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Rab6 mediates membrane organization and determinant localization during *Drosophila* oogenesis

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The *Drosophila melanogaster* body axes are defined by the precise localization and the restriction of molecular determinants in the oocyte. Polarization of the oocyte during oogenesis is vital for this process. The directed traffic of membranes and proteins is a crucial component of polarity establishment in various cell types and organisms. Here, we investigate the role of the small GTPase Rab6 in the organization of the egg chamber and in asymmetric determinant localization during oogenesis. We show that exocytosis is affected in *rab6*-null egg chambers, which display a loss of nurse cell plasma membranes. Rab6 is also required for the polarization of the oocyte microtubule cytoskeleton and for the posterior localization of *oskar* mRNA. We show that, in vivo, Rab6 is found in a complex with Bicaudal-D, and that Rab6 and Bicaudal-D cooperate in *oskar* mRNA localization. Thus, during *Drosophila* oogenesis, Rab6-dependent membrane trafficking is doubly required; first, for the general organization and growth of the egg chamber, and second, more specifically, for the polarization of the microtubule cytoskeleton and localization of *oskar* mRNA. These findings highlight the central role of vesicular trafficking in the establishment of polarity and in determinant localization in *Drosophila*.

KEY WORDS: *Drosophila* oogenesis, Rab6, *oskar* mRNA, Exocyst, Microtubules, Polarity

INTRODUCTION

In *Drosophila melanogaster*, the embryonic body axes are defined by the localization and restriction of determinants as mRNAs and proteins in the oocyte (Riechmann and Ephrussi, 2001). This asymmetry is the result of a succession of processes that take place during oogenesis. The ovary consists of ovarioles, which are composed of egg chambers of increasing developmental stages (Fig. 1B). In the germarium, germline stem cells divide asymmetrically, generating a new stem cell and a cystoblast (Huynh and St Johnston, 2004). The cystoblast undergoes four rounds of mitosis with incomplete cytokinesis, producing a germline cyst of 16 cells interconnected via 15 actin-rich cytoplasmic bridges, the ring canals. Each cystocyte displays a specific number of ring canals reflecting the mitosis at which it was generated: the two first-born cystocytes possess four ring canals, and the eight last-born possess one. Oocyte specification occurs via the progressive and selective concentration of cell fate determinants to the two four-branched cells and, finally, to just one of these cells, the oocyte. This process requires an organized and polarized microtubule (MT) network spanning the germline cyst and connecting the cystocytes (Grieder et al., 2000; Theurkauf et al., 1993; Vaccari and Ephrussi, 2002). The remaining 15 cells adopt the nurse cell fate and provide molecules required for oocyte development. The germarium also contains somatic stem cells. Some of these differentiate into follicular epithelial cells that encapsulate the germline cyst, thus forming the stage-1 egg chamber, which exits the germarium. Oogenesis has been subdivided into 14 stages (reviewed in King, 1970; Spradling, 1993). During the first six stages, the 16 cystocytes grow at roughly identical rates and contribute almost equally to the increase in size of the egg chamber. At stage 8, vitellogenesis begins and the increase in egg chamber size mainly reflects the major growth of the oocyte.

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Antero-posterior axis specification occurs at stage 7, with the secretion by the oocyte of the Gurken protein, a *Drosophila* TGFα homolog and EGF-receptor ligand (reviewed in Nilson and Schupbach, 1999). This signal induces a group of overlying follicle cells to adopt the posterior fate (Gonzalez-Reyes et al., 1995; Roth et al., 1995). In return, these cells emit an unknown signal, causing the disassembly of the unique MT-organizing center (MTOC) of the germline cyst. This MTOC is located at the posterior of the oocyte until stage 7. New MT then nucleate from the anterior and lateral cortex of the oocyte, whose nucleus moves in a MT-dependent manner from its posterior location to an anterior corner of the cell. Here, a second Gurken signal causes the overlying follicle cells to adopt a dorsal fate, thus specifying the dorsoventral axis. Concomitantly, MT-dependent localization of *bicoid* and *oskar* mRNAs to the anterior and posterior of the oocyte, respectively, establishes the antero-posterior axis of the future embryo (reviewed in Riechmann and Ephrussi, 2001).

Genetic analysis has revealed the involvement of proteins such as Bicaudal-D (BicD) and Dynein, a minus-end-directed MT motor, together with the MT network, in germline cyst development, oocyte specification, asymmetric mRNA localization within the oocyte and egg-chamber formation (Navarro et al., 2004; Oh and Steward, 2001; Suter and Steward, 1991; Swan et al., 1999; Vaccari and Ephrussi, 2002). Thus, in *BicD* mutant germaria, the MT cytoskeleton fails to maintain its polarization and none of the cystocytes enter meiosis, resulting in a cyst of 16 nurse cells (Huynh and St Johnston, 2000; Oh and Steward, 2001).

