Visinin-like Protein (VILIP) Is a Neuron-specific Calcium-dependent Double-stranded RNA-binding Protein*

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Double-stranded RNA-binding proteins function in regulating the stability, translation, and localization of specific mRNAs. In this study, we have demonstrated that the neuron-specific, calcium-binding protein, visinin-like protein (VILIP) contains one double-stranded RNA-binding domain, a protein motif conserved among many double-stranded RNA-binding proteins. We showed that VILIP can specifically bind double-stranded RNA, and this interaction specifically requires the presence of calcium. Mobility shift studies indicated that VILIP binds double-stranded RNA as a single protein-RNA complex with an apparent equilibrium dissociation constant of $9.0 \times 10^{-8}\text{ M}$. To our knowledge, VILIP is the first double-stranded RNA-binding protein shown to be calcium-dependent. Furthermore, VILIP specifically binds the 3′-untranslated region of the neurotrophin receptor, trkB, an mRNA localized to hippocampal dendrites in an activity-dependent manner. Given that VILIP is also expressed in the hippocampus, these data suggest that VILIP may employ a novel, calcium-dependent mechanism to regulate its binding to important localized mRNAs in the central nervous system.

RNA-binding proteins are crucial to a number of fundamental biological processes (1, 2). By comparing the amino acid sequences of different RNA-binding proteins, several classes of protein domains have been identified that mediate the RNA-protein interaction. One class of RNA-binding proteins specifically recognizes the double-stranded RNA (dsRNA)$^1$ A-form helix through a conserved motif called the dsRNA-binding domain (3). As opposed to the sequence-specificity of DNA-protein binding, which results from interactions with the DNA major groove and sugar-phosphate backbone, recognition of dsRNA often shows no sequence specificity. Rather, RNA-binding proteins recognize elements within the dsRNA minor groove, single-stranded RNA loops, and through tertiary interactions with different regions of the RNA molecule, thereby leading to a greater variety of binding possibilities for RNA-protein recognition (1, 4, 5).

Proteins with the dsRNA-binding domain have a wide range of expression and a diverse array of essential biological functions. For example, staufen, a protein required for localization of mRNAs during Drosophila development, contains three full-length dsRNA-binding domains (Type 1) and two truncated C-terminal domains (Type 2) (3). Type 1 and Type 2 dsRNA-binding domains are also found in human interferon-induced dsRNA-dependent protein kinase (PKR), which is activated during viral infections and has antiviral and antiproliferative properties (6–9). In addition, dsRNA-binding domains have been found in proteins isolated from Xenopus oocytes, viral-encoded proteins, and bacterial RNAse III (3, 10, 11).

In neurons of the central nervous system, the asymmetric distribution of specific proteins in response to extracellular signals can occur through the subcellular localization of the protein's cognate mRNA (for review, see Ref. 12). As a way to identify RNA-binding proteins that may be involved in the localization of neuronal mRNAs, we used PCR amplification of brain-derived cDNA with degenerative primers directed to conserved regions of the dsRNA-binding domain. Using this approach, we identified a dsRNA-binding domain in the neuron-specific member of the Ca$^{2+}$-binding EF-hand proteins, visinin-like protein (VILIP) and demonstrated that it specifically binds dsRNA in a Ca$^{2+}$-dependent manner. In the hippocampus, one localized mRNA encodes the high affinity neurotrophin receptor, trkB, which is localized to the dendrites in response to increased neuronal activity (13), and our studies demonstrated that Ca$^{2+}$ induces VILIP to bind to a specific region of the trkB mRNA. These studies suggest that VILIP can interact with important localized mRNA in neurons of the central nervous system and may be regulated by the intracellular availability of Ca$^{2+}$.

