The Expression of Poly(A)-binding Protein Gene Is Translationally Regulated in a Growth-dependent Fashion through a 5'-Terminal Oligopyrimidine Tract Motif*

(Received for publication, September 10, 1998, and in revised form, October 20, 1998)

Eran Hornstein‡, Anna Git§, Ilana Braunstein¶, Dror Avni§, and Oded Meyuhas**

From the Department of Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

Poly(A)-binding protein (PABP) is an important regulator of gene expression that has been implicated in control of translation initiation. Here we report the isolation and the initial structural and functional characterization of the human PABP gene. Delineation of the promoter region revealed that it directs the initiation of transcription at consecutive C residues within a stretch of pyrimidines. A study of the translational behavior of the corresponding mRNA demonstrates that it is translationally repressed upon growth arrest of cultured mouse fibroblasts and translationally activated in regenerating rat liver. Furthermore, transfection experiments show that the first 32 nucleotides of PABP mRNA are sufficient to confer growth-dependent translational control on a heterologous mRNA. Substitution of the C residue at the cap site by purines abolishes the translational control of the chimeric mRNA. These features have established PABP mRNA as a new member of the terminal oligopyrimidine tract mRNA family. Members of this family are known to encode for components of the translational apparatus and to contain an oligopyrimidine tract at the 5' terminus (5'TOP). This motif mediates their translational control in a growth-dependent manner.

PABP1 is the major cytoplasmic RNA-binding protein in eukaryotes that exhibits a preferential affinity for poly(A). This highly conserved protein has been implicated in regulating the initiation of translation (11, 2) and references therein), mRNA stability (3), regulation of poly(A) tail length during the polyadenylation reaction (4, 5), or poly(A) shortening (6, 7).

Study of PABP gene expression in various vertebrates has established the respective mRNA as translationally controlled. Thus, serum stimulation of quiescent Swiss 3T3 cells seems to up-regulate the translation of PABP mRNA, as indicated by the resistance of the induction to actinomycin D treatment (8) and the lack of change in the level of PABP mRNA (9). Likewise, PABP mRNA is essentially sequestered in messenger ribonucleoprotein in quiescent duck reticulocytes (10), in mouse testis (11), and during early Xenopus embryogenesis (12).

TOP mRNAs encode for various components of the translational apparatus, like ribosomal proteins (rp) and elongation factors 1a and 2 (EF1a and EF2). These mRNAs are candidates for growth-dependent translational control mediated through a translational cis-regulatory element. This approximately 30-nucleotide-long element is composed of a cytidine residue at the cap site followed by an uninterrupted stretch of up to 13 pyrimidines (13–15) and sequences immediately downstream (16, 17).

Growth-dependent translational control of an mRNA generally correlates with the presence of an oligopyrimidine stretch at its 5' terminus. Yet, the linkage between these functional and structural features is not an absolute one. Thus, human β-tubulin mRNA is refractory to growth arrest in all of the examined cell lines, although it contains a bona fide translational cis-regulatory element including a 5'TOP, which is able to confer translational control on heterologous mRNA (18). In contrast, the 6.0-kb transcript of insulin-like growth factor II is translationally regulated in a growth-dependent manner (19), yet it does not contain a 5'TOP (20). In light of these exceptions, the structural basis for the translational control of PABP mRNA could not be unequivocally predicted. Moreover, the cumulative experience in assessing the regulatory role of 5'TOP motifs in the expression of various TOP genes has underscored the prerequisite to delimit the corresponding promoter regions (16, 21–23). Hence, to establish the structural base for the translational behavior of PABP mRNA, we set out to clone and characterize the corresponding human gene. Our structural and functional analyses have established the transcription start site of PABP gene within a stretch of pyrimidine leading to the production of a TOP mRNA with an exceptionally long 5'-UTR. The translational efficiency of PABP mRNA tightly correlates with growth conditions of cultured fibroblasts as well as rat liver. The translational cis-regulatory element has been delimited to reside within the first 32 nucleotides of this mRNA and involves the 5'TOP motif.
Isolation of Genomic Clones—A human genomic library from W138 cell line of lung fibroblasts (Stratagene) was screened with a 32P-labeled probe (1588-bp Scar-I EcoRI fragment) derived from the human PABP cDNA (24). The initial screen of 2 × 10^6 plaques (one genome equivalence) yielded 33 positives, of which three different clones were isolated following a more stringent wash regime (0.3 M NaCl, 0.05 M sodium acetate at 67 °C) in the second and third cycles of plaque purification. Restriction enzyme mapping and sequencing of the corresponding inserts established that one is pseudogene 1 and the other two are processed pseudogenes 2 and 3. Processed pseudogene 2 contains the entire sequence of PABP cDNA, whereas the nucleotide sequence determined in processed pseudogene 3 corresponds to positions 1941 to 2548 in this cDNA. Pseudogene 1 contains a sequence that corresponds to nucleotides 1230 to 1953 of PABP cDNA and is flanked by a noncoding sequence.

