Complementarity between the 5'- and 3'-terminal sequences of rice stripe virus RNAs

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The 5' and 3' termini of four ssRNA species of rice stripe virus (RSV) isolate T were sequenced. The 3' termini of the three smallest ssRNAs, i.e. RNAs 2, 3 and 4, had the sequence 5' GACUUUGUGU 3'; that of ssRNA 1 had the sequence 5' GACAUUGUGU 3'. The 5'-terminal sequences of all four ssRNAs were 5' ACACAAAGUCC 3'. The 5'- and 3'-terminal sequences of about 20 bases of each ssRNA were almost complementary to each other. It is possible that RSV RNAs form panhandle structures characteristic of the RNA of negative-strand viruses.

Introduction

The filamentous particles of rice stripe virus (RSV; tenuivirus group), isolate T, contain four ssRNAs of Mr 3 × 10^6, 1.6 × 10^6, 1.1 × 10^6 and 0.9 × 10^6 and four dsRNAs of Mr 5 × 10^6, 2.5 × 10^6, 1.8 × 10^6 and 1.5 × 10^6, which contain the sequence of the ssRNA corresponding in size (Toriyama, 1982; Toriyama & Watanabe, 1989). A similar relationship between encapsidated ssRNA and dsRNA was shown for another isolate of RSV and for maize stripe tenuivirus (Ishikawa et al., 1989; Falk et al., 1989). RSV particles also contain a nucleocapsid protein of Mr 32000 and a minor protein of Mr 230000 (Toriyama, 1986). The nucleoproteins of RSV and rice grassy stunt tenuivirus are also associated with an RNA-dependent RNA polymerase activity (Toriyama & Watanabe, 1989). A similar relationship between encapsidated ssRNA and dsRNA was shown for another isolate of RSV and for maize stripe tenuivirus (Ishikawa et al., 1989; Falk et al., 1989). RSV particles also contain a nucleocapsid protein of Mr 32000 and a minor protein of Mr 230000 (Toriyama, 1986). The nucleoproteins of RSV and rice grassy stunt tenuivirus are also associated with an RNA-dependent RNA polymerase activity (Toriyama, 1986; 1987). Toriyama (1986) suggested that the genomic RNAs have negative polarity, and that the Mr 230000 protein may be an RNA polymerase.

The genome structure of tenuiviruses has not been studied in detail. In this paper we report 5'- and 3'-terminal sequences for the four genomic RNAs of RSV as a first step in defining the genome structure.

Methods

Preparation of viral RNA. Particles of RSV isolate T and each RNA species of the four ssRNAs and dsRNAs were prepared as described by Toriyama & Watanabe (1989), except that ssRNA 1 (Mr approx. 3 × 10^6), used for two-dimensional shift analysis, was partially purified by electrophoresis in low melting-point agarose.

Labelling of viral RNA molecules. The 3' end-labelling of each RNA species (1 to 3 µg) was done as described by Bruce & Uhlenbeck (1978).

For the 5' end-labelling, each purified RNA species (1 to 3 µg) was dephosphorylated with 0.01 of a unit of calf intestinal alkaline phosphatase (Boehringer Mannheim) in 160 mM-Tris-HCl pH 8.0 at 50 °C for 1 h. Then 2 µl of 50 mM-nitriotriacetic acid pH 7 was added to the mixture and heated at 95 °C for 2 min to inactivate the phosphatase. The dephosphorylated RNA was labelled with 2 to 3 MBq [γ-32p]ATP using 3 to 6 units of T4 poly (adenosine 3', 5'-diphosphate) kinase (3' phosphatase-free, Boehringer Mannheim) in 21 mM-mercaptopoethanol, 14 mM-MgCl2 and 120 µM-spermidine at 37 °C for 30 min (Chaconas & van de Sande, 1980).

Labelled RNA, prepared for two-dimensional shift analysis, was purified by electrophoresis in 1% agarose (LE, Nakarai Chemical) and recovered by electroelution.

Analysis of terminal nucleotides. The 5' end-labelled RNA was treated with 100 µg/ml of nuclease P1 (Wako Pure Chemical Industries) in 50 mM-ammonium acetate pH 5.3, containing 10 µg of wheatgerm tRNA, at 50 °C for 2 h. The 3' end-labelled RNA was treated with RNase T2 (Sankyo) in 20 mM-sodium acetate pH 4.5 containing 10 µg of tRNA.

The terminally labelled nucleotides in the digested samples were detected by thin-layer chromatography in cellulose layers (Polygram sheet, Nagel) in isobutyric acid/ammonia solution (25 to 28%)/H2O (57.7:3.8:38.5 v/v/v) followed by autoradiography.

