FEBS Letters 354 (1994) 271–273

FEBS 14739

Enhancing effect of the 3′-untranslated region of tobacco mosaic virus RNA on protein synthesis in vitro

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Received 23 September 1994

Abstract In order to test the enhancing effect of the 3′-terminal untranslated region (3′-UTR) of tobacco mosaic virus (TMV) RNA on protein synthesis in vitro we used a chimeric mRNA construct containing TMV 5′-UTR (Ω) and firefly luciferase mRNA. The addition of the TMV 3′-UTR to the chimeric mRNA construct results in a more than 3-fold stimulation of the synthesis of the functionally active protein in the wheat germ cell-free translation system. We have demonstrated that the proper length of the TMV 3′-terminal part is important for efficient translation; elongation of the TMV tail by 160 vector-derived nucleotides fully abolishes the stimulation effect of the TMV 3′-UTR in vitro.

Key words: Viral 3′-untranslated region; Recombinant mRNA; Cell-free translation; mRNA stability

1. Introduction

The addition of the 3′-untranslated region (UTR) of tobacco mosaic virus (TMV) RNA to the chimeric mRNA construct containing capped TMV 5′-UTR (Ω) and luciferase mRNA was reported to significantly increase their expression in vivo [1,2]. However, the stimulation of translation by the TMV 3′-UTR in vitro has not been reported. At the same time it was reported that the 3′-UTRs can stimulate translation of satellite tobacco necrosis virus RNA [3,4] and alfalfa mosaic virus RNA in vitro [5,6]. We have recently obtained evidence showing that the presence of the structured 3′-UTR in the AlMV RNA 4 is important for the in vitro viral mRNA expression at the initiation phase of translation [5].

In this study the gene encoding for firefly luciferase was used to analyze the ability of the 3′-UTR of TMV RNA to affect its expression in a wheat germ cell-free translation system. We have compared the translation efficiencies and stability of the chimeric RNA constructs differing in their 3′-terminal parts. The experiments have shown that the addition of the TMV 3′-UTR to chimeric mRNA increases their translation up to 3.5-fold, increasing the translational efficiency but not mRNA stability. We have demonstrated that the proper length of the TMV 3′-terminal part is important for the efficient translation.

2. Materials and methods

2.1. In vitro transcription

The Ω/LUC, Ω/LUC/TMV 3′-UTR and Ω/LUC/TMV 3′UTR/PL UTR transcripts (see section 3) were obtained from the Ω and TMV 3′-UTR-containing luciferase (LUC) gene construct [1] linearized with *Bam*H1, *Nde*1 and *Bgl*1, respectively. In vitro transcription with T7 RNA polymerase was carried out as described in [7]. The mRNA constructs were synthesized as uncapped mRNAs. Radiolabeled transcripts were synthesized in the same reaction mixture but with the UTP concentration reduced to 1.5 mM and [^{14}C]UTP with a specific activity of 51 mCi/mmol (Isotope, St. Petersburg, Russia) added to 1.25 μCi per 100 μl. The transcripts were isolated by chloroform deproteinization and subsequent precipitation with 3 M LiCl. The deproteinized samples were precipitated by 70% ethanol with 100 mM ammonium acetate and then analyzed by electrophoresis in 5% polyacrylamide gels in the presence of 8 M urea. The RNA was dissolved in water to a concentration of 1 mg/ml.

2.2. Cell-free translation reactions

Translation mixtures were incubated at 24°C for 90 min (or as denoted in the text) in a 50 μl volume of 24 mM HEPES (pH 7.6) containing 20 μl wheat germ extract [8], 9 pmol (or as denoted in the text) of chimeric mRNA, 3 μg creatine phosphokinase, 2.1 mM magnesium acetate, 110 mM potassium acetate, 100 μM ethylene diamine tetraacetate, 10 mM creatine phosphate, 1 mM ATP, 0.2 mM GTP, 2.4 mM dithiothreitol, 0.1 mM spermine, 20 μM [^{14}C]Leu, and 50 μM of each of the other 19 amino acids. 5 μl samples of the translation mixture were taken and the size and homogeneity of the protein products were determined by electrophoresis through 10–25% polyacrylamide gels run in a denaturing buffer [9]. The functional integrity of the protein products was determined by measuring luciferase activity in 5 μl of the translation mixture [1]. The maximum luciferase activity in the case of the Ω-LUC mRNA translation was taken as 100%.