It has been shown that the mammalian homologs of BicD (BICD1 and BICD2) physically interact with Dynamitin, a component of the Dynactin complex (the regulatory and cargo-loading subunit of Dynein), and with Dynein intermediate chain (Hoogenraad et al., 2001) to control Dynein-based motility (Hoogenraad et al., 2003). In addition, BICD1 and BICD2 bind RAB6a and RAB6a′ (Matanis et al., 2002; Short et al., 2002), members of the Rab family of small monomeric GTPases, which are among the major regulators of vesicular trafficking. Rab proteins segregate over the various organelle membranes in the cell, and their specific interactions with effectors promote asymmetric and polarized vesicle transport (van

Ijzendoorn et al., 2003; Zerial and McBride, 2001). RAB6a′ also directly binds the Dynactin complex via its p150^{Glued} subunit and promotes the recruitment of this complex to the trans-Golgi Network (TGN) (Short et al., 2002). Finally, RAB6a, RAB6a′, BICD2 and Dynactin appear to cooperate to mediate the recycling and retrograde transport of proteins from the TGN (Young et al., 2005), indicating that, in mammals, these three proteins are physically and functionally linked.

Polarized intracellular transport and vesicular trafficking can be used to create and/or maintain asymmetry during *Drosophila* development (Dudu et al., 2004; Gonzalez-Gaitan, 2003). However, little is known about the involvement of vesicular trafficking and of Rab proteins in cell polarization during oogenesis. One Rab protein, Rab11, has been shown to act in *oskar* mRNA localization at mid-oogenesis (Dollar et al., 2002; Jankovics et al., 2001). Given the interactions in mammalian cells of BICD1 and BICD2, and of the Dynactin-Dynein complex, whose homologs have essential functions in *Drosophila*, with RAB6a and RAB6a′, we investigated the involvement of this protein in *Drosophila* oogenesis. Our study reveals that Rab6 is required at several steps during oogenesis. Depletion of Rab6 in the germ line causes membrane-trafficking defects that affect egg-chamber plasma membranes and the proper organization of the germline cyst. At mid-oogenesis, lack of Rab6 results in an abnormally polarized oocyte MT network and in the mis-localization of *oskar* mRNA.

MATERIALS AND METHODS

Fly stocks and manipulations

Flies were grown according to standard procedures (Ashburner, 1989). For the generation of germline clones by FRT/FLP-mediated recombination, *rab6^{D23D}* (Purcell and Artavanis-Tsakonas, 1999) was recombined with [*w;FRT40A^{neoR}*]. *hs::rab6* is described by Purcell and Artavanis-Tsakonas (Purcell and Artavanis-Tsakonas, 1999). To generate *rab6^{D23D}* and *sec5^{E13}* somatic or germline clones, [*w;rab6^{D23D},FRT40A/Cyo;*] flies (some carrying the *KinlacZ* transgene) (Clark et al., 1994), or [*w;sec5^{E13},FRT40A/Cyo;*] flies (Murthy and Schwarz, 2004) were crossed with [*yw,hs::FLP;Ovo^{D},FRT40A/Cyo;*] or with [*w,f,hs::FLP;GFP,FRT40A/Cyo*] flies. Progeny were heat-shocked daily for 1 hour at 37°C during the third larval instar.

For rescue experiments, [*yw,hs::FLP/w;Ovo^{D},FRT40A/rab6^{D23D}, FRT40A;hs::rab6/+;*] females and [*yw,hs::FLP/w;Ovo^{D},FRT40A/ rab6^{D23D},FRT40A;*] control females were heat-shocked at 37°C for 1 hour each day during the third larval instar, and then three times for 30 minutes every 2 days until emergence.

Evaluation of hatching-rate, fertility and the embryonic requirement for *rab6*

Virgin *rab6* [*yw,hs::FLP/w;Ovo^{D},FRT40A/rab6^{D23D},FRT40A*] females and [*yw,hs::FLP/w;Ovo^{D},FRT40A/FRT40A*] and OreR control females were collected and crossed to wild-type OreR males or to males heterozygous for either *rab6^{D23D}* [*w;rab6^{D23D}/Cyo,UbiGFP;*] or for a genomic deletion covering the *rab6* locus [*;Df(2L)prd1.7,b^{1}Adh^{n2}pr^{1}cn^{1}sca^{1}/Cyo,UbiGFP;*] (Bloomington #3344). *rab6^{D23D}* eggs were obtained after Ovo^{D} selection. After aging the females for 3 days at 25°C, eggs were collected on apple juice plates and hatch rates were scored. From *rab6^{D23D}* eggs that hatched, both GFP-positive and GFP-negative second-instar larvae were observed; however, only GFP-positive third-instar larvae were observed, and all hatched flies displayed the CyO dominant marker, revealing the larval lethality of the *rab6* mutation. To test the fertility of flies hatched from *rab6^{D23D}* eggs that lacked the maternal contribution of Rab6, female offspring were mated with wild-type OreR males.

Whole-mount ovary staining

Antibody stainings were performed as previously described (Tomancak et al., 2000). Antibodies and dilutions were as follows: rabbit anti-Staufen (1:2000), rabbit anti-Oskar (1:3000), mouse anti-Sec5 (22A2; 1:200), mouse anti-Syx1A (8C3; 1:20), mouse anti-Gurken (1D12; 1:200), rat anti-Yolkless (1:100), mouse anti-BicD (1B11; 1:20) and rabbit anti-Lava lamp (1:500). Double-labeling of ovaries using RNA probes and antibodies was performed as previously described (Vanzo and Ephrussi, 2002).

