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Posterior Localization of Dynein and Dorsal-Ventral Axis Formation Depend on Kinesin in *Drosophila* Oocytes

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Summary

To establish the major body axes, late *Drosophila* oocytes localize determinants to discrete cortical positions: *bicoid* mRNA to the anterior cortex, *oskar* mRNA to the posterior cortex, and *gurken* mRNA to the margin of the anterior cortex adjacent to the oocyte nucleus (the "anterodorsal corner") [1–3]. These localizations depend on microtubules [4–7] that are thought to be organized such that plus end-directed motors can move cargoes, like *oskar*, away from the anterior/lateral surfaces and hence toward the posterior pole [8–10]. Likewise, minus end-directed motors may move cargoes toward anterior destinations [6, 11–13]. Contradicting this, cytoplasmic dynein, a minus-end motor, accumulates at the posterior [14]. Here, we report that disruption of the plus-end motor kinesin I causes a shift of dynein from posterior to anterior. This provides an explanation for the dynein paradox, suggesting that dynein is moved as a cargo toward the posterior pole by kinesin-generated forces. However, other results present a new transport polarity puzzle. Disruption of kinesin I causes partial defects in anterior positioning of the nucleus and severe defects in anterodorsal localization of *gurken* mRNA. Kinesin may generate anterodorsal forces directly, despite the apparent preponderance of minus ends at the anterior cortex. Alternatively, kinesin I may facilitate cytoplasmic dynein-based anterodorsal forces by repositioning dynein toward microtubule plus ends.

Results and Discussion

To better understand microtubule-based localization processes in *Drosophila* oocytes, we studied the localization of kinesin I with an antiserum that binds its motor subunit, kinesin heavy chain (Khc). An even distribution of Khc was seen throughout the germline cells of the germarium and early egg chambers (Figure 1). Staining was usually more intense in the somatic follicle cells that enclose the egg chambers and was particularly strong in polar follicle cells. Beginning in stage 8 and continuing through stage 10A, Khc was most concentrated at the posterior pole of the oocyte. A small concentration also appearedin the anterodorsal corner (Figures 1C and 1D). Disruption of Khc expression in clones of cells by mitotic recombination with a null allele of the *Khc* gene [10, 15, 16] showed that the posterior Khc was a product of the germline and not of the posterior follicle cells (Figures 1E and 2C).

Previous studies of microtubules in late-stage oocytes suggest that microtubule minus ends are most concentrated at the anterior and least concentrated at the posterior pole [12, 17]. In

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addition, tests of the localization of (β-galactosidase fused to the motor domains of Khc or Nod suggest that plus-end transport is directed toward the posterior pole and minus-end transport is directed toward the anterior margin [6]. This is consistent with posterior accumulation of Khc (Figures 1C-1E and 2A) and with the disruption of posterior *oskar* mRNA localization that we reported in *Khc* mutants [10]. However, in apparent contradiction, cytoplasmic dynein, which is minus end-directed, has also been shown to accumulate at the posterior pole in late-stage oocytes [14].

To test the possibility that dynein is carried toward the posterior pole by kinesin I, we compared the distribution of cytoplasmic dynein heavy chain (cDhc) and Khc in late-stage oocytes produced by *Khc* null germline clones (Figure 2). In the *Khc* mutants, cDhc staining showed little or no posterior localization; rather, it accumulated strongly at the anterior (Figure 2D). Anti-tubulin staining (see Figure S1 in the Supplementary Material available with this article online) and previous tests indicate that the anterior-posterior gradient of microtubules is not disrupted in *Khc* null oocytes [10]. Therefore, the shift of dynein to the anterior in *Khc* mutants suggests that kinesin I is responsible for moving cytoplasmic dynein away from minus ends at the anterior and thus moving it toward the posterior pole.

The chorions of eggs produced by *Khc* null germline clones suggested defects in dorsal-ventral axis formation (see Figure S2). Proper dorsal pole specification within the oocyte induces follicle cells to differentiate into a pair of dorsal respiratory appendages near the anterior end of mature eggs. Of 359 eggs from *Khc* null germline clones, only 1% had normal appendages. Of the remainder, 17% had fused appendages, 26% had a rudimentary dorsal bump, and 56% showed no dorsal material. These phenotypes were completely rescued by a wild-type *Khc* transgene. These results indicate that germline kinesin I has an important role in dorsal pole specification.

