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A homolog of FBP2/KSRP binds to localized mRNAs in *Xenopus* oocytes

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SUMMARY

A *Xenopus* oocyte expression library was screened for proteins that bind to the 340-nucleotide localization element of Vg1 mRNA. Four different isolates encoded a *Xenopus* homolog of the human transcription factor, FUSE-binding protein 2 (FBP2). This protein has been independently identified as the splicing regulatory factor KSRP. The only significant difference between the *Xenopus* protein, designated VgRBP71, and KSRP is the absence of a 58 amino acid segment near the N-terminal of the former. In vivo binding assays show that VgRBP71 is associated with mRNAs localized to either the vegetal or animal hemispheres, but was not found with control mRNAs. Unlike other factors that bind to the localization element of Vg1 mRNA, VgRBP71 does not accumulate at the vegetal cortex with the mRNA; rather, it is present in the nucleus and throughout the cytoplasm at all stages of oogenesis. Cytoplasmic VgRBP71 appears to be most concentrated at the cell cortex. VgRBP71 interacts with Prrp, another protein that binds to the Vg1 localization element; this association does not require the presence of Vg1 mRNA.

Key words: FUSE-binding protein, KSRP, Prrp, RNA localization, Vg1 mRNA, VgRBP71, ZBP2, *Xenopus*

INTRODUCTION

The strict temporal and spatial localization of maternal mRNA underlies the asymmetric development of oocytes and embryos (Kloc et al., 2002; Lipshitz and Smibert, 2000; Yaniv and Yisraeli, 2001). *Xenopus* Vg1 mRNA encodes a member of the transforming growth factor β (TGFβ) family that is needed for induction of dorsal mesoderm during embryogenesis (Dale et al., 1993; Thomsen and Melton, 1993; Weeks and Melton, 1987). Commencing at stage III of oogenesis, Vg1 mRNA, which is uniformly dispersed in the cytoplasm, is transported to the vegetal pole and accumulates in a condensed layer along the entire vegetal cortex (Melton, 1987). This process is determined by a *cis*-acting element spanning a 340 nucleotide (nt) region in the 3′UTR designated the Vg1 localization element (VLE) (Mowry and Melton, 1992).

It appears that the majority of localized mRNAs in a variety of cells from yeast to neurons are organized in large particles (Bertrand et al., 1998; Ferrandon et al., 1994; Hoek et al., 1998; Ross et al., 1997; Schroeder and Yost, 1996; Shen et al., 1998; Wilhelm et al., 2000). This observation suggests that several *trans*-acting factors are required to support this process, which usually requires concomitant translational repression of the mRNA, at least until localization is complete. For example, a minimum of five different proteins are required for the localization of *bicoid* mRNA in *Drosophila* oocytes and an equal number of *trans*-acting factors are involved in the localization of *Ash1* mRNA in yeast (Mohr and Richter, 2001). Six different polypeptides can be crosslinked to the localization element of Vg1 mRNA upon UV irradiation, suggesting that a multi-component complex also mediates transport of this RNA (Mowry, 1996). Three proteins that bind to the VLE have been identified. The first, alternatively called Vg1 RBP or Vera, is a *Xenopus* homolog of chicken zipcode-binding protein, which binds to and determines localization of β-actin mRNA in fibroblasts (Deshler et al., 1998; Havin et al., 1998). Vg1 RBP/Vera appears to be necessary for the association of Vg1 mRNA with microtubules (Elisha et al., 1995) and is itself associated with a subcompartment of the endoplasmic reticulum (Deshler et al., 1997). A second factor, which is a homolog of hnRNP I, binds to and colocalizes with Vg1 mRNA (Cote et al., 1999). Mutations in the VLE that prevent this interaction abolish localization of the RNA. A proline-rich protein, dubbed Prrp, binds to Vg1 mRNA and is concentrated in the vegetal cortex of stage III/IV oocytes (Zhao et al., 2001). It also interacts with the actin-associated protein profilin. Prrp can bind to other mRNAs (e.g., VegT, An1, An3) that use the late pathways for localization in *Xenopus* oocytes, but does not bind to mRNAs that use the early pathway. The exact functions of these three factors have not been determined.

Identification of all the components of the localization machinery is a necessary step towards understanding how a particular mRNA is delivered to its proper intracellular destination. We have screened a *Xenopus* oocyte expression library for proteins that can bind to the localization element of Vg1 mRNA and have identified the *Xenopus* homolog of a protein that has been implicated in an extraordinary number of activities. Human far upstream element (FUSE) binding protein, FBP, is a transcriptional activator of the *Myc* gene

(Duncan et al., 1994). Two paralogs, FBP2 and FBP3, have been subsequently identified, establishing the existence of a family of putative transcription factors that differ substantially at the nucleotide level, but show appreciable similarity in amino acid sequence (Davis-Smyth et al., 1996). Although FBP was first identified as a single-stranded DNA binding protein, it contains four K-homology (KH) RNA-binding domains, and members of the FBP family have now been found in a variety of complexes that regulate alternative splicing, stability and even editing of mRNA (Grossman et al., 1998; Irwin et al., 1997; Lellek et al., 2000; Min et al., 1997). Of particular significance, an FBP2 homolog binds to the zipcode element of β-actin mRNA and affects its localization in fibroblasts and neurons (Gu et al., 2002).

MATERIALS AND METHODS

Several of the experimental procedures have been described previously, including the screen of the λgt22A cDNA expression library, subcloning and sequencing of phage DNA, cRACE identification of the 5′ end of mRNA, RNA isolation and northern blot analysis (Zhao et al., 2001).

Plasmids and nucleic acids

The plasmid pGEX-4T-2-c4 carries the complete coding sequence of VgRBP71 inserted into the *Bam*HI and *Eco*RI restriction sites of the vector. This plasmid was used for the expression of VgRBP71 as a glutathione *S*-transferase fusion protein. A DNA fragment generated by PCR amplification, which encodes the first 449 amino acids of VgRBP71, was inserted into the *Nde*I and *Xho*I sites of pET-23b for expression of the truncated polypeptide with a C-terminal polyhistidine tag. The plasmid vectors used in the yeast two-hybrid assays were purchased from Clontech. The ^{32}P-labeled probe for the northern blot assay was prepared from the *Sal*I-*Not*I restriction fragment of a VgRBP71 cDNA clone (pGEMEX-λ4) using the DECAprime II Random Priming DNA Labeling Kit (Ambion). Plasmids containing the localization elements of VegT mRNA (nt 1849-2294) (Kwon et al., 2002) and Xcat-2 mRNA (nt 393-748) (Zhou and King, 1996) or the entire 3′UTR of An1 mRNA (nt 2268-2789) were constructed using RT-PCR reactions. Total oocyte RNA was isolated from mixed staged oocytes using RNAwiz (Ambion). First strand DNA synthesis used a combination of random hexamers (Promega) and T_{16} (Roche) primers along with Thermoscript reverse transcriptase (Invitrogen). The resulting cDNA was amplified with specific primers and Taq DNA polymerase. The PCR products were ligated into pCR2.1-TOPO (Invitrogen). The An1 fragment was subsequently subcloned into the *Bam*HI and *Xho*I sites of pBSKSII(+) (Stratagene). RNA was synthesized by run-off transcription using phage T7 RNA polymerase and plasmids linearized with either *Bam*HI (VegT and Xcat-2) or *Xho*I (An1).

