The 3′-untranslated region of brome mosaic virus RNA does not enhance translation of capped mRNAs in vitro

O.V. Karpova, V.A. Mavrodieva, O.L. Tomashevskaya, N.P. Rodionova, J.G. Atabekov*

Department of Virology, Moscow State University, Moscow 119899, Russian Federation

Received 13 January 1995

Abstract The translation enhancing ability of *cis*-acting 3′-terminal untranslated region (3′-UTR) of brome mosaic virus (BMV) was examined. Two chimeric mRNA constructs translated in rabbit reticulocyte lysates contained the BMV coat protein (CP) gene and NPTI gene, respectively. It was shown that the 3′-UTR of BMV RNA enhanced the translational efficiency of uncapped but not capped messages.

Key words: Plant viral 3′-UTR; Cap structure; Translational enhancement

1. Introduction

It has been well documented that the 5′- and 3′-untranslated sequences of eukaryotic mRNAs may contribute to the regulation of genes translation or/and to the message stability [1,2]. The genomic and subgenomic RNAs of several plus-sense RNA viruses contain 3′-terminal untranslated regions (3′-UTR) which terminate in so called tRNA-like structures and are similar enough to cellular tRNAs to be recognized by several tRNA-specific proteins (for review see [3]).

The 3′-UTRs of the three genomic RNAs (RNAs 1–3) and one subgenomic (sg) RNA 4 of brome mosaic virus (BMV) contain the 3′-terminal 134 nucleotides forming the tyrosine-accepting tRNA-like structure [4]. BMV RNA3 (2,117 nt) comprises the 5′-proximal 3a gene coding for the 32K movement protein [5], the coat protein gene (from 1,241 to 1,820 nt) and 3′-UTR (from 1,821 to 2,117); BMV sgRNA4 consists of the coat protein (CP) gene and 3′-UTR, i.e. is 3′-coterminal with RNA 3. The 3′-UTR of BMV RNAs 3 and 4 (about 300 nt) consist of five pseudoknots (four of them being located upstream of tRNA-like structure) and three nonpseudoknotted stem-loop structures in this upstream region.

It has been shown by [6] that deletion of tRNA-like structure from BMV RNA 3 had a minor (if any) effect on its stability in barley protoplasts and actually no difference in translational efficiency in vitro could be detected between wild-type RNA 3 and those bearing deletions within the 3′-UTR. The authors also reported that the deletions within the 3′-UTR did not affect translatability of BMV RNA 4 in vivo [6].

Somewhat different observations have been done by Gallie and co-workers on the role of TMV 3′-UTR in translational control [2,7,8]. They found that TMV 3′-UTRs enhanced the translation of capped mRNA but did little to increase translatability of uncapped mRNA in vivo. Addition of the TMV 3′-UTR to a reporter gene failed to increase translation in rabbit reticulocyte lysates regardless of whether the mRNA was capped or not [8], however it was reported recently [9] that considerable stimulation of uncapped mRNAs translation occurred in wheat germ extracts. This is in agreement with our results [10] showing that addition of the TMV or BMV 3′-UTR to the uncapped chimeric mRNAs containing the neomycin phosphotransferase (NPTI) gene enhanced sharply their translational efficiencies in reticulocyte lysates.

Thus, it has been reported that: (i) TMV or BMV 3′-UTR enhanced the translation of capped (but not uncapped) mRNAs in vivo [8,11]; (ii) BMV 3′-UTR did not influence the translatability of capped (cap^+) BMV RNA 3 in vitro or BMV RNA 4 in vivo [6] and (iv) both of TMV and BMV 3′-UTRs enhanced the translational efficiency of uncapped (cap^-) mRNAs in vitro [9,10]. In this study we examined the role of the BMV 3′-UTR in translation of the BMV CP gene and chimeric mRNAs in rabbit reticulocyte lysates (RRL) and found that 3′-UTR enhanced the translation of only uncapped (but not capped) messages.

