Development 134, 3419-3425 (2007) doi:10.1242/dev.008078

# Rab6 and the secretory pathway affect oocyte polarity in *Drosophila*

**Jens Januschke[1,2], Emmanuelle Nicolas[1], Julien Compagnon[1], Etienne Formstecher[3], Bruno Goud[4] and Antoine Guichet[1,*]**

The *Drosophila* oocyte is a highly polarized cell. Secretion occurs towards restricted neighboring cells and asymmetric transport controls the localization of several mRNAs to distinct cortical compartments. Here, we describe a role for the *Drosophila* ortholog of the Rab6 GTPase, Drab6, in establishing cell polarity during oogenesis. We found that Drab6 localizes to Golgi and Golgi-derived membranes and interacts with BicD. We also provide evidence that Drab6 and BicD function together to ensure the correct delivery of secretory pathway components, such as the TGFα homolog Gurken, to the plasma membrane. Moreover, in the absence of Drab6, *osk* mRNA localization and the organization of microtubule plus-ends at the posterior of the oocyte were both severely affected. Our results point to a possible connection between Rab protein-mediated secretion, organization of the cytoskeleton and mRNA transport.

**KEY WORDS: Rab6, Secretory pathway, RNA transport, Gurken, Microtubules, Bicaudal D, *oskar* mRNA**

## INTRODUCTION

By regulating the transport of proteins and lipids toward the plasma membrane, the secretory pathway plays an important function in cell polarity. Rab GTPases are important regulatory factors of vesicular traffic. Members of the Rab6 family regulate protein transport between the Golgi, endoplasmic reticulum, plasma membrane and endosome (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000). The role of Rab6 in establishing cell polarity was, however, unclear. We chose the *Drosophila* oocyte as a model to study cell polarity in vivo. The oocyte lies at the posterior of the egg chamber, which consists of a cluster of 16 interconnected germ cells surrounded by a monolayer of follicular epithelium. During egg chamber formation, the germ line forms a 16-cell cluster and in which one cell is singled out to become the oocyte, while its 15 sister cells develop into nurse cells (for a review, see Huynh and St Johnston, 2004). During early oogenesis, microtubules (MTs) are nucleated from the MT-organizing center at the posterior of the oocyte. Towards stage 7, an unidentified signal from the posterior follicle cells triggers the organization of perpendicular MT subsets controlling the dorsoventral axis (DV) and anteroposterior axis (AP) in the oocyte (Januschke et al., 2006; MacDougall et al., 2003). *bicoid*, *oskar* (*osk*) and *gurken* (*grk*) mRNAs, which determine the embryonic axes, are then localized, respectively, to the anterior, posterior and anterodorsal poles of the oocyte (Riechmann and Ephrussi, 2001).

Studies in cultured mammalian cells have revealed a molecular mechanism whereby BicD modulates MT-based Golgi trafficking by recruiting cytoplasmic Dynein to transport vesicles (Hoogenraad et al., 2001; Matanis et al., 2002). The recruitment of Dynein to vesicles is mediated by the interaction of BicD with the small Golgi-localized GTPase Rab6 (Matanis et al., 2002; Short et al., 2005). In *Drosophila*, and more specifically in the oocyte, the Golgi apparatus is not organized into stacked cisternae arranged into 'Golgi ribbons'. Instead, it is organized into mini-stacks of transitional endoplasmic reticulum (tER)-Golgi units evenly distributed throughout the cell (Herpers and Rabouille, 2004; Kondylis et al., 2001). Whereas three Rab6 isoforms have been characterized in mammals (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000), only one has been identified in *Drosophila* (Shetty et al., 1998). So far, *Drosophila* Rab6 (Drab6) has been shown to be involved in Rhodopsin transport in photoreceptor cells and bristle morphogenesis (Purcell and Artavanis-Tsakonas, 1999; Shetty et al., 1998). In this study, we present the characterization of the function of Drab6 during oogenesis and propose a possible connection between Rab protein-mediated secretion, the organization of the cytoskeleton and mRNA transport.

## MATERIALS AND METHODS

### Fly stocks

$w^{1118}$ used as wild type; $rab6^{D23D}$ (Purcell and Artavanis-Tsakonas, 1999) was recombined to FRT-40A (Bloomington). $rab6^{D23D}$ and $Khc^{7.288}$ germ line and follicle cells clones were generated as described previously (Januschke et al., 2002). *GFP-trap*, *GalT* and *PDI* (A. Debec, Institut Jacques Monod, Paris, France). *Khc-lacZ* (I. Clark, UCLA, Los Angeles, CA). *BicD-GFP* and $BicD^{mom}$ (B. Suter, University of Bern, Bern, Switzerland). Overexpression of *Dynamitin* and colchicine treatment was performed as described previously (Januschke et al., 2002).

### Transgenes, biochemistry and immunohistochemistry

Drab6 was cloned into maternally expressed *tub*GFP (Januschke et al., 2002) and Polyubiquitin mRFP vectors. Details of protocols for biochemistry experiments are available upon request. The yeast two-hybrid screen was carried out as described (Formstecher et al., 2005).

Electron microscopy and immunodetection were performed as described (Januschke et al., 2006). Antibodies: Stau (St Johnston et al., 1991); Osk (Hachet and Ephrussi, 2001); β-galactosidase (Roche); Grk (DSHB); Lva (W. Sullivan, University of California, Santa Cruz, CA); BicD, Syntaxin 5 (DSHB); KDEL (Stressgen); Dynactin (E. L. Holzbaur, Rensselaer Polytechnic, Troy, NY); GFP (Roche); LE lectin (Vector); WGA, phalloidin, LysoTracker (Molecular Probes).

[1]Institut Jacques Monod, Unité Mixte de Recherche 7592, CNRS, Universités Paris 6 et Paris 7, 2 place Jussieu, F-75251, Paris Cedex 05, France. [2]Cell Division Group, IRB, Parc Cientific de Barcelona, c/Josep Samitier 1-5, 08028 Barcelona, Spain. [3]Hybrigenics SA, 3-5 impasse Reille, 75014 Paris, France. [4]Unité Mixte de Recherche 144 CNRS/Institut Curie, Institut Curie, 26 rue d'Ulm, 75248, Paris Cedex 05, France.

