

Kinesin II Mediates *Vg1* mRNA Transport in *Xenopus* Oocytes

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Summary

The subcellular localization of specific mRNAs is a widespread mechanism for regulating gene expression [1]. In *Xenopus* oocytes microtubules are required for localization of *Vg1* mRNA to the vegetal cortex during the late RNA localization pathway [2–4]. The factors that mediate microtubule-based RNA transport during the late pathway have been elusive. Here we show that heterotrimeric kinesin II becomes enriched at the vegetal cortex of stage III/IV *Xenopus* oocytes concomitant with the localization of endogenous *Vg1* mRNA. In addition, expression of a dominant negative mutant peptide fragment or injection of a function-blocking antibody, both of which impair the function of heterotrimeric kinesin II, block localization of *Vg1* mRNA. We also show that exogenous *Vg1* RNA or *Xcat-2*, another RNA that can use the late pathway, recruits endogenous kinesin II to the vegetal pole and colocalizes with it at the cortex. These data support a model in which kinesin II mediates the transport of specific RNA complexes destined for the vegetal cortex.

Results and Discussion

Early and late pathways have been described for the localization of specific mRNAs to the vegetal cortex of *Xenopus* oocytes during stages I–III of oogenesis [3, 5]. The late pathway, also called the *Vg1* pathway, begins during stage II of oogenesis when the *Vg1* mRNA becomes localized to a wedge-shaped region [3, 6, 7] between the germinal vesicle and the vegetal cortex. Association of *Vg1* mRNA with the wedge region in stage II oocytes occurs in the absence of microtubules [6], but subsequent localization of the RNA from the wedge to

the vegetal cortex in later stage III/IV oocytes requires microtubules [3, 4]. Recently, we performed a computational search to identify new localized mRNAs in *Xenopus* [8], and one of the 20 top-scoring genes encodes *Xklp3b*, a subunit of *Xenopus* heterotrimeric kinesin II. This molecular motor protein consists of 80 kDa and 95 kDa motor-containing subunits, which are referred to as *Xklp3a* and *Xklp3b*, respectively, and a third 100 kDa subunit, KAP, that is tightly associated with the tail domain [9]. In addition to playing a role in cilia formation, intraflagellar transport, vesicle and protein trafficking, and melanosome transport [10], heterotrimeric kinesin II has been implicated in ER-to-Golgi transport in cultured epithelial cells [9]. Because the ER [6, 7] and an ER-associated protein, Vera/*Vg1* RBP [7, 11–13], have been implicated in *Vg1* mRNA localization, we decided to characterize the subcellular localization and function of heterotrimeric kinesin II in *Xenopus* oocytes.

Whole-mount in situ hybridizations in stage I oocytes showed that *Xklp3b* mRNA is present throughout the cytoplasm in a diffuse pattern and is slightly enriched in the mitochondrial cloud (Figure 1A). *Vg1* mRNA is not localized at this stage (Figure 1D), as has previously been reported [3, 5]. In stage II oocytes, the *Xklp3b* mRNA is diffuse throughout the cytoplasm and is also enriched to some extent in the wedge region between the germinal vesicle and vegetal cortex (Figure 1B), as is *Vg1* (Figure 1E). In stage III oocytes, *Xklp3b* mRNA is observed throughout the cytoplasm, with some enrichment observed in the vegetal cortex (Figure 1C), whereas, *Vg1* mRNA shows more extensive localization to the vegetal cortex (Figure 1F). After stage III, localization of the *Xklp3b* mRNA is not apparent (data not shown), suggesting that *Xklp3b* mRNA is not specifically retained at the vegetal cortex. Western-blot analysis shows that both the *Xklp3a* and *Xklp3b* subunits of heterotrimeric kinesin II are translated throughout oogenesis (Figure 1J). This is unlike *Vg1*, which is not translated until localization of the mRNA is complete in stage IV oocytes [14, 15]. Whole-mount immunocytochemistry to the *Xklp3b* subunit showed that heterotrimeric kinesin II is diffuse throughout the cytoplasm and is not preferentially localized along the animal-vegetal axis in stage I (data not shown) or stage II (Figure 1G) oocytes. In stage III oocytes, kinesin II is still abundant in the cytoplasm but also begins to accumulate at the vegetal cortex (Figure 1H), with an increased vegetal enrichment seen at the cortex in stage IV (Figure 1I). Quantitative image analysis indicates that the kinesin II signal in the vegetal cortex is enriched approximately 3 fold over the animal cortex of stage III/IV oocytes. The timing of kinesin II enrichment at the vegetal cortex is concomitant with *Vg1* mRNA localization (Figure 1F). Interestingly, as the mechanism of RNA localization to the vegetal cortex is evolutionarily conserved in chordates [8], the subcellular distribution of kinesin II is conserved in ascidian eggs, which also show a ubiquitous and vegetally enriched pattern at the cortex (Figure 1K).