**EXPERIMENTAL PROCEDURES**

*Degenerate PCR—Total brain RNA was isolated using Trizol™ (Life Technologies, Inc.) following the manufacturer’s directions and converted into cDNA using oligo(dT) as a primer and AMV reverse transcriptase (Amersham Pharmacia Biotech). PCR amplifications were performed using 3′ at dsR-1 and dsR-2 degenerate primers for 30 cycles with 0.6 °C/sec ramp speeds for 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. Resulting PCR products were cloned into pCR II (Invitrogen, Carlsbad, CA).

The dsR-1 and dsR-2 primers were directed to the conserved regions of the dsRNA-binding domain as indicated in Scheme 1 in the Dmstau-1 dsRNA-binding domain (also see Fig. 1B). The sequence and degenerate positions of dsR-1 and dsR-2 are indicated in Scheme 2.

Scheme 1

| dsR-1 | dsR-2 |
|-------|-------|
| FMCVWEWLRAYKITHQYRLTEERGPAPAHCCKTPTVTLMGDEEYSADCFKTKA |
cDNA Library Screening—A cDNA library prepared from post-natal day 20 mouse brain (Stratagene, La Jolla, CA) was high density screened with 32P-labeled gel-purified PCR product. Positive clones were plaque-purified, converted to plasmid DNA using ExAssist™/SOLR systems (Stratagene) (14) and sequenced at the Cleveland Clinic Molecular Biotechnology Core.

DNA and Protein Sequence Analysis—BLASTN version 2.0.3 was used to search the nonredundant GenBank™ data base. Clustal W (PAM 100) was used to align the dsRNA domains. Expression and Purification of Recombinant VILIP—The coding region of VILIP was cloned into the pQE32 vector expression as an N-terminal-tagged 6xHis fusion protein (pMhis-VILIP) (Qiagen, Valencia, CA) and was transformed into the Escherichia coli strain M15. Expression of the 6xHis-VILIP fusion protein was induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG) at mid-log bacterial cultures. Bacteria were then collected by centrifugation, washed with cold phosphate-buffered saline, and sonicated. Purification of recombinant VILIP used nickel-nitrilotriacetic acid (Ni-NTA) chromatography per the manufacturer's directions (Qiagen).

Northwestern Binding Analysis—RNA binding assays were performed as described previously with modifications (3). Ni-NTA agarose affinity-purified VILIP was electrophoresed on 15% Tris-HCl polyacrylamide gels (Bio-Rad) and electro-transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA). Membranes with transferred proteins were denatured in 8 M urea for 1 h and renatured by a series of 10 washes that were 2.3 dilutions of the previous wash. Dilutions were with TBS (20 mM Tris base, pH 7.6, 137 mM NaCl, 44 mM HCl, and either 10 mM CaCl₂ or 1 mM EDTA). After blocking for 1 h (25 mM NaCl, 10 mM MgCl₂, 10 mM HEPES, pH 8.0, 1 mM dithiothreitol, 5% nonfat dry milk, and 10 mM CaCl₂ or 1 mM EDTA), 32P-labeled RNA was allowed to bind for 4 h at 25 °C in binding buffer (50 mM NaCl, 10 mM MgCl₂, 10 mM HEPES, pH 8.0, 1 mM dithiothreitol, 2.5% nonfat dry milk, and 10 mM CaCl₂ or 1 mM EDTA) at 1 × 10⁶ cpm/ml. The membranes were washed with three changes of binding buffer, 10 min each, dried, exposed to Biomax MR film (Eastman Kodak), and quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Polyclonal (IgG) (Amersham Pharmacia Biotech) was digested with RNase T & A (Ambion, Austin, TX), ethanol precipitated, and end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. Poly C was also end-labeled with [γ-32P]ATP, except the RNase digestion was omitted.