Early steps in dorsal specification occur during stage 7. The posterior microtubule-organizing center (MTOC) disassembles, and the oocyte cortex takes on MTOC activity [17,18]. Microtubules become particularly abundant at the anterior and anterior margins and are least abundant at the posterior. This suggests an anterior-posterior gradient of cortical microtubule minus ends. The nucleus then shifts from the posterior pole to the anterior margin in a microtubule-dependent manner [19], and *gurken* mRNA becomes concentrated around the entire anterior margin. Subsequently, during stages 8–10, *gurken* disappears from most of the anterior margin and becomes concentrated between the nuclear envelope and the adjacent anterior-lateral cortex (the anterodorsal corner) in a microtubule-dependent manner [7, 20]. Gurken protein is expressed and secreted there, inducing dorsal fates in neighboring follicle cells (reviewed in [1]).

In *Khc* null stage-8 to -10 oocytes, anti-Gurken immunostaining revealed that anterodorsal accumulation was either weak or absent (see Figure S3). Consistent with poor Gurken expression, *kekkonI* mRNA, which is normally induced in anterodorsal follicle cells by Gurken signaling from the oocyte, was weak or absent (see Figure S3). These results indicate that Khc in the germline is required for normal anterodorsal Gurken expression and signaling.

The processes underlying anterodorsal Gurken expression were examined byin situ hybridization and light microscopy. During stages 6–8, *gurken* mRNA showed a normal transition from localization at the posterior to localization at the anterior margin (Figures 3D and 3E). The anterior signal in stage 8 appeared as a ring in both mutants and controls when oocytes were angled appropriately [20]. However, in stage-9 and -10 mutant oocytes, rather than localizing to the anterodorsal corner, the *gurken* signal was almost always spread evenly across the anterior in a broad diffuse band that had no ring-like profile (Figure 3F).

This indicates that kinesin I is critical for normal anterodorsal localization of *gurken* mRNA. Poor expression of Gurken from the mislocalized mRNA, and the consequent lack of dorsalization, is likely to reflect position-dependent translational repression [7].

The position of the oocyte nucleus on the anterior margin defines the site of *gurken* mRNA localization and thus is a critical part of the localization mechanism [21]. Nuclear positioning was defective in about 50% of stage-9 and -10 *Khc* null oocytes (Figures 3F and 4A). As we previously reported [10], nuclei appeared to accomplish the initial posterior to anterior shift during stage 7; however, a rigorous assessment of nuclear position is difficult in stage 7 because of the small size of the oocyte. To gain further insight, we compared nuclear positioning in wild-type and *Khc* null stage-8 to -10 oocytes (Figure 4A). Although some nuclei were mispositioned in stage-8 mutants, there was a marked shift away from the anterior margin in stages 9 and 10. While these data do not establish whether or not Khc has a minor role in initial anterior migration, the decline in normal positioning during stages 8–10 suggests that kinesin I does help keep the nucleus at the anterior. The poor retention in *Khc* mutants may reflect defects in the anchoring of the nucleus to the cortex of the anterior margin [22]. It could also reflect a decline in ongoing anterodorsal forces on the nucleus that may be needed to maintain its normal position.

Consistent with the nucleus acting as a target for anterodorsal *gurken* localization, mislocalized nuclei in *Khc* null oocytes sometimes had small patches of the *gurken* mRNA signal associated with them (Figure 3F). This highlights the possibility that the failure of anterodorsal *gurken* localization in null oocytes is a secondary consequence of failed anterodorsal nuclear positioning. Initial observations suggested that this was not true, since many oocytes that showed no elevated anterodorsal *gurken* accumulation did have normally positioned nuclei. Comparison of the frequencies of nuclear mispositioning and *gurken* mislocalization confirmed this (Figures 4A and 4B). Thus, the mechanism of anterodorsal *gurken* localization requires proper nuclear positioning [21, 22], microtubules [7], and kinesin I.

In summary, our results provide several insights into localization processes during mid-late oogenesis: 1) kinesin I colocalizes at the posterior pole with cytoplasmic dynein, 2) kinesin I is required for the posterior localization of cytoplasmic dynein, 3) kinesin I is required for the dorsal localization of *gurken* mRNA, and 4) kinesin I contributes to the proper anterior positioning of the oocyte nucleus. A role for kinesin in moving dynein toward the posterior pole provides a solution to the paradox of the accumulation of a minus-end motor in an area thought to be a destination for plus end-directed transport[6, 14]. However, a role for kinesin in anterodorsal localization is surprising because of evidence that minus ends are most concentrated there. In particular, a Nod:(β-galactosidase fusion protein that is targeted to microtubule minus ends accumulates around the nucleus and at the anterior margin during stages 8–10 [6]. How might a plus end-directed motor participate in localization toward an area dominated by microtubule minus ends?

