

## RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization

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***Vg1* mRNA translocation to the vegetal cortex of *Xenopus* oocytes requires intact microtubules, and a 3' UTR *cis*-acting element (termed VLE), which also mediates sequence-specific binding of several proteins. One protein, the 69-kD *Vg1* RBP, associates *Vg1* RNA to microtubules in vitro. Here we show that *Vg1* RBP-binding sites correlate with vegetal localization. Purification and cloning of *Vg1* RBP revealed five RNA-binding motifs: four KH and one RRM domains. Surprisingly, *Vg1* RBP is highly homologous to the zipcode binding protein implicated in the microfilament-mediated localization of  $\beta$  actin mRNA in fibroblasts. These data support *Vg1* RBP's direct role in vegetal localization and suggest the existence of a general, evolutionarily conserved mechanism for mRNA targeting.**

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Intracellular RNA localization leads to asymmetric protein synthesis, a necessary step in the process of pattern formation during early embryogenesis in many species (for review, see St. Johnston 1995; Gavis 1997). In *Xenopus* oocytes, morphological and molecular differences between the animal and vegetal hemispheres help define the primary axis around which subsequent development proceeds. Two temporally distinct pathways have been identified for vegetal RNA localization. The early pathway facilitates localization of several RNAs found to be localized during stages I-II of oogenesis (*Xcat-2*, *Xlsirts*, *Xwnt-11*), appears to be microtubule-independent, and is correlated with the migration of the mitochondrial cloud to a small region at the vegetal pole (Kloc et al. 1993; Mosquera et al. 1993; Forristall et al. 1995; Kloc and Etkin 1995; Zhou and King 1996a). The second pathway

occurs during late stage III-early stage IV, requires intact microtubules, and targets RNA to a tight shell along the entire vegetal cortex (Melton 1987; Yisraeli and Melton 1988; Yisraeli et al. 1990). So far, only one mRNA, *Vg1*, is known to localize via the second pathway in vivo, although *Xcat-2* RNA can employ this pathway when injected into stage III oocytes (Zhou and King 1996b). *VegT/Xombi/Apod/Brat* RNA is also localized to the vegetal cortex in a manner resembling the second pathway, but it is not yet known whether this localization requires intact microtubules; furthermore, it is released from the cortex in stage V/VI oocytes, significantly earlier than is *Vg1* RNA (Lustig et al. 1996; Stennard et al. 1996; Zhang and King 1996). *Cis*-acting elements mediating one or the other pathway have been mapped, by deletion analysis, to extensive regions of the 3' UTRs (Mowry and Melton 1992; Zhou and King 1996a,b; Gautreau et al. 1997). *Trans*-acting factors that bind these regions and may provide a link between the RNA and components of the cytoskeleton have been characterized only preliminarily, and the connection between these interactions and localization is just beginning to be defined (Schwartz et al. 1992; Mowry 1996; Deshler et al. 1997).

*Vg1* RNA-binding protein (RBP) is a 69-kD oocyte protein that binds specifically to the vegetal localization element (VLE) of *Vg1* RNA (Schwartz et al. 1992). Because it is enriched in the microtubule fraction of oocytes and mediates the association of VLE sequences to microtubules in vitro (Elisha et al. 1995), *Vg1* RBP is a good candidate for a *trans*-acting factor involved in *Vg1* RNA localization. In this paper we have mapped the *Vg1* RBP-binding sites in the *Vg1* VLE, and, using confocal microscopy to visualize injected wild-type and mutant VLEs in the same oocyte, we observe a direct correlation between the presence of two *Vg1* RBP-binding sites and vegetal localization. The unexpected finding that the microtubule-associated protein *Vg1* RBP is highly homologous to the microfilament-binding zipcode binding protein (ZBP-1) involved in  $\beta$ -actin mRNA localization suggests that microtubule- and microfilament-based systems of localization may be more related than originally anticipated.