Mobility Shift Assay—The mobility gel shift assay was adapted from Bass et al. (15). 32P-labeled poly(1-C) were electrophoresed, and appropriately sized fragments were eluted from nondenaturing polyacrylamide gels. Single-stranded RNA was prepared by boiling 32P-labeled, gel-eluted poly I(C) for 10 min and transferring immediately to ice. Known concentrations of recombinant VILIP were allowed to bind either poly(C) or single-stranded RNA (~2.0 × 10⁶ cpm/sample) in 100 μl Tris, pH 8.0, 250 mM KCl, 100 mM NaCl, 5 mM dithiothreitol, 100 μg/ml bovine serum albumin, 10% glycerol, and either 100 mM CaCl₂ or 100 mM MgCl₂, for 20 min at 25 °C. Resulting binding reactions were run on a 20-cm 4% polyacrylamide in 1× TB (100 mM Tris, pH 8.3, 100 mM boric acid) without sample buffer at 150 volts. Gels were dried and exposed to Biomax MR film (Eastman Kodak). Bound and unbound dsRNA were quantified using a PhosphorImager (Molecular Dynamics). The apparent equilibrium dissociation constant (the protein concentration at which half the free dsRNA is bound) was calculated as described previously (15, 16) by plotting the fraction bound (dsRNA-VILIP complex radioactivity/dsRNA-VILIP complex radioactivity + free dsRNA fraction radioactivity) versus protein concentration.

DNA templates used to synthesize trkB mRNAs were prepared by PCR amplification using the trkB cDNA (ATCC number 63055) and primers directed to the entire trkB coding region. Each 5′ and 3′ primer was flanked by either the promoter sequences for the bacteriophage T3 (5′-GCAATAAACCCTCTACATAAG-3′) or T7 (5′-CTTAATACGACTCACTATAG-3′) enzymes. The resulting amplification product was gel eluted and used directly for RNA synthesis using the Maxiscript transcription system (Ambion, Austin, TX) following the manufacturer's directions. The synthesized RNA was then incubated at 98 °C for 10 min in 250 mM KCl, followed by slow cooling to room temperature to ensure RNA base pairing (17).

RESULTS

VILIP Contains a dsRNA-binding Domain—To generate a PCR probe that could be used to identify a dsRNA-binding protein from the central nervous system, RNA isolated from P20 rat brains was converted into cDNA and used as a template for degenerate primers directed to the 5′ and 3′ most conserved regions of the Drosophila staufen dsRNA-binding domain (see “Experimental Procedures”). To avoid excessive degeneracy, primers were designed that took into account mammalian codon utilization (18). A PCR product was generated and cloned that corresponded in size with a dsRNA-binding domain. Sequencing of the clone revealed a similarity with the dsRNA-binding domain (data not shown).

A mouse cDNA library from P20 brains was screened with 32P-labeled PCR insert and two dsRNA clones were identified that both contained the same open reading frame that encoded a peptide of 22,140 kDa (Fig. 1A). Screening the National Institutes of Health nonredundant data base identified the coding region as visinin-like protein (VILIP) (GenBank™ accession number D21165) (19, 20). VILIP is a neural Ca²⁺-binding protein (NCaPs) that belongs to the superfamily of EF-hand Ca²⁺-binding proteins (19). As a member of the NCaPs family, VILIP contains four canonical EF-hand binding domains (sites I, II, III, and IV) that span amino acid residues 25–56, 66–92, 100–127, and 150–178, (Fig. 1A) (20).

To identify putative dsRNA-binding domains in VILIP, a sequence comparison of the VILIP amino acid sequence with the dsRNA-binding domains of a number of dsRNA-binding proteins was performed. A single region in VILIP spanning amino acid residues 110 to 182 was found to be similar to both the Type 1 (full-length) and Type 2 (C-terminal) dsRNA-binding domains (Fig. 1A). The average amino acid similarity of VILIP to Type 2 dsRNA-binding domain to other Type 1 dsRNA-binding domains was only 11%, compared with an average 28.6% similarity among all the dsRNA-binding proteins analyzed (Fig. 1B). When comparing the VILIP dsRNA-binding domain to other Type 2 dsRNA-binding domains the average amino acid similarity is 12.8%, whereas the overall similarity among the Type 2 domains analyzed was 17.9% (Fig. 1C).