2.3. RNA decay assay

The RNAs (27 pmol) labeled with [^{14}C]UTP (10,000 cpm/μg) were incubated in 150 μl of the wheat germ translation mixture at 26°C. 20 μl samples were removed after 0, 10, 20, 40 and 80 min of incubation and diluted with 80 μl of ice-cold 20 mM HEPES-KOH buffer, pH 7.6, with 120 mM KAc and 5 mM $MgAc_2$. The samples were extracted with 150 μl of deproteinization mixture consisting of 70 μl phenol and 70 μl chloroform. The deproteinized samples were precipitated by 70% ethanol with 100 mM ammonium acetate and then analyzed by electrophoresis in 5% polyacrylamide gels in the presence of 8 M urea with subsequent autoradiography.

3. Results and discussion

We used the following DNA constructs with the promotor of T7 RNA polymerase: (a) the coding region for luciferase and the Ω sequence of TMV (Ω/LUC); (b) the coding region for luciferase and both the Ω and 3′-UTR of TMV (Ω/LUC/TMV 3′-UTR); (c) the coding region for LUC, Ω, 3′-UTR of TMV and the additional downstream plasmid sequence resulting in a 163 base addition to the 3′-terminus (Ω/LUC/TMV 3′-UTR/PL UTR). The corresponding chimeric mRNAs were produced by T7 RNA polymerase (Fig. 1A). Electrophoretic analysis of the products of mRNA synthesis has demonstrated that each of the three transcripts has a different mobility and represents the major band in the electrophoretic pattern (Fig. 1B).

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SSDI 0014-5793(94)01126-5

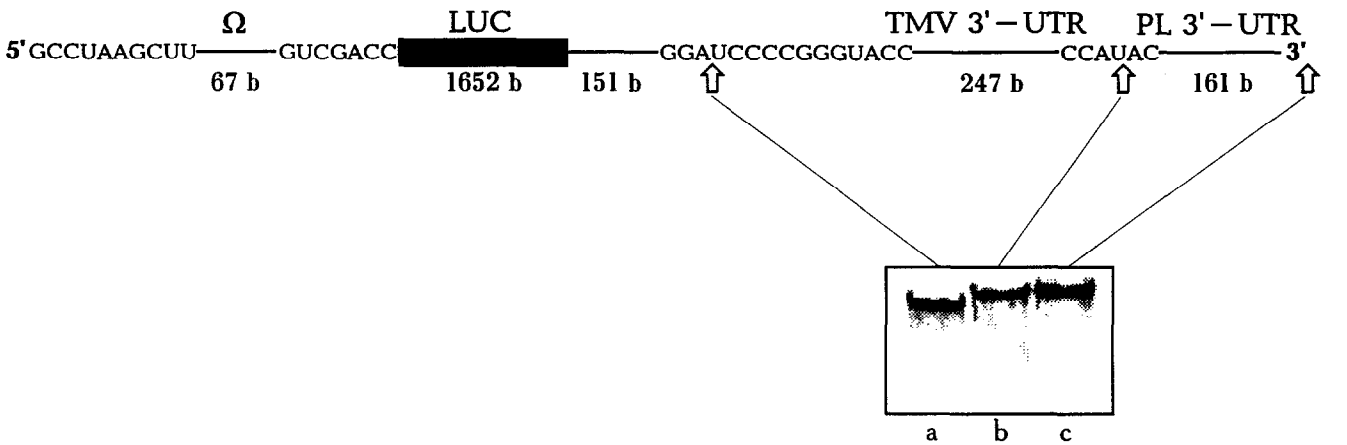

Fig. 1. mRNA constructs used to test the effect of the TMV 3'-UTR on gene expression. All the constructs contain the same LUC gene, the first 142 bases of the native LUC 3'-UTR and 67-base sequence representing the TMV 5'-UTR (Ω). Three 3'-UTRs were employed: (a) a 24-base sequence representing a polylinker sequence from the junction with the 142-base native LUC 3'-UTR to the *Bam*H1 site of pUC19; (b) a 31-base sequence containing a polylinker sequence and a 247-base 3'-UTR of TMV; (c) a 31-base sequence containing a polylinker sequence, a 247-base 3'-UTR of TMV and a 163-base sequence of the plasmid 3'-UTR (PL UTR). Electrophoretic patterns of RNAs differing in 3'-UTR length are given in the lower panel: (a) Ω/LUC mRNA, (b) Ω/LUC/TMV 3'-UTR mRNA, and (c) Ω/LUC/TMV 3'-UTR/PL UTR mRNA.

We compared the translation efficiencies of the obtained chimeric RNA constructs differing in their 3'-terminal parts. It can be seen in Fig. 2 that at saturating amounts of mRNA in the incubation mixture the Ω/LUC/TMV 3'-UTR mRNA provides a 350% higher level of the functionally active protein than the tail-less Ω/LUC mRNA and the Ω/LUC/TMV 3'-UTR/PL UTR mRNA with elongated TMV 3'-UTR tail (Fig. 2A). The electrophoresis autoradiogram (Fig. 2B) shows the yield of the full-sized luciferase synthesized in each reaction.

