MOLECULAR AND CELLULAR BIOLOGY, Sept. 1997, p. 5255–5268 Vol. 17, No. 9
0270-7306/97/$04.00+0

Control of the Translational Efficiency of β-F1-ATPase mRNA Depends on the Regulation of a Protein That Binds the 3′ Untranslated Region of the mRNA

JOSÉ M. IZQUIERDO AND JOSÉ M. CUEZVA*

Departamento de Biología Molecular, Centro de Biología Molecular "Severo Ochoa," Universidad Autónoma de Madrid—Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, 28049 Madrid, Spain

Received 10 March 1997/Returned for modification 21 April 1997/Accepted 23 June 1997

The expression of the nucleus-encoded β-F1-ATPase gene of oxidative phosphorylation is developmentally regulated in the liver at both the transcriptional and posttranscriptional levels. In this study we have analyzed the potential mechanisms that control the cytoplasmic expression of β-F1-ATPase mRNA during liver development. Remarkably, a full-length 3′ untranslated region (UTR) of the transcript is required for its efficient in vitro translation. When the 3′ UTR of β-F1-ATPase mRNA is placed downstream of a reporter construct, it functions as a translational enhancer. In vitro translation experiments with full-length β-F1-ATPase mRNA and with a chimeric reporter construct containing the 3′ UTR of β-F1-ATPase mRNA suggested the existence of an inhibitor of β-F1-ATPase mRNA translation in the fetal liver. Electrophoretic mobility shift assays and UV cross-linking experiments allowed the identification of an acutely regulated protein (3′βFBP) of the liver that binds at the 3′ UTR of β-F1-ATPase mRNA. The developmental profile of 3′βFBP parallels the reported changes in the translational efficiency of β-F1-ATPase mRNA during development. Fractionation of fetal liver extracts revealed that the inhibitory activity of β-F1-ATPase mRNA translation cofractionates with 3′-UTR band-shifting activity. Compared to other tissues of the adult rat, kidney and spleen extracts showed very high expression levels of 3′βFBP. Translation of β-F1-ATPase mRNA in the presence of kidney and spleen extracts further supported a translational inhibitory role for 3′βFBP. Mapping experiments and a deletion mutant of the 3′ UTR revealed that the *cis*-acting element for binding 3′βFBP is located within a highly conserved region of the 3′ UTR of mammalian β-F1-ATPase mRNAs. Overall, we have identified a mechanism of translational control that regulates the rapid postnatal differentiation of liver mitochondria.

The biogenesis of mitochondria is a complex cellular response that involves the concerted expression of the two genomes in which molecular components of the organelles are encoded (2). The rapid switch in the metabolic pathways relevant for energy provision that occurs in the mammalian liver during development provides a suitable in vivo experimental system for the analysis of the mechanisms that control the biogenesis of mitochondria. The development of the functional capabilities of mitochondria (37, 51, 68), as well as the expression patterns of nucleus- and mitochondrion-encoded genes of oxidative phosphorylation during liver development (11, 30, 39, 47, 48), indicates the existence of two main biological programs for controlling the biogenesis of liver organelles. These programs, defined as those responsible for organelle proliferation and differentiation, respectively, are differentially expressed throughout liver development and controlled at different levels of gene expression. Whereas proliferation of liver mitochondria, that is, the continuous accumulation of the organelles in hepatocytes (1, 13, 58), seems to be basically controlled at the level of transcription of nucleus-encoded genes (30) and by a concerted increase in the replication of mitochondrial DNA (11, 48), differentiation of the organelles, i.e., the acquisition of the ultrastructural, molecular, and functional capabilities of adult-type mitochondria (13, 68), is controlled at two posttranscriptional levels of gene expression that involve profound changes in the stability (30, 47) and translational efficiency (30, 39, 48) of the transcripts encoded in both genomes.

Nucleus- and mitochondrion-encoded transcripts of oxidative phosphorylation have been shown to accumulate in the fetal liver (30), despite the lower transcriptional activity of the nuclear gene (30) and the lower cellular representation of the mitochondrial genome (11, 48) at this stage of development. For transcripts encoded in both genomes, the accumulation of the mRNAs results from profound developmental changes in mRNA stabilities (30, 47).

Activation of the stored transcripts for oxidative phosphorylation proteins occurs at the time of birth (30, 39, 48) simultaneously with a generalized burst in the rate of global protein synthesis (39). However, the relative synthesis of mitochondrial proteins is preferential over the bulk of the cytosolic proteins, at least during the first postnatal hours (39, 48, 68). Therefore, the mammalian liver provides an additional example in which certain mRNAs are stored "masked" (31, 42, 70) until required to fulfill a predetermined differentiation program of the organism: the differentiation of mitochondria (30, 68).

Recently, high-resolution in situ hybridization experiments have revealed that nucleus-encoded β-F1-ATPase mRNA is localized in the hepatocytes of fetal rats in electron-dense structures called β-mRNA clusters (20). This finding contrasted with the cytoplasmic presentation of another transcript of oxidative phosphorylation (α-F1-ATPase mRNA) (20) and thus initially suggested possible involvement of the cluster structure in the masking of β-F1-ATPase mRNA during fetal development (30, 39). However, analysis of the presentation of β-F1-ATPase mRNA in the hepatocytes throughout develop-

* Corresponding author. Mailing address: Centro de Biología Molecular, Universidad Autónoma de Madrid, 28049 Madrid, Spain. Phone: 34-1-397 4866. Fax: 34-1-397 4799. E-mail: jmcuezva@trasto.cbm.uam.es.

TABLE 1. Oligonucleotides used in this study[a]

Oligonucleotide	Orientation	Sequence	Expt(s)
A	Antisense 41-mer	5′-CAC AAC GGC GCC GAT GAC TGC CAC AAT TTG CCC GGT GGC GG-3′	Primer extension, RNase H protection
B	Antisense 41-mer	5′-ACG CCC CAC AAG ACT CAA CAT GGC GGA GTC CGG GTG GAG AC-3′	Primer extension, RNase H protection
C	Antisense 30-mer	5′-CAT GTA GAA GGC TTG TTC CGG GAG ATG GTC-3′	RNase H protection
D	Antisense 42-mer	5′-CGA CCC ATG CTC CTC TGC CAG CTT GTC AGC CTT TGC CAC AGC-3′	RNase H protection
E	Antisense 30-mer	5′-CCC ACC CTT GGC GTA TGG GGC CAG CAG ATC-3′	RT-PCR
F	Sense 30-mer	5′-GCG CGG GTA CCC GAA TCC AGT CTC CAC CCG-3′	PCR
G	Antisense 30-mer	5′-GCG AGC TCC GCG AAG CTT TTT TTT TTT TTT-3′	RT-PCR
H	Sense 30-mer	5′-GGC AGT GAG CAT TAT GAT GTT GCT CGT GGG-3′	PCR
I	Sense 30-mer	5′-GCG CGG GTA CCA TGT TGA GTC TTG TGG GGC-3′	PCR
J	Sense 30-mer	5′-GCG CGG GTA CCA TGG GGA ATA TCT TTG CAA-3′	PCR
K	Antisense 30-mer	5′-GCG CGG GTA CCT CAT TTC TGG TTC GGG AGC-3′	PCR

[a] The sequences of oligonucleotides A through I were derived from the rat liver β-F1-ATPase cDNAs previously reported by Garboczi et al. (23) and Boulet et al. (8). The sequences of oligonucleotides J and K were derived from the pET11d plasmid.

ment revealed that the transcript is always presented in clusters (20). Furthermore, using a combination of in situ hybridization and immunocytochemical procedures, we have provided evidence that cytoplasmic β-mRNA clusters are the subcellular sites involved in the control of the translation of the transcript during development (54). In light of the close proximity between β-mRNA clusters and mitochondria, a role for this structure in fast-tracking the sorting of the precursor protein to its mitochondrial destination has been suggested (20, 54; for a review see reference 38).

Activation of masked mRNAs is usually mediated by covalent modification and/or *trans* activation of the stored mRNAs in response to a given stimulus (3, 9). In the case of nucleus-encoded β-F1-ATPase mRNA, the rapid and specific developmental changes in the translational efficiency of the transcript (39) suggested that changes in the poly(A)$^+$ RNA fraction, or in the reporter template itself, were responsible for the increased and preferential translation of β-F1-ATPase mRNA during mitochondrial differentiation (39). In this work, we have therefore undertaken a study aimed at identifying the potential mechanisms (14, 25, 41) that may control the cytoplasmic expression of β-F1-ATPase mRNA during liver development. We have found that changes in the translational efficiency of β-F1-ATPase mRNA are not the result of intrinsic differences in the 3′ or 5′ ends of the mRNA but rather are mediated by regulatory proteins of the liver that specifically inhibit β-F1-ATPase mRNA translation. Remarkably, the 3′ UTR of β-F1-ATPase mRNA is an essential *cis*-acting element involved in its translation. A protein (3′βFBP) that binds to the 3′ UTR of β-F1-ATPase mRNA has been identified in fetal rat liver extracts. It is shown that β-F1-ATPase mRNA binding activity is acutely regulated during liver development, paralleling the abrupt developmental changes in the translational efficiency of β-F1-ATPase mRNA (39). In addition, we report that the 3′βFBP and β-F1-ATPase mRNA translational inhibitory activities cofractionate and further show the same expression patterns in the tissues of adult rats. The mapping of an essential *cis*-acting element for binding of the regulatory protein is also provided. Altogether, the findings illustrate for the first time that regulatory proteins that bind the 3′ UTR of an oxidative phosphorylation transcript are involved in controlling the developmental and tissue-specific expression pattern of the gene. In other words, mechanisms regulating translation are also of relevance when considering the control of the biogenesis of mitochondria in mammals.

MATERIALS AND METHODS

Abbreviations. ARF, ADP ribosylation factor; TBE, 0.09 M Tris-borate–0.002 M EDTA; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; UTR, untranslated region; DTT, dithiothreitol; AMV, avian myeloblastosis virus; RT, reverse transcriptase; BSA, bovine serum albumin; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); EMSA, electrophoretic mobility shift assay; 3′βFBP, β-F1-ATPase mRNA 3′-UTR binding protein.

Analysis of liver β-F1-ATPase mRNA by RNase H protection assays. RNase H protection assays were carried out essentially as described previously (4). Specific oligonucleotides (250 pmol) (Table 1) and/or 500 pmol of oligo(dT)$_{12–18}$ (Pharmacia) was annealed to either 40 μg of total RNA from fetal, 1-h-old neonatal, and adult rat liver or 1 μg of poly(A)$^+$ RNA from fetal and 1-h-old neonatal rat liver. The oligonucleotide · β-F1-ATPase mRNA and/or oligo(dT) · poly(A)$^+$ tail hybrids formed were digested by addition of 0.8 U of RNase H (Pharmacia) at 21°C for 45 to 60 min. The digested RNA was analyzed by Northern blotting procedures with specific oligonucleotides (Table 1) (29).

Primer extension analysis of liver β-F1-ATPase mRNA. Primer extension experiments were carried out according to the method of Boorstein and Craig (7) using 100 fmol of labeled 41-mer oligonucleotide (Table 1) and 60 μg of total RNA samples obtained from the liver at different stages of development. After forming the oligonucleotide · β-F1-ATPase mRNA hybrids, 24 U of AMV RT (Boehringer) was added and incubated at 42°C for 60 min. The reaction products were loaded on an 8% polyacrylamide–7 M urea gel. After electrophoresis, the gel was dried and the ^{32}P-labeled products were visualized by exposure of the gels to X-ray films. DNA molecular weight marker V from Boehringer labeled with [γ-^{32}P]ATP and polynucleotide kinase was used as a size marker.

Obtainment of full-length β-F1-ATPase cDNA from rat liver and other plasmids used. Rat liver poly(A)$^+$ RNA (1 to 2 μg) was used as a template of the RT (Promega), as described in the Promega Protocols and Applications Guide. Specific primer sequences for the 5′ and 3′ ends of β-F1-ATPase cDNA from rat liver were derived from those described previously (8, 23) to generate the rat liver 3′ and 5′ β-F1-ATPase cDNA fragments used in PCR. The sequence and polarity of each primer is indicated in Table 1. The amplified 5′ and 3′ β-F1-ATPase cDNA products were digested with *Kpn*I-*Eco*RI and *Apa*I-*Hin*dIII, respectively. Restriction fragments, together with the *Eco*RI-*Apa*I cDNA fragment obtained from partial rat liver β-F1-ATPase cDNA, kindly supplied by P. L. Pedersen (23), were subcloned into pBluescript SK vector (Stratagene). The recombinant plasmid was named pJMI-β-F1.

