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The 5′ terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner

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ABSTRACT

TOP mRNAs are vertebrate transcripts which contain a 5′ terminal oligopyrimidine tract (5′ TOP), encode for ribosomal proteins and elongation factors 1α and 2, and are candidates for growth-dependent translational control mediated through their 5′ TOP. In the present study we show that elongation factor 2 (EF2) mRNA is translationally regulated in a growth-dependent manner in cells of hematopoietic origin, but not in any of three different non-hematopoietic cell lines studied. Human β1-tubulin mRNA is a new member of the family which contains all the hallmarks of a typical TOP mRNA, yet its translation is refractory to growth arrest of any of the examined cell lines. Transfection experiments indicate that the first 29 and 53 nucleotides of the mRNAs encoding EF2 and β1-tubulin, respectively, contain all the translational *cis*-regulatory elements sufficient for ubiquitously conferring growth-dependent translational control on a reporter mRNA. These results suggest that the distinct translational regulation of TOP mRNAs reflects downstream sequences which can override the regulatory features of the 5′ TOP in a cell type-specific manner. This notion is further supported by the fact that mutations within the region immediately downstream of the 5′ TOP of rpS16 mRNA confer onto the resulting transcripts growth-dependent translational control with a cell type specificity similar to that displayed by EF2 mRNA.

INTRODUCTION

The translation efficiency of mRNAs encoding many vertebrate proteins associated with the translational apparatus, such as ribosomal proteins (rps) (1) and elongation factors 1α (EF1α) and EF2 (2,3), is predominantly dependent on the cellular growth status. One common feature to all these mRNAs rigorously analyzed thus far, is the 5′ terminal oligopyrimidine tract (5′ TOP). This element is comprised of a cytidine residue at the cap site followed by an uninterrupted stretch of up to 13 pyrimidines (1,4,5) and is critical for the translational control as demonstrated for rp mRNAs (6). This mode of regulation strictly depends on the 5′ terminal location of the oligopyrimidine tract and the translational *cis*-regulatory element (TLRE) requires in addition the involvement of sequences immediately downstream of the 5′ TOP (7).

Previous studies have shown that rp mRNAs are translationally repressed upon growth arrest of any cell line examined (mammalian or amphibian) both in culture and *in vivo* [1 and references therein] and regardless of the mean used for halting cell proliferation. Assuming that such a general response is applicable for all TOP mRNAs we monitored the translational behavior of EF2 mRNA. Surprisingly, we repeatedly failed to demonstrate growth-dependent translational control of this mRNA in fibroblasts even though it had been previously shown to be translationally repressed in resting cells of hematopoietic origin (3,8). Likewise, we failed to show translational repression of human β1-tubulin upon growth arrest despite the fact that this mRNA appears to possess a typical 5′ TOP sequence (9). In the present report we show that: (i) the translational control of EF2 mRNA, unlike that of mRNAs encoding rps and EF1α, is confined to cells of hematopoietic origin; (ii) β1-tubulin mRNA has a bona fide TLRE, yet it does not confer a translational control when in its native context; (iii) the TLRE of both EF2 and β1-tubulin mRNAs are ubiquitously functional when linked to a reporter mRNA; and (iv) mutations within the TLRE of rpS16 mRNA can render an otherwise ubiquitously regulated transcript into one which exhibits cell type specificity similar to that of EF2 mRNA.

MATERIALS AND METHODS

Cell culture and DNA transfection

P1798.C7 mouse lymphosarcoma cells were grown as suspension cultures, arrested by treatment with 0.1 μM dexamethasone (Sigma) for 24 h and were transiently transfected by the DEAE–dextran method (7). NIH 3T3 mouse fibroblasts were grown as monolayer and arrested by 24 h treatment with 5 μg/ml aphidicolin (Sigma), a specific DNA polymerase inhibitor (7). NIH 3T3 cells were transiently or stably transfected by the DNA–calcium phosphate coprecipitation method and stable transfectants (polyclonal) were selected with geneticin (Sigma) (7). Friend mouse erythroleukemia clone 745 (MEL) (10) was grown and arrested as described (11). WHT 1249, a human cell

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line of Epstein–Barr virus-transformed lymphoblastoid and human skin fibroblasts were grown as described (7). Chinese hamster ovary (CHO-K1) cells were grown as monolayer culture in F-12 medium containing 5% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin, and arrested by 24 h treatment with 0.3 mM hydroxyurea. Human embryonic kidney 293 cells (12) and HeLa 229 cell line were grown as monolayer culture in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. The former were arrested by 24 h treatment with 5 μg/ml aphidicolin or 0.3 mM hydroxyurea and the latter by 24 h treatment with 25 μM nocodazol.

