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The structure of barley stripe mosaic virus double-stranded RNAs

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The terminal structures of the double-stranded replicative forms (RFs) of barley stripe mosaic virus (BSMV) RNAs 1–3 have been investigated. All three BSMV RFs have identical right-hand ends but unique left-hand ends. The plus (+) strands of RFs lack the 3′-ultimate A typical for the encapsidated BSMV RNAs. The 3′-termini of the minus (−) strands contain an unpaired G. It was demonstrated that the internal poly(A) tract of BSMV genome has an equivalent poly(U)-counterpart in the RF (−) strands. The possible role of these peculiarities of BSMV RF structure in RNA replication is discussed.

RNA; Barley stripe mosaic virus; double-stranded RNA; Poly(A) sequence; (Plant virus)

1. INTRODUCTION

Barley stripe mosaic virus (BSMV) is the type member of the hordeivirus group. Its genome consists of three messenger-sense RNAs: RNA 1 (4.0 kb), RNA 2 (3.3 kb) and RNA 3 (2.8–3.2 kb depending on the virus strain) [1]. Formally, hordeiviruses belong to the tricornaviruses, however their genomes are arranged in a substantially different way. Only RNA 1 in BSMV resembles RNA 1 of tricornaviruses: it codes for a single replication-related protein of 120 kDa [2,3]. The BSMV coat protein gene is located in the 5′-terminal region of RNA 2 [2,4]. Besides, RNA 2 contains three more open reading frames [5]. RNA 3 is dicistronic; it codes for the BSMV putative replicase (74–86 kDa), and a 17 kDa protein that is expressed in vivo through a subgenomic RNA [3,6,7]. A peculiarity of BSMV is a natural instability of the gene coding for 74–86 kDa replicase: it can be elongated by duplication of some 350 nucleotides or shortened by deletion of 185 nucleotides [2,8]. Finally, the 5′-ends of BSMV RNAs are capped [9], and the 3′-terminal regions of these RNAs contain a unique tandem of an internal poly(A) tract and a 3′-terminal tRNA-like structure accepting tyrosine [10,11].

Recently the detailed terminal structures of some plant viral double-stranded replicative form (RF) RNAs have been published [12–14]. These data gave grounds for suggesting that the RF structure can really reflect the replicative strategy of viral RNA. The present work describes the characterization of the 5′- and 3′-termini of the (+) and (−) strands of BSMV-specific RF molecules.

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2. MATERIALS AND METHODS

The BSMV strain North Dakota 18 was obtained from Dr A.O. Jackson. The preparation of pure virus and RNA has been described [2]. Double-stranded RNA from wheat leaves was isolated by two cycles of Whatman CF-11 chromatography as in [15]. The individual species of BSMV RNAs ^{32}P-labeled on their 5′- or 3′-termini were isolated from Bio-Rad low-gelling-temperature agarose gels as detailed in [14], which also describes RNase T_1 digestion of the gel-purified RNAs. The pCp-

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labeled T_1-oligonucleotides were electrophoresed in 20% PAG with 7 M urea and autoradiographed. The 3′- or 5′-terminal bases of labeled BSMV RNAs were identified by complete digestion with nucleases T_2 or P_1 followed by two-dimensional chromatography in thin layers of cellulose (Eastman Kodak).

To isolate the internal poly(A) tract from BSMV RF (or virion RNA) it was denatured by boiling and quick cooling and digested with RNases T_1 and A for 1 h at 37°C. The same protocol was used for poly(U) tract isolation except that RNase A was replaced with RNase U_2. The resulting digests were 3′-labeled after phosphatase treatment and analysed in 20% sequencing PAGs.

3. RESULTS

Agarose gel electrophoresis of the double-stranded (ds) RNA isolated from BSMV-infected wheat leaves revealed three bands corresponding in size to those expected for BSMV RFs 1–3. The 3′-termini of the (+) and (−) strands of RFs were characterized by the following procedures: (i) 3′-terminal labeling of virion RNAs and RFs; (ii) excision of individual bands from the agarose gel; (iii) their dilution and thermal denaturation of RNA; (iv) RNase T_1 digestion, purification and concentration of the resulting terminally labeled T_1-oligonucleotides; (v) their analysis in sequencing PAG. This experimental approach allowed separation of the two labeled 3′-terminal oligonucleotides owing to the different positions of the first internal G (from the 3′-end) on the two strands in each of the three BSMV RFs (see fig.1a–c). Since BSMV genomic RNAs have unique 5′-termini [5,8] but identical 3′-termini [10,11], we anticipated that the tetranucleotide common for RFs 1–3 (denoted in fig.1a–c) would represent the 3′-termini of their (+) strands. On the other hand, labeled T_1-oligonucleotides unique to each of the three RFs would represent the 3′-termini of their (−) strands. Notably, the same analysis performed with individual virion RNAs revealed a pentanucleotide identical for RNAs 1–3 (fig.1d–f). It is the pentanucleotide that should be formed after T_1 hydrolysis of the pCp-labeled 3′-terminal extremity of BSMV RNAs, since the sequence of this region is ...GACCA$_{OH}$ [11]. These data allowed us to conclude that the

