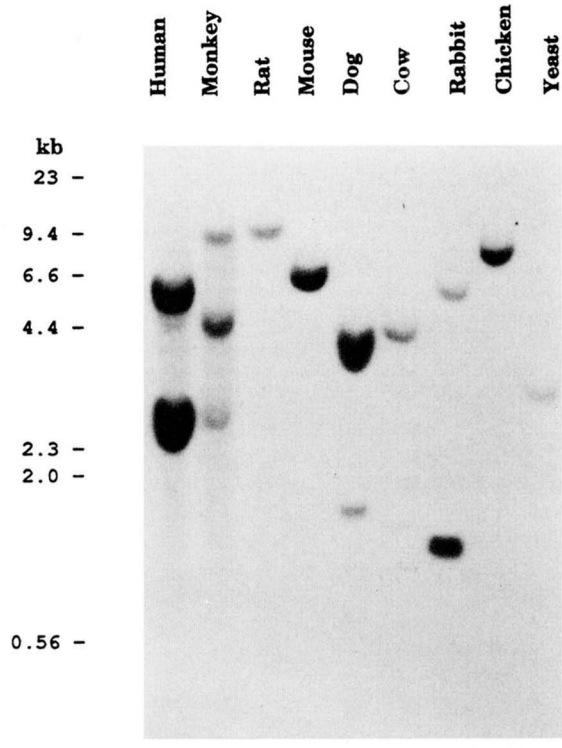


FIG. 5. **Southern analysis of the presence of sequences related to IF-2_{mt} in different species.**

similarity between human IF-2_{mt} and other IF-2s decreases remarkably at the COOH terminus, the region thought to be the tRNA binding domain. As shown in Table I, human IF-2_{mt} has an average of 35% identical residues with other IF-2s within this region. In contrast to other IF-2s, the region from Asp-429 to Glu-512 in the COOH terminus of human IF-2_{mt} has the highest surface probability in the whole molecule. Over 50% of the amino acid residues within this region are charged. It also has an extra 37 amino acid residues not found in any prokaryotic IF-2. These additional amino acids (Fig. 3) are clustered primarily in three closely spaced sections near the beginning of the COOH-terminal half of the protein. Two of these regions are also observed in yeast IF-2_{mt}. In general, human IF-2_{mt} shows an overall 36% sequence identity to IF-2 from prokaryotes and yeast mitochondria. The observations summarized above suggest that the G-domain of IF-2 has been highly conserved during evolution while other regions of the molecule have been allowed to diverge to a greater extent.

Abundance of IF-2_{mt} mRNA in Different Human Tissues—The abundance of the IF-2_{mt} transcript in various human tissues was determined by Northern blotting using the IF2G DNA (the G-domain) as a probe. As shown in Fig. 4, transcripts of IF-2_{mt} migrating at approximately 2.5 kb were detected in all of the tissues examined. The estimated size of the IF-2_{mt} mRNA is very close to the length of the cDNA cloned (2541 bp). Heart, liver, skeletal muscle, and kidney all had relatively abundant levels of the IF-2_{mt} transcript, with skeletal muscle having the highest abundance among the tissues tested. Lower amounts of the IF-2_{mt} mRNA were detected in brain, placenta, lung, and pancreas. The IF-2_{mt} message levels in these tissues are estimated to be 20–50-fold lower than in skeletal muscle. As expected, the IF-2_{mt} gene is expressed to some extent in all of the tissues tested with higher levels of expression observed in specialized tissues known to have a high demand for energy.

In addition to the major IF-2_{mt} mRNA observed at 2.5 kb, two other bands of approximately 3.5 and 1.5 kb, respectively, were observed on the Northern blot (Fig. 4). These bands are present at about 5% of the intensity of the prominent 2.5-kb transcript and may represent signals from a related gene. Alternatively, they could arise from a precursor and a degradation product, respectively, of the IF-2_{mt} mRNA.

Conservation of the IF-2_{mt} Sequence in Different Species—Since the G-domain of IF-2 appears to be highly conserved, we have used the human IF2G DNA as a probe for sequences homologous to IF-2_{mt} in the genomic DNAs of a wide range of species (Fig. 5). Between one and three restriction fragments of genomic DNA from eukaryotes as widely divergent as yeast and human hybridize with this probe at high stringency. This observation confirms the general idea that IF-2_{mt} is highly conserved throughout the eukaryotic kingdom, at least in the region encoding the G-domain.