A second screen of 8 × 10^6 plaques of the same library was carried out using replicate Nitran filters (Schleicher & Schuell) with either a 263-bp EcoRI-StuI 5'-terminal region of PABP cDNA or a 200-bp XhoI-PstI fragment derived from a region flanking the PABP insert in pseudogene 1. Sixteen positive clones were isolated, and their DNAs were subjected to restriction enzyme digestion and Southern blot hybridization with seven nonoverlapping probes spanning the entire PABP cDNA. Two overlapping phage clones were selected for further analysis: 3E, which hybridized with 5'-end and mid-region probes, and 60, which hybridized with mid-region and 3'-end probes.

After preliminary mapping with restriction enzymes, selected fragments were excised and subcloned into plasmid vectors. To determine the exon-intron junctions as well as the corresponding intron junctions, all exons were sequenced using primer walking. Upon identification of each exon-intron junction, a complementary primer was synthesized and used to sequence the opposite strand.

DNA Sequencing—Double-stranded plasmid DNA was sequenced either manually by the dideoxy method (25) using a Sequenase kit (U. S. Biochemical Corp., Cleveland, Ohio) or automatically by a DNA sequencing system (Applied Biosystems, Foster City, CA).

Primer Extension—Determination of the transcription start site in PABP cDNA (33), a 1.56-kb EcoRI fragment of the human PABP gene, was inserted between filled-in SacI site and SalI site of PABP cDNA. The resulting construct contains the PABP gene preceded by the human PABP sequence spanning positions 43 to +32.

The strategy used for the construction of pPABP-GH7 was similar to that described for pPABP-GH6 except for the usage of oligonucleotide PABP-42 (GCTTACAGGTGCGCGGCGGTT), which hybridized to nucleotides -43 to +28 of PABP gene and is flanked by an underlined XhoI recognition site and PABP-GH1 as a template. (ii) The PCR-generated fragment was cloned in blunt-ended and then cleaved from BamHI, and the resulting 80-bp fragment was cloned between the HindIII and BamHI sites of pG0H. The resulting construct contains the PABP gene preceded by a human PABP cDNA sequence spanning positions 43 to +32.

The strategy used for the construction of pPABP-GH8 was similar to that described for pPABP-GH6 except for the usage of oligonucleotide PABP-43 (GATGATCGCCGCGGGCCCGTT). Boldface letters correspond to nucleotides -165 to -141 of PABP gene and are preceded by an underlined XhoI recognition site instead of PABP-41. The resulting construct contains the PABP gene preceded by a human PABP cDNA sequence spanning positions 156 to +32.

Plasmid Constructions—Standard protocols were used for all recombinant DNA technology (30).

pPABP-GH1 was constructed through the following steps. (i) An 1.4-kb HindIII fragment, spanning positions -378 to +63 of human PABP gene, was inserted into EcoRI site of pUC18 to yield pPABP-5.1-f. (ii) A 450-bp fragment was generated by PCR (31) using oligonucleotide primers pUC-1(16) and PABP-8 (GGGGATCCACTCTCAGACTACC; boldface letters correspond to nucleotides +32 to +15 of PABP gene and are preceded by an underlined BamHI recognition site) and pPABP-5 fas a template. (iii) The PCR-generated fragment was cleaved by HindIII and BamHI. The resulting 410-bp fragment was cloned......
shown. Exons containing 5′ and 3′ splice sites are depicted as thin lines. b, an expanded map of the exons and adjacent intronic sequences are shown. Exons containing 5′- and 3′-UTRs as well as RNA binding domains (RBD 1 to 4) are marked.