2. Materials and methods

Preparative and analytical methods including virus accumulation, RNA isolation and aminoacylation, radioactivity assay, electrophoresis in agarose and polyacrylamide gels and isolation of RNA fragments from agarose were described previously [12,13].

Cell-free translation in RRL or Krebs 2 cells extracts was performed as described [14,15].

Site-specific cleavage of BMV RNA with RNase H and ligation of RNA fragments with T4 RNA ligase was performed according to [13,15]. Two synthetic oligodeoxynucleotides complementary to the 3′-UTR of BMV RNA 4 (and 3) were used for RNase H cleavage: oligonucleotide 1 (5′-GGGGTGAAGAAG-3′) and oligonucleotide 2 (5′-CAACCACGACT-3′). The 3′-UTR1 (about 300 nt) and 3′-UTR2 (about 160 nt) could be cleaved out from BMV RNAs in the presence of oligonucleotides 1 and 2, respectively.

Plasmid constructs and in vitro transcription: capped and uncapped forms of NPT1 gene transcripts were prepared by in vivo transcription of linearized plasmids as described by [16,17].

3. Results

3.1. Influence of the 3′-UTR of BMV RNA on translation of cap^- and cap^+ BMV CP gene

The method of site-specific cleavage of large RNAs with RNase H from *E. coli* in the presence of oligodeoxynucleotides complementary to given sites of RNA has been repeatedly applied to different viral RNAs [12,13,18,19].

In the first series of experiments the relative levels of translation of authentic BMV RNAs 3 and 4 and those deprived of their 3′-UTR were compared. To this end BMV RNA 4 and RNA 3 were treated with RNase H in the presence of two

*Corresponding author. Fax: (7) (095) 938 06 01.

