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# Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in *Xenopus* oocytes

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**Localized RNAs are found in a variety of somatic and developing cell types. In many cases, microtubules have been implicated as playing a role in facilitating transport of these RNAs. Here we report that Vg1 RNA, which is localized to the vegetal cortex of *Xenopus laevis* oocytes, is associated with microtubules *in vivo*. Because of the ubiquitous nature of tubulin, the association of specific RNAs with microtubules is likely to involve factors that recognize both RNA and microtubules. Vg1 RNA binding protein (Vg1 RBP), previously shown to bind with high affinity to the vegetal localization site in Vg1 RNA, appears to function in this capacity. Vg1 RBP is associated with microtubules: it is enriched in microtubule extracts of oocytes and is also co-precipitated by heterologous, polymerized tubulin. Furthermore, Vg1 RBP binding activity is required for the specific association of Vg1 RNA to microtubules *in vitro*. These data suggest a general model for how specific RNAs can be localized to particular sites via common cytoskeletal elements.**

*Keywords*: MAPs/polarity/RNA binding proteins/RNA localization/Vg1 RBP

## Introduction

Many types of cells demonstrate some form of an obvious asymmetrical morphology. On a molecular level, this asymmetry is detectable in the heterogeneous intracellular distribution of certain molecules. An increasing number of localized RNAs have been described in the last few years in a wide variety of organisms and cell types (Ding and Lipshitz, 1993; Wilhelm and Vale, 1993). Although cytoskeletal elements have been implicated in the localization process in a number of systems, the mechanism for localizing specific RNAs to particular intracellular destinations is not well understood.

In *Xenopus laevis*, Vg1 RNA is localized to the vegetal cortex of oocytes (Melton, 1987). Initially distributed homogeneously in stage I/II oocytes, Vg1 transcripts accumulate along the vegetal cortex during late stage III/early stage IV, where they remain until maturation (Melton, 1987; Weeks and Melton, 1987; Yisraeli and Melton, 1988). The use of cytoskeletal inhibitors allows dissection of the localization process into at least two distinct steps: translocation of the message, requiring intact microtubules (MTs), and anchoring of the message, requiring intact microfilaments (Yisraeli *et al.*, 1990). A 360 nucleotide (nt) fragment from the 3′ UTR of Vg1 RNA has been shown to contain all the information necessary and sufficient to confer vegetal localization to covalently linked sequences (Mowry and Melton, 1992). This region was independently found to bind with high affinity ($K_d$ = $3\times10^{-10}$ M) to a 69 kDa protein present in oocyte S100 extracts (Schwartz *et al.*, 1992). The protein, termed Vg1 RNA binding protein (RBP), appears to recognize a vegetal localization motif, present in vegetally localized transforming growth factor β-5 (TGFβ-5) RNA, but not in animally localized An2 RNA or other, non-localized RNAs (Schwartz *et al.*, 1992).

The ubiquitous nature of the cytoskeletal elements involved in Vg1 RNA localization suggests that other proteins provide the requisite specificity for both RNA–protein and protein–protein interactions. We have examined the role Vg1 RBP plays in this process. In taxol-stabilized MT preparations, Vg1 mRNA is selectively enriched as opposed to other, non-localized messages. Vg1 RBP is also highly enriched in these preparations and can be quantitatively co-precipitated from S100 extracts by the addition of purified, polymerized, taxol-stabilized tubulin. Moreover, the specific association of Vg1 mRNA to MTs requires the addition of an extract containing active Vg1 RBP. These data suggest that Vg1 RBP mediates the attachment of Vg1 RNA to MTs and in this way may enable its subsequent localization.