RESULTS

Organization of the Human PABP Gene—A human genomic library in λ Fix vector was screened as described under “Experimental Procedures.” We isolated two overlapping clones, ΦE and ΦI (Fig. 1a), which collectively span an ~25-kb region that encompasses the PABP gene. Restriction fragment mapping and Southern blot analysis of the phages and various subclones together with DNA sequencing of selected segments disclosed the complete organization of the PABP gene (Fig. 1b). The gene consists of 15 exons totaling 2.86 kb and 14 introns covering about 22 kb. The sequences flanking the exon–intron junctions conform to the consensus for 5′ and 3′ splice sites (Table I).

The entire exonic sequence is 12 nucleotides longer than that of the published cDNA sequence (24). This dissimilarity is part of several differences summarized in Table II, of which the major one is an insertion of 9 nucleotides leading to the addition of 3 amino acids (Lys-Phε-Gly) following amino acid residue 212 in the published sequence (24). The sequence and the relative location of these three residues is identical to that found in mouse PABP protein and is similar to the corresponding sequence in Xenopus laevis protein (Table II). The isolated human gene encodes for a protein that is identical in size to that deduced from the mouse cDNA and differ by only three conservative replacements: Lys to Arg, Glu to Asp, and Ser to Thr at positions 176, 259, and 576, respectively (35). In light of the apparent evolutionary conservation of PABP amino acid sequence, it is conceivable that the missing three residues in the original report (24) is because of a sequencing error, whereas other differences might simply reflect polymorphism in the human population.

Interestingly, the PABP genes from Xenopus and human not only show a high degree of conservation of the amino acid sequence (93% of identical residues) but also an identical distribution of the four RNA binding domains among the different exons. Each of these functional domains are divided between two or three exons, which are the same exons in both species (Fig. 1a and Ref. 36).

5′ and 3′ Boundaries of the PABP Gene—Primer extension analysis of human PABP mRNA was carried out twice using lymphoblastoid cells. The extended DNA was electrophoresed on a sequencing gel alongside with sequencing reaction of either the cDNA clone or the pPABP-GH2. In both cases, they comigrated with nucleotides corresponding to position −2 and −1 with respect to the published sequence of human PABP cDNA (data not shown). Circumstantial evidence concerning the transcription start site has been obtained from sequence analysis of PABP processed pseudogene 2. This gene is flanked on both ends by direct repeats. It has been previously shown that in many cases the 5′ repeat is separated by one nucleotide from the established cap site (37, 38). Indeed, the 5′ repeat is located just three nucleotides upstream of the 5′-end of the cDNA (Fig. 2). Taken together, we have concluded that the transcription of PABP gene initiates at two consecutive C residues, and the resulting mRNA contains a 5′-terminal oligopyrimidine tract of 12 or 11 residues (Fig. 2). Like most other TOP mRNAs, the distal cap site is preceded by a few pyrimidine residues, and the entire pyrimidine tract is flanked by GC-rich sequences (13). It appears, therefore, that PABP mRNA is a bona fide TOP mRNA.

The 3′-end of the PABP gene has been determined to reside at the nucleotide immediately preceding the poly(A) tail in both the PABP cDNA and the processed pseudogene 3 (Fig. 2).

It should be noted that scanning of the promoter and the intronic as well as the 3′ flanking sequences of the human PABP gene against the EMBL data base has revealed significant homology with two ESTs. Thus, a sequence of the antisense strand within the promoter region (positions +98 to −205) show 94.3% homology with a rat ovary mRNA of unknown function (accession number AI176738). Similarly, a sequence of the antisense strand spanning the first 108 nucleotides of exon 15 and the last 132 nucleotides of intron 14 exhibits 96.6% homology with a human testis mRNA of unknown function (accession number AI140680). Conceivably, these sequences result from reverse transcription of PABP mRNA molecules derived by the rare usage of an alternative upstream transcription start site (AI176738) or of a splicing intermediate (AI140680).

