# Short virion RNA in barley stripe mosaic virus

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## 1. INTRODUCTION

Barley stripe mosaic virus (BSMV) is the most studied representative of the group of hordeiviruses. BSMV strains have been described containing 2, 3, or 4 types of virion RNA. Length determination in agarose gels after denaturing with glyoxal [1] gave a value about 4000 residues for RNA 1, 3300 for RNA 2, 3000 for RNA 3, and 2600 for RNA 4 (V.V.D., unpublished). The genome of BSMV is functionally fragmented, comprising RNAs 1 and 2 in bipartite strains [2] and RNAs 1 to 3 in tripartite ones [3]. The functional significance of RNA 4 is obscure, the more so that it is readily lost when the virus is passaged [4].

An analysis has been carried out of the in vitro translation products of individual RNAs from several BSMV strains [5–7]. RNA 1 of all strains codes in vitro for a protein with $M_r$ of 120000 (p120). Translation of RNA 2 from a bipartite strain yields two main products: the virus coat protein ($M_r$ 23000), and a polypeptide of $M_r$ 85000 (p85). In certain ionic conditions a polypeptide of $M_r$ 25000 (p25) is also formed, which probably overlaps with the coat protein [7]. On the other hand, no p85 is formed upon translation of RNA 2 from 3 or 4-component BSMV strains. RNA 3 codes for a polypeptide of $M_r$ 75000 (p75), and RNA 4 for that of $M_r$ 55000 (p55). The amino acid sequences of p85, p75, and p55 overlap [7].

A recent paper [8] gave grounds for suggesting that the RNA 2 population of a bipartite BSMV strain is heterogeneous, comprising two types of molecules (RNA 2a and 2b) of similar length but different primary structures. Tripartite and quadripartite strains have no RNA 2b but possess RNA 3 homologous to RNA 2b.

One can see that in vitro translation expresses about 80% of information potentially coded for by RNA 1, 65% that of bipartite strain RNA 2b, only 20% of RNA 2a, some 60% of RNA 3, and about 55% of RNA 4. Since a considerable portion of the coding capacity of virion RNAs is not used during in vitro translation, one can suggest the existence of subgenomic RNAs which provide in vivo for the autonomization and realization of the information encoded in the 'closed' part of genomic RNAs. The present work describes a short virion RNA of BSMV that codes for a polypeptide with $M_r$ of about 17500 and appears to be subgenomic.

## 2. MATERIALS AND METHODS

The tripartite BSMV strain Norwich (Norwich III) was obtained from Dr L. Lane; its bipartite derivate (Norwich II) was isolated by Drs R.-M. Leiser and T. Stanarius. Preparation of pure viruses and individual virion RNAs, and RNA translation in rabbit reticulocyte lysates were described in [7]. RNA chromatography on oligo(dT)-cellulose, sucrose density gradient (10–40%) centrifugation, and electrophoresis in 4% polyacrylamide gels with 6 M urea were performed as in [9,10].

## 3. RESULTS AND DISCUSSION

In many plant viruses with either continuous or fragmented genomes, subgenomic RNAs are known to be capable of being coated by the coat protein and transferred into the viral progeny (for

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review see [1]). It was therefore natural to try to detect subgenomic RNAs in BSMV virion RNA preparations.

Comparison of the translation products of the total BSMV virion RNA and of individual genomic RNAs in the rabbit reticulocyte cell-free system revealed a substantial difference: the total virion RNA preparation coded for a protein with $M_r$ of 17500, whereas the individual genomic RNA preparations had no such ability [7]. These results were obtained for the bipartite strain Norwich II as well as for the tripartite strain Norwich III. These data gave grounds for suggesting that the total virion RNA preparation contains a special template coding for the protein of $M_r$ 17500 (p17), which is lost during preparative isolation of RNAs 1–4.

As shown in our previous work, BSMV individual RNAs contain an internal heterogeneous poly(A) sequence positioned at a distance of 210 nucleotides from the 3′-terminus which ends in a tRNA-like structure [9,10,12,13]. However, oligo-(dT)-cellulose chromatography of each RNA reveals two fractions: one is bound to the absorbent while the other is not. These fractions are designated as poly(A)$^+$ and poly(A)$^-$; their relative portions differ between individual RNAs. In fact, more than 90% of RNA 1 are poly(A)$^+$ molecules, while about 70% of RNA 2 is in the poly(A)$^-$ fraction [12].

To determine into which fraction the p17 coding mRNA goes, the total BSMV Norwich II virion RNA was subjected to oligo(dT)-cellulose chromatography. It turned out that p17 is observed exclusively among the translation products of the poly(A)$^+$ fraction of virion RNA of both 2 and 3-component BSMV strains (cf. fig.1a,1b). It must be noted that p120 is also translated mostly from the poly(A)$^+$ RNA since the latter contains almost all RNA 1.