## Results

### *Vg1 RNA is associated with MTs*

Vg1 RNA was previously shown to be associated with a detergent-insoluble fraction from oocytes (Pondel and King, 1988; Yisraeli *et al.*, 1990). Because MTs are known to be involved in the localization process (Yisraeli *et al.*, 1990), RNA was purified from taxol-stabilized MT extracts (Elinson, 1985) prepared from oocytes in the presence of GTP (a necessary cofactor for MT formation), and assayed for the presence of Vg1 RNA. Only 2–4% of the total RNA in oocytes is associated with MTs (Z.Elisha and J.K.Yisraeli, personal observation). This implies that if all of the Vg1 RNA in an oocyte were associated with MTs, a 25- to 50-fold enrichment of Vg1 RNA in the pellet over total RNA would have been expected. The 3-fold increase of Vg1 mRNA in MT as compared with total RNA (Figure 1, lanes 1 and 2) suggests that ~10% (3/50–3/25) of Vg1 RNA is associated with MTs under these conditions. This association is sequence specific, with Vg1 RNA highly over-represented in the MT RNA population as compared with another RNA: the nuclear protein-encoding message, max (Tonissen and Krieg, 1994) (Figure 1, lanes 2 and 5). Max RNA is approximately as

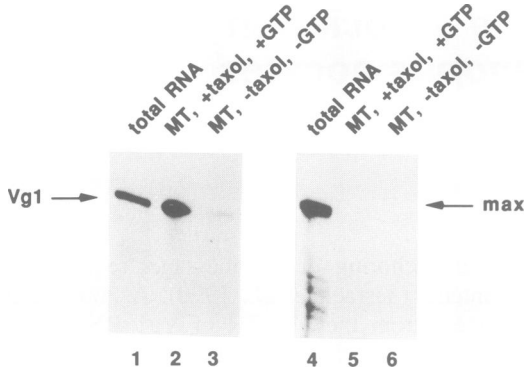


**Fig. 1.** Vg1 RNA is associated with MTs. MT extracts were prepared from oocytes under conditions conducive (1 mM GTP and 0.5 μM taxol, 25°C; lanes 2, 5) or non-conducive (minus GTP and taxol, 4°C; lanes 3, 6) to MT formation. Extracts were centrifuged through a sucrose cushion, and RNA was extracted from the clear pellet, or directly from oocytes (total RNA, lanes 1, 4). Twenty micrograms of each RNA were analyzed for the presence of Vg1 RNA or *Xenopus* max RNA, by RNase protection. The protected fragments are both 150 nt long. Note that Vg1 RNA is 3-fold enriched in the RNA extracted from MT pellets formed in the presence of taxol and GTP (lane 2) than in total RNA (lane 1); max RNA is not detectable in these pellets (lane 5).

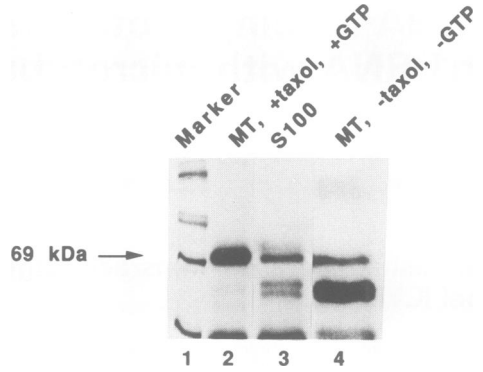


**Fig. 2.** Vg1 RBP is enriched in MT extracts. Microtubule (lane 2) and S100 (lane 3) extracts were UV crosslinked to a radioactive Vg1 3′ UTR RNA probe (as described in Materials and methods) and electrophoresed on a 10% SDS–polyacrylamide gel; the dried gel was exposed to X-ray film overnight. An extract non-conducive to MT formation, as described in Figure 1, was also compared (lane 4). Depending on the preparation, between 50 and 90% of the Vg1 RBP binding activity in oocytes associates with the MT pellet. Densitometry of this autoradiogram reveals that 8.2 times as much Vg1 RBP is labeled in the MT extract with taxol and GTP as is labeled in an S100 extract, and 7.2 times as much as in the MT extract without taxol or GTP. Mol. wt markers (Marker, lane 1) from top to bottom are 200, 97.4, 69 and 46 kDa.

abundant as Vg1 in total RNA extracts (lanes 1 and 4). Fibronectin RNA (Desimone *et al.*, 1992), also as abundant as Vg1 and max, is similarly absent from MT RNA (data not shown). When extracts are prepared at 4°C without GTP or taxol (conditions that induce depolymerization of most MTs), little of these mRNAs is found in the pellet (lanes 3 and 6). These results indicate that Vg1 RNA is associated with MTs in oocytes.

