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The 3′ untranslated region of bovine preprolactin contains a transferable non-poly(A) mRNA sequence that prolongs translation

Aikaterini Vassilakos, Martin J.G. Hughes, David W. Andrews*

Department of Biochemistry, 1200 Main St. West, Health Sciences Centre 4H41, McMaster University, Hamilton, Ont., L8N 3Z5, Canada

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Abstract Preprolactin transcripts, synthesized in vitro, were actively translated for a prolonged period when injected into *Xenopus* oocytes. As a result, prolactin continued to be secreted into the media for up to 6 days after injection of the transcript. To investigate the role of the preprolactin 3′ untranslated sequence in stabilizing transcripts, it was fused to coding regions derived from signal recognition particle receptor α-subunit or preproinsulin receptor. The translational half-life of the chimeric RNA was increased for both coding regions, suggesting that a sequence within the preprolactin 3′ untranslated region that prolongs translation is transferable. Deletion mutagenesis of this untranslated region demonstrated that a sequence of 98 nucleotides immediately following the prolactin stop codon was sufficient to prolong translation of RNAs injected into *Xenopus* oocytes.

Key words: Translation; 3′ untranslated region; Preprolactin mRNA; *Xenopus laevis*

1. Introduction

The translational efficiency of mRNA has been shown to be due to several factors, including the 5′ cap structure, the 5′ leader sequence, and sequences immediately surrounding the initiation codon (reviewed in [1,2]). These sequences largely determine both the rate and the fidelity of initiation of translation in vitro and in vivo (reviewed in [1,2]). More recently, sequences in the coding region have also been implicated in translational efficiency [3,4].

The relative abundance of an mRNA coding for a given gene product can also influence the amount of protein synthesized. The abundance of a specific mRNA species is determined both by the rate of transcript synthesis, processing, and transport, as well as the translational half-life of the molecule within the cytoplasm (reviewed in [5]). The translational half-life of an mRNA is determined by the kinetics of both mRNA inactivation (masking and unmasking) and degradation (chemical stability). The poly(A) tail, found on most eukaryotic mRNA species, has been shown to be the primary determinant of translational activity for many mRNAs (reviewed in [6]). While there is evidence for the involvment of polyadenylation in stabilizing transcripts in *Xenopus* oocytes, it has also been suggested that the poly(A) tail, together with poly(A) binding protein, may be involved in increasing the efficiency of translation initiation for some mRNAs (reviewed in [7]). In addition, recent evidence suggests that polyadenylation and de-adenylation mechanisms are involved in the activation and inactivation of mRNA translation in oocytes (reviewed in [8]).

*Corresponding author. Fax: (1) (905) 522 9033.
E-mail: AndrewsD@FHS.McMaster.CA

Since most mRNAs are polyadenlyated, other mechanisms may contribute to the observed differences in translational stability of certain mRNAs in eukaryotic cells. Evidence for gene-specific regulatory mechanisms comes from the characterization of de-stabilization sequences in the 3′ UTRs of cellular proto-oncogenes, and of stabilization sequences present in the α and β-globin 3′ UTRs (reviewed in [9,10]).

In addition to providing insight into the mechanisms of translation, the analysis of UTR sequences has proven useful for the development of expression vectors capable of increasing the synthesis of foreign coding regions in vitro and in vivo [11–14]. We have previously described a semi-synthetic 5′ leader sequence (termed UTK) containing the *Xenopus* β-globin 5′ UTR fused to an appropriately positioned consensus sequence for translation initiation. The UTK leader has been found to improve the translational efficiency of every coding region tested to date, in both reticulocyte lysate [15] and wheat germ extract (Falcone and Andrews, unpublished) cell-free translation systems.

To examine mRNA stability for actively translated mRNAs in vivo, we injected in vitro transcribed RNAs with the UTK 5′ leader into *Xenopus* oocytes. Protein synthesis was examined for four coding regions (bovine preprolactin, human insulin receptor, deletion mutants of bovine preprolactin, and the α-subunit of the signal recognition particle (SRP) receptor). Usually, RNA injected into oocytes is actively translated for less than 24 h. However, when the amount of prolactin secreted by microinjected oocytes was examined as an indication of steady- state synthesis, secretion was observed for up to 6 days following mRNA injection. Here we demonstrate that the 3′ UTR of bovine preprolactin contains transferable sequences responsible for maintaining the injected RNA in a translation-competent form in the cytosol of *Xenopus* oocytes. Furthermore, we provide evidence that at least one regulatory sequence is independent of the poly(A) tail and is located within the 98 bases immediately following the prolactin termination codon.