*Author for correspondence (e-mail: guichet@ijm.jussieu.fr)

**Accepted 22 July 2007**

## RESULTS AND DISCUSSION

In vertebrate cells, Rab6 is associated with the Golgi and the trans-Golgi network (TGN) membranes (Del Nery et al., 2006; Mallard et al., 2002; Martinez et al., 1997; Martinez et al., 1994; Opdam et al., 2000). To investigate the subcellular localization of Drab6 in the *Drosophila* germ line, we monitored the expression pattern of transgenic lines expressing Drab6 fused to GFP (Fig. 1A) and RFP (Fig. 1B). We observed that during oogenesis, the global distribution of Drab6 evolved. Drab6 first accumulated transiently in a central position during stages 7/8, then was uniformly distributed at the beginning of stage 9 to end up juxtaposed to the entire oocyte cortex (Fig. 1A). It is noteworthy that promoters of different strengths gave similar expression patterns. In addition, the genomic null allele *rab6*$^{D23D}$ (Purcell and Artavanis-Tsakonas, 1999) was fully rescued by the different lines expressing Drab6.

Drab6 did not colocalize extensively with ER membranes (labeled with PDI-GFP) (Bobinnec et al., 2003) (Fig. 1B). Instead, it seemed to be differentially associated with two types of Golgi unit (Fig. 1C). Lava Lamp (Lva), a cis-Golgi marker (Papoulas et al., 2005), colocalized with Drab6, mainly at the cortex of the oocyte and in nurse cells. A GFP trap protein corresponding to a UDP-galactose:beta-N-acetylglucosamine