### *Vg1 RBP is associated with MTs*

Vg1 RBP was first identified in S100 extracts of oocytes as a protein that binds with high affinity ($K_d$ = $3\times 10^{-10}$ M) to two vegetally localized RNAs, Vg1 and TGFβ-5, but not to an animally localized message, An2, or to other, unrelated sequences (Schwartz *et al.*, 1992). In order to determine the intracellular partitioning of Vg1 RBP, MT extracts were prepared, and Vg1 RBP was UV crosslinked to the vegetal localization/Vg1 RBP-binding sequence of Vg1 RNA. As seen in Figure 2, not only is Vg1 RBP present in the MT fraction, but this fraction is also enriched 8.2-fold in Vg1 RBP as compared with an S100 extract (lanes 2 and 3). Two non-specific RNA binding proteins, however, with mol. wts of 54 and 56 kDa [presumably mRNP3 and mRNP4/FRGY2/p56 (Darnbrough and Ford, 1981; Murray *et al.*, 1992; Wolffe *et al.*, 1992)], are clearly labeled in the S100 extract, but almost completely absent from the MT pellet. Furthermore, the enrichment of Vg1 RBP in the MT fraction is dependent on stabilizing MTs; extracts prepared on ice with the same buffer, but lacking taxol and GTP, show no enrichment of Vg1 RBP (cf. lanes 2 and 4). Thus, the association of Vg1 RBP with the MT pellet requires the formation and maintenance of MTs.

A more stringent test for MT association is the ability of polymerized, purified tubulin to co-precipitate the protein (Vallee and Collins, 1986). Polymerized, phosphocellulose-purified rabbit brain tubulin (Sloboda and Rosenbaum, 1982; Vallee, 1986b) was added to S100

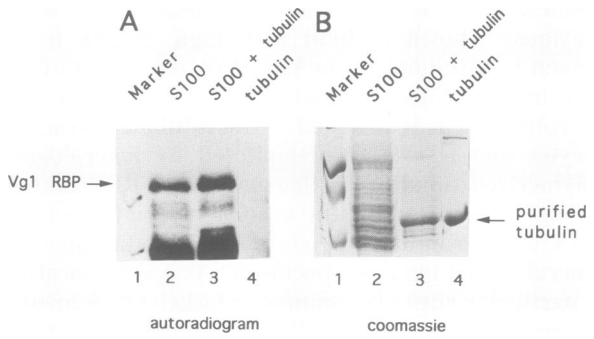


**Fig. 3.** Vg1 RBP is co-precipitated by purified, polymerized tubulin. Tubulin was extracted from rabbit brains and purified by phosphocellulose chromatography. Purified tubulin was polymerized *in vitro* and used to precipitate associated proteins from an S100 extract. The presence of Vg1 RBP was assayed by UV crosslinking of a domain B probe to 15 μg of S100 extract (lane 2), the proteins that co-precipitated with tubulin from 15 μg of S100 extract (lane 3) and purified tubulin alone (lane 4). (**A**) Autoradiogram and (**B**) Coomassie staining of the same gel. The position of purified tubulin is indicated. Note that the amount of Vg1 RBP present in lane 3 is not detectable by Coomassie staining. Mol. wts of the protein markers (lane 1) are 97.4, 69 and 46 kDa.

extracts, and MTs with associated proteins were sedimented by centrifugation (Vallee and Collins, 1986). As shown in Figure 3A, all of the Vg1 RBP is co-precipitated by polymerized tubulin under these conditions. This precipitation is highly specific, as demonstrated by the absence from the MT pellet of most of the prominently stained proteins of the S100 extract (Figure 3B). Taken together, these data strongly indicate that Vg1 RBP associates with MTs, both *in vitro* and *in vivo*.

