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Internal Translation Initiation on Poliovirus RNA: Further Characterization of La Function in Poliovirus Translation In Vitro

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Initiation of poliovirus RNA translation by internal entry of ribosomes is believed to require the participation of *trans*-acting factors. The mechanism of action of these factors is poorly defined. The limiting amount of one of these factors, La protein, in rabbit reticulocyte lysates (RRL) has been postulated to partially explain the inefficient translation of poliovirus RNA in this system. To further characterize La activity in translation and to identify other potential limiting factors, we assayed the ability of La protein as well as purified initiation factors, eIF-2, guanine nucleotide exchange factor (GEF), eIF-4A, eIF-4B, eIF-4F, and eIF-3, to stimulate the synthesis of P1, the capsid precursor protein, in poliovirus type 1 (Mahoney) RNA-programmed RRL. Of the proteins tested, only La, GEF, and to some extent eIF-2 stimulated the synthesis of P1. The enhanced translation of P1 in response to La occurred concomitantly with the inhibition of synthesis of most aberrant polypeptides, resulting from initiation in the middle of the genome. Deletion of the carboxy-terminal half (214 amino acids) of La did not decrease its binding to the poliovirus 5′ untranslated region but abrogated the stimulatory and correcting activity in translation. In contrast to La, GEF and eIF-2 stimulated the overall translation and increased the synthesis of aberrant products as well as P1. Neither La, GEF, nor any other factor stimulated translation of encephalomyocarditis virus RNA in RRL. The implications of these findings for the mechanism of internal translation initiation on picornavirus RNAs are discussed.

The genome of poliovirus, a member of the *Picornaviridae* family, is a single-stranded RNA molecule which is translated from a single initiator AUG to yield a 247-kDa precursor protein. The precursor protein is cleaved in a stepwise manner to generate the mature viral proteins (11). Translation of the poliovirus genome and subsequent steps of the viral replication cycle, including the final assembly of infectious virions from de novo-synthesized viral RNA and proteins, were recently reproduced in a cell-free system from HeLa cells (38). Despite these advances in modeling picornavirus replication in vitro, some important molecular mechanisms involved in this process are still obscure. Of particular interest is the mode by which picornaviruses initiate translation by internal ribosome binding (26, 59). This mechanism violates the ribosome scanning model according to which ribosomes can access the initiator AUG only by first binding to the 5′ end and then linearly scanning the mRNA until the appropriate initiator AUG is encountered (30). Picornavirus RNAs possess unique structural properties which allow them to employ cap- and 5′ end-independent internal ribosome binding in translation initiation. In contrast to cellular mRNAs (except organellar mRNAs), picornavirus RNAs are not capped. Unlike the majority of cellular mRNAs, they possess a long (600 to 1,200 nucleotides) 5′ untranslated region (UTR) with multiple cryptic AUGs (1). An internal portion of the 5′ UTR can direct internal ribosome binding, as demonstrated by in vitro and in vivo translation experiments in which this segment was placed as an intercistronic spacer in bi- and tricistronic mRNAs (3, 27, 28, 47).

Several studies defined the essential structural elements of the 5′ UTR required for internal ribosome binding (ribosome landing pad [RLP] or internal ribosome entry site). The RLP of poliovirus spans a sequence of more than 400 nucleotides, suggesting the involvement of secondary and tertiary structures in ribosome binding (21, 40, 48). Consistent with this are the findings that even minor destabilizations of a specific secondary structure element (52, 58) within the RLP by point mutations in the Sabin vaccine poliovirus genomes markedly diminished their translation compared with RNAs of their neurovirulent counterparts (39, 62, 63). In addition, the importance of two elements, the oligopyrimidine tract UUUCC and an appropriately spaced downstream AUG for internal ribosome binding, was documented in several picornavirus RNAs (33, 40, 50, 53).

The mechanism of internal translation initiation on poliovirus RNA is likely to require *trans*-acting initiation factors (59) in addition to the canonical initiation factors that are required for translation of all mRNAs (eIF-2, eIF-4A, eIF-4B, etc.). Several recently identified proteins are believed to be required for poliovirus translation (34, 50). One of them, p52, originally detected by its ability to bind to the nucleotide 559–624 segment of poliovirus type 2 Lansing RNA (RNA 559–624) (34), was recently purified to homogeneity and identified as the La autoantigen (35). This protein elicits an autoimmune response in patients with systemic lupus erythematosus and Sjögren's syndrome (65). A role of La protein in translation initiation on poliovirus RNA was demonstrated in a rabbit

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reticulocyte lysate (RRL), in which this protein is apparently limiting (35).

