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The 5′CL-PCBP RNP complex, 3′ poly(A) tail and 2A^{pro} are required for optimal translation of poliovirus RNA

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ABSTRACT

In this study, we showed that the 5′CL-PCBP complex, 3′ poly(A) tail and viral protein 2A^{pro} are all required for optimal translation of PV RNA. The 2A^{pro}-mediated stimulation of translation was observed in the presence or absence of both the 5′CL and the 3′ poly(A) tail. Using protein–RNA tethering, we established that the 5′CL-PCBP complex is required for optimal viral RNA translation and identified the KH3 domain of PCBP2 as the functional region. We also showed that the 5′CL-PCBP complex and the 3′ poly(A) tail stimulate translation independent of each other. In addition to the independent function of each element, the 5′CL and the 3′ poly(A) tail function synergistically to stimulate and prolong translation. These results are consistent with a model in which the 5′CL-PCBP complex interacts with the 3′ poly(A)-PABP complex to form a 5′–3′ circular complex that facilitates ribosome reloading and stimulates PV RNA translation.

Introduction

Poliovirus (PV) belongs to the *Picornaviridae* family of single-stranded positive-sense RNA viruses. The PV genome contains a large open reading frame that is flanked by the 5′ NTR, which includes the internal ribosome entry site (IRES), and the 3′ NTR and poly(A) tail. A small viral protein, VPg, is covalently linked to the 5′ end of the genome (Flanegan et al., 1977; Lee et al., 1977; Ambros & Baltimore, 1978; Pettersson et al., 1978; Wimmer et al., 1993). Translation of the viral RNA genome is directed by the IRES and takes place in the cytoplasm of the infected cell (Pelletier et al., 1988; Pelletier & Sonenberg, 1988; Pelletier & Sonenberg, 1989). Translation of the viral genomic RNA results in the synthesis of a polyprotein which is cleaved by the viral proteases, 2A^{pro} and 3C^{pro}/3CD^{pro} (Krausslich & Wimmer, 1988; Harris et al., 1990).

The terminal 5′ cloverleaf (5′CL), IRES, 3′ NTR and poly(A) tail are important *cis*-active RNA elements that regulate different steps in the PV life cycle. In general, these *cis*-active elements function in the form of ribonucleoprotein complexes (RNP) which contain both viral and cellular proteins. The 5′CL is organized into stem *a* and stem-loops 'b', 'c' and 'd,' where stem-loops 'b' and 'd' bind the cellular poly(C) binding proteins (PCBP) and viral protein 3CD^{pro}, respectively (Andino et al., 1990; Andino et al., 1993; Parsley et al., 1997). Recently, a PCBP binding site was also identified in the C-rich sequence adjacent to the 5′CL (Toyoda et al., 2007). Previous studies show that mutations in stem-loop 'b' including those that specifically disrupt PCBP binding to stem-loop 'b' result in the inhibition of PV RNA translation (Simoes & Sarnow, 1991; Parsley et al., 1997; Gamarnik & Andino, 1998; Lyons et al., 2001). However, these results are complicated by the fact that disrupting PCBP binding to the 5′CL also destabilizes PV RNA (Murray et al., 2001). Other studies report that the presence of a 3′ poly(A) tail stimulates IRES-driven translation of reporter RNA constructs, and the stimulation of translation is observed in the absence of 2A^{pro} (Bergamini et al., 2000; Michel et al., 2001; Svitkin et al., 2001; Dobrikova et al., 2006).