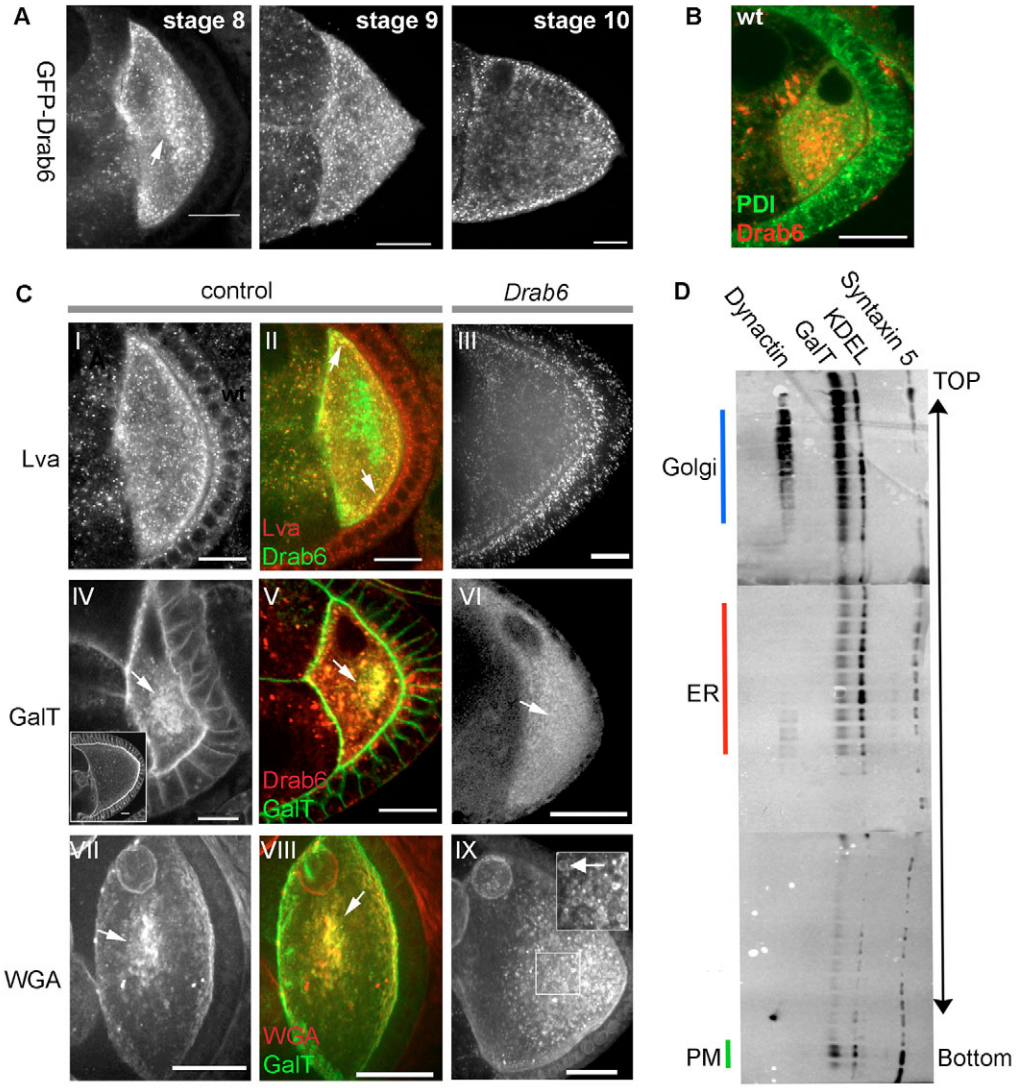



**Fig. 1. *Drosophila* Rab6 shows a dynamic localization and is enriched on Golgi membranes.** (**A**) Drab6 mutant oocytes rescued by GFP-Drab6 expression showed a stage-dependent distribution. Drab6 was central during stages 7 and 8 (arrow, in 40% of cases Drab6 expression was central, *n*=112), uniform during stage 9 (86%, *n*=81) and always juxtaposed to the oocyte cortex from the end of stage 9 onward (*n*=64). (**B**) RFP-Drab6 and PDI-GFP co-expressing egg chamber. (**C**) Colocalization of Drab6 and effects of its loss on different Golgi markers in control (**I**,**II**,**IV**,**V**,**VII**,**VIII**) and *rab6*$^{D23D}$ (**III**,**VI**,**IX**) oocytes. Lva (I) colocalized with Drab6 in a rescued egg chamber mainly at the cortex (II, arrows), but global Lva localization did not depend on Drab6 (III). GalT (IV) colocalized with RFP-Drab6 in the center during stage 8 (V, arrow; inset in IV is a stage 10 egg chamber), but did not accumulate in the center in *rab6*$^{D23D}$ (VI). WGA was central during stage 8 (VII arrow), colocalized with GalT (VIII, arrow), but formed abnormal ring-like aggregates in *rab6*$^{D23D}$ (IX, arrow in inset, which is a magnified view of the boxed area). (**D**) Immunoblots of fractions from a membrane density gradient of GalT-expressing ovaries tested with markers specific to the Golgi (Dynactin), the ER (KDEL and Syntaxin 5) and the plasma membrane (Syntaxin 5). GalT was predominantly enriched in fractions containing Golgi membranes, but was additionally found in fractions reflecting the plasma membrane. Vertical bars to the left indicate the sedimentation profile: ER, endoplasmatic reticulum; PM, plasma membrane. Scale bars: 20 μm.

beta-1,3-galactosyltransferase (GalT) (Morin et al., 2001), enriched predominantly in Golgi membranes (Fig. 1D), exhibited a distribution similar to that of GFP-Drab6: it accumulated in the center of the oocyte at stage 8, where it colocalized with Drab6, and was later confined to the cortex (Fig. 1C). Importantly, the distribution of Lva and GalT was similar in both $_{mat}\alpha$*tubGFP-Drab6*, *ubiRFP-Drab6* and control oocytes (see Fig. S3 in the supplementary material). Given that Lva and GalT markers are not present in the Golgi cisternae that are evenly distributed throughout the oocyte, as documented by electron microscopy (EM) analysis (Herpers and Rabouille, 2004), they might be the hallmark of distinct functional Golgi units, with Drab6 being able to interact with both types of Golgi. Unlike Lva, the distribution of which was only mildly affected (Fig. 1C), GalT and acetyl-glucosamine-modified proteins [detected by the wheat germ agglutinin lectin (WGA)] expressed by Golgi structures were abnormally distributed in *Drab6* mutants (Fig. 1C). Moreover, ultrastructural analysis by EM revealed that the ER was abnormally swollen in *Drab6* mutant oocytes (Fig. 2A′ versus B′), and that the Golgi mini-stacks were markedly curved, with partially inflated cisternae (Fig. 2A″ versus B″).

These morphological effects led us to investigate the role of Drab6 in the secretory pathway. We monitored the polarized secretion of the TGFα-like growth factor Grk (Neuman-Silberberg and Schupbach, 1993). Grk secretion is restricted to the anterodorsal corner through a rapid transit from the ER towards the Golgi apparatus (Herpers and Rabouille, 2004). In GFP-Drab6-rescued egg chambers, Grk and Drab6 colocalized (Fig. 2C). In Drab6 mutant oocytes, *grk* mRNA localization was the same as in wild type (see Fig. S1 in the supplementary material). Grk protein, however, was slightly more abundant than in controls and an important fraction extended ventrally (Fig. 2E). Polarized secretion of Grk led to the formation of two dorsal appendages on the egg shell (Fig. 2D). In the absence of Drab6, mislocalized Grk induced ventralization (Fig. 2E, inset; 22% absent dorsal appendages, 28% fused, *n*=199), instead of a dorsalization (multiple dorsal appendages on the egg

