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Translational enhancement of the poliovirus 5′ noncoding region mediated by virus-encoded polypeptide 2A

(translational regulation/dicistronic mRNA/T7 RNA polymerase-expressing cell line/firefly luciferase gene)

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ABSTRACT **Genetic and biochemical studies have revealed that the 5′ noncoding region of poliovirus mediates translation of the viral mRNA by an unusual mechanism involving entry of ribosomes in internal sequences of mRNA molecules. We have found that mRNAs bearing the 5′ noncoding region of poliovirus were translated at an enhanced rate in poliovirus-infected mammalian cells at a time when translation of cellular mRNAs was not yet inhibited. This translational enhancement of the poliviral 5′ noncoding region was mediated by the expression of virus-encoded polypeptide 2A. This indicates that 2A is a multifunctional protein involved directly or indirectly in the activation of viral mRNA translation, in addition to its known roles in viral polyprotein processing and in inhibition of cellular protein synthesis. Thus, 2A represents an activator of translation of a viral mRNA that is translated by an internal ribosome binding mechanism. A likely consequence of this role of 2A is the efficient translation of viral mRNAs early in the infectious cycle, when host cell mRNAs can still compete with viral mRNAs for the host cell translation apparatus.**

Translational regulation in eukaryotic organisms can be mediated by sequences and structures in the RNA as well as proteins, causing both repression and activation of mRNA translation (for review, see ref. 1). Repression of translation initiation is usually mediated by RNA hairpin structures present in the 5′ noncoding region (NCR) of mRNA molecules. One well-studied example is the translational repression of ferritin mRNA, where the binding of a 90-kDa protein to an RNA hairpin, the "iron responsive element," present in the 5′ NCR of ferritin mRNA represses translation (2). Activation of translation has been reported in *Saccharomyces cerevisiae*. For example, the regulation of translation of GCN4 mRNA by the *GCN* and *GCD* gene products is dependent on the availability of amino acids (3). In this case, some of the translational regulators (for example, the eukaryotic translation factor 2) have been shown to be components of the normal translation apparatus (4). Recently, translational transactivation of viral mRNAs by virus-encoded gene products has been reported. The translation of the 35S mRNA of cauliflower mosaic virus was found to be stimulated by the gene product of the viral 19S mRNA (5). This finding indicates that viruses can carry functions to enhance the translation of their genetic material, thus circumventing competition for the translation apparatus with host cell mRNAs.

Poliovirus, a positive-strand RNA virus, has been shown to inhibit host cell translation by cleavage of the p220 protein of the cap-binding complex eIF-4F (6). Subsequently, it was found that the virus-encoded protein 2A, together with the translation initiation factor eIF3, is responsible for p220 cleavage (7). As a result, eIF-4F is rendered nonfunctional, and capped host cell mRNAs cannot be translated by a cap-dependent scanning mechanism (8), while the uncapped viral mRNA can be translated by a cap-independent mechanism of internal ribosome binding (9). This mechanism involves the binding of ribosomes to RNA sequences, termed the internal ribosome entry site (IRES) (10) or the ribosome landing pad (9), present in the 5′ NCR of the viral RNA.

Here, we provide evidence that mRNAs containing the 5′ NCR of poliovirus are translated at an enhanced rate compared to mRNAs lacking the viral 5′ NCR in cells at very early times after infection with poliovirus. Importantly, the observed enhanced rate of translation of poliovirus 5′ NCR-containing RNAs occurred at a time when cellular translation was not inhibited, thus ruling out competition for the translation machinery as the only mechanism for the preferential translation of viral RNA in infected cells. Furthermore, genetic analysis has identified the transactivator function as the poliovirus-encoded protein 2A. This finding indicates that poliovirus encodes a translational transactivator function, possibly to jump-start viral gene expression very early in the infectious cycle, thereby circumventing competition with ongoing cellular translation.