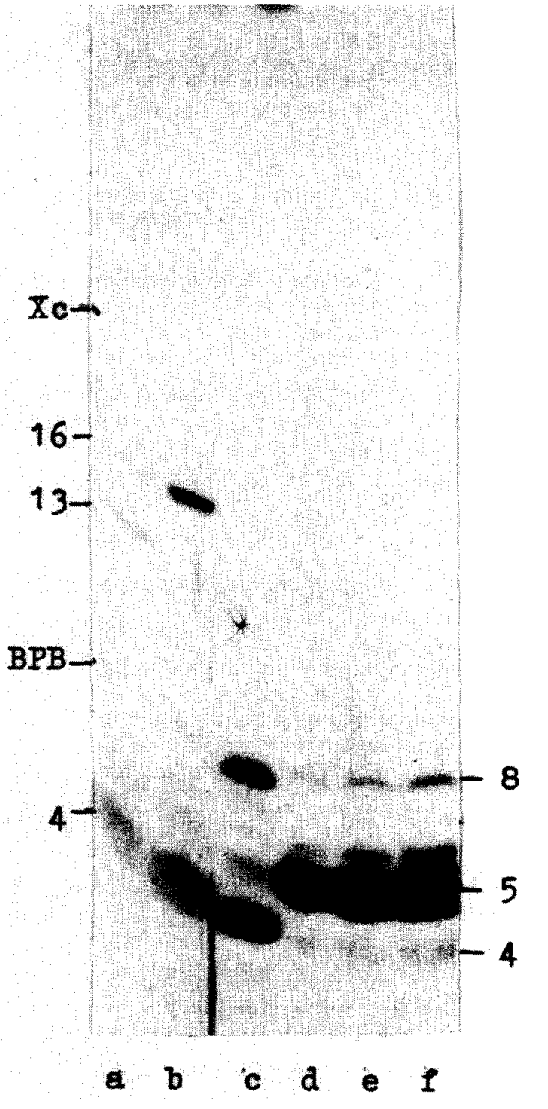

Fig.1. 20% PAG of RNase T_1 digests of pCp-labeled and agarose-gel-purified BSMV virion RNAs and RFs. (a) RF 1, (b) RF 2, (c) RF 3, (d) RNA 1, (e) RNA 2, (f) RNA 3. The positions of major labeled T_1-oligonucleotides are indicated.

3′-termini of the (+) strands isolated from RFs 1–3 were one base shorter than those isolated from virion RNAs 1–3. This conclusion was supported by determining the 3′-terminal bases of the tetra- and pentanucleotides. As expected these were A for virion RNAs and C for the (+) strands of RFs 1–3 (not shown). Thus, the positive strands of all

three BSMV RFs end in an identical sequence that lacks the 3′-ultimate A as compared to the encapsidated RNAs.

As seen in fig.1a–c, the labeled 3′-termini of the (−) strands after T_1 digestion yielded oligonucleotides with lengths of 13 bases for RF 1, 16 for RF 2, and 8 for RF 3. Identification of the 3′-terminal residues of these oligonucleotides gave the predominant G in all three cases. Only the oligonucleotide from RF 2 contained besides G some 3′-terminal C (not shown). Notably, these 3′-terminal Gs have no complements at the 5′-termini of single-stranded, encapsidated RNAs 2 [5] and 3 [8]. As deduced from the published sequences, the distances to the first internal G in the (−) strand should be 15 for RNA 2 [5] and 7 for RNA 3 [8], i.e. one base shorter than detected for RFs 2 and 3 (fig.1a–c). It was suggested that the 3′-ends of the BSMV RFs 2 and 3 (−) strands carry an unpaired G, as demonstrated for the RF molecules of cucumber mosaic virus (CMV) [12], brome mosaic virus (BMV) [16] and potato virus X [14].

Whereas encapsidated BSMV RNAs cannot be labeled at their 5′-termini without prior decapping [9], the ds RNAs 2 and 3 can, and therefore at least some are uncapped. Identification of the 5′-terminal nucleotides gave only G for the RF 2 and 3 molecules (not shown). This result was not surprising because (i) virion RNAs 2 and 3 (if decapped) carry G at their 5′-ends [5,8]; (ii) the 5′-terminal nucleotides of the (−) strands of RFs 2 and 3 should also be Gs if they are exact complements of the 3′-termini of the (+) strands (which end in C as demonstrated here).

RF 2

(+) 5′ p_xGUAAAAGAAAAGGAACAA...AAGACC$_{OH}$ 3′

(−) 3′ $_{HO}$(G)CAUUUUCUUUUCCUUGUU...UUCUGGp_x 5′

RF 3

(+) 5′ p_xGUUAUAGCUU...AAGACC$_{OH}$ 3′

(−) 3′ $_{HO}$GCAAUAUCGAA...UUCUGGp_x 5′

Fig.2. The terminal structures of the BSMV RFs 2 and 3. The sequences in ordinary type are taken from [5,8], and those determined in the present study are given in bold-face. The 3′-terminal G in RF 2 (−) strand is in parentheses because it is only present in about half of the RF 2 molecules.