### *Vg1 RBP mediates the association of Vg1 RNA to MTs*

It seems unlikely that Vg1 RNA is specifically recognized by MTs alone, given both the ubiquitous nature of tubulin

and the small subset of localized RNA molecules in oocytes (King and Barklis, 1985; Rebagliati *et al.*, 1985). Proteins that facilitate association of RNA to MTs could provide the required specificity. In order to determine whether oocyte S100 extract contains such an activity, an *in vitro* RNA co-sedimentation assay was devised, based on the tubulin co-precipitation experiment described above. Polymerized, purified tubulin was first incubated with an S100 extract, and then total oocyte RNA was added to the complexes. Microtubules as well as associated proteins and RNA were sedimented, and the RNA extracted from both supernatants and pellets was assayed for Vg1 and max RNA by RNase protection. Thirty-nine percent of Vg1 mRNA associates with the MT pellet under these conditions, while only 4% of max mRNA is present in the same RNA (Figure 4A, lane 2). In order to test whether this association requires Vg1 RBP binding activity, the S100 extract was replaced by an equivalent amount of oocyte protein depleted in Vg1 RBP binding activity by 66% (Figure 4B, lane 2). This protein mix represents the 150 mM NaCl wash from a phosphocellulose column fractionation of an oocyte S100 extract (see Materials and methods). This fraction contains the vast majority of the proteins bound to the column (Figure 4B, lane 4, and data not shown). When the Vg1 RBP-depleted extract was used in this reconstitution experiment, a clear shift in the fractionation of Vg1 mRNA is observed, with only 16% of this mRNA now associating with the MT pellet (Figure 4A, lane 3). This 16% represents a decrease of ~60% from the amount of Vg1 RNA associating with the pellet in the presence of the complete S100 extract (comparing lanes 2 and 3 in Figure 4A). The decrease in associated Vg1 RNA correlates well with the depleted Vg1 RBP binding activity in this fraction (66% depletion, Figure 4B).

In summary, an oocyte S100 extract is sufficient to associate Vg1 RNA to MTs. Decreasing Vg1 RBP binding activity results in a concomitant decrease in MT-associated Vg1 RNA. Furthermore, the major RNA binding activity in this extract that specifically recognizes both Vg1 RNA and MTs is Vg1 RBP. These data strongly suggest that Vg1 RBP is the 'go-between' that associates Vg1 RNA with MTs.