Primer extension

Determination of the transcription start site in hGH chimeric transcripts was carried out by primer extension as previously described (7).

DNA sequencing

Double-stranded plasmid DNA was sequenced by the dideoxy method (13) using a Sequenase kit (US Biochemical Corp., Cleveland, OH).

Polysomal fractionation and RNA analysis

Harvesting and lysis of cells as well as size fractionation of polysomes by sedimentation through sucrose gradients were performed as described (14). When polysomal gradients were divided into two fractions (polysomal and the subpolysomal) RNA was extracted from each fraction by RNAzol B (Biotecx Laboratories, Houston, TX) according to the supplier's instructions and the Poly(A)$^+$ mRNA was isolated as described (15). In all cases where polysomal gradients were divided into 12 fractions, RNA was extracted as described (16) and analyzed without further enrichment through oligo(dT) column. Northern blot analysis was performed as described (16). Quantification of the radioactive signals on the blots was carried out by a phosphorimager (Fujix BAS 1000, Fuji, Japan). To assess the effectiveness of the growth arrest treatment and the selectivity of the effect on rp mRNAs, we compared in each case (even if not shown) the polysomal association of a chimeric mRNA with that of endogenous rp mRNA and non-rp mRNA from the same polysomal gradient. Only experiments, in which both these controls exhibited their typical translational behavior (repressed and unrepressed, respectively) were included. A transcript has been considered translationally controlled if it is converted from mostly polysome-associated in growing cells into mostly subpolysome-associated in resting cells.

Plasmid constructions

Standard protocols were used for all recombinant DNA technology (17).

pEF2-GH was constructed through two steps: (i) a 464 bp *Bam*HI–*Kpn*I fragment spanning positions –335 to + 129 of Chinese hamster EF2 gene (5) was inserted in between the respective sites in pUC18 to yield pEF2a; (ii) a 394 bp *Hin*dIII–*Hae*II fragment containing EF2 sequence spanning positions –335 to +29 was excised from pEF2a and inserted after trimming (T4 DNA polymerase) the 5′ protruding end of the *Hae*II, in between *Hin*dIII and filled in *Sal*I sites of p0GH (18).

The pβ1Tub-GH chimera was constructed by digesting a subclone containing the 5′ region of the M40 gene, encoding the human β1-tubulin (9), by *Bgl*I. After blunting the ends with T4 DNA polymerase, the DNA was cut with *Hin*dIII and the resulting 0.75 kb fragment spanning positions –700 to +53 was inserted in between a *Hin*dIII site and filled in (Klenow enzyme) *Bam*HI site of p0GH.

pS16m(7–16)-GH was constructed through the following steps: (i) a synthetic oligonucleotide representing the –11 to +29 region of mouse rpS16 gene and containing substitution (AGCTGAAG-TC) of the sequence spanning positions +7 to +16 was used to replace the corresponding wild-type sequence (CCGGTCGCGG) in a construct of the rpS16 gene [c in ref. (19)] to yield pS16m(7–16); (ii) the rpS16 sequence spanning positions +30 to +2050 was excised from pS16m(7–16) by digestion with *Eco*RI and *Sac*I. The ends were made blunt by T4 DNA polymerase and ligated with the 2.1 kb *Bam*HI–*Eco*RI hGH gene fragment, which had been made blunt by filling in with Klenow enzyme.

The structure of all constructs described here was confirmed by DNA sequencing.