To observe membranes and membranous organelles in their native organization using lipophilic styryl compounds (FM-dyes, Molecular Probes), ovaries were dissected in PBS, fixed in 4% formaldehyde for 20 minutes and washed twice in PBS (10 minutes each). Next, they were incubated with rhodamine-conjugated phalloidin (1:500; Molecular Probes) in PBS for 6 hours at room temperature or overnight at 4°C. Subsequently, they were incubated for 10 minutes in PBS containing FM4-64 (1:2000), then for 5 minutes in PBS with DAPI (1:2500). They were then washed twice for 5 minutes in PBS and equilibrated in N-propylgallate medium before mounting. Confocal microscopy was performed on a Leica TCS-SP and images edited using Adobe Photoshop.

Cloning

N-terminal fusions of Myc-tag or GFP to Rab6 or Rab7 were constructed according to standard procedures (details available upon request).

Co-immunoprecipitation and western blotting

Ovarian extracts were prepared from 3-day-old well-fed flies expressing Myc-tagged Rab6 or Rab7 under maternal-tubulin promoter control [*;;UASp::Myc-Rab/Mattub::Gal4Vp16;*] using the Gal4/UAS system (Rorth, 1998). Ovaries were washed twice in 1:1 PBS:Extraction Buffer [20 mM HEPES pH 7.5, 400 mM NaCl, 1 mM PMSF, 1×Protein Inhibitor without EGTA/EDTA (Roche catalogue number 1873580) 10% Glycerol] and lysed in 5 μl extraction buffer per 20 ovaries. After centrifugation (10 minutes at 16,000 ***g*** at 4°C), the supernatant was collected and 9 μl of 2×IP buffer (20 mM HEPES pH 7.5, 300 mM NaCl, 5 mM $MgCl_2$, 2×Protein Inhibitor without EGTA/EDTA and 500 mM Sucrose) plus 2 μl PBS and 2 μl of mouse monoclonal anti-c-Myc (Sigma, Clone 9E10) was added per 5 μl supernatant, mixed and rocked for 2 hours at 4°C. In total, 40 μl of 1:1 Protein G beads:Washing Buffer (20 mM HEPES pH 7.5, 400 mM NaCl, 1×Protein Inhibitor without EGTA/EDTA) were added and samples rocked for 1 hour at 4°C. The bead pellet and supernatant were collected individually. Beads were washed four times in washing buffer and resuspended in SDS-buffer. Proteins were detected by 10% SDS-PAGE followed by western blotting, using either mouse monoclonal anti-c-Myc (9E10; 1:1000) or mouse anti-BicD (4C2; 1:100), following standard procedures.

RESULTS

In *Drosophila*, *rab6* is a unique and essential gene. The *rab6^{D23D}* allele is a homozygous-lethal genomic deletion of the entire *rab6* coding sequence (Purcell and Artavanis-Tsakonas, 1999). Therefore, to analyze the role of *rab6* in oogenesis, we generated homozygous-mutant *rab6* germline clones in otherwise heterozygous females. Upon mitotic recombination, *rab6^{D23D}* germline clones were identified by either the absence of nuclear GFP or were selected using the dominant female-sterile Ovo^{D} technique (Chou and Perrimon, 1996).

Loss of actin organization in *rab6*-null egg chambers

In wild-type egg chambers, each nurse cell is delimited by a plasma membrane and an underlying actin cytoskeleton, but communicates with its neighboring cells through the ring canals (see Fig. S1A in the supplementary material). In *rab6^{D23D}* mutant germaria, cyst formation and maturation appeared normal (data not shown). However, from stages 2 to 7, 80.6% (n=191) of *rab6^{D23D}* egg chambers displayed a progressive disappearance of the nurse cell cortical actin cytoskeleton, aberrantly spaced nurse cell nuclei (Fig. 1A), and a concomitant aggregation of ring canals and actin debris in the egg chamber (see Fig. S1B,C in the supplementary material). At stage 7, *rab6^{D23D}* egg chambers of two classes, based on the

integrity of the oocyte, are observed (see Fig. S1 in the supplementary material). The first class (32.5%) consisted of strongly affected egg chambers and is characterized by the absence of all cortical actin, which usually delimits the cystocytes, oocyte included. Such egg chambers continue to grow but ultimately degenerate, as none of this type is observed beyond stage 7. The second class (67.5%; see Fig. S1 in the supplementary material) is more heterogeneous. All egg chambers of this second class possess a delimited oocyte.

The mutant phenotypes observed in *rab6*^{D23D} egg chambers are caused by a lack of *rab6* function, because they are fully rescued by a heat-inducible *rab6* transgene (Fig. 1, also see Fig. S2 in the supplementary material). Therefore, *rab6* is required for proper actin organization in the egg chamber. The oocyte and the nurse cells may differ in their requirement for Rab6, as the oocyte is often the only cell in the cyst displaying an intact actin cytoskeleton. By contrast, *rab6* does not appear essential either for viability or for polarity of the follicle cells (see Fig. S3A in the supplementary material). Indeed, no difference was observed between *rab6*-null follicle cell clones and wild-type cells, either morphologically or with respect to the distribution of polarity markers, such as aPKC, Par-1 or DE-Cadherin (data not shown).

rab6-null egg chambers form open syncytia

The disappearance of the cortical actin cytoskeleton and abnormal spacing of the nurse cell nuclei in *rab6*^{D23D} egg chambers suggested the possible loss of plasma membranes within the mutant cysts. We therefore evaluated the integrity of the membranes, staining them with lipophilic styryl compounds (FM4-64, Molecular Probes) that fluoresce upon insertion into membranes. To resolve plasma membranes, membranous organelles and vesicles in their native organization, we used the rapid-diffusion property of the dye to mark all membranes of fixed egg chambers without permeabilization.