SSDI 0014-5793(95)00103-4

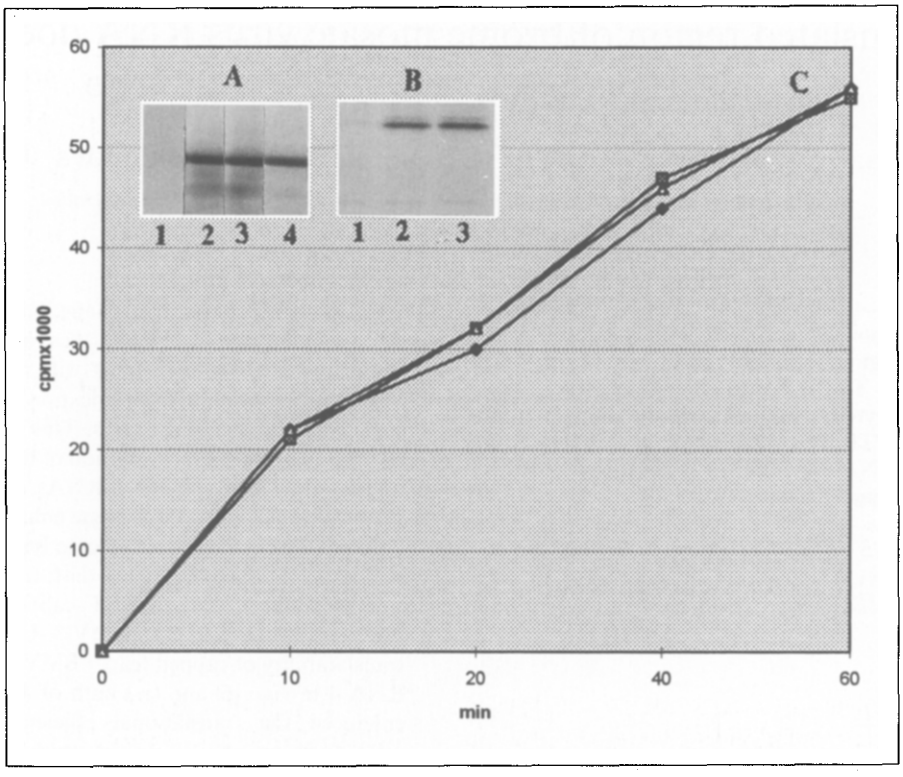

Fig. 1. Autoradiograph of SDS-PAGE (8–20%) of the [^{35}S]methionine-labeled translational products synthesized in RRL (60 min incubation). (A) BMV CP coded by: native BMV RNA4 (2); BMV RNA4 deprived of tRNA-like structure (BMV RNA4 3′-UTR$^-$) (3); short 3′-terminal CP gene carrying fragment of BMV RNA 3 (Sh-RNA3) (4); no RNA added (control) (1); 2 μg RNA was added in (2–4). (B) The BMV 3a gene-product (32K protein) coded by: native BMV RNA 3 (2); RNA 3 deprived of 3′-UTR (3); no RNA added (1); 2 μg RNA was added in (2–3). (C) Time course of translation in RRL of: intact BMV RNA 4 (—◆—), BMV RNA 4 deprived of tRNA-like structure (-▨-), and Sh-RNA 3 (—△—). Oligodeoxynucleotide 1 (see section 2) was used for RNase H directed cleavage of RNAs.

oligodeoxynucleotides 1 and 2 (see section 2). Translational efficiency of BMV RNAs 4 and 3 lacking their 3′-UTRs was compared with that of intact viral RNAs in RRL. Very similar results were obtained with both oligonucleotides, therefore only the data obtained with oligonucleotide 1 are presented. Fig. 1A (lanes 2 and 3) shows that the presence or absence of the 3′-UTR had no noticeable effect on the level of translation of capped monocistronic BMV RNA 4 (Fig. 1A) or bicistronic BMV RNA 3 (Fig. 1B). This observation was in agreement with our earlier results showing that removal of the 3′-terminal tRNA structure did not change neither coding specificity nor the translational efficiency of TMV [20] and barley stripe mosaic virus RNAs [19]. The results of comparative time course translation (Fig. 1C) showed that removal of the 3′-UTR from BMV RNA 4 did not influence its translational efficiency (and consequently, its stability). This was in apparent discrepancy with the results of in vivo translation [8,11] showing that TMV and BMV 3′-UTRs were dependent on the presence of cap to serve as translational enhancers.

BMV RNA 3 can be cleaved site-specifically by RNase H in the presence of oligo $d(T)_{10}$ producing two translatable fragments: the 5′-proximal long fragment containing the 3a gene and the 3′-proximal short (ShRNA3) fragment containing the CP gene and the 3′-UTR [12,13,21]. The Sh-fragment differs from authentic sgRNA 4 in two features: (i) it is uncapped and (ii) it contains 29–31 nontranslated 5′-terminal nucleotides upstream of the CP gene AUG codon whereas the RNA 4 leader sequence is only 9 nt. The Sh-fragment could be depleted of the 3′-UTR by RNase H cleavage in the presence of appropriate complementary oligodeoxynucleotide to obtain the BMV CP-gene uncapped and devoid of the 3′-UTR. The following four forms of CP gene have been derived thereby from BMV RNA 3 and 4: (i) Authentic (i.e. capped and containing 3′-UTR) RNA 4 (cap$^+$3UTR$^+$); (ii) Sh RNA3-fragment, i.e. uncapped but containing the 3′-UTR CP gene (cap$^-$3′-UTR$^+$); (iii) RNA 4 devoid of about 300 nt of the 3′-UTR by RNase H (cap$^+$3′-UTR$^-$); and (iv) Sh-fragment devoid of the 3′-UTR by RNase H (cap$^-$3′-UTR$^-$).

