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The La Autoantigen Contains a Dimerization Domain That Is Essential for Enhancing Translation

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The La autoantigen is an RNA-binding protein that is involved in initiation and termination of RNA polymerase III transcription. It also binds several viral RNAs, including those of poliovirus and human immunodeficiency virus (HIV). Binding of the La protein to these RNAs enhances their translation in vitro (K. Meerovitch, Y. V. Svitkin, H. S. Lee, F. Lejbkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg, J. Virol. 67:3798–3807, 1993, and Y. V. Svitkin, A. Pause, and N. Sonenberg, J. Virol. 68:7001–7007, 1994). Here, a functional domain in the carboxy-terminal half of La that is distinct from the RNA-binding domain is described. Deletion of this domain abrogated the ability of La protein to enhance translation of poliovirus RNA and a hybrid HIV *trans*-activation-response element–chloramphenicol acetyltransferase mRNA. Far-Western assays indicated that the La protein homodimerized in vitro, and the C-terminal deletions that caused a loss of activity in translation also abrogated the dimerization signal. Gel filtration chromatography of recombinant La protein confirmed that La protein exists as a dimer under native conditions. Addition of the purified dimerization domain resulted in a loss of translation stimulatory activity of La protein in cell-free-translation reactions.

The La autoantigen (also called SS-B and p52) is a cellular protein that binds with high affinity to sites within the 5′ untranslated regions (UTRs) of poliovirus (a member of the family *Picornaviridae*) (23) and human immunodeficiency virus (HIV) (8) mRNAs. Interaction of La with these viral mRNA 5′ UTRs stimulates translation initiation (23, 32, 34).

The La protein belongs to the RNA recognition motif (RRM) superfamily of RNA-binding proteins (6, 19). The cellular function of regulating initiation and termination of RNA polymerase III transcription has been ascribed to La (14, 15, 20, 21). In addition, a role for the La protein in viral replication (28) and translation initiation of some viral RNAs has been shown (for a review, see reference 3).

Previous studies indicate that the La protein regulates translation of poliovirus and HIV RNA (25, 32, 34). However, the 5′ UTRs of poliovirus and HIV are quite distinct in that poliovirus RNA is uncapped and contains an extensive secondary structure and multiple AUG codons that are not used for initiating translation (for a review, see reference 24) while the HIV 5′ UTR is capped and contains a stable stem-bulge-loop structure proximal to the 5′ cap called the *trans*-activation-response (TAR) element (2). In the case of poliovirus RNA, translation initiation is achieved by ribosome binding to an internal ribosome entry site (IRES) (30). The La autoantigen binds a site within the IRES of poliovirus and promotes internal initiation of translation (23, 25, 32).

The HIV TAR element serves as a binding site for the viral *trans*-activator Tat, which enhances transcription from the HIV long terminal repeat (for a review, see reference 18). The TAR element is also inhibitory to translation initiation (10, 13, 29, 31). Impaired ribosome binding is due to the inaccessibility of the cap structure to the cap-binding complex (29), and it is conceivable that this block must be overcome to allow for efficient expression of HIV proteins. In contrast to poliovirus, the HIV type 1 (HIV-1) 5′ UTR does not promote internal initiation of translation (27), and thus the mRNA 5′ cap structure of the mRNA is required for ribosome binding.

In this study, we have identified a functional domain in the C terminus of La that promotes homodimerization and is absolutely required for the function of La in enhancing translation of poliovirus RNA and HIV TAR-element-containing mRNAs in vitro. Gel filtration chromatography confirmed that the La protein forms a dimer under native conditions. Addition of the purified dimerization domain resulted in inhibition of La activity in cell-free-translation reactions programmed with poliovirus and HIV TAR-element-containing mRNAs.

MATERIALS AND METHODS

Plasmids and DNA constructs. pET-La cDNA (a gift from J. Keene, Duke University) (8) was used for the generation of the C-terminal deletions. The La 1-380, La 1-293, and La 1-275 fragments (encoding up to amino acids 380, 293, and 275 of La, respectively) were generated by digestion of pET-La with *Sau*96I/*Eco*RI, *Bst*EII/*Eco*RI, and *Dra*I/*Eco*RI, respectively. The 3′ overhangs were filled in with Klenow fragment (New England Biolabs [NEB]) and religated with T4 DNA ligase (NEB). The stop codons were derived from vector sequences. pET-La1-194 and pET-La1-250 were kindly provided by D. J. Kenan and J. Keene and were described previously (8).

For generation of a glutathione *S*-transferase (GST)–La wild type fusion construct, an *Nco*I/*Bam*HI fragment from pET-La was blunt ended with Klenow fragment (NEB) and ligated to *Sma*I-digested pGex2T. GST–La 112-408 (encoding amino acids 112 to 408 of La) was generated by digestion of pET-La with *Bgl*II/*Bam*HI, followed by ligation to pGex2T digested with *Bam*HI. Ligation of the *Sca*I fragment from pET-La to *Sma*I-digested pGex2T yielded GST–La 138-304. For generation of GST–La 172-348, pET-La was digested with *Bst*NI and the resulting 530-bp fragment was blunt ended and ligated to *Sma*I-digested pGex2T. The GST–La 226-348 mutant was constructed by digestion of pET-La with *Xba*I/*Bst*NI and pGex2T with *Eco*RI, followed by blunt ending and ligation. A portion of La cDNA was amplified by PCR (between nucleotides 568 and 1080) with a reverse primer that generated an *Eco*RI site at amino acid codon 329. This PCR product was digested with *Xba*I/*Eco*RI, and pGex2T was digested with *Eco*RI; this was followed by blunt ending and ligation, resulting in gener-

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ation of the GST–La 226-329 mutant. pET-La was digested with *Bst*EII/*Bst*NI, and pGex3X was digested with *Eco*RI; this was followed by blunt ending and ligation, thus generating GST–La 293-348. The 180-bp *Dra*I fragment from pET-La was ligated to *Eco*RI-digested and blunt-ended pGex2T, resulting in production of GST–La 276-336. Stop codons are derived from vector sequences.