The coincident enrichment of heterotrimeric kinesin

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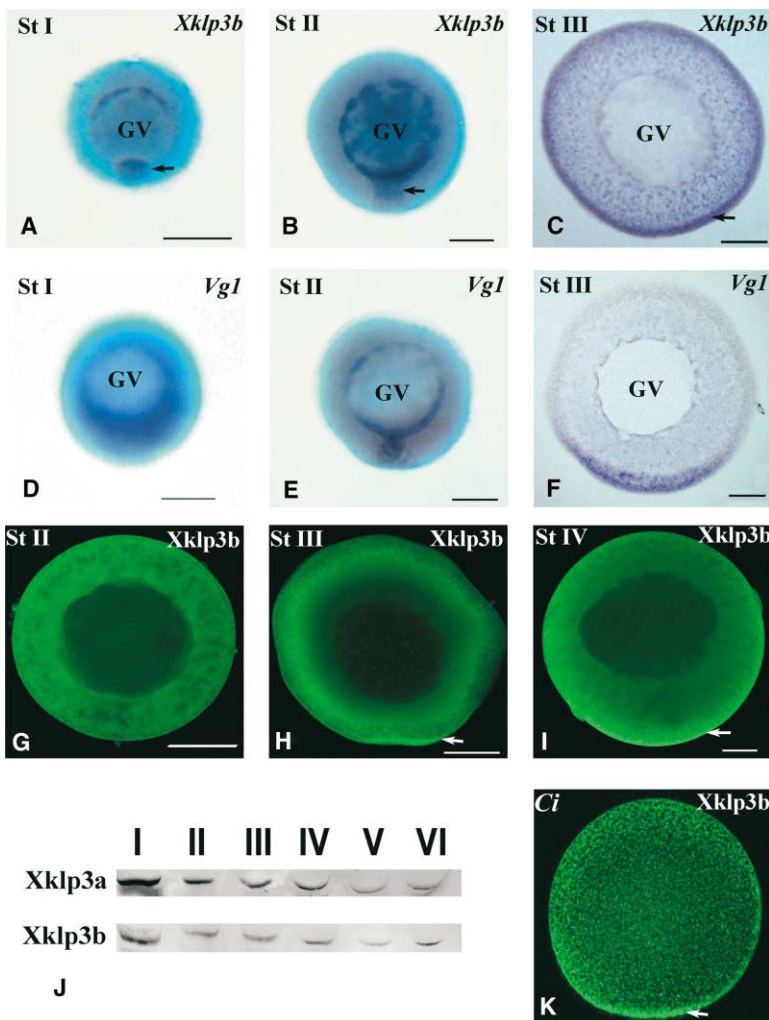


Figure 1. Expression and Localization of Heterotrimeric Kinesin II mRNA and Protein during Oogenesis

(A–F) Whole mount in situ hybridization to endogenous *Xklp3b* (A–C) and *Vg1* mRNA (D–F) in stage I (A and D), stage II (B and E), and stage III (C and F) oocytes. Arrows in (A)–(C) depict slight enrichment of *Xklp3b* mRNA in the mitochondrial cloud, wedge region, and vegetal cortex, respectively. GV is the germinal vesicle. The vegetal pole is oriented down in all images, and (C) and (F) are sectioned so that the cortex is visible. (G–I and K) Confocal images of Xklp3b-labeled *Xenopus* stage II (G), stage III (H), and stage IV (I) oocytes and an egg from *Ciona intestinalis* (K). Arrows in (H), (I), and (K) indicate enrichment of kinesin II at the vegetal cortex. (J) Western blots of whole-cell extracts prepared from stage I–VI oocytes via Xklp3a- and Xklp3b-specific antibodies. The scale bar represents 100 μ m.