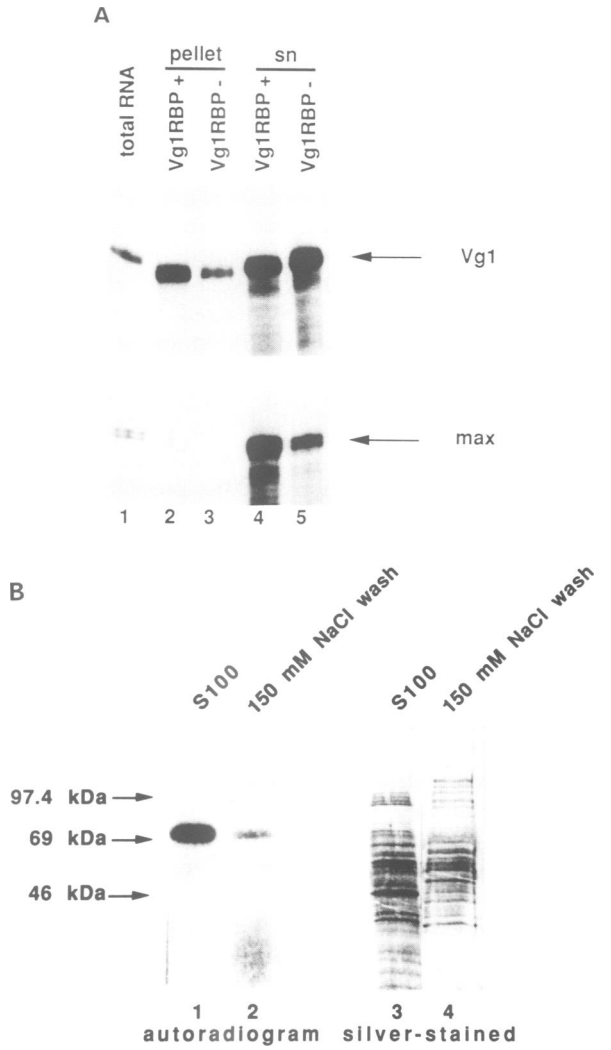


**Fig. 4.** Vg1 RBP binding activity is required for the association of Vg1 RNA to MTs. (**A**) 200 μg of total oocyte RNA were incubated with 20 μg of either an oocyte S100 extract active in Vg1 RBP binding ('Vg1RBP+', lanes 2 and 4) or the 150 mM NaCl wash fraction shown in (B) (lanes 2 and 4) that is diminished in Vg1 RBP binding ('Vg1RBP–', lanes 3 and 5). Phosphocellulose-purified tubulin was polymerized *in vitro* and then added to the extract, and the MTs and associated proteins were pelleted. RNA was extracted from the pellets ('pellets', lanes 2 and 3) and supernatants ('sn', lanes 4 and 5), and RNase protection assays were performed on equivalent fractions (one-quarter) of the RNA that was extracted from either the pellets or supernatants using Vg1 and max probes. The positions of the protected Vg1 and max probes are indicated by arrows. Five micrograms of total RNA were assayed for comparison (lane 1). The relative intensities of the protected bands were determined using a phosphoimager and are described in the text. (**B**) 0.5 μg of an S100 extract (lanes 1 and 3) or of the 150 mM NaCl wash of a phosphocellulose column fractionation of an S100 extract (lanes 2 and 4; see Materials and methods) were UV crosslinked to 12 fmol of Vg1 domain B probe. Densitometry of lanes 1 and 2 shows that the S100 extract contains 2.9-fold more Vg1 RBP binding activity per nanogram of protein than does the 150 mM NaCl wash. Lanes 1 and 2 are an autoradiogram of the UV crosslinking; lanes 3 and 4 show the pattern of proteins in the same lanes following silver staining of the gel. The ~45 kDa band seen in Figures 2 and 3 is absent from lane 1 due to the low amount of protein used in the UV crosslinking reaction, which emphasizes high-affinity RNA–protein binding.

## Discussion

RNA has been reported to be associated with MTs in a number of different cell types (Yisraeli *et al.*, 1990; Pokrywka and Stephenson, 1991; Theurkauf *et al.*, 1993; Bassell *et al.*, 1994; Clark *et al.*, 1994). Here we estimate that ~10% of Vg1 RNA pellets with MTs made from a mixed oocyte population. This association is not just RNA that adventitously pelleted with the MTs, because several other RNAs are not detectable in this pellet (Figure 1 and data not shown). In addition, MTs appear to be the major cytoskeletal element involved; conditions which specifically destabilize MTs cause a complete loss of Vg1 mRNA enrichment in the pellet (Figure 1). Almost all of Vg1 RNA appears to be localized to the vegetal cortex during oogenesis by the middle of stage IV, and this localization involves MTs (Yisraeli *et al.*, 1990). It is unclear whether the 10% of Vg1 RNA associated with MTs represents the majority of Vg1 RNA in a small population of oocytes (e.g. at a particular stage), or whether it is a function of available sites, binding proteins, MTs, etc., at any particular point during the process of

localization. Even when just stage III/IV oocytes are examined, the fraction of Vg1 RNA associated with MTs is similar (data not shown). Taken together, these results raise the possibility that not all of the RNA is transported at once, and that association with MTs may be a rate-limiting step.