On the other hand, the *Apa*I-*Hin*dIII cDNA product, containing the 3′ UTR of β-F1-ATPase mRNA from rat liver, was also subcloned into pBluescript SK vector (Stratagene). The recombinant plasmid was named pJMI3′UTR-β-F1. To obtain the plasmid containing 5′-UTR-deleted β-F1-ATPase cDNA (pJMI5′UTR-β-F1), a sense oligonucleotide containing a *Kpn*I site and a partial region of the presequence of the β-F1-ATPase precursor (oligonucleotide I, [Table 1]) was synthesized. The corresponding cDNA was obtained by PCR with sense oligonucleotide I and the antisense oligonucleotide E (Table 1), by using pJMI-β-F1 as a template. The construct pARF, containing bovine ARF-1 cDNA, was obtained by PCR from the pET11d plasmid, kindly provided by A. Alconada, with oligonucleotides J (sense) and K (antisense) (Table 1). The amplified cDNA product was digested with *Kpn*I and subcloned into the appropriately restricted pBluescript SK vector (Stratagene). The heterologous plasmid (pARF-3′βUTR) containing the coding sequence of ARF-1 and the 3′ UTR of β-F1-ATPase mRNA, was obtained by subcloning the *Apa*I-*Hin*dIII cDNA fragment from pJMIβ-F1 into pARF. Sequencing of plasmids with T7 and T3 primers was performed with the *fmol* DNA sequencing system (Promega).

In vitro synthesis of RNA transcripts. The pJMI-β-F1 and pARF-3′βUTR plasmids were linearized by cleavage at the *Hin*dIII site. Other plasmids used in this study were linearized with restriction endonucleases, as follows: pJMI-β-F1 with *Apa*I, *Sac*I, and *Dra*I to generate the deletions of the 3′ UTR of β-F1-ATPase mRNA; pJMI5′UTRβ-F1 with *Hin*dIII and *Apa*I to generate the Δ-5′UTR and Δ-5′,3′UTR deletion constructs, respectively; pJMI3′UTR-β-F1 with *Sac*I, *Dra*I, and *Hin*dIII to generate, respectively, the 3′-Δ1, 3′-Δ2, and wild-type riboprobes of the 3′ UTR; pARF with *Apa*I and with *Bam*HI and *Hin*dIII to generate, respectively, the ARF and the two ARF-3′RNA mRNAs. The in vitro transcription reaction was carried out with the mCap RNA capping kit (Stratagene) according to the protocol of the manufacturer. Radiolabeled RNA probes were prepared without adding cap structures, in the presence of 0.05 mM cold rUTP plus 50 μCi of [α-^{32}P]UTP (400 Ci/mmol).

In vitro translation of mRNAs. In vitro-synthesized mRNAs, derived from the corresponding plasmids (up 40 μg/ml), were utilized as templates for protein synthesis in a nuclease-treated rabbit reticulocyte lysate (Amersham). The reactions were performed in the presence of 40 μCi of L-[^{35}S]methionine (>1,000 Ci/mM) and 40 U of RNasin (39, 62). At different reaction times (up to 1 h), the products were analyzed by SDS-PAGE (29).

Preparation and fractionation of liver extracts. Liver homogenates and postmitochondrial supernatants from fetal, 1-h-old neonatal, and adult rats were prepared (29) and stored at −70°C until use. Postmitochondrial supernatants were centrifuged at 180,000 × *g* for 1 h, and the supernatants obtained were dialyzed against 10 mM K-phosphate buffer, pH 7.0 (molecular weight cutoff of the membrane, 6,000 to 8,000). Protein (2.5 mg) from the dialyzed liver extract was fractionated at a constant flow of 25 ml/h at 4°C through a Sephadex G-100 column with 50 mM Tris-HCl, pH 7.4. The column was calibrated previously with a mixture containing blue dextran (40 μg), BSA (200 μg), and cytochrome *c* (200 μg). Collected fractions (1 ml) were desalted with Centricon-10 concentrators (Amicon, Inc.) and combined into four main fractions (see Fig. 8). Heat-denatured liver extracts were also used. Protein concentrations were determined with the Bradford reagent (Bio-Rad) with BSA as the standard. Other tissue extracts from adult male rats were also prepared.

RNase protection assays. Liver poly(A)$^+$ RNA (2 μg) from fetal, 1-h-old neonatal, and adult rats were coprecipitated with 5 × 10^5 cpm of the β-F1-ATPase sense or antisense RNA probes. The sense and antisense RNA probes were obtained from the transcription of the pJMI-β-F1 and pJMI3′UTR-β-F1 plasmids digested with either *Hin*dIII or *Kpn*I by using T7 or T3 polymerase, respectively. Annealing was performed at 45°C for 16 to 18 h. After hybridization, the samples were digested with 50 μg of RNase A (Boehringer) per ml and 1,000 U of RNase T_1 (Boehringer) per ml at 37°C for 30 min. After treatment with 50 μg of proteinase K (Boehringer), samples were extracted with phenol-chloroform and analyzed on 6% denaturing polyacrylamide gels.

RNA EMSA and UV cross-linking assay. Band shift assays were carried out with the modifications previously described (74). Protein (25 μg) from liver extracts was incubated with radiolabeled probes (1 × 10^5 to 5 × 10^5 cpm) in 20 μl of 15 mM HEPES, pH 8.0, containing 4 μg of *Escherichia coli* tRNA, 10 mM KCl, 10% glycerol, and 1 mM DTT at 30°C for 10 min prior to the addition of 20 U of RNase T_1 (Boehringer). After a 30-min incubation at 37°C, the RNA-protein complexes were resolved on a 5% polyacrylamide–0.5× TBE native gel. For competition studies, an excess of unlabeled RNA was added 10 min before the addition of the radiolabeled RNA to liver extracts. UV cross-linking studies were performed essentially as described above. Reaction mixtures were exposed to 254 nm of UV light (Stratalinker 1800; Stratagene) for 6 min on ice. The RNA-protein complexes were resolved by SDS–12% PAGE. After electrophoresis, the gels were vacuum dried and the ^{32}P-labeled complexes were visualized by exposure of the gels to X-ray films with intensifying screens.

RNase T_1 mapping analysis. After drying and exposure of the RNA electrophoretic mobility shift gels, the retarded band was scissored from the gels. The bands obtained were hydrated and extracted overnight at 37°C in a medium containing 0.5 M ammonium acetate–10 mM magnesium acetate–0.1 mM EDTA–0.1% SDS. After phenol-chloroform extraction, the eluted RNA was precipitated with 20 μg of tRNA and 3 volumes of ethanol. The precipitated RNA was resuspended in H_2O and digested with 20 U of RNase T_1 for 20 min at 22°C. The digested products were resolved on a 12% PAGE–7 M urea denaturing gel. After drying, the gel was exposed to a phosphorimage plate and/or autoradiography.

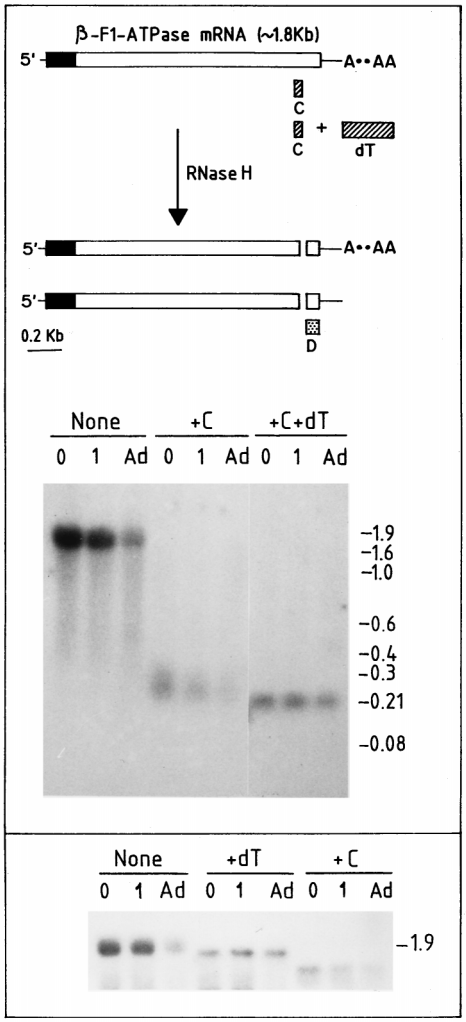
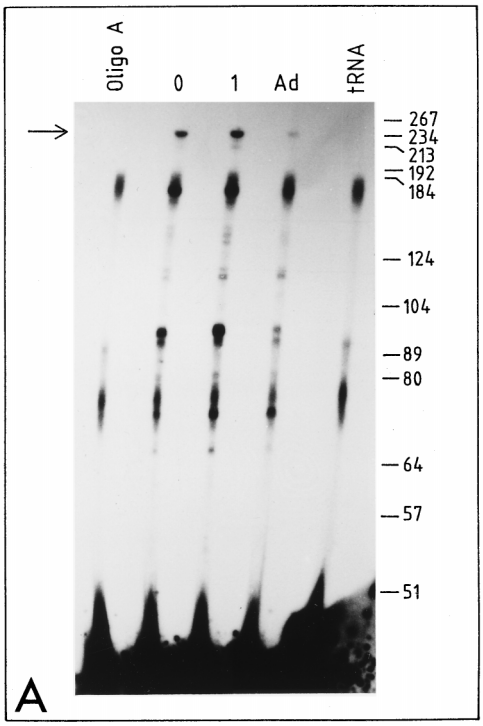


FIG. 1. RNase H analysis of the 3′ UTR and poly(A)$^+$ tail lengths of β-F1-ATPase mRNA during liver development. (Upper panel) Schematic representation of β-F1-ATPase mRNA. The thin lines indicate the relative lengths of the 5′ and 3′ UTRs, whereas the black box in the representation of the transcript illustrates the mRNA coding region of the presequence of the β-precursor. The hatched boxes illustrate the length and hybridization position of antisense oligonucleotide C (Table 1) and oligo(dT). The dotted box illustrates the length and hybridization position of antisense oligonucleotide D (Table 1), used for the detection of the generated 3′ β-F1-ATPase mRNA fragments. Total RNA (40 μg) from fetal (0), 1-h-old neonatal (1), and adult (Ad) rat liver was analyzed as described in Materials and Methods. Detection of the 3′ β-F1-ATPase mRNA fragments was carried out with ^{32}P-labeled oligonucleotide D. (Lower panel) Detection of the generated 5′ β-F1-ATPase mRNA fragment, after digestion with RNase H, with a *Pst*I-*Hin*dIII fragment of β-F1-ATPase cDNA. RNA molecular weight marker I (Boehringer) and *E. coli* tRNA were used as size markers.

RESULTS

The poly(A)$^+$ tail of liver β-F1-ATPase mRNA remains basically the same during development. The accumulation of β-F1-ATPase mRNA in the fetal liver in a translationally repressed state (30, 39) suggested the possibility that specific covalent modifications in the mRNA itself (31, 55, 70, 72) are involved in mediating the rapid changes in the translational efficiency of the transcript during development (30, 39). Since changes in the polyadenylation track of the β-F1-ATPase mRNA could escape detection by conventional Northern blotting procedures, we studied the 3′ end of β-F1-ATPase mRNA at different stages of liver development by RNase H analysis (4).

Incubation of the total RNA fraction, obtained from livers at different stages of development, with an antisense oligonucleotide (oligonucleotide C [Table 1]) of the 3′ end of β-F1-ATPase mRNA (Fig. 1, upper panel), plus or minus oligo(dT), and further incubation with RNase H resulted in the specific digestion of mRNA-DNA hybrids and the generation of large 5′ and small 3′ fragments of β-F1-ATPase mRNA, the latter fragments with or without the poly(A)$^+$ track (Fig. 1). Fractionation and further hybridization of the resulting RNA samples to a 3′-end antisense oligonucleotide (oligonucleotide D



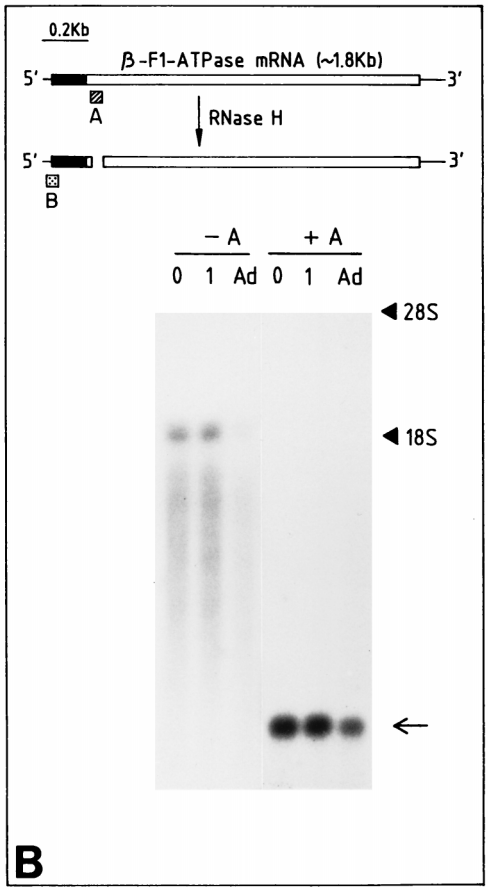


FIG. 2. The rat liver β-F1-ATPase gene has a unique transcription initiation site during development. (A) Primer extension analysis of β-F1-ATPase mRNA from fetal (0), 1-h-old neonatal (1), and adult (Ad) rat liver with oligonucleotide A (Table 1) was carried out as described in Materials and Methods. tRNA was used as a control for nonspecific elongation. The migration of ^{32}P-labeled DNA molecular weight marker V (Boehringer) is shown on the right. The arrow indicates the migration of the major elongated product. (B) RNase H analysis of the 5′ end of β-F1-ATPase mRNA during liver development. In the schematic representation of the β-F1-ATPase mRNA, the thin lines indicate the relative lengths of the 5′ and 3′ UTRs, whereas the black box in the representation of the transcript illustrates the mRNA coding region of the presequence of the β-precursor. The hatched box illustrates the length and hybridization position of antisense oligonucleotide A (Table 1). The dotted box illustrates the length and hybridization position of antisense oligonucleotide B (Table 1) used for the detection of the 5′ β-F1-ATPase mRNA fragment generated after incubation with RNase H. The arrow indicates the migration of the generated 5′ β-F1-ATPase mRNA fragment.