RRL programmed with poliovirus RNA synthesizes a set of aberrant (not found in virus-infected cells) polypeptides initiated in the middle of the viral genome rather than at the beginning of polyprotein reading frame. This translation deficiency can be relieved to yield authentic proteins by addition of ribosomal salt wash fractions from nucleated (HeLa or Krebs-2) cells (7, 14, 51, 63, 64). Similar to crude ribosomal salt wash from Krebs-2 cells, La protein stimulates the synthesis of the capsid precursor protein, P1, from the authentic initiation site of translation. In addition, La protein inhibits the accumulation of aberrant products, thus correcting poliovirus translation. It was suggested, therefore, that La represents a novel factor required for translation of poliovirus RNA (35). Earlier results demonstrated stimulation of poliovirus RNA translation in RRL by a high-molecular-weight complex of polypeptides, presumably eIF-2/guanine nucleotide exchange factor (GEF), that was termed initiation correction factor ICF (64). It should be noted, however, that the crude fractions contained correcting activity that was lost in the most purified fractions (64). Recent reports suggest that other RNA-binding proteins, such as p57 and p97, might play a role in initiation of picornavirus translation (4–6, 22, 29, 31, 50, 66).

In this study, we characterized the requirements for different initiation factors for optimal translation of poliovirus RNA in RRL. We compared the effects of La protein and highly purified initiation factors, eIF-2, GEF, eIF-4A, eIF-4B, eIF-4F, and eIF-3, on the pattern of poliovirus-specific polypeptides synthesized in RRL. We found that, in addition to La, GEF and to some extent eIF-2 can promote poliovirus RNA translation. However, in contrast to La, GEF and eIF-2 stimulated the overall synthesis of both correct and aberrant products.

MATERIALS AND METHODS

Preparation of viral RNAs. Poliovirus type 1, M-1-2p (a clone of the Mahoney strain), was grown and purified as described previously (63). RNA was isolated from purified virions and fractionated by centrifugation through a sucrose gradient (63). The RNA was precipitated with ethanol. Encephalomyocarditis (EMC) virus RNA was isolated from purified virions as described previously (61). Viral RNAs were analyzed by electrophoresis in 0.8% formaldehyde–agarose gel (62) followed by staining with ethidium bromide. Agarose gel analyses showed that the RNAs were intact.

Preparation of RNAs for UV cross-linking. [^{32}P]GTP-labeled RNAs corresponding to two segments of 5′ UTR of poliovirus type 2 (Lansing) were synthesized. (i) A segment corresponding to nucleotides 121 to 450 was synthesized from pP2CAT (46) by PCR. The sense primer consisted of a bacteriophage T7 promoter sequence (TAATACGACTCAC TATAG) followed by deoxyoligonucleotides corresponding to poliovirus sequences from 121 to 135. Two additional G residues were included in front of the poliovirus sequence to increase the efficiency of transcription by T7 polymerase. The antisense primer corresponded to sequences complementary to nucleotides 424 to 450. At the end of the reaction, primers were removed by centrifugation through a Centricon 100 according to the manufacturer's protocol. The DNA template was used to synthesize labeled RNA transcripts with T7 RNA polymerase (Promega) under standard reaction conditions. (ii) An *Acc*II-*Bal*I fragment derived from pP2CAT (46), which contains a portion of the poliovirus 5′ UTR from nucleotides 522 to 631, was blunt end ligated in the *Hin*cII site of the vector pGem 1. The resulting plasmid was called pGem 109. pGem 109 was linearized by digestion with *Bam*HI (in the polylinker downstream of the insert) and transcribed in vitro with SP6 RNA polymerase, using conditions supplied by the manufacturer.

UV cross-linking of RNAs. UV cross-linking reactions were performed essentially as described previously (34). RNA probes (2×10^5 cpm/pmol) labeled with [^{32}P]UTP during transcription were incubated for 10 min at 30°C with either 1 μg of La or 0.5 μg of La(1-194) in a total volume of 15 μl in the presence of 100-molar-excess calf liver tRNA. Samples were irradiated on ice for 30 min with a 254-nm germicidal lamp. Unbound RNA was digested with 20 μg of RNase A at 37°C for 30 min. Samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by autoradiography.