Molecular probes

The isolated fragment probes used in the Northern blot analysis were: a 1.15 kb *Pst*I fragment containing mouse α-actin cDNA (20); a 3.14 kb *Bam*HI fragment containing human eEF2 cDNA (21); a 0.97 kb fragment bearing the rpL32 processed gene 4A, joined to the 5′ and 3′ flanks of 3A (22); a 0.51 kb *Sac*II–*Xba*I fragment containing a mouse rpL30 processed gene derived from p1cXba (23); a 0.29 kb *Eco*RI–*Hin*dIII fragment containing the cDNA insert of mouse S16 derived from a subclone in pUC18 (24); a 0.2 kb *Eco*RI–*Bam*HI fragment containing human Dβ1-tubulin (M40)-specific sequence, corresponding to the 3′ untranslated region (UTR) (9); a 0.28 kb *Eco*RI–*Hin*dIII fragment containing human β2-tubulin-specific sequence, corresponding to the 3′ UTR (25); a 0.62 kb *Pst*I fragment containing human superoxide dismutase I (SOD) cDNA (24); a 0.95 kb *Pst*I fragment containing rat rpS4 cDNA (26); a 0.37 and 0.73 kb *Pst*I fragment spanning the entire rat rpL5 cDNA (27); a 1.8 kb *Bgl*I fragment containing mouse EF1α cDNA (kindly provided by L. I. Slobin); a 0.8 kb *Hin*dIII fragment containing a hGH cDNA (kindly provided by T. Fogel, Bio-Technology General).

RESULTS

Cell-specific translational control of EF2 mRNA

It has been recently shown that the mRNA encoding EF2, like those encoding rps and EF1α, is subject to growth-dependent translational control in human B-lymphoblastoid cells (3). The presence of a 5′ TOP has been established, however, only for Chinese hamster eEF2 mRNA (5). Hence, we set out to examine the translational behavior of this mRNA in CHO cells. Surprisingly, eEF2 mRNA has been completely refractory [79% (an average of two experiments) in polysomes from resting cells] to changes in growth status of these cells (Fig. 1a, CHO). We have assumed that the discrepancy between our observation with CHO cells, which are fibroblasts, or fibroblast-like cells (28) and those made with cells of lymphatic origin, might simply reflect differences among cell lineages. To examine this hypothesis, we compared the