Antibody

Antiserum was prepared against the GST-VgRBP71 fusion protein and antibody purified by affinity chromatography. A polypeptide containing the first 449 amino acids of VgRBP71 (10 mg) was coupled to a HiTrap column (Amersham Pharmacia); serum (10 ml) was applied to the column and bound antibody eluted as described previously (Harlow and Lane, 1988). The affinity-purified antibody showed very little cross-reaction with proteins in extract prepared from oocytes. Most importantly, there is no cross-reaction with the *Xenopus* homolog of FBP, which has a predicted molecular mass of 64.8 kDa. Anti-hemagglutinin antibody was purchased from Clonetech.

RNA-binding assays

In vivo assays were performed essentially as described before (Zhao et al., 2001), except that the VgRBP71 RNP complexes were immunoprecipitated from oocyte homogenate using affinity-purified, polyclonal antibody prepared against VgRBP71. In vitro RNA-binding assays (10 μl) were in buffer containing 40 mM HEPES (pH 7.5), 100 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 5% glycerol, 0.2 mg/ml yeast tRNA, 0.2 mg/ml heparin and 4 U RNasin (Promega). Each reaction contained 1 nM internally radiolabeled VLE RNA and the indicated amount of unlabeled competitor RNA. VgRBP71 and RNA was incubated for 2 hours on ice and then separated by electrophoresis on 7% polyacrylamide (40:1, acrylamide/bisacrylamide) gels at 200 V at ambient temperature.

Confocal microscopy

Albino oocytes were treated with type II collagenase (1 mg/ml) in OR2– solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM Na_2HPO_4, 5 mM HEPES, pH 7.8) (Opresko, 1991) for 1 hour at 28°C, and rinsed in Modified Barth's Solution-High Salt (MBSH: 110 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $NaHCO_3$, 0.5 mM Na_2HPO_4, 15 mM Tris-HCl, pH 7.6). The oocytes were fixed for at least two hours in MEMFA (2 mM EGTA, 1 mM $MgSO_4$, 3.7% formaldehyde, 0.1 M MOPS, pH 7.4) (Hemmati-Brivanlou et al., 1990) and rinsed with four changes of TBS (137 mM NaCl, 2.7 mM KCl, 25 mM Tris-HCl, pH 7.4). The oocytes were then equilibrated in TBSN (TBS + 0.2% NP-40) for 5 minutes. The equilibration buffer was removed and the oocytes were incubated in 0.5 ml TBSN containing 2% BSA and 10 μl rabbit anti-VgRBP71 antibody for 24 hours at room temperature with gentle shaking. Control oocytes were processed in the same fashion in the absence of the primary antibody. Following the incubation with antibody, oocytes were washed in TBS for 24 hours; the wash buffer was changed every 6-8 hours. The oocytes were then stained for 24 hours in 0.5 ml TBSN containing 2% BSA and 5 μl goat anti-rabbit antibody conjugated to Alexa Fluor 568 (Molecular Probes). Unbound secondary antibody was removed by washing the oocytes at room temperature with gentle shaking for a total of 24 hours in TBS with the buffer changed every 6-8 hours. The oocytes were then dehydrated using several changes of methanol, cleared with benzyl benzoate:benzyl alcohol (2:1, v/v) and analyzed by confocal microscopy (Dent et al., 1989). Oocytes to be bisected were collected, washed, fixed and rinsed as described above. Individual oocytes were placed on a glass slide and sliced in half with a #10 scalpel using a single quick motion. A drop of buffer was then added to the oocyte halves, which were then transferred to a 24-well plate containing TBS. The samples were rinsed to remove debris from the sectioning and then processed in the same fashion as whole oocytes. A BioRad MRC 1024 scanning confocal system attached to a Nikon Diaphot 200 inverted microscope was used to collect images of stained oocytes.

Yeast two-hybrid assays

Matchmaker Two-Hybrid System 3 was used to screen a *Xenopus* oocyte cDNA library cloned into the vector pACT2 (Clontech). The bait plasmids contained the complete, the N-terminal (amino acids 1-251) or the C-terminal (amino acids 242-360) coding sequences of Prrp inserted into the vector, pGBKT7. The complete coding sequence of VgRBP71, the N-terminal region encompassing the four KH domains (amino acids 1-449) or the C-terminal domain containing the tyrosine-rich repeats (amino acids 450-672) were inserted into the prey vector, pGADT7. Protein expression was verified in each case by western blot assays using the Myc or HA epitope encoded in the bait or prey vector, respectively. The bait plasmid carrying Prrp exhibited no autonomous activation of reporter genes on its own in the host strain (AH109). However, this plasmid does slow the growth of these cells, so bait and prey plasmids were transformed together. The transformation mixtures were spread on plates containing synthetic drop-out (SD) medium lacking adenine, histidine, leucine and tryptophan and kept at 30°C for 4 days. In the case of the library

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KSRP       MSDYSTGGPPPGPPPPAGGGGGAGGAGGGPPPGPPG-----AGDRGGGGPCGGGPGGGS- 54
ZBP2       MEI-STPDFGFG-TEDSSAQQSANRAIPQPVPAPAFPLKETASDTGGTAPTFGTLQDNIN 58
VgRBP71    MSDYPGAQS---------------------------------------------------- 9

KSRP       ---------AGGPSQPPGGGGP---GIRKDAFADAVQRARQIAAKIGGDAATTVNNSTPD 102
ZBP2       ELCLRYQTVCSEGRDGTGGGGPPGGGIRKDAFADAVQRARQIAAKIGGDAATTVNNSTPD 118
VgRBP71    ------------------------GNRKDAFADAVQRARQIAAKIGGDVTGGVNN-TPE 43

KSRP       FGFGGQKRQLEDG--------DQPESKKLASQGD------------------------- 128
ZBP2       FGFGGQKRQLEDG--------DQPESKKLAAQGDCEYGPRGGAPAAPPERSGPVGDPPGP 170
VgRBP71    FNFGGQKRQLEDGDMFGFPSPDQPECKKLATQPE------------------------- 77

KSRP       ---------------------SISSQLGPIHPPPR-TSMTEEYRVPDGMVGLIIGRGGEQ 166
ZBP2       PRAERGRRPPPALTGGALPSAALPPQLGPMHPPPRSTTVTEEYRVPDGMVGLIIGRGGEQ 230
VgRBP71    ---------------------SMPPQLAPVH-PPRSSSMTEEYRVPDGMVGLIIGRGGEQ 115

KSRP       INKIQQDSGCKVQISPDSGGLPERSVSLTGAPESVQKAKMMLDDIVSRGRGGPPGQFHDN 226
ZBP2       INKIQQDSGCKVQISPDSGGLPERSVSLTGSPEAVQKAKLMLDDIVSRGRGGPPGQFHDY 290
VgRBP71    INKIQQESGCKVQISPDSGGMPERIVSLTGNPDAVQKAKMLLDDIVLRGRGGPPSQFHDS 175

KSRP       ANGGQNGTVQEIMIPAGKAGLVIGKGGETIKQLQERAGVKMILIQDGSQNTNVDKPLRII 286
ZBP2       ANG-QNGTVQEIMIPAGKAGLVIGKGGETIKQLQERAGVKMIFIQDGSQNTNVDKPLRII 349
VgRBP71    SNG-QNGSLQEIMIPAGKAGLIIGKGGETIKQLQERAGVKMILIQDGSQNTNMDKPLRIV 234

KSRP       GDPYKVQQACEMVMDILRERDQGGFGDRNEYGSRIGGG-------IDVPVPRHSVGVVIG 339
ZBP2       GDPYKVQQACEMVMDILRERDQGGFGDRNEYGSRIGGG-------IDVPVPRHSVGVVIG 402
VgRBP71    GEPFKVQQACEMVMDLLKERDQPNF-DRNEYGTRGGGGGGGGGGGIDVPVPRHSVGVVIG 293