The compilation of the data presented here and the published sequences of BSMV RNAs 2 and 3 [5,8] allowed us to deduce the terminal structures of BSMV RFs 2 and 3 (see fig.2).

The question of the manner of replication of the internal poly(A) tract of varying length in the BSMV genome is of special interest. If it replicates in a template-dependent fashion, the (−) strands of the RF molecules must carry a poly(U) tract of

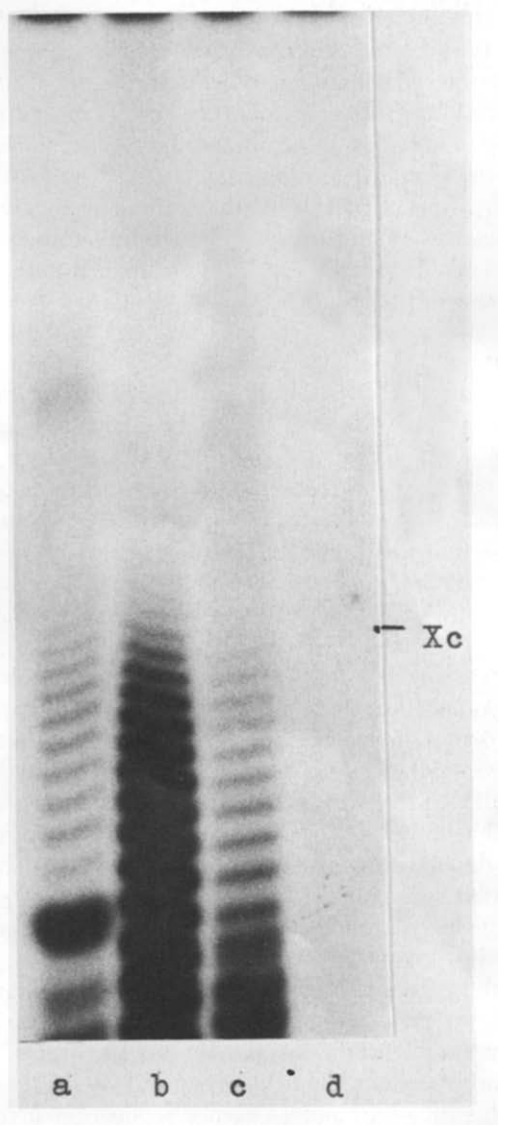

Fig.3. 20% PAG of the 3′-labeled RNase digests of denatured BSMV virion RNA or RF. (a) BSMV RNA, RNases A and T_1, (b) BSMV RF, RNases A and T_1, (c) BSMV RF, RNases U_2 and T_1, (d) BSMV RF, RNases A, U_2 and T_1 (control).

the same length as the poly(A) in the (+) strands. It is this suggestion that is confirmed by isolation and comparative analysis of the poly(A) tract from virion RNA and RF (+) strands, and of the poly(U) tract from RF (−) strands (fig.3a–c). Thus, BSMV RF molecules contain a perfect (or nearly perfect) poly(A)/poly(U) hybrid of length identical to that of the poly(A) tract in genomic RNAs.

4. DISCUSSION

Despite the substantial difference in genome organization between BSMV and other tyrosine-accepting RNAs of tricornaviruses such as BMV and CMV, the ds forms of their genomic RNAs carry identical terminal structures. The most obvious peculiarities of these structures are the presence of an unpaired G at the 3′-ends of (−) strands and the lack of a 3′-terminal A on the nascent (+) strands. The latter peculiarity is consistent with the demonstration that BMV RNA 3 (−) strand synthesis is initiated opposite the penultimate C [16]. The possibility cannot be excluded, however, that in the ds molecules containing the parental 3′-adenylated (+) strand, the 3′-ultimate unpaired A could participate in reinitiation of (−) strand synthesis. An analogous function was proposed for the unpaired G present at the other end of the RF molecule [12]. Since the RF molecules isolated from infected cells carry the unpaired nucleotide only on the (−) strand, they serve, possibly, only for (+) strand synthesis. This asymmetry could result from tight coupling of the addition of extra G with RNA replication. On the other hand, the posttranscriptional adenylation could be disconnected from RNA replication.

The internal poly(A) tract is one of the most intriguing elements in the BSMV genome. As shown earlier, BSMV RNA molecules with short oligoadenylates (up to 3 bases) during the infection of host plants can direct the synthesis of molecules with longer ones (up to 40 bases) [17]. These observations gave grounds for the hypothesis of non-template synthesis of BSMV poly(A) tracts. The results presented here clearly demonstrate that the real process of poly(A) tract replication involves direct template copying. The same situation takes place during replication of the 3′-terminal poly(A) sequence in potato virus X or cowpea mosaic virus genomes [13,14].

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