Functional Characterization of the PABP Promoter—Conceivably, the 5′ TOP identified in the PABP gene might explain the previous observation concerning growth-dependent translational control of PABP mRNA. However, examining this possibility is based on assessment of the translational efficiency of chimeric mRNAs containing the 5′ TOP of PABP mRNA. Formation of an mRNA with an authentic PABP 5′ TOP requires the construction of a chimeric gene containing PABP promoter and the first transcribed nucleotides of PABP gene followed by the sequence of a reporter gene. Previous such analyses of several other TOP genes have disclosed critical transcriptional regulatory elements within the first exon or even the first intron (21). Hence, a prerequisite for functional analysis of the 5′ TOP element is a functional characterization of the promoter region. To this end, we first sequenced the promoter region from position −378 (Fig. 3). PABP promoter includes a canonical TATAA box spanning positions −30 to −26. In that respect it is similar to the promoters of EF1α and EF2 genes but differs from those of most rp genes (13). Next, we constructed various chimeras harboring the hGH gene preceded by sequences con-
taining portions of various lengths of the 5' flanking sequences and 5'-UTR of the PABP gene. These constructs were transiently transfected into HeLa cells, and their expression was assessed by monitoring both the relative abundance of the resulting transcripts and the amount of hGH secreted into the medium.

Fig. 4 shows that the promoter activity was not affected when the 5'-flanking sequence was shortened from 378 to 234 nt (PABP-GH3). It decreased about 2-fold when further shortened to 156 nt (PABP-GH7), dropped abruptly when it was decreased to 103 nt (PABP-GH6), and was completely abolished when only 43 nt were left (PABP-GH8). The complete loss of the promoter activity apparent in the last construct might reflect the omission of sequences perfectly matching the binding sites for ATF and SP1, two ubiquitous transcription factors (Fig. 3).

Sequential deletion of sequences within the 5'-flanking region decreased the promoter activity from 52 to 0.4-fold when 5' of the transcription initiation site was shortened from 486 nt (PABP-GH1) to 32 nt (PABP-GH11). A further decrease by 3- to 5-fold was detected upon complete deletion of transcribed PABP sequence (compare PABP-GH1 and PABP-GH8 in Fig. 4). It appears that a regulatory element residing within the first 32 nt of the transcribed region modulates the abundance of the hGH transcript in an orientation-dependent manner, as its reinsertion in opposite orientation into pPABP-GH8 does not affect its activity (compare PABP-GH11(as) and pPABP-GH8 in Fig. 4). These results suggest that PABP promoter extends into the transcribed region, as has been previously shown for several other TOP genes (21, 39). Yet, we cannot formally exclude the possibility that the first 32 nucleotides of PABP mRNAs play a role in stabilization of the transcript. Whatever the mechanism, based on these observations, we used in subsequent experiments a promoter region spanning positions −378 to −32 or further downstream.

The Translational cis-Regulatory Element of PABP mRNA Resides within the First 32 Nucleotides and Is 5' TOP-dependent—Analysis of the polysomal distribution of PABP mRNA in NIH 3T3 cells reveals that this mRNA is subject to translation control in a growth-dependent manner. Thus, PABP mRNA is mostly associated with polysomes in growing cells and sequenced in subpolysomal fraction (messenger ribonucleoprotein particles) upon growth arrest (Fig. 5, endogenous PABP). Pre-

---

**TABLE I**

**Organization of human PABP gene**

| Exon/Intron | Exon size bp | Sequences at Exon/Intron junctions | Intron size bp | Protein interruption | RNA binding domain |
|-------------|-------------|-----------------------------------|----------------|---------------------|-------------------|
| I/1         | 698         | ttcctgcaacG GAG CTT GGT GAT G AT | CCG GCC GAC Ggtgagcgccg | 4.8                | Ala-65            |
| I/2         | 194         | ttcctgcaacG GAG CTT GGT GAT G AT | T TCA TGT AAAGtagcgaa | 0.2                | Lys-129/Val-130   |
| III/3       | 116         | ttcctgcaacG GAG CTT GGT GAT G AT | T ATG CCA AAA GTgtagttata | 2.2               | Val-168            |
| IV/4        | 140         | atctattagA AT TTA GGT GGT GAT G AG | GCC AAG TTT Ggtgattgtc | 2.5                | Gly-215            |
| V/5         | 95          | aatcttttagGG CCT GCC TT T GCA CAG | T GCA CAG AAAGtagggtgc | 3.1                | Lys-246/Ala-247    |
| VI/6        | 138         | aatcttttagGG CCT GCC TT T GCA CAG | A AGA TAC CAGgtcattt | 0.2                | Gin-292/Gly-293    |
| VII/7       | 96          | ttcctgcaacG GAG CTT GGT GAT G AT | T AGT GCA AAAGtaggaaac | 1.2                | Lys-324/Val-325    |
| VIII/8      | 273         | ttcctgcaacG GAG CTT GGT GAT G AT | T GCA CCA AAA GTgagggtttta | 2.6               | Gin-415/Thr-416    |
| IX/9        | 9           | ttcctgcaacG GAG CTT GGT GAT G AT | C CA AAG tagggtacgtt | 2.1                | Pro-446/Phe-447    |
| X/10        | 111         | aaaatagcagCAT TTC AAA A CAG GGT GTT gtagcttta | 0.1                | Ala-483            |
| XI/11       | 155         | ttcctgcaacG GAG CTT GGT GAT G AT | T ATG CCA AAA GTgtaggttttt | 1.0               | Gin-534/Prol-535   |
| XII/12      | 85          | ttcctgcaacG GAG CTT GGT GAT G AT | CAA ATG TTG GTgagtcctt | 0.5                | Gly-563            |
| XIII/13     | 131         | ttcctgcaacG GAG CTT GGT GAT G AT | C GTG TCT AAAGtaggtttaaa | 0.5               | Lys-606/Val-607    |
| XIV/14      | 94          | ttcctgcaacG GAG CTT GGT GAT G AT | C ATG TTA AAgtaggctttttt | 1.0               |                 |
| XV          | 43          | acatctattagA AT TTA GGT GGT GAT G AG | | | |