Expression of proteins. BL21(DE3) bacteria (Novagen) were transformed with the pET-La plasmids to enable expression of wild-type La and C-terminally truncated proteins. La proteins were purified as described previously (32). Briefly, extracts were passaged over a DEAE-Sepharose column and the flowthrough fractions (containing La proteins) were further chromatographed on heparin- and poly(U)-Sepharose columns. La proteins were eluted with either NaCl or KCl gradients.

For expression of GST-La fusion proteins, the bacterial strain BL21 (Novagen) was transformed with the various pGex plasmids (described above). Extracts of *Escherichia coli* expressing the GST-La wild type and GST–La 226-348 were lysed by sonication and purified with glutathione-S-Sepharose beads as recommended by the manufacturer (Pharmacia). *E. coli* extracts used for Western blot and Far-Western assays were obtained by lysis in sodium dodecyl sulfate (SDS) buffer.

RNA transcription and UV-induced cross-linking. pSP64/CAT and pSP64/TAR-CAT plasmids (29) were linearized with *Bam*HI, and capped mRNAs were generated in vitro as previously described (34).

The HIV-1 TAR+111 RNA probe was obtained by linearizing pSP64/TAR-CAT (29) with *Xho*I, followed by in vitro transcription with SP6 polymerase (Boehringer Mannheim) and [α-^{32}P]UTP, as described by the manufacturer (Promega). UV-induced-cross-linking assays were carried out as previously described (23). The TAR+111 RNA probe (50,000 cpm) was incubated for 10 min at 30°C with 1 μg of La wild-type or mutant proteins in a 15-μl reaction mixture containing a 100-fold molar excess of calf liver tRNA (Boehringer Mannheim). 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 30°C for 30 min. Samples were analyzed by SDS–12.5% polyacrylamide gel electrophoresis (PAGE), followed by autoradiography.

In vitro translation. Assays were carried out with micrococcal nuclease-treated rabbit reticulocyte lysate (RRL; Promega), as previously described (34). Poliovirus RNA was isolated from purified poliovirus type 1 (M-1-2p, a clone of the Mahoney strain) as described previously (33). RNA was fractionated by centrifugation through a sucrose gradient (33). Each translation reaction mixture (12.5 μl) contained 8.75 μl of the RRL and the following components at the indicated final concentrations: 75 mM potassium acetate, 0.75 mM magnesium acetate, 10 mM creatine phosphate, 50 μg of creatine phosphokinase per ml, 20 mM each unlabelled amino acid (except methionine), [^{35}S]methionine (5 μCi, 1,200 Ci/mmol), 2 mM dithiothreitol, 0.02 mM hemin, and 50 μg of calf liver tRNA per ml. mRNA was added to a final concentration of 32 μg/ml, and reaction mixtures were supplemented with 1.5 μg of purified proteins where indicated in the figure legends. Products of translation reactions were resolved by SDS–15% PAGE and detected by autoradiography. Quantitation of labelled protein products was performed with a model BAS-2000 phosphorimager (Fuji Corp.).

Western and Far-Western blots. Proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Western blots for detection of C-terminal deletion mutants of La were probed with the A2 monoclonal antibody (1:300 dilution) (7). GST-La fusions were detected with a rabbit polyclonal antibody raised against GST (1:1,000 dilution). Far-Western assays were carried out with duplicate membranes, as previously described (4). The Far-Western probe was generated by insertion of the La cDNA into the pET-HMK vector (4), followed by expression and purification from *E. coli* as described previously (32). Heart muscle kinase (HMK)-La protein (2 μg) was incubated with HMK (20 U; Sigma) in the presence of [γ-^{32}P]ATP (50 μCi) for 60 min at 37°C and passaged through a G-50 column, as described previously (4). The probe was added to the membrane at 250,000 cpm/ml in hybridization buffer for 12 to 16 h, followed by extensive washing and autoradiography.

Gel filtration chromatography. Gel filtration was carried out by fast-performance liquid chromatography (Pharmacia-LKB) with a Superose 6 column (Pharmacia). The column was equilibrated in buffer A (50 mM Tris-HCl [pH 7.5], 100 mM KCl, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol) at a flow rate of 0.25 ml/min. Elution peaks of standard proteins (200 μg; Pharmacia) were monitored by absorbance at 280 nm. Elution times were converted to elution volumes (V^e), and the elution coefficient (K_{av}) was calculated according to the equation $K_{av} = (V^e - V_0)/(V_t - V_0)$, where the total bed volume (V_t) and the void volume (V_0) of the Superose 6 column were measured as 25 and 7.25, respectively. A standard curve ($r = 0.96$, where r is the correlation coefficient, thus indicating a strong correlation between molecular weight and K_{av}) of log molecular weight versus K_{av} was obtained for ferritin, catalase, aldolase, bovine serum albumin (BSA), ovalbumin, and chymotrypsinogen A. The K_{av} were calculated for the La wild-type and La 1-194 proteins (0.46 and 0.54, respectively).

RESULTS

Characterization of the C-terminal domain of La. The C terminus of La functions as an effector domain in translation (32). To map and characterize this effector domain, several fragments with C-terminal deletions were generated by modification of La cDNA (Fig. 1A) and expression in *E. coli*; purification was as previously described (32). Coomassie blue staining showed that the La mutants were purified to apparent homogeneity (Fig. 1B). La wild-type and La 1-293 proteins contained small amounts of faster-migrating species (Fig. 1B, lanes 1 and 3) that likely represent partial breakdown products of La, since these polypeptides reacted with antibodies against La (see Fig. 3A, lanes 1 and 3).