KSRP       RSGEMIKKIQNDAGVRIQFKQDDGTGPEKIAHIMGPPDRCEHAARIINDLLQSLRSGPPG 399
ZBP2       RSGEMIKKIQNDAGVRIQFKQDDGTGPEKIAHIMGPPERCEHAARIINDLLQSLRSGPPG 462
VgRBP71    RSGDMIKKIQNDAGVRIQFKQDDGTGPDKIAHIMGPPDRCEHAASIISDLLQSLRTGPPG 353

KSRP       PPGGPGMPPGGRGRGRGQGNWGPPGGEMTFSIPTHKCGLVIGRGGENVKAINQQTGAFVE 459
ZBP2       PPG-HGMPPGGRGRGRGQGIWGPPGGEMTFSIPTHKCGLVIGRGGENVKAINQQRGAFVE 521
VgRBP71    PPG-PGMPPGGRGRGRGQGPWGPPGGEMTFSIPTHKCGLVIGRGGENVKAINQQTGAFVE 412

KSRP       ISRQLPPNGDPNFKLFIIRGSPQQIDHAKQLIEEKIEGPLCPVGPGPGG---PGPAGPMG 516
ZBP2       ISRQLPPNGDPNFKLFIIRGSPQQIEHAKQPIEEKIEGPLCPVGPGPGPGGPPGPAGPMG 581
VgRBP71    ISRQPPPNGDPNFKMFIIRGNPQQIDHAKQLIEEKIEGPLCPIGPGPPG---PGPAGPMG 469

KSRP       PFNPGPFNQGPPGAPPHAGGPPP--HQYPPQGWGNTYPQW-QPPAPHDPSKAAAAAADPN 573
ZBP2       PFNPGPFNQGPP------GGPPP--HQYPPQGWGNTYPQW-QPPAPHDPSK-AAAAADPN 631
VgRBP71    PYNPGPYQPGPPGGPPHQGPPPPAAHQYPPQGWGSTYPQWGQPPAAHDPTK-PPAPTDPS 528

KSRP       AAWAAYYSHYYQQPPGPVPGPAPAPAAPPAQGEPPQPPPTG-QSDYTKAWEEYYKKIGQQ 632
ZBP2       AAWAGYYSHYYQQPPGPVPGQPPAPTAPPVQGEPPQPPPTG-QSDYTKAWEEYYKKIGQQ 690
VgRBP71    AAWAAYYSHYYQQPSAPVPGQPPAVPAPPPQGEPPQQPPSANQPDYTKAWEEYYKKMGQQ 588

KSRP       PQQPGAPPQQDYTKAWEEYYKKQ--------AQVATGGGPGAPPGSQPDYSAAWAEYYRQ 684
ZBP2       PQQPGAPPQQDYTKAWEEYYKKQA-------AQVATGGGPGAPPGPQPDYSAAWAEYYRQ 743
VgRBP71    TPQPAGQP--DYTKAWEEYYKKQAATPGAPAAAVAAAAATAAPPAAQPDYSAAWAEYYRQ 646

KSRP       QAAYYGQTPGPGGPQPPPTQQGQQQAQ 711
ZBP2       QAAYYGQTPGAAGPAPPPTQQGQQ-AQ 769
VgRBP71    QAAYYGQAPGAPPTQPPSAQPGPQ-AQ 672
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Fig. 1. The predicted amino acid sequence of VgRBP71. The sequence of the *Xenopus* protein is compared with human KSRP and chicken ZBP2. The KH domains are underlined and the four repeated tyrosine motifs are indicated in bold. The entire nucleotide sequence, including flanking UTRs, can be found at DDBJ/EMBL/GenBank Accession Number, AF533513.

screen, colonies were transferred onto SD medium lacking leucine and tryptophan in order to eliminate multiple prey plasmids within any given colony. After this second round of selection, colonies were spotted onto SD plates for a colony lift assay to measure β-galactosidase activity (Breeden and Nasmyth, 1985). Colony lift assays were performed in triplicate, in order to estimate the strength of the interaction between individual pairs of proteins. Prey plasmids identified in the screen were initially classified based upon restriction enzyme digestions. Representative clones from these groups were sequenced, and from this latter group several were chosen and used as probes in southern blot assays. Several of the positives clones identified by southern hybridization were confirmed by DNA sequencing.

Coimmunoprecipitation assays

VgRBP71 carrying an HA epitope (pGADT7-KSRP) and Prrp carrying a *Myc* epitope (pGBKT7-Prrp) were synthesized using the TnT T7 Coupled Reticulocyte Lysate System (Promega). The reactions were run at 30°C for 90 minutes and used directly for immunoprecipitation reactions. Aliquots of each protein synthesis reaction (15 μl) were added to 150 μl of NET-2 buffer (150 mM NaCl, 0.05% NP-40, 50 mM Tris-HCl, pH 7.4) containing protein-A Sepharose coated with rabbit anti-HA antibody (Clontech). Negative controls lacked either VgRBP71 or anti-HA antibody. The effect of RNA on the VgRBP71:Prrp interaction was tested by treating aliquots of the two proteins with 1 μl RNase A (10 mg/ml) for 10 minutes at 37°C prior to incubation with the antibody coated protein-A Sepharose beads. The protein mixtures were incubated with the beads for 1.5 hours at 4°C with end-to-end rotation. The beads were collected by brief centrifugation, washed three times with 200 μl NET-2 buffer containing 1 mM PMSF and 1 μg/ml pepstatin. The samples were suspended in SDS/dye solution, placed at 100°C for 5 minutes, and separated on a 10% SDS polyacrylamide gel. The proteins were transferred to a nitrocellulose filter that was exposed to X-ray film. For co-immunoprecipitation assays from oocyte extract, [^{35}S]-labeled Prrp was synthesized from pGBKT7-Prrp in reticulocyte lysate. Approximately 100 stage III/IV oocytes were injected with 25 nl of reticulocyte lysate and incubated overnight in MBSH solution at 18°C. Oocytes were homogenized in NET-2 buffer and cleared in a microcentrifuge. Equal amounts of lysate were incubated with protein A-Sepharose beads carrying affinity-purified VgRBP71 antibody or with protein A-Sepharose beads alone for 2 hours at 4°C with gentle rotation. The supernatant was removed and the beads were washed four times with NET-2 buffer. The samples were processed as described for the in vitro assays.

RESULTS

A homolog of FBP2/KSRP binds to the localization element of Vg1 mRNA

A 453 nucleotide transcript containing the vegetal localization element (VLE) of Vg1 mRNA, labeled internally with [^{32}P], was used to screen an oocyte expression library for proteins that can specifically bind to this RNA. Five of the resulting ten positive clones encode a novel proline-rich protein that can interact with profilin and, perhaps, other members of the cytoskeleton (Zhao et al., 2001). Four of the remaining clones are independent isolates of FUSE-binding protein 2 (FBP2) and one clone corresponds to the founding member of this family, FBP. The open reading frame (ORF) of the *Xenopus* FBP2 homolog encodes a protein of 672 amino acids with a predicted molecular mass of 71 kDa (Fig. 1). The *Xenopus* and human sequences have 76% identity and 82% similarity. A 43

nucleotide leader sequence precedes the ORF in the *Xenopus* transcript.

FBP was first identified as a single-stranded DNA-binding protein that binds to an upstream control element (FUSE) of the human *MYC* gene (Duncan et al., 1994). The central region of the protein has four KH domains, which are capable of binding to either RNA or single-stranded DNA. The C-terminal region of FBP2 has four repeats of a tyrosine-rich motif that can function independently as a transcriptional activation domain (Davis-Smyth et al., 1996). There are at least three members of the FBP family that exhibit appreciable similarity in amino acid sequence, but that differ substantially at the nucleotide level due to differences in codon usage (Davis-Smyth et al., 1996).