**TABLE II**

**Sequence diversity between human PABP cDNA and gene**

| Position* | Region | Nucleotides in cDNA | Nucleotides in gene | Amino acids cDNA/gene | Amino acids in mouse (10) | Amino acids in Xenopus (75) |
|-----------|--------|---------------------|---------------------|-----------------------|--------------------------|---------------------------|
| -1, –2    | 5'-UTR | CC                  | CC                  | Lys-606/Val-607       | Lys-606/Val-607           |
| 479/480   | 5'-UTR | G                   | G                   | Lys-606/Val-607       | Lys-606/Val-607           |
| 922       | CS     | C                   | T                   | Tyr/Thr               | Tyr/Thr                  |
| 1137/1138 | CS     | TTTGGCAAG           | TTTGGCAAG           | LysPheGly             | LysPheGly                |
| 1775      | CS     | G                   | A                   | Val/Ile               | Ile                       |
| 2406      | 3'-UTR | A                   | A                   |                        |                          |
| 263/2622  | 3'-UTR | A                   | A                   |                        |                          |

* The coordinates refer to those published for the cDNA (accession number Y00345).

* The sign [ depicts a site of insertion.

---

**Fig. 2. The 3' and 5' boundaries of PABP gene.** Sequences at the 5' and 3' termini of human PABP cDNA were aligned with the corresponding genomic sequences of the active PABP gene, processed pseudogene 2, and processed pseudogene 3. White letters represent conserved nucleotides. Dotted sequences not included in this alignment, and boxed sequences are repetitive elements delimiting the processed pseudogene. The 5'-end was determined by primer extension analysis of PABP mRNA, and the 3'-end was determined by the nucleotide preceding the poly(A) sequence in both PABP cDNA and processed pseudogene 3.
vious attempts to delimit the translational ci-regulatory ele-
ment of various TOP mRNAs have shown that it is confined to
within their first 30 nt (16, 18, 23). However, the 5'-UTR of
PABP mRNA (505 nt long) is considerably larger than those of
other TOP mRNAs (an average of 40 nt). Hence, to delineate
the corresponding region in PABP mRNA, we set out to ex-
tamine the translational behavior of two mRNAs containing the
first 265 nt (PABP-GH2) or just 32 nt (PABP-GH1) of PABP
mRNA. A prerequisite for such an experiment with chimeric
TOP mRNAs is the establishment of the transcription start site
by primer extension analysis. Fig. 6 shows indeed that the
major cap sites of the mRNA encoded by PABP-GH1 are at two
consecutive C residues, coinciding with positions +2 and +3 in
the human PABP gene. Likewise, the same start sites were
identified for the PABP-GH2 construct, although they are em-
bedded within a much larger PABP sequence and their identi-
fication required the use of a different primer (Fig. 6).