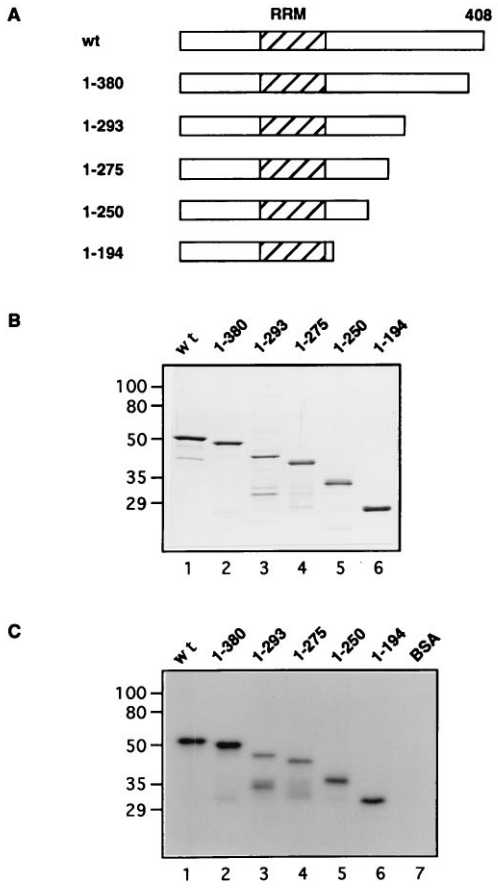

FIG. 1. C-terminal deletions of the La autoantigen. (A) A schematic representation of wild-type (wt) La and C-terminal deletions in La is shown. The positions of the deletions and the RRM are shown. (B) Coomassie blue staining of 1 μg of purified wild-type (lane 1) and mutant (lanes 2 to 6) La proteins after SDS–12.5% PAGE. (C) UV-induced cross-linking of La proteins (lanes 1 to 6) and BSA (lane 7) to a ^{32}P-labelled TAR element RNA (encoding nucleotides +1 to +111 of HIV-1 [29]) was performed as described previously (23). Molecular mass markers (in kilodaltons) are noted at the left.

To ascertain the RNA-binding activities of the La mutants, the mutants were analyzed for TAR element RNA binding in a UV-induced-cross-linking assay with a radiolabelled RNA corresponding to nucleotides +1 to +111 of HIV-1 (Fig. 1C). Wild-type La (lane 1) and all the C-terminally truncated proteins (lanes 2 to 6) interacted with the TAR element RNA, whereas bovine serum albumin (BSA) protein did not (lane 7). These results show that deletion of as much as 214 amino acids from the C terminus did not affect RNA-binding activity, which is in agreement with the results of previous studies (8, 32).

A C-terminal domain of La is required for activity in translation. Translation of poliovirus RNA in RRL is inefficient and generates a number of aberrant products which are not syn-

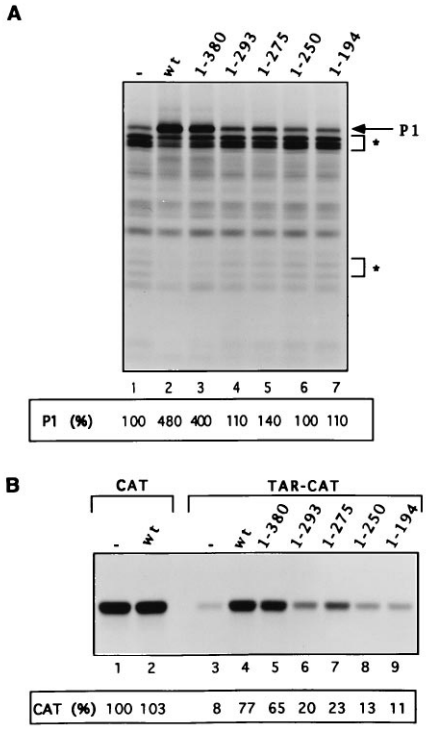

FIG. 2. Effect of La fragments on translation. (A) Translation extracts (RRL) were programmed with poliovirus RNA in the absence of La (buffer control; lane 1) or in the presence of purified wild-type La (lane 2) or La deletion mutants (lanes 3 to 7). The position of poliovirus P1 polyprotein is indicated by an arrow, and aberrant translation products are marked by brackets and asterisks to the right. The amounts of P1 protein synthesized were quantitated, and values are given relative to the amount in the control (lane 1; set at 100%). (B) Translation extracts were programmed with CAT mRNA (lanes 1 and 2) or a hybrid HIV TAR element-CAT mRNA (encoding nucleotides +1 to +111 of HIV-1 upstream of the CAT reporter gene [29]) (lanes 3 to 9). Reactions were carried out in the absence of added protein (buffer control; lanes 1 and 3), in the presence of wild-type La protein (lanes 2 and 4), or with C-terminal deletion mutants of La (lanes 4 to 9). The amounts of CAT protein were quantitated and are given as percentages of the amount of CAT mRNA in the control (lane 1).

thesized in poliovirus-infected cells (9, 25, 33). When RRL was supplemented with purified La protein, the amount of aberrant products was reduced and synthesis of the poliovirus P1 polyprotein was enhanced (25, 32). However, addition of a C-terminal truncated La protein (La 1-194) had no stimulatory effect on translation, suggesting a requirement of the C terminus for La function (32). Here, we used the activity of La in translation to identify the effector domain in the C terminus of La. Translation extracts (RRL) were programmed with poliovirus RNA alone (Fig. 2A, lane 1) or in the presence of purified recombinant wild-type La (lane 2) or truncated La proteins (lanes 3 to 7). Translation of the precursor P1 polyprotein was enhanced 4.8-fold upon addition of wild-type La protein (compare lanes 1 and 2). Also, the amounts of aberrant translation products were reduced in the presence of La (compare lanes 1 and 2), consistent with the results of previous studies (25, 32). Addition of the La 1-380 fragment also caused enhanced translation of P1 (fourfold greater stimulation; lane 3), while the other truncated proteins showed no significant effect on P1 translation (lanes 4 to 7). Thus, the region between amino acids 293 and 380 is the amino-terminal boundary of the translation activation domain.