2. Materials and methods

2.1. Recombinant DNA constructs

Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). SP6 RNA polymerase was from Epicentre Technologies (Madison, WI); the RNase inhibitor RNA Guard was from Pharmacia Biotech (Baie d'Urfe, Canada). [^{35}S]Methionine was from Dupont-New England Nuclear (Mississauga, Canada).

A plasmid encoding the α-subunit of the SRP receptor was modifed by introducing a termination codon (in an *Xba*I linker) into the coding region at codon 426. The plasmid was then cut with *Xba*I, end-repaired with the Klenow fragment of polymerase, and a *Sac*I linker was added. These manipulations resulted in a plasmid encoding the deletion mutant SR1, containing the first 426 amino acids of SRP receptor α-subunit with a *Sac*I site immediately following the termination codon. Thus, the

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SR1 3′ UTR contains 636 nucleotides of coding sequence and 406 nucleotides of the SRP receptor α-subunit 3′ UTR. The insulin receptor cDNA clone was a kind gift from A. Ullrich. This plasmid contains 180 nucleotides of the endogenous 3′ UTR ending with an *Spe*I restriction site. For cloning purposes the plasmid was cut with *Spe*I, end-repaired using the Klenow fragment of DNA polymerase, and a *Sac*I linker was inserted.

Construction of plasmids, encoding either preprolactin or the preprolactin deletion mutant Pt with the SD, KD, UTR and UTK leaders, were described previously [15]. The complete 3′ UTR and coding region of Pt is similar to that of preprolactin except that amino acids 2–58 of the mature prolactin domain have been deleted. In addition, a *Sac*I restriction site was introduced immediately following the termination codon of Pt using the naturally occurring *Esp*I site overlapping the termination codon. The translation start site in the UTK leader is contained within an *Nco*I site. Therefore, by first removing the *Sac*I site in the polylinker sequence at the 3′ end of the Pt construct, it was possible to replace the Pt coding region in this plasmid with other coding sequences by digestion with *Nco*I and *Sac*I. The resulting plasmids contained the desired coding region flanked with the UTK leader and the bovine preprolactin 3′ UTR. Plasmids containing the insulin receptor and SR1 coding regions were assembled in this way. To construct plasmids with the UTK leader and the endogenous 3′ UTR we made use of a plasmid (pSPUTK; available from Stratagene) containing the UTK leader followed by a multiple cloning site. The 3′ and 5′ ends of the constructs were sequenced using the NEB vent polymerase sequencing system according to the manufacturer's instructions. Complete details for any of the constructs are available from the authors.

2.2. Transcription in vitro

All of the plasmids were linearized by digestion with a suitable restriction enzyme before transcription in vitro [16]. In most cases an *Eco*RI restriction site within the polylinker at the 3′ end of the 3′ UTR was used for linearization. Restriction sites within the preprolactin 3′ UTR were used to produce transcripts truncated at different positions within the 3′ UTR. Because *Sph*I and *Alw*NI leave 3′ overhangs, the DNA was end-repaired using the Klenow fragment of DNA polymerase prior to transcription in vitro [16]. SP6 polymerase reactions generating capped transcript were as described previously except that glutathione buffer (50 mM reduced glutathione, 10 mM oxidized glutathione and 20 mM HEPES, pH 7.5) was used in place of DTT [17]. Transcripts were normalized for RNA content using a fluorometric assay described previously [15]. An aliquot of each transcript was translated in a reticulocyte lysate reaction prior to injection to ensure that full-length molecules were synthesized from the RNA. Reticulocyte lysate translations were as described previously [15] and contained 1 μl of the transcription reaction and 10 μCi [^{35}S]methionine.

2.3. Xenopus oocyte microinjection

Adult female breeding *Xenopus laevis* were purchased from Boreal (St. Catharines, Canada). Ovarian fragments were surgically removed from anaesthetized animals, stage VI oocytes were manually dissected and stored at 19°C in ND96 media (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.6) supplemented with antibiotics. Oocytes were injected using a model NA-1 injection system (Sutter Instrument Co. Novato, CA). Borosilicate micropipettes (ID 0.5 mm, OD 1.0 mm) were pulled using a K.T. Brown Type Puller and bevelled using a model BV-10 beveller (both Sutter Instrument Co., Novato, CA).

