Short communication

The 5′-proximal region of Potato virus X RNA involves the potential cap-dependent “conformational element” for encapsidation

E.K. Petrova a,*, N.A. Nikitin a, E.A. Trifonova a, A.D. Protopopova b, O.V. Karpova a,
J.G. Atabekova c

a Biology Department, Lomonosov Moscow State University, Moscow 119234, Russia
b Scientific Research Institute of Physical-Chemical Medicine, Moscow 119435, Russia
c A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia

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ABSTRACT

Filamentous helical Potato virus X (PVX) can be regarded as one of the well-studied viruses. Nevertheless, some aspects of the PVX assembly remained obscure. Previously, we have shown that the presence of a cap structure at the 5′ end of PVX RNA is indispensable for assembly of viral ribonucleoprotein (vRNP) particles varying in length. Here, most significantly, removal of the cap structure from previously capped PVX RNA did not affect the efficiency of decapped RNA molecules to be assembled into vRNP. This result provided evidence that the cap structure by itself does not act as a signal for initiation of vRNP assembly. These observations allowed to presume that the capping triggers some spatial changes in the 5′-proximal site of PVX RNA creating a “conformational encapsidation signal for vRNP assembly”, which is capable of triggering vRNP assembly in the absence of cap structure. Apparently, during capping the 5′-proximal segment of PVX RNA acquires a unique conformation which is stable to be retained even after cap removal.

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1. Introduction

Potato virus X (PVX) is a type member of the Potexvirus genus (Alphaflexiviridae family). The genomic 5′-capped positive-strand RNA, 6435-nt long, is encapsulated in a flexuous filamentous particle about 515 nm long and 13.5 nm in diameter.

PVX was the first flexuous plant virus reassembled in vitro from RNA and coat protein (CP) [1–3]. The majority of the reassembled helical particles was shorter than native PVX and termed “viral ribonucleoproteins” (vRNPs) or “virus-like particles”.

Reassembly of PVX starts with the CP binding to the 5′ terminus of genomic RNA [4–7]. However, it was difficult to propose that the cap structure by itself can serve as a signal for a viral particle assembly, since it is a widespread RNA modification. On the other hand, there is evidence that functions of the viral cap structure are more diverse than those of cellular mRNAs [8,9]. We have presumed that capping promotes spatial changes at the RNA 5′-proximal site resulting in a local conformation favourable for interaction with the CP. To further understand the effect of the cap structure, the vRNPs assembly from native capped PVX RNA, RNAs capped with Vaccinia virus capping system or decapped by Tobacco Acid Pyrophosphatase (TAP) was examined.

2. Materials and methods

2.1. Purification of Potato virus X (PVX), PVX CP and RNA

Russian strain of PVX was isolated from infected plants (Datura stramonium L.) according to Miroshnichenko et al. [10]. PVX CP and PVX RNA were isolated according Karpova et al. [6].

2.2. In vitro vRNP assembly was performed as described [6,7].

2.3. Transmission electron microscopy (TEM)

Samples were prepared using standard procedures [11]. The images were obtained as described by Petrova et al. [7].

2.4. Atomic force microscopy (AFM)