Further, poly(A)$^+$ and poly(A)$^-$ RNA preparations were compared by polyacrylamide gel electrophoresis (not shown). The poly(A)$^+$ fraction, unlike the poly(A)$^-$ one, proved to contain a minor zone of a short RNA with $M_r$ of about $0.25 \times 10^6$. The content of this RNA in the preparation was fairly low, not exceeding 1%. The RNA was further purified and concentrated by sucrose density gradient centrifugation (fig.2A). Fractions 9 ($M_r$ $0.15–0.40 \times 10^6$ RNA) to 15 (full-size genomic RNA) were collected. For comparison, the BSMV

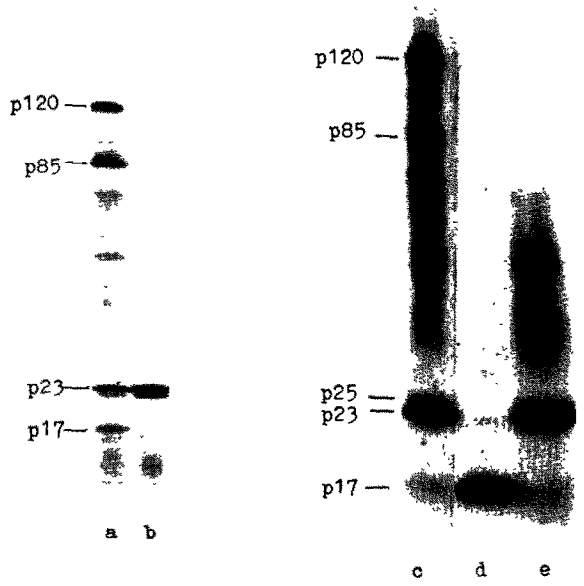


Fig.1. Electrophoretic analysis of the products of BSMV strain Norwich II RNA translation in reticulocyte lysates. (a) Virion RNA poly(A)$^+$ fraction; (b) Virion RNA poly(A)$^-$ fraction; (c) Total virion RNA; (d.e) Virion RNA with $M_r$ of $0.15–0.40 \times 10^6$ taken from the sucrose density gradient fractions 9–10 (see fig.2): (d) Poly(A)$^+$; (e) Poly(A)$^-$.

RNA poly(A)$^-$ fraction was centrifuged in a twin tube. Electrophoretic analysis of appropriate gradient fractions showed that on the background of a tail apparently formed by genomic RNA breakdown products the poly(A)$^+$ preparation contains an RNA zone with $M_r$ of about $0.25 \times 10^6$ which is lacking in the poly(A)$^-$ preparation (fig.2). This RNA seems to be a probable candidate for the part of a p17-coding template.

Preparations of RNA with $M_r$ of $0.15–0.40 \times 10^6$ obtained from the gradient were translated in a rabbit reticulocyte cell-free system. Analysis of the translation products showed that the poly(A)$^-$ fraction codes for the virus coat protein, a small amount of p25, and a heterogeneous set of products with $M_r$ up to 50000 (fig.1e). This result seems to be only natural: the fraction contains mostly the 5′-terminal and internal fragments of genomic RNAs up to $0.4 \times 10^6$. As the coat protein gene be-

gins about 80 nucleotides from the 5′-terminus of RNA 2 [14] and has dimensions of about $0.2 \times 10^6$, it can be wholly contained in the 5′-terminal fragments of such size and actively translated. The same considerations may also hold true for p25. On the other hand, neither p85 nor p120 genes can completely fit into such RNA fragments and are therefore only partly translated, yielding a heterogeneous set of products. As to the respective fraction from BSMV poly(A)$^+$ RNA, it codes exclusively for p17; i.e., the mRNA for p17 is indeed present in this fraction (see fig.1d). The 3′-terminal fragments of genomic RNA going into the poly(A)$^+$ fraction are not translated, apparently because they contain no functional initiating codons. An identical RNA coding for p17 was isolated from the total virion RNA of Norwich III (not shown).

Two most plausible suggestions can be made about the nature of the p17-coding RNA. It can be either a satellite or a subgenomic BSMV RNA. To test the first idea, the following experiment was performed: wheat plants were infected with an artificial mixture of individual genomic RNAs 1 and 2 of the bipartite BSMV strain Norwich II, which had been isolated from polyacrylamide gels and hence certainly contained no $0.25 \times 10^6$ RNA. The viral progeny was propagated to obtain the total virion RNA preparation which was then subjected to all the above-listed procedures. After gradient centrifugation, the corresponding fraction of poly(A)$^+$ RNA again contained the same quantity of the $0.25 \times 10^6$ species. Thus the satellite nature of the p17-coding RNA seems to be ruled out, and in all probability the RNA is subgenomic.