Previous reports and recent results suggest that dorsal pole specification requires the minus end-directed motor, cytoplasmic dynein. Hypomorphic mutations that impair the function of *Drosophila* Lis1, which is known to be required in various systems for dynein/dynactin function in nuclear migration and other motility processes [23, 24], can cause ventralization of chorions, mislocalization of the nucleus, and failure of anterodorsal *gurken* localization [13, 25]. More recently, conditional overexpression of a protein that disrupts the dynein/dynactin complex has been shown to cause equivalent, though more severe, defects in those same dorsal specification processes ([26]; Jason Duncan and Rahul Warrior, personal communication). The fact that the same dorsal pathway phenotypes are caused by germline

Khc disruptionsuggests that kinesinIand cytoplasmic dynein both are required for nuclear positioning and anterodorsal *gurken* mRNA localization.

We suggest the following model to explain these results. Dynein, which is synthesized in nurse cells, walks along microtubules from nurse cells through connecting ring canals toward microtubule minus endsatthe oocyte posterior until stage 4 [14]. After the microtubule cytoskeleton reorganizes during stage 7, concentrating minus ends at the anterior cortex, dynein-generated movements are redirected away from the posterior. This drives the nucleus and *gurken* mRNA to the anterior margin. Materials like dynein and determinant mRNAs, moved by unknown forces, continue to enter the oocyte from nurse cells through the anterior ring canals [27]. Those that need to be distributed toward the posterior and are too large to diffuse efficiently are moved by kinesin I, either directly or by means of cytoplasmic flows [28]. As the oocyte enlarges during late stages, diffusion of the large cytoplasmic dynein/dynactin complex away from anterior minus ends becomes limiting. Thus, active transport of dynein away from the anterior by kinesin or by kinesin-generated cytoplasmic flows becomes critical. In stage-9 and -10 *Khc* mutant oocytes, dynein is trapped near minus ends at the anterior cortex. Anterior-directed dynein-based forces that act on *gurken* mRNA, the nucleus, and/or nuclear anchors are reduced, disrupting their normal positioning mechanisms.

If this dynein recycling model is correct, why does a loss of Khc influence nuclear position and disrupt anterodorsal *gurken* localization but not other putative dynein functions, such as the anterior localization of *bicoid* mRNA [10]? As with the initial localization of *gurken* mRNA, dynein-based forces toward the anterior margin may not be sensitive to poor recycling while the oocyte is small. Subsequent anterior localization of *bicoid* as the oocyte enlarges may be relatively insensitive to a decline in long-range, anterior-directed forces because its requirements for such forces are less than those of the nucleus and *gurken* mRNA.

Although the dynein recycling model provides a unifying explanation, it is quite speculative, and other possibilities for independent influences of kinesin I on dynein positioning and dorsal specification should also be seriously considered. Perhaps dynein diffusion is not limiting and posterior dynein localization is a simple consequence of kinesin-driven posterior accumulation of organelles or complexes that have dynein binding sites. Several possibilities for a nonrelated function of Khc in anterodorsal localization processes come to mind. Khc may influence the dynein/dynactin complex in certain dynein-based transport processes because of shared regulatory factors [29, 30]. Alternatively, kinesin may have amore direct rolein generating forces directed into the anterodorsal corner. For example, kinesin I linked to the cortexinthe anterodorsal corner couldcreate pulling forces on minus ends and essentially “reel in” microtubules and attached materials. Examples of this sort of movement generated by cortically anchored dynein are well known [31, 32], and we did observe a small concentration of Khc in the anterodorsal corner (Figures 1C and 1D). In another model, kinesin could be linked to *gurken* RNPs and the nuclear membrane, or perhaps nuclear anchor complexes, and directly transport them along a subset of microtubules oriented with their plus ends toward the anterodorsal corner. Present information on microtubule organization does little to address the possibility of specialized subsets of microtubules in the oocyte, but it would not be surprising if they were present. A full understanding of microtubule motor function in axis specification will require further investigation of microtubule organization, motor-cargo and motormotor relationships, and the dynamics of localization processes in living oocytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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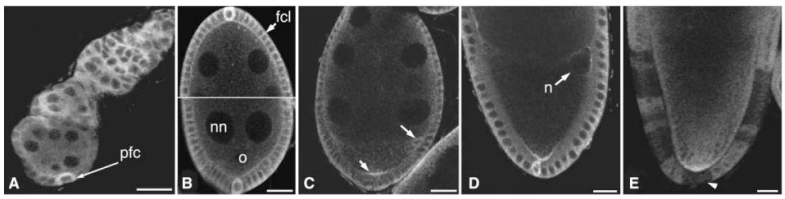