The viral protease 2A^{pro} is a critical protein involved in many aspects of the PV life cycle. 2A^{pro} is a cysteine protease which catalyzes the primary cleavage of the polyprotein, separating the capsid protein precursor (P1) from the replication protein precursor (P23) (Toyoda et al., 1986; Hellen et al., 1989, 1992). Aside from its function in viral polyprotein processing, 2A^{pro} inhibits host cell protein synthesis through the cleavage of the eukaryotic initiation factor eIF4G, which leads to the inactivation of the cap-binding complex (eIF4F) (Etchison et al., 1982; Lamphear et al., 1995; Borman et al., 1997). Although cap-dependent translation of cellular mRNAs is inhibited, viral translation does not require intact eIF4G and therefore, is not inhibited by 2A^{pro} (Ziegler et al., 1995; Belsham & Sonenberg, 1996). In addition to the inhibition of host protein synthesis, 2A^{pro} has been shown to stimulate the translation of both entero- and rhinovirus IRES-driven translation (Hambidge & Sarnow, 1992; Ziegler et al., 1995; Borman et al., 1997; Roberts et al., 1998; Svitkin et al., 2001; Dobrikova et al., 2006). In a previous study from our laboratory, we showed that

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proteolytically active $2A^{pro}$ directly stimulates and prolongs PV RNA translation, in addition to its ability to stabilize PV RNA. Furthermore, we showed that $2A^{pro}$ and the $2A^{pro}$ containing precursor proteins, 2AB and P2, were the only PV encoded proteins that were able to stimulate and prolong PV RNA translation (Jurgens et al., 2006).

In the present study, we used HeLa S10 translation–replication reactions to define the role of the 5′CL-PCBP complex, 3′NTR, poly(A) tail and $2A^{pro}$ in the translation of PV RNA. We found that the presence of the 5′CL-PCBP complex, the 3′ poly(A) tail and $2A^{pro}$ stimulated PV RNA translation. In contrast, the 3′ NTR had no effect on translation independent of the poly(A) tail. By tethering PCBP2 to the 5′CL, we further established the importance of the 5′CL-PCBP complex in enhancing viral RNA translation. Taken together, the 5′CL-PCBP complex, 3′ poly(A) tail and $2A^{pro}$ were all required to observe optimal levels of PV RNA translation. In addition, our results support a model in which the formation of a 5′–3′ circular RNP complex facilitates ribosome reloading and enhances translation.

Results

In this study, we used a poliovirus subgenomic transcript RNA, PV1p50 RNA, to examine the requirements for optimal translation of PV RNA (Fig. 1). PV1p50 RNA (p50 RNA) contains an in-frame deletion in the coding region in PV RNA and encodes a 50-kDa nonfunctional protein (p50), which serves as a reporter protein for monitoring the translation of PV RNA. A significant advantage of using p50 RNA to characterize the translation of PV RNA is the inclusion of the authentic 5′ and 3′ NTRs and the authentic viral translation initiation and termination sequences. In addition, the labeled protein synthesized in these reactions can be easily quantitated. Protein synthesis was measured by pulse-labeling for 1-h intervals over a period of 4 h and the amount of labeled p50 synthesized during each hour was determined by gel electrophoresis and autoradiography (Fig. 2A). The amount of labeled p50 protein synthesized during each hour was quantitated and is shown in Fig. 2B. This allowed us to measure both the rate of protein synthesis during each hour of the reaction and the total amount of protein synthesized in each reaction.

Role of 5′CL in PV RNA translation

To characterize the role of the 5′CL in translation of PV RNA, we used a 5′CL mutation (C24A) which is known to inhibit the binding of PCBP to stem-loop 'b' (Andino et al., 1993; Murray et al., 2001; Lyons et al., 2001). Since the C24A mutation destabilizes PV RNA (Murray et al., 2001), we used RNA transcripts with a 5′ cap to restore the stability of this mutant RNA as described in Materials and methods. In the reaction which contained p50 RNA, p50 protein synthesis continued for the entire 4 h reaction (Fig. 2A, lanes 1–4 and B). The largest amount of p50 synthesized was observed between 1 and 2 h and gradually decreased between 2–3 h and 3–4 h (Fig. 2A, lanes 1–4 and B). In contrast, in reactions containing C24A RNA, p50 was only synthesized in significant amounts from 0–1 h and 1–2 h (Fig. 2A, lanes 5–8 and B). There was a dramatic decrease in the rate of protein synthesis after 2 h and only very small amounts of p50 were

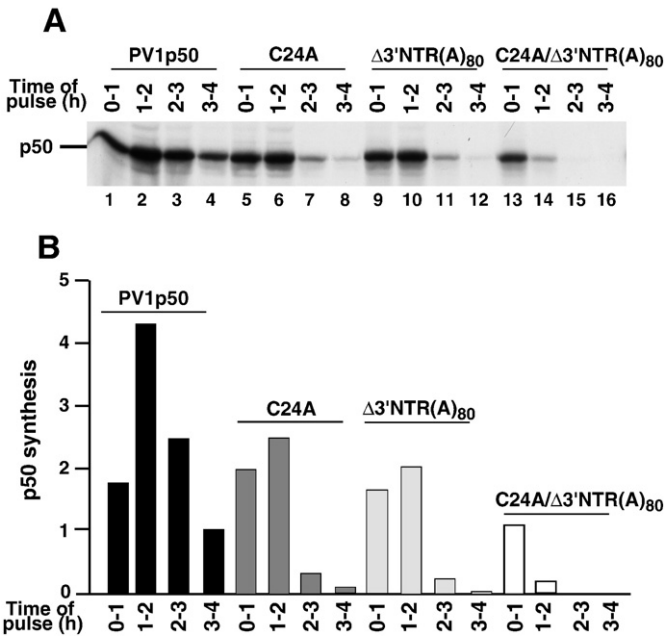

Fig. 2. Requirement of the 5′CL-PCBP complex and 3′NTR$(A)_{80}$ for efficient PV RNA translation. (A) Translation of either PV1p50, PV1p50(C24A), PV1p50(Δ3′NTR$(A)_{80}$) or PV1p50(C24A/Δ3′NTR$(A)_{80}$) RNA at a concentration of 34 μg/ml was measured by pulse-labeling for 1 h over a period of 4 h at 34 °C. The reactions were pulse-labeled with 15 μCi [^{35}S]methionine for 1 h at the indicated time points. At the end of the pulse, 4 μl of the translation reaction was solubilized in 40 μl SDS sample buffer. Labeled p50 protein synthesized was analyzed by 9–18% SDS-PAGE and visualized by autoradiography. (B) The amount of labeled p50 synthesized during each hour of the pulse was quantitated using a PhosphorImager.

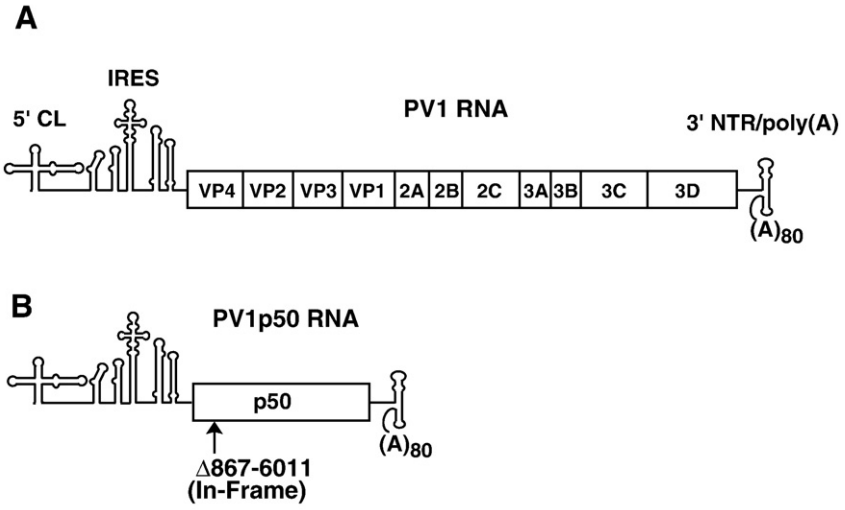

Fig. 1. Schematic of poliovirus RNAs utilized in this study. (A) Diagram of the full-length PV1 RNA which encodes all of the viral proteins. (B) Diagram of PV1p50 RNA in which nucleotides 867–6011 from PV1 RNA are deleted. This RNA contains the authentic 5′ NTR, IRES, 3′ NTR and poly(A) tail of PV1 RNA. It also contains the authentic initiation and stop codons of the viral polyprotein.