FBP2 has been independently identified in a human neural cell line as a splicing regulatory factor called KSRP, which is a component of a multiprotein complex that forms on an intronic splicing enhancer in *Src* pre-mRNA (Min et al., 1997). KSRP has a segment of 58 amino acids beginning at residue 9 that is not present in the *Xenopus* sequence. Whether this is a true difference between the human and *Xenopus* FBP2 genes or is a consequence of alternative splicing needs to be established, as the presence or absence of this sequence could determine the specialized functions of these proteins. The 58 amino acid sequence specific to KSRP was not found in a search of the *Xenopus* EST database. The complete sequence of human FBP2 has not been reported, so it is not known whether this N-terminal segment is present in this form of the transcription factor. Because of this difference between the *Xenopus* protein and KSRP, we refer to the former as VgRBP71 (Vg1 RNA-binding protein 71) in accordance with the convention used to designate other proteins that bind to the VLE.

Gu et al. (Gu et al., 2002) recently reported that a chicken homolog of FBP2/KSRP binds to the localization element (the zipcode) of β-actin mRNA in fibroblasts and neurons. Similar to the case with VgRBP71, the only substantial differences between the chicken protein, called zipcode binding protein 2 (ZBP2), and KSRP occur in the N-terminal regions. Whereas VgRBP71 is missing a 58 amino acid segment relative to KSRP, ZBP2 has a 47 amino acid insertion (Fig. 1). The amino acid sequences of VgRBP71 and ZBP2 have 73% identity and 78% similarity.

A search of the sequence databases using BLAST2 revealed that a rat protein, which binds to the localization element in the mRNA encoding microtubule-associated protein 2 (MAP2), is also a homolog of FBP2. MARTA1, along with a second protein, binds to a targeting element in the 3′UTR of MAP2 mRNA that mediates localization to dendritic regions in primary neurons (Rehbein et al., 2000). The rat sequence has 97% and 78% identity with the human and *Xenopus* sequences, respectively. The important conclusion to be drawn from these observations is that the cell-type specific localization of mRNAs encoding unrelated proteins, nonetheless, uses some conserved factors common to this process.

The remaining single clone isolated in the screen of the library is 81% identical to human FBP, the founding member of this family. The nucleotide sequence of the *Xenopus* FBP homolog identified in this work can be found at DDBJ/EMBL/GenBank accession number AF533514. Whereas the overall amino acid identity of *Xenopus* FBP and FBP2 is only 57%, the sequences within the four KH domains of the two proteins average about 70% identity. This may explain why a clone of the former was identified in the VLE-binding screen: FBP and FBP2 possibly share some overlapping RNA-binding specificity.

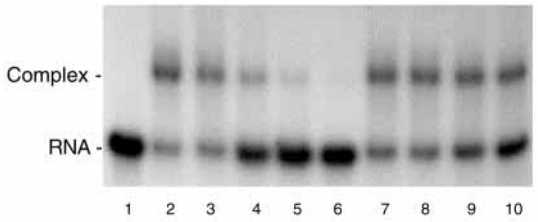

Fig. 2. Mobility shift assays for binding of VgRBP71 to VLE RNA. GST-VgRBP71 fusion protein (8 nM) and internally radiolabeled VLE RNA (1 nM) were incubated with 0, 1, 5, 10 or 25 nM unlabeled VLE RNA as a specific competitor (lanes 2-6, respectively), or 5, 25, 50 or 100 nM noncognate RNA (lanes 7-10, respectively). Lane 1 contains VLE RNA only.

VgRBP71 binds to the VLE with high affinity and specificity

The goal of the screen was to identify proteins that bind to the localization element of Vg1 mRNA. The affinity and specificity of binding to VLE RNA was determined using mobility shift assays with VgRBP71 expressed as a fusion protein with glutathione S-transferase. The dissociation constant for the VgRBP71-VLE complex was estimated from the concentration of protein needed to reach half-saturation in binding titration assays (data not shown). The apparent K_d in optimized buffer conditions is ~5 nM. The specificity of binding was tested by incubating a constant amount of VgRBP71 and radiolabeled VLE RNA with increasing concentrations of competing, unlabeled VLE RNA (Fig. 2, lanes 2-6) or a 355 nucleotide non-cognate RNA (lanes 7-10) transcribed from the plasmid pBSIISK. VLE RNA competes efficiently against itself in this assay; whereas, in the presence of a 100-fold excess of non-cognate RNA, ~55% of the VgRBP71-VLE complex still remains intact. These assays establish that the interaction is specific and that binding affinity is comparable with other RNA-binding proteins that have KH domains (Buckanovich and Darnell, 1997; Chkheidze et al., 1999; Lin et al., 1997).

Temporal expression of VgRBP71

There are two temporally distinct pathways for the localization of vegetal RNAs in *Xenopus* oocytes (Forristall et al., 1995; Kloc and Etkin, 1995). The early pathway operates during stages I and II of oogenesis; whereas, the late pathway, used by Vg1 mRNA, is active during stages III and IV. Oocytes were separated into three groups according to the Dumont stages (Dumont, 1972) and the levels of mRNA encoding VgRBP71 measured by northern hybridization (Fig. 3A). The amount of VgRBP71 mRNA in stage I to stage II oocytes is low; however, there is a substantial increase at stage III to stage IV that is maintained for the remainder of oogenesis. We observed a similar increase in the level of Prrp mRNA at stage III (Zhao et al., 2001). The single transcript seen in the northern blot is approximately 2700 nucleotides, which matches the length expected from sequencing and cRACE determinations.

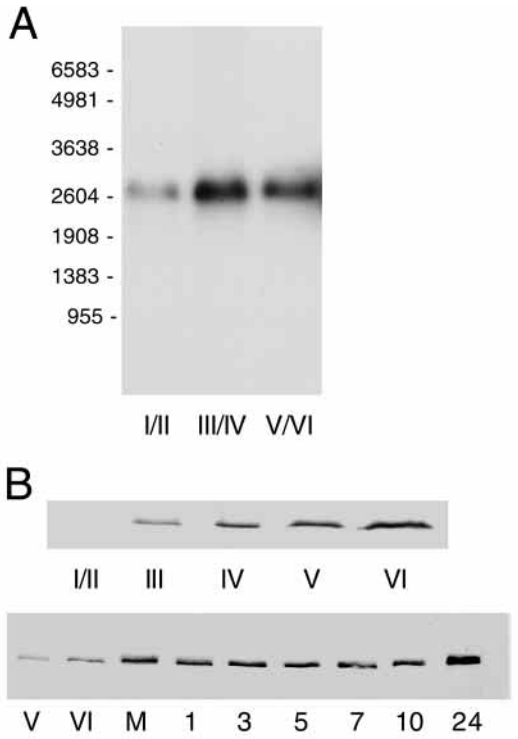

Fig. 3. Temporal expression of VgRBP71. (A) Oocytes were separated into three groups according to the designated Dumont stages and total RNA isolated for northern blot analysis. Each lane contains 10 oocyte-equivalents of RNA. The positions of RNA size standards (nt) are indicated. (B) Staged oocytes or embryos were homogenized and two oocyte/embryo equivalents were run per lane in a western blot assay. The numbers below each lane refer to the developmental stage of the oocytes (Dumont, 1972) or embryos (Nieuwkoop and Faber, 1956). M indicates stage VI oocytes matured by treatment with progesterone.

