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Protein–RNA tethering: The role of poly(C) binding protein 2 in poliovirus RNA replication

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Abstract

The exploitation of cellular functions and host proteins is an essential part of viral replication. The study of this interplay has provided significant insight into host cell processes in addition to advancing the understanding of the viral life-cycle. Poliovirus utilizes a multifunctional cellular protein, poly(C) binding protein 2 (PCBP2), for RNA stability, translation and RNA replication. In its cellular capacity, PCBP2 is involved in many functions, including transcriptional activation, mRNA stability and translational silencing. Using a novel protein–RNA tethering system, we establish PCBP2 as an essential co-factor in the initiation of poliovirus negative-strand synthesis. Furthermore, we identified the conserved KH domains in PCBP2 that are required for the initiation of poliovirus negative-strand synthesis, and showed that this required neither direct RNA binding or dimerization of PCBP2. This study demonstrates the novel application of a protein–RNA tethering system for the molecular characterization of cellular protein involvement in viral RNA replication.
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Keywords: Poliovirus; PCBP2; KH domain; RNA replication; Tethering

Introduction

Poly(C) Binding Protein 2 (PCBP2; also called hnRNP E2 and αCP2) is one of a family of poly(rC/dC) binding proteins which include hnRNP K and PCBPs 1–4 (Matunis et al., 1992; Leffers et al., 1995; Kiledjian et al., 1995; Makeyev and Liebhaber, 2000). In addition to their nucleic acid binding specificity, this protein family is characterized by the presence and positioning of three, highly homologous, KH domains (hnRNP *K H*omology domains) (Gibson et al., 1993; Siomi et al., 1993). In the case of the PCBPs, the first and third KH domains contain the primary nucleic acid binding activity, although the second domain may enhance binding affinity and/or specificity (Dejgaard and Leffers, 1996; Du et al., 2007). The structure of the KH domain is highly conserved, regardless of surrounding sequence context, acting as an independent cassette which can be evolutionarily tuned to a specific function. Although initially characterized as RNA binding proteins involved in pre-mRNA metabolism, more recent work has described an increasingly globalized set of essential cellular processes in which PCBPs participate. As yet, the most extensively studied family members are hnRNP K, PCBP1 and PCBP2. Current work has firmly established the involvement of the PCBP protein family in mRNA stabilization, transcriptional regulation, translational control and apoptotic program activation (reviewed by Makeyev and Liebhaber, 2002). The mRNAs targeted by these proteins are diverse as well, including α-globin, 15-lipoxygenase, collagen αI and androgen receptor (Ostareck et al., 1997; Stefanovic et al., 1997; Chkheidze et al., 1999; Yeap et al., 2002).

Poliovirus (PV) possesses a single-stranded positive sense RNA genome and is a prototypic member of the family *Picornaviridae*. Many members of this family, including PV, utilize the PCBPs during their replication, and one such example is the use of PCBP1 and/or PCBP2 in the cap-independent initiation of translation mediated by type I Internal Ribosomal Entry Sites (IRES) (Dildine and Semler, 1992; Blyn et al., 1996). In addition to the IRES, the PV genome contains a 5′-terminal cloverleaf (5′CL) structure that is essential for RNA replication and is conserved among all members of the *Enterovirus* genus (Andino et al., 1990, 1993; Xiang et al., 1995; Zell and Stelzner, 1997; Herold and Andino,

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2001; Teterina et al., 2001; Barton et al., 2001; Lyons et al., 2001). The 5′CL is divided into four domains: stem *a* and stem-loops *b*, *c* and *d* (Fig. 1b). Stem-loop d binds a viral protein, $3CD^{pro}$, and stem-loop b binds PCBP1 or PCBP2 (Fig. 3, top) (Andino et al., 1990, 1993; Parsley et al., 1997; Gamarnik and Andino, 1997). While PCBP1/2 bind to the 5′CL in the absence of viral proteins, the concomitant binding of 3CD results in a nearly 100-fold increase in PCBP binding affinity (Gamarnik and Andino, 2000). In addition, PV RNA replication is inhibited in PCBP-depleted HeLa S10 extracts, which suggests that PCBP binding to the 5′ cloverleaf is required in one or more steps of the viral RNA replication cycle (Walter et al., 2002).

In the current study, we investigated the role of the PCBP2/5′CL RNP complex in PV RNA replication. Herein, we show that either direct binding or tethering of PCBP2 to the 5′CL was required to form a functional replication complex that could initiate PV negative-strand synthesis. We describe a novel protein–RNA tethering system that was used in a functional analysis of PCBP2 relative to its role in PV RNA replication. Using the protein–RNA tethering system, we identified and characterized the domains in PCBP2 that were required for negative-strand synthesis independent of their ability to bind to the 5′ CL. The protein–RNA tethering system provides a novel experimental approach to perform a genetic analysis of PCBP2 as well as other cellular proteins that are required for specific steps in the viral replication cycle.