In a second assay for La function, a hybrid HIV TAR element-chloramphenicol acetyltransferase (CAT) mRNA (corresponding to nucleotides +1 to +111 of HIV-1 fused upstream of CAT [29]) was used. Capped CAT and TAR element-CAT mRNAs were translated in RRL in the absence or presence of La proteins (Fig. 2B). As reported previously (29, 34), TAR element-CAT mRNA was translated much less efficiently (approximately 10-fold less) than CAT mRNA (compare lane 3 to lane 1). Addition of wild-type La protein to extracts programmed with CAT mRNA had no effect on translation (compare lanes 1 and 2). In contrast, addition of wild-type La enhanced translation of TAR element-CAT mRNA by 10-fold compared to that of the control (compare lanes 3 and 4). The La 1-380 protein showed activity comparable to that of wild-type La (compare lanes 4 and 5), while the La 1-293 and La 1-275 proteins exhibited a small effect on translation (2.5-fold higher) (lanes 6 and 7). The addition of La 1-250 and La 1-194 proteins had only a slight effect on translation (compare lane 3 with lanes 8 and 9). The results were similar for both assays and indicate that residues between amino acids 293 and 380 of La are required for the efficient stimulation of translation of poliovirus and TAR element-CAT RNAs.

The C terminus of La contains a dimerization domain. Since all the C-terminal deletion mutants of La retained RNA-binding activity (Fig. 1C) but only the smallest deletion mutant (La 1-380) retained the translation stimulatory activity of wild-type La (Fig. 2), it was conceivable that a distinct effector domain is located in the C terminus. The presence of protein-protein interaction domains in several ribonucleoproteins (12, 16, 22, 26), as well as the formation of multiple La-RNA complexes in gel-mobility shift assays (25), prompted us to test whether the La protein can homodimerize. We employed a Far-Western assay (4) in which a purified Flag HMK-La fusion was labelled and used to probe a nitrocellulose membrane containing purified wild-type or mutant La proteins (Fig. 3A, right panel). A duplicate membrane was probed with a monoclonal antibody against La (A2 [7]) (Fig. 3A, left panel), which detected wild-type La (lane 1) and the C-terminal deletion mutants (lanes 2 to 5). The full-length La and La 1-380 proteins interacted with the La protein probe (right panel, lanes 1 and 2), whereas the other deletion mutants did not (lanes 3 to 5). Therefore, the La protein can homodimerize in vitro, and dimerization is dependent on a region between amino acids 293 and 380. In addition, the C-terminal sequences required for activity (Fig. 2) are also required for dimerization (Fig. 3A).

To further demarcate the dimerization domain, a panel of GST-La fusion proteins was generated and expressed in *E. coli*. Purified GST and the GST-La wild type or extracts expressing the various GST-La fusion proteins were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Parallel membranes were incubated either with an antibody against GST (Fig. 3B, top panel) or with ^{32}P-labelled La protein (Fig. 3B, bottom panel). While both GST and the GST-La wild type were readily detected by Western blotting (top panel, lanes 1 and 2), only the GST-La fusion bound to the radiolabelled La protein in a Far-Western assay (bottom panel, lanes 1 and 2). Deletion of the N-terminal 111 amino acids of La (GST–La 112-408; lane 3) had no effect on La dimerization. Further truncated proteins lacking the N terminus up to amino acid 293, which eliminates the whole RRM, retained the ability to dimerize (GST–La 172-348, GST–La 226-348, and GST–La 293-348; lanes 5, 6 and 8, respectively). The C-terminal boundary of these proteins was at amino acid 348. In contrast, deletion of the C terminus extending to amino acid 336 (GST–La 276-336; lane 9) and further to amino acid 329 (GST–La 226-329; lane 7) or 304 (GST–La 138-304; lane 4) resulted in a loss of dimerization capacity. Thus, the C terminus of La (between amino acids 293 and 348) contains a dimerization domain. The