MATERIALS AND METHODS

Cells and Viruses. Human HeLa cells and monkey COS cells (provided by R. Schneider, New York University) were grown on Petri dishes in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) calf serum (GIBCO); OST7-1 cells (provided by O. Elroy-Stein and B. Moss, Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD) were grown in modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum and G418 sulfate (Geneticin; GIBCO) at 0.5 mg/ml. Preparation of poliovirus type 1 stocks and protocols for viral infections were as described (11).

Plasmids. Plasmid pSV-TK/P2/CAT was provided by N. Sonenberg (McGill University, Montreal). The constructions of plasmids T7-5ncpolio-LUC and T7-5ncluc-LUC (12), T7-POLIO (13), T7-3NC-202 (14), and T7-2B-201 (11) have been described. Plasmid T7-OILOP was obtained from K. Kirkegaard (University of Colorado, Boulder). Plasmid T7-TMEV (strain BeAn) was provided by A. Pritchard and H. Lipton (Department of Neurology, University of Colorado Health Science Center, Denver). Plasmid T7-2A-1 was constructed by ligation of three fragments: an *Ava* I/*Bgl* II fragment (base pairs 2978–5601) from pSV2-polio (2A-1) (15), a *Bgl* II/*Eco*RI fragment (base pairs 5601–7498) from pSV2-polio (15), and a 5.3-kilobase *Eco*RI/*Ava* I fragment from T7-POLIO (13). T7-P1/2A was constructed by deleting base pairs 3915–7000

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Abbreviations: NCR, noncoding region; IRES, internal ribosome entry site(s); CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; BiP, immunoglobulin heavy-chain binding protein.

(a *Hin*cII fragment) from T7-POLIO. Subsequently, T7-5ncpolio-2A was made by deleting base pairs 865–3082 (a *Bst*BI fragment) from T7-P1/2A. The vectors pSV-CAT/5ncpolio/LUC and pSV-CAT/5ncbip/LUC have been described (16). pCMV-5ncbip-LUC contains an immediate early promoter/enhancer element from human cytomegalovirus upstream of the 5′ NCR of immunoglobulin heavy-chain binding protein (BiP) and the luciferase (LUC) coding region (a gift from D. G. Macejak, University of Colorado Health Science Center, Denver).

Preparation of Radiolabeled Extracts from Cells Transfected with Plasmids. Five micrograms of supercoiled plasmid DNA was transfected into 10^6 COS cells by using a calcium phosphate precipitation method as described (17). Forty hours after transfection, the cells were mock-infected or infected with poliovirus at a multiplicity of infection of 50. After an incubation for an additional 2.125 hr, the cells were radiolabeled by replacing the medium with methionine-free medium containing [^{35}S]methionine [400 μCi/ml (1 Ci = 37 GBq); New England Nuclear] and incubated for an additional hour. Cells were then washed three times with ice-cold phosphate-buffered saline and lysed into 500 μl of ice-cold lysis buffer (50 mM Tris·HCl, pH 7.9/5 mM EDTA/150 mM NaCl/1% Nonidet P-40). Extracts were subjected to centrifugation (12,000 × *g* for 5 min), and the soluble supernatants were used as the source for labeled proteins.

Cotransfections of Plasmids into Mouse OST7-1 Cells. Dishes containing 10^6 OST7-1 cells were transfected as described above, except that 1 μg of reporter DNA (see *Results*) was used either alone or with 10 μg of effector DNA (see *Results*). The cells were shocked with 10% (vol/vol) glycerol (in Dulbecco's modified Eagle's medium) for 90 sec at 4 hr after transfection and harvested 2 hr later by scraping the cells with a rubber policeman into 1 ml of phosphate-buffered saline without calcium and magnesium. Five hundred microliters of the sample was sedimented and resuspended in 50 μl of 1 M potassium phosphate (pH 7.8) followed by three cycles of freeze/thawing, and the lysate was used for luciferase assays (12); the second half of the sample was sedimented, subsequently lysed in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.5% Nonidet P-40, and used for RNase protection analysis.

