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Translational in vitro activity of the 3a gene and the coat protein gene derived from brome mosaic virus RNA 3 by site-specific cleavage with RNase H

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Two translationally active fragments were derived from the dicistronic Brome Mosaic Virus (BMV) RNA 3 by site-specific cleavage with RNase H from *E.coli*: the 5′-proximal (L) fragment encoding the 32 kDa protein and the 3′-proximal (Sh) fragment carrying the coat protein gene. The translational efficiency of the L- and Sh-fragments was compared with those of the native BMV RNA 3 and RNA 4, encoding the 32 kDa and coat proteins, respectively. The Sh-fragment template activity was similar to that of RNA 4, although it was uncapped and contained 20–22 additional 5′-terminal nucleotides in comparison with BMV RNA 4.

RNA cleavage, site specific; Translation; Nontranslated leader sequence

1. INTRODUCTION

The BMV genome consists of three RNA species (RNAs 1–3). RNAs 1 and 2 are monocistronic and encode the putative components of viral RNA polymerase. RNA 3 is dicistronic: the 5′-proximal gene (3a) encodes the 32 kDa protein (tentative transport protein), the 3′-proximal gene encoding the coat protein. BMV RNA 3 is functionally monocistronic: the coat protein gene is translationally silent. A separate subgenomic RNA (RNA 4), 3′-coterminal with RNA 3, is produced to express the coat protein gene [1]. There is an intercistronic noncoding region (about 250 nucleotides long) between the 3a and coat protein genes, containing an internal poly(A) tract located 29 bases 5′ to the AUG codon of the coat protein gene [2].

BMV RNA 3 can be cleaved site-specifically by RNase H from *E. coli* in the presence of oligo $d(T)_{10}$ producing two functionally active fragments: the 5′-proximal (L) fragment containing the 3a gene and the 3′-proximal (Sh) fragment containing the coat protein gene and the 3′-terminal tRNA-like structure accepting tyrosine [3]. It should be noted that the Sh-fragment differs from authentic subgenomic RNA 4 in two features: (i) the Sh-fragment is uncapped and (ii) it contains 29–31 nontranslated 5′-terminal nucleotides upstream to the coat protein gene AUG codon [3], whereas the RNA 4 leader sequence is only 9 nucleotides long.

Here, the translational efficiency of the L- and Sh-fragments derived from BMV RNA 3 has been examined in comparison with native BMV RNAs 3 and 4.

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2. MATERIALS AND METHODS

BMV strain Russian was propagated in wheat plants (var. Mironovskaya 808) and isolated as described [4]. Viral RNAs were obtained by phenol extraction. BMV RNA 3 cleavage with RNase H in the presence of oligo $d(T)_{10}$ was performed as described by Karpova et al. [3]. Isolation of RNA fragments from agarose was performed according to Tyulkina et al. [5].

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Cell-free translation in rabbit reticulocyte or Krebs-2 was carried out according to [6,7]. [^{35}S]Methionine-labelled cell-free translation products were analyzed in 8–20% SDS-polyacrylamide gels as in [6].

3. RESULTS AND DISCUSSION

The method of site-specific cleavage with RNase H has been applied to different positions of diverse high molecular mass RNAs [3,5,8–10].

The L-fragment produced upon BMV RNA 3 cleavage with RNase H in the presence of oligo $d(T)_{10}$ contains the 3a gene and about 200 additional nucleotides downstream to the termination codon [excluding the poly(A) sequence] derived from the nontranslated intercistronic region [3]. On the other hand, the Sh-fragment is 5′-phosphorylated and contains two or three adenylates at the 5′-terminus (fig.1). Therefore, its nontranslated leader sequence is 29–31 nucleotides long whereas the authentic subgenomic BMV RNA 4 is capped and contains a 9-nucleotide-long nontranslated leader sequence.

It can be seen from fig.2 that the L-fragment derived from BMV RNA 3 directs in vitro synthesis of the 32 kDa protein. It should be noted that the translation efficiency of the L-fragment was similar to that of its natural counterpart (RNA 3), which follows from a comparison of lanes B and D.