II protein and *Vg1* mRNA at the vegetal cortex of stage III oocytes suggested a role for kinesin II in *Vg1* mRNA transport. To test this, we preinjected stage III/IV oocytes with an Xklp3a-specific function-blocking monoclonal antibody [16], K2.4, and then microinjected an X β G-VgLE (*Xenopus* β -globin-Vg1 localization element) fusion RNA to monitor RNA localization. The K2.4 antibody specifically inhibits kinesin II-driven motility but does not affect transport by conventional kinesin [17]. Oocytes preinjected with PBS (Figure 2A) or 50 ng of mouse IgG (Figure 2B) demonstrate robust localization in roughly 90 percent of oocytes (Table 1). However, localization was severely impaired in oocytes preinjected with 50 ng of the K2.4 antibody (Figure 2C) with only 38 percent (Table 1) of oocytes showing decipherable localization. Injection of 10 ng of the K2.4 antibody also impaired localization, but to a lesser degree (58 percent) (Table 1), and thus showed a concentration-dependent effect. This indicates that the antibody concentrations used in these experiments were in the linear range of their inhibitory activity, minimizing the possibility of nonspecific effects. The inhibition of RNA localization observed with the K2.4 antibody strongly suggested that kinesin II is required for *Vg1* mRNA transport.

To test further for a functional role of heterotrimeric kinesin II in *Vg1* mRNA localization, we coinjected X β G-VgLE RNA and an mRNA encoding an HA-tagged Xklp3b tail domain polypeptide fragment that functions as a dominant-negative mutant for heterotrimeric kinesin II [9]. This construct has been shown to incorporate into heterotrimeric kinesin II complexes [9], and expression of this mutant peptide fragment blocks kinesin II-dependent functions but does not block processes dependent on conventional kinesin in *Xenopus* [18]. An identical transcript containing a point mutation in the AUG start codon was used as a negative control. Robust localization was detected in oocytes injected with the X β G-VgLE transcript alone (Figure 2D) or coinjected with the AUG⁻ mutant transcript (Figure 2E). However, localization was severely impaired in oocytes coinjected with the HA-Xklp3b dominant-negative mRNA (Figure 2F), with detectable localization dropping from approximately 90 percent with no coinjected transcript or the AUG⁻ mutant mRNA to 28 percent (Table 1). Western-blot analysis using an anti-HA-specific antibody shows expression of the HA-tagged Xklp3b tail domain polypeptide fragment in oocytes injected with the Xklp3b dominant-negative mRNA but not in oocytes injected