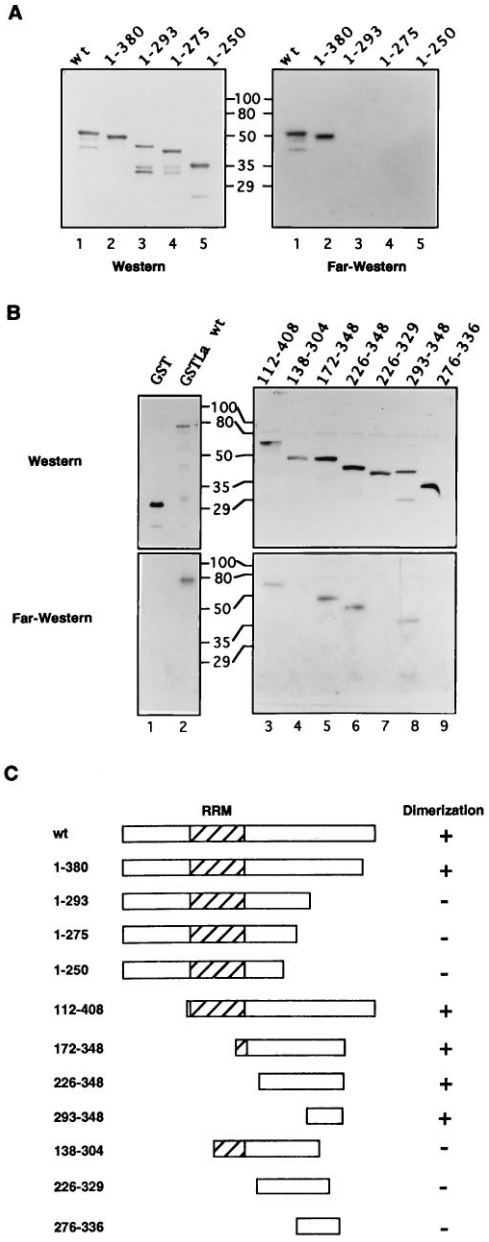

FIG. 3. Dimerization of the La autoantigen. (A) Wild-type (wt) La and C-terminal deletion mutants were resolved by SDS–12.5% PAGE, and duplicate gels were transferred to nitrocellulose membranes. (Left panel) One membrane was probed with a monoclonal antibody which recognizes La protein (A2, 1:300 dilution [7]), followed by detection by enhanced chemiluminescence (Amersham). (Right panel) The other membrane was subjected to a Far-Western assay (4) using a ^{32}P-labelled Flag HMK-La protein probe. (B) Mapping of the dimerization domain by Far-Western analysis with GST-La fusion proteins. Purified GST (lane 1) or a GST-La wild-type fusion protein (lane 2) was resolved by SDS–10% PAGE, transferred to duplicate nitrocellulose membranes, and probed with a polyclonal antibody that recognizes GST (top panel) or was subjected to a Far-Western assay (bottom panel), as described above. Several GST-La fusion proteins were expressed in *E. coli* and resolved by SDS–12.5% PAGE (lanes 3 to 9). Duplicate gels were transferred to nitrocellulose membranes and either probed with polyclonal antiserum that recognizes the GST moiety (top panel) or subjected to a Far-Western assay (bottom panel, as described above). Molecular mass markers (in kilodaltons) are noted between the gels. (C) Schematic representation of La mutants and summary of dimerization results.

positions of the various deletions and dimerization capacities are depicted schematically in Fig. 3C.

Gel filtration chromatography of recombinant La protein. In order to confirm that the La protein forms dimers under native conditions, gel filtration chromatography was performed. Protein standards were employed to calibrate the column (Fig. 4A). Purified recombinant La protein eluted from the column in a single peak, which corresponded to a molecular mass of ~98 kDa (Fig. 4A). This size is consistent with a dimer, since the predicted molecular mass of La protein is 46.8 kDa (6). The La elution peak was asymmetrical, and a portion extended to a V_e comparable to that of ovalbumin (data not shown). This suggests that a portion of La protein eluted close to its monomer size. Since the Far-Western experiments show that the La 1-194 truncated protein lacks the dimerization domain (Fig. 3A), it is predicted to exist as a monomer under native conditions. Indeed, the purified La 1-194 protein eluted from the column as a 35-kDa protein (Fig. 4A), which is close to its size after migration on denaturing gels as an ~28-kDa polypeptide (Fig. 1B). The integrity of the eluted wild-type La protein was verified by Coomassie blue staining of the peak fraction, yielding a major species of 52 kDa and trace amounts of a 42-kDa partial proteolytic fragment of La (Fig. 4B, lane 2). A small amount of a 75-kDa polypeptide was also detected and likely corresponds to residual BSA present in the chromatography injection loop from the previous run. The eluted protein reacted with a monoclonal antibody against La (A2 [7]) (Fig. 4C, lane 2) and comigrated with native La protein from HeLa cell extracts (lane 1). To determine whether the peak fraction from the column for wild-type La exhibits translation stimulatory activity, RRL was programmed with capped CAT (Fig. 4D, lanes 1 and 2) or TAR element-CAT (lanes 3 and 4) mRNAs in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of the La protein that was eluted from the Superose column. La protein had little effect on translation of CAT mRNA (Fig. 4D, compare lanes 1 and 2) but stimulated CAT synthesis from the TAR element-CAT mRNA to levels comparable to that of the control (compare lanes 1 and 4). Since the peak fraction contained predominantly La dimers (with only low levels of the monomer form), these results show that it is likely the dimer form of La protein that is active in translation.

The dimerization domain exhibits a dominant-negative effect on La activity. To further address the importance of dimerization of La for its function in translation initiation, a portion of La (La 226-348) containing the dimerization domain but lacking the RNA-binding domain was purified and its effect on translation was examined. Addition of wild-type La protein enhanced poliovirus P1 translation (7.7-fold) (Fig. 5A, lane 2). However, when an increasing amount of La 226-348 protein was mixed with the wild-type protein, the stimulatory effect of wild-type La on P1 translation was reduced in a dose-dependent manner (lanes 3 to 5) and was abolished at a 3:1 molar ratio of La 226-348 to wild-type protein (compare lanes 2 and 5). Addition of the La 226-348 protein alone also reduced (2.5-fold) the residual synthesis of P1 polyprotein (compare lanes 1 and 6). Thus, the purified dimerization domain inhibits the activity of La protein in translation of poliovirus RNA and acts in a dominant-negative manner.

Similar experiments were carried out to investigate the effect of the La 226-348 fragment on translation of capped CAT and TAR element-CAT mRNAs (Fig. 5B). Addition of wild-type La or La 226-348 proteins had no significant effect on translation of CAT mRNA (lanes 1 to 4). However, enhancement of TAR element-CAT mRNA translation by La (compare lanes 5 and 6) was dramatically reduced (up to sixfold) when an increasing amount of La 226-348 protein was added (compare