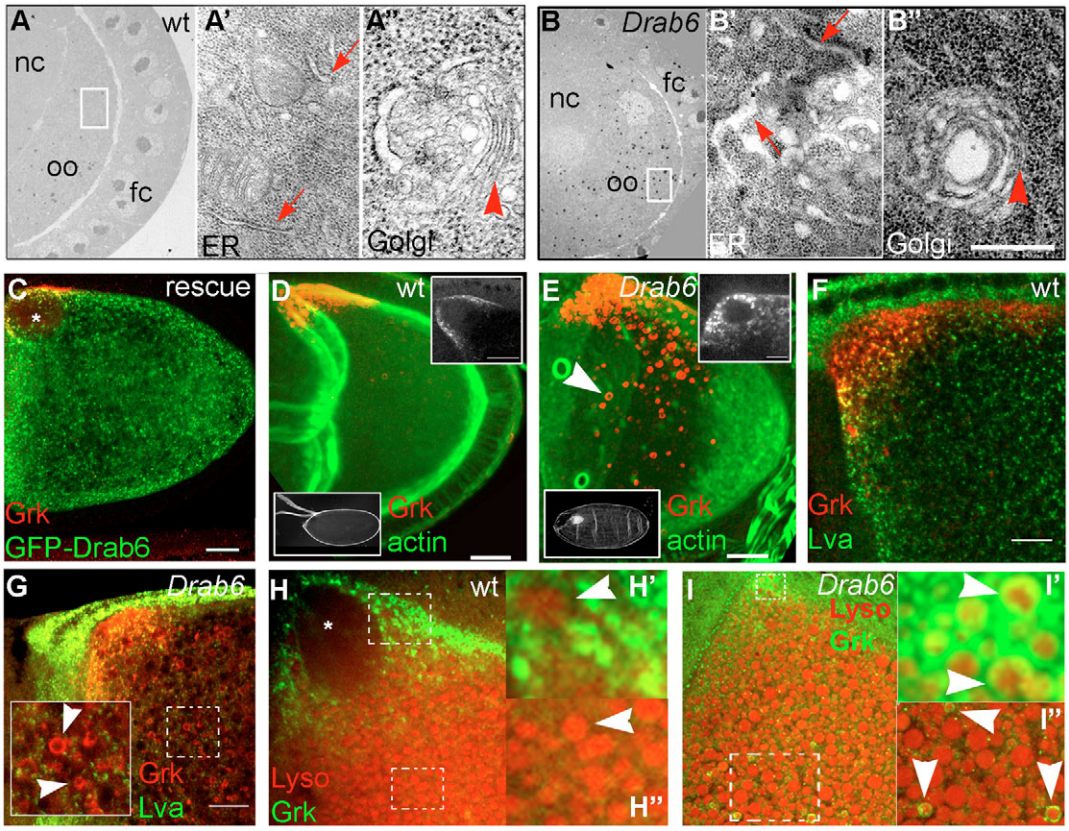



**Fig. 2. Grk is mislocalized to post-Golgi compartments in *Drosophila* Rab6 mutants.** (**A**,**B**) Low magnification electron micrographs of control (A) and *rab6*$^{D23D}$ mutant (B) egg chamber. (**A′**,**A″**) Magnified view of boxed area from A. (**B′**,**B″**) Magnified view of boxed area from B. nc, nurse cells; oo, oocyte; fc, follicle cells. ER morphology in control (arrows in A′) and in *rab6*$^{D23D}$ (arrows in B′). Normal tER-Golgi unit morphology in the control (A″) and onion-like shaped morphology in mutant (B″). Cisternae (arrowheads) were not altered in number, although severely swollen, in the mutant. (**C**) Drab6 and Grk colocalization in GFP-Drab6-rescued oocyte. (**D**,**E**) Projection of optical sections (~20 μm) of control (D) and *rab6*$^{D23D}$ (E) stage 9 oocytes, stained for Grk and F-actin. Upper insets, confocal sections at the nucleus. Lower insets, control egg and ventralized egg in the mutant. In contrast to the control, Grk formed larger particles close to and ring-like structures remote from the nucleus (arrowhead) in the mutant. (**F**,**G**) Grk and Lva colocalized marginally to small particles close to the nucleus in the control (F) and in *rab6*$^{D23D}$ (G) oocytes. Colocalization was not observed for the large Grk-positive ring-like structures (G, arrowheads in enlarged view shown in inset) in the mutant. (**H**,**I**) Control and Drab6 mutant egg chamber, respectively, labeled with Grk and LysoTracker (Lyso) to reveal endosomal compartments and lysosomes. (**H′**,**I′**) Magnified view of upper boxed area from H and I, respectively. (**H″**,**I″**) Magnified view of lower boxed area from H and I, respectively. Grk colocalized in a ring-like manner with Lyso-positive structures close to and distal to the nucleus only in *rab6*$^{D23D}$. Asterisk, oocyte nucleus. Scale bars: 20 μm; 500 nm in B″.

shell) as observed when Grk is ectopically secreted (Neuman-Silberberg and Schupbach, 1994). Hence, this argues for a specific failure of Grk delivery to the plasma membrane. This phenotype is specific to Drab6 because it could be fully rescued by the GFP-Drab6 transgene (Fig. 2C).

Next, we analyzed the intracellular localization of Grk in the absence of Drab6. Grk accumulated frequently in large ring-like particles in the *Drab6* mutant, but not in control oocytes (Fig. 2D,E). These Grk 'rings', similar to those of yolk granules (Bokel et al., 2006; Queenan et al., 1999), did not contain Lva (Fig. 2G), suggesting that Grk is not blocked in the Golgi. Grk actually accumulated in *Drab6* mutants on vesicles stained by LysoTracker (Fig. 2H versus I), which labels either lysosomes or late endosomes containing yolk granules (Dermaut et al., 2005). Hence, two independent approaches suggest that Grk is not blocked in the Golgi, but is mislocalized to post-Golgi compartments, probably endosomes.

Interestingly, the secretory impairment was also confirmed by *Lycopersicon esculentum* tomato lectin (LE) detecting modified proteins in the Golgi. In the absence of Drab6, LE revealed abnormal vesicular structures in the oocyte and nurse cells that had failed to reach the cortex (see Fig. S2 in the supplementary material). EM analysis also demonstrated rupture of the plasma membrane between neighboring nurse cells (see Fig. S2 in the supplementary material). Finally, we observed that GFP-Drab6-rescued egg chambers exhibited an accumulative enrichment of Drab6 at the plasma membrane during oogenesis, which was particularly evident in nurse cells (see Fig. S2 in the supplementary material). This is consistent with the involvement of Drab6 in secretion towards the plasmalemma.

We have established the existence of three important and novel aspects of Drab6 function during oogenesis, as follow.

First, consistent with its localization in vertebrate cells, Drab6 is predominantly localized to the Golgi complex in *Drosophila*, but overlaps with Golgi markers that have distinct localizations, suggesting that Drab6 might associate with distinct functional Golgi units. Drab6 might also play a role in membrane exchange between Golgi and ER and in Golgi organization, according to our EM analysis, which is again consistent with known functions of mammalian Rab6 (Del Nery et al., 2006; Martinez et al., 1997; Young et al., 2005).

Second, by controlling the migration of Golgi units towards the cell cortex, Drab6 controls the delivery of membrane to the plasmalemma, as shown in *Drab6* mutants in which glycosylated proteins labeled by WGA and LE lectins accumulate in large vesicular structures. This pattern is similar to the mislocalization profile of Grk in the absence of Drab6.

Third, in the oocyte, Drab6 is required for the anterodorsal secretion of Grk, which leads to the differentiation of the follicle cells required for the morphogenesis of the dorsal appendages of the egg shell. In the absence of Drab6, we observed that Grk is mislocalized to late endosomal or lysosomal compartments, demonstrating that Drab6 also affects post-Golgi traffic. In vertebrates, one of the Rab6 isoforms (Rab6A′) is also involved in endosome-to-Golgi transport (Del Nery et al., 2006; Utskarpen et al., 2006). Additionally, a role for Ypt6p (the only copy of Rab6 in the yeast *S. cerevisiae*) has also been documented as being involved in fusion of endosome-derived vesicles with the late Golgi (Siniossoglou and Pelham, 2001). It remains to be established whether Drab6 functions directly in the secretory pathway or if the effects observed in *Drab6* mutants on post-Golgi trafficking are a consequence of defects in endosome-to-Golgi trafficking.