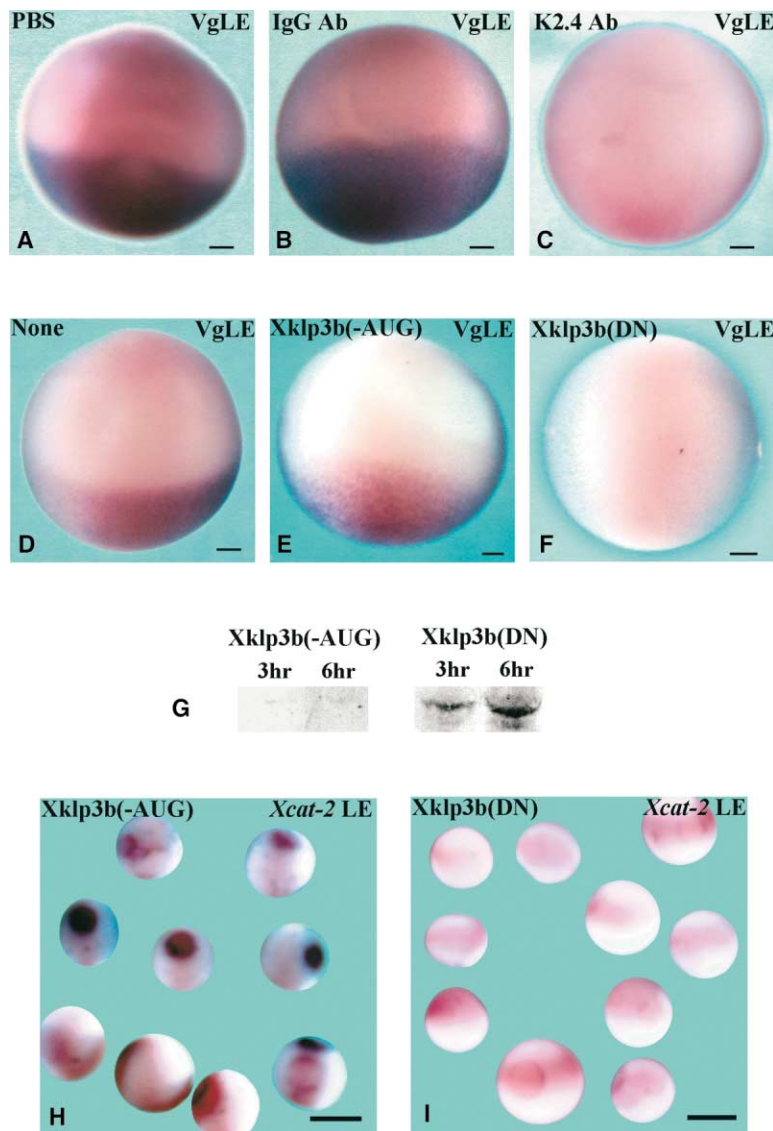


Figure 2. Heterotrimeric Kinesin II Is Required for Localization of *Vg1* mRNA

(A–C) Stage III/IV oocytes were preinjected with PBS (A), mouse IgG (B), or the K2.4 monoclonal heterotrimeric function-blocking antibody (C) and assayed for *Vg1* mRNA localization by in situ hybridization to the injected XβG-VgLE fusion transcript. (D–F) Stage III/IV oocytes were injected with the XβG-VgLE RNA alone (D) or coinjected with the XβG-VgLE and AUG⁻ mutant (E), or the Xklp3b dominant-negative mutant mRNA (F). (G) Western-blot analysis (using an anti-HA specific antibody) of oocytes injected with the HA-tagged Xklp3b dominant-negative construct (right) or the AUG⁻ mutant (left). A protein fragment of approximately 50 kDa was detected in extracts prepared 3–6 hr after injection of the Xklp3b dominant-negative RNA but not in extracts prepared from oocytes injected with the AUG⁻ mutant RNA. (H and I) Oocytes coinjected with digoxigenin-labeled *Xcat-2* LE and the AUG⁻ mutant (H) or the Xklp3b dominant-negative mRNA (I). Localization frequencies of the *Xcat-2* LE were 89% (n = 37) for the AUG⁻ mutant and 23% (n = 35) for the dominant-negative construct. The scale bar represents 100 μm in (A)–(F) and 500 μm in (H) and (I).

Table 1. Inhibition of *Vg1* mRNA Localization

Pre- or Co-Injection	Percent Localization (n = Number of Oocytes)
PBS	88 (n = 119)
50 ng IgG	87 (n = 91)
50 ng K2.4	38* (n = 107)
10 ng K2.4	58* (n = 19)
None	89 (n = 62)
Xklp3b DN AUG mutant	100 (n = 50)
Xklp3b DN	28* (n = 47)

Stage III/IV oocytes were preinjected with PBS, mouse IgG, or the K2.4 monoclonal antibody or coinjected with the HA-Xklp3b dominant-negative encoding mRNA along with the XβG-VgLE transcript to monitor RNA localization. The percent localization reflects the number of oocytes demonstrating localization to the vegetal cortex from multiple experiments. An asterisk indicates that oocytes that had reduced frequencies of localization also showed weaker localization at the vegetal cortex.

with the AUG⁻ mutant RNA (Figure 2G). Taken together, the results obtained with the K2.4 function-blocking antibody and HA-Xklp3b dominant-negative mRNA demonstrate that heterotrimeric kinesin II is required for efficient localization of *Vg1* mRNA during the late pathway.