All these RNAs were electrophoresed in 1% agarose gel and the appropriate bands were cut out as described earlier [12,13]. Then RNA was isolated from agarose and translated in RRL producing a major product, the CP with M_r of 20 K. In parallel experiments all RNAs were tested for their ability of being aminoacylated with tyrosine in the presence of wheat germ aminoacyl-tRNA synthetase; the efficiency of aminoacylation of authentic BMV RNAs 3 and 4 was taken as 100%. The data of electrophoretic analysis (not presented, see [13]) taken together with the results of aminoacylation experiments (Fig. 2) show that the 3′-UTRs have been cleaved out from both Sh RNA3-fragment and RNA 4 resulting in production of (i) non-aminoacylatable fragment: (ShRNA cap$^-$3′-UTR$^-$, RNA 4 cap$^+$3′-UTR$^-$ and short aminoacylatable 3′-UTRs containing tRNA-like structures (3′-UTR1 or 3′-UTR2 consisting of about 300 nt and 160 nt, respectively).

Translation of the BMV RNAs-derived CP-gene-carrying RNA fragments in RRL allowed to conclude that: (i) efficiency

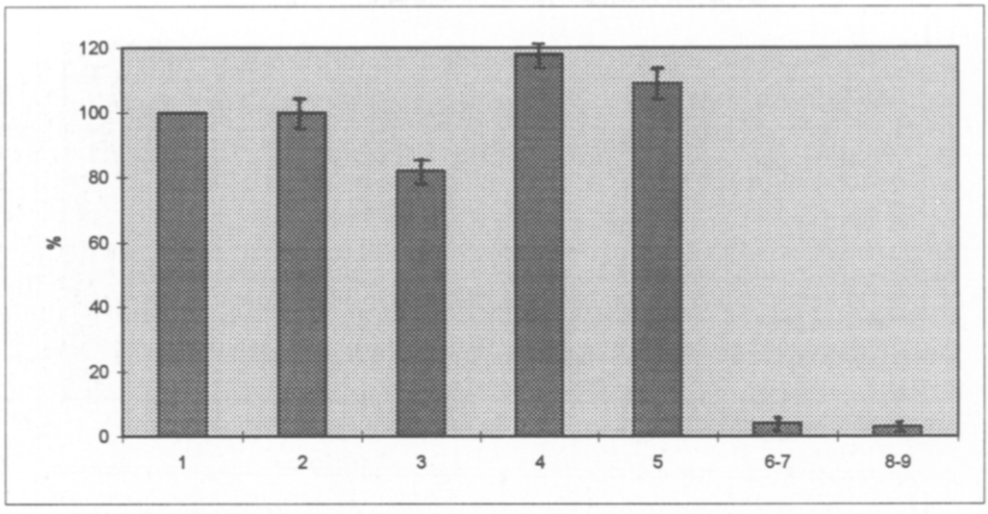

Fig. 2. Relative levels of aminoacylation of BMV RNAs 4 and 3 and the fragments derived from them by RNase H cleavage: (1) RNA 3, (2) RNA 4 (cap^+, 3′-UTR^+); (3) Sh RNA 3 (cap^-, 3′-UTR^+); (4) 3′-UTR 300 nt-long (3′-UTR1); (5) 3′-UTR 160 nt-long (3′-UTR2); (6,7) Sh RNA deprived of 3′-UTR1 or 3′-UTR2 (Sh RNA cap^-3′-UTR^-); (8,9) RNA 4 deprived of 3′-UTR1 or 3′-UTR2 (RNA 4 cap^+3′-UTR^-). The 3′-UTR1 and 3′-UTR2 were cleaved out from BMV RNA; by RNase H in the presence of oligonucleotide 1 and 2 (see section 2). Standard error bars are presented, results are averages of at least four independent experiments.

of translation of authentic (cap^+3′-UTR^+) RNA 4 and BMV RNA 3-derived CP gene carrying Sh-fragment (cap^-3′-UTR^+) were relatively equal (Fig. 3); comparative time course translation of these two RNAs (Fig. 1C) supported this conclusion; (ii) removal of 3′-UTR from BMV RNA 4 did not decrease its template activity (Figs. 1 and 3); (iii) on the other hand, removal of the 3′-UTR from Sh-fragment (uncapped CP-gene) resulted in about two-fold reduction of its translational efficiency (Fig. 3).