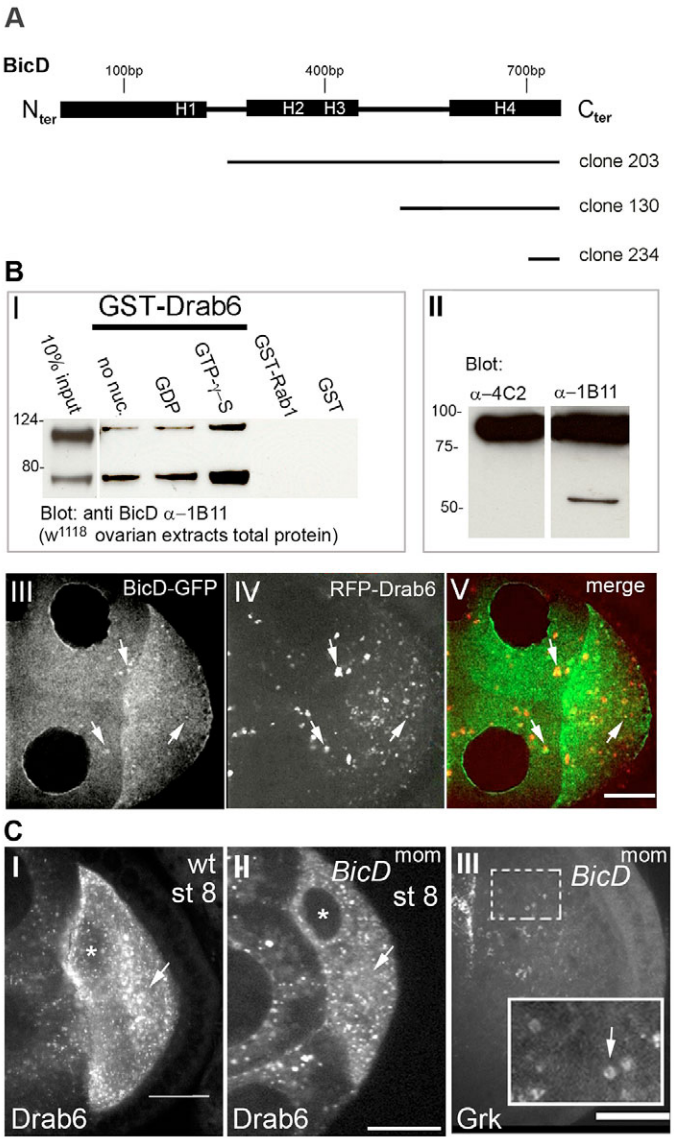
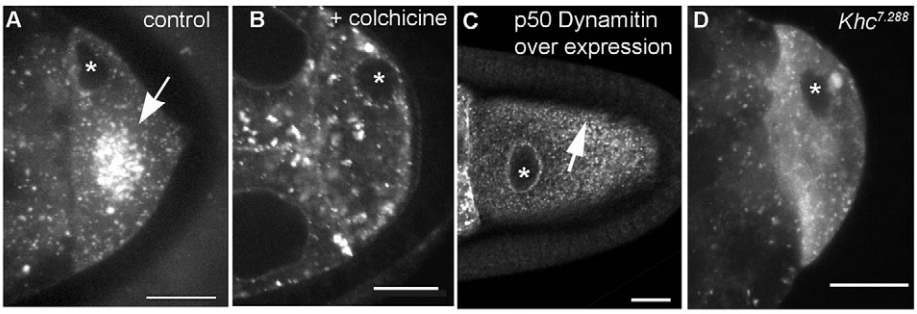



**Fig. 3. Drab6 interacts with BicD in *Drosophila*.** (**A**) Schematic of BicD. Exemplary truncated BicD clones containing the C-terminal H4 coiled-coil domain that interacted with Drab6 in the two-hybrid screen. (**B**) Interaction of Drab6 and BicD. (**I**) Western blot. GST-Drab6 specifically retained BicD. Preloading GST-Drab6 with the non-hydrolyzable GTP analog, GTP-γ-S, yielded an improved interaction. In addition to BicD (~89 kDa), a polypeptide of lower molecular weight (probably a degradation product, ~60 kDa) was specifically retained on GST-Drab6 beads and could be revealed with the 1B11 but not the 4C2 antibody (**II**). (**III-V**) Frames taken from time-lapse recording of BicD-GFP (III) and RFP-Drab6 (IV) co-expressing egg chamber, which colocalized to several aggregates in nurse cells and the oocyte (V). (**C**) Drab6 accumulates in the center (arrow) in GFP-Drab6-rescued egg chambers (**I**). In *BicD*$^{mom}$ egg chambers of equal age (**II**), GFP-Drab6 accumulation in the center was abolished (arrow). In *BicD*$^{mom}$ oocytes, Grk protein accumulated in ring-like structures remote from the nucleus (**III**, arrow in inset, which is a magnification of the upper boxed area). Asterisk, position of oocyte nucleus. Scale bars: 20 μm.

In order to identify potential Drab6-binding proteins, we performed a yeast two-hybrid screen (Formstecher et al., 2005) using as bait Drab6Q71L, a GTPase-deficient mutant. Sixty-two distinct truncated clones of BicD, lacking parts of the amino-




| A' | B' | C' | D' |
|---|---|---|---|
| **control** | **+ colchicine** | **++p50/Dynamitin** | **Khc$^{7.288}$ mutants** |
| vesicle speed [µm/sec]±S.E.: | vesicle speed [µm/sec]±S.E. | vesicle speed [µm/sec]±S.E. | vesicle speed [µm/sec]±S.E. |
| 0.33±0.06 | 0.03±0.01 | 0.253±0.016 | 0.10±0.01 |
| (max/min): | (max/min): | (max/min): | (max/min): |
| 0.99/0.13 | 0.06/0.013 | 0.95/0.06 | 0.25/0,05 |
| *n*: 62 | *n*: 51 | *n*: 97 | *n*: 92 |