When injected into *Drosophila* embryos, localized RNAs have been shown to recruit factors that are required for their localization [19, 20]. To determine if endogenous kinesin II could be recruited ectopically into the late RNA localization pathway, we injected fluorescently labeled VgLE into stage II oocytes, which normally do not show enrichment of kinesin II in the wedge or vegetal cortex (Figure 1G). We then assessed the distribution of kinesin II by immunocytochemistry. To enhance detection of any potential enrichment of kinesin II, we also used the *Xcat-2* localization element (*Xcat-2* LE) because it can localize during the late pathway [2] and consistently produces stronger localization signals than the VgLE (data not shown). Furthermore, late localization of the *Xcat-2* LE is also dependent on kinesin II

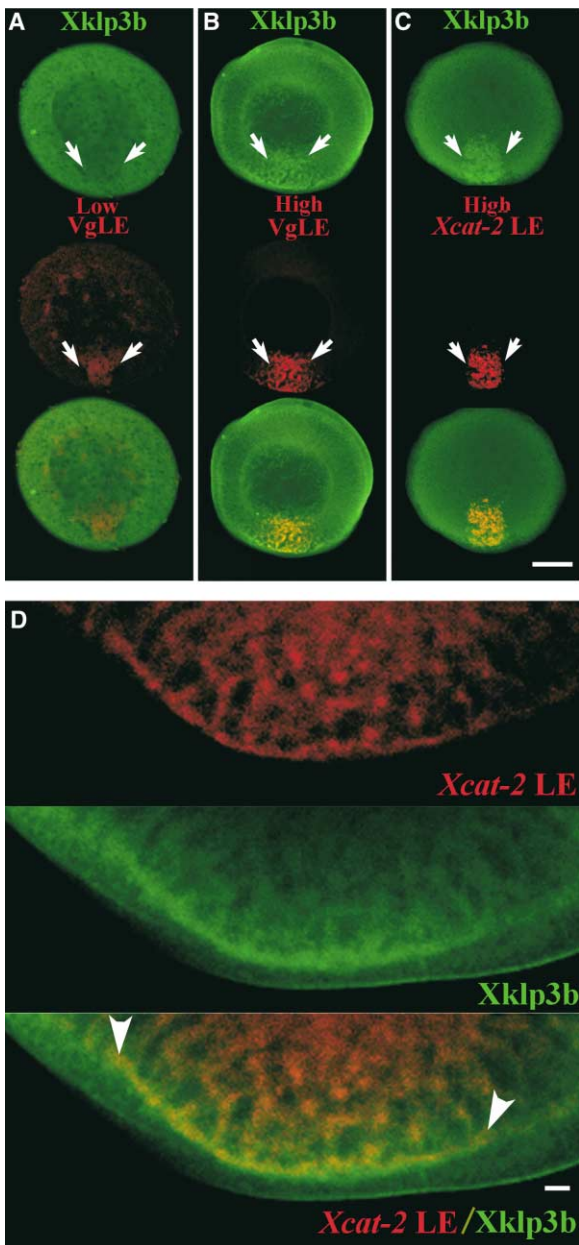


Figure 3. Exogenous Vegetal RNA Recruits Endogenous Heterotrimeric Kinesin II

(A–C) Stage II oocytes were injected with low (5 fmol) or high (35 fmol) amounts of alexa fluor 546-labeled RNA (red), and immunocytochemistry was then performed with an anti-Xklp3b primary and Cy-2-labeled (green) secondary antibody for detection of kinesin II. Panels (A)–(C) show confocal images of oocytes injected with low amounts of the VgLE (A), high amounts of the VgLE (B), or high amounts of the Xcat-2 LE (C). The top image in all panels represents kinesin II, the middle panel shows the labeled RNA, and the bottom panel is an overlay of the two images. The RNA labels the wedge in all three oocytes (arrows) (A). (D) Higher magnification of the wedge and vegetal cortex from a late stage II oocyte shows colocalization (yellow) of the Xcat-2 LE and kinesin II most extensively in the vegetal cortex at the base of the wedge (arrowheads). The scale bar represents 100 μm in (A)–(C) and 10 μm in (D).