In wild-type cysts, the actin cytoskeleton and the plasma membrane appear to colocalize (Fig. 2A, arrow) and delimit each nurse cell. The majority of *rab6*^{D23D} egg chambers, rather than forming a compartmentalized cyst, form 'open' syncytia (Fig. 2B,C) in which some or all nurse cells lack delimiting plasma membranes and the nuclei are encompassed within a single actin and plasma membrane boundary (Fig. 2B, arrow). In strongly affected *rab6*^{D23D} egg chambers, all nuclei are found within a single open syncytium (Fig. 2C). In these chambers, the nuclei are found at the periphery of a common cytoplasm containing, in its center, a single mass of membranes, ring canals and actin debris (Fig. 2C, arrowhead). Noticeably, the oocyte is consistently the last cell of such *rab6*-null germline cysts to display plasma membrane loss. *rab6*^{D23D} egg chambers in which membrane loss was observed, but that nevertheless developed past stage 7, displayed a stereotypic organization at mid-oogenesis. In these chambers, 12 out of the 15 nurse cell nuclei were reproducibly contained within two open syncytia, the first comprising eight, and the second four, nuclei (see Fig. S4A in the supplementary material).

Lack of Rab6 does not seem to affect oocyte fate, because the enrichment and restriction of BicD, Par-1 and Staufen observed from the germarium stage until mid-oogenesis (Shulman et al., 2000; St Johnston et al., 1991; Suter and Steward, 1991; Tomancak et al., 2000) occurred in *rab6*^{D23D} oocytes the same as in wild-type (see Fig. S2A″ in the supplementary material, and data not shown). In addition, no egg chamber with 16 nurse cells and no oocyte, which would indicate a lack of oocyte specification or a loss of oocyte fate, was observed in *rab6*-null mutants. Finally, in strongly affected *rab6*^{D23D} egg chambers in which no oocyte was observed, the oocyte-specific markers BicD and Par-1 were detected around the central cluster of ring canals (see Fig. S3B″ in the supplementary material, and data not shown). This ectopic enrichment suggests the prior existence of a correctly specified oocyte whose membrane integrity was impaired. Thus *rab6* function is dispensable for oocyte specification, but is required to maintain the organization of egg chambers and of their internal plasma membranes.

rab6-null egg chambers display defects in Yolkless and Gurken trafficking

The loss of plasma membrane in *rab6*^{D23D} germline clones, together with the known role of Rab6 homologs in vesicular traffic through the Golgi, led us to examine whether membrane trafficking is affected in *rab6*^{D23D} egg chambers. As a readout of this process, we first made use of the vitellogenin receptor Yolkless, which is translated in the oocyte and secreted before being actively recycled for re-exocytosis (Schonbaum et al., 2000).

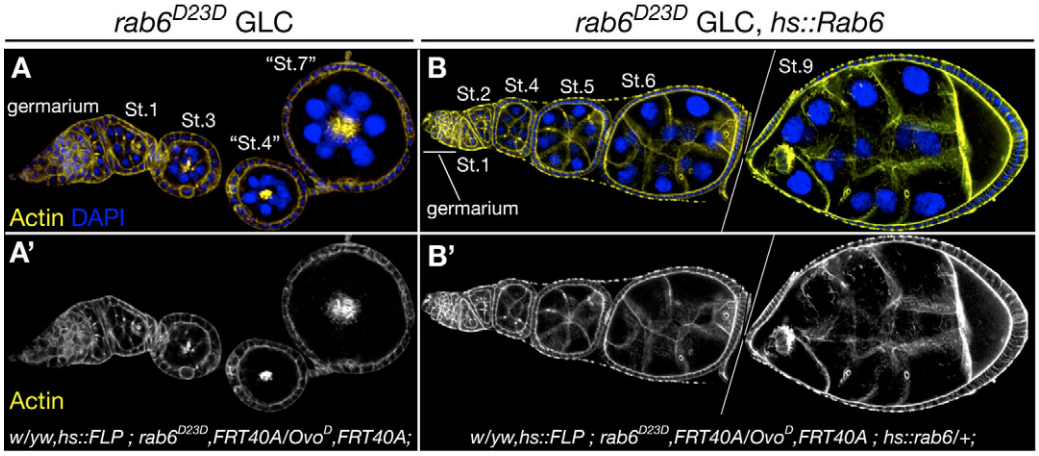

Fig. 1. Loss of actin organization in *rab6*^{D23D} egg chambers. *rab6*^{D23D} egg chambers (Ovo^{D}-selected germline clones) with or without a *hs::rab6* transgene were stained to reveal DNA (DAPI, blue) and F-actin (phalloidin, yellow). In *rab6*^{D23D} egg chambers (**A**), the actin cytoskeleton disappears progressively between stage 2 and stage 7. Expression of *hs::rab6* (**B**) fully rescues the loss-of-actin phenotype of the *rab6*^{D23D} mutant (compare A and B).

