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cis-Acting sequences required for coat protein binding and in vitro assembly of *Potato virus X*

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Abstract

The 5′ region of *Potato virus X* (PVX) RNA containing an AC-rich single-stranded region and stem–loop 1 (SL1) has been shown to be important for PVX replication (Miller, E.D., Plante, C.A., Kim, K.-H., Brown, J.W., Hemenway, C., 1998. Stem–loop structure in the 5′ region of potato virus X genome required for plus-strand RNA accumulation. J. Mol. Biol. 284, 591–608.). Here, we describe the involvement of SL1 for binding to the PVX coat protein (CP) using an in vitro assembly system and various deletion mutants of the 5′ region of PVX RNA. Internal and 5′ terminal deletions of the 5′-nontranslated region of PVX RNA were assessed for their effects on formation of assembled virus-like particles (VLPs). Mutant RNAs that contain the top region of SL1 or sequences therein bound to CP to form VLPs. In contrast, transcripts of mutants that disrupt SL1 RNA structure were unable to form VLPs. SELEX was used to further confirm the specific RNA recognition of PVX CP using RNA transcripts containing randomized sequences of the upper portion of SL1. Wild-type (wt) sequences along with many other sequences that resemble SL1 structure were selected after fourth and fifth rounds of SELEX (27.0% and 44.4%, respectively). RNA transcripts from several SELEX winners that are predicted to form stable stem–loop structures very closely resembling wt PVX SL1 VLPs. RNA transcripts not predicted to form secondary structures similar to SL1 did not form VLPs in vitro. Taken together, our results suggest that RNA secondary structural elements within SL1 and/or sequences therein are crucial for formation of VLPs and are required for the specific recognition by the CP subunit.

Keywords: PVX; Assembly; OAS; SELEX; Stem–loop structure

Introduction

Virus assembly is an essential component of the infection cycle of all viruses. Specific recognition of viral RNA(s) by capsid protein (CP) plays a crucial role in encapsidation of the viral RNA genomes of plus-strand RNA viruses. Also, for several plant RNA viruses, RNA packaging has been shown to be essential for cell-to-cell movement (Dolja et al., 1995; Van der Vossen et al., 1994) and systemic spread (Dolja et al., 1994; Vaewhongs and Lommel, 1995). The sequence elements involved in the specific packaging of viral RNAs have been identified for several viruses including *Tobacco mosaic virus* (TMV; Zimmern, 1977; Zimmern and Butler, 1977), *Brome mosaic virus* (BMV; Choi and Rao, 2000, 2003), *Hepatitis B virus* (Junker-Niepmann et al., 1990), *Sindbis virus* (Tellinghuisen et al., 2001; Weiss et al., 1994), *Human immunodeficiency virus* (HIV) (Harrich et al., 2000; Russell et al., 2003; Shubsda et al., 2002), and the *Saccharomyces cerevisiae virus L-A* (Fujimura et al., 1990).

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Despite the importance of these RNA–protein recognition events in plant RNA virus movement and systemic spread, the specific packaging signal or origin of assembly (OAS) has not been well characterized for many of these viruses. For a few plant RNA viruses, assembly has been studied both in vitro and in vivo. The specific interaction between a 69-nucleotide (nt) TMV RNA sequence including a stem–loop structure and the TMV CP leads to the formation of virion assembly (Butler, 1984). RNA elements that act as specific packaging signals were also reported for *Turnip crinkle virus* (Qu and Morris, 1997) and BMV (Duggal and Hall, 1993). The in vitro assembly studies revealed that the ionic strength and pH alter the type of particle formed, presumably by altering RNA–protein and protein–protein interactions. In the *Cowpea chlorotic mottle virus* (CCMV), for example, under low pH (<6.0) and low ionic strength (i = 0.2) conditions, particles assemble without RNA, forming empty particles. At higher pH (>7.0) and in the presence of viral RNA, RNA-containing capsids form (Zhao et al., 1995).