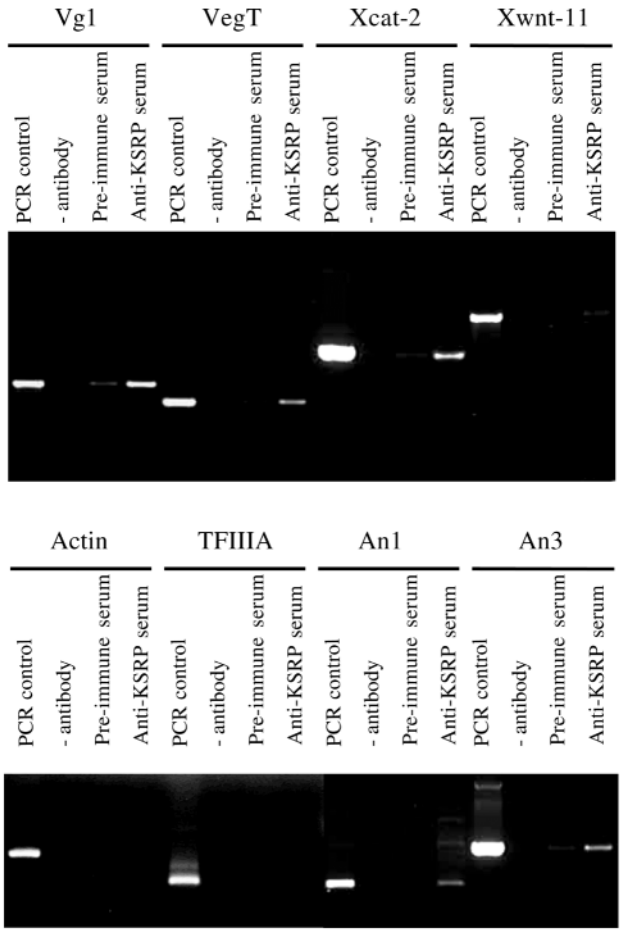

Fig. 4. In vivo RNA binding assays. VgRBP71 was immunoprecipitated from whole cell extract. RNA in the precipitate was converted to cDNA by reverse transcription and amplified by PCR using gene-specific primers denoted by the bars above the relevant lanes of the agarose gels. For each mRNA tested, a standard was generated using total oocyte mRNA as the template for RT-PCR (PCR control). Control reactions included precipitation with protein A-Sepharose resin (–antibody) and protein A-Sepharose resin that had been adsorbed with pre-immune serum (Pre-immune serum).

The levels of VgRBP71 protein were measured in western blot assays and they mirror those of the mRNA (Fig. 3B). There is very little VgRBP71 detected prior to stage III, at which time the amount of protein increases substantially and then remains fairly constant. Thus, there is a correlation between the temporal expression of VgRBP71 and the time at which localization of Vg1 mRNA takes place. Expression of VgRBP71 continues beyond oogenesis. There is another increase in the level of the protein upon oocyte maturation, after which it remains constant within the developing embryo. This observation is consistent with the assignment of other functions to orthologs of this protein that are not limited to oocytes, such as roles in alternative splicing in neurons (Min et al., 1997), as well as mRNA localization in fibroblasts and neurons (Gu et al., 2002). It is important to note that the antibody used in these studies was affinity purified and showed no detectable cross-reaction with *Xenopus* FBP.

VgRBP71 is associated with other localized mRNAs

We tested for the in vivo association of VgRBP71 with Vg1 mRNA and other localized mRNAs using an immunoprecipitation assay. Oocytes were manually disrupted and whole cell extract incubated with anti-VgRBP71 antibody immobilized on protein A-Sepharose. RNA specifically retained on these beads after multiple washes was detected by an RT-PCR assay using gene-specific primers (Fig. 4). A standard for each RNA was generated by amplifying cDNA prepared from total oocyte RNA (lanes marked PCR control). In no case did protein A-Sepharose beads alone yield a PCR product (–antibody). However, beads adsorbed with pre-immune serum in some cases did give a small amount of product that, nonetheless, could be distinguished from a specific signal.

These assays detected an association of VgRBP71 with Vg1 mRNA as well as with VegT mRNA, which also uses the late pathway for localization. Interestingly, there is also a distinct PCR product for *Xcat2* mRNA. This mRNA is normally localized through the early pathway; however, when it is injected into stage IV oocytes, it can be localized through the late pathway (Zhou and King, 1996). This latter observation indicates that *Xcat2* mRNA can associate with at least one or more *trans*-acting factors of the late pathway, one of which is

apparently VgRBP71. Xwnt11 mRNA, which is also localized to the vegetal pole by the early pathway, gave an extremely weak signal in this assay. Because the levels of VgRBP71 are low during the operation of the early pathway, it seems unlikely that it would be found with *Xwnt11* mRNA. The latter result brings up an important caveat concerning this assay. It cannot be assumed that immunoprecipitation in this case is quantitative. Recovery of individual complexes is likely to be influenced by several factors, including subcellular location, association with other cellular structures and accessibility of epitopes in any given complex to the antibody.

VgRBP71 also binds to two mRNAs (An1 and An3) that are localized to the animal hemisphere. Thus, the possibility arises that VgRBP71 is an RNA-binding protein that is generally associated with mRNA in *Xenopus* oocytes. To address this point, we tested for binding of the protein to mRNAs encoding actin and transcription factor IIIA (Fig. 4) and to ribosomal protein L5 and profilin (data not shown), which are not known to be localized. These mRNAs are abundant in *Xenopus* oocytes, making it likely that we could detect any association with VgRBP71. There was no PCR product generated for these four mRNAs. Together, these results indicate that VgRBP71 is associated with localized mRNAs in *Xenopus* oocytes, but that it is not specific to a particular directional (vegetal/animal) pathway.

The apparent association of VgRBP71 with several different localized mRNAs in these assays presents the question of whether this factor binds directly to all of them or whether, in some cases, it is due to protein-protein interactions within a larger RNP complex. The only other *cis*-acting elements, which control localization through the late pathway, that have been identified are for VegT (Bubunenko et al., 2002; Kwon et al., 2002) and Xcat2 (Zhou and King, 1996) mRNAs. We synthesized RNAs containing these segments as well as the entire 3′UTR of An1 mRNA (579 nt) and used them for in vitro binding assays. In order to compare directly the binding of each of these RNAs to VgRBP71, we once again used competition assays (Fig. 5). The competition strength of the VegT and An1 localization elements are very similar to the VLE, establishing that VgRBP71 binds directly to these RNAs with high affinity. The apparent affinity of VgRBP71 for Xcat2 is somewhat less than its affinity for the other RNAs, but is distinctly greater than the nonspecific RNA control. This result may reflect the fact that Xcat2 is normally transported through the early pathway, but can use the late pathway when injected into stage III oocytes. It is possible that the interaction of VgRBP71 with Xcat2 mRNA in vivo may be facilitated by other factors that bind to the localization element of the latter. Although we find that the binding of VgRBP71 to these other localization elements and to VLE RNA is mutually exclusive, this does not mean that the complexes are identical or even similar. Alternative combinations of KH domains within the protein could mediate interactions with the different RNAs. Moreover, these results do not exclude the possibility that an interaction with other proteins helps to recruit VgRBP71 to these mRNAs in vivo.