[Table 1]) of β-F1-ATPase cDNA allowed the visualization of the undigested β-F1-ATPase mRNA and the resulting small 3′-end fragments of the mRNA (Fig. 1, upper panel). With a similar strategy, the hybridization of digested RNA samples to a *Pst*I-*Hin*dIII fragment of β-F1-ATPase cDNA (23) allowed the visualization of the undigested, larger 5′ fragment of β-F1-ATPase mRNA (Fig. 1, lower panel). The results obtained by these approaches revealed (i) that the relative abundance of β-F1-ATPase mRNA in fetal and neonatal liver is higher than in the adult (Fig. 1), in agreement with previous findings with different probes used for the detection of β-F1-ATPase mRNA levels (30, 39); (ii) that the poly(A)$^+$ tail of β-F1-ATPase mRNA is heterogeneous in size at all stages of liver development, ranging from ~10 to 100 bases (Fig. 1, upper panel), which is in agreement with the size of the poly(A)$^+$ tail for other mature eukaryotic mRNAs; and (iii) that the 3′ end and the majority of the poly(A)$^+$ track of β-F1-ATPase mRNA has the same length during liver development (Fig. 1), although it cannot be excluded that as development proceeds the lengths of the longest poly(A)$^+$ tails decrease. Therefore, it appears that changes in the translational efficiency of the transcript are not accompanied by major changes in the poly(A)$^+$ tail of the mRNA. These results were confirmed by using the same experimental approach with the poly(A)$^+$ RNA fraction of fetal and neonatal samples (data not shown).

A unique transcription start site for the rat liver β-F1-ATPase gene during development. It has been reported that differences in the 5′ UTR of eukaryotic mRNAs are expected to influence the translational efficiency of the transcripts (25, 41, 50, 73). The human β-F1-ATPase gene has been shown to contain, in various studies, one (44) or two (45) transcription start sites. The situation for the rat gene is not known and, in case of the existence of two transcription start sites, it is conceivable that developmental regulation of transcription initiation at different sites in the promoter of the gene would result in β-F1-ATPase mRNAs with different 5′ leaders that could, therefore, express different translational efficiencies. Thus, we studied whether the 5′ end of rat liver β-F1-ATPase mRNA changes throughout development.

Primer extension analysis of RNA fractions obtained at different stages of development (Fig. 2A), by using an antisense oligonucleotide of the 5′ end of β-F1-ATPase cDNA (oligonucleotide A [Table 1]) (23, 29), revealed a major elongated product (~240 nucleotides) of the RT reaction (Fig. 2A). Differences in the amount of the major elongated product between the samples most likely reflect the different cellular representations of β-F1-ATPase mRNA during liver development (30, 39). Other intermediate-size products were also detected (Fig. 2A). These most likely corresponded to noncompleted products of the transcriptase reaction, as previously suggested by others (23), because of the extensive secondary

structure detected in the mRNA coding region of the presequence of the β-subunit precursor. Interestingly, primer extension reactions with antisense oligonucleotide B (Table 1), which is complementary to the most 5′ end of β-F1-ATPase cDNA, revealed no elongated products (data not shown).

The length of the 5′ leader of β-F1-ATPase mRNA during liver development was further studied by RNase H analysis (Fig. 2B). Incubation of total RNA fractions from different stages of liver development with oligonucleotide A, digestion of the RNA-DNA hybrids with RNase H, and further detection of the generated fragments with oligonucleotide B revealed the presence of a unique ~200-nucleotide 5′ fragment of the β-F1-ATPase mRNA during development. Thus, the results (Fig. 2A and B) suggest that the rat liver β-F1-ATPase gene is transcribed during liver development from a single transcription start site, which is located ~27 bp upstream of the initiator codon AUG (8), in agreement with findings of a unique transcription start site on the human gene (44).

In the view of the absence of differences in the 3′ and 5′ ends of β-F1-ATPase mRNA during liver development (Fig. 1 and 2), which could suggest mechanisms controlling developmental changes in the translational efficiency of the transcript (30, 39), we next explored whether developmentally regulated molecules of the liver could be involved in controlling the translational efficiency of the transcript (39).

Cloning full-length β-F1-ATPase cDNA from rat liver. In order to characterize putative *trans*-acting factors involved in the regulation of β-F1-ATPase mRNA translation during liver development, it was first necessary to obtain full-length β-F1-ATPase cDNA from rat liver. In brief, the 5′ and 3′ ends of β-F1-ATPase mRNA were obtained by RT coupled to PCRs with specific oligonucleotides (E and F for the 5′ ends and G and H, for the 3′ ends [Table 1]). The sequences of the 5′ and 3′ ends of the recombinant plasmid, named JMI-β-F1, matched the previously published sequences of the 5′ and 3′ ends of β-F1-ATPase cDNAs from rat liver (8, 23). The JMI-β-F1 plasmid was efficiently transcribed in vitro, producing β-F1-ATPase mRNA of the expected size (data not shown).

RNase protection assays provided no indication of the existence of β-F1-ATPase mRNA antisense RNAs in the liver. The fact that translation of β-F1-ATPase mRNA from the fetal poly(A)$^+$ RNA fraction is less efficient than that carried out from the poly(A)$^+$ RNA fraction of the neonatal liver (39) suggested that the molecular nature of the putative effector of β-F1-ATPase mRNA translation could be a polyadenylated RNA and/or the protein product of an RNA contained in the fraction (39). Examples of regulatory RNAs involved in the translation of specific mRNAs have been provided previously (25, 36, 52, 53, 64, 69, 71). Therefore, we studied whether the poly(A)$^+$ RNA fraction of rat liver contains, depending on the stage of development, some kind of polyadenylated RNA able to protect the labeled β-F1-ATPase mRNA probe from degradation with a mixture of RNase A plus T_1. The results obtained revealed that in vitro-generated β-F1-ATPase mRNA is completely degraded irrespective of the presence in the assay of poly(A)$^+$ RNA from fetal, neonatal, or adult rat liver (Fig. 3, lanes 3 to 5). In contrast, an antisense β-F1-ATPase mRNA probe, used as a control in the RNase protection assay, revealed the presence of a protected RNA fragment of the expected size when incubated in the presence of poly(A)$^+$ RNA from rat liver (Fig. 3). These results most likely exclude the possibility that antisense RNAs regulate β-F1-ATPase mRNA translation during liver development.

Developmentally regulated *trans*-acting factors of the liver modulate the rate of translation of β-F1-ATPase mRNA. In line with previous findings (Fig. 1 and 2) and suggestions (39),

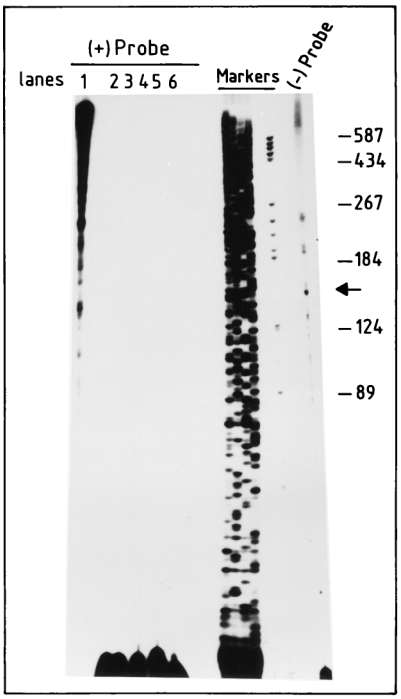


FIG. 3. RNase protection analysis of in vitro-synthesized β-F1-ATPase mRNA. The *Hin*dIII-digested pJMI-β-F1 plasmid was transcribed in vitro with T7 viral RNA polymerase to generate β-F1-ATPase mRNA (lane 1). Two micrograms of poly(A)$^+$ RNA from fetal (lane 3), 1-h-old neonatal (lane 4), and adult (lane 5) rat liver were annealed to the radiolabeled β-F1-ATPase mRNA probe. The nonprotected fragments were digested with RNases A plus T_1, and the products were fractionated through 6% polyacrylamide sequencing gels. Lanes 2 and 6 contained the β-F1-ATPase mRNA probe with no further additions and 2 μg of *E. coli* tRNA, respectively. The marker lanes are a sequencing ladder of unrelated DNA and ^{32}P-labeled DNA molecular weight marker V from Boehringer, respectively. In the lane labeled (−) Probe, a positive control for the RNase protection analysis is shown. Two micrograms of fetal poly(A)$^+$ RNA was annealed to the antisense 3′ UTR of β-F1-ATPase mRNA. The antisense RNA probe was synthesized from the *Kpn*I-digested pJMI3′UTR-β-F1 plasmid by using T3 viral RNA polymerase. The size of the protected fragment is indicated by an arrow.

the observed differences in translational efficiency of β-F1-ATPase mRNA during development may thus result from the presence of specific regulatory proteins in the liver which inhibit (in the fetal stage) or activate (in the neonatal stage) the translation of β-F1-ATPase mRNA (39). Therefore, we next studied whether the translational efficiency of the in vitro-generated transcript could be modified by the presence of exogenously added extracts from rat liver obtained at different stages of development. The translational efficiency of the transcript was assessed in reticulocyte lysates, an in vitro system previously shown to reflect differences in the translational efficiency of the transcript (39) that basically mimic those observed in vivo (30). In vitro-synthesized β-F1-ATPase mRNA was efficiently translated in vitro with rabbit reticulocyte lysates (Fig. 4A). A single 52-kDa polypeptide was obtained, in contrast with previous results by others (8) but in agreement with the size of the precursor protein of the β-subunit of the F1-ATPase complex from rat liver (62).

Addition of liver extracts to the assay system always promoted a decline in the amount of the synthesized β-precursor compared to those lacking any addition (Fig. 4A). However, it is remarkable to observe that the inhibition of translation of β-F1-ATPase mRNA, promoted by the addition of 1-h-old neonatal and adult liver extracts, was much less than that triggered

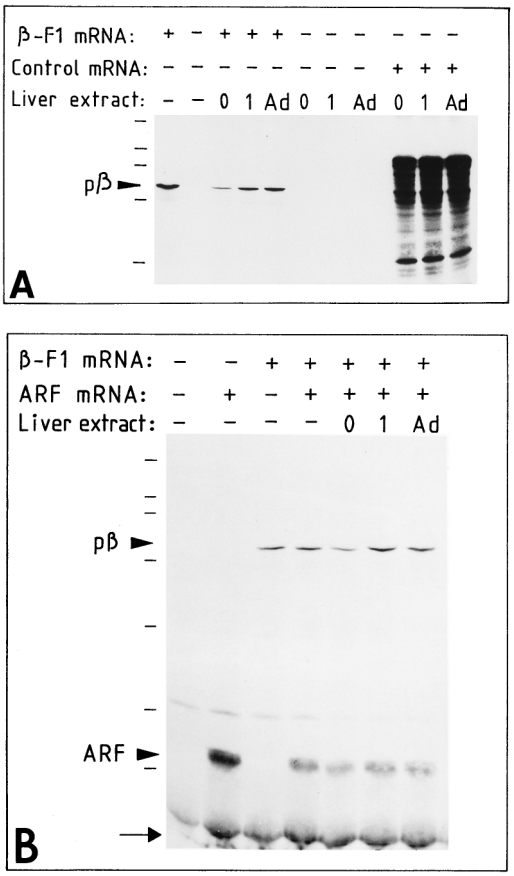


FIG. 4. Effects of liver extracts on the translation efficiency of in vitro-synthesized β-F1-ATPase mRNA. (A) Twenty micrograms of in vitro-synthesized β-F1-ATPase mRNA (β-F1 mRNA) or control B RNA (Amersham) per milliliter was added to nuclease-treated rabbit reticulocyte lysates. Where indicated, 25 μg of protein from fetal (0), 1-h-old neonatal (1), or adult (Ad) rat liver extracts was used to determine the translational efficiency of β-F1-mRNA. Translation was carried out for 30 min at 30°C. The [^{35}S]methionine-labeled products were fractionated by SDS–12% PAGE and processed by fluorography. The autoradiogram of the gel of a representative experiment is shown. The migration of the synthesized β-subunit precursor (pβ) and molecular mass markers (200, 97, 69, 46, and 30 kDa, from top to bottom) are indicated. For quantitation of the inhibitory effect of fetal extracts on the translational efficiency of β-F1-ATPase mRNA, see the text. (B) Simultaneous translation of the mixture of β-F1-ATPase and ARF mRNAs reveals the translational advantage of the former transcript. Ten micrograms of in vitro-synthesized β-F1-ATPase and ARF mRNAs were added alone or in combination to the lysate. Where indicated, 20 μg of protein from fetal (0), 1-h-old neonatal (1), or adult (Ad) rat liver extracts was added. The migration of the synthesized pβ and ARF protein is indicated by arrowheads. Molecular mass markers (200, 97, 69, 46, 30, 21, and 14 kDa, from top to bottom) are shown on the left. The arrow indicates the front of the gel.

by the addition of fetal liver extracts (Fig. 4A). From the mean of two independent experiments, addition of fetal extracts promoted 80% inhibition whereas neonatal or adult extracts promoted only 30 or 20% inhibition, respectively. Interestingly, fetal liver extracts did not show such inhibitory effect for the translation of a polycistronic mRNA used as a control (Fig. 4A), suggesting that fetal extracts specifically inhibit translation of β-F1-ATPase mRNA.