Purification of La and the La(1-194) deletion mutant. Recombinant La protein was purified according to the following protocol. An overnight culture of *Escherichia coli* BL21(DE3)pLysS was inoculated with pET-La and grown in the presence of ampicillin and chloramphenicol at 37°C to an optical density at 600 nm of between 0.8 and 1.0. Isopropylthiogalactopyranoside (IPTG) was added to a final concentration of 0.4 mM, and the cells were harvested 3 h after induction. Pelleted cells were suspended in buffer (25 mM Tris-HCl [pH 8.0], 3 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiothreitol, 100 mM NaCl) containing 125 μg of phenylmethylsulfonyl fluoride per ml and sonicated two times for 30 s each time. Cell debris was removed by centrifugation (12,000 × *g*, 30 min), and the supernatant was applied directly to a DEAE column. The flowthrough from the DEAE column containing most of La was applied directly to a heparin-agarose column. The bound protein was eluted by a linear gradient of 0.1 to 1.0 M NaCl. La begins to elute at about 300 mM NaCl. Fractions were analyzed by gel electrophoresis, and the fractions containing most of La were pooled and dialyzed against 25 mM morpholineethanesulfonic acid (MES; pH 6.5)–3 mM $MgCl_2$–0.1 mM EDTA–0.5 mM dithiothreitol–100 mM NaCl. The protein was loaded onto a 20-ml S-Sepharose column. After the column was washed with the buffer described above, the bound La protein was eluted with a linear gradient of 0.1 to 1.0 M NaCl. Fractions were analyzed by gel electrophoresis. Peak fractions were pooled, dialyzed against 25 mM Tris-HCl (pH 8.0)–3 mM $MgCl_2$–0.1 mM EDTA–0.5 mM dithiothreitol–100 mM NaCl and loaded onto 5-ml poly(U)-Sepharose column. Bound La was eluted with a linear gradient of 0.1 to 1.0 M NaCl. Fractions were analyzed on a gel, and those containing La were pooled and dialyzed against buffer A (20 mM Tris-HCl [pH 7.5], 10% [vol/vol] glycerol, 0.1 mM EDTA, 1 mM dithiothreitol, 100 mM KCl). Purified La protein was stored at −70°C at concentration of 1 mg/ml. La(1-194), with a molecular mass of 22 kDa, was expressed in *E. coli* and purified by the protocol used for La except that chromatography on an S-Sepharose column was omitted and a stepwise (between 1.0 and 1.5 M NaCl) rather than gradient elution was used in the chromatography on poly(U)-Sepharose.

Purification of initiation factors. Rabbit reticulocyte eIF-2 and GEF were purified to apparent homogeneity from the 0.5 M KCl wash of ribosomes and postribosomal supernatant, respectively, as previously described (12). The activity of eIF-2 was assayed by ternary complex (eIF-2 · GTP · [^{35}S]Met-$tRNA_f$) formation, and that of GEF was assayed by monitoring the release of [^{3}H]GDP from an isolated eIF-2 · [^{3}H]GDP binary complex. Recombinant murine eIF-4A and recombinant human eIF-4B proteins were purified to apparent homogeneity as described previously (44). RNA helicase assay of

eIF-4A and eIF-4B was performed by using a ^{32}P-labeled RNA substrate consisting of a 10-bp duplex region and two 3′-terminal single-stranded tails (44). The reaction mixtures were analyzed on an SDS–15% polyacrylamide gel, and RNAs were visualized by autoradiography. eIF-4A and eIF-4B were active in RNA unwinding. Highly purified reticulocyte eIF-4F and eIF-3 were the generous gift of W. C. Merrick. Procedures for purification, characterization, and testing of eIF-4F and eIF-3 in a reconstituted globin synthesis assay are described in references 19 and 36, respectively. Initiation factor preparations were stored at −70°C at a concentration of 1 mg/ml in buffer A. A 0.5 M KCl ribosomal wash fraction was obtained from Krebs-2 cells and fractionated by precipitation at between 40 and 70% saturation of ammonium sulfate (63). The fraction had a protein concentration of 15 mg/ml.