Analysis of the polypoidal distribution of the mRNAs encod-
ing PABP-GH1 and PABP-GH2 demonstrates that they are
both translationally repressed upon growth arrest of NIH 3T3,
as do endogenous mRNAs encoding PABP and EF1α (Fig. 5).
These results indicate that the first 32 nucleotides of PABP
mRNA include all the regulatory elements required for confer-
ing growth-dependent translational control on a heterologous
mRNA. Our primer extension analyses have demonstrated that
endogenous PABP mRNA and mRNAs encoding PABP-GH1
and PABP-GH2 do not start at the same nucleotide. However,
in all cases, the major transcription start sites are at one or two
C residues within a stretch of four consecutive pyrimidines. To
examine whether substitution of the C residue at the cap site
by purines affects the translational control, we constructed the
PABP-GH11s gene. This gene is similar to that of PABP-GH1,
except for the replacement of the C residues at positions +1
and +2 by the tetranucleotide GGAT. This change leads to the
selection of the new A residue as the major transcription start
site (Fig. 6). The fact that the A at the cap site is followed by a
stretch of 11 pyrimidines is not sufficient to render this mRNA
translationally regulated (Fig. 5). It appears, therefore, that
translational control of PABP mRNA is strictly dependent on
the location of the oligopyrimidine tract at the very 5'-end.

PABP mRNA Is Translationally Regulated during Liver Re-
generation—To study the translational behavior of PABP
mRNA in whole animals, we exploited the fact that when the
liver reaches the adult stage it becomes quiescent, yet it retains
the capacity to resume proliferation after partial hepatectomy.
Hence, polysomes from sham-operated and regenerating liver
were size-fractionated by sucrose gradient centrifugation, and
the polysomal association of various mRNAs was assessed by
Northern blot analysis of each gradient fraction (Fig. 7). Our

FIG. 3. Nucleotide sequence of the promoter and the first exon
of PABP gene. The canonical TATA element is underlined, and the
consensus recognition sequence for transcription factors SP1 and ATF
are overlined. The transcription start sites are marked by arrows, and
the initiation codon is boxed. The sequence of exon 1 is framed, and the
first nucleotides of intron 1 are presented in lowercase letters. Left and
right brackets delimit sequences included in PABP-GH (PG) clones, and
the numbers refer to the specific chimeric clones schematically depicted
in Fig. 5.

FIG. 4. Delimitation of the PABP
gene promoter. HeLa cells were tran-
siently transfected with 4 μg of various
PABP-GH constructs and incubated for
24 h, and then the medium was replaced.
The amount of hGH secreted during the
next 3 h was measured in an aliquot of the
medium. The relative abundance of hGH
mRNA was assessed by Northern blot hy-
bridization of mRNAs extracted from the
cells immediately after aliquoting the
medium. The radioactive signals were
quantified by phosphorimaging. In the
schematic presentations of PABP-GH
constructs, the 5'-flanking sequence was
denoted as a thin line, and exon 1 was
denoted as a dotted box. The relative pro-
moter activity of the chimeric genes was
expressed either as the amount of se-
creted hGH or as the abundance of the
corresponding mRNA. The results were
normalized to those obtained for PABP-
GHS and are presented as average ± S.E.
of the number of measurements indicated
in parenthesis.
results demonstrate that the proportion of PABP mRNA associated with polysomes increased from 37% in sham-operated liver to 72% within 15 h after partial hepatectomy (Fig. 7). A similar recruitment into polysomes (from 40% in control to 88% in regenerating liver) is apparent also for EF1α mRNA, another TOP mRNA. In contrast, albumin mRNA was efficiently translated under both these conditions, despite a slight unloading from polysomes following the operation (from 94% in polysomes to 83%). It should be noted that the translational behavior of albumin mRNA is not exceptional, as any of the other four non-TOP mRNAs previously examined in the regenerating liver exhibited no translational activation (40).