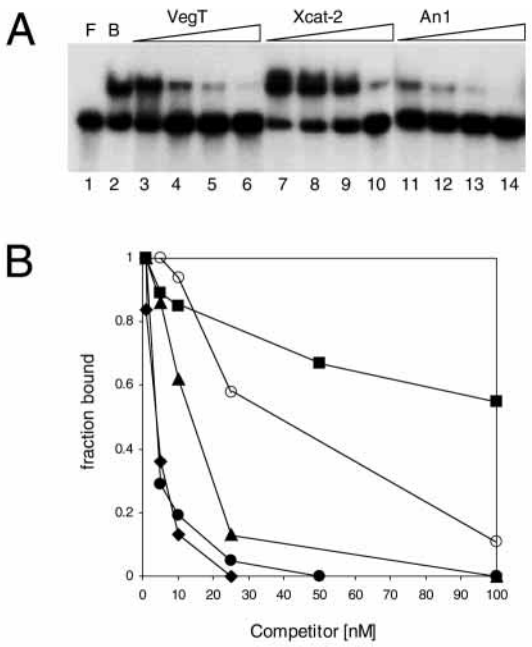

Fig. 5. VgRBP71 binds directly to other localization elements. (A) The binding of VgRBP71 was measured in competition assays using internally radiolabeled VLE RNA (1 nM) in the presence of increasing concentrations of the designated unlabeled competitor RNA. Lane 1, VLE RNA alone; lane 2, VLE RNA with VgRBP71 and no competitor RNA; lanes 3, 7, 11 contain 5 nM competitor RNA; lanes 4, 8, 12 contain 10 nM competitor RNA; lanes 5, 9, 13 contain 25 nM competitor RNA; lanes 6, 10 and 14 contain 100 nM competitor RNA. (B) Autoradiographs of the mobility shift assays were scanned with a laser densitometer to quantitate the fraction of bound VLE RNA at each concentration of competitor RNA relative to that in the absence of competitor. The isotherms correspond to (◆) VLE RNA, (●) An1 RNA, (▲) VegT RNA, (○) Xcat-2 RNA and (■) nonspecific RNA control. The data for VLE and nonspecific RNAs are taken from the assays presented in Fig. 2.

Intracellular location of VgRBP71 during oogenesis

The nuclear function of human FBP is well documented (Davis-Smyth et al., 1996; Liu et al., 2001) and the protein appears to have three distinct nuclear localization elements (He et al., 2000), which are also present in VgRBP71. All of the FBP proteins contain four KH domains, an RNA-binding motif that was first identified in hnRNP K (Siomi et al., 1993). The latter belongs to a family of hnRNP proteins that shuttle between the nucleus and cytoplasm. Human hnRNP K contains a bipartite-basic nuclear localization signal (NLS) as well as a second domain, termed KNS, that mediates bi-directional movement of the protein across the nuclear envelope (Michael et al., 1997). VgRBP71, however, does not contain a KNS domain or other known shuttling sequences such as M9 (Michael et al., 1995) or HNS (Fan and Steitz, 1998). Additionally, there is no identifiable nuclear export sequence (NES) in the protein.

Confocal fluorescence microscopy was used to examine the intracellular distribution of VgRBP71 during oogenesis and, most importantly, to determine whether any amount of the protein is located in the cytoplasm, consistent with its binding to localized mRNAs (Fig. 6). In accord with the northern (Fig. 3A) and western (Fig. 3B) assays, there is little VgRBP71 detected in stage I and II oocytes. The protein that is present in early stage oocytes is positioned evenly around the cell cortex and uniformly through the nucleus. The marked increase

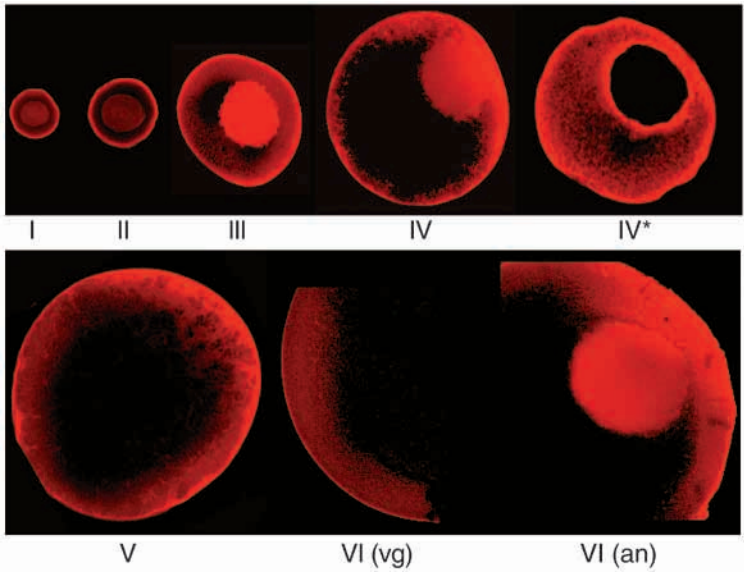

Fig. 6. Distribution of VgRBP71 during oogenesis. Staged oocytes were processed for immunocytochemical analysis using affinity purified antibody prepared against VgRBP71 and a secondary antibody conjugated with Alexa Fluor 568. Stage III through V oocytes are oriented with the vegetal pole at the lower left corner. The stage IV oocyte marked with an asterisk (IV*) was bisected along the animal-vegetal axis prior to immunochemical staining. The images marked VI(vg) and VI(an) are the vegetal and animal hemispheres, respectively, of a stage VI oocyte.

of VgRBP71 at stage III is most conspicuous in the nucleus; however, there is a concomitant increase in the cytoplasm as well. Although still enriched in the cortex, VgRBP71 is found in appreciable amounts throughout the cytoplasm of stage III oocytes. Although much of the protein remains nuclear at stage IV, the cytoplasmic distribution has changed somewhat. There is an enrichment of the protein in the animal hemisphere in the region between the nucleus and the cortex. In order to improve the immunochemical staining, a stage IV oocyte was bisected along the animal-vegetal axis prior to incubation with the primary antibody (Fig. 6, IV*). Although the nucleus is lost during this procedure, the manipulation improves the staining of the oocyte and yields considerably greater detail. The distinct cortical enrichment is still observed, but a punctate cytoplasmic distribution is more apparent. By stages V and VI, cytoplasmic VgRBP71 is concentrated at the periphery of the oocyte with a modest excess in the animal hemisphere relative to the vegetal. The amount of protein in the nucleus remains high. A western blot assay with manually dissected, staged oocytes has confirmed the nucleocytoplasmic distribution of VgRBP71 during oogenesis (results not shown). The confocal images establish that a significant amount of VgRBP71 resides in the cytoplasm, consistent with its ability to bind to localized mRNAs. Correspondingly, about 10% of total cellular ZBP2 is located in the cytoplasm of fibroblasts and neurons (Gu et al., 2002).

The cytoplasmic distribution of VgRBP71 is different from the other three VLE-binding proteins that have been characterized so far. Vg1 RBP/Vera, VgRBP60/hnRNP I, and Prrp all exhibit a clear co-localization with Vg1 mRNA at the vegetal cortex in stage IV oocytes, although VgRBP60 also shows diffuse staining in the animal hemisphere (Cote et al., 1999). VgRBP71, however, ultimately accumulates in a band around the entire periphery of the oocyte. This disposition is in accordance with the ability of the protein to bind to mRNAs that are localized to either hemisphere of the oocyte, further supporting the idea that VgRBP71 does not confer directional specificity. In fact, these results suggest that it functions in some general aspect of localization, which could include associated activities such as translational control or stabilization of localized mRNAs, rather than transport itself.