These observations suggest that the 3′-UTR of BMV RNA does not influence the translational efficiency of capped mRNAs being active as translational enhancer only within uncapped mRNA. To verify this hypothesis a chimeric mRNAs were constructed and the influence of the BMV RNA 3′-UTR on translational efficiency of NPTI reporter gene in capped (cap^+) and uncapped (cap^-) form was examined.

3.2. *Influence of the 3′-UTR of BMV on translation of chimeric cap^- and cap^+ mRNAs*

We have previously demonstrated that the 5′-nontranslated sequence of potato virus X RNA ($\alpha\beta$-sequence and its deletion derivative $\Delta\alpha\beta$) strongly enhanced the translational efficiency

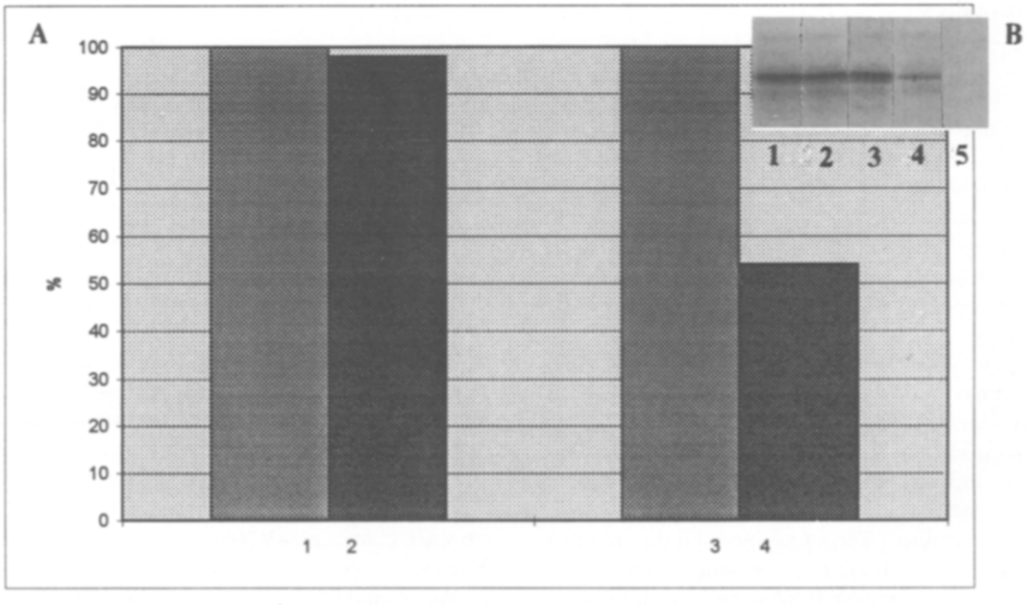

Fig. 3. (A) Relative translation levels of: (1) BMV RNA 4 (RNA 4 cap^+3′-UTR^+); (2) RNA 4 deprived of 3′-UTR1 (RNA 4 cap^+3′-UTR^-); (3) Sh RNA3 (Sh RNA 3 cap^-3′-UTR^+); (4) Sh RNA deprived of 3′-UTR1 (Sh RNA cap^-3′-UTR^-). Results are averages of at least three independent experiments. (B) Autoradiograph of SDS-PAGE (8–20%) of the [^{35}S]methionine-labeled translational products synthesized in RRL under the direction of: (1) BMV RNA 4 cap^+3′-UTR^+; (2) RNA 4 cap^+3′-UTR^-; (3) Sh RNA 3 cap^-3′-UTR^+; (4) Sh RNA cap^-3′-UTR^-; (5) no RNA added; 1 μg of RNA was added in (1–4).