The data of Fig. 3A show the amount of the functionally active protein synthesized as a function of the incubation time in the reactions with the in vitro synthesized mRNAs. The

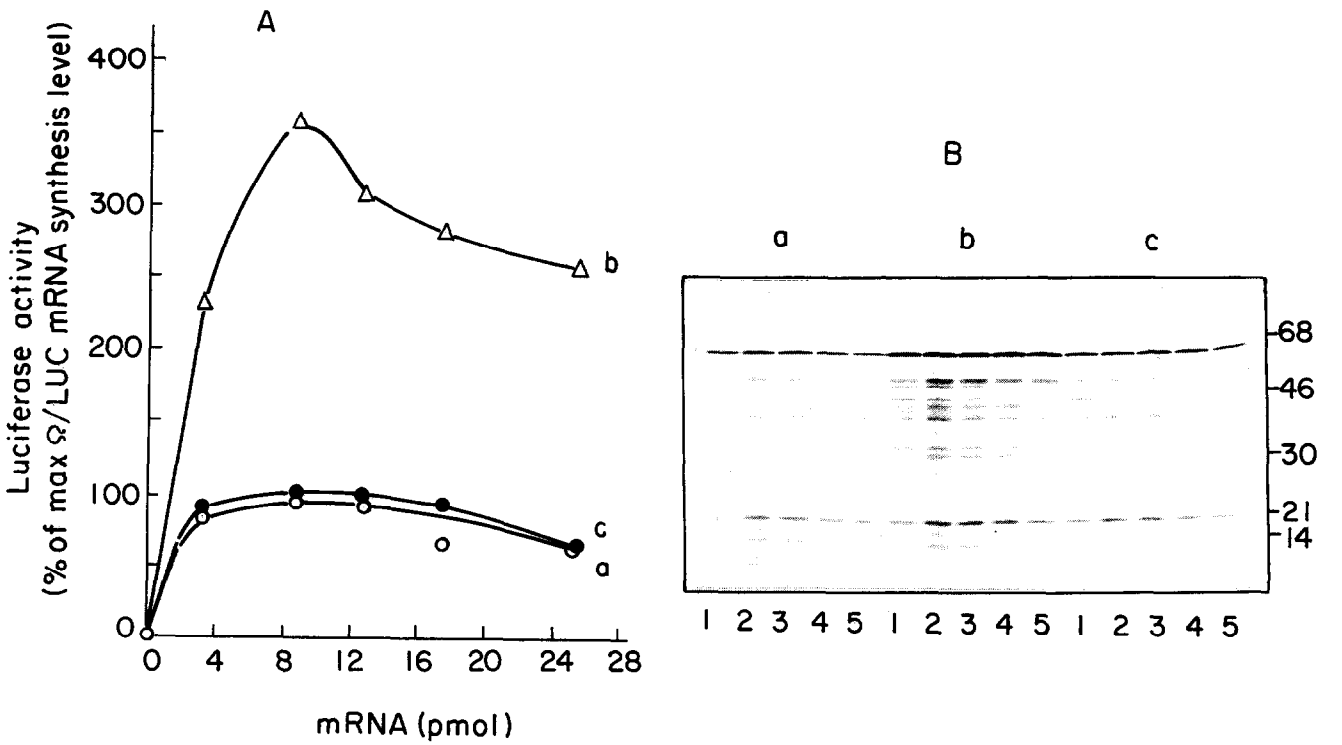

Fig. 2. Comparison of the template efficiency of the chimeric mRNAs differing in the length of their 3'-terminal portions in the course of translation. (A) The graph shows the amount of functionally active protein synthesized as a function of the amount of chimeric mRNA. (B) The autoradiograph shows the electrophoretic patterns of the [^{14}C]proteins synthesized in the wheat germ cell-free system with (1) 3.5 pmol, (2) 9 pmol, (3) 13 pmol, (4) 18 pmol and (5) 26 pmol of chimeric mRNA. Three variants of chimeric mRNA were used for translation: (a) Ω/LUC mRNA, (b) Ω/LUC/TMV 3'-UTR mRNA, and (c) Ω/LUC/TMV 3'-UTR/PL UTR mRNA.