Two-dimensional mobility shift analysis. Samples of labelled RNA were mixed with 24 µg tRNA in 15 µl of 0.5 mM-EDTA in 50 mM-NaHCO3-NaOH pH 9.1 and kept at 95 °C for 5 min, 10 min or 20 min. Three samples were combined, lyophilized and dissolved in 2 µl of H2O. The digested RNA was spotted onto cellulose acetate (Separax, Fuji Film) and subjected to electrophoresis (HEP 406 Advantec, Japan) for 40 min at 3000 V in 5% acetic acid, 1 mM-EDTA and 0.5% pyridine pH 3.5.

After electrophoresis, the cellulose acetate strips were blotted onto DEAE-cellulose thin-layer plates (Polygram sheet, Nagel). The second dimension was homochromatography as described previously (Jay et al., 1974; Wengler & Wengler, 1981).
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ssRNA 1
5' (p) ACACAAAGUCCACAGGAACAAACAGUGUUUUUGUUUUUAUAAA

3' HO UGGUGAUCAG UCUCCUUUUAUAAAACAGCUAAGAGGCUUUCUUAACUCCUCGUGGUUGUGUAGGAGCAGACCCGAA

ssRNA 2
5' (p) ACACAAAGUCCUGGGUUAUAAAGRNNAACUAUAIUUAAANUUACACGAAANUG

3' HO UGGUGUUCAG ACCEUAUUGAGAACUCCUACCCAUAUUUGAUUAAGAAGAUUGCUUGGAAGAAGGAUAGUGAGAACGU

ssRNA 3
5' (p) ACACAAAGUCCAGGGUAAAAGUGUUUAUAAAANUUACAGAAnAAACG

3' HO UGGUGUUCAG ACCCAAUUGAGAACUCCUACCCAUAUUUGAUUAAGAAGAUUGCUUGGAAGAAGGAUAGUGAGAACGU

ssRNA 4
5' (p) ACACAAAGUCCAGGGCAUUGUACANGAUHCAAUUAACGAAANUGU

3' HO UGGUGUUCAG UCCCGUUAAGAAACCUUAUUGCUAGUUAAGAAGAUUGCUUGGAAGAAGGAUAGUGAGAACGU

Fig. 1. Terminal sequences and possible panhandle structures of the four ssRNA species. N indicates nucleotides which could not be determined unambiguously. The positions of hydrogen bonds forming the panhandle are indicated by * and other complementary base pairs are indicated by .. Conserved sequences are underlined.

RNA sequencing. RNA sequencing by chemical modification was performed as described by Peattie (1979). Sequencing using RNase T1, RNase U2, RNase PhyM and Bacillus cereus RNase was done by using an RNA sequencing enzyme kit (Pharmacia) according to the manufacturer's instructions (Donis-Keller et al., 1977).

Results

The 3' termini of the four ssRNA species and the four dsRNAs of RSV were efficiently labelled with [5'-32P]pCp using T4 RNA ligase. The 5' termini of RSV RNA molecules could only be labelled with [7-32P]ATP using T4 polynucleotide kinase after the RNA termini had been dephosphorylated with alkaline phosphatase. The 5' termini of dsRNA molecules were also labelled after dephosphorylation. Treatment with tobacco acid pyrophosphatase prior to dephosphorylation did not result in the enhancement of 32P incorporation which suggests that the 5' ends of the RNA molecules were not capped. These results indicate that all the RSV RNAs had phosphorylated 5' ends and 3'-OH ends. The 3'-terminal nucleotide of each of the four ssRNAs was U and the 5'-terminal nucleotide of each ssRNA was A (data not shown).

The 3'- and 5'-terminal sequences determined for each ssRNA are shown in Fig. 1. The first 18 bases in each sequence were determined using two-dimensional mobility shift analysis (Fig. 2 and 3) while the rest of each sequence was determined using the chemical modification and enzymic methods. The 3'- and 5'-terminal sequences of each of the four ssRNA species were complementary for 18, 19 or 20 nucleotides (Fig. 1).

The first 10 bases of the 3' ends of the four ssRNA species were identical except that there was a base change (U to A) at the 6th position from the 3' end of ssRNA 1 (Fig. 2a and b). The first 11 bases at the 5' end of the four ssRNA species were identical (Fig. 3). These 3'- and 5'-terminal sequences flanked sequences which were unique for each species of ssRNA. The regions of the four ssRNA species sequenced were A + U-rich.