**Fig. 4. Drab6 is actively transported along microtubules.** Frames taken from time-lapse recordings of GFP-Drab6-expressing *Drosophila* egg chambers. Untreated (**A**) GFP-Drab6-rescued egg chamber and (**B**) colchicine-treated egg chamber. (**C**) GFP-Drab6-expressing egg chamber overexpressing Dynamitin and (**D**) *Khc*$^{7.288}$ germ line clone expressing GFP-Drab6. Below each panel, parameters of vesicle movement derived from the time-lapse recordings are indicated (**A′-D′**). Particle parameters were determined using ImageJ. Particles in oocytes and nurse cells were traced in a single optical plane in three different egg chambers for each: control, *Khc* clones and overexpression of Dynamitin. (A,B) Colchicine treatment abolished accumulation in the center as seen in controls (arrow) and particles seemed to form bigger clusters. (C) Overexpressing Dynamitin reduced accumulation of Drab6 at the cortex (arrow). (D) Stage 8 *Khc*$^{788}$ oocyte expressing GFP-Drab6. Drab6 did not accumulate in the center and formed clusters close to the oocyte nucleus (asterisks). Scale bars: 20 µm.

terminus, interacted with Drab6Q71L (data not shown). The intersection of all identified fragments defined a minimal interacting domain, mapping to amino acids 699-772 in the coiled-coil motif H4 of BicD (Fig. 3A), shown for murine BicD to interact with the mammalian Rab6 (Matanis et al., 2002). In order to validate this interaction, we performed glutathione S-transferase (GST) pull-down assays, using lysates from wild-type ovaries. GST-Drab6 specifically retained BicD, as GST alone and GST-Rab1 did not bind BicD. Furthermore, preloading GST-Drab6 with the non-hydrolyzable GTP analog, GTP-γ-S, yielded an improved interaction with BicD (Fig. 3B). We conclude, therefore, that in vitro, BicD interacts through its carboxy-terminus preferentially with the active form of Drab6 (GTP-bound), as has been shown for mammalian Rab6 (Matanis et al., 2002; Short et al., 2002).

Time-lapse recording showed that in the oocyte and nurse cells, RFP-Drab6 and BicD-GFP (Pare and Suter, 2000) colocalize to multiple large aggregates with low dynamics (Fig. 3B). Further GFP-Drab6 accumulation in the center depended on the presence of BicD during stage 8, as observed in a *BicD*$^{mom}$ background (see Swan and Suter, 1996) (Fig. 3C). Interestingly, in such *BicD*$^{mom}$ oocytes, Grk was found in ring-like structures remote from the nucleus, as observed in Drab6 mutant oocytes (Fig. 3C).

Since BicD and Rab6 have been shown to be involved in MT-based transport, we checked whether Drab6-positive structures require MTs to move. Time-lapse microscopy revealed that large aggregates were less dynamic than the highly motile small particles. Colchicine MT depolymerization severely reduced the movement of Drab6 particles, which formed large clusters (Fig. 4B), indicating that Drab6 is actively transported along MTs. The MT motors Kinesin I [Kinesin heavy chain (Khc)] and Dynein have been shown to be involved in polarizing the *Drosophila* oocyte (Brendza et al., 2000; Brendza et al., 2002; Duncan and Warrior, 2002; Januschke et al., 2002). Inactivating the Dynein complex by the overexpression of Dynamitin (Januschke et al., 2002) prevented accumulation of Drab6 at the oocyte cortex (Fig. 4C), but did not significantly reduce Drab6 movements (Fig. 4C′). By contrast, in *Khc*$^{7.288}$ germ line clones, Drab6 did not localize in the center of the oocyte during stage 7/8 but formed abnormal aggregates around the mispositioned nucleus. For reasons we currently do not fully understand, the speed of Drab6 particles was significantly reduced compared with controls or Dynamitin-overexpressing oocytes (Fig. 4D).

We observed that Drab6 and BicD interact in a yeast two-hybrid screen and in GST pull-down assays and colocalize in vivo. Moreover, there were indications that Drab6 requires BicD for correct subcellular localization, which suggests that Drab6 interacts with BicD in *Drosophila* as it does in mammals. Strikingly, we found that lack of each protein compromises Grk secretion in a very similar way. Overexpression of Dynamitin, to impair Dynein function, induces ectopic accumulation of Grk and ventralization of the egg shell (Januschke et al., 2002). Therefore, in *Drosophila*, BicD/Dynein and Drab6 are likely to be involved together in Grk secretion to the anterodorsal corner of the oocyte.

It is important to mention that colocalization of the two proteins was limited. Moreover, lack of BicD or Drab6 yields different phenotypes. BicD mutation affects oocyte determination and the position of the oocyte nucleus (Swan et al., 1999), but has no impact on MT organization in mid-oogenesis (Swan and Suter, 1996), which is not the case in the *Drab6* mutant (see Fig. S1 in the supplementary material). A genetic interaction between BicD's co-factor Egalitarian and Kinesin I has already been demonstrated (Navarro et al., 2004), suggesting that Drab6 might interact with Dynein and Kinesin I via BicD.

Interestingly, we noticed that in the absence of Drab6, *osk* mRNA was not correctly localized in the oocyte (see Fig. S1 in the supplementary material) (42% dot, 31% diffuse, 10% undetectable, remainder wild type, *n*=75). *gurken* and *bicoid* mRNAs were, however, unaffected (see Fig. S1 in the supplementary material), and *osk* mRNA localization to the oocyte center is frequent when the MT network is not correctly polarized (St Johnston, 2005). In *Drab6* mutant oocytes, the defective posterior localization of the MT plus-end marker Khc–β-Gal (Clark et al., 1997) indicates a defect in MT organization (see Fig. S1 in the supplementary material). Similar observations have been reported recently (Coutelis and Ephrussi, 2007).