A protein can be empirically defined as a MT-associated protein if it satisfies at least one of two criteria: that it sediments with MTs through repeated polymerization–depolymerization (reassembly) cycles, or that it co-precipitates with purified, polymerized tubulin (Vallee, 1986b; Vallee and Collins, 1986). In cases where the endogenous tubulin concentration is not high enough for reassembly, taxol has been used to stabilize MTs (Vallee and Collins, 1986). In these instances, it is necessary to show that sedimentation of the associated protein is dependent on polymerization (i.e. the presence of taxol and GTP). Vg1 RBP satisfies both of these criteria. First, the enrichment of Vg1 RBP in the MT pellet is dependent on taxol and GTP (Figure 2). The amount of Vg1 RBP present in the pellet without taxol and GTP (Figure 2, lane 4) may be the result of a small fraction of stable oocyte MTs (Gard, 1991); alternatively, this small amount of pelleted Vg1 RBP (14% of the amount present in the pellet with taxol and GTP) may suggest that the protein can associate with other insoluble cytoskeletal elements as well. Second, all of the Vg1 RBP in an S100 extract pellets with purified exogenous brain tubulin induced to polymerize by taxol and GTP (Figure 3A). This co-sedimentation is very specific, as judged by the absence of most of the Coomassie-stained bands from the initial S100 extract (Figure 3B). Thus, Vg1 RBP associates with MTs. Cloning of Vg1 RBP should indicate whether it contains a MT binding site, or interacts indirectly with MTs via additional factors.

Microtubule-associated proteins have been found in both eukaryotic and prokaryotic cells, and have generally been attributed structural roles in initiating, stabilizing and even stiffening MTs (Edson *et al.*, 1993; Hirokawa, 1994). In addition, some MT-associated proteins (MAPs) are specific for particular cell types and can even demonstrate intracellular compartmentalization (Matus, 1991). In *Xenopus* oocytes, another protein of ~45 kDa co-sediments with MTs (Figure 2, lane 2 and Figure 3A, lane 3). This protein binds RNA and recognizes virtually any probe used in the crosslinking reactions (J.K.Yisraeli, unpublished data). Judging from its size, ubiquitous binding and association with MTs, this protein may be EF-1α, which has been shown to bind to MTs in other systems (Durso and Cyr, 1994). Vg1 RBP appears to be a novel MAP in that it is capable of binding specific RNAs as well as MTs.

The RNA co-sedimentation studies shown here indicate that Vg1 RBP plays a central role in specifically associating Vg1 RNA with MTs. Whether this association is enough to provide the directionality for Vg1 RNA localization is not yet known. In *Drosophila* stage 8 oocytes, MTs are arranged in an anterior–posterior direction, with their plus ends at the posterior pole (Theurkauf *et al.*, 1992). A kinesin–β gal construct inserted into these oocytes by P-element transformation is localized to the posterior pole in precisely the same manner and at the same time as *oskar* RNA, suggesting that perhaps the same sort of mechanism may be localizing both molecules (Clark *et al.*, 1994). Gard (1994) has shown that, in *Xenopus* oocytes, γ-tubulin is localized to the vegetal cortex around the same period of time that Vg1 RNA undergoes localization, and MTs can be seen to grow from the vegetal cortex inward following depolymerization in the cold. The presence of MTs with an organized polarity emanating from the vegetal pole could generate the requisite directionality to explain localization, provided that the RNA can be associated with the MTs. In fact, a part of the 3′ UTR of rat tau RNA that contains a Vg1 RBP binding site is localized to the vegetal pole when injected into stage III *Xenopus* oocytes (Litman *et al.*, manuscript submitted). Thus, Vg1 RBP may facilitate vegetal localization in oocytes by associating RNAs containing a Vg1 RBP binding site with the appropriately organized MTs.