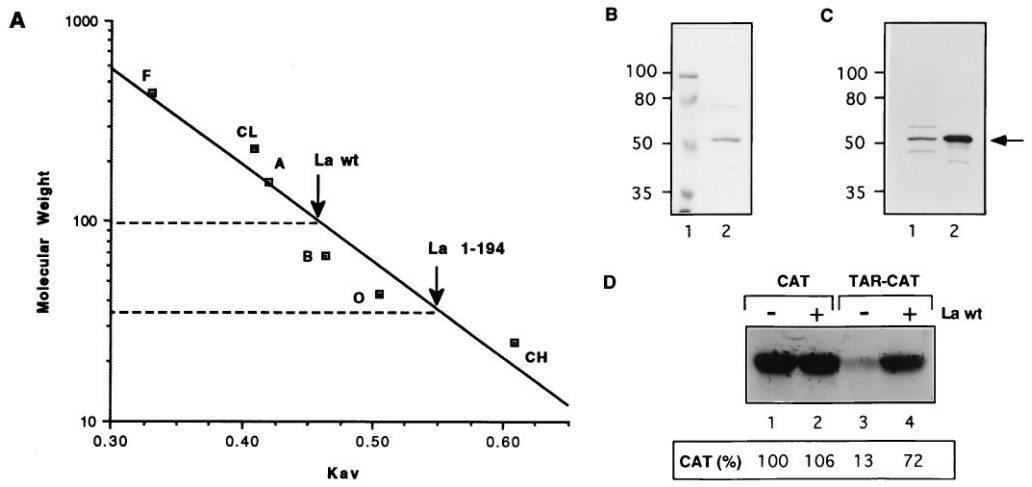

FIG. 4. Gel filtration chromatography of recombinant La protein. (A) Gel filtration was carried out by fast-performance liquid chromatography (Pharmacia-LKB) with a Superose 6 column (Pharmacia). Protein standards (Pharmacia) were used to calibrate the column. K_{av} for ferritin (F), catalase (CL), aldolase (A), BSA (B), ovalbumin (O), and chymotrypsinogen A (CH) were calculated (see Materials and Methods) and used to generate a standard curve. The K_{av} of wild-type (wt) La and La 1-194 proteins are indicated by arrows, and the corresponding molecular weights (in thousands) are marked by dashed lines. (B) Protein from the elution peak of wild-type La was resolved by SDS–10% PAGE and stained with Coomassie blue. Lane 1, prestained molecular weight markers (in thousands) (Bio-Rad); lane 2, protein from the column elution peak for wild-type La. (C) Total HeLa cell proteins (25 μg; lane 1) and protein contained in the wild-type La elution peak (1 μg) were resolved by SDS–10% PAGE, transferred to a nitrocellulose membrane, incubated with a monoclonal antibody directed against La protein (A2, 1:300 dilution [7]) and detected by enhanced chemiluminescence (Amersham). (D) RRL programmed with capped CAT (lanes 1 and 2) and TAR element-CAT (lanes 3 and 4) mRNA was incubated with buffer A (lanes 1 and 3) or with wild-type La protein from the peak fraction from the column (1.4 μg) (lanes 2 and 4). The amounts of CAT protein were quantitated and are given as percentages of the amount from the CAT mRNA control reaction (lane 1).

lane 6 with lanes 7 to 9). In addition, the inefficient translation of TAR element-CAT mRNA (lane 5) was further reduced (fivefold) in the presence of the La 226-348 fragment (lane 10). These findings show that addition of the dimerization domain inhibits the activity of the wild-type protein. Also, the ability of the La 226-348 fragment to specifically inhibit poliovirus RNA and TAR element-CAT mRNA translation in the absence of exogenous wild-type La protein suggests that the dimerization domain sequesters endogenous La protein, which is required for the translation of these mRNAs.

DISCUSSION

In this study, we have identified and characterized a C-terminal effector domain of the La autoantigen which is distinct from its RNA-binding domain. This effector domain is required for La activity in translation (Fig. 2) (32) and was identified as a dimerization domain (Fig. 3). Indeed, La protein exists predominantly as a dimer under native conditions. The ability of the purified dimerization domain to specifically inhibit translation of poliovirus RNA and a hybrid HIV TAR element-CAT mRNA (Fig. 5) suggests that it has dominant-negative activity against the function of wild-type La protein. Also, this implies that formation of functional La dimers requires both monomers to retain RNA-binding activity.

The important features or amino acids within the dimerization domain of La protein are not known. This portion of La spanning amino acids 293 to 348 contains 39% hydrophobic residues and 41% charged amino acids. Secondary-structure predictions (6) suggest that this region possesses both α-helical and nonhelical structures. A potential leucine zipper motif located close to the N terminus of the *Drosophila* La homolog, which is conserved in other species, has been postulated to be a dimerization domain (1). However, the N-terminal region of the human La protein is not involved in homodimerization (Fig. 3). It is possible that this leucine zipper motif mediates interaction between La and other proteins, but there is currently no evidence for this.

In addition to La protein, several other RNA-binding proteins form homo- or heterodimers (12, 16, 22, 26). The abundant RNA-binding protein p50 from reticulocytes also forms multimers and is a major component of messenger RNP particles (11). These proteins are involved in several RNA metabolic processes, including RNA splicing, mRNA masking, and translation. Protein-protein as well as protein-RNA interactions likely mediate adoption of the correct RNA structure. In fact, RNA chaperone activity has been ascribed to a number of RNA-binding proteins, including heterogeneous nuclear ribonucleoprotein particle A1 and nucleocapsid protein p7 of HIV-1 (for a review, see reference 17). Interestingly, p7 and heterogeneous nuclear ribonucleoprotein particle A1 also dimerize (5, 35), although it is not known whether dimerization is required for this activity.

The stimulatory effect of La protein on translation of poliovirus and HIV TAR element-CAT RNAs shows that La protein modulates two distinct mechanisms of translation initiation, cap-independent and cap-dependent ribosome binding, respectively. Although the mechanism by which this occurs is not known, it is possible that binding of La dimers to the IRES element of poliovirus and the HIV TAR element stem-loop structure promotes formation of RNA structures which favor ribosome binding. Alternatively, the enhanced translation of these RNAs may be due to possible interactions between La protein and translation initiation factors. However, no interactions were detected between purified initiation factors and La protein in Far-Western assays (19a).