Cell-free protein synthesis. Micrococcal nuclease-treated RRL was purchased from Promega Corp. Conditions for protein synthesis in vitro were similar to those recommended by the manufacturer. Each reaction mixture (12.5 μl) contained 8.75 μl of lysate and the following components at the indicated final concentrations: 80 mM potassium acetate, 30 mM KCl, 0.5 mM magnesium acetate (unless indicated otherwise), 10 mM creatine phosphate, 20 μM each unlabeled amino acids minus methionine, 5 μCi of [^{35}S]methionine (1,200 Ci/mmol), and 50 μg of calf liver tRNA per ml. Poliovirus RNA was added to a final concentration of 24 μg/ml (unless indicated otherwise). Reaction mixtures were supplemented with 1 or 2 μg of initiation factor preparations in buffer A and incubated at 30°C for 60 or 180 min as indicated. Products of in vitro translation were analyzed on SDS–15% polyacrylamide gels (100:1 acrylamide-bisacrylamide). After electrophoresis, the gels were treated with En^{3}Hance, dried, and fluorographed. The intensity of polypeptide bands was determined with a BAS-III PhosphoImager (Fuji Corp.).

RESULTS

Stimulation and correction of poliovirus RNA translation in RRL by La. The function of La protein as an enhancing factor for poliovirus translation was demonstrated earlier (35). Here, we further characterized this activity. Figure 1 shows the effect of La on translation at different RNA concentrations. At mRNA concentrations of 0.3 μg (24 mg/ml) and higher, the pattern of products synthesized in RRL is represented largely by a set of aberrant polypeptides which are known to result from anomalous initiation in the middle of the genome (14). Little of the capsid precursor protein, P1, is formed, indicating the low efficiency of utilization of the correct initiation site under these conditions. The addition of 1 μg of purified La alters the pattern of products synthesized. P1 appears as a strong band and typically is the most abundant product accumulating after 60 min of translation (lanes 1 to 12). Long (3-h) incubations of La-supplemented reactions yielded increased amounts of the processed capsid proteins, VP0, VP1, and VP3 (compare lanes 13 and 14), as is also seen in the presence of Krebs-2 cell ribosomal wash fraction (lane 15). La also inhibits synthesis of most aberrant products (those affected most strongly are Y and Z). At low concentrations of RNA (0.1 μg), the pattern of poliovirus polypeptides synthesized is authentic, as the synthesis of aberrant products was not evident (see also reference 25) and the response to exogenous La was not seen (lanes 1 and 2). At 0.2 μg of poliovirus RNA, the stimulation of P1 synthesis by La was only marginal (up to 2-fold), compared with 8- to 10-fold stimulation seen at RNA concentrations of 0.6 to 0.8 μg.

The stimulatory effect of La protein on P1 expression was

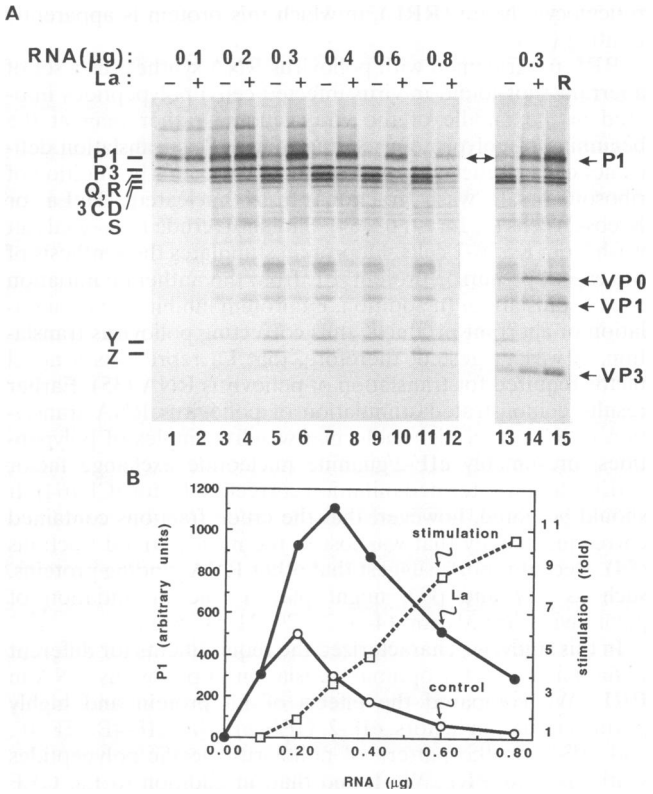

FIG. 1. Effects of La at different RNA concentrations on poliovirus RNA translation in RRL. (A) Indicated amounts of poliovirus RNA were translated for 60 min (lanes 1 to 12) or 180 min (lanes 13 to 15) in the absence or presence of 1 μg of La protein or 15 μg of Krebs-2 cell ribosomal wash fraction (R; lane 15). Conditions of translation and SDS-polyacrylamide gel electrophoresis of the products are described in Materials and Methods. The positions of P1, mature capsid proteins, and aberrant in vitro products named as specified by Dorner et al. (14) are indicated. (B) Quantification of the P1 band in lanes 1 to 12 of the gel in panel A. Quantification was done as described in Materials and Methods. Values are given as arbitrary units. The dashed line represent stimulation of P1 synthesis by La.