Localized RNAs have been identified in a large number of organisms and in a wide variety of cell types (Lipshitz, 1995). In general, RNA trafficking in these systems is thought to involve molecules that mediate the association of specific RNAs with cytoskeletal elements (Garner *et al.*, 1988; Yisraeli *et al.*, 1990; Pokrywka and Stephenson, 1991; Theurkauf *et al.*, 1993; Clark *et al.*, 1994). Such ribonucleoprotein complexes may appear as 'particles' under the appropriate conditions (Ainger *et al.*, 1993; Wang and Hazelrigg, 1994; Oberman and Yisraeli, 1995). Additional molecules may well be required either to direct these complexes to their ultimate intracellular destination and/or to provide the necessary locomotive force. Recent results in our laboratory indicate that a number of localized RNAs from different organisms contain a conserved, Vg1 RBP binding site (manuscript in preparation). Taken together, these data raise the interesting possibility that a common mechanism for associating specific RNAs to ubiquitous cytoskeletal elements may have been adapted over time to the specific needs of different systems.

## Materials and methods

### *Preparation of protein extracts*

*Xenopus laevis* frogs were obtained from Xenopus 1, Ann Arbor, MI. Oocytes were defolliculated by incubation at room temparature for 1–2 h in 0.3% collagenase (Sigma) in phosphate-buffered saline (PBS) initially warmed to 37°C. S100 extracts were prepared by homogenizing the defolliculated oocytes in TGKED buffer [50 mM Tris (pH 7.5)/25% glycerol/50 mM KCl/0.1 mM EDTA/0.5 mM dithiothreitol (DTT)/1 mM phenyl methyl sulfonyl fluoride (PMSF)/10 μg/ml leupeptin/2 μg/ml pepstatin]. The cleared extracts were then centrifuged in a Beckman type TLS55 rotor at 4°C for 1 h at 100 000 *g*.

MT extracts were prepared basically as described by Elinson (1985) by homogenizing oocytes in MT buffer [100 mM PIPES (pH 6.9)/10 mM EGTA/1 mM $MgCl_2$/30% glycerol/2 mM PMSF/10 μg/ml leupeptin/2 μg/ml pepstatin] on ice. Extracts were cleared of yolk and cortical debris by spinning for 10 min in a microcentrifuge. The concentration of the cleared extracts was then adjusted to 2 mg/ml by the addition of more MT buffer. Protein concentrations were determined using a Bio-Rad assay kit. Taxol was added (1 μM), as was GTP (1 mM) (Sigma), and the extracts were warmed to 30°C for 10 min so that the MTs would polymerize. The extracts were spun in a Beckman type TI50 rotor at 35 000 r.p.m. The MT pellets were resuspended in cold $Ca^{2+}$ medium (2 mM $CaCl_2$/1 mM $MgSO_4$/100 mM PIPES). Aliquots of both extracts were stored at −80°C.

### *RNase protection*

Antisense RNA probes to Vg1 domain B (Schwartz *et al.*, 1992) and to the *Apa*LI–*Hin*dIII fragment of *Xenopus* Max2 cDNA (Tonissen and Krieg, 1994) were synthesized at a specific activity of $6\times10^8$ c.p.m./μg and purified by electrophoresis on a 6% polyacrylamide/urea gel. Full-

length transcripts were eluted in 0.5 M amonium acetate (pH 6.5)/1 mM EDTA/0.1% SDS and hybridized at 37°C, as described previously (Ausubel *et al.*, 1992). RNase digestion was at 37°C for 20 min.