Translational efficiency in *Xenopus* oocytes was determined by co- injection of in vitro synthesized transcription products and [^{35}S]methionine. Normalized SP6 transcription products were mixed with an equal volume of [^{35}S]methionine (10 mCi/ml) and 50 nl were injected into each oocyte. Groups of 40–50 oocytes were injected with each transcript and incubated in ND96, for the time indicated. To assess the period of translational activity of the injected RNA, transcript was injected alone and cells were pulse-labelled at the indicated times by placing 5–10 oocytes in 0.5 ml ND96 containing [^{35}S]methionine (0.05 mCi/ml) for 4 or 6 h for Pt and SR1 transcripts, respectively. To follow insulin receptor expression, [^{35}S]methionine was present during the entire 40 h incubation.

After incubation, oocytes were homogenized and the labelled proteins were recovered by immunoprecipitation as described [18]; except for SR1 translation products where the solubilization and initial wash buffer contained 1.5% Triton X-100, 500 mM NaCl, 100 mM Tris-Cl, pH 8.0, 10 mM EDTA, the mouse IgG1 monoclonal antibody [19] was precipitated using an equal mixture of protein A and protein G agarose, and the initial pelleting step was over a 0.5 M sucrose cushion. Antibodies to ovine prolactin and human insulin receptor (AB-1) were obtained from United States Biochemicals (Cleveland, OH), and Oncogene Science (Uniondale, NY), respectively. Protein A coupled to agarose was purchased from Bio-Rad Laboratories (Mississaugua, Canada) and protein G coupled to agarose was from Oncogene Science (Uniondale, NY).

Immunoprecipitation products were separated by SDS-PAGE using the Tris-tricine buffer system, fluorographed and exposed to film [20]. Relative protein synthesis was quantified by densitometric scanning of the fluorograms using a Hoefer Scientific GS300 scanning densitometer.

3. Results and discussion

3.1. The UTK leader increases translation in vivo

Previously we reported that the UTK leader increased the rate of translation initiation for a variety of coding regions in vitro [15]. To examine the role of 3′ UTR sequences in the context of this efficient 5′ UTR it was first necessary to extend our previous results to a whole-cell system. To this end, transcription products from SP6 polymerase reactions were injected into *Xenopus* oocytes and protein synthesis was examined by immunoprecipitation of the prolactin deletion mutant Pt, from cells labelled with [^{35}S]methionine. The 5′ leader sequences used were the same as examined previously [15], and were selected to permit comparison of the effect on translation of the UTR sequence with that of the consensus sequence for translation initiation [21]. The complete sequence of each leader has been published [15]. The SD and KD leaders contain a plasmid-derived sequence between the SP6 promoter and the translation start site, and therefore are typical of the untranslated sequences obtained using most standard cloning vectors. The translation start sites for the SD and KD leaders are the relatively unfavourable initiation sequence CCCAUGG and the strong initiation sequence ACCAUGG, respectively [21]. The UTR and UTK leaders contain the *Xenopus* β-globin 5′ UTR linked to the same compromised and strong initiation sequences, respectively.

To assay the translational efficiency of the different leaders in a whole-cell system, transcription products were injected into *Xenopus* oocytes. Microinjection permits direct comparison of different RNAs, independent of transcription. In addition, the differences observed are unlikely to be influenced by rates of maturation or transport because the RNA is injected directly into the cytoplasm. Microinjection of RNA containing the UTK leader into oocytes resulted in more synthesis of Pt during a 4 h pulse label than was obtained for RNA with the other leaders (Fig. 1A). Moreover, both the UTR and the consensus sequence for translation initiation contribute to the increase in protein synthesis observed (Fig. 1A, compare lanes 2 and 3 with 4). Densitometric results for five separate experiments suggests that the increase in translation efficiency due to addition of the consensus initiation sequence was approximately the same as that due to the UTR sequence (3- to 4-fold). The net increase due to the two sequences together was greater than 7-fold, suggesting that the two sequences independently promote efficient translation. Therefore, to study the effect of the 3′ UTR on efficiently translated RNAs, the UTK leader was included in the constructs assayed below.