RESULTS

Rationale for Seeking a Poliovirus-Induced Factor That Can Act in Trans to Enhance Translation of Viral mRNAs. We showed previously that a cell line harboring a mutated poliovirus cDNA, 3NC202, stably integrated in the host chromosome could effectively complement the growth of poliovirus mutant 2A-1 (14). Mutant 2A-1 bears a lesion in polypeptide 2A that is involved in cleavage of host cell protein p220, leading to inactivation of the cap-binding protein complex eIF–4F (15). Mutant 2A-1-derived 2A protein fails to induce cleavage of p220 in infected cells (15). Thus, the observed complementation of 2A-1 in the 3NC202-containing cell line, under conditions where cellular protein synthesis was not inhibited, suggested that a factor, provided or induced by the integrated viral genome, could rescue the translation of the mutant 2A-1 RNA by a mechanism other than inhibition of cellular translation.

Enhanced Translation of mRNAs Containing the Poliovirus 5′ NCR in Poliovirus-Infected Cells during Ongoing Translation of Cellular mRNAs. To test for translational enhancement of mRNAs containing the 5′ NCR of poliovirus early in poliovirus-infected cells, we transfected plasmid pSV-TK/P2/CAT (9) into monkey COS cells. This plasmid could be used in these cells as a template for the synthesis of capped dicistronic mRNAs containing thymidine kinase (TK) as a first cistron, followed by the 5′ NCR of poliovirus and chloramphenicol acetyltransferase (CAT) as a second cistron. The first cistron, TK, should therefore be translated by a cap-dependent scanning mechanism, whereas the second cistron should be translated by an internal ribosome binding mechanism mediated by the polioviral 5′ NCR (9).

The pSV-TK/P2/CAT construct was expressed as predicted (9), and transfected cells were either mock-infected or infected with poliovirus and labeled with [^{35}S]methionine. Soluble extracts were prepared and analyzed by SDS/PAGE. Fig. 1 shows that the lysate from mock-infected cells (lane 1) displayed the same rate of translation of cellular mRNAs as the lysate obtained from infected cells (lane 2). Thus, this experimental protocol permitted us to test the translational efficiency of mRNAs under conditions in which overall host cell translation was not yet inhibited due to poliovirus infection. Next the lysates were immunoprecipitated with an antibody directed against TK protein. Again, similar amounts of TK protein were present in lysates from mock-infected (lane 3) and poliovirus-infected cells (lane 4), indicating that the cap-dependent translation of the first cistron in the dicistronic reporter mRNA was not affected under these conditions.

In poliovirus-infected cells, however, translation of the second cistron, CAT, mediated by internal ribosome binding to the 5′ NCR of poliovirus, was markedly enhanced in infected extracts compared to mock-infected extracts (lanes 6 and 5, respectively). Analysis by scanning densitometry revealed that 8-fold more CAT protein was labeled in infected extracts than in mock-infected extracts. Since the enhanced translation of the second cistron mediated by the 5′ NCR of