**DISCUSSION**

Structural analysis of the human PABP gene as well as of chimeric genes driven by PABP promoter revealed that the transcription starts mainly at one or two C residues within an oligopyrimidine stretch. The presence of 12-nucleotide long TOP appears to provide the structural basis for its growth-dependent translational control in fibroblasts (Fig. 5 and Refs. 8 and 9) or adult liver (Fig. 7), and thus, has established PABP mRNA as a new member of the TOP mRNA family. Interestingly, PABP 5' TOP sequence is unique among the TOP mRNAs, as it contains an exceptionally high ratio of C to T (3 to 1). In comparison, the average ratio in 31 vertebrate rp mRNAs is 1.03 ± 0.08, and in five non-rp TOP mRNAs, it is 0.76 ± 0.19 (13). Whether this high C content has an additional regulatory role is yet to be determined. Nevertheless, shifting the first C of PABP 5' TOP into position +3 completely abolished the translational response to growth arrest (PABP-GH11s, Fig. 5). This is consistent with our previous report that the pyrimidine tract fails to exert its effect even when preceded by a single A residue (16). Formally, we cannot rule out the possibility that the loss of translational control is because of the concomitant shortening of the pyrimidine stretch from 12 to 11 residues. However, the latter explanation is less likely, as most 5' TOP motifs contain eight pyrimidines. Moreover, several 5' TOPs with just five pyrimidines have been shown to suffice the translational control mechanism (16–18).

RNA-protein binding experiments have recently provided some clues concerning putative specific trans-acting factors that might be involved in the translational control of TOP mRNAs (41–46). Nevertheless, the relevance of proteins that specifically bind to the 5' TOP or the adjacent downstream
sequences is still unclear, as the binding activity remains unchanged under various growth conditions, at which the translational efficiency of rp mRNAs is repressed or derepressed. In contrast, a recent report has demonstrated that direct interaction of repressor molecules with pyrimidine-rich sequences within the 3’ UTR of 15-lipoxygenase mRNA mediates its translational silencing (47).

Another putative component of the translational control mechanism, which has recently attracted much attention, is p70 S6 kinase (p70(s6k)). Numerous studies have shown that mitogenic or hormonal stimulations induce phosphorylation of rpS6 by this enzyme with a concomitant derepression of the translation of TOP mRNAs (48). Furthermore, inhibition of p70(s6k) by the immunosuppressant rapamycin or by expression of a dominant-negative p70(s6k) mutant selectively repressed the synthesis of oligonucleotides.

PABP cDNA and to Evelyne Segall and the late Elias Froimovitch for the provision of repressor molecules with pyrimidine-rich sequences therein). The identification of this motif led to the hypothesis of translational repression of PABP mRNA at the translational level through binding of the resulting protein to the 5’-UTR of PABP mRNA (505 nt) is substantially larger than the average length (44 ± 4) of this region in other 36 vertebrate TOP mRNAs rigorously analyzed (13). An intriguing possibility is that sequences downstream of the 5’ TOP serve an additional regulatory function(s). Indeed, human PABP mRNA contains an A-rich region spanning position 73 to 123, which is evolutionarily conserved from yeast (Ref. 51 and references therein). The identification of this motif led to the hypothesis that PABP mRNA is autogenously regulated at the translational level through binding of the resulting protein to the 5’-UTR (52). In vitro experiments have shown that in the addition of PABP to a cell-free translation system selectively inhibits the translation of PABP mRNA and that this repression is mediated through the A-rich region (51, 53). Nevertheless, inactivation of poly(A) polymerase in yeast, which is followed by the loss of poly(A) and consequently to an increase in the ratio of PABP to poly(A), had only a minor effect on the total level of PABP (54). The regulatory role, if any, of this conserved motif in mammalian cells has yet to be determined.

Acknowledgments—We are grateful to Thierry Grange for the human PABP cDNA and to Evelyne Segall and the late Elias Froimovitch for the synthesis of oligonucleotides.