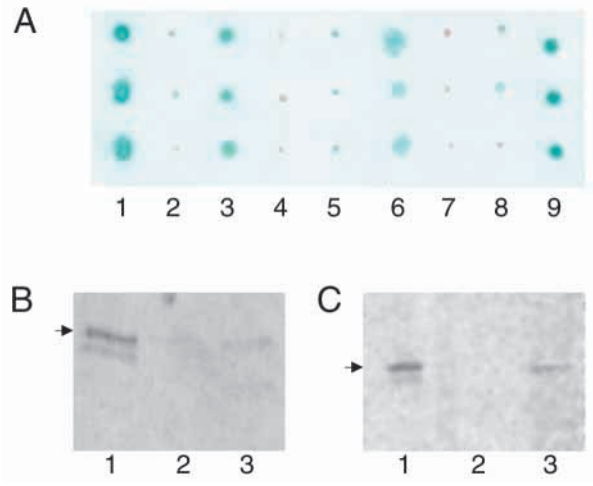

Fig. 7. VgRBP71 interacts with Prrp. (A) A yeast two-hybrid screen of a *Xenopus* oocyte cDNA library using the VLE-binding protein Prrp as bait retrieved approximately 15 preys containing VgRBP71. This interaction was characterized using a colony-lift filter assay. Each column represents three independently selected colonies with the following bait-prey combinations. 1, positive control (p53/T antigen); 2, negative control (lamC/T antigen); 3, Prrp/VgRBP71; 4, Prrp/prey vector; 5, bait vector/VgRBP71; 6, RNA binding domain of Prrp (amino acids 1-251)/KH domains of VgRBP71 (amino acids 1-449); 7, RNA-binding domain of Prrp/C terminus of VgRBP71 (amino acids 450-672); 8, proline-rich domain of Prrp (amino acids 242-360)/KH domain of VgRBP71; 9, proline-rich domain of Prrp/C terminus of VgRBP71. (B) The interaction between Prrp and VgRBP71 was also tested using a co-immunoprecipitation assay. VgRBP71, carrying an HA epitope, and Prrp were expressed individually in rabbit reticulocyte lysate; Prrp was labeled with [^{35}S]. Samples of the proteins were incubated together for 1.5 hours and then VgRBP71 retrieved with protein A-Sepharose beads coupled with HA antibody. The immunoprecipitate was analyzed by SDS-PAGE followed by autoradiography. Lane 1, VgRBP71 and Prrp; lane 2, VgRBP71 and Prrp incubated with beads not coupled to HA antibody; lane 3, Prrp alone incubated with beads coupled to HA antibody. (C) Co-immunoprecipitation of VgRBP71 and Prrp from oocyte extract. [^{35}S]-labeled Prrp was injected into stage III/IV oocytes, which were then kept overnight. Oocytes were disrupted manually and incubated with protein A-Sepharose alone (lane 2) or protein A-Sepharose coupled to anti-VgRBP71 antibody (lane 3). The immunoprecipitate was analyzed as described above. Lane 1 contains total extract prepared from injected oocytes and serves as a standard. Arrows indicate the position of Prrp.

VgRBP71 interacts with Prrp

The first protein identified in the screen of the expression library with VLE RNA was a novel proline-rich hnRNP protein called Prrp (Zhao et al., 2001). This protein co-localizes with Vg1 mRNA in stage IV oocytes and can bind to other mRNAs that use the late pathway for localization (e.g. VegT). The proline-rich domain of Prrp interacts with profilin, an actin-associated protein that appears to regulate microfilament assembly (Schlüter et al., 1997), suggesting that the protein may play a role in anchoring Vg1 mRNA to the vegetal cortex. We have used Prrp as the bait in a yeast two-hybrid screen of a *Xenopus* oocyte library in an effort to identify other components of the RNP complex that forms on the VLE or factors that otherwise associate with this complex. A screen of 3.5×10^6 colonies yielded 59 that exhibited β-galactosidase activity. Of these positive clones, at least 15 encode FBP2 (VgRBP71) and three encode FBP. Thus, two proteins, Prrp and VgRBP71, identified independently by their ability to bind to the localization element of Vg1 mRNA, appear to interact with each other. Neither Vg1 RBP/Vera nor VgRBP60 were found in this screen. However, Prrp itself was recovered three times, suggesting that it may form dimers or other higher order structures that are typical of many hnRNP proteins (Kim et al., 2000). Most of the remaining positive clones contain unique sequences (based upon restriction enzyme digestions and southern blot analysis) that are either not found in the sequence databases or that we have not yet sequenced.

The two-hybrid assay was also used to determine which domains in Prrp and VgRBP71 mediate their association. The results reveal that two segments in each protein are involved in this interaction. The N-terminal half of Prrp (amino acids 1-251) contains two RNP domains that interact with the N-terminal segment of VgRBP71 (amino acids 1-449) that extends through the four KH domains of that protein (Fig. 7A, lane 6). In addition, the C-terminal of Prrp (amino acids 242-360), which contains several polyproline repeats, and the C-terminal of VgRBP71 (amino acids 450-672), also interact (Fig. 7A, lane 9). The other combinations of bait and prey were negative.

We also tested the association of Prrp with VgRBP71 using immunoprecipitation assays (Fig. 7B). VgRBP71, carrying a hemagglutinin (HA) epitope, and Prrp were expressed individually in rabbit reticulocyte lysate; Prrp was labeled with [^{35}S]. Aliquots of both proteins were mixed together and then incubated with protein A-Sepharose beads coated with anti-HA antibody. The precipitate was analyzed by SDS gel electrophoresis followed by autoradiography. The immunoprecipitation of VgRBP71 also brings down Prrp confirming an interaction between the two (lane 1). Prrp was not recovered with protein A-Sepharose beads that had not been coated with antibody (lane 2) nor was it recovered in the absence of VgRBP71 (lane 3). Because both Prrp and VgRBP71 bind to the VLE, there is some possibility that their apparent association is mediated by RNA and is not direct. This seemed unlikely, as the C-terminal segments of the two proteins, which lack RNA-binding domains, give a positive signal in the two-hybrid assay. Nonetheless, this possibility was tested in the co-immunoprecipitation assays. Samples of Prrp and VgRBP71 were treated with RNase A prior to mixing together. This treatment had no effect on the recovery of Prrp with VgRBP71 (results not shown).

An immunoprecipitation assay was also used to determine whether these two proteins interact in oocytes (Fig. 7C). Immunoprecipitation of VgRBP71 from oocyte homogenate followed by a western blot using Prrp antiserum was inconclusive because of interference from the first (VgRBP71-specific) antibody. In order to circumvent this problem, stage III/IV oocytes were injected with [^{35}S] labeled Prrp and kept overnight. VgRBP71 was retrieved from extract prepared from these oocytes using affinity-purified antibody bound to protein A-Sepharose. The immunoprecipitate was analyzed by electrophoresis on an SDS polyacrylamide gel followed by autoradiography. Prrp co-precipitated from oocyte extract with VgRBP71 (Fig. 7C, lane 3), demonstrating an association of these two factors in vivo.

DISCUSSION

Multicomponent complexes appear to form on the *cis*-acting sequences that direct the localization of mRNAs to their proper intracellular destination. Six polypeptides can be crosslinked by UV irradiation to the localization element of Vg1 mRNA, which provides a minimum estimate for the number that associate with this region of the mRNA (Mowry, 1996). Three proteins that bind to the VLE have been identified. Vg1 RBP/Vera and VgRBP60, a homolog of hnRNP I, correspond to the UV crosslinked proteins p69 and p60, respectively. However, preliminary experiments in this laboratory indicate that the third protein, Prrp, which has an apparent molecular mass of 39 kDa, is not the crosslinked protein p40.