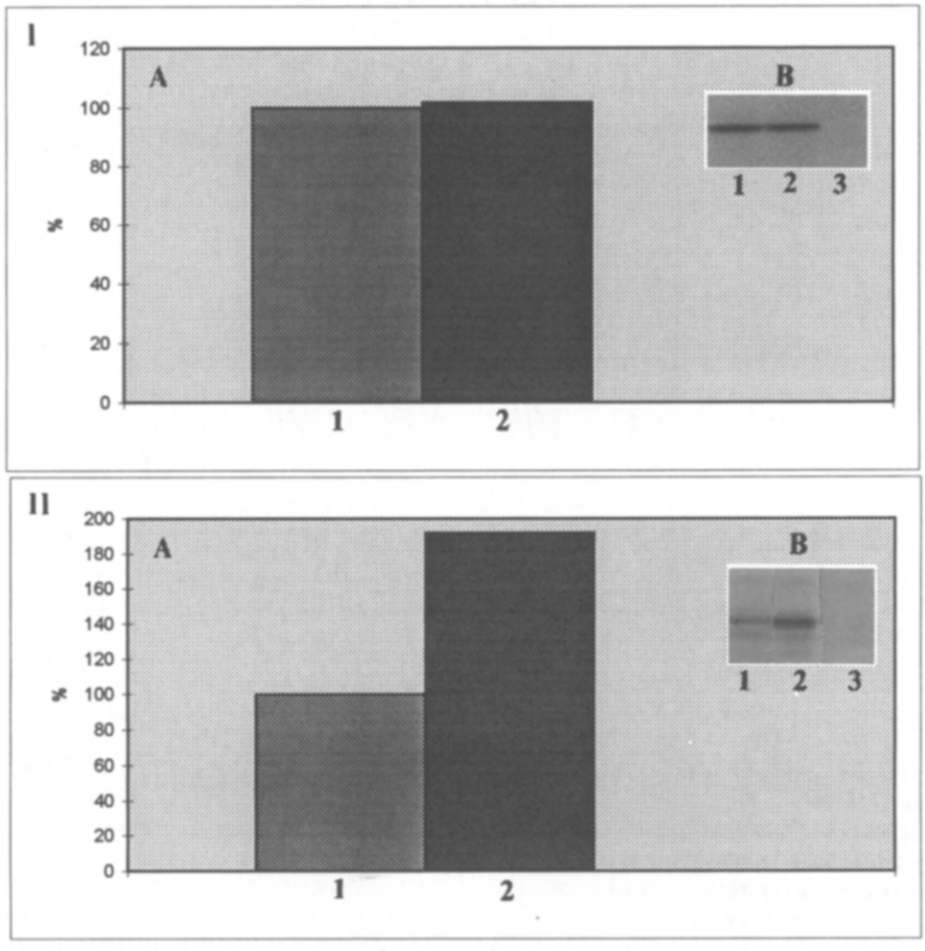

Fig. 4. Enhancing of uncapped $\Delta\alpha\beta$NPTI mRNA translation by the 3′-UTR of BMV. (A) Relative translation levels of cap$^+$ (exp. No. I) and cap$^-$ (exp. No. II) mRNAs containing or lacking the 3′-UTR of BMV RNA: Exp. I, cap$^+$ $\Delta\alpha\beta$NPT 3′-UTR$^-$ (1); cap$^+$ $\Delta\alpha\beta$NPT 3′-UTR$^+$ (2); Exp. II, cap$^-$ $\Delta\alpha\beta$NPT1 3′-UTR$^-$ (1); cap$^-$ $\Delta\alpha\beta$NPT1 3′-UTR$^+$ (2). (B) Autoradiograph of SDS-PAGE (8–20%) of the [^{35}S]methionine-labeled translation products synthesized in RRL. For abbreviations, see 'A'; lane 3 corresponds to endogeneous translation (no RNA added); 2 μg and 0.8 μg of RNA was added in Exp. I and II, respectively.