### Results and Discussion

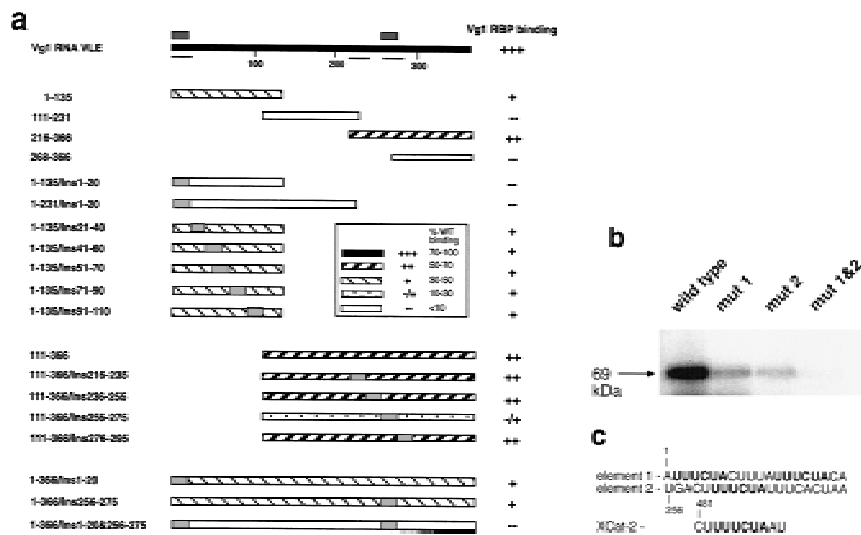
To determine whether *Vg1* RBP binding to the VLE correlates with vegetal localization, we first used UV cross-linking to precisely map *cis*-acting *Vg1* RBP-binding sites in the 366-nucleotide-long VLE. Initial mapping experiments indicated that two nonoverlapping regions at each end of the VLE can both bind *Vg1* RBP (Fig. 1a). We chose to pinpoint the *Vg1* RBP-binding sites using a linker-scan (LS) approach, which allowed us to compare the effects of relatively minor sequence changes (unaltered in length by deletions) to wild-type sequences. Twenty-nucleotide segments of VLE sequence were sequentially replaced with a 20-nucleotide linker sequence that does

[Key Words: RNA localization; *Vg1* RBP; *Xenopus*; oocytes; microtubules; KH domain]

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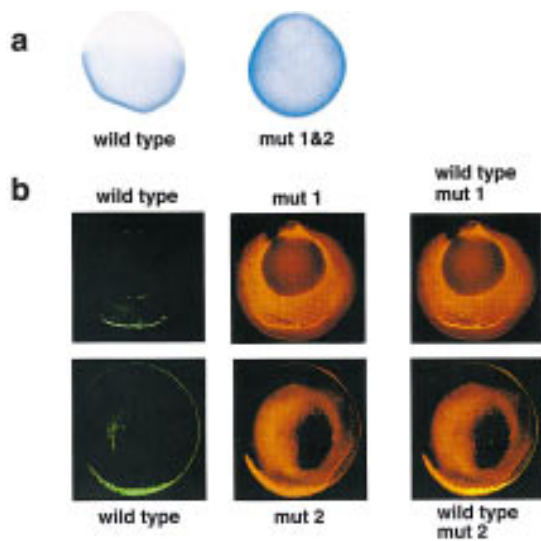
**Figure 1.** *Cis*-acting binding elements in the VLE and their role in localization. (a) The ability of different fragments of the VLE, as well as of the LS, substituted VLE fragments, to bind Vg1 RBP is schematically represented. The coordinates of the fragments and the substitution used for each binding assay (with the first nucleotide of the VLE numbered 1 and the substitution indicated as *Ins*) are listed at the left. The two regions identified as being important for Vg1 RBP binding are indicated by the boxes at the top. Domains protected by oocyte proteins from RNase degradation, mapped and labeled by Mowry (1996) as C, A/B, and D, respectively, are shown as lines beneath the full-length VLE. The degree of binding is indicated in the inset. (b) A representative UV cross-linking experiment (summarized in a) is shown for wild-type VLE, 1-366/ins1-20 (*mut 1*), 1-366/ins256-275 (*mut 2*), and 1-366/ins1-20&256-275 (*mut 1&2*) RNAs. (c) The nucleotide sequences of the first and second binding elements in the VLE are compared with a sequence from the 3' UTR of *Xcat-2* RNA from the region mapped as containing a late pathway localization element (Zhou and King 1996b). The hexanucleotide UUUCUA present in all these sequences is indicated in boldface type.