Our observation that regions in the 5′ UTR increase transla-

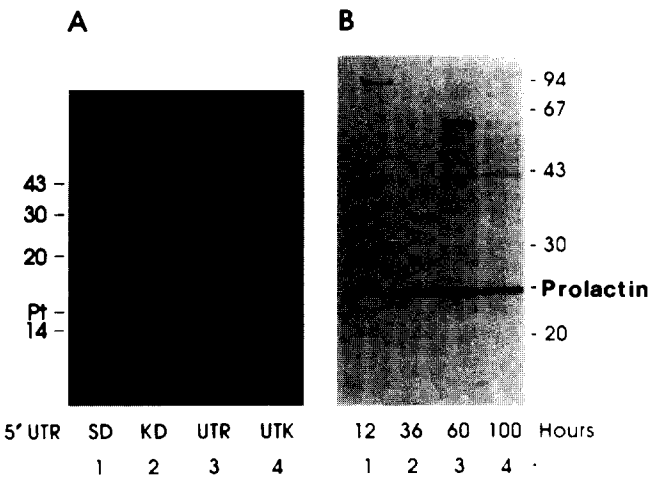

Fig. 1. (A) Translation of Pt with different leader sequences in *Xenopus* oocytes. In vitro synthesized transcripts were normalized for RNA content by fluorometry, mixed with an equal volume of [^{35}S]methionine and injected into *Xenopus* oocytes. Oocytes were incubated for 4 h in ND96 medium, homogenized and subjected to immunoprecipitation with anti-ovine prolactin antiserum. The products of the immunoprecipitation were separated by SDS-PAGE and fluorographed. The 5′ untranslated region (5′ UTR) is indicated below each lane. The migration positions of Pt and molecular weight markers are indicated to the left of the panel. (B) Prolactin is secreted from oocytes for 5 days post-injection into *Xenopus* oocytes. Oocytes were injected with 50 nl of preprolactin transcription reaction. After 2 h, groups of 10 viable oocytes were placed in separate containers. At the times indicated below the lanes, media was collected, replaced, and non-viable oocytes discarded. Proteins in the media were concentrated by evaporation in a SpeedVac Concentrator (Savant Instruments, Farmingdale, NY), separated by SDS-PAGE, and visualized by staining the gel with Coomassie brilliant blue. Media samples collected from one group of 10 oocytes are shown. The migration position of molecular weight markers (in kDa) and of prolactin is indicated at the right of the figure.

tion efficiency independently of the consensus sequence for efficient initiation (Fig. 1A) is not predicted by the scanning model for initiation [21]. The scanning model states that the nucleotide sequence immediately surrounding the initiator AUG is the primary determinant of translational efficiency and does not allow for regions of the 5′ UTR being involved in enhancing translation [21]. However, the *Xenopus* 5′ UTR clearly increases translational efficiency, even for mRNAs with a compromised initiation sequence, in diverse systems, including rabbit reticulocyte lysate [15], wheat germ extract (Falcone and Andrews, unpublished data) and *Xenopus* oocytes, suggesting that this region acts as a general enhancer of eukaryotic translation.

After microinjection of preprolactin RNA containing the UTK leader into *Xenopus* oocytes, the amount of protein secreted into the medium during a 24 h period was as high as 125 ng per cell. Therefore, it was possible to detect the secretion of prolactin from oocytes by Coomassie blue staining of total culture medium after SDS-PAGE (Fig. 1B). Most of the preprolactin labelled in a 4 h pulse is secreted as prolactin from the oocytes within 6 h [18]. Therefore, we were surprised that prolactin secretion could be detected by Coomassie blue staining of total oocyte media up to 6 days following injection of the RNA (Fig. 1B). Typically, the half-life for protein synthesis from an efficiently translated RNA after injection into *Xenopus* oocytes is a few hours ([22,23]; and unpublished data). In contrast, densitometry of the data obtained from three independent experiments similar to that shown in Fig. 1B revealed that functional RNA encoding preprolactin persists for at least 100 h. Because microinjected oocytes survive in culture for only 5–6 days it is possible that cell viability, rather than RNA stability, eventually limits the synthesis of preprolactin in these cells.