observed under a wide range of ionic conditions (data not shown). A suboptimal concentration (1.5 mM) of Mg^{2+} partially suppressed abnormal initiation and resulted in less complex pattern of polypeptides synthesized (control lanes in Fig. 2A and B). Yet under these conditions, La stimulated accumulation of P1 in a dose-dependent manner (Fig. 2B).

Effect of carboxy-terminal truncated La on poliovirus translation. The ability of La to bind to an appropriate region of poliovirus 5′ UTR may be attributed to the function of the La RNA recognition motif (RRM), which spans 76 amino acids and resides in the amino-terminal half of the molecule (8). However, it is likely that the effector domain in La is separate from the RNA binding domain. To examine this possibility, we used a La deletion mutant, La(1-194), which lacks the carboxy-terminal half of the molecule and retains the RRM (Fig. 3A). The results of UV cross-linking experiments show that La and La(1-194) possess similar RNA-binding specificities. Both La and La(1-194) cross-linked to the nucleotide 522–631 segment of the poliovirus 5′ UTR much more efficiently (10- and 7-fold, respectively) than to the nucleotide 121–450 segment (Fig. 3B).

Notwithstanding its affinity for the 5′ UTR, La(1-194) did not stimulate the synthesis of P1 in a poliovirus RNA-pro-

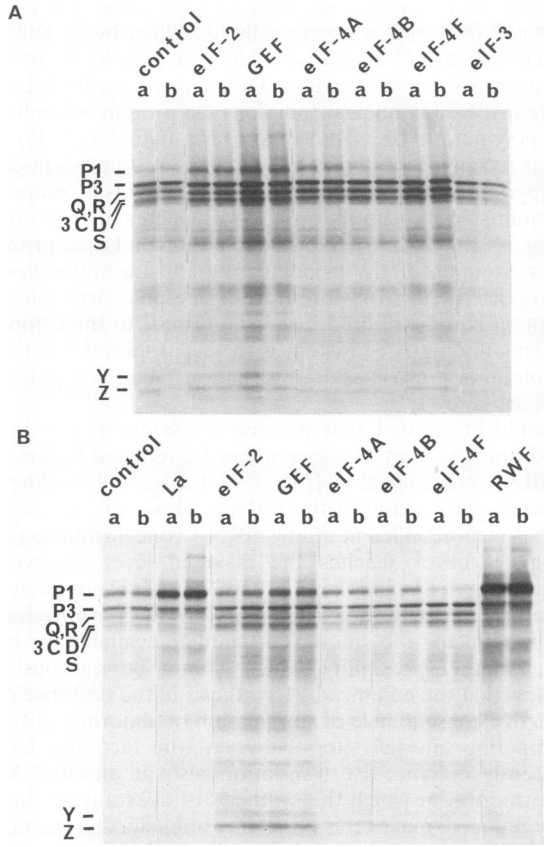

FIG. 2. Effects of initiation factors on the efficiency and polypeptide pattern of poliovirus RNA translation in RRL. Translations were carried out at the standard concentration (0.5 mM) of Mg^{2+} (A) or at a suboptimal concentration (1.5 mM) (B). The samples were supplemented with 1 μl (lane a) or 2 μl (lane b) of buffer A (control), 1 μg (lane a) or 2 μg (lane b) of La protein or the indicated preparations of initiation factors, or 15 or 30 μg of Krebs-2 cell ribosomal wash fraction (RWF). Other conditions were as described in Materials and Methods.

