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Replication and 3'-end repair of a subviral RNA associated with turnip crinkle virus

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Abstract

Replication of plus (+)-strand RNA viruses proceeds through minus (-)-strand intermediates. Satellite RNA C (satC), one of the nonessential subviral RNAs of *Turnip crinkle virus* (TCV), is dependent on the TCV-encoded RdRp for its replication. Earlier work showed that a stem-loop structure at the 3' end of (+)-strand satC is required for synthesis of (-)-strands (Song and Simon, 1995a). Using an *in vitro* RdRp assay, I defined two separate *cis*-acting elements on satC (-)-strands that can promote complementary strand synthesis. One element comprises 11 bases and is located near the 3' end (3'-proximal), and the other consists of 14 bases and is located 41 bases from the 5' end (5'-proximal). Both elements contain multiple consecutive C residues followed by multiple consecutive purines. ^ *In vivo* mutagenesis and genetic selection (SELEX) studies were carried out to investigate the functional significance of the two elements as well as the satC (-)-strand 3' terminus (3' OH-CCCUAU), which contains the (-)-strand 3'-end sequence 3' OH-CC₁₋₂ (A/U)(A/U)(A/U) found in all *carmovirus* RNAs (named the *carmovirus* consensus sequence or CCS). My results indicate that the 3'-terminal CCS and the 5'-proximal element are highly conserved and required for satC (+)-strand synthesis. Although mutations introduced into the 3'-proximal element were tolerable, this element preferentially contains a sequence similar to the CCS and/or polypurines, suggesting that this element may also contribute to satC accumulation *in vivo*. ^ All RNAs associated with TCV terminate with the motif CCUGCCC-3' at the 3' end. Transcripts of satC containing a deletion of the motif, or the 3'-terminal 6 bases, are nearly always repaired to wild-type *in vivo* by RdRp-mediated primer extension of oligoribonucleotides synthesized by abortive initiation and complementary to the 3' end of TCV genomic RNA (Nagy *et al.*, 1997). In this thesis, I provide evidence that two additional

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mechanisms are used by the TCV RdRp to repair shorter deletions of the 3'-end motif of satC. Deletion of the 3'-terminal CCC residues along with addition of 8 non-template bases is repaired *in vivo* mainly by homologous recombination between the similar 3'-ends of satC and TCV. Deletion of the 3'-terminal 4 or 5 bases, in the presence or absence of non-template bases, led to recovery of progeny containing a mixture of wild-type 3'-ends and non-wild-type 3'-ends that included base alterations, deletions and insertions. Assays using an *in vitro* RdRp transcription system indicate that the TCV RdRp is likely able to polymerize nucleotides in a template-independent, non-random fashion before initiating transcription of deletion-containing satC. The existence of 3 different repair mechanisms associated with a single virus suggests an intrinsic need for 3'-end reconstruction in the cellular environment. ^

Subject Area

Biology, Molecular

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