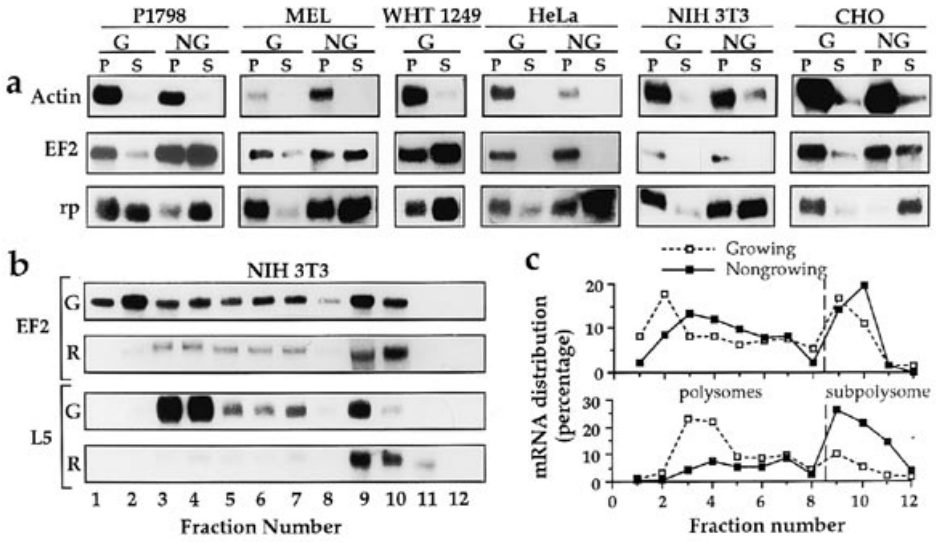

Figure 1. Cell type-specific translational control of EF2 mRNA. (**a**) Polysomes–subpolysomes distribution of EF2 mRNAs under different growth conditions. Cytoplasmic extracts were prepared from untreated (G) or nongrowing cells (NG) due to 24 h dexamethasone treatment of P1798 cell, 96 h hexamethylene bisacetamide treatment of MEL cells, 24 h hydroxyurea treatment of CHO cells or 24 h aphidicolin treatment of NIH 3T3 and HeLa cells. These extracts were centrifuged through sucrose gradients and separated into polysomal (P) and subpolysomal (S) fractions. Poly(A)$^+$ mRNA from equivalent aliquots of these fractions was analyzed by Northern blot hybridization with the probes indicated at the left. rp refers to the probes used to detect rpL32 mRNA in P1798, rpL30 mRNA in MEL, rpS4 mRNA in WHT 1249 and rpS16 mRNA in HeLa, NIH 3T3 and CHO cells. (**b**) Distribution of EF2 mRNA in polysomal and subpolysomal fractions. Postnuclear supernatants from untreated (G) or hydroxyurea-treated (R) NIH 3T3 cells were size fractionated by centrifugation through sucrose gradients which were subsequently divided into eight polysomal (1–8) and four (9–12) subpolysomal fractions. RNA was isolated from each fraction and subjected to Northern blot hybridization with the probes indicated at the left. (**c**) The autoradiographic signals presented in (b) were quantified by phosphorimager and the relative amounts of the mRNAs in each fraction are graphically depicted.

translational behavior of EF2 mRNA in NIH 3T3, HeLa cells and three cell lines of hematopoietic origin: P1798 lymphosarcoma cells, MEL cells and human WHT 1249 lymphoblastoids. The latter cell line is an exception in that its rp mRNAs are translationally repressed even in proliferating cells [Fig. 1a and (7)]. Our results clearly show that EF2 mRNA is translationally repressed in all three cell lines of hematopoietic origin [45 ± 5% (five experiments), 27% (one experiment) and 43% (two experiments) in polysomes from resting cells, respectively] under conditions where the rp mRNAs, but not actin mRNA, were unloaded from polysomes (Fig. 1a). In contrast, EF2 mRNA, like actin mRNA, was efficiently translated in growth arrested NIH 3T3 [68 ± 4% in polysomes (three experiments)] and HeLa cells [83% in polysomes (one experiment)] (Fig. 1a). Based on these results we could not exclude the possibility that our failure to detect translational repression of EF2 mRNA in CHO cells might reflect the poor resolution associated with partitioning the gradient into only two fractions (polysomal and subpolysomal). Thus, translational repression, which does not result from a complete unloading of ribosomes from the mRNA, but rather is the outcome of a considerable shift from heavy to light polysomes, might be missed. To examine this possibility, we analyzed the polysomal distribution of EF2 mRNA in gradients divided into 12 fractions. The results obtained in this experiment show that the translation of EF2 mRNA in NIH 3T3 cells is only slightly affected by growth arrest and to a much lesser extent than rpL5 mRNA (Fig. 1b and c). It should be noted that the proportion of EF2 mRNA distributed among the eight polysomal fractions in resting NIH 3T3 (71%) is similar to that measured in gradients divided into only two fractions (68 ± 4%).

β1-tubulin mRNA, even though it contains a 5′ TOP, is not a subject for a growth-dependent translational control

β1-tubulin gene (M40), like other TOP genes, has a major transcription start site within an uninterrupted stretch of pyrimidines (9). The transcription of β2-tubulin gene starts at a C residue which is followed by three pyrimidines ($_{+1}$CUCU$_{+4}$) (25). The polysomal association of the corresponding mRNAs was examined in three human cell lines of different embryonal origins. The two species of β1-tubulin mRNAs (2.6 and 1.8 kb) and the 1.8 kb β2-tubulin mRNA were efficiently translated even under conditions in which rpS4 mRNA was translationally repressed (Fig. 2a). Similar results have been obtained also with human skin fibroblasts (data not shown). Furthermore, the resistance of the β1-tubulin mRNAs to growth-dependent translational repression is also evident when the polysomal gradients were divided into 12 fractions (Fig. 2b and c). It appears therefore, that the presence of a 5′ TOP does not suffice for efficiently rendering β1- or β2-tubulin mRNAs into translationally regulated ones, in any of the four human cell lines examined by us.

The TOP and adjacent sequences from EF2, and β1-tubulin can confer translational control on a heterologous mRNA in any of the examined cell lines

One plausible explanation for the results presented in Figures 1 and 2 is that the TLRE in EF2 and β1-tubulin mRNAs has a suboptimal structure which is recognized in only a subset of cell types. Alternatively, downstream sequences override, in a cell type-specific manner, the effect of an otherwise typical TLRE. To