not bind Vg1 RBP (Figs. 1 and 2; data not shown). In each of the regions that binds Vg1 RBP independently, one 20-nucleotide element was found to be essential for Vg1 RBP binding. Although substitution of each of these elements individually (leaving the other intact) in the context of the full-length VLE partially reduces Vg1 RBP binding, loss of both elements abolishes binding almost completely (Fig. 1b). Thus, Vg1 RBP appears to bind independently at two distinct sites in the VLE. We note that a hexanucleotide repetitive sequence, UUUCUA, is found in two copies in the first element, once in the second element, and nowhere else in *Vg1* RNA (Fig. 1c; see below).

Localization of VLE RNAs with substitutions in either one or both of the Vg1 RBP-binding elements was compared with the localization of wild-type VLE using the oocyte culture system described previously (Yisraeli and Melton 1988). Microinjected VLE RNA with both sites substituted was homogeneously distributed throughout the oocyte after 5 days in culture, in contrast to wild-type VLE, which shows clearly defined vegetal localization (Fig. 2a). The effects of substituting either site alone were assayed by simultaneously injecting wild-type VLE RNA, labeled with digoxigenin-UTP, and substituted VLE RNA, labeled with fluorescein-UTP; their localization was visualized by confocal microscopy using rhoda-

mine and Cy-5-labeled antibodies, respectively (Fig. 2b). Substitution of either site alone significantly impairs localization, as indicated by the presence of the substituted RNA throughout most of the oocyte (red channel). Significantly, a low level of cortical localization of the singly substituted VLEs can be detected colocalizing (in yellow) with the wild-type VLE RNA (green channel) (Fig. 2b), but this colocalization was not detected with the double substitutions (data not shown). This technique allows us to quantify the degree of partial localization of the substituted RNAs with respect to the internal wild-type VLE controls. For both singly substituted RNAs, we estimate this degree of partial localization to be on the order of 20%.

Initial characterization of the VLE performed by Mowry and Melton (1992) showed that deletions removing either 5' or 3' sequences from the VLE significantly impair localization; these deletions included, respectively, either the upstream or downstream elements we have pinpointed here. More recently, Mowry (1996) identified, by RNase footprinting, three distinct regions of the VLE protected by *Xenopus* oocyte proteins; two of these regions fall precisely at the sites identified here for Vg1 RBP binding (see Fig. 1a). As mentioned above, a 6-nucleotide sequence present in two copies in the first element is also present in one copy in the second element (Fig. 1c). This sequence is also present in the region of *Xcat-2* RNA, which is important for its localization via the late pathway (Fig. 1c) (Zhou and King 1996b). In UV cross-linking assays, Vg1 RBP binds *Xcat-2* RNA (Z. Elisha, pers. comm.). An oocyte protein, termed Vera, reported to be associated with the ER and of a slightly larger size than Vg1 RBP, has been recently described as also binding the VLE (Deshler et al. 1997). Deletion of two 9-nucleotide repeats from the first 20 nucleotides of the VLE (containing the hexanucleotide sequence mentioned above) also impairs localization significantly, as well as reducing Vera binding. (These investigators also found that additional combinations of deletions can affect localization and protein binding.) A very recent set of experiments by Gautreau et al. (1997) further emphasizes the importance of the Vg1 RBP-binding sites we have mapped here. They identified two subelements, at the 5' and 3' ends of the VLE, whose deletion severely impairs localization (the 5' subelement is 1-135 and contains the first Vg1 RBP-binding site, and the 3' subelement is 201-340 and contains the second Vg1 RBP-binding site; see Fig. 1, a and c). Each of these subelements is incapable, on its own, of vegetal localiza-