Proteolytic cleavage by one of the domains of the precursor polypeptide encoded in the control RNA yields several polypeptides during the translation reaction (Fig. 4A). Since one of these polypeptides has the same electrophoretic migration as that of the β-precursor (Fig. 4A), the effect of liver extracts was further assessed in a translation mixture containing β-F1-ATPase mRNA and bovine ARF-1 mRNA. In the absence of added extracts, and under the translation conditions assayed, it was predicted that both mRNAs would compete for the available translational machinery, resulting in less-synthesized products than when translated separately. However, the results obtained revealed that the synthesis of ARF was very much inhibited (60 and 70%) whereas that of the β-precursor was not affected (Fig. 4B). This finding suggested the existence of an intrinsic characteristic of β-F1-ATPase mRNA that is able to provide to the transcript a translational advantage for competing for the translational machinery of the lysate. In agreement with previous findings (Fig. 4A), the addition of fetal liver extract to the translation reaction mixture repressed the synthesis of the β-precursor (70%) and marginally affected the synthesis of ARF (0 and 15%, from two independent experiments) (Fig. 4B).

The 3′ UTR of β-F1-ATPase mRNA is an essential *cis*-acting element of mRNA translation. In order to map the apparent *cis*-acting element providing β-F1-ATPase mRNA its in vitro translational advantage, we generated different deletion constructs of the mRNA and assayed their translational efficiency in the reticulocyte system (Fig. 5A). The results revealed that the deletion of the 5′ UTR of the mRNA (Δ-5′UTR) promoted almost a twofold increase in the amount of synthesized β-precursor compared to the wild-type construct (Fig. 5A). In contrast, deletion of the 3′ UTR (Δ-3′UTR) promoted almost complete inhibition of the synthesis of the β-precursor (Fig. 5A). In agreement with the partial in vitro inhibitory effect of the 5′ UTR of the mRNA on the synthesis of the β-precursor, the amount of β-precursor synthesized from the double deletion construct (Δ-5′,3′UTR) was slightly recovered compared to the synthesis from the Δ-3′UTR construct (Fig. 5A). These findings indicate that the 3′ UTR of β-F1-ATPase mRNA is an

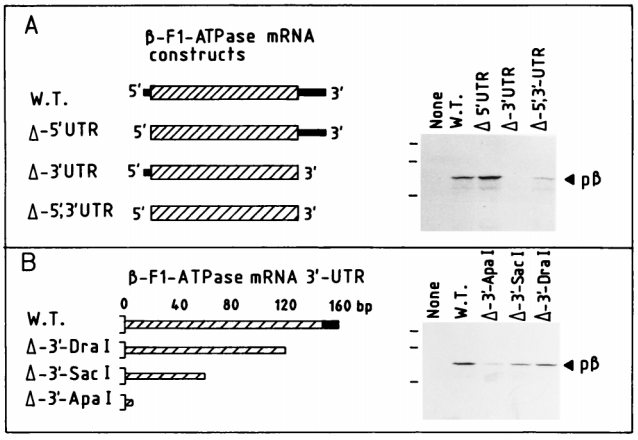


FIG. 5. A full-length 3′ UTR of β-F1-ATPase mRNA is required for efficient translation of the transcript. (A) Synthetic β-F1-ATPase mRNAs (W.T., Δ-5′UTR, Δ-3′UTR, and Δ-5′,3′UTR) were obtained as described in Materials and Methods. The closed and hatched boxes represent the UTRs and coding regions of β-F1-ATPase mRNA, respectively. (B) Schematic representation of the size of the 3′ UTR of β-F1-ATPase mRNA in different 3′-UTR deletion mutants. The mutants are indicated by Δ-3′ followed by the restriction endonuclease used to linearize templates prior to in vitro transcription. The hatched boxes represent the β-F1-ATPase 3′ UTRs of the mRNAs. The closed boxes represent the 13 adenosines at the 3′ end. In both panels, 0 or 500 ng of each construct was translated in the lysates for 30 min at 30°C. Aliquots (4 μl) of the in vitro-synthesized protein were processed to determine the translational efficiency of each mRNA construct. Representative autoradiograms are shown. The migration of the synthesized β-subunit precursor (pβ) is indicated by an arrowhead. The migration of molecular mass markers (97, 69, and 46 kDa, from top to bottom) is shown on the left.

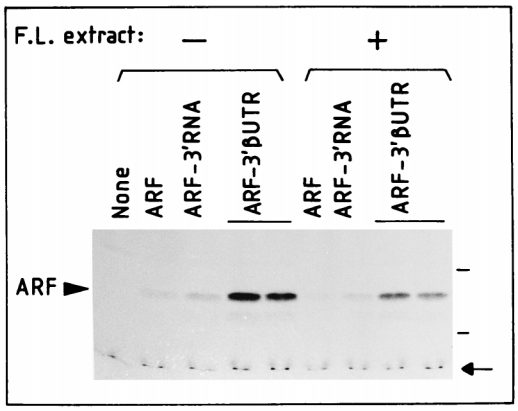


FIG. 6. Translation-enhancing effect of the 3′ UTR of β-F1-ATPase mRNA on a chimeric reporter transcript. The chimeric RNAs (ARF-3′RNA and ARF-3′βUTR) were generated as described in Materials and Methods. Zero or 400 ng of each RNA was translated in a standard reaction assay. Where indicated (+), 25 μg of protein from fetal rat liver extract was added. The migration of the synthesized ARF is indicated by an arrowhead. Molecular mass markers (21 and 14 kDa, from top to bottom) are shown on the right. The arrow indicates the front of the gel.

essential *cis*-acting element for efficient translation of the transcript. Progressive 3′ deletions of the 3′ UTR of the mRNA, generated by digestion of the pJMI-β-F1 plasmid with the *Hin*dIII (wild type), *Dra*I, *Sac*I, and *Apa*I restriction endonucleases (Fig. 5B), resulted in a gradual decrease in the amount of synthesized β-precursor (30, 44, and 80% of the synthesized β-precursor from the wild-type mRNA, respectively) (Fig. 5B), further indicating that β-F1-ATPase mRNA translation is dependent on a complete 3′ UTR.

The 3′ UTR of β-F1-ATPase mRNA enhances translation of a chimera and contains the target site for developmental regulation of the transcript. To confirm the essential role of the 3′ UTR of β-F1-ATPase mRNA in translational regulation, we subcloned the 3′ βUTR downstream of the ARF mRNA and tested the translational efficiency of the reporter construct. As additional controls for the experiment, sequences of the pBluescript plasmid (*Apa*I-*Hin*dIII [ARF-3′RNA] or *Apa*I-*Bam*HI [data not shown]) were also placed 3′ downstream of the ARF mRNA (Fig. 6). The results obtained confirmed an autonomous translational enhancing activity of the 3′ UTR of β-F1-ATPase mRNA because the amount of ARF synthesized from the ARF-3′βUTR chimera was 5- to 10-fold above that synthesized from any of the other ARF constructs (Fig. 6). Furthermore, when translation of the ARF-3′βUTR construct was carried out in the presence of fetal liver extracts, a specific recapitulation of the inhibitory effect of the extract on the synthesis of ARF (55% inhibition) was observed (Fig. 6). These findings strongly suggest that the 3′ UTR of β-F1-ATPase mRNA is the target *cis*-acting element involved in translational regulation of the expression of the transcript during development.

Developing liver reveals profound differences in the activity of 3′βFBP. mRNA regulatory sequences in certain transcripts are found in the 3′ UTR of the mRNAs (17, 25, 41, 50). The finding that β-F1-ATPase mRNA is localized (20) and translated (54) within cluster structures of the hepatocyte, together with the essential (Fig. 5) and target (Fig. 6) roles played by the 3′ UTR of β-F1-ATPase mRNA in controlling its translation, led us to search for proteins that could bind the 3′ UTR of β-F1-ATPase mRNA (3′βFBP). To distinguish between proteins that could be involved in localization of the transcript (20) and those involved in controlling its translation, we focused on those proteins that could display a different expression pattern during liver development, i.e., that could show different RNA binding activities in fetal and neonatal extracts, the two stages of development that show profound differences in the translational efficiency of the transcript (Fig. 4; also reference 39).

The EMSAs depicted in Fig. 7A illustrate the existence in rat liver extracts of a set of proteins with varying degrees of binding activity for the 3′ UTR of β-F1-ATPase mRNA. Remarkably, fetal extracts contained a binding activity that is much less represented in extracts from neonatal and adult rat liver (Fig. 7A). The specificity of the binding activity of this protein, on average 52 kDa in the native RNA-complexed state, is illustrated by the lack of binding activity in heat-denatured extracts and in competition experiments with a molar excess of unlabeled 3′ UTR of β-F1-ATPase mRNA (Fig. 7A).

UV cross-linking of the 3′ UTR of β-F1-ATPase mRNA with liver extracts from various stages of development (Fig. 7B) confirmed the above findings. The results obtained by this approach further illustrated that the major developmentally regulated RNA binding activity corresponded to RNA-protein complexes of ~52 kDa in the denatured state (Fig. 7B). As previously illustrated, the binding activities were heat sensitive and competed by an excess of unlabeled 3′ UTR of β-F1-ATPase mRNA (Fig. 7B).

Cofractionation of the mRNA binding and translational inhibitory activities of β-F1-ATPase mRNA. An additional correlation between translational inhibitory activity and β-F1-ATPase mRNA binding activity was obtained by fractionation of fetal liver extracts and demonstration that both activities cofractionate (Fig. 8). Since the results of EMSA and UV cross-linking experiments (Fig. 7) indicated that the RNA-protein complex migrated at ~52 kDa, we selected for fractionation of the fetal liver extract a gel filtration chromatographic procedure in which cellular proteins could be separated according to the expected molecular weight of the inhibitor protein. The proteins eluted from the G-100 column were grouped in four fractions, each of them enriched in cytosolic proteins of different molecular mass according to the calibration of the column (>70 kDa, between 40 and 70 kDa, between 30 and 40 kDa, and <30 kDa for fractions 1 to 4, respectively) (Fig. 8A). The percentage of protein recovered in the fractions was 58, 24, 12, and 6% of the total protein loaded for fractions 1 to 4, respectively. As hypothesized, the EMSAs of the 3′ UTR of β-F1-ATPase mRNA with the fetal extract and with the resulting fractions (Fig. 8B) revealed the enrichment of the binding activity (approximately fourfold) in the fraction containing proteins of the 40- to 70-kDa range (fraction 2).