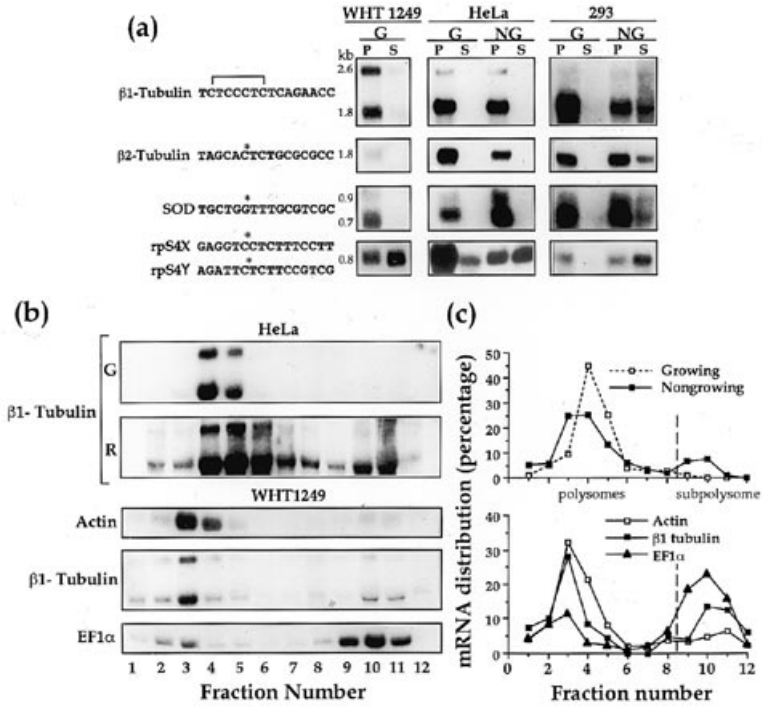

Figure 2. The translational behavior of mRNAs encoding human β1- and β2-tubulin. Cytoplasmic extracts were prepared from untreated (G) or nongrowing (NG) due to 24 h aphidicolin treatment of HeLa cells and 24 h nocodazol treatment of 293 cells. Preparation of polysomal fractions and their analysis as in Figure 1. The sequences around the transcription start site of the corresponding human genes and the size (in kb) of the resulting transcripts are indicated at the left of the autoradiograms. The transcription start region is bracketed in β1-tubulin (9) and the transcription start site in the human genes encoding β2-tubulin (25), SOD (40) and the sex chromosome-specific rpS4X and rpS4Y (41) are marked by asterisks. The two rpS4 mRNA species are of a similar size and equally well detected by the S4 probe. (**b** and **c**) Distribution of EF2 mRNA in polysomal and subpolysomal fractions. Postnuclear supernatants from untreated (G) or aphidicolin-treated (R) HeLa cells, untreated (G) and from untreated WHT 1249 cells, were size fractionated and manipulated as described in Figure 1b and c, respectively.

distinguish between these two possibilities, we examined the ability of the first 29 or 53 nucleotides of Chinese hamster EF2 and human β1-tubulin (M40) mRNAs, respectively, to confer translational control on human growth hormone mRNA. The two chimeric constructs were transfected into various cell lines and the transcription start sites were analyzed by primer extension using poly(A)$^+$ mRNA from NIH 3T3 cells. Figure 3 shows that EF2-GH mRNA starts at the site previously reported for the endogenous Chinese hamster EF2 mRNA [Fig. 3 and (5)]. Likewise, β1Tub-GH mRNA starts at a C residue followed by four pyrimidines within the previously identified transcription start region [Fig. 3 and (9)]. Analysis of polysomal distribution of these mRNAs (Fig. 4) demonstrates that EF2-GH mRNA exhibits a ubiquitous translational control as it translationally repressed upon growth arrest of both P1798 cells [29% (one experiment) in polysomes] and NIH 3T3 cells [43% (one experiment) in polysomes] even though the endogenous mRNA responds to this treatment only in cells of hematopoietic origin (compare with the polysomal distribution of EF2 mRNA in both these cell lines in Fig. 1a). Likewise, β1Tub-GH mRNA is translationally repressed upon growth arrest of P1798 [45% (two experiments) in polysomes] or NIH 3T3 cells [51% (one experiment) in polysomes] even though the endogenous human β1-tubulin remains efficiently translated in any of the cell lines examined (compare with the polysomal distribution of endogenous β1 tubulin mRNA in Fig. 2a). These results suggest that the 5′UTR of both mRNAs includes all the regulatory elements required for conferring growth-dependent translational control on a heterologous mRNA, but failed to do so in the context of the native mRNA, at least in some cell lines.