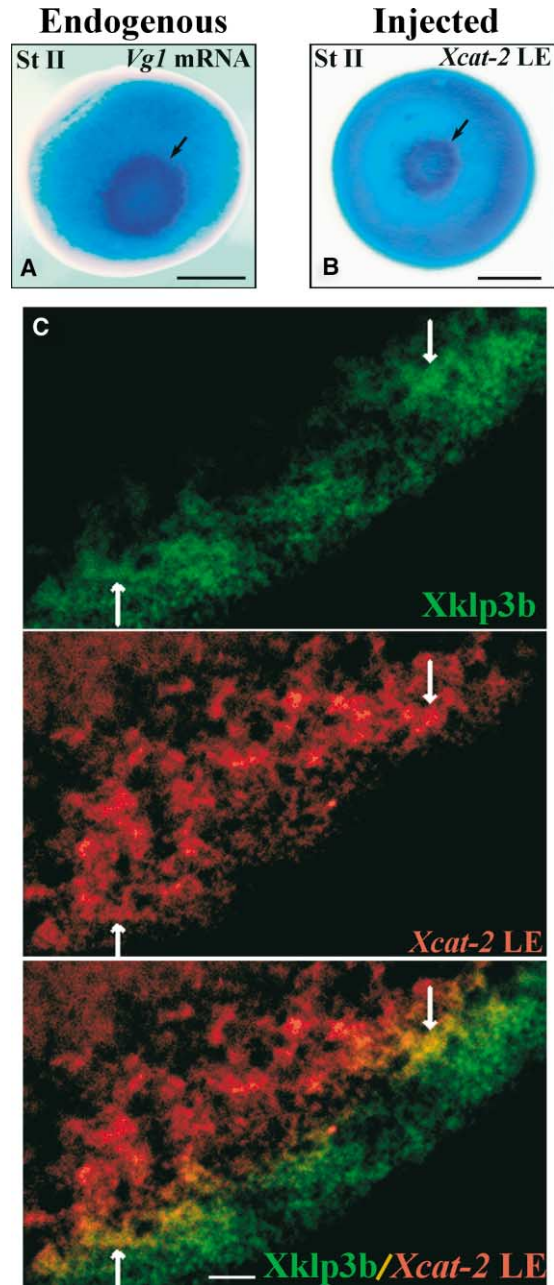


Figure 4. Colocalization of Kinesin II and RNA in the Ring Structure at the Vegetal Pole

(A) In situ hybridization to endogenous *Vg1* mRNA in a stage II oocyte showing the RNA localizing to a ring structure (arrow) at the vegetal pole. (B) Injected digoxigenin-labeled Xcat-2 LE localizes to a similar structure. (C) Optical cross-section of the wedge and ring structure from a stage II oocyte injected with alexa fluor 546-labeled Xcat-2 LE (red) and detection of endogenous kinesin II as in Figure 3. The most extensive overlap in labeling occurs at the extremity of the wedge (yellow) in the cortex, corresponding to a ring structure (arrows) at the vegetal pole. Note that kinesin II recruited to the wedge appears to travel deeper into the cortex than the RNA. The vegetal pole is oriented toward the bottom right of each panel in (C). The scale bar represents 100 μm in (A) and (B) and 10 μm in (C).