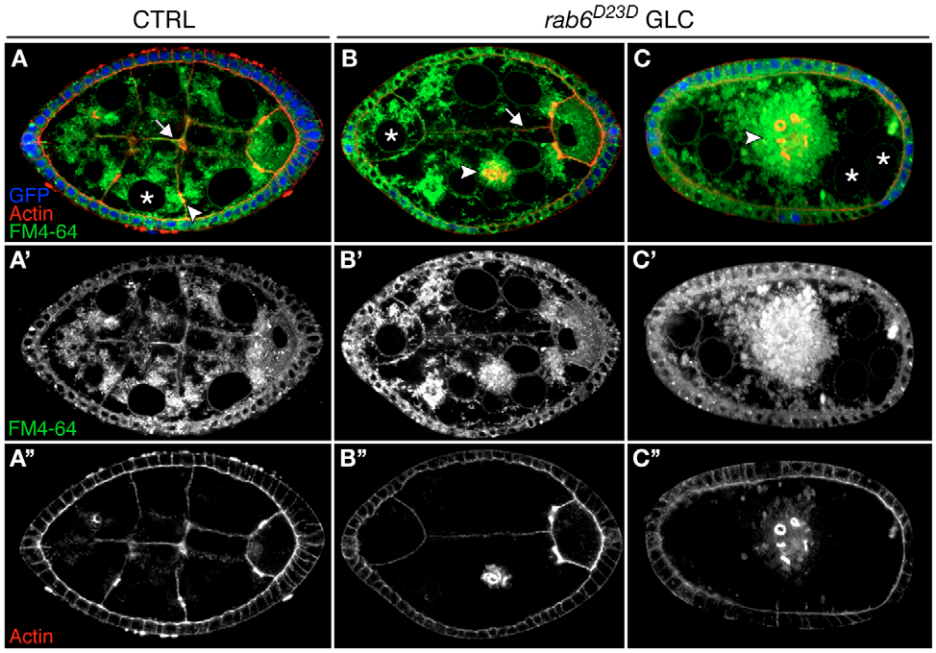

Fig. 2. *rab6^{D23D}* egg chambers form open syncytia. (**A-C**) Egg chambers stained to reveal F-actin (phalloidin, red) and all membranes (FM4-64, green). *rab6^{D23D}* clones are distinguished by the absence of nuclear GFP (blue). In wild-type stage-7 egg chambers (A), actin cytoskeleton and plasma membranes overlap (arrow). A membranous continuum decorating a nucleus (*) appears to span the ring canals, linking adjacent cells (arrowhead). (B,C) In mildly affected *rab6^{D23D}* egg chambers (B), remaining plasma membranes and actin delimit two nurse cell 'open' syncytia (arrow). Within these syncytia, clusters of actin debris and ring canals are embedded in dense membranous material (arrowhead in bottom syncytium; not visible in the upper syncytium in this focal plane). The membranous continuum passes through the ring canals in the remaining plasma membranes separating the large open syncytia and the oocyte. In strongly affected *rab6^{D23D}* egg chambers (C), all compartmentalization by actin and plasma membrane is lost, and nurse cell nuclei lie in a common cytoplasm containing a single central cluster of ring canals, membranes and actin debris (arrowhead). The nurse cell nuclei (*) found in the syncytia away from the membranes appear disconnected from the membranous continuum (B,B′) and are occasionally stripped of vesicular material (C,C′).

In young wild-type egg chambers, Yolkless is uniformly distributed throughout the ooplasm (Fig. 3A, arrowhead). At stage 8, with the onset of vitellogenesis, Yolkless switches from a cytoplasmic to a cortical localization. By stage 10, Yolkless is essentially restricted to the oocyte membrane (Fig. 3A). Yolkless goes through cycles of endocytosis, recycling and exocytosis to import vitellogenin from the perivitelline space into the oocyte (Schonbaum et al., 2000). In young affected *rab6^{D23D}* egg chambers, Yolkless localization in the oocyte appeared normal (Fig. 3B, arrowhead). At mid-oogenesis, however, although some Yolkless was detected at the oocyte cortex, a significant proportion of the protein remained cytoplasmic (Fig. 3B). Thus, Rab6 is required for correct Yolkless trafficking at mid-oogenesis, but not for its early enrichment in the oocyte.