Cell type-specific sequence requirement for growth-dependent translational control of rpS16 mRNA

Results presented in the previous sections suggest that sequences downstream of the TLRE in EF2 and β1-tubulin mRNAs can abolish the translational control of the respective mRNAs in one or more cell types. In contrast, all rp mRNAs studied thus far exhibit ubiquitous translational control [Figs 1 and 2 and (1)]. If this feature depends on a unique context of their TOP sequences and downstream elements, then modification in the latter might selectively affect the translational control in distinct cell types. To examine this hypothesis we compared the translational control of a GH chimeric mRNA which starts with the first 29 nucleotides of rpS16 mRNA [S16wt(1–29)-GH], with that of two mutants. S16wt(1–29)-GH mRNA like the endogenous rpS16 mRNA is translationally repressed upon growth arrest of both P1798 and NIH 3T3 cells [Fig. 5 and (7)]. S16m(7–16)-GH mRNA is similar to S16wt(1–29)-GH mRNA except for a random replacement of 10 nucleotides (spanning positions +7 to +16) within the rpS16 sequence, including pyrimidine to purine replacements at positions +7 and +8. This change rendered this mRNA refractory to the growth arrest in fibroblasts [67% in polysomes (two experiments)] but did not affect the repression in dexamethasone-treated lymphosarcoma cells [33 ± 4% in polysomes (three experiments)] (Fig. 5). This apparent differential translational control cannot be

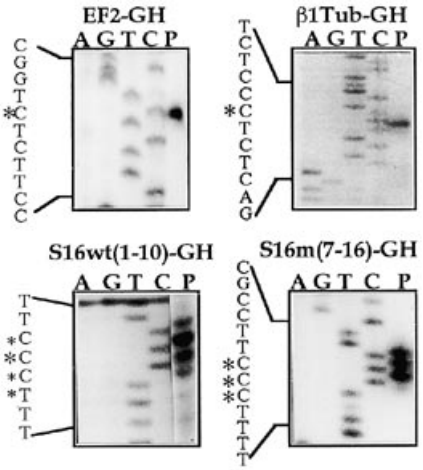

Figure 3. Determination of the transcription start site of various hGH chimeric mRNAs by primer extension. A 5′-end-labeled synthetic oligonucleotide complementary to nucleotides +31 to +12 of hGH gene was annealed to 5 µg of poly(A)$^+$ mRNA from stably transfected NIH 3T3 and extended with AMV reverse transcriptase. The extended product (P) was analyzed on a 6% acrylamide–urea gel alongside with a dideoxy sequencing reaction (A, C, G, T), in which the same primer (unlabeled) was used. Large and small asterisks indicate major and minor transcription start sites, respectively. The relative mobility of the primer extended DNAs corresponding to β1Tub-GH and the two S16-GH chimera are retarded due to a higher NaCl concentration relative to that in the sequencing reaction (7), and therefore should be regarded as initiating at the C residue positioned 1/2 nucleotide lower.

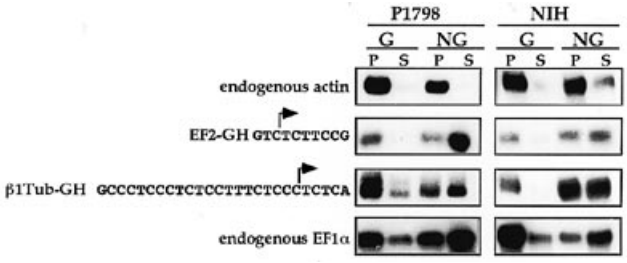

Figure 4. P1798 cells were transiently transfected while NIH 3T3 cells were stably transfected with the indicated hGH chimeric genes. Cytoplasmic extracts were prepared from untreated (G) or growth arrested (NG) cells which were manipulated as described in the legend to Figure 1a. Poly(A)$^+$ mRNA from polysomal (P) and subpolysomal (S) fractions was analyzed by Northern blot hybridization with hGH cDNA for detection of the chimeric transcripts and the cDNAs for actin and EF1α for the corresponding endogenous mRNAs.