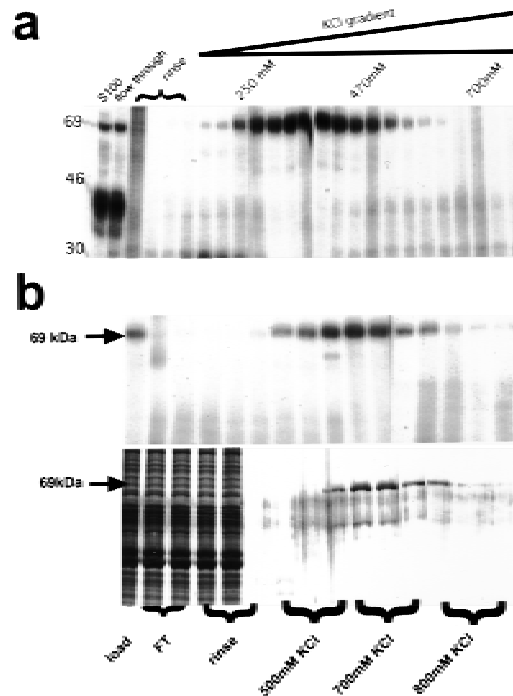
**Figure 2.** Localization of wild-type and mutant VLEs in oocytes. (a) The distribution of wild-type VLE RNA or double substituted VLE *mut 1&2* RNA (1-366/ins1-20&256-275, see Fig. 1a for map) following injection into late stage III oocytes. *mut 1&2* RNA appears to be fairly uniformly distributed throughout the oocyte; wild-type VLE shows strong cortical localization to the vegetal hemisphere alone. (b) Confocal micrographs of late stage III oocytes co-injected with wild-type VLE RNA (green channel) and either *mut 1* RNA (1-366/ins1-20, see Fig. 1a) or *mut 2* RNA (1-366/ins256-275) (red channel). Although the majority of both the mutant RNAs is not localized, some colocalization with the wild-type VLE RNA (yellow, third column) is observable.

tion, but a duplicated 5' subelement localizes well. This first subelement contains two copies of the hexanucleotide repeat, and mutations in this repeat eliminate vegetal localization. These results complement those presented here, in which we have preserved the basic structure and length of the VLE. Taken together, these data strongly suggest that the two Vg1 RBP-binding sites represent a *cis*-acting element required for localization via the late pathway and that cooperative Vg1 RBP binding is required for proper localization.

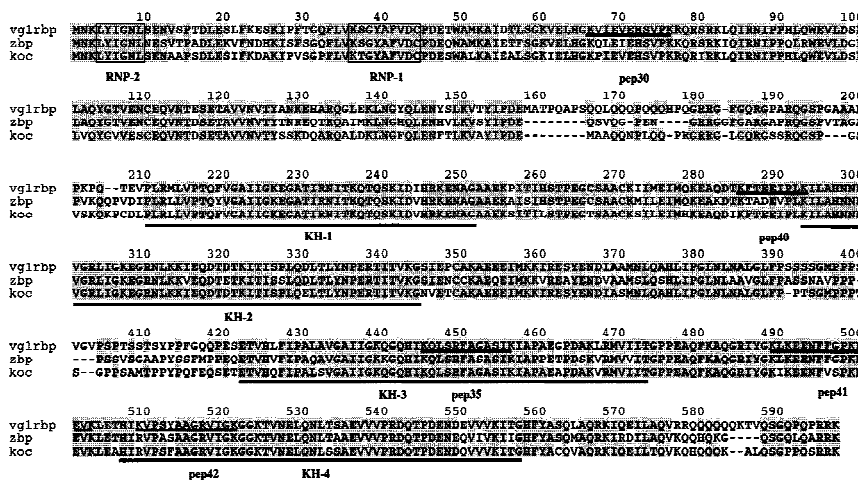
To clone Vg1 RBP, we first purified it from a *Xenopus* oocyte S100 lysate by sequential column chromatography. As shown in Figure 3a, Vg1 RBP, assayed by UV cross-linking, elutes off a heparin-Sepharose column in the range of 250–500 mM KCl. Then, these fractions were passed over an RNA affinity column, consisting of in vitro-transcribed VLE RNA attached to CNBr-activated Sepharose (Kaminski et al. 1995). Here, Vg1 RBP activity, which elutes from this column between 500 and 700 mM KCl, coincides with a principal silver-stained band migrating at 69 kD (Fig. 3b). The purified protein does not bind VLE sequences with both binding elements substituted, indicating that it has the same RNA-binding specificity as the oocyte extract protein (data not shown). Microsequencing of the gel-purified polypeptide generated five peptide sequences of 9–15 residues each. Subsequent RT-PCR of total *Xenopus* oocyte RNA using degenerate primers made to two “back-translated” pep-