The functional half-life of a coding region is often determined by sequences within the 3′ UTR (reviewed in [5,6]). The nucleotide sequence, predicted secondary structure and other salient features deduced from the DNA sequence of the prolactin 3′ UTR are shown in Fig. 2. DNA sequencing of this region revealed that the poly(A) sequence is followed by a run of 10 C's. Although this sequence is not part of the authentic preprolactin 3′ UTR, as it was added during the initial cDNA cloning of preprolactin, it was part of the 3′ UTR assayed above, and was therefore included in the constructs described below. The preprolactin 3′ UTR contains a nuclear polyadenylation sequence but lacks the cytoplasmic polyadenylation element necessary for addition of A residues in the cytoplasm of *Xenopus* oocytes [24]. Other previously characterized motifs, such as the AU-rich motif known to destabilize a wide variety of mRNAs [7], are absent from the preprolactin 3′ UTR. However, the sequence does contain 4 repeats of the sequence CCAU.

To determine if the preprolactin 3′ UTR could prolong the translation of coding regions other than prolactin, protein synthesis was measured for chimeric RNAs encoding either a deletion mutant of the SRP receptor α-subunit, termed SR1, or

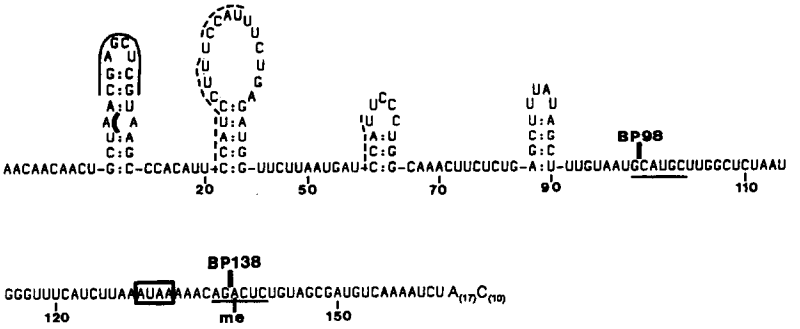

Fig. 2. The sequence and predicted secondary structure of the bovine preprolactin 3′ UTR. The positions of the truncation sites are indicated above the sequence. Numbering (below the sequence) begins after the TAA termination codon indicated by (. CCAU repeats are indicated with the dashed lines. Restriction endonuclease recognition sequences are indicated with a solid bar. The polyadenylation sequence is boxed, and **me** indicates the position of the major site of methylation in vivo.

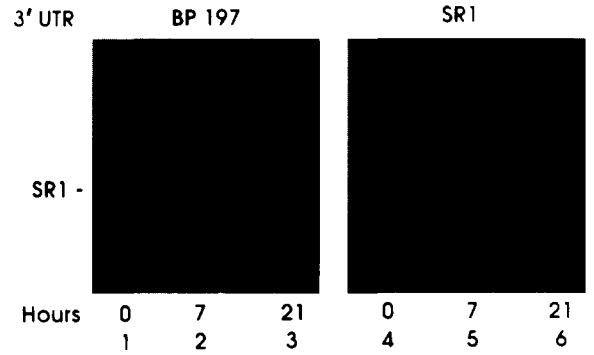

Fig. 3. Replacing the SR1 UTR with the BP197 UTR prolonged translation of SR1 transcripts. In vitro synthesized transcript of SR1 coding sequences with either the SR1 3′ UTR or the BP197 3′ UTR were injected into oocytes. At the time of injection (0) and at 7 and 21 h after injection, sets of 10 oocytes were removed and incubated for 6 h in media containing [^{35}S]methionine (0.5 mCi/10 ml). SR1 translation products were isolated by immunoprecipitation from homogenized oocytes and separated by SDS-PAGE. The gels were fluorographed, dried and exposed to film. The migration position of SR1 is indicated to the left of the figure.

preproinsulin receptor. Both coding regions were flanked by the UTK leader sequence and the complete 197 nucleotide 3′ UTR (BP197). The SR1 3′ UTR contains coding sequences, as well as 406 nucleotides of the SRP receptor α-subunit 3′ UTR. When the BP197 sequence was fused to the SR1 coding sequence in place of this 3′ UTR, translation of the microinjected RNA was extended by approximately 20 h (10-fold; Fig. 3, compare lanes 1–3 with 4–6). Although the SR1 3′ UTR contains coding sequences, the period during which the injected RNA is translated (Fig. 3, lanes 4–6) is similar to that of other injected RNAs. This result is consistent with a previous report demonstrating that the presence of coding sequences 3′ from a termination codon can destabilize nuclear but not cytoplasmic transcripts (reviewed in [9]). Therefore, it is likely that the apparent stability of the chimeric RNA is due to prolonged translation of the species with the BP197 3′ UTR rather than rapid degradation of the RNA with the SR1 3′ UTR.