attributed to the shortening of the TOP sequence, as a similar change occurred with S16wt(1–10)-GH mRNA (7), which contains the 8 nucleotide-long TOP followed by two additional authentic nucleotides of rpS16 mRNA [58% (two experiments) in polysomes from non growing NIH 3T3 cells versus 38% (two experiments) in resting P1798 cells] (Fig. 5). This differential translational control does not reflect a specific change in the transcription start site in NIH 3T3 cells, as the major cap sites in the two mutant mRNAs has been assigned in fibroblasts to the same three C residues as in S16wt(1–29)-GH mRNA [Fig. 3 and (7)]. Clearly, the difference in the mode of transfection into NIH 3T3 cells (stable) or P1798 cells (transient) cannot account for the loss of translational control of S16m(7–16)-GH mRNA in NIH 3T3 cells, as a similar loss was observed also when these cells

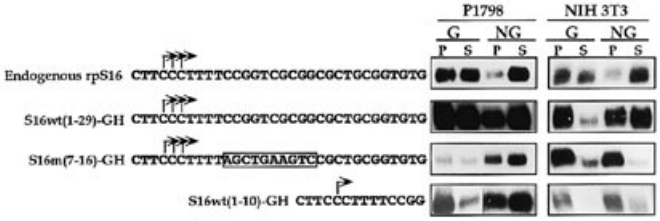

Figure 5. Mutations in sequences downstream of the rpS16 5′ TOP affect the translational control in a cell type-specific manner. P1798 and NIH 3T3 cells were transiently and stably transfected, respectively, with the indicated hGH chimeric genes. Cytoplasmic extracts were prepared from growing (G) cells (untreated P1798 and NIH 3T3 cells) and from nongrowing (NG) cells (24 h dexamethasone-treated P1798 cells and 24 h aphidicolin-treated NIH 3T3 cells), centrifuged through sucrose gradients and separated into polysomal (P) and subpolysomal (S) fractions. Poly(A)$^+$ mRNA from equivalent aliquots of these fractions was analyzed by Northern blot hybridization. Analysis of the endogenous rpS16 mRNA and the chimeric hGH mRNA was performed with the respective probes. The sequences at the left of the autoradiograms represent the nucleotides around the major transcription start sites (marked by arrows). Boxed letters represent nucleotides differing from the wild type sequence.

were transiently transfected [69% (two experiments) in polysomes from resting cells, data not shown].

DISCUSSION

There are only a few documented cases of cell- or tissue-specific variations in translational efficiency and these involve essentially two mechanisms: (i) differential utilization of upstream AUGs (29–31); and (ii) tissue-specific preference of polyadenylation site leading to distinct length of 3′ UTR (32). In the present report, however, we describe a novel mode of cell specificity in which the translational efficiency of the mRNA encoding EF2 is differentially modulated by altered growth status in cells of different lineages.

It appears that the common denominator of mRNAs which are translationally controlled in a growth-dependent manner is the involvement of their protein products in the translational apparatus (ribosomal proteins and elongation factors EF1α and EF2). Accumulating data concerning the translational behavior of mRNAs encoding EF1α and various rps, suggest ubiquitous translational repression of these mRNAs upon growth arrest, regardless of the cell type examined and the mean used to induce quiescence [the present study and (1)]. Likewise, we show here that EF2 mRNA is translationally regulated like rp mRNAs in three different hematopoietic cell lines (P1798, MEL and WHT 1249). However, monitoring the polysomal distribution of these two classes of mRNAs in non-hematopoietic cell lines (CHO, NIH 3T3 and HeLa) has demonstrated a selective resistance of the translation of EF2 mRNA to growth arrest. This differential regulation cannot be attributed to artefactual properties of these three cell lines or the mode of their arrest, as they represent different lineage [fibroblasts (the first two) and epithelial cells] and different organisms (hamster, mouse and human, respectively), as well as growth arresting by different drugs [hydroxyurea or aphidicolin (the last two)]. Furthermore, the simultaneous examination of the distribution of mRNAs encoding both EF2 and rps in the same polysomal gradients (Fig. 1), have ruled out erroneous conclusions due to mistakes in the experimental design. Nevertheless, the selective translational behavior of EF2 mRNA has raised an intriguing question of why it escapes the coordinate translational regulation, at least in some cell lines. One

plausible explanation is that coordinate alterations in the activity of the respective proteins might be achieved by employing different regulatory mechanisms. Thus, EF2 is inactivated by phosphorylation (33) and therefore, in some cells repression of its activity is carried out by translational repression of the respective mRNA, whereas in others (hematopoietic cells) it might reflect phosphorylation event at the protein level.