In summary, the La protein requires a C-terminal dimerization domain, in addition to RNA-binding activity, for its activity in translation. It is not known whether the dimerization domain is essential for the nuclear function of La protein in RNA polymerase III transcription initiation and termination or whether it is required only for the La protein's translational

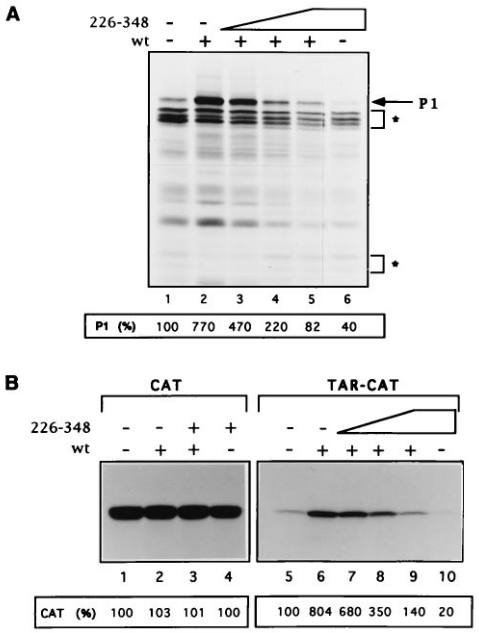

FIG. 5. Inhibition of La activity by the purified dimerization domain. (A) Translation extracts (RRL) were programmed with poliovirus RNA in the absence (buffer control; lane 1) or presence (1.5 μg; lane 2) of wild-type (wt) La protein. Lanes 3 to 5, wild-type La protein (1.5 μg) with increasing amounts of GST–La 226-348 protein (3:1, 1:1, and 1:3 molar ratios of wild-type to mutant proteins in lanes 3 to 5, respectively) (amino acids 226 to 348 of La contain the dimerization domain [Fig. 3]); lane 6, GST–La 226-348 protein alone (4.5 μg). Aberrant products are indicated by brackets and asterisks to the right of the figure. Quantitations of P1 protein (arrow to right) are given as percentages of the amount in the control (lane 1). (B) Translation reactions with CAT mRNA (lanes 1 to 4) were carried out in the absence of added protein (lane 1) or in the presence of wild-type La protein (lane 2). Lane 3 contains wild-type La and GST–La 226-348 (1:1 molar ratio), and lane 4 contains added GST–La 226-348 protein alone (1.5 μg; lane 4). Reactions programmed with TAR element-CAT mRNA (lanes 5 to 10) contained no added protein (lane 5) or wild-type La protein (1.5 μg; lane 6). In lanes 7 to 9, wild-type La protein (1.5 μg) and increasing amounts of GST–La 226-348 protein were added (3:1, 1:1, and 1:3 molar ratios of wild-type to mutant proteins, respectively). Lane 10 contains GST–La 226-348 protein alone (4.5 μg). Quantitations of CAT protein are given as percentages of the amount in the control (lanes 1 and 5).

role. It is known that phosphorylation of La protein is restricted to the C-terminal half of the protein (6), which contains the dimerization domain. Since the role of phosphorylation of La protein is not known, it will be of interest to determine whether phosphorylation regulates La dimerization and activity.

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REFERENCES

1. **Bai, C., Z. Li, and P. P. Tolias.** 1994. Developmental characterization of a *Drosophila* RNA-binding protein homologous to the human systemic lupus erythematosus-associated La/SS-B autoantigen. Mol. Cell. Biol. **14:**5123–5129.
2. **Baudin, F., R. Marquet, C. Isel, J. L. Darlix, B. Ehresmann, and C. Ehresmann.** 1993. Functional sites in the 5′ region of human immunodeficiency virus type 1 RNA form defined structural domains. J. Mol. Biol. **229:**382–397.
3. **Belsham, G. J., N. Sonenberg, and Y. V. Svitkin.** 1995. The role of the La autoantigen in internal initiation. Curr. Top. Microbiol. Immunol. **203:**85–98.
4. **Blanar, M. A., and W. J. Rutter.** 1992. Interaction cloning: identification of a helix-loop-helix zipper protein that interacts with c-Fos. Science **256:**1014–1018.
5. **Casas-Finet, J. R., J. Smith, Jr., A. Kumar, J. G. Kim, S. H. Wilson, and R. L. Karpel.** 1993. Mammalian heterogeneous ribonucleoprotein A1 and its constituent domains. Nucleic acid interaction, structural stability and self-association. J. Mol. Biol. **229:**873–879.
6. **Chambers, J. C., D. Kenan, B. J. Martin, and J. D. Keene.** 1988. Genomic structure and amino acid sequence domains of the human La autoantigen. J. Biol. Chem. **263:**18043–18051.
7. **Chan, E. K., and E. M. Tan.** 1989. Epitopic targets for autoantibodies in systemic lupus erythematosus and Sjogren's syndrome. Curr. Opin. Rheumatol. **1:**376–381.
8. **Chang, Y.-N., D. J. Kenan, J. D. Keene, A. Gatignol, and K.-T. Jeang.** 1994. Direct interactions between autoantigen La and human immunodeficiency virus leader RNA. J. Virol. **68:**7008–7020.
9. **Dorner, A. J., B. L. Semler, R. J. Jackson, R. Hanecak, E. Duprey, and E. Wimmer.** 1984. In vitro translation of poliovirus RNA: utilization of internal initiation sites in reticulocyte lysate. J. Virol. **50:**507–514.
10. **Edery, I., R. Petryshyn, and N. Sonenberg.** 1989. Activation of double-stranded RNA-dependent kinase (dsI) by the TAR region of HIV-1 mRNA: a novel translational control mechanism. Cell **56:**303–312.
11. **Evdokimova, V. M., C. L. Wei, A. S. Sitikov, P. N. Simonenko, O. A. Lazarev, K. S. Vasilenko, V. A. Ustinov, J. W. Hershey, and L. P. Ovchinnikov.** 1995. The major protein of messenger ribonucleoprotein particles in somatic cells is a member of the Y-box binding transcription factor family. J. Biol. Chem. **270:**3186–3192.
12. **Fresco, L. D., D. S. Harper, and J. D. Keene.** 1991. Leucine periodicity of U2 small nuclear ribonucleoprotein particle (snRNP) A′ protein is implicated in snRNP assembly via protein-protein interactions. Mol. Cell. Biol. **11:**1578–1589.
13. **Geballe, A. P., and M. K. Gray.** 1992. Variable inhibition of cell-free translation by HIV-1 transcript leader sequences. Nucleic Acids Res. **20:**4291–4297.
14. **Gottlieb, E., and J. A. Steitz.** 1989. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. EMBO J. **8:**851–861.
15. **Gottlieb, E., and J. A. Steitz.** 1989. The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription *in vitro*. EMBO J. **8:**841–850.
16. **Hermann, H., P. Fabrizio, V. A. Raker, K. Foulaki, H. Hornig, H. Brahms, and R. Luhrmann.** 1995. SnRNP Sm proteins share two evolutionary conserved sequence motifs which are involved in Sm protein-protein interactions. EMBO J. **14:**2076–2088.
17. **Herschlag, D.** 1995. RNA chaperones and the RNA folding problem. J. Biol. Chem. **270:**20871–20874.
18. **Jones, K. A., and B. M. Peterlin.** 1994. Control of RNA initiation and elongation at the HIV-1 promoter. Annu. Rev. Biochem. **63:**717–743.
19. **Kenan, D. J., C. C. Query, and J. D. Keene.** 1991. RNA recognition: towards identifying determinants of specificity. Trends Biochem. Sci. **16:**214–220.