The level of similarity of the VILIP dsRNA-binding domain with other dsRNA-binding domains is not particularly high, yet important amino acid similarities were identified in key positions that are conserved among all dsRNA-binding domains (Fig. 1, B and C). For example, a pair of alanines found in all Type 1 and 2 dsRNA-binding domains is conserved in the VILIP dsRNA-binding domain (VILIP amino acid positions 174 and 175). There is also conservation of specific valine and threonine residues (VILIP positions 119 and 146, respectively). Taken together, these data suggest that VILIP dsRNA-binding domain is similar to the dsRNA-binding domain at the most conserved residues.

VILIP Specifically Binds dsRNA in a Ca²⁺-dependent Manner—Because of the low level of homology of the VILIP dsRNA-binding domain to other dsRNA-binding domains, it was important to determine whether the VILIP dsRNA-binding domain was functional. Recombinant VILIP protein was expressed and purified from E. coli (see “Experimental Procedures”). Polyacrylamide gel electrophoresis analysis shows that after purification, the recombinant VILIP is the only detectable peptide (Fig. 2A).

To determine whether VILIP binds to dsRNA, a Northwestern RNA-binding analysis was performed. Crude uninduced extract and purified recombinant VILIP were electrophoresed and transferred to membranes. The membrane-bound proteins...
were then denatured, slowly renatured, and allowed to bind 
$^{32}$P-labeled dsRNA (poly(I-C)) or single-stranded RNA 
(poly(C)).

Initially, Northwestern analysis showed very low levels of 
RNA binding to VILIP. However, EF-hand proteins undergo 
conformational transitions upon binding with Ca$^{2+}$ that often 
increase their a helical content, a conformation conducive for 
binding dsRNA (21–23). We therefore reasoned that Ca$^{2+}$ may 
be required by VILIP to assume a secondary structure that 
would allow dsRNA binding. Upon the addition of Ca$^{2+}$ to the 
renaturing step of the Northwestern assay, VILIP bound 
 dsRNA although there was no binding in the presence of EDTA
(Fig. 2B). VILIP binding of RNA was specific for dsRNA, be-
cause single-stranded poly(C) did not bind to VILIP in condi-
tions with or without Ca$^{2+}$ (Fig. 2B). There was no detectable 
binding of dsRNA in crude extracts from cells that were not 
induced to express recombinant VILIP.

Native Ca$^{2+}$-free VILIP Can Bind dsRNA upon the Addition of Ca$^{2+}$—During Northwestern binding assays, membrane-bound VILIP is denatured then renatured in either the presence or absence of Ca$^{2+}$ and subsequently allowed to bind dsRNA. Upon binding of Ca$^{2+}$, VILIP undergoes specific conformational changes (24). Therefore, we decided to determine whether the conformational change in native VILIP induced by Ca$^{2+}$-binding is accompanied by the ability to bind dsRNA.

VILIP was renatured under Ca$^{2+}$-free conditions and then 
allowed to bind $^{32}$P-labeled dsRNA in the presence of Ca$^{2+}$.

VILIP bound dsRNA when Ca$^{2+}$ was added during dsRNA 
binding at levels comparable to when Ca$^{2+}$ was added earlier 
during renaturation (Fig. 3). Therefore, Ca$^{2+}$-binding can acti-
vate native Ca$^{2+}$-free VILIP in such a way that it assumes a 
conformation that allows binding of dsRNA.

A VILIP Protein-dsRNA Complex Is Detected by Mobility 
Shift Assays—To further characterize dsRNA binding by 
VILIP, mobility shift analysis was performed. Increasing 
amounts of VILIP were allowed to bind to a constant concen-
tration of size-fractionated$^{32}$P-labeled dsRNA in the presence 
of Ca$^{2+}$. The resulting VILIP-dsRNA complexes were analyzed 
on native polyacrylamide gels.