Whatever the mechanism for this selective regulation, it appears that it is not due to an exceptional composition of the 5′ TOP in this mRNAs as it starts with a C residue followed by a similar proportion of C and T residues as in 5′ TOP of ubiquitously regulated rp mRNAs (1). It remains, therefore, to identify the downstream sequences which might be involved in the cell type-specific translational control of EF2 mRNA.

Currently, the identity of the *trans*-acting factor(s) involved in the translational control of TOP mRNAs is still enigmatic, yet clues concerning a putative specific *trans*-acting factor have been derived from RNA–protein binding experiments (34–37). Moreover, the possibility that TOP mRNAs are translationally regulated via a specific repressor has been suggested by demonstrating that the translation of EF1α mRNA is selectively repressed *in vitro* by a salt wash of RNP (38). It should be noted, however, that the relevance of the oligopyrimidine-binding proteins 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.

Numerous studies have shown that mitogenic or hormonal stimulations induce the activity of $p70^{s6k}$ with a concomitant derepression of the translation of 5′ TOP containing mRNAs (39). Furthermore, inhibition of $p70^{s6k}$ by the immunosuppressant rapamycin selectively repressed the translation of mRNAs encoding ribosomal proteins and elongation factors (3). This correlation has led to the assumption that $p70^{s6k}$ activity might be a determinant in the regulation of the translational efficiency of TOP mRNAs. An intriguing possibility is that the cell type-specific translational control of EF2 mRNA might reflect parallel variations in the activities of $p70^{s6k}$ or of the putative repressor in cells of different lineage. However, such a simple model can not be of a general nature, as we have recently observed that poly(A)-binding protein, a new member of the TOP mRNA family, is translationally controlled with a different cell specificity (D. Avni and O. Meyuhas, unpublished data).

Conceivably, the 5′ TOP interacts with a *trans*-acting factor and the avidity of this interaction depends on the structure of the TLRE, which varies among different TOP mRNAs. Previously, we have shown that purine to pyrimidine substitution within the 5′ TOP of rp S16 mRNA renders the translation of the resulting transcript insensitive to growth arrest (6). In the present report we add a new dimension to this mode of regulation by demonstrating that substitution or deletion of the sequence immediately downstream of the 5′ TOP of rpS16, abolish its growth control in fibroblasts but not in lymphosarcoma cells (Fig. 5). If the abundance of the *trans*-acting factor differs between hematopoietic cells and fibroblasts and if downstream mutations affect the affinity of this factor to the 5′ TOP, then these mutations will be more critical in one cell line than in the other.

The ability of an intact 5′ TOP to confer translational control might be affected not only by the structure of its immediate downstream element, but also by interaction with further downstream sequences. Thus, our experiments with the endogenous human β1-tubulin mRNA and the chimeric β1Tub-GH mRNA demonstrate a case of an mRNA having a bona fide TLRE, yet it does not confer translational control when in its native context. One plausible explanation for these results is that sequences downstream of the TLRE within the native β1-tubulin mRNA might neutralize the regulatory properties of the TLRE under all circumstances, rendering this mRNA refractory to translational control. It is more likely, however, to assume that β1-tubulin mRNA might represent a class of mRNAs, which are subject to translational regulation with even higher cell type specificity than that of EF2 mRNA, or only during a specific developmental stage, which is yet to be disclosed.

Due to the lack of detailed information concerning the 5′ terminal structure of mRNA encoding β1-tubulin in other species, we cannot assess the extent of evolutionary conservation of this β1-tubulin-associated TLRE sequence and its possible regulatory role. It should be noted, however, that the lack of translational regulation of β2-tubulin mRNA does not necessarily reflect the presence of downstream overriding elements, but rather the presence of only four pyrimidines in its 5′ TOP (25) or the lack of essential downstream elements. Whatever the mechanism involved in the lack of translational regulation of the endogenous β1- or β2-tubulin, our observations clearly indicate that the presence of a 5′ TOP, *per se*, cannot be used as an ultimate diagnostic tool to seek out mRNAs endogenously regulated at the translational level.

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