19a. **Lee, H.** Unpublished observations.

20. **Maraia, R. J.** 1996. Transcription termination factor La is also an initiation factor for RNA polymerase III. Proc. Natl. Acad. Sci. USA **93:**3383–3387.
21. **Maraia, R. J., D. J. Kenan, and J. D. Keene.** 1994. Eukaryotic transcription termination factor La mediates transcript release and facilitates reinitiation by RNA polymerase III. Mol. Cell. Biol. **14:**2147–2158.
22. **Marello, K., J. LaRovere, and J. Sommerville.** 1992. Binding of Xenopus oocyte masking proteins to mRNA sequences. Nucleic Acids Res. **20:**5593–5600.
23. **Meerovitch, K., J. Pelletier, and N. Sonenberg.** 1989. A cellular protein that binds to the 5′-noncoding region of poliovirus RNA: implications for internal translation initiation. Genes Dev. **3:**1026–1034.
24. **Meerovitch, K., and N. Sonenberg.** 1993. Internal initiation of picornavirus RNA translation. Semin. Virol. **4:**217–227.
25. **Meerovitch, K., Y. V. Svitkin, H. S. Lee, F. Lejbkowicz, D. J. Kenan, E. K. L. Chan, V. I. Agol, J. D. Keene, and N. Sonenberg.** 1993. La autoantigen enhances and corrects aberrant translation of poliovirus RNA in reticulocyte lysate. J. Virol. **67:**3798–3807.
26. **Methot, N., M. S. Song, and N. Sonenberg.** 1996. A region rich in aspartic acid, arginine, tyrosine, and glycine (DRYG) mediates eukaryotic initiation factor 4B (eIF4B) self-association and interaction with eIF3. Mol. Cell. Biol. **16:**5328–5334.
27. **Miele, G., A. Mouland, G. P. Harrison, E. Cohen, and A. M. Lever.** 1996. The human immunodeficiency virus type 1 5′ packaging signal structure affects translation but does not function as an internal ribosome entry site structure. J. Virol. **70:**944–951.
28. **Pardigon, N., and J. H. Strauss.** 1996. Mosquito homolog of the La autoantigen binds to Sindbis virus RNA. J. Virol. **70:**1173–1181.
29. **Parkin, N. T., E. A. Cohen, A. Darveau, C. Rosen, W. Haseltine, and N. Sonenberg.** 1988. Mutational analysis of the 5′ non-coding region of human immunodeficiency virus type 1: effects of secondary structure on translation. EMBO J. **7:**2831–2837.

30. **Pelletier, J., and N. Sonenberg.** 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature (London) **334:**320–325.
31. **SenGupta, D. N., B. Berkhout, A. Gatignol, A. M. Zhou, and R. H. Silverman.** 1990. Direct evidence for translational regulation by leader RNA and Tat protein of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **87:**7492–7496.
32. **Svitkin, Y., K. Meerovitch, H. S. Lee, J. N. Dholakia, D. J. Kenan, V. I. Agol, and N. Sonenberg.** 1994. Internal translation initiation on poliovirus RNA: further characterization of La function in poliovirus translation in vitro. J. Virol. **68:**1544–1550.
33. **Svitkin, Y. V., S. V. Maslova, and V. I. Agol.** 1985. The genomes of attenuated and virulent poliovirus strains differ in their in vitro translation efficiencies. Virology **147:**243–252.
34. **Svitkin, Y. V., A. Pause, and N. Sonenberg.** 1994. La autoantigen alleviates translational repression by the 5′ leader sequence of the human immunodeficiency virus type 1 mRNA. J. Virol. **68:**7001–7007.
35. **Tanchou, V., C. Gabus, V. Rogemond, and J. L. Darlix.** 1995. Formation of stable and functional HIV-1 nucleoprotein complexes in vitro. J. Mol. Biol. **252:**563–571.