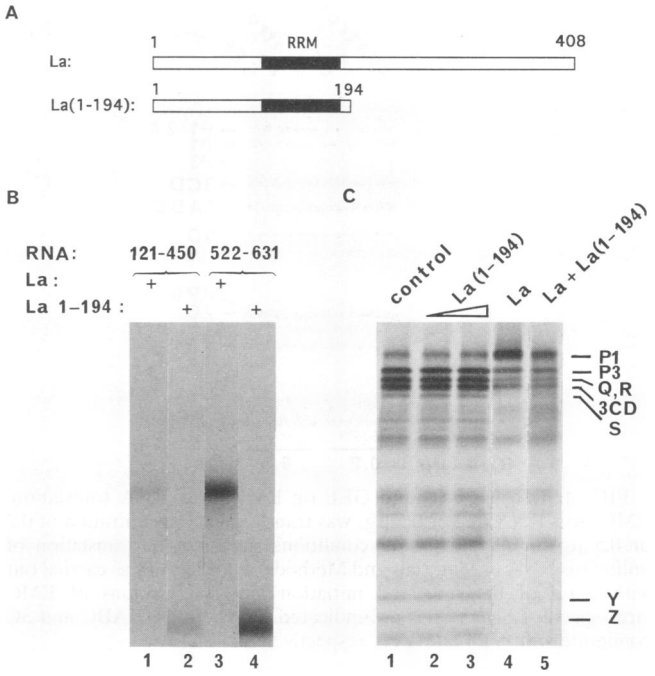

FIG. 3. Effects of the La(1-194) deletion mutant on RNA binding and poliovirus translation. (A) Schematic representations of La (8) and La(1-194). The position of the RRM is indicated. (B) Cross-linking of La and La(1-194) to fragments of the poliovirus 5′ UTR. UV cross-linking of La (lanes 1 and 3) or La(1-194) (lanes 2 and 4) to [^{32}P]UTP-labeled transcripts corresponding to nucleotides 121 to 450 (lanes 1 and 2) or nucleotides 522–631 (3 and 4) of poliovirus was as described in Materials and Methods. Proteins were resolved by electrophoresis on an SDS–15% polyacrylamide gel. (C) Translation of poliovirus RNA (0.3 μg) in RRL without (lane 1) or with 1 μg (lane 2) or 2 μg (lane 3) of La(1-194), 1 μg of La (lane 4), or 1 μg of La and 2 μg of La(1-194) (lane 5). The positions of P1, mature capsid proteins, and aberrant in vitro products are indicated.

grammed RRL, nor did it correct aberrant translation (Fig. 3C, lanes 1 to 3). Moreover, La(1-194) suppressed the stimulation of P1 synthesis in La-supplemented reactions (threefold; compare lanes 4 and 5 [note, however, that the correction activity of La was not suppressed]). These data suggest that interaction of the La RRM with the poliovirus 5′ UTR is necessary but not sufficient for its stimulatory and correcting activity on poliovirus translation and emphasize the importance of the structural elements in the La carboxy-terminal half for these functions. In addition, these results could suggest that the La(1-194) RRM competes with La for binding and thus acts as a dominant negative suppressor.

Effects of canonical initiation factors on poliovirus translation. Translation of poliovirus RNA in RRL might also be restricted by the availability of canonical initiation factors. Indeed, the synthesis of authentic poliovirus products is stimulated to a greater extent by the crude ribosomal wash fraction than by optimal amounts of La protein (Fig. 1A, lanes 13 to 15; Fig. 2B). Candidates for such factors are eIF-2 (10), GEF (64), eIF-4A (9, 55), eIF-4B, and eIF-4F (2, 57). Consequently, we examined the effects of these factors on the efficiency and pattern of poliovirus RNA translation in RRL (Fig. 2). The factors were assayed at standard (0.5 mM) Mg^{2+} (Fig. 2A) and at elevated (1.5 mM) Mg^{2+} (Fig. 2B), a condition in which aberrant translation is considerably suppressed. The following conclusions can be drawn. (i) None of the canonical initiation factors stimulated the correct translation of poliovirus RNA (accumulation of P1) to the level displayed by La or crude ribosomal salt wash (Fig. 2B). (ii) GEF, and to a lesser extent eIF-2, increased the overall efficiency of poliovirus RNA translation (2.5- to 3.0- and 1.5- to 2.0-fold, respectively, as assayed by incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble material), enhancing accumulation of both P1 and aberrant products. The effects of GEF and eIF-2 were more pronounced at the standard than at the elevated Mg^{2+} concentration. (iii) eIF-4F also stimulated poliovirus RNA translation (about 1.5-fold, as determined by the [^{35}S]methionine incorporation into trichloroacetic acid-insoluble material), but this stimulation was due to the increased synthesis of aberrant products rather than P1. In fact, the accumulation of P1 was reproducibly inhibited by eIF-4F. (iv) The efficiency and pattern of poliovirus RNA translation were not significantly affected by eIF-4A, eIF-4B, and eIF-3, although a slight increase or decrease (~25%) in synthesis of P1 by eIF-4A or eIF-3, respectively, was observed at the standard Mg^{2+} concentration. Also, the addition of elongation factors eEF-1α and eEF-2 from rabbit reticulocytes had no effect on translation (data not shown).