The human insulin receptor has been expressed previously in oocytes but was only detectable on SDS-PAGE gels after in vitro labelling of the β-subunit with [γ-^{32}P]ATP [25]. To improve expression of this molecule we replaced the endogenous 5′ leader with either the UTR or UTK leader and added the BP197 3′ UTR to the 3′ UTR of the prepro-insulin receptor sequence. As expected, in continuously labelled cells, addition of the UTK leader dramatically improved the synthesis of the insulin receptor. As a result, the early translation product (proreceptor) and the processed α- and β-subunits are easily detected by immunoprecipitation of homogenates prepared from microinjected oocytes (Fig. 4, compare lanes 2 and 3). Although addition of the BP197 3′ UTR did not significantly change the amount of the processed subunits observed, the amount of proreceptor detected 40 h post-injection was increased by 3-fold (Fig. 4, compare the top bands in lanes 3 and 4). The simplest explanation for the increase in proreceptor synthesis is that the mRNA encoding preproinsulin receptor is translated for a longer time when fused to the BP197 3′ UTR.

To localize the sequences within the BP197 3′ UTR responsible for augmenting translation, we examined a series of mutants containing progressive deletions. The plasmid encoding Pt with the UTK leader and BP197 3′ UTR was used for these experiments as Pt is not secreted from oocytes, thereby simplifying the measurement of protein synthesis at different time points. This plasmid was digested with restriction enzymes that cut the plasmid within the 3′ UTR at the positions indicated in Fig. 2, and transcript was synthesized in vitro. At different times after injection, oocytes were pulse-labelled with [^{35}S]methionine for 4–5 h. The relatively long pulse time ensured that the label completely equilibrated within the cell [18].

Comparision of the amount of Pt synthesis 30 h after RNA injection revealed that deletion of the 3′ end of the sequence reduced the translational half-life of the injected RNA somewhat (Fig. 5, lanes 3, 6 and 9). In this experiment the half-lives for translation of the RNA with the BP138 and BP98 3′ UTRs were both approximately 30 h. In contrast, only about 20% of the RNA with the BP197 3′ UTR was inactive 30 h post-injection. The additional sequences within the BP197 3′ UTR included a sequence of 17 A's followed by 10 C's, and the predominant site of methylation in vivo [21]. It remains to be determined whether any of these sequences contributes significantly to translation. Based on previous observations that a poly(A) tail containing 32 A's but not 17 A's was sufficient for mRNA stability, it is unlikely that the poly(A) sequence contributes significantly to RNA stabilization [23].

Molecules with the BP98 3′ UTR do not contain the poly(A) sequence, methylation site, or a polyadenylation signal, yet the RNA was remarkably stable (measured translational half-life 30 h). In contrast, other RNAs microinjected into *Xenopus* oocytes are found only in non-polysomal fractions within 4 h of deadenylation [24]. Consistent with these results, translation of molecules with the BP13 3′ UTR could not be detected in oocytes 2 h post-injection (Fig. 5, lane 11). The construct