Starting at a VILIP protein concentration of 100 ng/ml, a 
single large dsRNA-protein complex was observed binding to a 
200-bp dsRNA (Fig. 4A). By 500 ng/ml of VILIP, no unbound 
dsRNA was detected. Increasing amounts of VILIP caused a 
further increase of the dsRNA-protein complex signal but pro-
duced no additional complexes.

Quantification of $^{32}$P-labeled dsRNA in VILIP bound and 
unbound fractions allowed calculation of an apparent equilib-
rium dissociation constant ($K_d$) of $9.3 \times 10^{-6}$ M (Fig. 4B). In 
marked contrast, there was no VILIP binding to a 50-bp dsRNA and only low level binding to a 100-bp dsRNA indicating that

**FIG. 1.** VILIP contains one RNA-binding domain. A, mouse VILIP amino acid sequence showing the VILIP dsRNA-binding domain (boxed region). Underlining and Roman numerals indicate EF-hands. A comparison of the amino acid sequence of VILIP dsRNA with the Type 1 (B) and Type 2 (C) dsRNA-binding domains from other dsRNA-binding proteins show similarities in important residues. Boxed regions indicate sequence similarities with VILIP dsRNA-binding domain. Consensus sequence for VILIP dsRNA-binding domain is shown. Identical residues are listed in uppercase and similar residues in lowercase. The dsRNA-binding domains are: Dmstau-1–5, Drosophila staufen domains are required for maternal RNA localization; Hstrba-1–3, human TAR (trans-activating region)-binding protein domain binds to the HIV TAR RNA stem-loop; Pkr1 and 2, human dsRNA-dependent protein kinase is induced during viral defense; MmTIK-1 and -2, mouse TTK gene domains may be the mouse homolog of the human Pkr; Prvns34, porcine group C rotavirus ns34 protein is part of the viral replication complex; Sppac1 dm, Schizosaccharomyces pombe pac1 protein is a meiotic suppressor in the fission yeast S. pombe; Xrba-1–3, Xenopus RNA-binding protein domains are of unknown function isolated from a Xenopus laevis ovary cDNA library.
VILIP is a Ca\(^{2+}\)-dependent dsRNA-binding Protein

FIG. 2. Northwestern analysis of Ca\(^{2+}\)-dependent binding of dsRNA binding by VILIP. A, bacterial expression of VILIP. E. coli harboring the VILIP-expression construct were cultured and induced to express VILIP with isopropyl-1-thio-β-D-galactopyranoside. Cells were lysed and the recombinant VILIP purified with Ni-NTA chromatography (induced purified). Approximately 20 μg of recombinant VILIP was loaded. The amount of protein loaded on to the gel in the uninduced control (lane 1) corresponds to similar levels that would be present in the induced extract (lane 2) if not column purified. Lane 2 represents purified recombinant VILIP protein. VILIP was absent from unpurified and uninduced bacterial cultures (uninduced crude). B, Northwestern analysis of recombinant VILIP. Identical polyacrylamide gels with recombinant VILIP (induced purified) and uninduced crude bacterial extract (uninduced crude) were transferred to membranes and allowed to bind dsRNA (poly I-C) or single-stranded RNA (poly C), with or without Ca\(^{2+}\). Binding was detected only with dsRNA in the presence of Ca\(^{2+}\).

FIG. 3. Native Ca\(^{2+}\)-free VILIP can bind dsRNA upon the addition of Ca\(^{2+}\). VILIP was unable to bind dsRNA under Ca\(^{2+}\)-free renaturing and binding conditions (lanes 1 and 2). However, VILIP bound dsRNA with the same efficiency whether VILIP was renatured without or with Ca\(^{2+}\) (lanes 3 and 4, and 5 and 6, respectively) as long as Ca\(^{2+}\) was present during dsRNA binding.

VILIP requires dsRNA at least 200-bp long for efficient binding (Fig. 4, C and D). Similar experiments using in vitro synthesized RNA of various lengths also demonstrated VILIP’s dependence on RNA length for binding (data not shown).

