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Evolution of virus-derived sequences for high-level replication of a subviral RNA

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Abstract

Turnip crinkle virus (TCV) and its 356-nt satellite RNA satC share 151 nt of 3′-terminal sequence, which contain 8 positional differences and are predicted to fold into virtually identical structures, including a series of four phylogenetically inferred hairpins. SatC and TCV containing reciprocal exchanges of this region accumulate to only 15% or 1% of wild-type levels, respectively. Step-wise conversion of satC and TCV 3′-terminal sequences into the counterpart's sequence revealed the importance of having the cognate core promoter (Pr), which is composed of a single hairpin that differs in both sequence and stability, and an adjacent short 3′-terminal segment. The negative impact of the more stable TCV Pr on satC could not be attributed to lack of formation of a known tertiary interaction involving the 3′-terminal bases, nor an effect of coat protein, which binds specifically to TCV-like Pr and not the satC Pr. The satC Pr was a substantially better promoter than the TCV Pr when assayed in vitro using purified recombinant TCV RdRp, either in the context of satC or when assayed downstream of non-TCV-related sequence. Poor activity of the TCV Pr in vitro occurred despite solution structure probing indicating that its conformation in the context of satC is similar to the active form of the satC Pr, which is thought to form following a required conformational switch. These results suggest that evolution of satC following its initial formation generated a Pr that can function more efficiently in the absence of additional TCV sequence that may be required for full functionality of the TCV Pr.

Keywords: Turnip crinkle virus; RNA conformational switch; RNA virus evolution; Satellite RNA; RNA replication promoters

Introduction

Replication of genomic and subviral RNAs requires specific interactions between the replicase complex and RNA *cis*-acting elements. While core promoters located near the 3′ ends of plus (+)- and minus (−)-strands can independently recruit replication complexes resulting in low levels of de novo synthesized complementary strands (Dreher, 1999), additional elements throughout viral genomes aid in enzyme complex assembly or enhance or repress transcription (e.g., French and Ahlquist, 1987; Frolov et al., 2001; Herold and Andino, 2001; Khromykh et al., 2001; Klovins and van Duin, 1999; McCormack and Simon, 2004; Monkewich et al., 2005; Nagashima et al., 2005; Nagy et al., 1999; Panavas and Nagy, 2003; Panaviene et al., 2005; Ray and White, 2003; Vlot and Bol, 2003; Zhang and Simon, 2003). In addition, recent evidence suggests that RNA conformational rearrangements play key roles in coordinating translation and replication, regulating subgenomic RNA synthesis or producing asymmetric levels of (+)- and (−)-strands by masking or exposing elements required for a particular process (Barry and Miller, 2002; Isken et al., 2004; Khromykh et al., 2001; Olsthoorn et al., 1999; Koev et al., 2002; Na and White, 2006; Pogany et al., 2003; van den Born et al., 2005; Zhang et al., 2004a, 2006). For example, conformational changes at the 3′ ends of *Barley yellow dwarf virus* (Koev et al., 2002) and *Tomato bushy stunt virus* (TBSV; Na and White, 2006; Pogany et al., 2003) may control accessibility of the RNA-dependent RNA polymerase (RdRp) to the initiation site for (−)-strand synthesis.

The complexities inherent in RNA virus replication has led to the use of untranslated subviral RNAs such as defective interfering (DI) RNAs or satellite (sat) RNAs, as models for

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their larger, multifaceted helper viral genomic RNAs. Many (+)-strand RNA viruses are naturally associated with subviral RNAs, which depend on their helper virus for replication and host trafficking components (David et al., 1992; White and Morris, 1999; Simons et al., 2004). While DI RNAs are mainly derived from 5′ and 3′ portions of viral genomic RNAs, most satRNAs share little consecutive sequence similarity with their helper virus genomes and may have arisen from an unrelated RNA or a series of recombination events joining short segments of viral and non-viral RNAs that further evolved into a functional molecule (Carpenter and Simon, 1996). An unusual satRNA, satC (356 bases), is associated with *Turnip crinkle virus* (TCV; single (+)-strand RNA of 4054 bases), a member of the family *Tombusviridae*, genus *Carmovirus* (Simon, 2001). SatC has features of both DI and satRNAs with its 5′ 190 bases originating from nearly full-length TCV satRNA satD (194 bases) and its 3′ 166 bases derived from two regions at the 3′ end of TCV genomic RNA (Fig. 1A; Simon and Howell, 1986). TCV is also naturally associated with DI RNAs, such as diG, whose sequence is mainly derived from 5′ and 3′ regions of the genomic RNA (Fig. 1A; Li et al., 1989).