Given that Drab6 is required for late Grk signaling at the anterodorsal corner of the oocyte, it might also be involved in early germ line to soma signaling mediated by Grk, which controls MT organization (Gonzalez-Reyes et al., 1995; Roth et al., 1995). We think that this is unlikely. In the absence of this signaling, posterior follicle cells differentiate into anterior follicle cells and, as a consequence, the posterior structure of the egg shell, the aeropyle, is substituted with an anterior structure, the micropyle (Gonzalez-Reyes et al., 1995; Roth et al., 1995). We always observed an aeropyle at the posterior of eggs derived from *Drab6* mutant oocytes (see Fig. S2 in the supplementary material). Additionally, removing Drab6 from the posterior follicle cells did not affect oocyte polarity. Hence, Drab6 is possibly involved in MT organization at the posterior pole. Interestingly, Rab6 family interactors such as Rab6IP2/ELKS (Monier et al., 2002) are capable of interacting with CLASPs at the cortex of HeLa cells (Lansbergen et al., 2006), suggesting a link between Rab6 protein and MT organization at the cortex.

J.J. is very grateful to C. González in whose laboratory this work was finished. We thank S. Lepanse for EM analysis; E. Poisot for her work on BicD; C. Braendle and P. Dominguez for comments on the manuscript; A. Kropfinger for correcting the English language; the Bloomington Stock Center and the Developmental Studies Hybridoma Bank for providing reagents. J.J. was supported by the Association pour la Recherche sur le Cancer (ARC). This work was supported by ARC (grants 4446 and 3297), a GenHomme Network Grant (02490-6088) to Hybrigenics and Institut Curie, ACI 'Biologie cellulaire', 'Jeune Chercheur' grant 035117, ANR 'Blanche' (grant Cymempol, Blan06-3-139786).

**Supplementary material**

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/19/3419/DC1

**References**

**Bobinnec, Y., Marcaillou, C., Morin, X. and Debec, A.** (2003). Dynamics of the endoplasmic reticulum during early development of Drosophila melanogaster. *Cell Motil. Cytoskeleton* **54**, 217-225.

**Bokel, C., Dass, S., Wilsch-Brauninger, M. and Roth, S.** (2006). Drosophila Cornichon acts as cargo receptor for ER export of the TGF-like growth factor Gurken. *Development* **133**, 459-470.

**Brendza, R. P., Serbus, L. R., Duffy, J. B. and Saxton, W. M.** (2000). A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science* **289**, 2120-2122.

**Brendza, R. P., Serbus, L. R., Saxton, W. M. and Duffy, J. B.** (2002). Posterior localization of dynein and dorsal-ventral axis formation depend on kinesin in Drosophila oocytes. *Curr. Biol.* **12**, 1541-1545.

**Clark, I. E., Jan, L. Y. and Jan, Y. N.** (1997). Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the Drosophila oocyte, epithelium, neuron and muscle. *Development* **124**, 461-470.

**Coutelis, J. B. and Ephrussi, A.** (2007). Rab6 mediates membrane organization and determinant localization during Drosophila oogenesis. *Development* **134**, 1419-1430.

**Del Nery, E., Miserey-Lenkei, S., Falguieres, T., Nizak, C., Johannes, L., Perez, F. and Goud, B.** (2006). Rab6A and Rab6A' GTPases play non-overlapping roles in membrane trafficking. *Traffic* **7**, 394-407.

**Dermaut, B., Norga, K. K., Kania, A., Verstreken, P., Pan, H., Zhou, Y., Callaerts, P. and Bellen, H. J.** (2005). Aberrant lysosomal carbohydrate storage accompanies endocytic defects and neurodegeneration in Drosophila benchwarmer. *J. Cell Biol.* **170**, 127-139.

**Duncan, J. E. and Warrior, R.** (2002). The cytoplasmic dynein and kinesin motors have interdependent roles in patterning the Drosophila oocyte. *Curr. Biol.* **12**, 1982-1991.

**Formstecher, E., Aresta, S., Collura, V., Hamburger, A., Meil, A., Trehin, A., Reverdy, C., Betin, V., Maire, S., Brun, C. et al.** (2005). Protein interaction mapping: a Drosophila case study. *Genome Res.* **15**, 376-384.

**Gonzalez-Reyes, A., Elliott, H. and St Johnston, D.** (1995). Polarization of both major body axes in Drosophila by gurken-torpedo signalling. *Nature* **375**, 654-658.

**Hachet, O. and Ephrussi, A.** (2001). Drosophila Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport. *Curr. Biol.* **11**, 1666-1674.

**Herpers, B. and Rabouille, C.** (2004). mRNA localization and ER-based protein sorting mechanisms dictate the use of transitional endoplasmic reticulum-golgi units involved in gurken transport in Drosophila oocytes. *Mol. Biol. Cell* **15**, 5306-5317.

**Hoogenraad, C. C., Akhmanova, A., Howell, S. A., Dortland, B. R., De Zeeuw, C. I., Willemsen, R., Visser, P., Grosveld, F. and Galjart, N.** (2001). Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes. *EMBO J.* ***20***, 4041-4054.

**Huynh, J. R. and St Johnston, D.** (2004). The origin of asymmetry: early polarisation of the Drosophila germline cyst and oocyte. *Curr. Biol.* **14**, R438-R449.

**Januschke, J., Gervais, L., Dass, S., Kaltschmidt, J. A., Lopez-Schier, H., Johnston, D. S., Brand, A. H., Roth, S. and Guichet, A.** (2002). Polar transport in the Drosophila oocyte requires Dynein and Kinesin I cooperation. *Curr. Biol.* **12**, 1971-1981.

**Januschke, J., Gervais, L., Gillet, L., Keryer, G., Bornens, M. and Guichet, A.** (2006). The centrosome-nucleus complex and microtubule organization in the Drosophila oocyte. *Development* **133**, 129-139.

**Kondylis, V., Goulding, S. E., Dunne, J. C. and Rabouille, C.** (2001). Biogenesis of Golgi stacks in imaginal discs of Drosophila melanogaster. *Mol. Biol. Cell* **12**, 2308-2327.