It has been reported that BMV RNA 4 is an efficient template for in vitro translation [11] and that

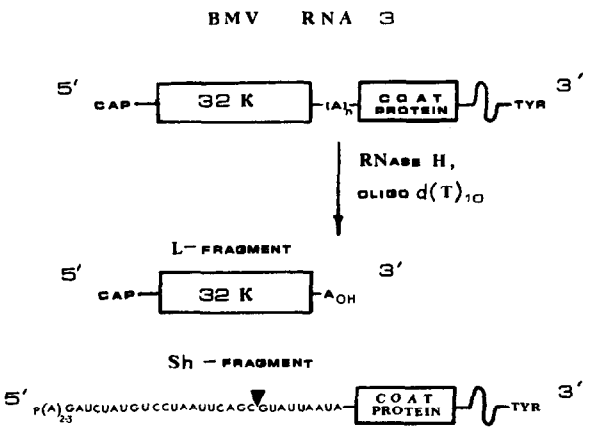

Fig.1. Structure of Brome Mosaic Virus RNA 3 and of L- and Sh-fragments derived from RNA 3 by site-specific cleavage with RNase H in the presence of oligo $d(T)_{10}$. (▼) Position of the 5′-terminal residue of BMV RNA 4.

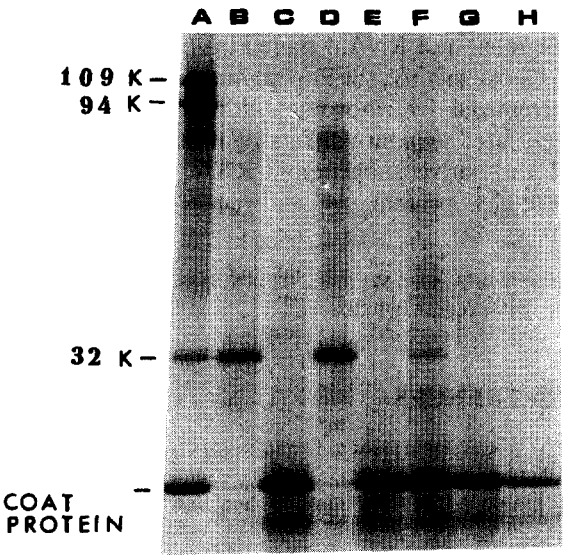

Fig.2. BMV-specific translation in rabbit reticulocyte lysate. A, virion BMV RNAs (3 μg); B, RNA 3 (2 μg); C, RNA 4 (1 μg); D, L-fragment (1 μg); E, Sh-fragment (1 μg); F, L-fragment (1 μg) + Sh-fragment (1 μg); G, RNA 3 (2 μg) + RNA 4 (1 μg); H, L-fragment (1 μg) + RNA 4 (1 μg). Numbers in parentheses indicate the amount of exogenous RNA added to 25 μl cell-free system.

its 5′-leader sequence ensures high affinity to ribosomes [12,13]. A considerable decrease in translational activity in wheat germ extracts after RNA decapping was reported for BMV RNA 4 by Shih et al. [14]. On the other hand, it was shown that 5′-extra nucleotides reduce the translation efficiency of transcripts from alfalfa mosaic virus RNA 4 DNA copies [15] and from some potato virus X gene DNA copies (Miroshnichenko et al., in preparation).

Although the Sh-fragment is uncapped and its 5′-nontranslated sequence carries 20–22 additional nucleotides in comparison to RNA 4 (fig.1), it can be translated efficiently in cell-free systems from rabbit reticulocyte (fig.2) or Krebs-2 cells (not shown), producing a protein which migrates like the BMV coat protein (fig.2, lanes C,E). Moreover, translation of the L-fragments is noticeably inhibited in the presence of equimolar Sh-fragment (fig.2, lanes D,F), which appears to be due to competition between these templates. Translation of the L-fragment was completely inhibited in the presence of a 2-fold excess of Sh-fragment (not shown). Similar competition occurs between BMV

RNAs 3 and 4 (fig.2, lane G) and between L-fragment and RNA 4 (lane H) upon translation.

Consequently, the Sh-fragment can be efficiently recognized by ribosomes although their 5'-non-translated sequence differs significantly from those of the native subgenomic RNA 4, encoding the viral coat protein.

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