There is only circumstantial information on the function of these proteins. Vg1 RBP/Vera is required for the association of Vg1 mRNA with microtubules (Elisha et al., 1995), which may either be direct or mediated through a specialized region of the endoplasmic reticulum (Deshler et al., 1997). The ability of Prrp to bind to profilin suggests that this protein may be involved in the actin-dependent anchoring of Vg1 mRNA in the vegetal cortex (Zhao et al., 2001). A complete description of Vg1 mRNA localization will require the identification of all the factors that assemble on the VLE. By extension, identification of the binding partners of these proteins should provide clues to their individual functions. Thus, the indications that Vg1 RBP/Vera is microtubule associated and Prrp is microfilament associated suggest roles in translocation and anchoring, respectively.

We have identified a member of the FBP family of proteins, specifically a homolog of FBP2, that binds to the VLE. This protein is a general factor that can associate with mRNAs localized to either the animal or vegetal hemispheres of the oocyte. Interestingly, the sequence of the DNA-binding site for FBP, found upstream of the human *MYC* gene, is present near the 3′ end of the VLE (nt 243-269); however, this sequence does not occur in the 3′UTR of other localized mRNAs to which VgRBP71 binds. ZBP2, which is a chicken homolog of VgRBP71, binds to the 54 nt zipcode element in β-actin mRNA (Gu et al., 2002). A comparison of the sequence of the zipcode with the VLE did not reveal any appreciable alignment. The binding of VgRBP71 to its cognate sites, then, may be structure rather than sequence-dependent. It is also possible that different RNAs utilize different KH domains in VgRBP71 and, thus, present different identity elements for recognition by the

protein. For example, the binding of human FBP to FUSE DNA is determined primarily by KH domains 3 and 4 (Braddock et al., 2002; Duncan et al., 1994).

What constitutes a functional localization signal in the mRNAs that are sorted in *Xenopus* oocytes is not yet apparent. The localization element of VegT mRNA, which also uses the late pathway for movement to the vegetal cortex, has recently been delineated by two groups (Bubunenko et al., 2002; Kwon et al., 2002). Despite little apparent conservation in primary sequence or predicted secondary structure, the localization signals of Vg1 and VegT mRNAs seem to have a similar functional organization and bind the same set of proteins (Bubunenko et al., 2002). The 3′UTR of Vg1 mRNA contains four different repeated sequence elements (Deshler et al., 1997); repeats of one of these sequences, the 5-nt E2 element, also occur in the 3′UTR of VegT mRNA (Kwon et al., 2002). The E2 sequence is the recognition element used by Vera/Vg1 RBP and these repeats are necessary for the localization of Vg1 and VegT mRNAs (Bubunenko et al., 2002; Kwon et al., 2002). A second sequence element, VM1, which is the binding site for hnRNP I (Cote et al., 1999), may also be needed for efficient localization of these mRNAs to the vegetal cortex (Bubunenko et al., 2002). The sequences required for translocation and anchoring of *Xcat2* mRNA to the vegetal cortex through the late pathway are within the first 150 nt and the last 120 nt of the 3′UTR. The former sequence has a single E2 and two VM1 motifs; otherwise, the 3′UTR of Vg1 and Xcat-2 mRNAs exhibit no segments of notable sequence identity. Surprisingly, the 3′UTR of An1 contains three repeats of the short E2 element and nine variants of the VM1 motif. Bubunenko et al. (Bubunenko et al., 2002) have suggested that localization elements may not be defined so much by nucleotide sequence or secondary structure, but rather the strategic placement of multiple protein-binding motifs that assemble a common group of *trans*-acting factors. This scenario has the potential to account for the binding of the same set of proteins to localization signals that have very little sequence similarity. This model also implies that protein-protein interactions also play an important role in the assembly of the RNP complexes that form on the localization element. Thus, simple comparative sequence analysis may not immediately reveal the localization signals used by the multiple *trans*-acting factors that participate in the localization process. In accordance with these ideas, the in vitro binding assays demonstrate that VgRBP71 can bind directly to several different localization signals. In the case of *Xcat2*, other factors may strengthen the association of VgRBP71 with this RNA when it anomalously uses the late pathway.

Members of the FBP family have been implicated in an extraordinary array of disparate activities. The founding member was first identified as a transcriptional activator of *Myc* (Duncan et al., 1994) and subsequent members, FBP2 and FBP3, also contain tyrosine-rich C-terminal domains that are potent activation domains (Davis-Smyth et al., 1996). KSRP/FBP2 is part of a complex of proteins that binds to an element that regulates the tissue-specific splicing of *Src* mRNA (Min et al., 1997). Interestingly, another component of this splicing complex is polypyrimidine tract binding protein (PTB), a homolog of hnRNP I (Markovtsov et al., 2000). Thus, two proteins that bind to the Vg1 VLE are found in this splicing complex. Although there is no evidence for a direct interaction between hnRNP I and KSRP, binding of the latter to the splicing enhancer element required hnRNP H and hnRNP I. Similarly, a multi-protein complex that regulates the alternative splicing of β-tropomyosin also contains PTB/hnRNP I and FBP (Kaminski and Jackson, 1998). These observations raise the question whether some components of nuclear hnRNP complexes involved in RNA processing can, in some cases, have a distinct second role in the cytoplasm. The localization of *fushi tarazu* mRNA in *Drosophila* embryos requires prior exposure of the transcripts to nuclear components, which lead to the proposal that cytoplasmic localization uses hnRNP proteins that become associated with the mRNA in the nucleus (Lall et al., 1999). It is possible to imagine that these hnRNP complexes are dynamic with their composition changing as the mRNA is processed, exported from the nucleus, translocated and positioned in the cytoplasm, yet with some subset of proteins remaining associated with the RNA through several, if not all, steps.

Members of the FBP family have been identified in other nuclear and cytoplasmic processes. An FBP homolog binds to an element in the 3′UTR of the mRNA encoding the neuronal phosphoprotein, GAP43; this sequence controls the stability of the RNA (Irwin et al., 1997). Once again, homologs of FBP and hnRNP I are functionally connected. However, in this case, binding of the two proteins to the stability element appears to be mutually exclusive, suggesting that they have counteracting activities. Perhaps the most surprising discovery is that a FBP2/KSRP ortholog is a component of the enzyme complex that edits apolipoprotein B mRNA (Lellek et al., 2000). It is not obvious how these various activities are related or whether they need to be. However, sequencing of peptides derived from purified human DNA helicase V revealed that it is FBP; notably, this protein preparation also worked as an RNA helicase (Vindigni et al., 2001). If FBP or any of its homologs are truly RNA helicases, it may be that the protein is involved in the assembly or remodeling of RNP complexes on mRNA. This might then explain the widespread nucleocytoplasmic distribution of this protein, at least in *Xenopus* oocytes.

A search of the sequence databases revealed that VgRBP71 is also a homolog of rat MARTA1, which is one of two *trans*-acting factors that bind to a localization element in the 3′UTR of rat MAP2 mRNA, which directs it to dendritic compartments (Rehbein et al., 2000). As is the case with *Xenopus* VgRBP71, MARTA1 is found in the cytoplasm and the nucleus; additionally, the dissociation constant of the MARTA1:MAP2 mRNA complex is the same as that determined here for the VgRBP71:VLE complex. These similarities imply that VgRBP71 participates in some general aspect of mRNA localization and may account for its association with several different localized mRNAs in *Xenopus* oocytes, irrespective of their final intracellular destination. This idea is reinforced by the recent identification of ZBP2, which mediates localization of β-actin mRNA to the leading edge of embryonic fibroblasts and the growth cones of developing neurons (Gu et al., 2002). The two identified factors that bind to the zipcode element, ZBP1 and ZBP2, are orthologs of two of the proteins that bind to the VLE, Vg1 RBP/Vera and VgRBP71, respectively. Thus, there is an extraordinary conservation of proteins involved in RNA localization that extends across different cell types and species.

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