PVX, the type member of the *Potexvirus* group, is single-stranded, plus-sense RNA virus. The flexuous rod-shaped particles contain a 6.4-kb genomic RNA that is capped and polyadenylated (Bercks, 1970; Hiebert and Dougherty, 1988). The PVX genome is composed of an 84 nt 5′-nontranslated region (NTR), five open reading frames (ORFs), and a 72-nt 3′-NTR (Fig. 1A) (Bercks, 1970; Huisman et al., 1988; Skryabin et al., 1988). ORFs 1–5 encode viral RNA-dependent RNA polymerase (RdRp), three polypeptides (triple gene block; TGB) involved in virus movement, and the CP, respectively (Baulcombe et al., 1995; Chapman et al., 1992). During PVX infection, two major subgenomic RNAs (sgRNAs) are used to express ORF2 and CP, respectively, while ORF3 and ORF4 of the TGB are expressed from a third, less-abundant sgRNA (Morozov et al., 1991; Verchot et al., 1998).

The importance of the 5′ region of genomic RNA in the PVX replication has been shown through several studies. Two regions of the PVX 5′-NTR α (nt 1-41) and β (nt 42–84), were found to enhance translational efficiency of reporter genes in vitro (Smirnyagina et al., 1991) and in vivo (Pooggin and Skryabin, 1992; Zelenina et al., 1992). Multiple sequence and structural elements in the 5′-NTR of PVX RNA affect both genomic and sgRNA synthesis and contain multiple *cis*-acting regulatory signals (Kim and Hemenway, 1996, 1997; Miller et al., 1998). A stem–loop 1 (SL1) secondary structure was found to be important for plus-strand RNA accumulation. Miller et al. (1998) pro-

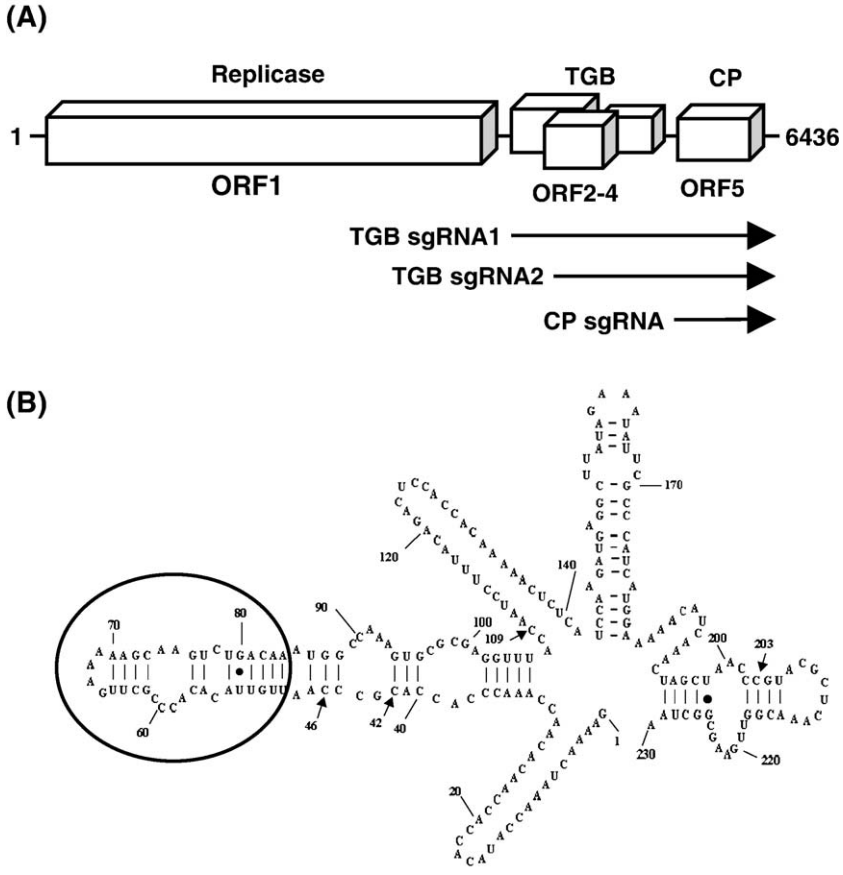

Fig. 1. Schematic diagram of the PVX genome (A) and the RNA SL1 present in the 5′ region (B) of PVX RNA (Miller et al., 1998). Five open reading frames are depicted as open boxes and three subgenomic RNAs are as bold arrowed lines. SELEX randomized sequence within RNA SL1 that occur in stem C (SC), loop C (LC), stem D (SD) and the tetra-loop (TL) are marked as circle and nucleotide locations are noted as lines and numbers.