After anterior migration of the oocyte nucleus, *gurken* mRNA is restricted to a tight space between the nucleus and the plasma membrane (Neuman-Silberberg and Schupbach, 1993). At this location, the mRNA is associated with local antero-dorsal ER-Golgi units and is translated; subsequently, Gurken protein is processed for secretion (Herpers and Rabouille, 2004). In wild-type egg chambers at stage 10, Gurken is detected as a sharp signal between the oocyte nucleus and the proximal plasma membrane (Fig. 3C) (Neuman-Silberberg and Schupbach, 1996), as well as in the cytoplasm of the overlying follicle cells (Fig. 3C″, arrow) (Peri et al., 1999). By contrast, the perinuclear signal appeared diffuse and most Gurken protein was detected in droplet-like structures dispersed throughout the ooplasm in the majority of *rab6^{D23D}* egg chambers (Fig. 3D,E). In some egg chambers (29.8%), Gurken was nevertheless detected in the cytoplasm of the apposed follicle cells (Fig. 3D″, arrow). However, in others (25.7%), the protein was not detected beyond the oocyte cytoplasm (Fig. 3E″). Remarkably, no ventralized eggs were observed, indicating that affected *rab6^{D23D}* egg chambers degenerate before egg-laying. As *gurken* mRNA localization and translation are normal in *rab6^{D23D}* egg chambers (data not shown), Gurken mislocalization is most likely the consequence of impaired exocytic trafficking of the protein.

Sec5 and Syx1A membrane localization is differentially impaired in *rab6^{D23D}* egg chambers

To characterize further the exocytosis defects observed in *rab6* egg chambers, we examined the localization of different proteins involved in this process. In eukaryotic cells, proteins of the SNARE family mediate the fusion of intracellular membranes (for a review, see Chen and Scheller, 2001). Syntaxin-1A (Syx1A), a t-SNARE that is found uniformly distributed on target membranes, promotes the fusion of docked vesicles of the regulated secretory pathway (Schulze and Bellen, 1996; Sollner et al., 1993).

In young wild-type egg chambers, Syx1A is detected on all membranes of the germline cyst (Fig. 4A′) and, at mid-oogenesis, predominantly localizes to nurse cell membranes (Fig. 4A, arrow). In strongly affected *rab6^{D23D}* egg chambers at early stages, and in mildly affected *rab6^{D23D}* egg chambers at mid-oogenesis, Syx1A is detected around the cluster of ring canals and actin debris (Fig. 4B, arrowheads). However, in these two classes of egg chambers, it is

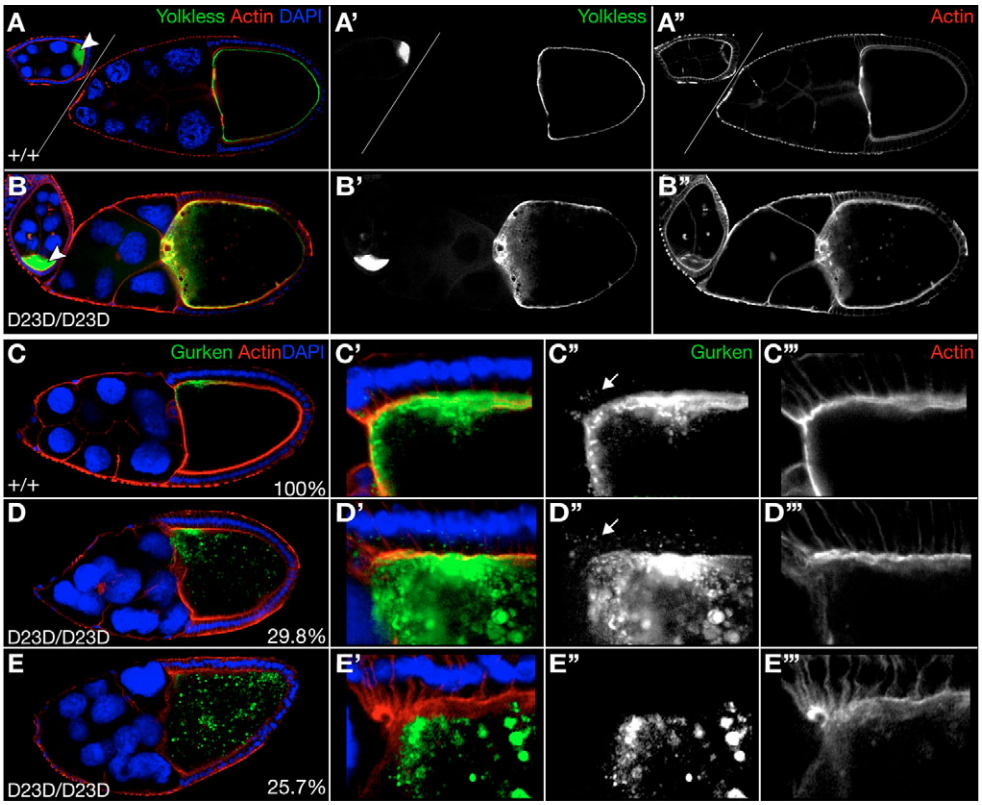

Fig. 3. Trafficking of Yolkless and Gurken is impaired in *rab6^{D23D}* oocytes. Egg chambers stained for Yolkless (**A**,**B**, green) or Gurken (**C-E**, green), F-actin (phalloidin, red) and DNA (DAPI, blue). In wild type (A), Yolkless concentrates in the oocyte cytoplasm before stage 8 (arrowhead). At stage 10, Yolkless is restricted to the oocyte membrane (A′). In *rab6^{D23D}* egg chambers (B), Yolkless concentrates in the oocyte normally (arrowhead). However, at stage 10, an important proportion of Yolkless remains cytoplasmic, indicating that trafficking is impaired (B′). In wild type (C), Gurken is detected as a small arc between the oocyte nucleus and the antero-dorsal plasma membrane. Secreted Gurken is detected in the cytoplasm of the overlying follicle cells (C″, arrow). In the majority of affected *rab6^{D23D}* stage-10 egg chambers (55.5%), large amounts of ectopic Gurken protein are detected in the ooplasm (D,E). In some (29.8%) affected *rab6^{D23D}* egg chambers, secreted Gurken is still detected in the cytoplasm of the overlying follicle cells (D″, arrow). In others (25.7%), Gurken is detected exclusively in the ooplasm and is not detected in the follicle cell cytoplasm (E″).