A recent work [8] also reports a short BSMV virion RNA with $M_r$ of $0.27 \times 10^6$. This RNA was shown to be homologous in its nucleotide sequence

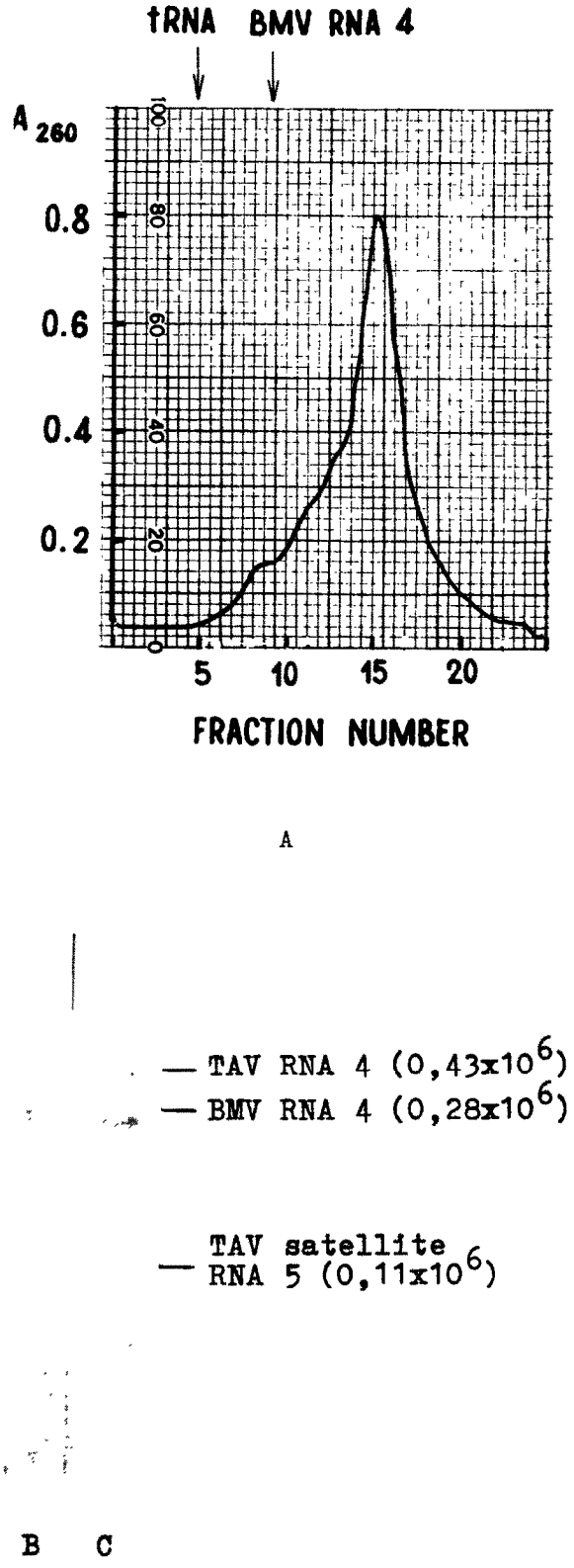


Fig.2. (A) Sucrose density gradient (10–40%, w/v) centrifugation of a poly(A)$^+$ BSMV RNA. Arrows indicate the positions of markers: total tRNA from wheat embryos, and brome mosaic virus (BMV) RNA 4 ($M_r$ of $0.28 \times 10^6$), centrifuged in sister gradients. Sedimentation from left to right; (B) Polyacrilamide gel analysis of poly(A)$^-$ BSMV RNA fractions with $M_r$ of $0.15–0.40 \times 10^6$ obtained by density gradient centrifugation (fractions 9–10 in A); (C) Poly acrilamide gel analysis of the same fractions of poly(A)$^+$ BSMV RNA. Positions and $M_r$ of RNA markers from BMV and tomato aspermy virus (TAV) are indicated on the right.

to RNA 3 of a tripartite BSMV strain and probably to RNA 2b of a bipartite one. It can be suggested that the poly(A)-containing, p17-coding RNA described here, and the $0.27 \times 10^6$ RNA found in [8] are one and the same subgenomic RNA of BSMV.

Further, translation of poly(A)$^+$ but not poly(A)$^-$ RNA from fraction 11 ($M_r$ up to $0.7 \times 10^6$) yielded a polypeptide of $M_r$ about 35000-p35 (not shown). The amount of p35 was several-times lower than of p17. It is interesting to note that BSMV-infected barley protoplasts, beside the $M_r$ $0.25 \times 10^6$ RNA, also contain a $M_r$ $0.65 \times 10^6$ RNA carrying a 3′-terminal tRNA-like structure [15]. It cannot be excluded that such RNA is present in trace amount in the virion RNA preparation and produces p35.

Thus, reconciliation of our data with those in [8] and in [15] gives grounds for suggesting that BSMV replication is attended by formation of at least two poly(A)-containing subgenomic RNAs homologous to the 3′-terminal region of genomic RNAs:

(i) RNA of $M_r$ $0.25 \times 10^6$ coding for p17;

(ii) RNA of $M_r$ about $0.7 \times 10^6$ coding for p35.

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