Addition of the fetal extract to translation assays containing β-F1-ATPase mRNA promoted a decline in the amount of in vitro-synthesized β-precursor (Fig. 8C), in agreement with previous findings (Fig. 4). Remarkably, inhibition of the synthesis of the β-precursor was much higher when translation reactions were carried out in the presence of proteins contained in fraction 2 (Fig. 8C), the fraction that contained 3′βFBP (Fig. 7B). Proteins of fraction 1 slightly affected the synthesis of the β-precursor (Fig. 8C), and proteins of fractions 3 and 4, which lack binding activity (Fig. 8B), revealed a higher synthesis of the β-precursor than when translation of β-F1-ATPase mRNA was carried out in the presence of the fetal extract (Fig. 8C). It is difficult to explain why translation of β-F1-ATPase mRNA was not fully restored when assayed in the presence of fractions devoid of β-F1-ATPase mRNA binding activity (Fig. 8), a situation also occurring when translation of β-F1-ATPase mRNA was carried out in the presence of neonatal or adult extracts (Fig. 4A). It is possible that other factors could account for

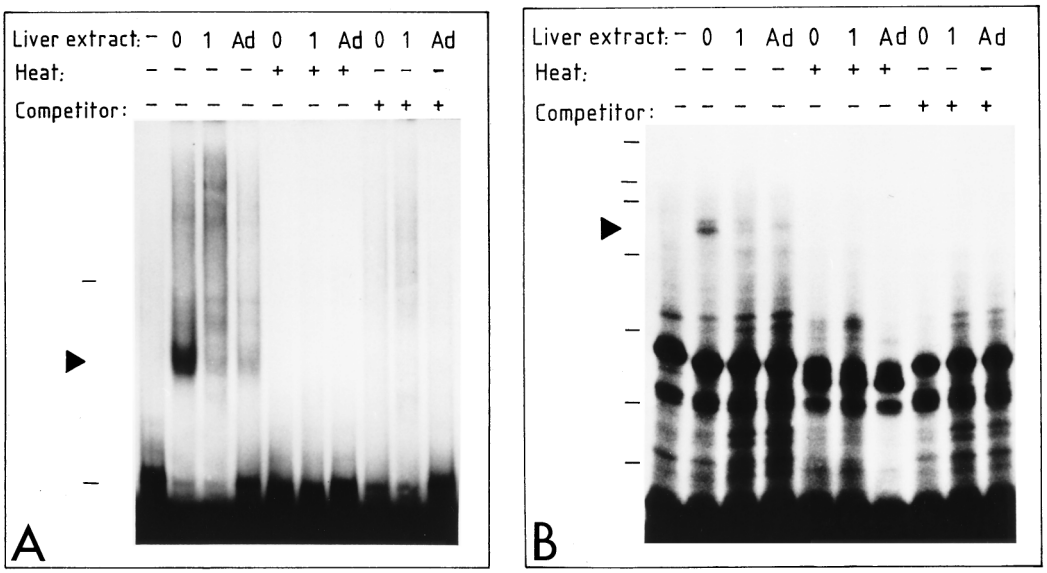


FIG. 7. Identification of a protein that binds to the 3′ UTR of β-F1-ATPase mRNA. The sense 3′ UTR of β-F1-ATPase mRNA was synthesized from the *Hin*dIII-digested pJMI3′UTR-β-F1 plasmid by using T7 viral RNA polymerase. The ^{32}P-labeled β-F1-ATPase mRNA probe was incubated with extracts (25 μg of protein) from fetal (0), 1-h-old neonatal (1), and adult (Ad) rat liver and analyzed by gel retardation in low-ionic-strength nondenaturing polyacrylamide gels (A) or by SDS–12% PAGE after UV cross-linking (B). β-F1-ATPase mRNA probes were also incubated with heat-denatured liver extracts. For competition experiments, an excess of unlabeled 3′-UTR β-F1-ATPase mRNA probe (300 ng) was added prior to the addition of the labeled probe. (A) Migration of the major specific RNA-protein complex (arrowhead) is shown in a representative gel retardation assay. The migration of the 69- and 46-kDa markers, from top to bottom, is shown on the left. (B) After incubation as described above, samples were subjected to UV cross-linking and digested with RNase T_1 as described in Materials and Methods. The RNA-protein complexes were resolved by SDS–12% PAGE. The migration of the major RNA-protein complex (arrowhead) is shown in a gel from a representative UV cross-linking experiment. The migration of protein standards of 200, 97, 69, 46, 30, 21, and 14 kDa (from top to bottom) is indicated on the left.

such an effect. Altogether, the results suggest that the 3′βFBP present in fetal liver extracts exerts translational repression on the transcript. Its lower binding activity, or perhaps its absence in neonatal and adult liver extracts (Fig. 7), could explain the lower repression of β-F1-ATPase mRNA translation observed in vitro (Fig. 4) and, therefore, the activation of translation of β-F1-ATPase mRNA in neonatal liver shortly after birth (30, 39).

Expression of the activity of 3′βFBP in adult rat tissues. In contrast to other nuclear genes of oxidative phosphorylation (for examples see reference 30), the rat genome was found to contain a single-copy β-F1-ATPase gene (30) that is tran-

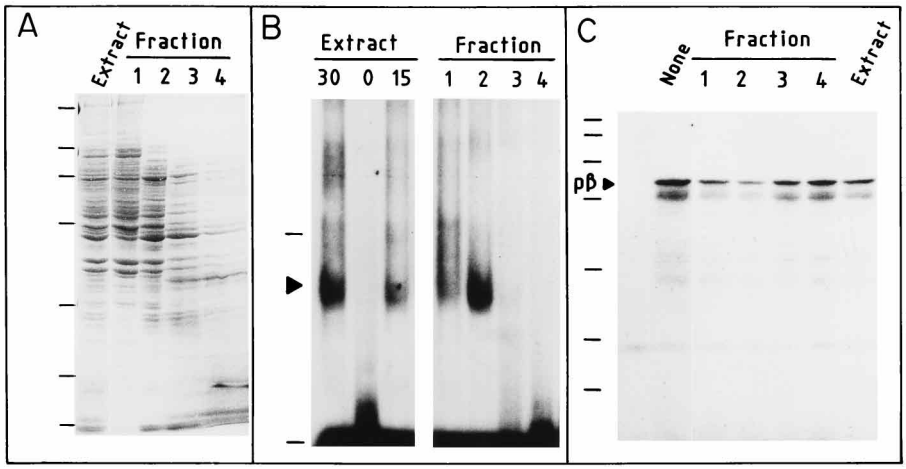


FIG. 8. Cofractionation of β-F1-ATPase mRNA protein binding and translation-inhibiting activities. (A) Fetal liver extract fractionated by gel filtration chromatography with a Sephadex G-100 column as described in Materials and Methods. Protein (30 μg) from fetal liver extract and the collected fractions was resolved by SDS–12% PAGE and Coomassie blue stained. Molecular mass markers (200, 97, 69, 46, 30, 21, and 14 kDa, from top to bottom) are shown on the left. (B) EMSA of the sense ^{32}P-labeled 3′ UTR of β-F1-ATPase mRNA incubated with 15 μg (3 μl) or 30 μg (6 μl) of protein from fetal liver extract or 15 μg (4 μl) of protein from each of the Sephadex G-100 fractions. The RNA-protein complexes were resolved on 5% 0.5× TBE native polyacrylamide gels. The migration of the 46- and 69-kDa markers is shown on the left. (C) Cell-free translation assays. Five hundred nanograms of in vitro-synthesized β-F1-ATPase mRNA was translated in vitro by using nuclease-treated rabbit reticulocyte lysates in the absence of fetal liver extract (none) or in the presence of 4 μl of each fraction (fractions 1 through 4) or 20 μg of protein from the fetal liver extract. The [^{35}S]methionine-labeled products were fractionated by SDS–12% PAGE and processed by fluorography. The migration of the in vitro-synthesized β-subunit precursor (pβ) is indicated. Molecular mass markers are the same as in panel A.

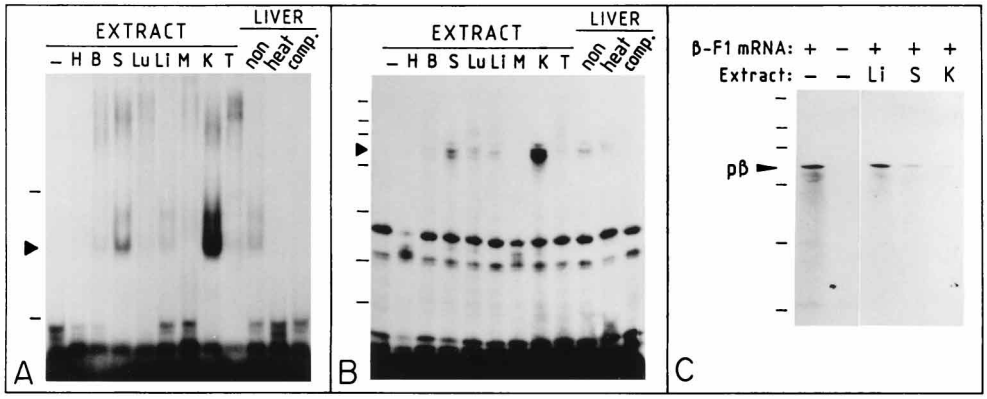


FIG. 9. Tissue-specific expression of the β-F1-ATPase mRNA 3′ UTR binding protein (3′βFBP). (A and B) The ^{32}P-labeled β-F1-ATPase mRNA probe was incubated in the absence (−) or presence of 25 μg of protein from different tissue extracts of adult rats, as follows: heart (H), brain (B), spleen (S), lung (Lu), liver (Li), muscle (M), kidney (K), and testis (T). Incubation of the adult liver extract (non) with heat denaturation (heat) and an excess of unlabeled probe (comp.) (see details in the legend to Fig. 7) is shown. The RNA-protein complexes formed were analyzed by gel retardation assays in low-ionic-strength nondenaturing gels (A) or by SDS–12% PAGE after UV cross-linking and digestion with RNase T_1 (B). The migration of the major 3′βFBP (arrowhead) is indicated in each panel. The migration of molecular mass markers of 69 and 46 kDa (from top to bottom in panel A) and of 200, 97, 69, 46, 30, 21, and 14 kDa (from top to bottom in panel B) is shown. (C) Twenty micrograms of in vitro-synthesized β-F1-ATPase mRNA (β-F1 mRNA) per milliliter was added to nuclease-treated rabbit reticulocyte lysates. Where indicated, 20 μg of protein from adult liver (Li), spleen (S), or kidney (K) extract was used to determine the translational efficiency of in vitro synthesized β-F1-ATPase mRNA. For other details see the legend to Fig. 4A. The autoradiogram of the gel of a representative experiment is shown. The migration of the synthesized β-subunit precursor (pβ) and of the protein standards used (200, 97, 69, 46, 30, and 21 kDa, from top to bottom) are indicated on the left.

scribed in all tissues examined as an ~1.8-kb mRNA (28). These findings suggested a lack of heterogeneity in β-F1-ATPase mRNA between different tissues and allowed the study of the expression of the activity of 3′βFBP in different adult rat tissues (Fig. 9A and B). The findings obtained may provide an additional correlation between β-F1-ATPase mRNA binding and translational inhibitory activities and a further indication of the biological relevance of the inhibitor protein in putative tissue-specific expression of the β-F1-ATPase gene (28). The results obtained by both EMSA (Fig. 9A) and UV cross-linking (Fig. 9B) illustrated that kidney (~12-fold) and spleen (~3-fold) have much higher levels of β-F1-ATPase mRNA binding activity (3′βFBP) than does adult liver. Consistent with the role ascribed to 3′βFBP, translation of β-F1-ATPase mRNA in the presence of kidney and spleen extracts was almost completely abolished compared to translation in the presence of adult liver extracts (Fig. 9C). These findings reinforce a translational-inhibitor role for the β-F1-ATPase mRNA binding activity and suggest the involvement of this activity in controlling the expression of the β-F1-ATPase gene in adult kidney and spleen.

Mapping the *cis*-acting element of the 3′ UTR of β-F1-ATPase mRNA. As a preliminary approach to identifying the RNA binding domain of the putative inhibitor of β-F1-ATPase mRNA translation (3′βFBP), a computer search of the 3′ UTR of mammalian β-F1-ATPase mRNAs was undertaken. The results (Fig. 10) illustrated a high degree of sequence conservation in the last two-thirds of the 3′ UTR of the mRNAs. Riboprobes for different regions of the 3′ UTR of β-F1-ATPase mRNA were generated by digestion of the pJMI3′UTR-β-F1 plasmid (Fig. 10) in order to map the protein binding region in the RNA sequence. Kidney extracts were used for the purpose of mapping the mRNA *cis*-acting element because they express the highest β-F1-ATPase mRNA binding activity (Fig. 9).

EMSAs with the generated riboprobes illustrated binding activity of the extracts only when the 3′ UTR of β-F1-ATPase mRNA was complete (3′-w.t.) (Fig. 10). In fact, none of the deleted riboprobes, those not containing (3′-Δ1) and those containing (3′-Δ2) a long stretch of conserved nucleotides, revealed binding activity or competed the binding of the protein to the full-length 3′ UTR riboprobe (Fig. 10). Similar results were obtained by UV cross-linking experiments (Fig. 10). These results suggest that an essential mRNA sequence for protein binding is located within the last 26 nucleotides plus the 13 adenosines of the 3′ UTR of β-F1-ATPase mRNA.

These findings were further confirmed by RNase T_1 footprinting. Theoretical analysis (FINGERPRINT [19]) of the number and length of the fragments obtained after digestion of the β-F1-ATPase 3′-UTR mRNA with RNase T_1 predicted that the largest fragment obtained, when the 3′ UTR of β-F1-ATPase mRNA contained a track of 13 adenosines, would consist of 23 nucleotides. This fragment corresponds to the terminal part of the RNA compatible with the digestion of the 3′ UTR of β-F1-ATPase mRNA riboprobe at the G (Fig. 10A) following the polyadenylation signal (Fig. 10 and data not shown). T_1 footprinting analysis of the retarded riboprobe generated a fragment of 23 nucleotides (Fig. 10), in agreement with the largest RNA product obtained after experimental digestion of the 3′ UTR of β-F1-ATPase mRNA with RNase T_1 (Fig. 10) and with computer analysis data. These results, therefore, support the theory that the last 26 nucleotides of the 3′ UTR of β-F1-ATPase mRNA are protected from degradation with T_1 by the binding of the regulatory protein(s).