The 3′-terminal 100 bases shared by TCV and satC differ at only eight positions ("positions" refers to particular locations where one or more consecutive bases may differ) and are predicted to be structurally similar by mFold (Fig. 1B; Zhang et al., 2004b; Zuker, 2003). This observation suggested that satC would be a good model for determining the function of TCV *cis*-acting sequences within this region in the replication process. A combination of in vivo studies using *Arabidopsis thaliana* protoplasts, in vitro assays for transcription initiation using purified recombinant TCV RdRp, and in vitro RNA solution structure probing revealed that this region in satC assumes two very different RNA conformations: an unresolved preactive conformation stabilized by extensive tertiary structure that

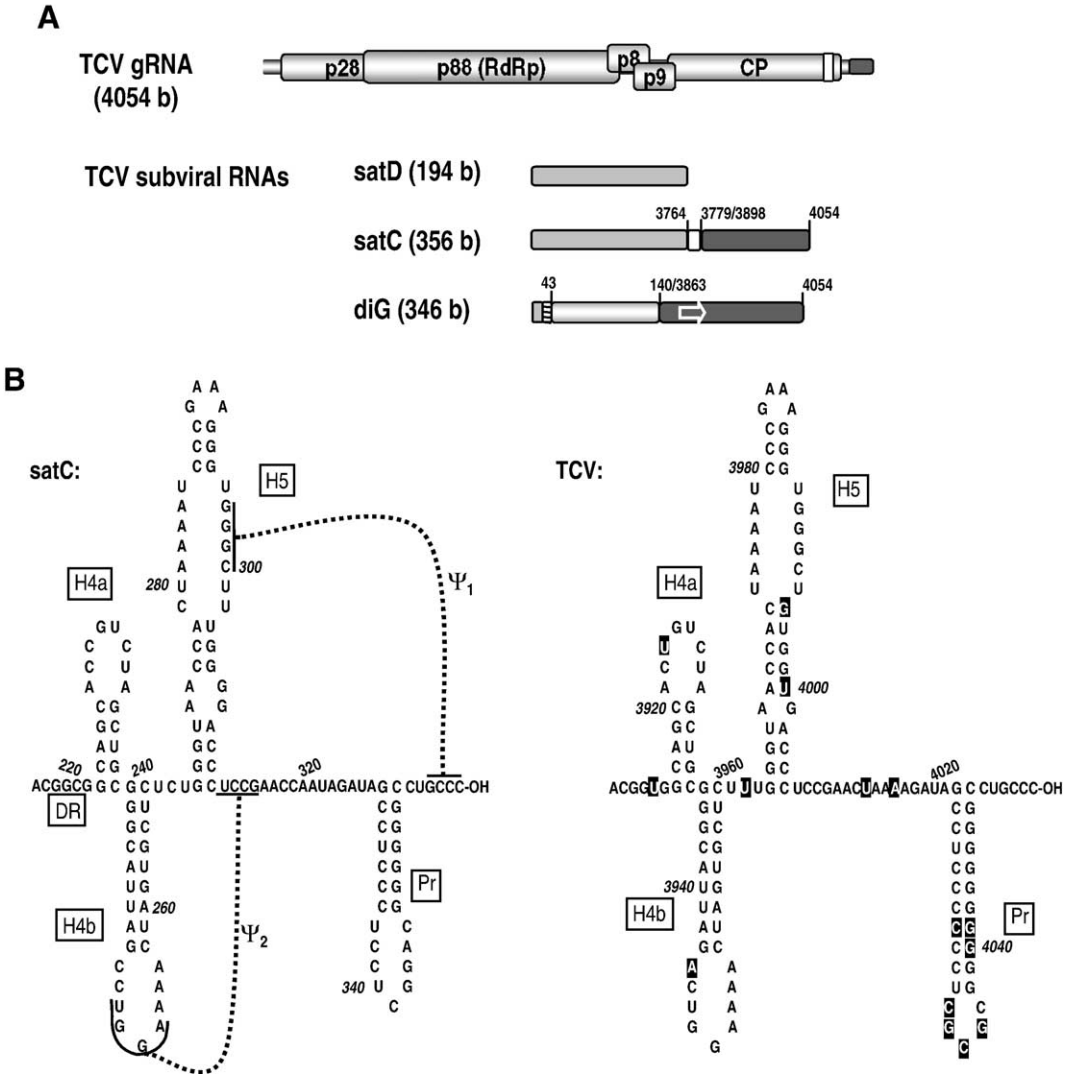

Fig. 1. Genomic and subviral RNAs in the TCV system. (A) Schematic representation of TCV genomic (g)RNA and subviral RNAs satC, satD and diG. Names of the TCV-encoded proteins are shown. Similar regions are shaded alike. Positions of TCV gRNA-derived sequence in satC and diG are given. diG contains a short repeated segment indicated by an arrow. (B) Sequence and structure of the hatched portions in TCV and satC. Base differences between satC and TCV are boxed in the TCV structure. Structures presented are phylogenetically conserved and predicted by mFold computer modeling (Zuker, 2003). Names of the hairpins are indicated. Two pseudoknots experimentally confirmed in satC are also shown.