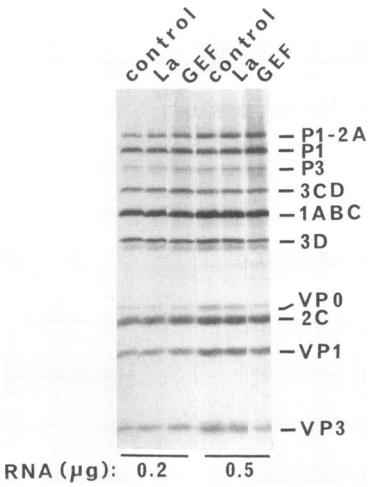

FIG. 4. Effects of La and GEF on EMC virus RNA translation. EMC virus RNA (0.2 or 0.5 μg) was translated at concentration of 0.2 or 0.5 μg for 60 min under conditions specified for translation of poliovirus RNA in Materials and Methods. Translation was carried out with 1 μg of the indicated initiation factors. Positions of EMC virus-specific polypeptides are indicated. Polypeptides 3ABC and 3C comigrate with VP0 and VP3, respectively.

Failure of La and GEF to stimulate translation of EMC virus RNA in RRL. RRL possesses all components needed for efficient and accurate translation of EMC virus RNA (24, 42, 45). This finding is further confirmed by the results showing that translation of EMC virus RNA in RRL is not stimulated either by La or by GEF even at a high (0.5 μg) RNA concentration (Fig. 4). No effect of the addition of eIF-2, eIF-4A, eIF-4B, eIF-4F, or eIF-3 was observed (data not shown), nor could the efficiency of EMC RNA translation be increased by crude initiation factor preparations, such as Krebs-2 ribosomal salt wash (data not shown). GEF and eIF-2 only marginally (less than 1.5-fold) stimulated the translation of several other mRNAs, such as tobacco mosaic virus, brome mosaic virus, and luciferase mRNAs, in the RRLs (data not shown).

DISCUSSION

Recent studies identified a number of cellular proteins which interact with the picornavirus RLP (5, 6, 10, 15, 29, 31, 34, 50, 66). Proteins binding to the poliovirus RLP include La (p52) (34), eIF-2 (10), p57 (50), p38, p48, and p54 (15). Earlier results suggested the existence of a high-affinity binding site for La in the poliovirus 5′ UTR (34, 35; this report). Poliovirus RNA 559–624 and the overlapping segments (RNAs 522–631, 556–591, and 575–609) bind La (p52) with high affinity (K_d, 3.8 to 5.7 nM), whereas segments spanning nucleotides upstream and downstream of RNA 559–624 do not bind La (p52) or do so with much lower affinity (32a). This finding suggests that a high-affinity binding site for La resides approximately between nucleotides 559 and 591 of the poliovirus 5′ UTR, i.e., within the region containing the highly conserved polypyrimidine tract/AUG motif. Binding of La to several RNA species has previously been attributed to 3′-terminal oligouridylates (32, 60). However, RNAs 556–591 and 522–631, which terminate with UGG, also bind La with high affinity (Fig. 3B and unpublished data). It is likely, therefore, that La is able to bind RNAs containing internal stretches of uridines as previously proposed (54).

Another reported *trans*-acting factor for poliovirus translation is p57 (50). p57 was originally identified by its ability to interact with the 5′ UTRs of EMC virus (5, 6, 29, 66) and foot-and-mouth disease virus (31). Interestingly, both La and p57 are involved in nuclear functions. La protein is involved in RNA polymerase III transcription termination (17, 18), and p57 was recently reported to be very similar, if not identical, to a polypyrimidine tract-binding protein, which is a component of a multisubunit complex that participates in pre-mRNA splicing (4, 16, 22, 43). However, although La is primarily nuclear, staining of the cytoplasm by anti-La antibodies has been reported (20, 23). Moreover, during poliovirus infection, La protein is redistributed from the nucleus to the cytoplasm (35). This finding is consistent with the proposed function of La protein as a cytoplasmic translation factor for poliovirus RNA (see below).