The lack of significant inhibition (15%) in the synthesis of β-precursor from a deletion construct of β-F1-ATPase mRNA that lacks the *cis*-acting element for binding of the regulatory protein (3′-Δ2 β-F1 mRNA) (Fig. 11), compared to the inhibition of synthesis from β-F1-ATPase mRNA (60%) in the presence of fetal liver extract (Fig. 11A), further confirmed the role of the deleted sequence as the binding site for the regulatory protein. It should be kept in mind that differences in the synthesis of β-precursor between these mRNAs, in the absence of inhibitor, are due to the requirement of a complete 3′ UTR (Fig. 5B). In any case, our findings cannot exclude that other 5′ upstream sequences and/or structures of the 3′ UTR of the mRNA may influence the binding of the protein to this region.

Is there a role for the 3′ UTR of β-F1-ATPase mRNA in the recruitment of the translational machinery? The lack of recovery of ARF synthesis when fetal liver extracts were added to

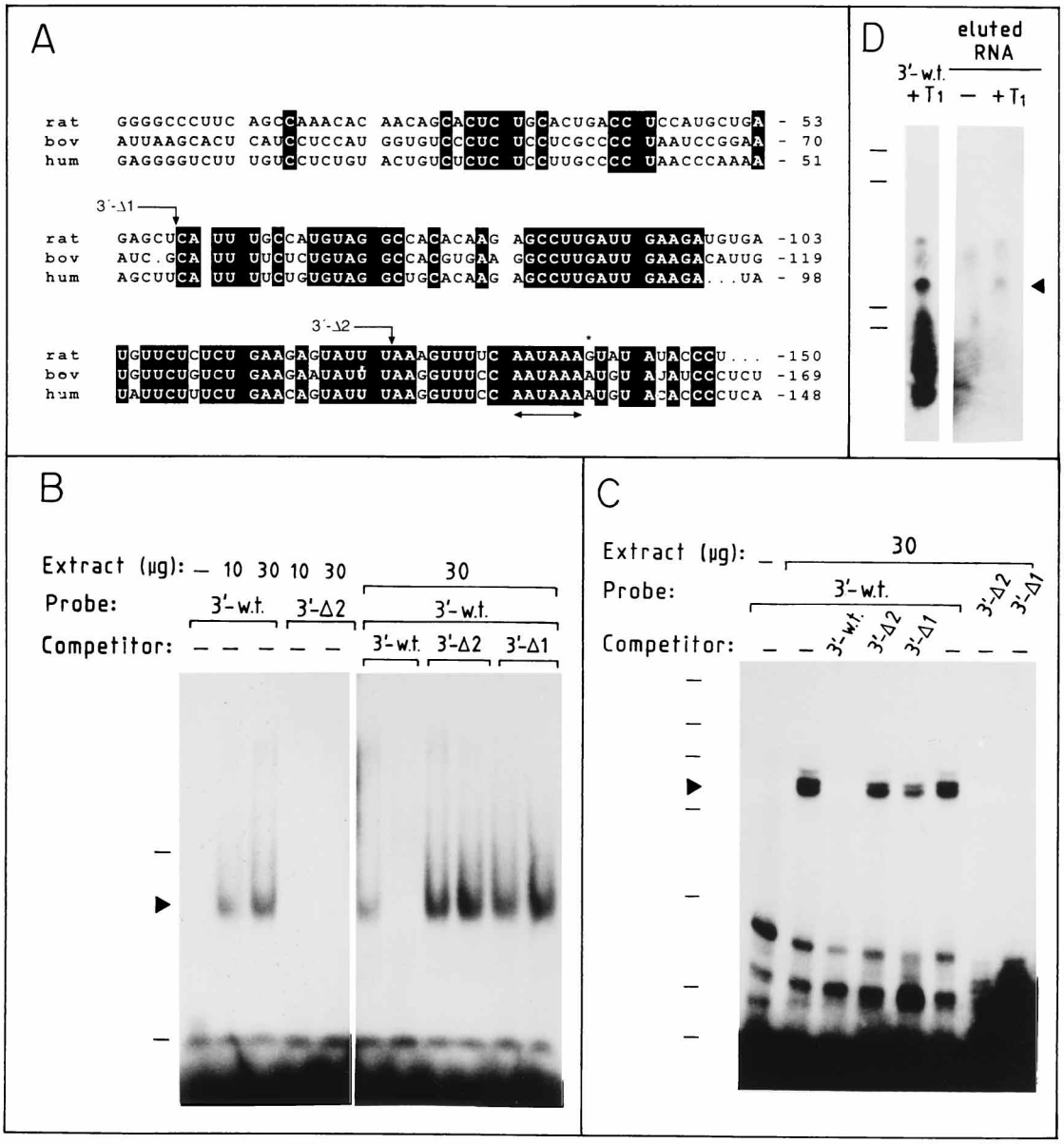


FIG. 10. Mapping of the protein binding site within the 3′ UTR of β-F1-ATPase mRNA. (A) Comparison of the 3′ UTRs of the rat (23), bovine (10), and human (44, 45) β-F1-ATPase mRNA sequences. Multiple sequence alignment was carried out with the PileUp program of the University of Wisconsin Genetics Computer Group software package (19). Nucleotide identities between the three sequences are shown in white letters over black boxes. Numbers indicate the nucleotide position after the U (+1) of the UGA stop codon in each sequence. The 3′-Δ1 and 3′-Δ2 arrows indicate the 3′ ends of the riboprobes synthesized when the pJMI3′UTR-β-F1 plasmid was digested with the *Sac*I and *Dra*I restriction endonucleases, respectively. The underlined AAUAAA sequence is the nuclear polyadenylation signal. The asterisk above the rat sequence indicates the position of the putative G nucleotide protected from degradation by RNase T_1 in the 3′-UTR β-F1-ATPase riboprobe containing a track of 13 adenosines. EMSA (B) and UV cross-linking (C) of the 3′ UTR of β-F1-ATPase mRNA with adult kidney extracts illustrate that the last 26 nucleotides of the sequence are essential for protein binding. Formation of specific mRNA-protein complexes was observed only with the probe containing the complete sequence of the β-F1-ATPase 3′ UTR (3′-w.t.). The specific binding was competed with the nonlabeled riboprobe (3′-w.t.) but not with two deleted probes (3′-Δ2 and 3′-Δ1). Arrowheads denote the migration of the RNA-protein complexes. The migration of molecular mass markers of 69 and 46 kDa (in panel B) and of 200, 97, 69, 46, 30, 21, and 14 kDa (in panel C) are indicated (from top to bottom) on the left of each panel. The labeled probes, 3′-w.t., 3′-Δ1, and 3′-Δ2, respectively, were generated from transcription of the *Hin*dIII-, *Sac*I-, and *Dra*I-digested pJMI3′UTR-β-F1 plasmid. The amounts of the competitor probes used in panel B are 30 and 300 ng for the 3′-w.t., and 40 and 400 ng for 3′-Δ1 and 3′-Δ2, for the first and second lane under each competitor, respectively. In panel C the amounts of the competitor probes used were 300 ng for 3′-w.t. and 400 ng for 3′-Δ1 and 3′-Δ2, respectively. (D) RNase T_1 mapping of the retarded RNA-protein complexes. After EMSA the retarded band was eluted and digested (+T_1) or not (−) with RNase T_1. The RNase T_1 digest of the β-F1-ATPase 3′-UTR RNA probe is also shown in the left lane. The migration of DNA markers (marker V; Boehringer) (18, 21, 51, and 57 nucleotides, from bottom to top) is indicated on the left. The arrowhead indicates migration of the protected fragment.

translation assays containing the mixture of β-F1-ATPase–ARF mRNAs (Fig. 4B), together with the translational enhancing activity of the 3′ UTR of the transcript (Fig. 5 and 6), suggested that the 3′ UTR of β-F1-ATPase mRNA might directly interact with components of the translational machinery. Furthermore, the interaction seems not to be hampered by the presence of the fetal inhibitor. To test this possibility, β-F1-ATPase mRNA was translated in the absence and presence of

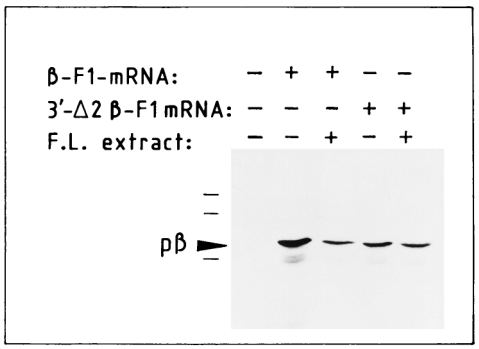


FIG. 11. Translation of a deletion mutant of the 3′ UTR of β-F1-ATPase mRNA is not inhibited by the fetal liver extract. β-F1-ATPase and Δ-3′-*Dra*I β-F1-ATPase (3′-Δ2 β-F1 mRNA) mRNAs were synthesized from plasmid pJMI-β-F1 digested with *Hin*dIII and *Dra*I, respectively, by using T7 viral RNA polymerase. The synthesized mRNAs (500 ng) were in vitro translated in the absence (−) or presence (+) of 25 μg of protein from fetal liver (F.L.) extract. The arrowhead indicates the migration of the synthesized β-subunit precursor (pβ). Molecular mass markers (97, 69, and 46 kDa, from top to bottom) are shown on the left.

kidney extracts, plus or minus increasing concentrations of the 3′ UTR of β-F1-ATPase mRNA (Fig. 12). The results obtained revealed that the inhibition brought about by the extract was not overcome when the 3′ UTR was added. In fact, the more 3′ UTR added to the assay, either alone or in combination with the extract, the less β-precursor was synthesized (Fig. 12). These results suggest, therefore, that the 3′ UTR of β-F1-ATPase mRNA, in addition to providing a binding site for the inhibitor, also interacts with some components of the translational machinery. It appears that when the 3′ UTR is present, either alone or attached to a transcript, it limits the translational machinery available for the translation of other transcripts.

DISCUSSION

We have analyzed potential mechanisms that may mediate, during liver development, the rapid translational activation of β-F1-ATPase mRNA (39), used as a reference marker of a nucleus-encoded transcript responsible for the development of mitochondrial oxidative phosphorylation. We report that, remarkably, the 3′ UTR of β-F1-ATPase mRNA is an essential *cis*-acting element required for in vitro translation of the transcript (Fig. 5). When this element is placed in *cis* on other mRNAs, the synthesis of the reporter protein is significantly increased (Fig. 6). In addition, the results show that translational control of β-F1-ATPase mRNA during liver development is apparently exerted by *trans*-acting inhibitory proteins of the liver (Fig. 4 and 6) that bind the 3′ UTR of the mRNA (Fig. 6 to 8). This study is the first to highlight such a mechanism of translational regulation for controlling the biogenesis of mammalian mitochondria, a mechanism that might be also operative in other mammalian cell types of the adult animal (Fig. 9).

One of the best-characterized mechanisms of cytoplasmic regulation of the expression of certain transcripts during early development is the control of their poly(A)$^+$ tail lengths (31, 55, 70, 72). In most reported cases, an increase in the length of the poly(A)$^+$ tail is associated with an increase in translation (27, 43, 49, 57), whereas in other situations, translational activation has been associated with deadenylation of the transcript (5, 18, 34). The lack of major differences in the poly(A)$^+$ tracks of β-F1-ATPase mRNA during liver development (Fig. 1) suggests that the translation control experienced by this transcript (30, 39) is exerted independently of the length of the poly(A)$^+$ tail. The minor changes in the lengths of the longest poly(A)$^+$ tails that might accompany the transition from the fetal to neonatal stage of development may result from the activation of the translation and subsequent degradation of the transcript (31, 61).

It has been recently reported that cytochrome *c* mRNAs have different translational efficiencies as a result of different 5′ UTRs of the mRNAs (73). Heterogeneity in this part of the cytochrome *c* transcripts is correlated with the existence of multiple transcription start sites on the promoter of the gene, most likely because it lacks a TATA box (73). Many nuclear genes encoding mitochondrial proteins, including the human β-F1-ATPase gene (44, 45), are devoid of TATA-containing promoters. Thus, it is conceivable that developmental regulation of the site of transcription initiation in the promoters of these genes could contribute to the regulated expression of the resulting transcripts by their different 5′ UTRs. However, the finding of the lack of heterogeneity in the 5′ UTR of rat β-F1-ATPase mRNA during liver development strongly suggests that this mechanism of translational regulation is not operative for the expression of β-F1-ATPase mRNA.

Several examples in which mRNA-RNA interactions are able to modulate translation of specific mRNAs have been provided (25, 33, 36, 52, 53, 63, 64, 69, 71). Interestingly, many of the inhibitory RNAs have been characterized from embryonic sources (reference 69 and references therein). Perhaps the best characterized are certain antisense RNAs, whose mechanism of action involves the hindering of translation by hybridization to regulatory regions of the sense mRNA, such as those recently described involved in the controlled expression of Lin-14 during *Caenorhabditis elegans* development (36, 71). We previously provided evidence suggesting that nonpolyadenylated RNAs were not involved in controlling β-F1-ATPase mRNA translation (39). The absence of β-F1-ATPase mRNA fragments, protected from degradation with RNases when incubated in the presence of poly(A)$^+$ RNA fractions (Fig. 3), appears to exclude a role for antisense mRNAs in controlling the translation of β-F1-ATPase mRNA during liver development.