tides (40 and 41; see Materials and Methods) yielded a product that encompassed the sequence of a third peptide (35). Screening of a *Xenopus* oocyte cDNA library with this PCR fragment resulted in a 2.4-kb cDNA, containing an ORF of 594 amino acids. All five peptides obtained from the purified Vg1 RBP were found in the conceptually translated cDNA protein sequence (see Fig. 4). By Northern blot analysis, Vg1 RBP mRNA appears to be ~2.8–3.0 kb long, implying that this clone is missing some noncoding region sequences (data not shown).

Database and literature searches with the Vg1 RBP peptides and, subsequently, the complete ORF, revealed a striking sequence conservation with an RBP implicated in mRNA localization in polarized somatic cells. Localization of  $\beta$ -actin mRNA to the leading edge of chick fibroblasts requires the presence of conserved elements in its 3' UTR, included in a 54-nucleotide sequence termed the zipcode (Kislauskis et al. 1993, 1994). The 68-kD ZBP-1 that binds to wild-type, but not to mutant zipcode sequences inactive in RNA localization, recently has been cloned (Ross et al. 1997). Vg1 RBP and ZBP-1 share 78% identical amino acids (Fig. 4). The searches also revealed that a human protein of ~65 kD, of unknown function, called KOC [KH-domain-containing protein overexpressed in cancer (Mueller-Pillasch et al. 1997)] is a very close relative of the localization element-binding proteins. Alignment of all three proteins, shown



**Figure 3.** Biochemical purification Vg1 RBP. (a) Fractions of the heparin-Sepharose column were assayed for Vg1 RBP cross-linking activity. KCl concentrations (as determined by conductivity) of selected fractions are indicated. (b) Equivalent fractions of the RNA affinity column were assayed for Vg1 RBP cross-linking activity (autoradiograph, top) and presence of protein (silver-stained gel, bottom). (Arrows) The location of Vg1 RBP.



**Figure 4.** Vg1 RBP protein sequence and its homology to ZBP-1 and KOC. The protein sequence for Vg1 RBP is shown with the four KH (underlined) and one RRM (boxed) domains indicated. The five peptides that were obtained from the microsequencing of the purified Vg1 RBP are underlined and labeled. ZBP-1 and KOC sequences are also shown, aligned to Vg1 RBP, and identical amino acids are shaded. Percent identities/homologies between the proteins are Vg1 RBP/ZBP-1, 78%/84%; Vg1 RBP/KOC, 83%/87%; ZBP-1/KOC, 76%/80%.

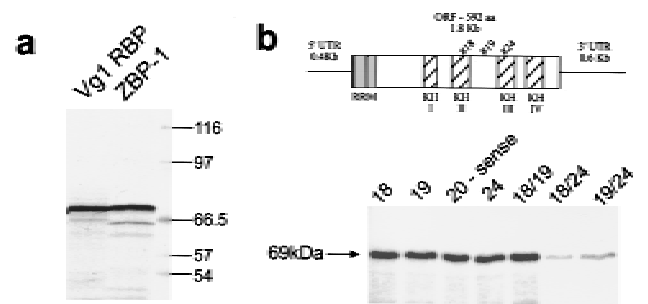
in Figure 4, indicates the high sequence conservation between Vg1 RBP, ZBP-1, and KOC, which extends throughout their length. A prominent feature of all these polypeptides is their multiple predicted RNA-binding motifs, including one RNA recognition motif (RRM) domain and four tandem KH domains. The RRM, characterized by its RNP2 and RNP1 motifs, and the KH (hnRNP K homology) domain are responsible for the RNA-binding ability of a variety of snRNA, pre-mRNA, mRNA, and rRNA-binding proteins, including some that play an important role in early development (Siomi and Dreyfuss 1997). The RRM and KH domains are the most highly conserved regions of the three proteins. We note that a REV-like nuclear export sequence is also present and conserved in all three proteins (325–333 in Vg1 RBP), but the functional importance, if any, of this motif is not clear. Our database searches failed to identify any obvious homologs of Vg1 RBP in yeast, *Drosophila*, or *Caenorhabditis elegans*. On the other hand, we did find other vertebrate counterparts as partial EST clones in mouse (accession nos. aa073514 and aa117282) and zebrafish (aa495472).