It could be argued that because P1 synthesis can be enhanced and aberrant translation can be reduced by lowering the mRNA concentration (Fig. 1A), La acts by binding and sequestering a fraction of the mRNA. However, although P1 synthesis is more efficient at low mRNA concentrations (up to 0.2 μg), it never reaches the maximal level observed in La-supplemented reactions (Fig. 1B). Thus, it is not likely that La changes the pattern of poliovirus products synthesized in RRL by reducing the amount of template available for translation. Another possibility, which cannot be rigorously excluded, is that the enhanced P1 synthesis in the presence of La is a passive consequence of suppression of abnormal initiation at competing internal sites. However, the fact that La can significantly enhance P1 translation also at elevated Mg^{2+} concentrations, at which the synthesis of aberrant products is greatly reduced, makes this possibility unlikely. Our results are most consistent with a model whereby La binds to a specific region within the 5′ UTR and actively promotes initiation of poliovirus translation at the correct AUG. La-mediated inhibition of aberrant translation may be a secondary effect, resulting from more efficient direction of the ribosomes to the authentic initiation site.

Another protein that stimulates poliovirus translation is GEF. GEF promotes the exchange of the nucleotide moiety of eIF-2 · GDP for GTP to recycle eIF-2 (13, 37). It consists of five polypeptides with M_rs of 82,000, 65,000, 55,000, 40,000, and 34,000 and is present in cells as a 1:1 complex with eIF-2. A possible role of eIF-2/GEF in poliovirus translation was originally suggested on the grounds that its subunit composition is similar to that of initiation correction factor purified from Krebs-2 cells (64). In addition, Del Angel et al. showed that eIF-2 is part of a protein complex that interacts with the 5′ UTR of poliovirus RNA (10). One characteristic feature of GEF, as was described for the initiation correction factor (64), is its ability to stimulate translation of aberrant poliovirus products only slightly less efficiently than P1. eIF-2 similarly increases the overall translation efficiency of poliovirus RNA, albeit to a lesser extent than GEF. This is in sharp contrast to La protein, in which case the enhanced synthesis of correct products is accompanied by inhibition of aberrant translation. The observed stimulation of poliovirus translation in RRLs by GEF and eIF-2 may result from their binding to the 5′ UTR. Alternatively, eIF-2 can stimulate translation by its well-characterized function, i.e., by promoting the formation of the ternary complex, eIF-2 · GTP · Met-$tRNA_f$, and GEF can act by promoting the recycling of eIF-2. It has been shown that GEF is limiting for translation in extracts from nucleated cells (49). However, translation of EMC virus RNA and other RNAs in the RRL is not stimulated by the addition of GEF or eIF-2. Therefore, these results suggest that GEF and eIF-2 are

not limiting for general translation in RRL but are limiting for poliovirus RNA. Experiments are in progress to establish whether GEF plays a direct role in the stimulation of poliovirus RNA translation or acts indirectly by increasing the supply of active eIF-2.

What is the mechanism by which La exerts its stimulatory effect? One possibility is that the binding of La to the poliovirus 5′ UTR is a prerequisite for binding of eIF-4A (or modified eIF-4F) and eIF-4B, which promote unwinding of the mRNA 5′ secondary structure, rendering the mRNA competent for interaction with ribosomes (55). Other factors, such as the polypyrimidine tract-binding protein (22) and p97 (4), either alone or in a complex with other proteins, may also facilitate internal ribosome binding. Since the deletion of the carboxy-terminal half of La results in the loss of its ability to stimulate poliovirus translation but not RNA binding, it is possible that this region of La contains an effector domain that is involved in important protein-protein interactions. We also considered the possibility that La and GEF cooperate in promoting translation of poliovirus RNA. However, La and GEF when present together exhibited only an additive stimulatory effect on translation of poliovirus RNA (60a), suggesting that their stimulatory effects are mediated through independent pathways.

An intriguing question is whether La and/or GEF play a role in initiation of translation on other picornavirus RNAs or cellular mRNAs exploiting the mechanism of internal ribosome binding (41). Our results show that initiation of translation on EMC virus RNA in RRL is not La stimulated. However, we cannot rule out the possibility that the small amount of endogenous La present in RRL is sufficient to promote the efficient initiation of EMC virus translation. Interaction of eIF-2/GEF with the 5′ UTR of EMC virus RNA was previously suggested (56). It is clear that definite answers concerning the involvement of La and other factors in the internal translation initiation on different mRNAs must await the elaboration of highly fractionated cell-free systems consisting of pure components of the protein-synthesizing machinery.

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