also detected on the remaining membranes that individualize nurse cell syncytia (Fig. 4B, arrows). These results indicate that Syx1A transport, delivery and/or recycling to the remaining plasma membranes of the egg chamber are not abolished by the lack of Rab6.

During the course of this study, mutations in *sec5* and *sec6*, which encode two exocyst-complex proteins, were shown to affect *Drosophila* oogenesis. *sec5* and *sec6* egg chambers display a disorganization and loss of plasma membranes similar to *rab6* egg chambers (Murthy et al., 2005; Murthy and Schwarz, 2004). The exocyst is a conserved complex of proteins implicated in exocytosis and vesicle trafficking (reviewed in Lipschutz and Mostov, 2002). It plays a crucial role in new membrane addition and marks regions of active secretion and cell growth in yeast and mammalian cells. During *Drosophila* oogenesis, Sec5 enrichment correlates with the zone of active growth, where activity of the protein is required (Murthy and Schwarz, 2004). In amorphic *sec5^{E10}* mutants, oogenesis arrests early and clusters of ring canals are found in the cytoplasm of those egg chambers. Hypomorphic *sec5^{E13}* mutant egg chambers develop further and present a variety of actin phenotypes ranging from wild-type-like, to a lack of cortical actin associated with cytoplasmic ring canal clusters. In those egg chambers, the distribution of Yolkless and Gurken proteins is abnormal (Murthy and Schwarz, 2004).

The striking similarity of the *sec5* and *rab6* mutant phenotypes led us to examine Sec5 distribution in *rab6^{D23D}* egg chambers. Sec5 was present on nurse cell and oocyte plasma membranes in wild-type egg chambers (Fig. 4C,C′) (Murthy and Schwarz, 2004), where it appeared to colocalize with the actin cytoskeleton when examined by confocal microscopy (Fig. 4C, arrow). By contrast, Sec5 was absent from the residual membranes separating the open nurse cell syncytia of *rab6^{D23D}* egg chambers (Fig. 4D, arrow), suggesting that the trafficking and/or membrane maintenance of Sec5 was impaired. Remarkably, Sec5 was readily detected on the oocyte plasma membrane of these *rab6* egg chambers (Fig. 4D′). Hence, there is an important, yet differential, requirement for Rab6 in Sec5 localization to membranes within the germline cyst.

Our finding that loss of *rab6* in the germline does not hamper localization of the t-SNARE protein Syx1A, but affects plasma membrane localization of exocyst component Sec5, suggests that some of the *rab6* phenotypes we observe might be due to Sec5 mislocalization. However, the observed defects in Gurken secretion by *rab6^{D23D}* oocytes, in which Sec5 is still correctly localized, suggest that Rab6 might act directly, independent of Sec5, in Gurken exocytosis.

Furthermore, investigation of the distribution of Golgi and ER markers by immunofluorescence and electron microscopy revealed an enlargement of the Golgi, but not of the ER compartment,

indicating an impairment of exocytosis at the Golgi level in *rab6*^{D23D} egg chambers (see Fig. S5 and Fig. S6 in the supplementary material, and data not shown). These results are consistent with the characterized role of mammalian Rab6 in the trafficking of Golgi-derived membranes (Antony et al., 1992; Martinez et al., 1994; Matanis et al., 2002; Short et al., 2002; White et al., 1999; Young et al., 2005).

Posterior localization of *oskar* mRNA is affected in *rab6*-null egg chambers

Two other regulators of vesicular trafficking, Rab11 and Ter94, are required for the localization of the posterior determinant *oskar* during oogenesis (Dollar et al., 2002; Jankovics et al., 2001; Ruden et al., 2000). We therefore wished to determine if *rab6* might also play a role in this process. To this end, we assessed the distribution of *oskar* mRNA either directly or by using the RNA-binding protein Staufen as a reporter (Ephrussi et al., 1991; Kim-Ha et al., 1991; St Johnston et al., 1991). During early oogenesis, *oskar* mRNA concentrated in *rab6*^{D23D} oocytes as it did in wild type (data not shown). At mid-oogenesis, Staufen/*oskar* localized to the posterior pole of wild-type oocytes, where they form a tight crescent from stage 9 onwards (Fig. 5A,E) (Ephrussi et al., 1991; Kim-Ha et al., 1991). By contrast, at stages 9 and 10, approximately 70% of affected *rab6*^{D23D} egg chambers showed mislocalized Staufen/*oskar* (n=268, Fig. 5C,D,G-H).