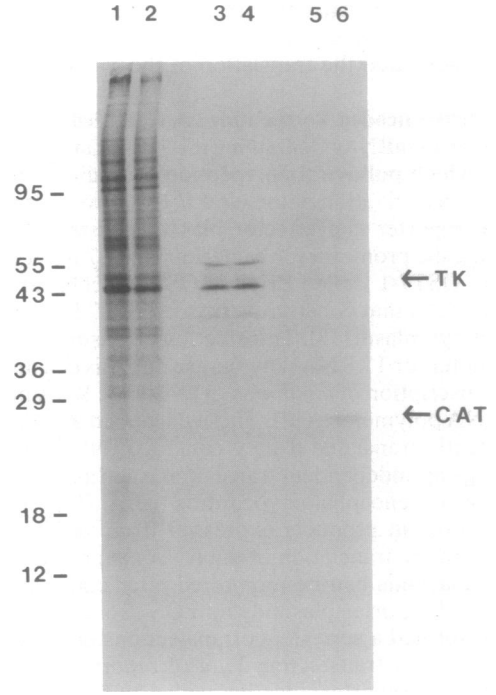

FIG. 1. Translation of dicistronic mRNAs in mock-infected and poliovirus-infected cells. Cells were transfected with plasmid pSV-TK/P2/CAT and subsequently mock-infected (lanes 1, 3, and 5) or infected with poliovirus (lanes 2, 4, and 6). Radiolabeled soluble extracts were prepared as described in *Materials and Methods*. Similar amounts of extract were analyzed by SDS/PAGE either directly (lanes 1 and 2) or after immunoprecipitation with anti-TK (lanes 3 and 4) or anti-CAT (lanes 5 and 6) antibodies. An autoradiograph of the gel is shown. Migration of prestained protein markers (in kDa) is shown at left, and the positions of thymidine kinase (TK) and chloramphenicol acetyltransferase (CAT) are indicated on the right. None of the poliovirus-encoded proteins comigrates with TK or CAT (not shown).

Table 1. Translational transactivation of luciferase mRNAs containing the poliovirus 5′ NCR by coexpression of individual poliovirus gene products in OST7-1 cells

Reporter plasmid	Effector plasmid	Units of light × 10^{-3}	Fold stimulation	Mean fold stimulation
T7-5ncluc-LUC	—	0.04	—	
T7-5ncpolio-LUC	—	3.4	—	
T7-5ncpolio-LUC	T7-POLIO	23.3	6.8	7.5 ± 0.6
T7-5ncpolio-LUC	T7-OILOP	4.6	1.3	1.5 ± 0.1
T7-5ncpolio-LUC	T7-2B-201	36.5	11.0	14.0 ± 3.3
T7-5ncpolio-LUC	T7-3NC-202	35.5	10.0	14.0 ± 3.7
T7-5ncpolio-LUC	T7-TMEV	1.6	0.5	0.5 ± 0.1
T7-5ncpolio-LUC	T7-2A-1	4.6	1.3	1.3 ± 0.1
T7-5ncpolio-LUC	T7-5ncpolio-P1/2A-1	5.7	1.7	1.3 ± 0.3
T7-5ncpolio-LUC	T7-5ncpolio-2A-1	4.9	1.4	1.3 ± 0.3
T7-5ncpolio-LUC	T7-5ncpolio-P1/2A	19.9	6.0	7.0 ± 2.4
T7-5ncpolio-LUC	T7-5ncpolio-2A	56.1	16.5	13.4 ± 3.2*

Cotransfections were performed as described in *Materials and Methods*. The amount of luciferase produced in 2×10^5 cells is shown in units of lights ($\times 10^{-3}$). All light values have been corrected for the amount of reporter mRNA expressed, as determined by RNase protection assays as shown in Fig. 3. Experiments were performed three times, and the mean fold stimulation is indicated.
*Experiments were performed twice.

poliovirus in infected cells was not simply due to an inhibition of cap-dependent translation (Fig. 1, lanes 1–4), this result suggests that poliovirus-infected cells provided a function that transactivated the translation of the 5′ NCR of poliovirus.

Poliovirus-Encoded Polypeptide 2A Can Mediate Enhanced Translation of mRNAs Containing the 5′ NCR of Poliovirus. To identify which polioviral polypeptide(s) mediated the translational transactivation of the viral 5′ NCR, we cotransfected different reporter and effector plasmids (listed in Table 1) containing the promoter for bacteriophage T7 RNA polymerase into OST7-1 cells. Briefly, OST7-1 cells are mouse fibroblast cells that constitutively express T7 RNA polymerase in the cytoplasm (18). Transfection of plasmids containing the promoter for T7 RNA polymerase into this cell line results in the transcription of *uncapped* cytoplasmic RNA molecules by T7 RNA polymerase (18). These uncapped RNAs can only be efficiently translated if they contain 5′ NCRs capable of mediating cap-independent translation, such as the 5′ NCR of poliovirus or encephalomyocarditis virus (18). Thus, the effect of protein products expressed from various effector plasmids on the translation of the RNAs expressed from the reporter plasmids can be monitored after cotransfection of effector and reporter plasmids into OST7-1 cells.