**Lansbergen, G., Grigoriev, I., Mimori-Kiyosue, Y., Ohtsuka, T., Higa, S., Kitajima, I., Demmers, J., Galjart, N., Houtsmuller, A. B., Grosveld, F. et al.** (2006). CLASPs attach microtubule plus ends to the cell cortex through a complex with LL5beta. *Dev. Cell* **11**, 21-32.

**MacDougall, N., Clark, A., MacDougall, E. and Davis, I.** (2003). Drosophila gurken (TGFalpha) mRNA localizes as particles that move within the oocyte in two dynein-dependent steps. *Dev. Cell* **4**, 307-319.

**Mallard, F., Tang, B. L., Galli, T., Tenza, D., Saint-Pol, A., Yue, X., Antony, C., Hong, W., Goud, B. and Johannes, L.** (2002). Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. *J. Cell Biol.* **156**, 653-664.

**Martinez, O., Schmidt, A., Salamero, J., Hoflack, B., Roa, M. and Goud, B.** (1994). The small GTP-binding protein rab6 functions in intra-Golgi transport. *J. Cell Biol.* ***127***, 1575-1588.

**Martinez, O., Antony, C., Pehau-Arnaudet, G., Berger, E. G., Salamero, J. and Goud, B.** (1997). GTP-bound forms of rab6 induce the redistribution of Golgi proteins into the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* **94**, 1828-1833.

**Matanis, T., Akhmanova, A., Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F., Goud, B., De Zeeuw, C. I. et al.** (2002). Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex. *Nat. Cell Biol.* **4**, 986-992.

**Monier, S., Jollivet, F., Janoueix-Lerosey, I., Johannes, L. and Goud, B.** (2002). Characterization of novel Rab6-interacting proteins involved in endosome-to-TGN transport. *Traffic* **3**, 289-297.

**Morin, X., Daneman, R., Zavortink, M. and Chia, W.** (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **98**,15050-15055.

**Navarro, C., Puthalakath, H., Adams, J. M., Strasser, A. and Lehmann, R.** (2004). Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nat. Cell Biol.* **6**, 427-435.

**Neuman-Silberberg, F. S. and Schupbach, T.** (1993). The Drosophila dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF-alpha-like protein. *Cell* **75**, 165-174.

**Neuman-Silberberg, F. S. and Schupbach, T.** (1994). Dorsoventral axis formation of Drosophila depends on the correct dosage of the gene gurken. *Development* **120**, 2457-2463.

**Opdam, F. J., Echard, A., Croes, H. J., van den Hurk, J. A., van de Vorstenbosch, R. A., Ginsel, L. A., Goud, B. and Fransen, J. A.** (2000). The small GTPase Rab6B, a novel Rab6 subfamily member, is cell-type specifically expressed and localised to the Golgi apparatus. *J. Cell Sci.* **113**, 2725-2735.

**Papoulas, O., Hays, T. S. and Sisson, J. C.** (2005). The golgin Lava lamp mediates dynein-based Golgi movements during Drosophila cellularization. *Nat. Cell Biol.* **7**, 612-618.

**Pare, C. and Suter, B.** (2000). Subcellular localization of Bic-D::GFP is linked to an asymmetric oocyte nucleus. *J. Cell Sci.* **113**, 2119-2127.
**Purcell, K. and Artavanis-Tsakonas, S.** (1999). The developmental role of warthog, the notch modifier encoding Drab6. *J. Cell Biol.* **146**, 731-740.
**Queenan, A. M., Barcelo, G., Van Buskirk, C. and Schupbach, T.** (1999). The transmembrane region of Gurken is not required for biological activity, but is necessary for transport to the oocyte membrane in Drosophila. *Mech. Dev.* **89**, 35-42.
**Riechmann, V. and Ephrussi, A.** (2001). Axis formation during Drosophila oogenesis. *Curr. Opin. Genet. Dev.* **11**, 374-383.
**Roth, S., Neuman-Silberberg, F. S., Barcelo, G. and Schupbach, T.** (1995). Cornichon and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in Drosophila. *Cell* **81**, 967-978.
**Shetty, K. M., Kurada, P. and O'Tousa, J. E.** (1998). Rab6 regulation of rhodopsin transport in Drosophila. *J. Biol. Chem.* **273**, 20425-20430.
**Short, B., Preisinger, C., Schaletzky, J., Kopajtich, R. and Barr, F. A.** (2002). The Rab6 GTPase regulates recruitment of the dynactin complex to Golgi membranes. *Curr. Biol.* **12**, 1792-1795.
**Short, B., Haas, A. and Barr, F. A.** (2005). Golgins and GTPases, giving identity and structure to the Golgi apparatus. *Biochim. Biophys. Acta* **1744**, 383-395.
**Siniossoglou, S. and Pelham, H. R.** (2001). An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J.* **20**, 5991-5998.
**St Johnston, D.** (2005). Moving messages: the intracellular localization of mRNAs. *Nat. Rev. Mol. Cell Biol.* **6**, 363-375.
**St Johnston, D., Beuchle, D. and Nusslein-Volhard, C.** (1991). Staufen, a gene required to localize maternal RNAs in the Drosophila egg. *Cell* **66**, 51-63.
**Swan, A. and Suter, B.** (1996). Role of Bicaudal-D in patterning the Drosophila egg chamber in mid-oogenesis. *Development* **122**, 3577-3586.
**Swan, A., Nguyen, T. and Suter, B.** (1999). Drosophila Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. *Nat. Cell Biol.* **1**, 444-449.
**Utskarpen, A., Slagsvold, H. H., Iversen, T. G., Walchli, S. and Sandvig, K.** (2006). Transport of ricin from endosomes to the Golgi apparatus is regulated by Rab6A and Rab6A'. *Traffic* **7**, 663-672.
**Young, J., Stauber, T., del Nery, E., Vernos, I., Pepperkok, R. and Nilsson, T.** (2005). Regulation of microtubule-dependent recycling at the trans-Golgi network by Rab6A and Rab6A'. *Mol. Biol. Cell* **16**, 162-177.