REFERENCES

1. Sachs, A., Sarnow, P., and Hentze, M. (1997) Cell 89, 831–838
2. Craig, A., Haghighat, A., Yu, A., and Sonenberg, N. (1998) Nature 392, 520–523
3. Bernstein, P., Pelte, S. W., and Ross, J. (1989) Mol. Cell. Biol. 9, 659–670
4. Amriani, N., Minet, M., Le Gouar, M., Lacroix, F., and Wyers, F. (1997) Mol. Cell. Biol. 17, 3694–3701
5. Minvielle-Sebastia, L., Preker, P., Wiederkehr, T., Strahm, Y., and Keller, W. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6593–6597
6. Thomas, G., Thomas, G., and Luther, H. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5712–5716
7. Thomas, G., and Thomas, G. (1986) J. Cell Biol. 103, 2177–2144
8. Maundrell, K., Imaiizumi-Scherrer, M. T., Maxwell, E. S., Civelli, O., and Scherrer, K. (1988) J. Biol. Chem. 258, 1387–1390
9. Gu, W., Kwon, Y., Oko, R., Herms, L., and Hecht, N. B. (1995) Mol. Reprod. Dev. 40, 273–285
10. Zhang, B. D., Giebelhaus, D. M., El, W. D., Kenner, K. A., and Moon, R. T. (1989) Mol. Cell. Biol. 9, 2756–2760
11. Meyuhas, O., Avni, D., and Shama, S. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 363–384, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Uetsuki, T., Naito, A., Nagata, S., and Kaziro, Y. (1989) J. Biol. Chem. 264, 5791–5798
13. Nakanishi, T., Kohto, K., Ishiura, M., Ohashi, H., and Uchida, T. (1988) J. Biol. Chem. 263, 6384–6391
14. Avni, D., Shama, S., Lorenzi, F., and Meyuhas, O. (1994) Mol. Cell. Biol. 14, 3822–3833
15. Biberman, Y., and Meyuhas, O. (1997) FEBS Lett. 405, 333–336
16. Avni, D., Biberman, Y., and Meyuhas, O. (1997) Nucleic Acids Res. 25, 995–1001
17. Nielsen, F., Ostergaard, L., Nielsen, J., and Christiansen, J. (1997) Nature 377, 358–362
18. Raizus, A., Eccles, M., and Reeve, A. (1993) Biochem. J. 299, 133–139
19. Harharan, N., and Perry, R. P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1526–1530
20. Levy, S., Avni, D., Harharan, N., Perry, R. P., and Meyuhas, O. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3319–3323
21. Grange, T., de Sa, C. M., Odds, J., and Pietz, R. (1987) Nucleic Acids Res. 15, 4771–4786
22. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
23. Higgins, G. M., and Anderson, R. M. (1931) Arch. Pathol. 12, 186–202
24. Wieder, M., Searfoss, A. M., and Hurney, C. A. (1996) in Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Moleculer Cloning: A Laboratory Guide to RNA: Isolation, Analysis, and Synthesis (Krieg, P., ed) pp. 65–81, Wiley-Liss, Inc., New York
26. Scheller, U., Marcu, K. B., and Perry, R. P. (1997) Cell 85, 1495–1509
27. Meyuhas, O., Thompson, A. E., and Perry, R. P. (1987) Mol. Cell. Biol. 7, 2691–2699
28. Raizus, A., Eccles, M., and Reeve, A. (1993) Biochem. J. 299, 133–139
29. Meyuhas, O., Thompson, A. E., and Perry, R. P. (1987) Mol. Cell. Biol. 7, 2691–2699
30. Pellizzoni, L., Lotti, F., Maras, B., and Pierandrei-Amaldi, P. (1993) Nucleic Acids Res. 21, 2301–2308
31. Severson, W. P., Macek, I., and White, M. W. (1995) Eur. J. Biochem. 229, 426–432
32. Pellizzoni, L., Cardinali, D., Biswas, M., Mercanti, D., and Pierandrei-Amaldi, P. (1996) J. Mol. Biol. 259, 904–915
33. Pellizzoni, L., Lotti, F., Maras, B., and Pierandrei-Amaldi, P. (1997) J. Mol. Biol. 264, 267–275
34. Pellizzoni, L., Lotti, F., Ruitjes, S., and Pierandrei-Amaldi, P. (1998) J. Mol. Biol. 281, 563–608
35. Ostareck, D. H., Ostareck-Lederer, A., Wilm, M., Thiele, B. J., Mann, M., and Hentze, M. W. (1997) Cell 89, 597–606
36. Jefferies, H. B. J., and Thomas, G. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B., and Sonenberg, N., eds) pp. 389–409, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
37. Terada, N., Patel, H. R., Takase, K., Kohto, K., Nairn, A. C., and Gelfand, E. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11477–11481
38. Jefferies, H., Fumagalli, S., Dennis, P., Reinhard, C., Pearson, R., and Thomas, G. (1997) EMBO J. 16, 3693–3704
39. de Mello Neto, O., Standart, N., and de Sa, C. (1995) Nucleic Acids Res. 23, 2189–2205
40. Sachs, A., Bond, M., and Korenberg, R. (1986) Cell 45, 827–835
41. Bag, J., and Wu, J. (1996) Eur. J. Biochem. 247, 145–152
42. Proweller, A., and Butler, J. (1998) J. Biol. Chem. 273, 10859–10865