Finally, the VILIP-dsRNA complex did not form with single-stranded RNA, because there was no shift when the dsRNA was denatured prior to the gel shift analysis (Fig. 5). The formation of the VILIP-dsRNA complex was specifically dependent on Ca\(^{2+}\) because it was undetectable in the presence of EDTA (no Ca\(^{2+}\)) or another divalent cation (Mg\(^{2+}\)) (Fig. 5).

VILIP binds Neurotrophin Receptor trkB mRNA in a Ca\(^{2+}\)-dependent Manner—The mRNA for the high affinity neurotrophin receptor (trkB) has been shown to be localized to the dendrites of hippocampal neurons in an activity-dependent manner (13). Given that such neuronal activity generates a concomitant increase in intracellular Ca\(^{2+}\) and VILIP is also expressed in the hippocampus (25), we decided to determine whether VILIP can interact with trkB mRNA. Gel shift assays showed that a VILIP-trkB mRNA complex is formed with increasing amounts of VILIP and that this interaction requires Ca\(^{2+}\) (Fig. 6, A and B).

In many localized mRNAs, cis-acting signals in the mRNA 3’-UTR interact with RNA-binding proteins and mediate mRNA localization (for review, see Ref. 26). To determine whether VILIP binds the trkB 3’-UTR, gel shift experiments using RNA containing only the trkB mRNA 3’-UTR were performed. VILIP binds to the trkB mRNA 3’-UTR in a Ca\(^{2+}\)-dependent manner (Fig. 6C), but does not interact with the trkB mRNA coding region (Fig. 6D). In addition, the VILIP-trkB mRNA complex will not form when trkB mRNA is denatured, and it specifically requires Ca\(^{2+}\) as the divalent cation for binding (data not shown). Taken together, these data indicate that VILIP in the presence of Ca\(^{2+}\) binds trkB mRNA by interacting specifically with dsRNA sequences in the trkB mRNA 3’-UTR, whereas it does not bind the trkB mRNA coding region.

DISCUSSION

In this study, we showed that the EF-hand Ca\(^{2+}\)-binding protein, VILIP, contains a copy of the dsRNA-binding domain characteristic of such dsRNA-binding proteins as Drosophila staufen and human PKR. Despite the low amino acid similarity of the VILIP dsRNA-binding domain with other dsRNA-binding domains, we demonstrated that VILIP specifically binds to dsRNA in a Ca\(^{2+}\)-dependent manner. Because two EF-hands (III and IV) overlap the dsRNA-binding domain, the low amino acid conservation in the VILIP dsRNA-binding domain may result from selective pressure to maintain the ability to bind both dsRNA and Ca\(^{2+}\). Therefore, VILIP dsRNA-binding domain may represent a minimal dsRNA-binding domain that still remains functional.

VILIP is unique, because it contains both Ca\(^{2+}\)-binding EF-hands and a dsRNA-binding domain. One possible mechanism for the Ca\(^{2+}\) induction of VILIP binding of dsRNA is through conformational changes in the EF-hands Ca\(^{2+}\)-binding domains. EF-hands are helix-loop-helix motifs that are typically
paired to interact and produce a single globular domain (for review, see Ref. 21). Binding Ca\(^{2+}\) by the EF-hands of VILIP causes significant conformational changes (24). Furthermore, Ca\(^{2+}\) binding of recoverin, another EF-hand protein, increases recoverin \(\alpha\) helical content (22). RNA binding has been shown to be mediated by \(\alpha\) helical-enriched proteins, and \(\alpha\) helices have been demonstrated for the structure of the dsRNA-binding domain (27–30). Furthermore, mutations that destabilize the \(\alpha\) helices eliminate dsRNA binding (8). Therefore, Ca\(^{2+}\)-binding by VILIP may cause conformational changes that involve increased \(\alpha\) helical content that can then mediate dsRNA-binding. Because two EF-hand domains overlap with the VILIP dsRNA-binding domain, Ca\(^{2+}\) binding may affect the conformation of the VILIP dsRNA-binding domain directly resulting in binding dsRNA. Site-directed mutagenesis will be needed to dissect the precise roles of the Ca\(^{2+}\)-binding EF-hands and the dsRNA-binding domain in the formation of the VILIP-dsRNA complex.

