Mutagenic analysis of *Potato Virus X* movement protein (TGBp1) and the coat protein (CP): *in vitro* TGBp1–CP binding and viral RNA translation activation

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SUMMARY

Previously, we have shown that encapsidated *Potato virus X* (PVX) RNA was non-translatable *in vitro*, but could be converted into a translatable form by binding of the PVX movement protein TGBp1 to one end of the virion or by coat protein (CP) phosphorylation. Here, a mutagenic analysis of PVX CP and TGBp1 was used to identify the regions involved in TGBp1–CP binding and translational activation of PVX RNA by TGBp1. It was found that the C-terminal (C-ter) 10/18 amino acids region was not essential for virus-like particle (VP) assembly from CP and RNA. However, the VPs assembled from the CP lacking C-ter 10/18 amino acids were incapable of TGBp1 binding and being translationally activated. It was suggested that the 10-amino-acid C-ter regions of protein subunits located at one end of a polar helical PVX particle contain a domain accessible to TGBp1 binding and PVX remodeling. The non-translatable particles assembled from the C-ter mutant CP could be converted into a translatable form by CP phosphorylation. The TGBp1–CP binding activity was preserved unless a conservative motif IV was removed from TGBp1. By contrast, TGBp1-dependent activation of PVX RNA translation was abolished by deletions of various NTPase/helicase conservative motifs and their combinations. The motif IV might be essential for TGBp1–CP binding, but insufficient for PVX RNA translation activation. The evidence to discriminate between these two events, i.e. TGBp1 binding to the CP-helix and TGBp1-dependent RNA translation activation, is discussed.

INTRODUCTION

*Potato Virus X* (PVX) is the type member of the genus Potexvirus, family Flexiviroidae. About 1300 identical protein subunits in a filamentous PVX virion form a helical array with the positive RNA packed between the turns of the helix (Tollin and Wilson, 1988). The PVX coat protein (CP) consists of 237 amino acids and is glycosylated (Baratova et al., 2004; Tozzini et al., 1994). The PVX RNA contains five genes (Huisman et al., 1988; Skryabin et al., 1988). The 5′-proximal gene codes for the 165-kDa replicase and is glycosylated (Baratova et al., 2004; Tozzini et al., 1994). The PVX RNA contains five genes (Huisman et al., 1988; Skryabin et al., 1988). The 5′-proximal gene codes for the 165-kDa replicase, and the 3′-proximal CP gene is preceded by three partially overlapping genes termed the ‘triple gene block’ (TGB) coding for three movement proteins (MPs) referred to as TGBp1, TGBp2 and TGBp3, respectively. The TGB-coded MPs together with CP are involved in PVX cell-to-cell movement (Chapman et al., 1992; Verchot et al., 1998; for reviews, see Batten et al., 2003; Morozov and Solovyev, 2003; Verchot-Lubicz, 2005).

Two different models have been proposed to explain the nature of the infectious potexvirus transport that moves from cell to cell over the infected plant: (1) it has been suggested (Allison and Shalla, 1974; Oparka et al., 1996; Santa Cruz et al., 1998) that filamentous virions are involved in cell-to-cell movement of PVX; (2) by contrast, it has been reported that *in vitro* assembled non-virion RNP (CP-RNA-TGBp1) complexes moved from cell to cell in microinjection experiments, *in vivo* (Lough et al., 2000). Recently, we examined the structure of complexes assembled *in vitro* from PVX RNA, TGBp1 and CP. The single-tailed particles (STPs) with the 5′-terminal region of PVX RNA encapsidated in a helical head-like structure and TGBp1 bound to the end of the head were revealed (Karpova et al., 2006a). Strong evidence was provided on structural similarity between the native virions and STP heads. Apparently, the STPs represent incompletely assembled PVX virions. We suggested that translatable complexes of TGBp1 with the extremity of the