Overall, it appears that translational control of β-F1-ATPase

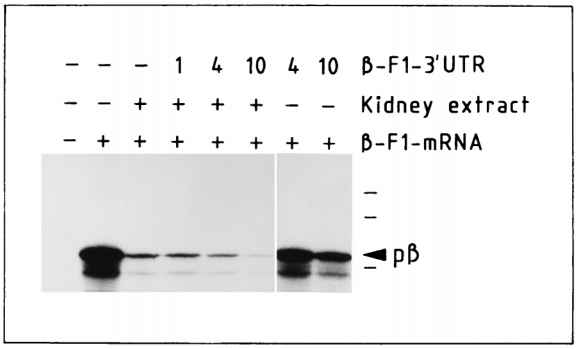


FIG. 12. The 3′ UTR of β-F1-ATPase mRNA inhibits in *trans* the translation of β-F1-ATPase mRNA. The 3′ UTR of β-F1-ATPase mRNA was synthesized from pJMI3′UTRβ digested with the *Hin*dIII restriction endonuclease. Full-length β-F1-ATPase mRNA (β-F1-mRNA) (500 ng) was translated in the presence (+) or absence (−) of 25 μg of kidney extract plus or minus increasing amounts of the 3′ UTR of β-F1-ATPase mRNA. The numerals 1, 4, and 10 indicate the molar ratio between the 3′ UTR and β-F1-ATPase mRNA. The arrowhead indicates the migration of the synthesized β-subunit precursor (pβ). Molecular mass markers (69, 46, and 30 kDa, from top to bottom) are shown on the right.

mRNA during liver development and, thus, rapid postnatal mitochondrial differentiation (68), is mechanistically closer to the examples provided by the expression of protamine 2 during spermiogenesis (35), the expression of fibroblast growth factor receptor 1 during meiotic maturation of frog oocytes (56), the repression of maternal mRNAs by FRGY2 in *Xenopus* oocytes (40), and the stage-specific control of 15-lypoxygenase expression during erythroid cell differentiation (46). However, it should be stressed that, in addition to this type of regulation, the expression of β-F1-ATPase mRNA also shows similarities with enhancing elements found in the 3′ UTR of certain viral mRNAs (16, 24).

No definitive indication has been provided yet to explain the mechanistic cross talk between the 5′ and 3′ ends of an mRNA in translational control (21, 22, 65–67). The findings reported herein regarding (i) the translational stimulatory effect of the 3′ UTR of β-F1-ATPase mRNA (Fig. 5 and 6), (ii) the inhibition of synthesis of the β-precursor (Fig. 4) and of ARF from the chimera (Fig. 6) in the presence of fetal liver extracts, (iii) the identification of a developmentally regulated protein that binds the 3′ UTR of β-F1-ATPase mRNA (Fig. 7), and (iv) the copurification and correlation existing between the binding and translational inhibitory activities (Fig. 8, 9, and 11) strongly suggest that translational control of the transcript in the liver, and perhaps in other mammalian tissues (Fig. 9), is exerted by the *trans*-acting inhibitory protein(s) that blocks the essential functional role of the 3′ UTR in translation upon interaction with the binding site on the 3′ regulatory element (Fig. 10 and 11). This is in line with previous suggestions by others (14, 25, 41).

We should stress that, remarkably, β-F1-ATPase mRNA is localized in certain electron-dense structures of the hepatocyte (20). These structures have been shown to contain 60S ribosomal proteins as well as molecular chaperones (54). Likewise, the stability of β-F1-ATPase mRNA is controlled throughout liver development (30). Most *cis*-acting elements involved in mRNA localization and control of the stability of eukaryotic mRNAs have been mapped onto the 3′ UTR of the mRNAs (6, 12, 14, 17, 25, 41, 59). Therefore, it may not be surprising if, within the conserved 3′ UTR of β-F1-ATPase mRNA (Fig. 10), there is a certain degree of overlapping of *cis*-acting elements involved in controlling the translation, stability, and subcellular localization of the transcript. This is compatible with the finding that a full-length 3′ UTR of the transcript is required to confer complete translational efficiency to β-F1-ATPase mRNA (Fig. 5).

In recent years, several RNA binding proteins that are able to repress translation of specific mRNAs in response to different morphogenetic, developmental, and/or metabolic responses of the organism have been identified (for reviews see references 14, 25, and 41). These proteins bind *cis*-acting elements located in both the 5′ and 3′ UTRs of the mRNAs. Our findings (Fig. 10 and 11) strongly suggest that the highly conserved AU-rich region (77%) present in the last 26 nucleotides of the 3′ UTR of β-F1-ATPase mRNA is involved in the binding of the developmentally regulated and tissue-specifically expressed inhibitory protein. Interestingly, this region of β-F1-ATPase mRNA is highly conserved within the 3′ end of the 3′ UTR of many other mammalian nucleus-encoded mitochondrial proteins (32), which might suggest a broader functional role for this inhibitory activity in exerting concerted translational control of the cytoplasmic expression of the genes involved in mitochondrial function.

The abrupt developmental decrease in the activity of the β-F1-ATPase mRNA binding protein (Fig. 7) strongly suggests that either the protein itself or its binding activity is acutely regulated during development. Regulation of the RNA binding activity of the protein may be exerted by specific covalent modification and/or interaction of the protein with other regulated components of the liver as a result of undetermined mRNA unmasking signals. Examples of both types of regulatory mechanisms for promoting the rapid inactivation of mRNA-inhibitory proteins have already been provided in other cellular systems (15, 26, 35, 60). However, it is also possible that acute regulation of the expression of the gene encoding the putative inhibitory protein may also accomplish the same rapid regulation of the translational efficiency of the transcript during development. The different translational efficiencies of β-F1-ATPase mRNA, manifested in in vitro translations of fetal and neonatal rat liver RNA fractions (39), seem to point in this direction.

Recently, biochemical evidence in yeast illustrating that the poly(A)$^+$ tails of the mRNAs interact with the 5′ cap structure through the translational initiation factor eIF-4G has been provided (67). Based on the proximity for both mRNA ends suggested by these results (67) and on our observation that the 3′ UTR of β-mRNA (Fig. 4B and 12) inhibits translation of other mRNAs, apparently by competition for the available

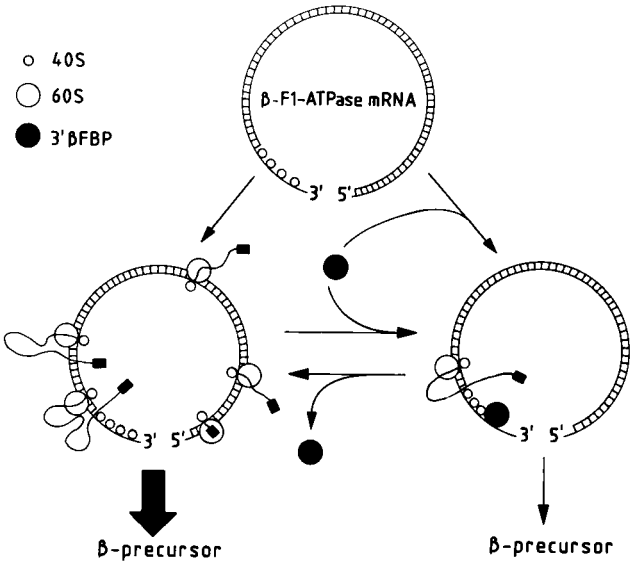


FIG. 13. Schematic illustration of the putative mechanisms controlling the translation of β-F1-ATPase mRNA. The 3′ UTR of β-F1-ATPase mRNA is required for efficient translation of the transcript (Fig. 5). When the 3′ UTR of the transcript is placed downstream of a reporter, it acts as a translational enhancer (Fig. 6). Presumably, its effect is exerted by interaction with components of the translational machinery (40S ribosomal subunit) because the translation of transcripts containing the 3′ UTR is preferential over transcripts lacking the 3′ UTR (Fig. 4B). Besides, when the 3′ UTR is added in *trans* to the translation assay, it inhibits the synthesis of the β-subunit precursor in both the absence and presence of the inhibitor protein (3′βFBP) (Fig. 12). In the absence of 3′βFBP (the left side of the schematic), the translation of the transcript is very efficient because the cross talk between the 3′ and 5′ ends of the mRNA is not impeded. This situation in the transcript resembles that of the translational efficiency of β-F1-ATPase mRNA in the neonatal liver, characterized by an increased output of β-subunit precursor concurrent with differentiation of liver organelles. In the presence of 3′βFBP (the right of the schematic), the translation of the transcript is very inefficient because the cross talk between the 3′ and 5′ ends of the mRNA is presumably hampered by steric constraints imposed by the binding of 3′βFBP. In both situations, the 3′ UTR is still able to interact with components of the translational machinery (Fig. 12 and reference 54). This situation in the transcript resembles that of the translational efficiency of β-F1-ATPase mRNA in the fetal liver, characterized by low output of β-subunit precursor. Regulation of the binding activity and/or expression of 3′βFBP allows a rapid switch in the translational efficiency of the transcript.

translational machinery, we suggest the following working hypothesis (Fig. 13). The 3′ UTR of β-F1-ATPase mRNA acts as a translation enhancer by promoting the recruitment of the translational machinery for providing reinitiation events due to the physical proximity and cross talk of both mRNA ends. In the absence of the inhibitor protein, the mRNA is efficiently translated, providing the required β-precursor (Fig. 13). However, in the presence of the inhibitor (Fig. 13), β-F1-ATPase mRNA translation is carried out inefficiently because the binding of the repressor to the most 3′ end of the 3′ UTR of the mRNA sterically represses the reinitiation events and, therefore, the amount of β-precursor synthesized is much lower. It is obvious that future investigations are required to elucidate the posttranscriptional mechanisms that control the expression of this essential gene of mitochondrial oxidative phosphorylation. In this regard, the characterization of the mechanisms by which the 3′ UTR interacts with the translational machinery and the identification of 3′βFBP deserve the most attention.

ACKNOWLEDGMENTS

P. L. Pedersen and A. Alconada are gratefully acknowledged for providing the partial rat liver β-F1-ATPase and bovine ARF cDNAs used in this study. We are grateful to J. Satrústegui for critical review of the manuscript. We thank L. K. Ostronoff for the preparation and fractionation of liver extracts and M. Chamorro and D. Jelenic for technical and secretarial assistance, respectively.

This work was supported by grant PB94-0159 from Dirección General de Investigación Científica y Técnica and by an institutional grant from Fundación Ramón Areces, Spain.