of different reporter genes [16,17,22]. The cDNA constructs comprising T7 bacteriophage RNA polymerase promoter, $\Delta\alpha\beta$ sequence and the NPTI gene were transcribed by T7 polymerase [17]. The mRNAs were synthesized in uncapped or capped forms (cap$^-$ $\Delta\alpha\beta$ NPTI and cap$^+$ $\Delta\alpha\beta$NPTI). At the next step, the 3′-UTR1 or 3′-UTR2 derived from BMV RNAs by RNase cleavage was ligated with cap$^-$ or cap$^+$ forms of NPTI gene by T4 bacteriophage RNA ligase as described earlier [12,13] producing (cap$^-$$\Delta\alpha\beta$NPTIBMV3′-UTR) and (cap$^+$$\Delta\alpha\beta$NPTIBMV3′-UTR) chimeric RNAs, respectively. The material subjected to incubation with RNA ligase and ATP was analyzed by electrophoresis in a 1% agarose gel; after isolation from agarose the chimeric mRNAs were quantified and then translated in RRL. Radioactive bands corresponding to NPTI protein were excised from the dried gel and their radioactivity was determined. The translational data obtained with chimeric mRNAs (Fig. 4) are in agreement with results of authentic BMV CP-gene-carrying RNAs translation (Fig. 3) showing that the 3′-UTR of BMV enhanced translation of only uncapped (but not capped) mRNAs in vitro. In separate experiments it was found that chimeric mRNAs used were reasonably stable in cell-free system: no significant changes in electrophoretic mobility or integrity were observed during 60 min translation in RRL (data not presented).

4. Discussion

The 3′-UTRs of several plant viral mRNAs were shown to increase their translational efficiency which was not correlated with the ability of 3′-UTR to stabilize and increase the half-life of mRNA molecules [2,8,11,23,24].

The results reported on the role of BMV and TMV 3′-UTRs in cell-free translation systems were rather fragmentary. Lasher et al. [6] found that no important relationship existed between the presence of the 3′-UTR and translatability of capped BMV RNA 3 in wheat germ system. On the other hand, it was shown [9,10] that addition of TMV and BMV 3′-UTRs to the uncapped chimeric mRNAs enhanced their translational efficiency.

We examined the role of BMV 3′-UTR in translation of authentic BMV CP-gene-carrying RNA 4 (cap$^+$3′-UTR$^+$) or its derivatives (cap$^-$3′-UTR$^+$; cap$^+$3′-UTR$^-$; cap$^-$3′-UTR$^-$) and influence of the BMV 3′-UTR on translation of cap$^+$ or cap$^-$ NPTI gene in vitro. Our experiments have shown that in RRL

the BMV 3′-UTR enhanced the translation of only cap⁻ messages having no impact on translation of capped mRNAs (Fig. 3 and 4). Our data demonstrate that the cap and 3′-UTR of BMV RNA are not interdependent and no synergism between these two elements could be revealed in RRL. Moreover, the 3′-UTR could somewhat compensate the cap absence within the cap⁻ mRNAs (Figs. 3 and 4). It should be noted that the RRL cell-free system is an order of magnitude less cap-dependent than wheat germ extracts (e.g. see Timmer et al. [23]) and in vivo translation systems [2].

Our observations allowed to suggest that the cap and 3′-UTR may compete for a hypothetical *trans*-acting factor(s) which is limiting in RRL. However in numerous experiments we did not detect any inhibiting effect of exogeneously added BMV (or TMV) 3′-UTRs on translation of cap⁺ or cap⁻ mRNAs in RRL or Krebs 2 cells extracts (data not presented). One can speculate that the cap and 3′-UTR should be positioned within a single physically intact mRNA molecule for such competition to occur.

Acknowledgements: This work was supported by the Russian Foundation for Basic Research and partially by International Scientific Foundation.