We performed a series of cotransfections of plasmids into OST7-1 cells by transfecting 1 μg of reporter plasmid (T7-5ncpolio-LUC reporter) together with a 10-fold molar excess of various effector plasmids (Table 1). In each experiment, the steady-state amount of reporter mRNA in the cells was monitored by an RNase protection assay. This served to control for DNA transfection efficiencies, transcription efficiencies by T7 RNA polymerase, and stability of the resulting uncapped RNAs. Fig. 2 shows that the steady-state amounts of reporter mRNAs in individual transfection experiments varied only 3- to 4-fold between experiments. Thus, after quantitation of the steady-state levels of reporter mRNA by computing densitometry, the amount of luciferase produced could be used to measure the translation efficiency of the reporter mRNA.

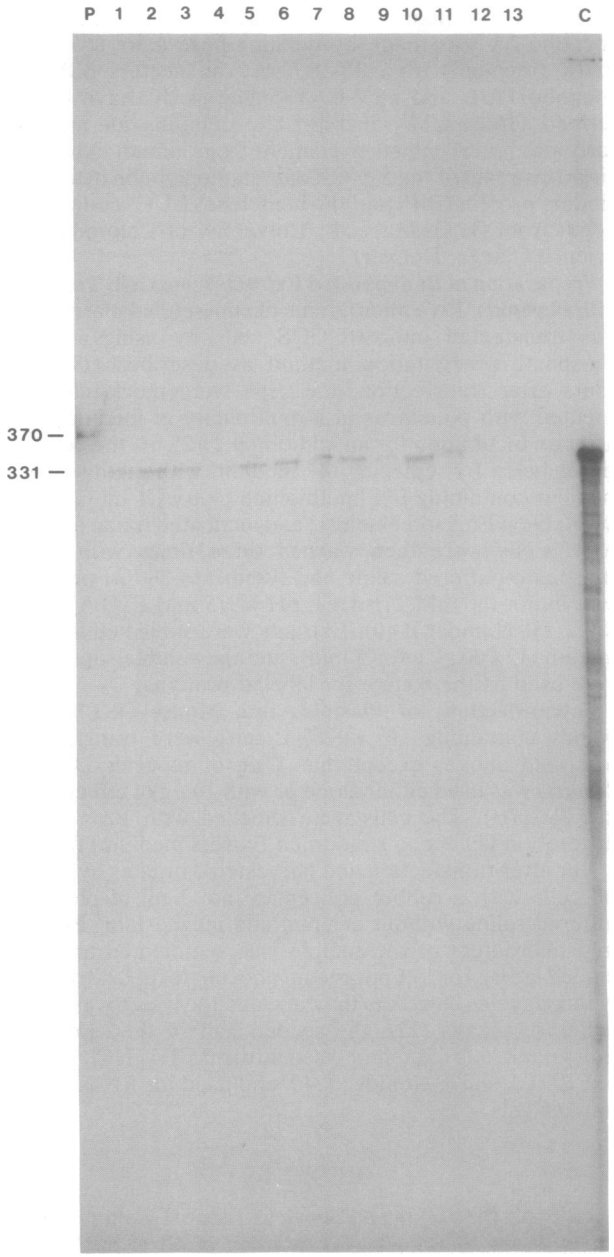

FIG. 2. Quantitation of luciferase reporter mRNA in transfected OST7-1 cells. The relative steady-state amounts of luciferase mRNAs in transfected cells were determined by an RNase protection assay (17) using a radiolabeled 370-nucleotide RNA, complementary to the 3′ end of luciferase mRNA, as a probe. Protected RNA was analyzed in a urea-containing polyacrylamide gel. An autoradiograph of the gel is shown. Lane P, undigested radiolabeled probe; lanes 1–13, probe after incubation with extracts from OST7-1 cells transfected with various plasmids followed by digestion with ribonucleases. Reporter plasmids were T7-5ncpolio-LUC (lanes 1 and 3–12) and T7-5ncluc-LUC (lane 2). Effector plasmids were T7-POLIO (lane 3), T7-OILOP (lane 4), T7-2A-1 (lane 5), T7-3NC-202 (lane 6), T7-2B-201 (lane 7), T7-P1/2A (lane 8), T7-P1/2A-1 (lane 9), T7-5ncpolio-2A (lane 10), and T7-5ncpolio-2A-1 (lane 11). Lane 12, treatment of cell extract, transfected with T7-5ncpolio-LUC, with ribonucleases prior to protection analysis; lane 13, nontransfected OST7-1 extract; lane C, ribonuclease protection of *in vitro*-synthesized luciferase mRNA. The amount of luciferase mRNA present in cells transfected with T7-TMEV was determined from an autoradiograph obtained from another polyacrylamide gel (not shown). Size markers (in nucleotides) are shown at left.