Efficient labelling of both the 3' and 5' ends of dsRNAs of RSV facilitated confirmation of their terminal sequences by two-dimensional mobility shift analysis. For example, Fig. 2(c) and (d) show the 5' terminal sequences of dsRNA 2 and dsRNA 4. One strand was identical to the 5'-terminal sequences of ssRNA 2 and ssRNA 4, respectively (see Fig. 3b and d) and the other was entirely complementary to the 3'-terminal sequence of each ssRNA. The wandering spots of these dsRNA samples were identical until the 10th base because ssRNA 2 and ssRNA 4 have complementary sequences at both termini. At the 11th position, however, the wandering spots diverge into two parallel lines shifted in the one cytidine residue direction (one
Fig. 2. Autoradiograms of wandering spots produced by two-dimensional mobility shift analysis. (a and b) Wandering spots of 3' end-labelled RNA 1 and RNA 2, respectively. Arrows show the base change (U to A) at the sixth base of ssRNA 1 (a) and the corresponding base U in ssRNA 2 (b). (c and d) Wandering spots of 5' end-labelled dsRNA 2 and dsRNA 4. Two parallel lines, started at the 10th or 11th base from the terminal base, are shifted in the one cytidine residue direction.

Fig. 3. Autoradiograms of wandering spots of 5' end-labelled ssRNAs. (a) RNA 1, (b) RNA 2, (c) RNA 3 and (d) RNA 4. The ssRNA 1 sample was contaminated with dsRNA 2, this after the 10th base a parallel line of spots is visible on the right-hand side (see also Fig. 2c).
strand is missing a cytidine at position 11) from this position until the 19th base and 18th base in dsRNA 2 and dsRNA 4, respectively. This shift pattern was also observed for dsRNA 3. Due to the change of U to A at the sixth base in ssRNA 1, the wandering spots of dsRNA 1 show two sets of parallel lines. The first, at the sixth base, was unique to the 3′ end of ssRNA 1 and the second at the 11th position was identical to those of the other dsRNAs. These results indicate that the terminal sequence of each dsRNA of RSV obtained from purified RSV preparations (Toriyama & Watanabe, 1989) are perfectly complementary, over the 3′- and 5′-terminal regions, to each ssRNA of RSV analysed.

Discussion

Sequences at the 3′ end and the 5′ end of each ssRNA of RSV are complementary to each other, although a mismatched cytosine exists at the 10th or 11th position from the 5′ ends. Terminal complementary sequences are present in negative-strand viruses such as influenza virus, vesicular stomatitis virus and snowshoe hare virus (Skehel & Hay, 1978; Keene et al., 1979; Bishop et al., 1982). The terminal sequences of influenza virus RNAs have been shown recently to form panhandle structures (Honda et al., 1987; Hsu et al., 1987). We suggest that the terminal complementary sequences cause RSV RNAs to form panhandles.

Another resemblance to negative-sense viruses is that particles of RSV and rice grassy stunt virus are associated with a highly active RNA-dependent RNA polymerase (Toriyama, 1986, 1987).

In contrast, Falk et al. (1987) reported that genomic RNAs of maize stripe virus have positive polarity, because the nucleocapsid protein and major non-capsid protein products can be translated directly from viral RNAs. RSV RNAs stimulate the incorporation of radioactive leucine in in vitro translation systems (Toriyama, 1985) but little of the translation products were precipitable with the antiserum to the nucleocapsid of RSV. A possible explanation for these contrasting properties would be an ambisense structure (Bishop, 1986a), which has been suggested for RSV RNA (Toriyama, 1986; Hayano et al., 1990). Indeed the complete sequence of ssRNA 3 of RSV isolate T (Y. Zhu, T. Hayakawa, S. Toriyama & M. Takahashi, unpublished results) shows that it contains the nucleocapsid protein gene, one of two large putative open reading frames, in the 5′-proximal region of the complementary sequence to the viral ssRNA 3.

Honda et al. (1987) showed by using a footprinting technique that the RNA polymerase of influenza virus binds to the base-paired part of the panhandle structure. The base-paired region of the proposed panhandle structure of RSV could also act as a recognition site for RNA polymerase. RNA 1 is less abundant than RNAs 2, 3 or 4 in purified preparations of RSV (Toriyama, 1982, 1986). Possibly the mismatch unique to ssRNA 1 at the sixth base from the 3′ end may be involved in replicative and transcriptional control and/or assembly of this RNA into the filamentous particles.

The 3′-terminal sequences of RSV RNAs are homologous with those of two genera of Bunyaviridae. One genus, the phleboviruses, is transmitted by sandflies, mosquitoes or gnats and the 3′-terminal sequence of their three RNAs is 5′ GCUUUGUGU 3′. The 3′-terminal sequence of the other genus, uukuviruses, transmitted by ticks, is 5′ GAGGUCUUUGUGU 3′. Moreover, the 3′-terminal RNA sequences of viruses in these two genera are complementary to each of their 5′-terminal sequences (Ihara et al., 1984; Bishop, 1986b). Although bunyaviral particles are spherical, they contain internal nucleocapsids (Bishop, 1986b) similar to the filamentous viral particles of tenuiviruses. The similarities between RSV and the members of the Bunyaviridae which include both RNA sequence features and persistent transmission by arthropods (including transovarial transmission) may suggest a measure of evolutionary relationship.

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References


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