RNA was extracted (Yisraeli *et al.*, 1990) either directly from oocytes or from MT pellets, prepared as described above, with the addition of vanadyl ribonucleoside complex (New England Biolabs) to inhibit RNase activity. These extracts were then centrifuged through a 10% sucrose cushion (Vallee, 1986a).

### *UV crosslinking assay*

All of the UV crosslinking experiments employed sense RNA encoding the Vg1 domain B region synthesized in the presence of [$^{32}$P]UTP and bromo-UTP (Schwartz *et al.*, 1992). Unless otherwise specified in the figure legend, 6 fmol of radioactive RNA were incubated with 15 μg of protein extract. Following a 30 min incubation at room temperature, heparin was added to 5 mg/ml and the samples were UV irradiated at 1 J/cm$^2$ with a Stratalinker XL-1000 UV crosslinker at 260 nm wavelength. After a 15 min incubation at 37°C in 1 mg/ml RNase A, samples were denatured at 65°C and then electrophoresed on a discontinuous SDS–10% polyacrylamide gel. Dried gels were exposed to Kodak X-AR film overnight.

### *Protein co-sedimentation with polymerized tubulin*

Tubulin was extracted from either rabbit or bovine brains and purified by phosphocellulose chromatography (PC-tubulin), as described previously (Sloboda and Rosenbaum, 1982; Vallee, 1986b). To determine what oocyte proteins co-sediment with MTs, 300 μg of PC-tubulin were polymerized by the addition of 40 μM taxol and 1 mM GTP, warmed to 30°C for 5 min. To 1 mg of S100 extract, 20 μM taxol and 1 mM GTP were added together with the pre-polymerized PC-tubulin and placed on ice for 15 min. In order to stay above the critical concentration for PC-tubulin assembly, care was taken that the tubulin concentration should not fall below 0.5 mg/ml. The sample was then spun in a bench-top centrifuge at room temperature for 30 min. The pellet was resuspended in cold $Ca^{2+}$ medium (see above).

### *RNA co-sedimentation with polymerized tubulin*

Twenty micrograms of either complete S100 extract or a fraction of the extract depleted in Vg1 RBP binding activity (the 150 mM NaCl eluate from a phosphocellulose column; Figure 4B, and see the following section on phosphocellulose chromatography of an S100 extract) were incubated with 200 μg of total oocyte RNA for 30 min, followed by the addition of 20 μM taxol and 1 mM GTP. Separately, PC-tubulin was polymerized *in vitro* as described above and then added to the RNA–protein mix. Following a 15 min incubation on ice, the solution was pelleted by centrifugation for 30 min at room temperature in a bench-top centrifuge. The pellets were resuspended in 50 mM Tris (pH 7.5)/ 50 mM NaCl/5 mM EDTA/0.5% SDS. Proteinase K (Boehringer) was added to both the resuspended pellets and the supernatants to a concentration of 200 μg/ml and incubated for 30 min at 50°C. After phenol/chloroform extraction, the RNA was precipitated using the Quick Precip kit (AGTC).

### *Phosphocellulose chromatography of an S100 extract*

Phosphocellulose chromatography of an S100 extract was performed as described previously (Sloboda and Rosenbaum, 1982; Vallee, 1986b), with the following modifications. Elution off the column was limited to 2 ml/h. Following collection of the flow through and two washes (4 column volumes each) with buffer containing no NaCl, protein bound to the column was eluted by washing, stepwise, with increasing NaCl concentrations (150, 300 and 600 mM). A total of 80–90% of the protein that initially bound to the column was eluted in the 150 mM NaCl wash. Vg1 RBP binding activity was found predominantly in the flow through, although a small amount was eluted in the 150 mM NaCl wash (Figure 4B, lane 2, and data not shown).

### *Quantitation*

Quantitative analysis was performed either by phosphoimaging the dried gels (Figure 4A) or by scanning the autoradiograms on a flatbed scanner and analyzing the band intensity using digital imaging software (IMAGE 1.41, National Institutes of Health).

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