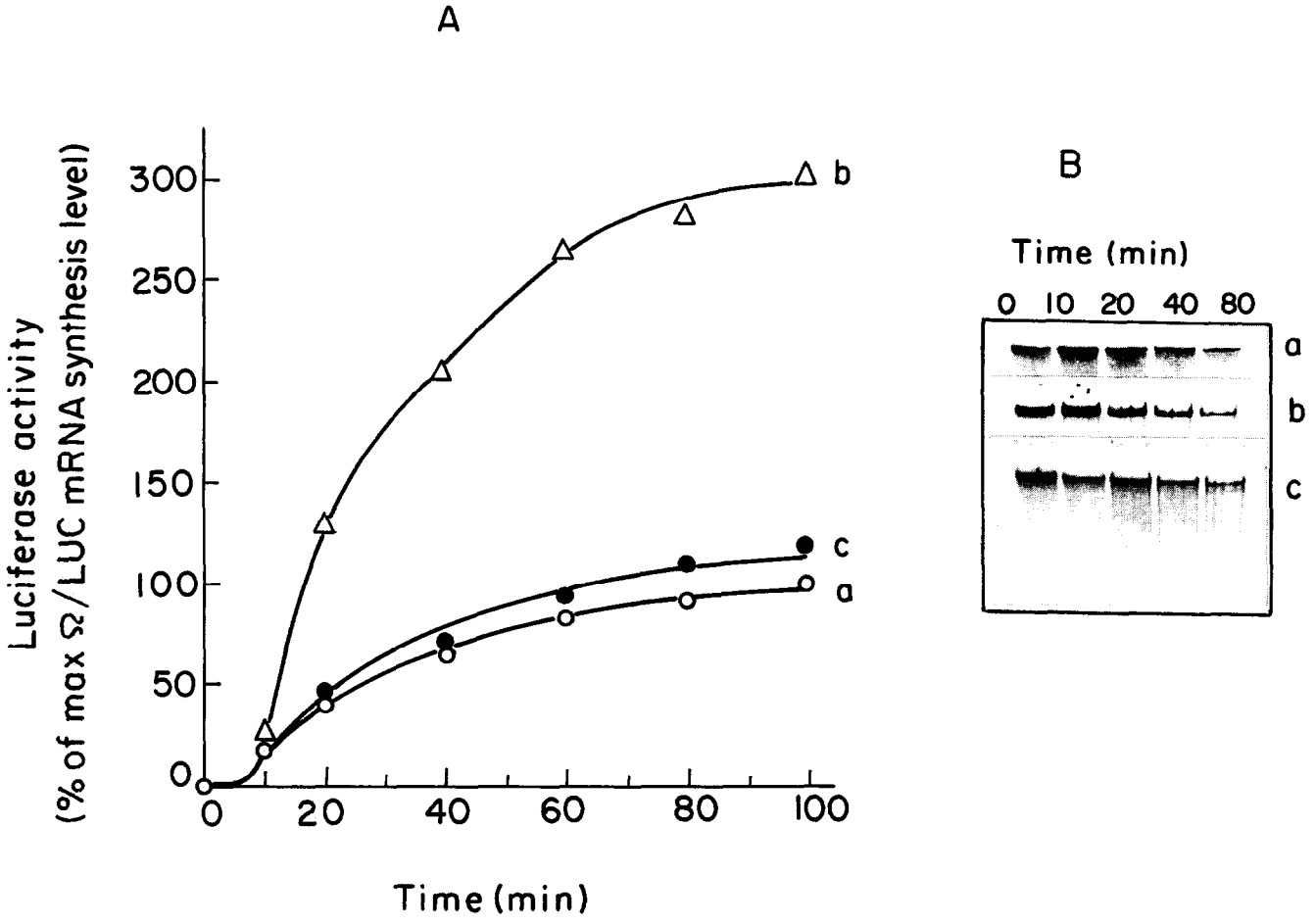

Fig. 3. (A) Time-course of synthesis of functionally active protein in the wheat germ cell-free system and (B) degradation during translation, programmed with (a) Ω/LUC mRNA, (b) Ω/LUC/TMV 3′-UTR mRNA, and (c) Ω/LUC/TMV 3′-UTR/PL UTR mRNA.

amount of protein synthesized by each time point was apparently higher in the reaction with the Ω/LUC/TMV 3′-UTR mRNA during all the translation periods. Thus, a significantly lower level of protein synthesis was seen in the absence of the trailer. Second, the proper length of the TMV 3′-terminal part is important for efficient translation: elongation of the TMV tail by 160 vector-derived nucleotides fully abolishes the stimulation effect of the TMV 3′-UTR in vitro. Electrophoretic analysis of [^{14}C]RNAs in the course of translation (Fig. 3B) demonstrated that the low level of the tail-less Ω-LUC mRNA translation is not caused by its preferential degradation. Approximately the same level of degradation was shown in the case of each RNA during the 80 min incubation time.

Acknowledgements: This work was supported by the Russian Academy of Sciences and by Grant 93-04-6638 from the Russian Foundation for Basic Research.

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