because its localization is abolished in stage III/IV oocytes coinjected with the HA-Xklp3b dominant-negative mRNA but not the AUG⁻ mutant (Figures 2H and 2I). In stage II oocytes injected with low amounts of the VgLE (5 fmol), localization of the RNA to the wedge is seen, but no recruitment of kinesin II can be detected (Figure 3A). Oocytes injected with high amounts (35 fmol) of the VgLE, however, show much stronger labeling of the wedge by the RNA and a corresponding enrichment of kinesin II at the wedge and vegetal cortex (Figure 3B). This enrichment was seen in 93% (n = 27) of oocytes that showed RNA localization. Oocytes injected with high amounts (35 fmol) of the *Xcat-2* LE show even stronger enrichment of kinesin II at the wedge and cortex (Figure 3C) (96 percent [n = 47] of the oocytes examined). Enrichment of kinesin II to the wedge and vegetal cortex was never observed in uninjected oocytes (data not shown) or those injected with low amounts of the VgLE (Figure 3A). Higher magnification shows that the most significant enrichment of kinesin II in stage II oocytes often occurs at the vegetal cortex just beneath the wedge (Figure 3D, middle panel).

In addition to being recruited to the wedge region and vegetal cortex, kinesin II exhibits extensive colocalization with the injected RNAs at the cytoplasmic side of the cortex during different stages of the localization process. In late stage II oocytes, this colocalization pattern extends continuously along the vegetal cortex at the base of the wedge (Figure 3D, lower panel). In early stage II oocytes, however, colocalization occurs at the vegetal pole in a ring structure that is often observed via *in situ* hybridization to endogenous *Vg1* mRNA (Figure 4A). We believe this structure represents the first signs of *Vg1* mRNA reaching the vegetal cortex. Like endogenous *Vg1*, digoxigenin-labeled *Xcat-2* LE also forms a ring structure when it is injected into early stage II oocytes (Figure 4B). An optical cross-section of the wedge and vegetal cortex at this stage shows that kinesin II colocalizes most extensively with the injected RNA in the ring structure of the cortex (Figure 4C). The distinct colocalization patterns of kinesin II and vegetal RNAs in the cortex of early (Figure 4C) and late (Figure 3D) stage II oocytes strongly suggest that kinesin II plays a direct role in the transport of RNAs to the vegetal cortex. Interestingly, kinesin II recruited into the vegetal pathway is detected further into the cortex than the RNA (Figure 4C). This suggests that the RNA “rides” with the motor in the wedge but is released the moment it enters the vegetal cortex, where it then becomes anchored [3, 4]. We propose that RNA-kinesin II interactions are highly dynamic *in vivo*, which could facilitate the release and anchoring steps. These dynamic interactions may also explain why it takes two days for vegetal RNAs to localize in a 400 μm cell *in vivo* even though kinesin II can travel at a velocity of 0.4 $\mu\text{m}/\text{s}$ along microtubules *in vitro* [17].

Although conventional kinesin has been implicated in the localization of RNAs in germ cells of *Drosophila* and in somatic cells of vertebrates [21], our work represents the first identification of a molecular motor that is responsible for RNA localization in the germ cells of a vertebrate. Kinesin II-driven RNA localization to the vegetal cortex of oocytes appears to be a general mecha-

nism in vertebrates because the expression pattern of kinesin II is conserved in ascidians (Figure 1K). These organisms are thought to be the best living representatives of the ancestral chordate and also have a number of RNAs localized at the vegetal cortex [22–25]. A role for kinesin II in germ cells of nonchordate metazoa has not yet been reported to the best of our knowledge and, therefore, remains to be explored. Interestingly, kinesin II appears to be involved in the localization of *tau* mRNA in the axons of neurons [26]. Because *tau* mRNA localizes to the vegetal cortex when injected into *Xenopus* oocytes [27], the mechanism responsible for transporting mRNAs within vertebrate axons and oocytes may be similar. Our data also suggest that vegetal RNAs have the ability to recruit and direct kinesin II to their destination rather than simply hitching a ride with motor molecules already en route. A similar situation exists for conventional kinesin, which can be recruited and “steered” by a cargo protein that interacts directly with its heavy chains [28]. Because kinesin II [29, 30] and vegetal RNAs such as *Vg1* [31–33] affect left-right asymmetry and mesoderm formation, pursuing the mechanism by which this motor recognizes, transports, and releases cargo RNA should enhance our understanding of early development in vertebrates.

Supplemental Data

Supplemental Experimental Procedures are available with this article online at <http://www.current-biology.com/cgi/content/full/14/3/219/DC1>.

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