Results

A mutation in stem-loop b of the 5′ cloverleaf inhibits negative- but not positive-strand synthesis

To clarify the role of PCBP binding to the 5′CL in PV RNA replication, we used a subgenomic PV RNA transcript (P23 RNA) which encodes all of the essential viral replication proteins and forms functional RNA replication complexes in cell-free reactions (Fig. 1A). We compared the replication of wild-type P23 RNA with the replication of the same RNA with a C24A mutation in stem-loop b of the 5′CL (P23–5′CL(C24A) RNA) (Figs. 2A and B). The 5′CL(C24A) mutation was shown in previous studies to inhibit the binding of PCBP to the 5′CL and the formation of the 5′ RNP complex (Andino et al., 1993;

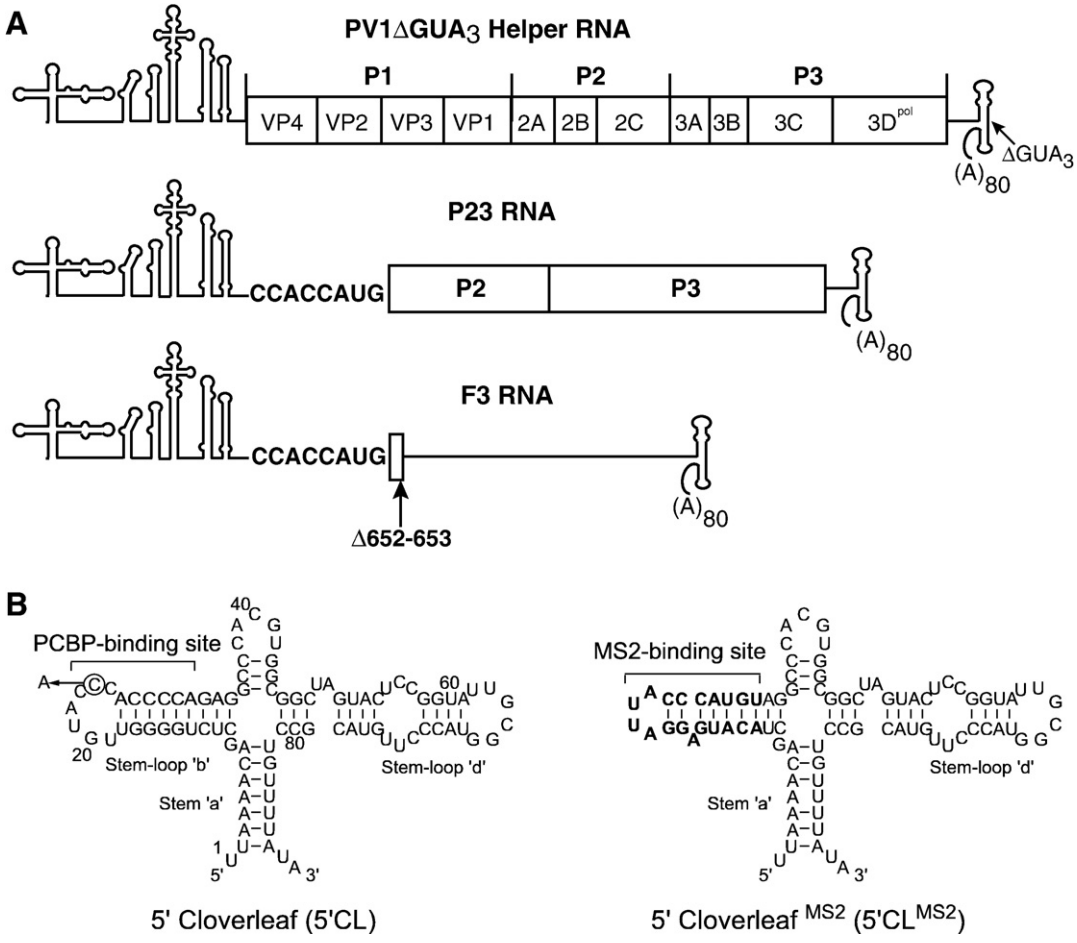

Fig. 1. Schematic of poliovirus RNAs used in this study. (A) PV1ΔGUA$_3$ RNA contains the entire poliovirus genomic RNA sequence with a five nucleotide deletion in the 3′NTR, rendering it incapable of RNA replication. P23 RNA contains a deletion of the P1 capsid coding region, however, it does encode all essential viral replication proteins. F3 RNA contains a deletion of both the P1 and P2 coding regions. In addition, F3 RNA contains a two nucleotide deletion at the beginning of the P3 coding region, producing a frameshift which results in premature termination of translation. (B) Sequence diagrams of both the wild-type 5′ cloverleaf with the C24A mutation (left) and the MS2 5′ cloverleaf (right).

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