REFERENCES

1. **Aprille, J. R.** 1986. Perinatal development of mitochondria in rat liver, p. 66–99. *In* G. Fiskum (ed.), Mitochondrial physiology and pathology. Van Nostrand Reinhold Co., Inc., New York, N.Y.
2. **Attardi, G., and G. Schatz.** 1988. Biogenesis of mitochondria. Annu. Rev. Cell Biol. **4:**289–333.
3. **Bachvarova, R. F.** 1992. A maternal tail of poly(A): the long and the short of it. Cell **69:**895–897.
4. **Baker, C., D. Holland, M. Edge, and A. Colman.** 1990. Effects of oligo sequence and chemistry on the efficiency of oligodeoxyribonucleotide-mediated mRNA cleavage. Nucleic Acids Res. **18:**3537–3543.
5. **Ballantine, J. E. M., and H. R. Woodland.** 1985. Polyadenylation of histone mRNA in *Xenopus* oocytes and embryos. FEBS Lett. **180:**224–228.
6. **Beck, S. C., and A. De Maio.** 1994. Stabilization of protein synthesis in thermotolerant cells during heat shock. Association of heat shock protein-72 with ribosomal subunits of polysomes. J. Biol. Chem. **269:**21803–21811.
7. **Boorstein, W. R., and E. A. Craig.** 1989. Primer extension analysis of RNA. Methods Enzymol. **180:**347–369.
8. **Boulet, D., J. Poirier, and C. Coté.** 1989. Studies on the biogenesis of the mammalian ATP synthase complex: isolation and characterization of a full-length cDNA encoding the rat F_1-β-subunit. Biochem. Biophys. Res. Commun. **159:**1184–1190.
9. **Bouvet, P., and A. P. Wolffe.** 1994. A role for transcription and FRGY2 in masking maternal mRNA within Xenopus oocytes. Cell **77:**931–941.
10. **Breen, G. A. M., P. L. Holmans, and K. E. Garnett.** 1988. Isolation and characterization of a complementary DNA for the nuclear-coded precursor of the β-subunit of bovine mitochondrial F1-ATPase. Biochemistry **27:**3955–3961.
11. **Cantatore, P., P. L. Polosa, F. Fracasso, Z. Flagella, and M. N. Gadaleta.** 1986. Quantitation of mitochondrial RNA species during rat liver development: the concentration of cytochrome oxidase subunit I (Col) mRNA increases at birth. Cell Differ. **19:**125–132.
12. **Chen, C.-Y. A., and A.-B. Shyu.** 1995. AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem. Sci. **20:**465–470.
13. **Cuezva, J. M., C. Valcarce, A. M. Luis, J. M. Izquierdo, A. Alconada, and M. Chamorro.** 1990. Postnatal mitochondrial differentiation in the newborn rat, p. 113–135. *In* J. M. Cuezva, A. M. Pascual-Leone, and M. S. Patel (ed.), Endocrine and biochemical development of the fetus and neonate. Plenum Press, New York, N.Y.
14. **Curtis, D., R. Lehmann, and P. D. Zamore.** 1995. Translational regulation in development. Cell **81:**171–178.
15. **Danon, A., and S. P. Mayfield.** 1994. ADP-dependent phosphorylation regulates RNA-binding *in vitro*: implications in light-modulated translation. EMBO J. **13:**2227–2235.
16. **Danthinne, X., J. Seurinck, F. Meulewaeter, M. Van Montagu, and M. Cornelissen.** 1993. The 3′ untranslated region of satellite tobacco necrosis virus RNA stimulates translation in vitro. Mol. Cell. Biol. **13:**3340–3349.
17. **Decker, C. J., and R. Parker.** 1995. Diversity of cytoplasmic functions for the 3′ untranslated region of eukaryotic transcripts. Curr. Opin. Cell Biol. **7:**386–392.
18. **Dehlin, E., A. von Gabain, G. Alm, R. Dingelmaier, and O. Resnekov.** 1996. Repression of beta interferon gene expression in virus-infected cells is correlated with a poly(A) tail elongation. Mol. Cell. Biol. **16:**468–474.
19. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
20. **Egea, G., J. M. Izquierdo, J. Ricart, C. San Martín, and J. M. Cuezva.** 1997. mRNA encoding the β-subunit of the mitochondrial F1-ATPase complex is a localized mRNA in rat hepatocytes. Biochem. J. **322:**557–565.
21. **Gallie, D. R.** 1991. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. Genes Dev. **5:**2108–2116.
22. **Gallie, D. R., and R. Tanguay.** 1994. Poly(A) binds to initiation factors and increases cap-dependent translation *in vitro*. J. Biol. Chem. **269:**17166–17173.
23. **Garboczi, D. N., A. H. Fox, S. L. Gerring, and P. L. Pedersen.** 1988. β subunit of rat liver mitochondrial ATP synthase: cDNA cloning, amino acid sequence, expression in *Escherichia coli*, and structural relationship to adenylate kinase. Biochemistry **27:**553–560.
24. **Hann, L. E., A. C. Webb, J.-M. Cai, and L. Gehrke.** 1997. Identification of a competitive translation determinant in the 3′ untranslated region of alfalfa mosaic virus coat protein mRNA. Mol. Cell. Biol. **17:**2005–2013.
25. **Hentze, M. W.** 1995. Translational regulation: versatile mechanisms for metabolic and developmental control. Curr. Opin. Cell Biol. **7:**393–398.
26. **Hentze, M. W., S. W. Caughman, T. A. Rouault, J. G. Barriocanal, A. Dancis, J. B. Harford, and R. D. Klausner.** 1987. Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. Science **238:**1570–1573.
27. **Huarte, J., D. Belin, A. Vassalli, S. Strickland, and J.-D. Vassalli.** 1987. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. Genes Dev. **1:**1201–1211.
28. **Izquierdo, J. M., and J. M. Cuezva.** 1993. Evidence of post-transcriptional regulation in mammalian mitochondrial biogenesis. Biochem. Biophys. Res. Commun. **196:**55–60.
29. **Izquierdo, J. M., A. M. Luis, and J. M. Cuezva.** 1990. Postnatal mitochondrial differentiation in rat liver. Regulation by thyroid hormones of the β-subunit of the mitochondrial F1-ATPase complex. J. Biol. Chem. **265:**9090–9097.
30. **Izquierdo, J. M., J. Ricart, L. K. Ostronoff, G. Egea, and J. M. Cuezva.** 1995. Changing patterns of transcriptional and post-transcriptional control of β-F1-ATPase gene expression during mitochondrial biogenesis in liver. J. Biol. Chem. **270:**10342–10350.
31. **Jackson, R. J., and N. Standart.** 1990. Do the poly(A) tail and 3′ untranslated region control mRNA translation? Cell **62:**15–24.
32. **Juretic, N., and M. Theus.** 1991. Analysis of the polyadenylation consensus sequence context in the genes of nuclear encoded mitochondrial proteins. FEBS Lett. **290:**4–8.
33. **Kleene, K., and J. Flynn.** 1987. Translation of mouse testis poly $(A)^+$ mRNAs for testis specific protein, protamine-1, and the precursor for protamine 2. Dev. Biol. **123:**125–135.
34. **Kleene, K. C.** 1989. Poly(A) shortening accompanies the activation of translation of five mRNAs during spermiogenesis in the mouse. Development **106:**367–373.
35. **Kwon, Y. K., and N. B. Hecht.** 1993. Binding of a phosphoprotein to the 3′ untranslated region of the mouse protamine 2 mRNA temporally represses its translation. Mol. Cell. Biol. **13:**6547–6557.
36. **Lee, R. C., R. L. Feinbaum, and V. Ambros.** 1993. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell **75:**843–854.
37. **Levy, M., and R. Toury.** 1970. Study of biogenesis of mitochondrial enzymes of the hepatocyte during development of rat. Bull. Soc. Chim. Biol. **52:**578–580.
38. **Lithgow, T., J. M. Cuezva, and P. A. Silver.** 1997. Highways for protein delivery to the mitochondria. Trends Biochem. Sci. **22:**110–113.
39. **Luis, A. M., J. M. Izquierdo, L. K. Ostronoff, M. Salinas, J. F. Santaren, and J. M. Cuezva.** 1993. Translational regulation of mitochondrial differentiation in neonatal rat liver. Specific increase in the translational efficiency of the nuclear-encoded mitochondrial β-F1-ATPase mRNA. J. Biol. Chem. **268:**1868–1875.
40. **Matsumoto, K., F. Meric, and A. P. Wolffe.** 1996. Translational repression dependent on the interaction of the *Xenopus* Y-box protein FRGY2 with mRNA. J. Biol. Chem. **271:**22706–22712.
41. **McCarthy, J. E. G., and H. Kollmus.** 1995. Cytoplasmic mRNA-protein interactions in eukaryotic gene expression. Trends Biochem. Sci. **20:**191–197.
42. **Meric, F., A. M. Searfoss, M. Wormington, and A. P. Wolffe.** 1996. Masking and unmasking maternal mRNA. The role of polyadenylation, transcription, splicing, and nuclear history. J. Biol. Chem. **271:**30804–30810.
43. **Muschel, R., G. Khoury, and L. M. Reid.** 1986. Regulation of insulin mRNA

abundance and adenylation: dependence on hormones and matrix substrate. Mol. Cell. Biol. **6:**337–341.
44. **Neckelmann, N., C. K. Warner, A. Chung, J. Kudoh, S. Minoshima, R. Fukuyama, M. Maekawa, Y. Shimizu, N. Shimizu, J. D. Liu, and D. C. Wallace.** 1989. The human ATP synthase β subunit gene: sequence analysis, chromosome assignment, and differential expression. Genomics **5:**829–843.
45. **Ohta, S., H. Tomura, K. Matsuda, and Y. Kagawa.** 1988. Gene structure of the human mitochondrial adenosine triphosphate synthase β subunit. J. Biol. Chem. **263:**11257–11262.
46. **Ostareck-Lederer, A., D. H. Ostareck, N. Standart, and B. J. Thiele.** 1994. Translation of 15-lipoxygenase mRNA is inhibited by a protein that binds to a repeated sequence in the 3′ untranslated region. EMBO J. **13:**1476–1481.
47. **Ostronoff, L. K., J. M. Izquierdo, and J. M. Cuezva.** 1995. mt-mRNA stability regulates the expression of the mitochondrial genome during liver development. Biochem. Biophys. Res. Commun. **217:**1094–1098.
48. **Ostronoff, L. K., J. M. Izquierdo, J. A. Enríquez, J. Montoya, and J. M. Cuezva.** 1996. Transient activation of mitochondrial translation regulates the expression of the mitochondrial genome during mammalian mitochondrial differentiation. Biochem. J. **316:**183–191.
49. **Paek, I., and R. Axel.** 1987. Glucocorticoids enhance stability of human growth hormone mRNA. Mol. Cell. Biol. **7:**1496–1507.
50. **Pain, V. M.** 1996. Initiation of protein synthesis in eukaryotic cells. Eur. J. Biochem. **236:**747–771.
51. **Pollak, J. K.** 1975. The maturation of inner membrane of fetal mitochondria. Biochem. J. **150:**477–488.
52. **Rastinejad, F., and H. M. Blau.** 1993. Genetic complementation reveals a novel regulatory role for 3′ untranslated regions in growth and differentiation. Cell **72:**903–917.
53. **Rastinejad, F., M. J. Conboy, T. A. Rando, and H. M. Blau.** 1993. Tumor suppression by RNA from the 3′ untranslated region of α-tropomyosin. Cell **75:**1107–1117.
54. **Ricart, J., G. Egea, J. M. Izquierdo, C. San Martín, and J. M. Cuezva.** 1997. The subcellular structure containing β-F1-ATPase mRNA in rat hepatocytes is translationally active. Biochem. J. **324:**635–643.
55. **Richter, J. D.** 1991. Translational control during early development. Bioessays **13:**179–183.
56. **Robbie, E. P., M. Peterson, E. Amaya, and T. J. Musci.** 1995. Temporal regulation of the *Xenopus* FGF receptor in development: a translation inhibitory element in the 3′ untranslated region. Development **121:**1775–1785.
57. **Robinson, B. G., D. M. Frim, W. J. Schwartz, and J. A. Majzoub.** 1988. Vasopressin mRNA in the suprachiasmatic nuclei: daily regulation of polyadenylate tail length. Science **241:**342–344.
58. **Rohr, H. P., A. Wirz, L. C. Henning, V. N. Riede, and L. Bianchi.** 1971. Morphometric analysis of the rat liver cell in the perinatal period. Lab. Invest. **24:**128–139.
59. **Ross, J.** 1996. Control of messenger RNA stability in higher eukaryotes. Trends Genet. **12:**171–175.
60. **Rouault, T. A., C. D. Stout, S. Kaptain, J. B. Harford, and R. D. Klausner.** 1991. Structural relationship between an iron-regulated RNA-binding protein (IRE-BP) and aconitase: functional implications. Cell **64:**881–883.
61. **Sachs, A., and E. Whale.** 1993. Poly(A) metabolism and function in eukaryotes. Cell **268:**22955–22958.
62. **Santarén, J. F., A. Alconada, and J. M. Cuezva.** 1993. Examination of processing of the rat liver mitochondrial F1-ATPase β subunit precursor protein by high-resolution 2D-gel electrophoresis. J. Biochem. **113:**129–131.
63. **Sarkar, S.** 1984. Translational control involving a novel cytoplasmic RNA and ribonucleoprotein. Prog. Nucleic Acid Res. Mol. Biol. **31:**267–293.
64. **Strickland, S., J. Huarte, D. Belin, A. Vassalli, R. J. Rickles, and J.-D. Vassalli.** 1988. Antisense RNA directed against the 3′ noncoding region prevents dormant mRNA activation in mouse oocytes. Science **241:**680–684.
65. **Tanguay, R. L., and D. R. Gallie.** 1996. Translational efficiency is regulated by the length of the 3′ untranslated region. Mol. Cell. Biol. **16:**146–156.
66. **Tarun, S. Z., Jr., and A. B. Sachs.** 1995. A common function for mRNA 5′ and 3′ ends in translation initiation in yeast. Genes Dev. **9:**2997–3007.
67. **Tarun, S. Z., Jr., and A. B. Sachs.** 1996. Association of the yeast poly(A) tail binding protein with translation initiation factor eIF-4G. EMBO J. **15:**7168–7177.
68. **Valcarce, C., R. M. Navarrete, P. Encabo, E. Loeches, J. Satrustegui, and J. M. Cuezva.** 1988. Postnatal development of rat liver mitochondrial functions. The roles of protein synthesis and of adenine nucleotides. J. Biol. Chem. **263:**7767–7775.
69. **Vanderburg, C. R., and M. A. Nathanson.** 1988. Posttranscriptional control of embryonic rat skeletal muscle protein synthesis. Control at the level of translation by endogenous RNA. J. Cell Biol. **107:**1085–1097.
70. **Wickens, M.** 1990. In the beginning is the end: regulation of poly(A) addition and removal during early development. Trends Biochem. Sci. **15:**320–324.
71. **Wightman, B., I. Ha, and G. Ruvkun.** 1993. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. Cell **75:**855–862.
72. **Wormington, M.** 1993. Poly(A) and translation: development control. Curr. Opin. Cell Biol. **5:**950–954.
73. **Yiu, G. K., W. Gu, and N. B. Hecht.** 1994. Heterogeneity in the 5′ untranslated region of mouse cytochrome c_T mRNAs leads to altered translational status of the mRNAs. Nucleic Acids Res. **22:**4599–4606.
74. **Zaidi, S. H. E., and J. S. Malter.** 1994. Amyloid precursor protein mRNA stability is controlled by a 29-base element in the 3′-untranslated region. J. Biol. Chem. **269:**24007–24013.