Figure 1.
Kinesin I Localization
Confocal fluorescence images of fixed egg chambers stained with *anti-Drosophila* Khc are oriented with posterior oriented downward.
(A) Germarium and early egg chambers.
(B) Split focal planes from a stage-7 egg chamber.
(C) A stage-8 egg chamber showing early accumulation of Khc at the posterior pole (short arrow) and the anterodorsal corner (long arrow) of the oocyte.
(D) A stage-9 egg chamber showing Khc accumulation at the posterior pole and at the anterodorsal corner adjacent to the oocyte nucleus (n).
(E) A stage-10A egg chamber with clones of Khc^{27}/Khc^{27} follicle cells, one of which eliminates Khc expression in the posterior polar follicle cells (arrowhead). For a through-focus series of (D), see the Supplementary Material available with this article online (Movie 1). The scale bars represent 15 μm. (fcl, follicle cell layer; pfc, polar follicle cells; nn, nurse cell nucleus; o, oocyte).

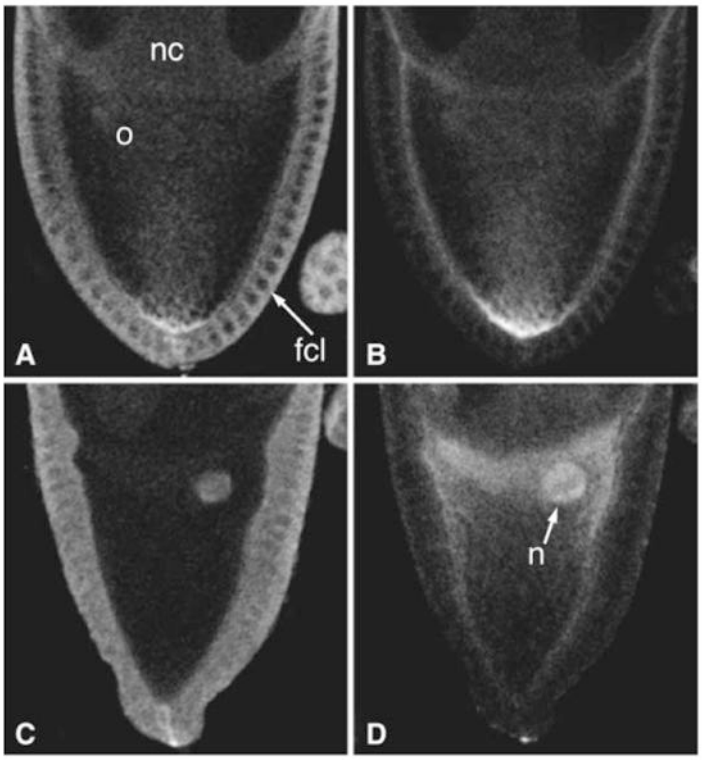

Figure 2.

Effects of Germline *Khc* Disruption on Dynein Distribution Fixed egg chambers were dual stained with anti-Khc and anti-cytoplasmic dynein heavy chain antibodies.

(A) A stage-10A wild-type egg chamber showing Khc distribution.

(B) The same egg chamber showing dynein distribution.

(C) A stage-10A chamber with a *Khc* null germline (Khc^{27}/Khc^{27}) showing little Khc staining in the oocyte or nurse cells.

(D) The same egg chamber showing dynein distribution. For unknown reasons, both antisera stained the nucleus in *Khc* null oocytes. This phenomenon has been seen previously with anti-cDhc staining in other mutant backgrounds [13]. (nc, nurse cell cytoplasm; o, oocyte; fcl, follicle cell layer; n, oocyte nucleus).