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REFERENCES

1. Gold, L. (1988) *Annu. Rev. Biochem.* **57,** 199–233
2. Gualerzi, C. O., and Pon, C. L. (1990) *Biochemistry* **29,** 5881–5889
3. Pain, V. M. (1986) *Biochem. J.* **235,** 625–637
4. Hershey, J. W. B. (1987) in *Escherichia coli and Samonella typhimurium* (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 613–647, American Society for Microbiology, Washington, D. C.
5. Hershey, J. W. B. (1991) *Annu. Rev. Biochem.* **60,** 717–755
6. O'Brien, T. W., and Matthews, D. E. (1976) in *Handbook of Genetics* (King, R. C., ed) Vol. 5, pp. 535–580, Plenum Publishing Corp., New York
7. Matthews, D. E., Hessler, R. A., Denslow, N. D., Edwards, J. S., and O'Brien, T. W. (1982) *J. Biol. Chem.* **257,** 8788–8794
8. Van Holde, K., and Hill, W. (1974) in *Ribosomes* (Nomura, M., Tissières, A., and Lengyel, P., eds) pp. 53–91, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
9. Cammarano, P., Romeo, A., Gentile, M., Felsani, A., and Gualerzi, C. (1972) *Biochim. Biophys. Acta* **281,** 597–624
10. Liao, H.-X., and Spremulli, L. L. (1990) *J. Biol. Chem.* **265,** 13618–13622
11. Liao, H.-X., and Spremulli, L. L. (1991) *J. Biol. Chem.* **266,** 20714–20719
12. Sacerdot, C., Dessen, P., Hershey, J. W. B., Plumbridge, J. A., and Grunberg-Manago, M. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 7787–7791
13. Shazand, K., Tucker, J., Chiang, R., Stansmore, K., Sperling-Petersen, H. U., Grunberg-Manago, M., Rabinowitz, J. C., and Leighton, T. (1990) *J. Bacteriol.* **172,** 2675–2687
14. Brombach, M., Gualerzi, C. O., Nakamura, Y., and Pon, C. L. (1986) *Mol. & Gen. Genet.* **205,** 97–102
15. Friedrich, K., Brombach, M., and Pon, C. L. (1988) *Mol. & Gen. Genet.* **214,** 595–600

16. Ernst, H., Duncan, R. F., and Hershey, J. W. (1987) *J. Biol. Chem.* **262,** 1206–1212
17. Pathak, V. K., Nielsen, P. J., Trachsel, H., and Hershey, J. W. (1988) *Cell* **54,** 633–639
18. Gaspar, N. J., Kinzy, T. G., Scherer, B. J., Humbelin, M., Hershey, J. W., and Merrick, W. C. (1994) *J. Biol. Chem.* **269,** 3415–3422
19. Vambutas, A., Ackerman, S. H., and Tzagoloff, A. (1991) *Eur. J. Biochem.* **201,** 643–652
20. Roth, M. J., Tanese, N., and Goff, S. P. (1985) *J. Biol. Chem.* **260,** 9326–9335
21. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 615–619
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
23. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5463–5467
24. Feinberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13
25. Adams, M., Soares, M., Kerlavage, A., Fields, C., and Venter, J. (1993) *Nature Genet.* **4,** 373–380
26. Kozak, M. (1991) *J. Biol. Chem.* **266,** 19867–19870
27. Hinnebusch, A. G. (1990) *Trends Biochem. Sci.* **15,** 148–152
28. Marth, J. D., Overell, R. W., Meier, K. E., Krebs, E., and Perlmutter, R. M. (1988) *Nature (Lond.)* **332,** 171–173
29. Arrick, B., Lee, A., Grendell, R., and Derynck, R. (1991) *Mol. Cell. Biol.* **11,** 4306–4313
30. Wickens, M., and Stephenson, P. (1984) *Science* **226,** 1045–1051
31. Hartl, F., Pfanner, N., Nicholson, D., and Neupert, W. (1989) *Biochim. Biophys. Acta* **988,** 1–45
32. Chou, P. Y., and Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276
33. Cenatiempo, Y., Deville, F., Dondon, J., Grunberg-Manago, M., Sacerdot, C., Hershey, J. W., Hansen, H. F., Petersen, H. U., Clark, B. F., Kjeldgaard, M., la Cour, T. F., Mortensen, K. K., and Nyborg, J. (1987) *Biochemistry* **26,** 5070–5076