Table 1 shows that OST7-1 cells transfected with plasmids that direct the synthesis of uncapped luciferase mRNAs

containing only the 5′ NCR of luciferase (T7-5ncluc-LUC), as expected, did not direct the synthesis of luciferase. In contrast, cells expressing uncapped luciferase mRNAs containing the 5′ NCR of poliovirus (T7-5ncpolio-LUC) yielded levels of active luciferase 80- to 90-fold higher. Cotransfection of T7-5ncpolio-LUC with T7-POLIO (containing full-length poliovirus cDNA), T7-2B201 (containing full-length viral cDNA with a mutation in sequences encoding 2B), or T7-3NC202 (containing full-length viral cDNA with a mutation in the 3′ NCR), respectively, further stimulated the translation of the polio–luciferase reporter mRNAs by 7- to 11-fold. Coexpression of full-length viral negative strands, using the effector plasmid T7-OILOP, or full-length positive strands of Theiler's murine encephalomyocarditis virus (from plasmid T7-TMEV) did not enhance translation of the reporter mRNAs. This indicates that the observed translational transactivation (Fig. 1) was mediated by a poliovirus-encoded gene product.

Interestingly, mRNAs expressed from plasmid T7-2A-1, which carries a full-length viral cDNA bearing a mutation in the gene encoding protein 2A, were unable to direct the synthesis of the transactivator, suggesting that protein 2A was involved in translation transactivation. To test this more rigorously, we constructed plasmids that could direct the synthesis of wild-type and mutant forms of 2A. Mutant forms of 2A (2A-1), expressed either as its precursor with P1 sequences (from plasmid T7-5ncpolio-P1/2A-1) or in isolation (from plasmid T7-5ncpolio-2A-1), did not enhance translation of the reporter mRNA molecules. In contrast, the wild-type forms of 2A, expressed from plasmids T7-5ncpolio-P1/2A and T7-5npolio-2A, respectively, stimulated translation of the reporter mRNAs 6- and 16.5-fold, respectively.

Of course, because only a subpopulation of OST7-1 cells received transfected DNA encoding 2A, any inhibition of translation in those cells was not measured directly. However, as can be seen in Fig. 1, cells infected with poliovirus (multiplicity of infection of 50) did not show significant inhibition of host cell translation between 2.125 and 3.125 hr after infection. Instead, the data in Table 1 together with the data presented in Figs. 1 and 3 (see below) suggest that protein 2A mediated directly or indirectly the translational transactivation of mRNAs containing the 5′ NCR of poliovirus and that this function is independent of 2A's known role in the inhibition of cellular protein synthesis (6, 7).

Protein 2A Cannot Transactivate the Translation of mRNAs Containing the IRES from the BiP mRNA. We tested whether 2A expression could enhance translation of an mRNA containing an IRES from a cellular mRNA, the mRNA encoding the BiP (16). Monkey COS cells were transfected with plasmids pSV-CAT/5ncpolio/LUC or pSV-CAT/5ncbip/LUC, containing the IRES elements of poliovirus and BiP between the first (CAT) and the second (LUC) cistrons, respectively. Transfected cells were mock-infected or infected with poliovirus and radiolabeled with [^{35}S]methionine. Soluble extracts were prepared, luciferase polypeptides were immunoprecipitated, and the collected immunoprecipitates were analyzed by SDS/PAGE. Fig. 3 shows that in all lysates, obtained from both mock-infected and infected cells, the rates of translation were indistinguishable (lanes 1, 2, 5, 6, 9, and 10). As shown in Fig. 1, however, dicistronic mRNAs containing the poliovirus IRES were translated at an enhanced rate, producing ≈3 times more luciferase protein from the second cistron in infected cells (Fig. 3, lane 4) as in mock-infected cells (Fig. 3, lane 3). In contrast, the dicistronic mRNAs containing the BiP IRES between the CAT and luciferase cistrons did not mediate enhanced translation of the second (luciferase) cistron under the same conditions (Fig. 3, lanes 7 and 8). To ensure that a possible determinant in the BiP IRES crucial for transactivation was not masked in the dicistronic mRNA, we constructed a plasmid (pCMV-5ncbip-LUC) that could direct the synthesis of monocistronic mRNAs containing the 5′ NCR of BiP linked to luciferase. As was the case for the dicistronic 5′ CAT-5ncbip-LUC mRNAs, the monocistronic 5ncbip-LUC mRNAs did not display enhanced translation of luciferase in infected cells (Fig. 3, lanes 9–12).