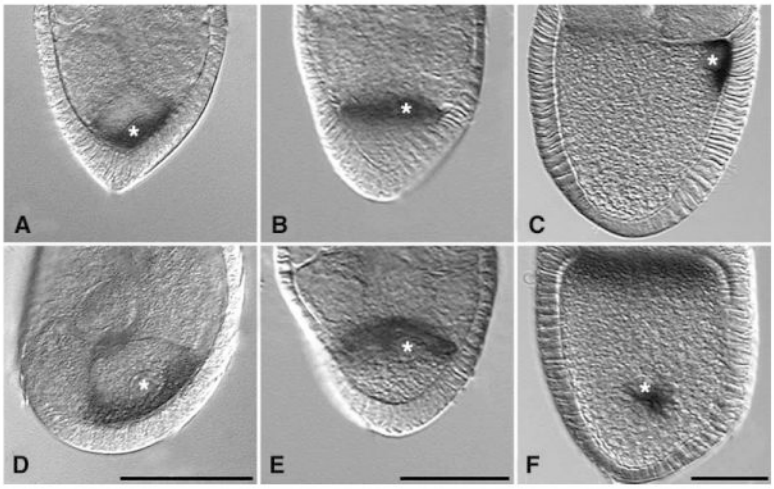

Figure 3.

Effects of Germline *Khc* Disruption on *gurken* mRNA Localization and Nuclear Position

(A-F) In situ hybridization with a *gurken*-specific RNA probe is shown for egg chambers from wild-type (top row) or from *Khc* null germline clones (bottom row). Posterior is oriented downward. (A) and (D) show stage-6 egg chambers, (B) and (E) show stage-8 egg chambers, and (C) and (F) show stage-10 egg chambers. The positions of oocyte nuclei are marked with white asterisks. Nuclear mislo-calization as seen in (F) was not a penetrant phenotype, being observed in about 50% of stage-9 and -10 oocytes (see Figure 4). The scale bars represent 50 μm.

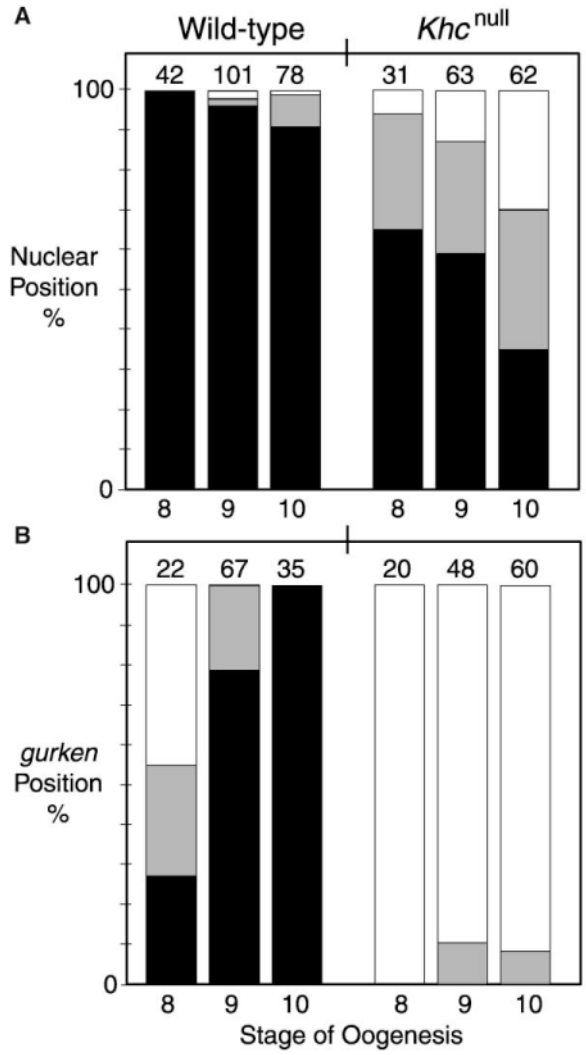

Figure 4.

Frequencies of Mislocalization for *gurken* mRNA and the Oocyte Nucleus in Egg Chambers from Wild-Type and *Khc* Null Germline Clones

(A) Nuclear position: black bar, nucleus in apparent contact with the anterior margin of the oocyte; shaded bar, nucleus close to the anterior margin, but not clearly in contact with it; open bar, nucleus at least one nuclear diameter away from the anterior margin.

(B) Localization of *gurken* mRNA: black bar, strong localization to one spot along the anterior margin (e.g., Figure 3C); shaded bar, widely distributed anterior but with a focused concentration somewhere along the anterior margin; open bar, widely distributed anterior with no focused areas (e.g., Figures 3B, 3E, and 3F). Sample sizes are noted above each bar.