As posterior localization of *oskar* mRNA requires a correctly polarized MT cytoskeleton, we assessed this polarity in *rab6*^{D23D} egg chambers using the Kin:β-gal reporter, a fusion of β-galactosidase to the motor domain of Kinesin (Clark et al., 1994). In contrast to wild-type stage 9-10 oocytes, a large proportion of *rab6*^{D23D} oocytes displayed an abnormal distribution of Kin:β-gal and Staufen. To ensure that these phenotypes were not the consequence of defective egg-chamber organization (actin cytoskeleton and membranes), we restricted our evaluation to egg chambers presenting the stereotypical pattern of two major open syncytia and an individualized oocyte, described above.

In close to one third of affected *rab6*^{D23D} egg chambers, Kin:β-gal and Staufen localized at the posterior of the oocyte (Fig. 5B-B″), as in wild-type (Fig. 5A-A″). In the remaining two-thirds, the crescent of Kin:β-gal and Staufen was either dramatically reduced (Fig. 5C) or entirely absent (Fig. 5D), and the two proteins were detected as a blob away from the oocyte cortex (Fig. 5C,D; see Fig.

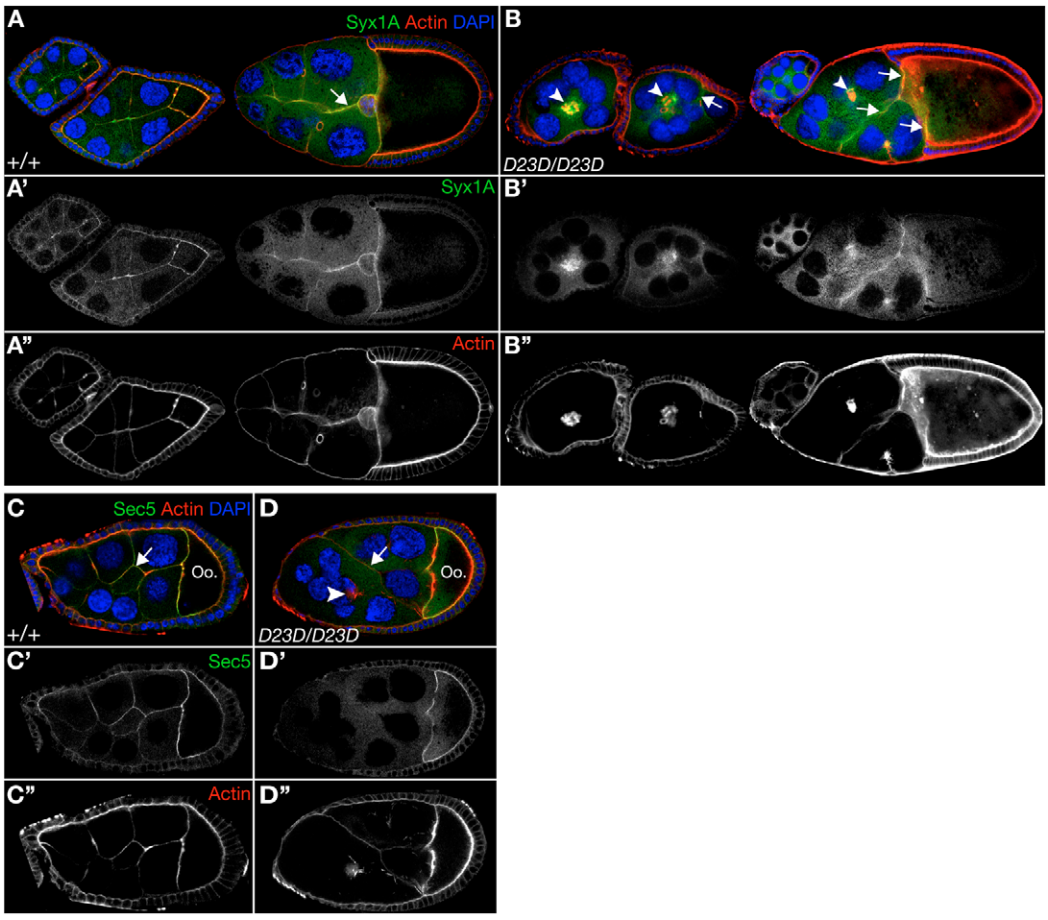

Fig. 4. Sec5 and Syx1A localization on membranes is differentially impaired in *rab6* egg chambers. Egg chambers stained to reveal Syx1A (**A**,**B**, green) or Sec5 (**C**,**D**, green), F-actin (phalloidin, red) and DNA (DAPI, blue). In wild type (A), Syx1A is detected on nurse cell and oocyte membranes (arrows). After stage 9, Syx1A is predominantly detected on nurse cell membranes. In *rab6*^{D23D} egg chambers (B), Syx1A is detected on the remaining plasma membranes (arrow) and around the ring canal cluster (arrowheads), in both strongly and in mildly affected egg chambers. In stage-8 wild-type egg chambers (C), Sec5 and actin colocalize at nurse cell (arrow) and oocyte (Oo) plasma membranes. In *rab6*^{D23D} egg chambers at these stages, Sec5 is absent from residual membranes that delimit nurse cell 'open' syncytia (D, arrow) but is readily detected on the oocyte plasma membrane (Oo). Sec5 is not detected in the aggregates of ring canals and actin debris (D, arrowhead).

S7 in the supplementary material for detailed percentages). