Demonstration of Functional Requirement of Polypyrimidine Tract-binding Protein by SELEX RNA during Hepatitis C Virus Internal Ribosome Entry Site-mediated Translation Initiation*  

Received for publication, July 18, 2000, and in revised form, August 6, 2000  
Published, JBC Papers in Press, August 10, 2000, DOI 10.1074/jbc.M006343200  

Adil Anwar‡, Naushad Ali§, Rasheeda Tanveer, and Aleem Siddiqui¶  

From the Department of Microbiology, University of Colorado Health Sciences Center, Denver, Colorado 80262  

Polypyrimidine tract-binding protein (PTB) has been previously shown to physically interact with the hepatitis C virus (HCV) RNA genome at its 5'-and 3'-noncoding regions. Using high affinity SELEX RNA molecules, we present evidence for the functional requirement of PTB during HCV internal ribosome entry site (IRES)-controlled translation initiation. This study was carried out in rabbit reticulocyte translation lysates in which the HCV IRES-driven reporter RNA was introduced alone with the PTB-specific SELEX RNA molecules. The SELEX RNAs specifically inhibited the HCV IRES function in the context of mono- and dicistronic mRNAs. The cap-dependent translation of a reporter (chloramphenicol acetyltransferase) RNA or naturally capped brome mosaic virus RNA, however, was not affected by the presence of SELEX during in vitro translation assays. The SELEX-mediated inhibition of the HCV IRES is shown to be relieved by the addition of recombinant human PTB in an add-back experiment. The in vivo requirement of PTB was further confirmed by cotransfection of Huh7 cells with reporter RNA and PTB-specific SELEX RNA. The HCV IRES activity was inhibited by the SELEX RNA in these cells, but not by an unrelated control RNA. Together, these results demonstrate the functional requirement of cellular PTB in HCV translation and further support the feasible use of SELEX RNA strategy in demonstrating the functional relevance of cellular protein(s) in complex biological processes.

Human hepatitis C virus (HCV), a member of the family Flaviviridae, is the leading cause of chronic hepatitis (≥80%). HCV infection has been strongly linked with the development of hepatocellular carcinoma (1–3). HCV contains a single-stranded RNA genome (9600 nucleotides) with plus polarity that encodes a single polyprotein of 3010 amino acids (1). The polyprotein is processed into three structural (C (p21), E1 (p31), and E2 (p72)) and several nonstructural (NS2 (p23), p7, NS3 (p70), NS4A (p6), NS4B (p27), NS5A (p58), and NS5B (p65)) polypeptides. Analysis of the conserved and divergent regions of the HCV genomes thus far cloned from various geographical locations indicates that the 5'-noncoding region (5'-NCR) that precedes the open reading frame and the 3' terminus of the genome are highly conserved among all subtypes (1, 4). These elements function as promoters of HCV gene expression.

The translation initiation of the HCV RNA genome occurs by internal ribosome entry located in the 5'-NCR (5, 6). It has been suggested that HCV RNA translation is regulated by at least three distinct elements: 1) the global structure of the viral internal ribosome entry site (IRES), which includes almost the entire sequence (except nucleotides 1–40) within the 5'-NCR and a few nucleotides downstream of the AUG initiator codon (see Fig. 1A) (5–13); 2) the 3'-terminal region, which has been shown to enhance IRES activity (14) (but the exact role of the entire 3'-NCR during translation regulation is still unclear); and 3) trans-acting cellular factors that interact with the viral IRES element and assist in internal initiation of translation (15–18). Several subunits of eukaryotic initiation factor-3 have been shown to bind the apical half of domain III of the 5'-NCR (18, 34). Notable among the non-canonical translation initiation factors that regulate the viral IRES elements are the polypyrimidine tract-binding protein (PTB) and La antigen (for review, see Ref. 19). PTB (p57 or hnRNP-1) has been shown to exist as a homodimer in solution and presents an oligomeric array of eight RNA recognition motifs (four in each monomer) (20). Structural analysis suggests that PTB possesses unusual features of RNP-1 and RNP-2 motifs within its RNA recognition motifs (35). The protein binds polypyrimidine tract near the 3'-splice site of many introns and can act as a repressor of splicing (21). In the cytoplasm, PTB has been shown to physically interact with viral and cellular 5'-NCRs (15, 22, 25, 32, 36). Furthermore, it has been shown to facilitate translation directed by picornaviral IRES elements in the rabbit reticulocyte translation system (22, 32). The overexpression of PTB was recently shown to have a considerable stimulatory effect on translation directed by IRES elements derived from hepatitis A virus, poliovirus, and HCV in different types of cells (24). We have previously shown interaction of PTB with the HCV 5'-NCR at multiple sites using a UV cross-linking assay (15). Interestingly, Ito and Lai (33) have demonstrated an additional PTB-binding site at the end of the core coding region that appears to have a regulatory effect on the HCV IRES activity. Our immunodepletion experiment suggested that PTB or PTB-associated factors may be required for the HCV IRES function (15). This study was undertaken to address the biological requirement of PTB for the HCV IRES function using high affi-
ity SELEX (systematic evolution of ligands by exponential enrichment) RNA ligands. Here, we demonstrate that the SELEX RNA ligands raised against human PTB specifically interfered with HCV IRES-directed translation under conditions in which cap-dependent translation of the in vitro transcribed RNA or naturally capped BMV RNA was unaffected. The PTB requirement for HCV IRES function was established by schemes of RNA cotransfections and add-back experiments.

MATERIALS AND METHODS

In Vitro Transcription—RNA transcripts were synthesized in vitro from linearized plasmid DNA that was purified by elution of the desired fragments from agarose gels after digestion with an appropriate restriction endonuclease. The plasmids pT7C1-341 and pT7DC1-341 (6) were linearized by HpaI and transcribed by T7 RNA polymerase to produce HCV 5′-NCR-containing monocistronic luciferase reporter and dicistronic mRNAs, respectively. The plasmids encoding SELEX 53.3, 63.1, and 63.6 or the pGEM-4 vector were linearized with HindIII or EcoRI, respectively, and transcribed with T7 RNA polymerase. Capped T7DC1-341 was synthesized in the presence of the m7G(5′)ppp(5′)G cap analogue using an Ampliscribe T7 transcription kit (Epigenic Technologies Corp.). The transcripts were visualized on formaldehyde-agarose gel, and the concentrations were determined.

Partition of Recombinant PTB—The plasmid GST-2TK/PTB, which encodes GST-human PTB fusion protein (15), was a generous gift of Dr. M. A. Garcia-Blanco. The expression of GST-PTB was induced with 1 mM isopropyl-β-D-thiogalactopyranoside in Escherichia coli (JM101) and affinity-purified on glutathione-Sepharose beads (Amer sham Pharmacia Biotech). The final preparation was dialyzed against 5 mM Hepes (pH 7.6), 1 mM EDTA, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 10% (v/v) glycerol. GST was also purified by a similar procedure and used as a control.

UV Cross-linking and Immunoprecipitation Assay—4-Thio-UDP (Sigma) was phosphorylated with nucleoside-5′-diphosphate kinase to prepare 4-thio-UTP. The SELEX RNA probes synthesized in the presence of 4-thio-UDP and [32P]CTP were incubated with the purified PTB or translation lysates at 30 °C for 30 min in RNA binding buffer as described previously (15). UV cross-linking was carried out in a Stratallinker (Stratagen) for 30 min at 4 °C. The ribonucleoprotein complexes were treated with RNase A (10–20 units; U. S. Biochemical Corp.) and analyzed by SDS-12% polyacrylamide gel electrophoresis followed by autoradiography. The immunoprecipitation assay was carried out with the UV-cross-linked product(s) between SELEX RNA probe and translation lysates using polyclonal anti-PTB antibodies or normal human IgG (control) (15).

Translation—In vitro translation was performed with rabbit reticulocyte lysates (Promega) according to the manufacturer’s protocol. One microgram of template RNA (T7C1-341) was translated in the presence of varying amounts (1–5 μg) of SELEX RNAs, and the proteins were radiolabeled with [35S]-methionine (ICN). The reaction was carried out for 1.5 h at 30 °C, and luciferase activity was assayed from a 2-μl aliquot as described by de Wet (23). Two-microliter translation reactions were subjected to SDS-12% polyacrylamide gel electrophoresis. The gels were treated with Fluoro-Hance (Research Products International Corp.), dried, and used for phosphoimage analysis (Bio-Rad) and autoradiography. During the PTB add-back experiments, varying amounts of purified recombinant GST-PTB (1–5 μg) or GST (control) were added to the translation reactions containing 5 μg of SELEX RNA. Translation of capped T7DC1-341 was carried out using 10 μl of rabbit reticulocyte lysates (RLAs), 2 μl of HeLa initiation factors (37), 1 μl of [35S]methionine, 1 μl of amino acid mixture without methionine, and 20 units of RNAsin in a total volume of 25 μl for 1 h at 30 °C. Translation was carried out in the absence (control) or presence of varying amounts of SELEX 53.3.

Transfection of Huh7 Cells—A liver-derived cell line (Huh7) was used for the cotransfection assay. The cells were transfected with in vitro transcribed T7C1-341 RNA (1 μg) using the Lipofectin transfection protocol (Life Technologies, Inc.). To demonstrate the inhibitory activity of SELEX RNA on HCV IRES-controlled translation, the cells were directly cotransfected with T7C1-341 RNA along with SELEX 53.3. An RNA derived from the pGEM-4 multiple cloning site served as a control during similar cotransfection experiments. Each experiment was repeated three to four times to confirm the results. Luciferase activity was assayed as described above.

RESULTS

The HCV IRES Requires PTB in Cell-free Translation Lysates—Sequence analysis of the HCV 5′-NCR identified three putative polypyrimidine tracts: Py-I (UCACCUCCCUGU, nucleotides 37–48), Py-II (CCCCCUCCCCGG, nucleotides 120–132), and Py-III (AUCUUACCUGG, nucleotides 191–202) (Fig. 1B) (15). All three polypyrimidine tracts appear to contain the core of the PTB consensus binding site (CYYYYCYYYGG, where Y is pyrimidine) that was identified by the SELEX procedure (Fig. 1B) (21). We previously reported the interaction between PTB and the HCV IRES at three distinct domains, each of which harbors one of the three polypyrimidine tracts (15). In an attempt to understand the biological significance of such unusual binding, we demonstrated dramatic reduction in the HCV IRES activity in HeLa and rabbit reticulocyte translation lysates that were immunodepleted of PTB (15). In a recent study, Lemon and co-workers (24) showed that PTB contributes to the HCV IRES activity. This was demonstrated by the dicistronic expression vector containing human PTB and the HCV IRES-driven reporter gene in BS-C-1 and Huh7 cells. The presence of multiple PTB consensus binding sites within the HCV IRES and their interaction with PTB, including the functional assays described above, argue for a role of PTB in HCV RNA translation. However, Kaminski et al. (25) observed that PTB depletion from HeLa lysates passed through an en-
The amounts of SELEX added in the lysates are as indicated. Preparation in the absence (lanes 2) or presence of SELEX was translated in RRLs supplemented with HeLa S100 lysates enriched for PTB. The translation products of BMV RNAs are shown on the right, and luciferase coding region is dictated by the HCV IRES (6). The mechanism, whereas the translation of the downstream luciferase (LUC) RNA is translated by a cap-dependent transferase) of this mRNA is translated by a cap-dependent mechanism, whereas the translation of the downstream luciferase coding region is dictated by the HCV IRES (6). The capped dicistronic RNA was translated in a reticulocyte translation system supplemented with HeLa S100 lysates enriched for PTB. The translation products of MV RNAs were translated as described above. The BMV RNA preparation consists of at least three capped RNAs that are translated into four viral proteins, 1a (110 kDa), 2a (97 kDa), 3a (35 kDa), and the coat protein (20 kDa). As shown in Fig. 2B (lanes 5–7), the translation of these RNAs was not significantly affected in the presence of SELEX molecules. However, the HCV IRES activity was dramatically inhibited under these conditions (lanes 2 and 3). In a control experiment, the SELEX RNA was also found to be an effective inhibitor of the polioviral IRES (data not shown).

The ability of SELEX RNA to inhibit the HCV IRES activity was also tested in the context of a capped dicistronic mRNA (T7DC1-341). The upstream cistron (chloramphenicol acetyltransferase) of this mRNA is translated by a cap-dependent mechanism, whereas the translation of the downstream luciferase coding region is dictated by the HCV IRES (6). The capped dicistronic RNA was translated in a reticulocyte translation system supplemented with HeLa S100 lysates enriched for PTB. We used SELEX strategy to sequester PTB during the active translation process. A similar approach has been successfully used to clarify conflicting issues on the role of PTB during regulation of RNA splicing (21). Three SELEX RNAs (63.1, 63.6, and 53.3), all of which harbor similar PTB consensus binding motifs but differ in their overall base composition (Fig. 1B), were used during the translation assays. These SELEX RNAs were shown to bind human PTB with high affinity (21). The SELEX binding to PTB in the translation lysates used during these studies was confirmed by UV cross-linking and immunoprecipitation assay (data not shown). HCV IRES-luciferase RNA (T7C1-341), in which the HCV IRES directs translation of the luciferase reporter gene, was translated in RRLs either in the absence or presence of varying amounts of SELEX RNAs. The resulting luciferase activity was assayed, and the protein bands were visualized by autoradiography (Fig. 2A). All of the SELEX RNAs were found to be inhibitory for the HCV IRES activity. However, the extent of inhibition for each SELEX RNA was variable. For example, SELEX 53.3 was found to be the most potent inhibitor of the HCV IRES activity (compare lanes 7 and 8 with lanes 3–5). This differential inhibitory ability of the SELEX RNAs could be due to the differences in sequence composition and their affinity for PTB. An unrelated RNA of similar length and stability that was derived from a pGEM-4 vector failed to inhibit IRES activity at a higher concentration (lane 9). To further confirm the specificity of inhibition by SELEX 53.3, naturally 5′-capped BMV RNAs were translated as described above. The BMV RNA preparation consists of at least three capped RNAs that are translated into four viral proteins, 1a (110 kDa), 2a (97 kDa), 3a (35 kDa), and the coat protein (20 kDa). As shown in Fig. 2B (lanes 5–7), the translation of these RNAs was not significantly affected in the presence of SELEX molecules. However, the HCV IRES activity was dramatically inhibited under these conditions (lanes 2 and 3). In a control experiment, the SELEX RNA was also found to be an effective inhibitor of the polioviral IRES (data not shown).

The ability of SELEX RNA to inhibit the HCV IRES activity was also tested in the context of a capped dicistronic mRNA (T7DC1-341). The upstream cistron (chloramphenicol acetyltransferase) of this mRNA is translated by a cap-dependent mechanism, whereas the translation of the downstream luciferase coding region is dictated by the HCV IRES (6). The capped dicistronic RNA was translated in a reticulocyte translation system supplemented with HeLa S100 lysates enriched for PTB. We used SELEX strategy to sequester PTB during the active translation process. A similar approach has been successfully used to clarify conflicting issues on the role of PTB during regulation of RNA splicing (21). Three SELEX RNAs (63.1, 63.6, and 53.3), all of which harbor similar PTB consensus binding motifs but differ in their overall base composition (Fig. 1B), were used during the translation assays. These SELEX RNAs were shown to bind human PTB with high affinity (21). The SELEX binding to PTB in the translation lysates used during these studies was confirmed by UV cross-linking and immunoprecipitation assay (data not shown). HCV IRES-luciferase RNA (T7C1-341), in which the HCV IRES directs translation of the luciferase reporter gene, was translated in RRLs either in the absence or presence of varying amounts of SELEX RNAs. The resulting luciferase activity was assayed, and the protein bands were visualized by autoradiography (Fig. 2A). All of the SELEX RNAs were found to be inhibitory for the HCV IRES activity. However, the extent of inhibition for each SELEX RNA was variable. For example, SELEX 53.3 was found to be the most potent inhibitor of the HCV IRES activity (compare lanes 7 and 8 with lanes 3–5). This differential inhibitory ability of the SELEX RNAs could be due to the differences in sequence composition and their affinity for PTB. An unrelated RNA of similar length and stability that was derived from a pGEM-4 vector failed to inhibit IRES activity at a higher concentration (lane 9). To further confirm the specificity of inhibition by SELEX 53.3, naturally 5′-capped BMV RNAs were translated as described above. The BMV RNA preparation consists of at least three capped RNAs that are translated into four viral proteins, 1a (110 kDa), 2a (97 kDa), 3a (35 kDa), and the coat protein (20 kDa). As shown in Fig. 2B (lanes 5–7), the translation of these RNAs was not significantly affected in the presence of SELEX molecules. However, the HCV IRES activity was dramatically inhibited under these conditions (lanes 2 and 3). In a control experiment, the SELEX RNA was also found to be an effective inhibitor of the polioviral IRES (data not shown).

The ability of SELEX RNA to inhibit the HCV IRES activity was also tested in the context of a capped dicistronic mRNA (T7DC1-341). The upstream cistron (chloramphenicol acetyltransferase) of this mRNA is translated by a cap-dependent mechanism, whereas the translation of the downstream luciferase coding region is dictated by the HCV IRES (6). The capped dicistronic RNA was translated in a reticulocyte translation system supplemented with HeLa S100 lysates enriched for PTB. We used SELEX strategy to sequester PTB during the active translation process. A similar approach has been successfully used to clarify conflicting issues on the role of PTB during regulation of RNA splicing (21). Three SELEX RNAs (63.1, 63.6, and 53.3), all of which harbor similar PTB consensus binding motifs but differ in their overall base composition (Fig. 1B), were used during the translation assays. These SELEX RNAs were shown to bind human PTB with high affinity (21). The SELEX binding to PTB in the translation lysates used during these studies was confirmed by UV cross-linking and immunoprecipitation assay (data not shown). HCV IRES-luciferase RNA (T7C1-341), in which the HCV IRES directs translation of the luciferase reporter gene, was translated in RRLs either in the absence or presence of varying amounts of SELEX RNAs. The resulting luciferase activity was assayed, and the protein bands were visualized by autoradiography (Fig. 2A). All of the SELEX RNAs were found to be inhibitory for the HCV IRES activity. However, the extent of inhibition for each SELEX RNA was variable. For example, SELEX 53.3 was found to be the most potent inhibitor of the HCV IRES activity (compare lanes 7 and 8 with lanes 3–5). This differential inhibitory ability of the SELEX RNAs could be due to the differences in sequence composition and their affinity for PTB. An unrelated RNA of similar length and stability that was derived from a pGEM-4 vector failed to inhibit IRES activity at a higher concentration (lane 9). To further confirm the specificity of inhibition by SELEX 53.3, naturally 5′-capped BMV RNAs were translated as described above. The BMV RNA preparation consists of at least three capped RNAs that are translated into four viral proteins, 1a (110 kDa), 2a (97 kDa), 3a (35 kDa), and the coat protein (20 kDa). As shown in Fig. 2B (lanes 5–7), the translation of these RNAs was not significantly affected in the presence of SELEX molecules. However, the HCV IRES activity was dramatically inhibited under these conditions (lanes 2 and 3). In a control experiment, the SELEX RNA was also found to be an effective inhibitor of the polioviral IRES (data not shown).
during the SELEX 53.3, which displayed the highest inhibitory ability. However, an unrelated RNA failed to inhibit IRES activity inhibited by the SELEX RNA in a dose-dependent manner. The pattern of HCV IRES-mediated translation is shown in Fig. 4. The HCV IRES activity was shown in Fig. 4. The HCV IRES activity was demonstrated above is that the SELEX molecules could sequester PTB in the translation lysates with high affinity (Fig. 2), rendering PTB unable to support the HCV IRES activity. In this case, the PTB requirement may be due to its direct interaction with the 5'-NCR and/or its indirect inhibition of the interaction of other proteins needed for the IRES function. One such example is the known PTB-binding protein hnRNP L, which has been shown to bind to the 3'-boundary of the HCV IRES (26, 27). This binding was shown to correlate with HCV IRES efficiency (27). In either case, the addition of an excess amount of recombinant PTB should rescue the efficiency of the HCV IRES that was inhibited by SELEX RNA molecules during translation. To test this hypothesis, an add-back experiment was carried out in which purified GST-human PTB was added to translation lysates containing HCV IRES-luciferase RNA and an excess amount of the SELEX RNA inhibitory for translation (Fig. 3, lane 3). Interestingly, the inhibition was considerably relieved by the supplemented GST-PTB in a dose-dependent manner (lanes 4–6). However, the addition of similar amounts of GST protein failed to rescue the HCV IRES activity (lanes 7 and 8). These results clearly demonstrate a direct involvement of PTB during HCV IRES-controlled translation initiation.

The HCV IRES Requires PTB in Vivo—Next, we investigated the PTB requirement for the HCV IRES activity in vivo using SELEX 53.3, which displayed the highest inhibitory ability during the in vitro translation assay (Fig. 2A). A liver-derived cell line (Huh7) that expresses PTB at levels similar to those expressed by the widely used HeLa cell line (24) was cotransfected with HCV IRES-luciferase RNA along with varying amounts of SELEX 53.3. The pattern of HCV IRES-mediated translation is shown in Fig. 4. The HCV IRES activity was inhibited by the SELEX RNA in a dose-dependent manner. However, an unrelated RNA failed to inhibit IRES activity even at higher concentrations during the cotransfection experiment. Consistent results were obtained during several transfection assays. These data together provide further evidence that the HCV IRES requires PTB for efficient translation initiation in vivo.

DISCUSSION

There is a growing body of evidence that supports the biological significance of non-canonical translation factors in the regulation of viral IRES-mediated translation initiation (15, 19, 22, 24, 25, 27, 32, 38, 39, 40, 43, 44). The majority of these studies have reported non-canonical translation initiation factors belonging to the hnRNP family of proteins (e.g., PTB, poly(rC)-binding protein, and hnRNP L) or a nucleocytoplasmic phosphoprotein designated as La antigen. These proteins have been thought to alter IRES activity either by their physical interaction with the IRES elements or by protein-protein interactions. In this study, we focused on PTB because of its ability to bind to the 5'- and 3'-NCRs (15, 28–30) and its reported HCV IRES stimulatory activity in a cell-based assay (24). The SELEX RNA-based strategy has been shown to be highly effective in demonstrating diverse functions of target proteins involved in complex biological pathways, including that of La antigen (21, 38, 41, 42). An additional advantage of using SELEX RNA is that it may not interfere with the nuclear functions of its target protein during transient transfection assays similar to that used in this study. We employed SELEX RNA technology to establish the functional requirement of PTB during HCV IRES-controlled translation initiation. The results presented here strongly suggest that SELEX RNA raised against PTB attenuates the HCV IRES activity most probably by sequestering cellular PTB in Huh7 cells or translation lysates. Thus, a direct interaction of PTB with the 5'-NCR that might be an essential ritual during recruitment of the 43 S initiation complex to the HCV IRES in vivo is prevented. In addition to this requirement, PTB interaction may also facilitate recruitment of its cellular partners such as hnRNP L or other proteins that may be needed for the assembly of an initiation complex. In either case, PTB binding to the 5'-NCR appears to have important biological consequences on translation of the HCV RNA.

The molecular mechanism(s) of the PTB requirement during internal initiation of translation of the HCV RNA is not known. Available evidence suggests a physical interaction of PTB with the following: (i) multiple structural domains of the HCV 5'-NCR (15), (ii) an internal polypyrimidine-rich motif at the 3'-end of the core coding sequence (33), and (iii) a region near the end of the HCV genome (within the 3'-NCR) (28–30). Interestingly, these viral cis-elements appear to bind PTB with different affinities. These differential kinetics may play critical role(s) in regulating the HCV IRES activity (33). Another im-
important feature of PTB is its intrinsic ability to homodimerize and/or multimerize in solution (20). Because of these unusual features, it is tempting to speculate a plausible role of PTB in establishing a dialogue between the 5'- and 3'-NCRs by bringing the ends in close proximity. Such RNA topology is now believed to be a prerequisite for an efficient translation initiation process (31). PTB may also function as a molecular chaperon because of its ability to bind multiple domains within the 5'-NCR. These interactions were found to be independent of La antigen,2 which binds the initiator AUG codon of the HCV RNA in the context of an ordered structure (16).

Recently, Riekh et al. (45) demonstrated functionally relevant ion-dependent tertiary folds of the HCV IRES. In light of these observations, it appears that these non-canonical translation initiation factors including PTB might be required for the stabilization of IRES conformation(s) in vivo. Amid all these possibilities, the requirement of PTB for efficient HCV IRES activity is unambiguously supported by the data presented here. Because PTB binds to the 3'-NCR, which functions as the assembly site for the preinitiation replication complex, the SELEX strategy has the potential to reveal its role during (--) strand synthesis. How PTB affects the HCV IRES activity and RNA replication via its interaction with the 5'- and 3'-NCRs is the subject of future investigation.

Acknowledgments—We thank Drs. David Barton (of this institution) for HeLa lysates and Ravinder Singh (University of Colorado, Boulder, CO) for plasmids encoding SELEX RNA against PTB.

REFERENCES
1. Houghton, M. (1996) in Fields Virology (Fields, B. N., Knipe, D. M., and Howley, P. M., eds) pp. 1035–1058, Lippincott-Raven Publishers, Philadelphia.
2. Saito, I., Miyamura, T., Ohbayashi, A., Harada, H., Katayama, T., Kikuchi, S., Watanabe, Y., Koi, S., Onji, M., Obia, Y., Chou, Q.-L., Houghton, M., and Kuo, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 97, 6547–6549.
3. Tsukuma, H., Hiyama, T., Tanaka, S., Nakao, M., Yabuuchi, T., Kitamura, T., Nakanishi, K., Fujimoto, I., Inoue, A., Yamazaki, H., and Kawashima, T. (1995) N. Engl. J. Med. 332, 1797–1801.
4. Kolykhlov, A., Feinestone, S. M., and Rice, C. M. (1996) J. Virol. 70, 3363–3371.
5. Tsukiyama-Kohara, K., Iizuka, N., Kohara, M., and Nomoto, A. (1999) J. Virol. 66, 1476–1483.
6. Wang, C., Sarnow, P., and Siddiqui, A. (1993) J. Virol. 67, 3338–3344.
7. Rijinbrand, R., Brenchley, P., van der Straaten, T., Whetter, L., Inchauspe, G., Lemon, S., and Spaan, W. (1995) FEBS Lett. 365, 115–119.
8. Fukushi, S., Katayama, K., Kurihara, C., Ishiyama, N., Hoshino, F. B., Ando, T., and Oya, A. (1994) Biochem. Cell Biol. 199, 425–432.
9. Honda, M., Pint, L.-H., Rijinbrand, R. C. A., Amphlett, E., Clarke, B., Rowlands D., and Lemon, S. M. (1996) Virology 222, 31–42.
10. Reynolds, J. E., Kaminski, A., Kettinin, H. J., Grace, K., Clarko, B. E., Carroll, A. R., Rowlands, D. J., and Jackson, R. J. (1995) EMBO J. 14, 6010–6020.
11. Lu, H.-H., and Wimmer, E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1412–1417.
12. Wang, C., Sarnow, P., and Siddiqui, A. (1994) J. Virol. 68, 7301–7307.
13. Wang, C., Le, S.-Y., Ali, N., and Siddiqui, A. (1995) RNA 1, 526–537.
14. Rijinbrand, R., van der Straaten, T., Whetter, L., Inchauspe, G., Lemon, S. M., and Spaan, W. (1995) J. Virol. 69, 6367–6375.
15. Ali, N., and Siddiqui, A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2249–2254.
16. Kanoshita, N., Tsukiyama-Kohara, K., Kohara, M., and Nomoto, A. (1997) Virology 233, 9–18.
17. Sizova, D. V., Kulupaeva, V. G., Pestova, T. V., Shatsky, I. I., and Hellen, C. U. T. (1998) J. Virol. 72, 4775–4782.
18. Krecic, A., and Swanson, M. S. (1996) in Adv. Virus Res. (Kapikian, A. Z., and Howley, P. M., eds) pp. 1035–1058, Lippincott-Raven Publishers, Philadelphia.
19. Kolykhlov, A., Feinestone, S. M., and Rice, C. M. (1996) J. Virol. 70, 1583–1595.
20. Kaminski, A., Hunt, S. H., Patton, J. G., and Jackson, R. J. (1995) RNA 1, 924–938.
21. Hahn, B., Cho, O. H., Kim, J.-E., Kim, Y. K., Kim, J. H., Oh, Y. L., and Jeong, S. K. (1998) FEBS Lett. 425, 401–406.
22. Hahn, B., Kim, Y. K., Kim, J. H., Kim, J. Y., and Jeong, S. K. (1998) J. Virol. 72, 8782–8788.
23. Ito, T., and Lai, M. M. C. (1997) J. Virol. 71, 8696–8706.
24. Tsukhihara, K., Tanaka, T., Hikjikata, M., Ruge, S., Toyoda, H., Nomoto, A., Yamamoto, N., and Shimotohno, K. (1997) J. Virol. 71, 6720–6726.
25. Gortarek, R. R., Geshell, L. H., Herold, K. M., Tsai, J., Sathe, G., Mao, J., Prescott, C., and Vechio, A. M. D. (1999) Nucleic Acids Res. 27, 1457–1463.
26. Sache, A. B., Sarnow, P., and Hentze, M. W. (1997) Cell 89, 831–838.
27. Hellen, C. U. T., Witter, G. W., Schmid, M., Shin, S. H., Pestova, T. V., Gil, A., and Wimmer, E. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7424–7426.
28. Ito, T., and Lai, M. M. C. (1999) Virology 254, 288–296.
29. Buratti, E., Tsimetnetsky, S., Zotti, M., and Baralle, F. S. (1998) Nucleic Acids Res. 26, 3179–3187.
30. Cazzou, M. R., Grune, T., Bhuma, J., Kelly, G., Ladas, A., Mathews, S., and Curry, S. (2000) EMBO J. 19, 3132–3141.
31. Sickinger, S., and Schreiber, M. (1999) Biol. Chem. Hoppe-Seyer 380, 1217–1223.
32. Barton, D. J., Morascono, B. J., and Planegan, J. B. (1996) Methods Enzymol. 275, 35–57.
33. Ali, N., Prijut, G. J. M., Kenan, D. J., Keene, J. D., and Siddiqui, A. (2000) J. Biol. Chem. 275, 27521–27529.
34. Isoyama, T., Kamoshita, N., Yasui, K., Iwai, A., Shirakiy, K., Toyoda, H., Yamada, A., Takasuki, Y., and Nomoto, A. (1999) J. Gen. Virol. 526–537.
35. Garsark, A. V., and Andino, A. (1998) Genes Dev. 12, 2283–2304.
36. Gold, L., Polisky, B., Uhlenbeck, O., and Yarus, M. (1995) Annu. Rev. Biochem. 64, 763–797.
37. Chen, H., Wang, R., and Gold, L. (2000) Methods Enzymol. 275, 505–520.
38. Das, S., Ott, M., Yamane, A., Tsai, W., Gromeier, M., Lahser, F., Gupta, S., and Dasgupta, A. (1998) J. Virol. 72, 6538–6547.
39. Spanberg, K., and Schwartz, S. (1999) J. Gen. Virol. 79, 1371–1376.
40. Kief, J. S., Zou, K., Rubin, R., Murray, M. G., Lai, Y. J. N., and Doudna, J. A. (1999) J. Mol. Biol. 292, 513–529.