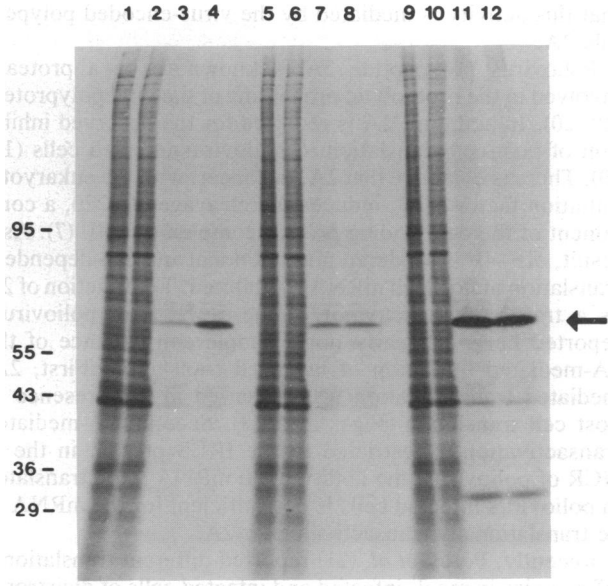

FIG. 3. Translation of dicistronic mRNAs containing either viral or cellular IRES. Cells were transfected with plasmid pSV-CAT/5ncpolio/LUC (lanes 1–4), pSV-CAT/5ncbip/LUC (lanes 5–8), and pCMV-5ncbip-LUC (lanes 9–12), respectively, and subsequently either mock-infected (odd lanes) or infected with poliovirus (even lanes). Labeled extracts (see legend to Fig. 1) were analyzed directly (lanes 1 and 2, 5 and 6, and 9 and 10) by SDS/PAGE or after immunoprecipitation with an antibody directed against luciferase (lanes 3 and 4, 7 and 8, and 11 and 12). An autoradiograph of the gel is shown. Migration of prestained protein markers (in kDa) is shown at left. The arrow denotes the position of luciferase protein.

These results argue that the translational transactivation of the poliovirus 5′ NCR mediated by 2A is specific for the polioviral IRES element. This idea is substantiated by the observation that an IRES element from the hepatitis B virus genome, composed of sequences present between the core and polymerase genes in the pregenomic RNA, was also not transactivated by poliovirus (data not shown). These experiments offer the strongest evidence that the increased rate of translation of poliovirus IRES-containing RNAs in poliovirus-infected cells results in part from positive activation and not simply from the inhibition of host cell translation. If the latter were the case, one would expect any mRNA capable of internal ribosome binding to display enhanced translation in poliovirus-infected cells.

DISCUSSION

Experiments presented here demonstrate the translational transactivation of the poliovirus 5′ NCR during poliovirus infection. Expression of dicistronic mRNAs containing the poliovirus IRES between two cistrons, followed by infection with poliovirus, resulted in the enhanced translation of the second cistron even at a time when cap-dependent translation of neither the first cistron nor host cell mRNAs was inhibited. This effect was also seen in monocistronic transcripts containing the poliovirus IRES expressed in uncapped form from T7 promoters by bacteriophage T7 RNA polymerase in OST7-1 cells. Expression of poliovirus mutant RNAs encoding various parts of the viral genome has provided evidence

that this activity is mediated by the virus-encoded polypeptide 2A.