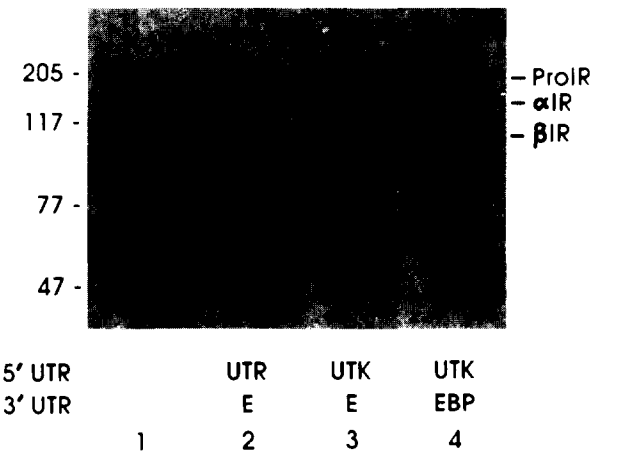

Fig. 4. Effect of 5′ UTR and 3′ UTR on insulin receptor synthesis in *Xenopus* oocytes. Oocytes were injected with 50 nl/oocyte in vitro synthesized transcript and incubated in 1 mCi [^{35}S]methionine/10 ml ND96 for 24 h, the media was replaced with fresh media and incubation continued for another 16 h. Insulin receptor translation products were analyzed for sets of 6 oocytes after immunoprecipitation by SDS-PAGE and fluorography. Lane 1, control oocytes injected with water. The 3′ untranslated regions are indicated as: E, endogenous 3′ UTR; EBP, fusion of the endogenous and BP197 UTRs. The UTR and UTK 5′ leader sequences are defined in the text. The migration positions of the proreceptor, α-subunit and β-subunit of the insulin receptor are indicated to the right of the figure. The migration positions of molecular weight markers (in kDa) are indicated at the left of the figure.

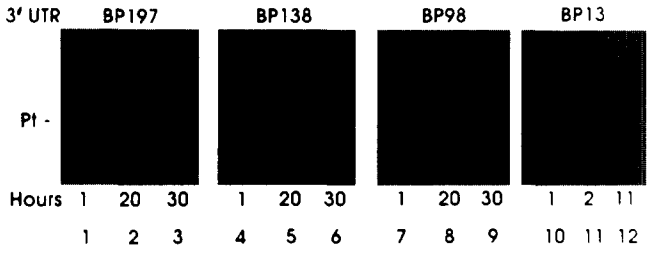

Fig. 5. Translational activity of Pt transcripts containing truncated 3′ UTRs. The truncation point is indicated above the panels, numbering starts with the termination codon. Normalized transcript was injected into cells and then at the time point indicated below each lane, a set of 5 oocytes was placed in media containing [^{35}S]methionine (0.5 mCi/10 ml) and labelled for 5 h. After labelling, oocytes were homogenized and translation products were immunoprecipitated with anti-ovine prolactin antiserum and separated by SDS-PAGE. The gels were fluorographed, dried and exposed to film. The migration position of Pt is indicated to the left of the figure.

containing the BP13 3′ UTR, ends with an *Eco*RI restriction site, therefore the last 5 bases in the UTR are identical to the last five bases of BP197. RNA from another plasmid containing a 38 nucleotide 3′ UTR between the stop codon and an *Eco*RI linearization site was also translated for less than 2 h (data not shown), suggesting that the short length of the BP13 3′ UTR does not account for the differences observed in Fig. 5. Taken together, these results suggest that there is a sequence within the first 98 bases of the BP197 3′ UTR that prolongs translation of RNAs in *Xenopus* oocytes.

Since the RNA is synthesized in vitro it is unmodified prior to injection. However, it is possible that the RNA acquires modifications that may affect functional stability after injection into the cytoplasm of the oocyte. Experiments to determine whether or not the BP98 sequence is sufficient to stabilize coding regions in cell types other than *Xenopus* oocytes are in progress.

4. Concluding remarks

Our observation that an mRNA can be both highly translated and stable is unusual. Nevertheless, the BP98 and BP197 3′ untranslated regions dramatically prolong translation for RNAs encoding Pt, SR1 and insulin receptor. A small number of other 3′ untranslated sequences have been demonstrated to prolong mRNA translation, including that of α- and β-globin [27] and β-actin [28]. Similar to these untranslated regions, the BP98 UTR sequence contains little predicted secondary structure (Fig. 2). In contrast to these sequences, mRNA stabilization by the BP98 UTR is independent of polyadenylation.

The relatively short period of translation observed with other RNAs may be due to inactivation (masking) of these RNAs rather than degradation. Consistent with this view, poly(A)$^+$ and poly(A)$^-$ transcripts coding for several different proteins were shown to be chemically stable over a 24 h time period after injection into *Xenopus* oocytes. However, the poly(A)$^+$ transcripts were translated more efficiently [22]. These and other similar results have led to the conclusion that *Xenopus* oocytes may lack the mRNA degradation system found in mammalian cells (reviewed in [7]). It is possible, therefore, that the prolonged translation observed for mRNA with the BP98 3′ UTR may result from a block in masking rather than increased chemical stability of the RNA.

Xenopus oocytes have proven to be a useful model system in many areas, including developmental biology, protein trafficking, and signal transduction. Many of these studies involve the expression of cloned genes in oocytes by micro-injection of transcript and subsequent analysis of the protein produced. Our results suggest that optimizing the 5′ and 3′ UTRs flanking the coding regions for these molecules may greatly facillitate such studies.

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