References

[1] Sleat, D.E. and Wilson, T.M.A. (1992) in: Genetic Engineering with Plant Viruses (Wilson, T.M.A. and Davies, J.W., Eds.) pp. 55–114, CRC Press, Boca Raton.
[2] Gallie, D.R., Feder, J.N., Schimke, R.T. and Walbot, V. (1991) Nucleic Acids Res. 19, 5031–5036.
[3] Prochiants, A. and Haenni, A.L. (1973) Nature 241, 168–170.
[4] Miller, W.A., Bujarsky, J.J., Dreher, T.W. and Hall, T.C. (1986) J. Mol. Biol. 187, 537–546.
[5] Allison, R., Tompson, C. and Ahlquist, P. (1990) Proc. Natl. Acad. Sci. USA 87, 1820–1824.
[6] Lahser, F.C., Morsh, L.E. and Hall, T.C. (1993) J. Virol. 63, 3295–3303.
[7] Gallie, D.R. and Walbot, V. (1990) Genes Dev. 4, 1149–1157.
[8] Leathers, V., Tanguay, R., Kobayashi, M. and Gallie, D.R. (1993) Mol. Cell. Biol. 13, 5331–5347.
[9] Zeyenko, V.V., Ryabova, L.A., Gallie, D.R. and Spirin, A.S. (1994) FEBS Lett. 354, 271–273.
[10] Mavrodieva, V.A. (1994) Ph.D. Thesis Moscow State University.
[11] Gallie, D.R. and Kobayashi, M. (1994) Gene 142, 159–165.
[12] Tyulkina, L.G., Karpova, O.V., Rodionova, N.P. and Atabekov, J.G. (1987) Virology 159, 312–320.
[13] Karpova, O.V., Tyulkina, L.G., Rodionova, N.P., Atabekov, K.J. and Atabekov, J.G. (1989) J. Gen. Virol. 70, 2287–2297.
[14] Morozov, S.Yu., Miroshnichenko, N.A., Zelenina, D.A., Fedorkin, O.N., Solovyev, A.G., Lukashova, L.I. and Atabekov, J.G. (1991) Biochimie 72, 677–684.
[15] Karpova, O.V., Tyulkina, L.G., Rodionova, N.P. and Atabekov, J.G. (1989) Arch. Phytopathol. Pflanzenschutz, Berlin 251, 15–26.
[16] Smirnyagina, E.V., Morozov, S.Yu., Rodionova, N.P., Miroshnichenko, N.A., Solovyev, A.G., Fedorkin, O.N. and Atabekov, J. (1991) Biochimie 73, 998–1011.
[17] Tomashevskaya, O.L., Solovyev, A.G., Karpova, O.V., Fedorkin, O.N., Rodionova, N.P., Morozov, S.Yu. and Atabekov, J.G. (1993) J. Gen. Virol. 74, 2717–2724.
[18] Stepanova, O.B., Metelev, V.G., Chichkova, N.V., Smirnov, V.D., Rodionova, N.P., Atabekov, J.G., Bogdanov, A.A. and Shabarova, Z.A. (1979) FEBS Lett. 103, 197–199.
[19] Agranovsky, A.A., Dolja, V.V. and Atabekov, J.G. (1982) Virology 119, 51–58.
[20] Rodionova, N.P., Karpova, O.V., Metelev, V.G., Bogdanova, S.L., Shabarova, Z.A. and Atabekov, J.G. (1983) Mol. Biol. (Moscow) 17, 809–817.
[21] Smirnyagina, E.V., Karpova, O.V., Miroshnichenko, N.A., Rodionova, N.P. and Atabekov, J.G. (1989) FEBS Lett. 254, 66–68.
[22] Zelenina, D.A., Kulaeva, O.I., Smirnyagina, E.V., Solovyev, A.G., Miroshnichenko, N.A., Fedorkin, O.N., Rodionova, N.P., Morozov, S.Yu. and Atabekov, J.G. (1992) FEBS Lett. 296, 267–270.
[23] Timmer, R.T., Benkowski, L.A., Schodin, D., Lax, S.R., Metz, A.M., Ravel, J.M. and Browning, K.S. (1993) J. Biol. Chem. 268, 9504–9510.
[24] Ryabova, L.A., Torgashov, A.F., Kurnasov, O.V., Bubunenko, M.C. and Spirin, A.S. (1993) FEBS Lett. 326, 264–268.