Two independent lines of evidence suggest that the cloned gene is indeed Vg1 RBP. First, the in vitro translation product made from the cloned gene is the same size as Vg1 RBP in S100 extracts or purified by RNA affinity chromatography; in vitro-translated ZBP-1 comigrates with this band as well (Fig. 5a). Second, to address the question of whether the cloned gene encodes the binding activity defined in S100 extracts as Vg1 RBP, antisense oligodeoxynucleotides directed against coding sequences were injected into stage III oocytes. The ability of the oligonucleotides, following overnight incubation, to cause a specific loss of binding activity as a result of RNase H-directed degradation of Vg1 RBP mRNA was monitored by extracting protein and then by performing

UV cross-linking (Fig. 5b). Although none of the oligonucleotides causes a loss of Vg1 RBP-binding activity when injected singly, a combination of two oligonucleotides directs a clear drop in binding activity (70% loss of control activity). This effect is very specific, as neither the sense oligonucleotide nor another combination of two antisense oligonucleotides influences Vg1 RBP binding. Northern blot analysis of RNAs extracted in parallel from the injected oocytes, probed with a Vg1 RBP DNA probe, shows precisely the same effect (data not shown). These results strongly suggest that the cloned gene encodes Vg1 RBP.

The identification of Vg1 RBP, shown here to mediate Vg1 mRNA localization in *Xenopus* oocytes, as a close homolog of ZBP-1, a protein implicated in targeting  $\beta$ -actin mRNA in fibroblasts, is startling. It clearly

suggests that this specific 3' UTR-RBP is a common component in localization pathways hitherto considered to be individual for mRNA and cell type, and raises several interesting questions. Despite the high degree of sequence similarity, significant functional differences exist between Vg1 RBP and ZBP-1. The zipcode motif that ZBP-1 binds includes a 6-nucleotide tandem repeat, ACACCC, which, when mutated, abrogates ZBP-1 binding (Kislauskis et al. 1993, 1994; Ross et al. 1997). The Vg1 RBP-binding sites described here contain a different hexanucleotide repeat, UUUCUA, and are AU rich (see



**Figure 5.** The cloned gene encodes Vg1 RBP. (a) In vitro translation of Vg1 RBP and ZBP-1 RNA. Both Vg1 RBP and ZBP-1 translation products migrate close to 69 kD, according to the molecular mass markers (right). (b) UV cross-linking of S100 extracts from oocytes injected with the indicated oligonucleotides was performed. Using a PhosphorImager to quantify the band intensities, we observed a 70% reduction in cross-linking activity (as compared to the control sense oligonucleotide 20) when oligonucleotides 18 and 24 are injected simultaneously, and a 50% reduction when 19 and 24 are injected together. The third pair of oligonucleotides (18 and 19) had no effect on Vg1 RBP-binding activity. The schematic drawing indicates the position of the oligonucleotides relative to the RNA-binding domains.