Poliovirus polypeptide 2A is known to be a protease involved in the proteolytic processing of the viral polyprotein (19, 20). In addition, 2A is required for the observed inhibition of host-cell translation in poliovirus-infected cells (15, 20). There is evidence that 2A, in concert with the eukaryotic initiation factor eIF3, induces the cleavage of p220, a component of the cap binding protein complex eIF-4F (7). As a result, eIF-4F is rendered nonfunctional and cap-dependent translation of host cell mRNA is inhibited. The function of 2A as a translational activator of the 5′ NCR of poliovirus, reported here, is clearly not a simple consequence of the 2A-mediated inhibition of host cell translation. First, 2A-mediated transactivation was observed in the presence of host cell translation (Figs. 1 and 3). Second, 2A-mediated transactivation is restricted to the IRES present in the 5′ NCR of poliovirus; the ability of an mRNA to be translated in poliovirus-infected cells is not sufficient for the mRNA to be translationally transactivated by 2A.

Recently, Percy *et al.* (21) reported different translational efficiencies in mock-infected and infected cells of dicistronic mRNAs containing various sequence elements of the 5′ NCR of poliovirus between two cistrons. The authors concluded that these effects on translation were due to poliovirus-induced cleavage of p220 and the subsequent inhibition of cap-dependent translation as opposed to a transactivation mechanism. In contrast to the studies presented here, Percy *et al.* (21) used a recombinant vaccinia virus to deliver T7 RNA polymerase to cells transfected with plasmids harboring the T7 promoter. However, it is known that recombinant as well as wild-type vaccinia virus can induce the nonspecific replication of transfected plasmids (18) and, more importantly, the cap-dependent and cap-independent translation of mRNAs (18). Any of such effects may have resulted in the failure to observe the transactivation reported here.

The finding that the BiP IRES, derived from the mRNA encoding BiP (16), could not be transactivated by 2A was surprising, because the translation of BiP mRNA has been shown to be enhanced in poliovirus-infected cells (22). However, the latter observations were only made after partial inhibition of host cell translation. Therefore, it is likely that the previously observed enhanced translation of BiP mRNA in poliovirus-infected cells was due to the diminished competition with other cellular mRNAs for the translational apparatus. That the translational transactivation function of poliovirus polypeptide 2A seems to be specific for polioviral 5′ NCRs may suggest that there are additional cellular factors stimulating translation of IRES-containing cellular mRNAs such as BiP. In this respect, it is noteworthy that the IRES elements of picornaviruses fail to show any striking resemblance to the BiP IRES (23, 24).

What is the mechanism of translational transactivation by 2A? 2A could bind directly to RNA sequences in the viral 5′ NCR, perhaps enhancing the binding of ribosomal subunits to the IRES. Alternatively, 2A could modify a cellular function, which could then function as a translational transactivator. In this respect, it is interesting that the p220 cleavage products were found to stimulate, although moderately (1.4-fold), the translation of poliovirus mRNA *in vitro* (25), which raises the possibility of a dual function for the cleavage products of p220. The first function would be to render eIF-4F nonfunctional for the translation of capped cellular mRNA, and the second function would be to activate translation of polioviral mRNA. Thus, low amounts of cleaved p220 could transactivate viral mRNA translation even when sufficient levels of intact eIF-4F were still present to support cellular translation. In this regard, we have observed that translational transactivation in poliovirus-infected cells coincides with the commencement of p220 cleavage (data not shown).

Why does poliovirus need a translational transactivator? It is known that the viral 5′ NCR is capable of initiating translation in the absence of any viral polypeptides (26), albeit much more poorly *in vitro* than *in vivo* (12). However, it has also been shown that the translational efficiency of the viral 5′ NCR differs in different cell types; in particular, viral mRNAs from distinct viral isolates were translated less well in cultured neuronal cells than in cell lines such as HeLa (27). Thus, it may be that a translational transactivator is required not only early in infection before host-cell translation is inhibited but also to extend the range of cell types that can be productively infected.

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