It is interesting that VILIP requires at least 200-bp dsRNA for stable binding, whereas other dsRNA-binding proteins can bind dsRNA as small as 16 bp (31). It is unlikely that VILIP can only recognize a minimum binding site 200-bp long, because we

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**Fig. 4.** VILIP forms a single large complex with dsRNA in mobility gel shift assay. A, by increasing the amounts of recombinant VILIP with a constant concentration of \(^{32}\)P-labeled 200-bp dsRNA, a VILIP-dsRNA complex was detected during polyacrylamide gel electrophoresis. B, the apparent equilibrium dissociation constant was calculated by plotting the radioactivity in the bound RNA (bound RNA/bound RNA + unbound RNA) and the log of the concentration of VILIP. Mobility gel shift assays with dsRNAs of 50 bp (C) and 100 bp (D) and increasing amounts of VILIP protein. VILIP does not bind the 50-bp dsRNA and binds the 100-bp dsRNA weakly, indicating that VILIP requires at least 200-bp dsRNA for efficient binding.

**Fig. 5.** Formation of the VILIP-dsRNA complex is dependent on Ca\(^{2+}\) and dsRNA. A, mobility gel shifts performed without (EDTA) or with Ca\(^{2+}\) and increasing amounts of VILIP protein shows that the VILIP-dsRNA complex is formed only in the presence Ca\(^{2+}\). B, the VILIP-dsRNA complex does not form with denatured RNA (ssRNA) or with Mg\(^{2+}\).
demonstrated VILIP binding to 100-bp dsRNA. Mobility gel shift experiments showed that VILIP appears to bind to 200-bp dsRNA as one complex. Even at low VILIP concentrations, there were no additional smaller complexes. VILIP concentrations may need to be significantly higher enough to allow VILIP-VILIP interactions that would then result in binding dsRNA. Although the VILIP-VILIP complex can bind a 100-bp dsRNA, a larger dsRNA may be able to interact with more of the dsRNA-binding domains in the complex and form a more stable VILIP-dsRNA complex. Although the measured dissociation constant for VILIP-dsRNA binding is low, the in vivo situation encountered by VILIP may be different from the in vitro assay. For example, other proteins may be involved in the formation of the VILIP-dsRNA complex as part of a ribonucleoprotein complex that often forms with localized RNAs (32).

We have demonstrated that VILIP interacts with the neuronal trkB mRNA in a Ca\(^{2+}\)-dependent manner. Because VILIP does not bind to the trkB mRNA coding region or other mRNAs (data not shown), the trkB mRNA-VILIP interaction apparently does not result from binding a nonspecific dsRNA structure. VILIP may be recognizing specific sequences present in the trkB mRNA 3\'-UTR to mediate its binding. It is interesting to note that sequence alignment of human, rat, and mouse trkB mRNA 3\'-UTRs reveals a region of high homology about 600 nucleotides in length in all three mRNAs that contains significant RNA stem-loop formation as determined by Mfold computer analysis (data not shown). Further study will determine whether this region of the trkB mRNA 3\'-UTR is functional in VILIP binding.

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**FIG. 6.** VILIP binds the 3'-UTR of the trkB mRNA in a Ca\(^{2+}\)-dependent manner. A, increasing amounts of recombinant VILIP formed a mobility gel shift complex with a constant concentration of 32P-labeled full-length trkB mRNA (B). Formation of the VILIP-full-length trkB mRNA complex requires Ca\(^{2+}\) (C). VILIP specifically interacts with the trkB mRNA 3'-UTR in a Ca\(^{2+}\)-dependent manner, but does not bind an mRNA encoding only the trkB mRNA coding region (D).