Fig. 1c). The source of the different binding specificities of these proteins remains to be determined; certainly, as in the case of the fragile X *FMR-1* gene, single-amino-acid substitutions in a KH domain can have profound effects (Siomi et al. 1994). An additional intriguing difference between the proteins lies in their cytoskeletal associations.  $\beta$ -Actin mRNA localization requires intact microfilaments (and not microtubules), and ZBP-1 co-elutes from the RNA affinity column with other proteins including actin (Sundell and Singer 1991; Ross et al. 1997). Vg1 RBP, however, associates with microtubules, both in vivo and in vitro, and can mediate the specific association of *Vg1* RNA to microtubules (Elisha et al. 1995). Furthermore, *Vg1* mRNA localization requires intact microtubules in oocytes (Yisraeli et al. 1990). Importantly, localized  $\beta$ -actin mRNA in neurons appears to be complexed in large granules that are associated with microtubules rather than microfilaments (Bassell et al. 1998). Interestingly, several reports have suggested that microtubule- and microfilament-based localization mechanisms may be capable of interacting. For example, in squid axons, vesicles can move along either microtubules or microfilaments (Kuznetsov et al. 1992). In *Drosophila* oocytes, *oskar* mRNA localization to the posterior pole, which is sensitive to microtubule inhibitors and is mediated by Stauf protein (which can bind to microtubules), is disrupted by mutants in microfilament-binding cytoplasmic tropomyosin II (Erdelyi et al. 1995). In fact, microtubule-interacting sites and actin-binding domains may be topologically very similar, as suggested by a comparison of kinesin and myosin 3-D structures (Woehlke et al. 1997) and by the observation that in yeast, a kinesin-related protein can suppress some aspects of a myosin mutant phenotype (Lillie and Brown 1992). The presence of a highly conserved protein involved in the movement of both microtubule- and microfilament-based localized mRNAs suggests that the mechanism for RNA localization may be a very fundamental one that has been adapted by different cells for specific needs. It will be interesting to see what provides the cytoskeletal specificity for Vg1 RBP and ZBP-1 and how additional, cell-specific proteins might mediate these interactions.

## Materials and methods

### UV cross-linking

For the LS and antisense experiments, UV cross-linking was performed using either 3 or 4  $\mu$ g of an oocyte S100 extract, respectively, as described previously (Elisha et al. 1995). UV cross-linking was also used to assay for the presence of Vg1 RBP activity in the column fractions; following normalization of the salt concentrations (as measured by conductivity), equivalent aliquots of each fraction were tested. Full-length VLE RNA was used as a radioactive probe for the column and antisense assays. Templates to synthesize the probes for the LS analysis were constructed by PCR (Schwartz 1996), with the 5' primer containing a T3 RNA polymerase promoter and the linker, which replaced successive 20-nucleotide segments of the VLE, consisting of the sequence GGAACAAAAGCT-TGCATGC. Substitutions were verified by sequencing. Probes for the LS analysis were synthesized in the presence of cap analog to prevent increased degradation as a result of the absence of polylinker sequence at the 5' end of the RNA (Schwartz 1996). Although bromo-UTP was used initially for synthesizing probes for the LS analysis, unsubstituted UTP was found to give identical results and used for subsequent experiments.

In the LS and antisense experiments, binding was quantitated using a PhosphorImager, relative to internal controls.

### Oocyte injections

Late stage III oocytes were injected with 1 ng of RNA (localization experiments) or oligodeoxynucleotides (antisense experiments) in 10 nl of water. For localization experiments, oocytes were cultured for 5 days, as described (Yisraeli and Melton 1988). For the whole mount immunohistochemical visualization of RNA, exogenous digoxigenin-labeled RNA was detected using alkaline phosphatase-conjugated anti-digoxigenin antibody and BM-Purple substrate (Boehringer Mannheim). The coinjection experiments (performed with wild-type oocytes to allow for orientation) were visualized by confocal microscopy: Wild-type VLE RNA, labeled with digoxigenin-UTP, was detected by a rhodamine-labeled anti-digoxigenin antibody (green channel), and the substituted VLE, labeled with fluorescein-UTP, was detected by a mouse anti-fluorescein first antibody and a donkey Cy5 anti-mouse second antibody (red channel). No fluorescence was detected with the fluorescein-UTP-labeled RNA alone, and autofluorescence of the yolk was not detectable under the conditions used (data not shown). In Figure 2, the degree of partial localization of the mutant RNA, relative to the wild-type RNA, was determined by calculating the fraction of all the mutant RNA fluorescent signal that was localized to the cortex and comparing that to the fraction of all the wild-type RNA that was localized to the cortex.

Antisense oligodeoxynucleotides consisted of the following sequences: (18) 5'-AAGTCCTGTAGTGGAGATAT; (19) 5'-ACTCCAACAGAAG-TGGTGG; and (24) 5'-TGCTTCCAATAATCGCTCC. The sense oligodeoxynucleotide control (20) was the complementary sequence to number 18. All of the oligonucleotides were synthesized with the first four and last four nucleotides consisting of phosphorothioate links, to minimize degradation. When combinations of oligonucleotides were injected, 0.5 ng of each was used. Oocytes injected with oligonucleotides were incubated overnight at 19°C in 1 $\times$  modified Barth's saline, and S100 protein extracts were prepared the following morning. Oocyte equivalents (1.2) of S100 extract (~3  $\mu$ g) were used for UV cross-linking.

### Purification and cloning of Vg1 RBP

S100 oocyte extract (200 mg) was applied to a heparin-Sepharose column and eluted with a salt gradient. The fractions containing the 69-kD Vg1 RBP-binding activity were pooled and concentrated using a Centricon filter. Six micrograms of the concentrated protein was loaded onto a CNBr-activated Sepharose column bound with 300  $\mu$ g of VLE RNA synthesized in vitro (Kaminski et al. 1995). After allowing the sample to flow through the column, the column was washed with 100 mM KCl and eluted with a salt gradient of 250 mM-1 M KCl. UV cross-linking was performed on aliquots from representative fractions. Pooled fractions containing Vg1 RBP were electrophoresed on a preparative gel. The Coomassie blue-stained Vg1 RBP band (of the same size) was excised from the gel, degraded with the endoprotease LysC, and the peptide products were then eluted and subjected to HPLC. Five clearly separated peaks were microsequenced by Edman degradation (see Fig. 3).

One microgram of total *Xenopus*-mixed oocyte RNA was reverse transcribed into single-stranded cDNA using the First Strand cDNA Synthesis Kit (Boehringer Mannheim) and random primers. An aliquot of the RT reaction was amplified by cloned *Pfu* DNA polymerase (Stratagene) with degenerate oligonucleotides (5'-AARTTYACTGARGARATHCC-3' based on peptide 40 KFTEEIPLK and 5'-CCRAARAARTTYTCYTCYTT-3' based on peptide 41 KLKEENFFGPKKEEVK). The 600-bp PCR product was gel-purified and, following verification by automated sequencing, labeled by random priming. Screening of a *Xenopus* oocyte cDNA library in Bluescript pBRN3 (generous gift of Nigel Garrett and John Gurdon, Wellcome/CRC Institute, Cambridge, UK) resulted in five clones that represented two allelic variants of Vg1 RBP (97% identical). In this work we used a full-length clone of allele D, containing an ORF of 594 amino acids. The first AUG encountered in this cDNA was predicted to serve as the initiator codon due to the 4 in-frame stop codons in the unusually long 378-nucleotides 5' UTR.

### In vitro translation

In vitro-transcribed capped mRNAs of 100 ng (Vg1-RBP) or 400 ng (ZBP-1) were translated in 10  $\mu$ l of the reticulocyte cell-free lysate (Promega) in the presence of [<sup>35</sup>S]Met, and labeled products were separated by 15% SDS-PAGE. Loading volumes were adjusted to give similar intensity of products, to compensate for the lower efficiency of translation of ZBP-1 mRNA compared to that encoding Vg1 RBP.

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## Note added in proof

Two genes highly related to Vg1 RBP have appeared in GenBank while this paper was in press. B23 (accession no. AF042353) is a transcriptional activator of the TFII $\alpha$  gene in stage I *Xenopus* oocytes and is identical to an allelic variant of Vg1 RBP isolated during this cloning process (see Materials and Methods). Vera (accession no. AF055923) is a Vg1 RBP that has been implicated in Vg1 RNA localization. It appears to be identical to Vg1 RBP, although its recognition site is reported to be different than that of Vg1 RBP (Deshler et al. 1998, *Curr. Biol.* **8**: 489–496). The functional significance of these proteins remains to be determined.

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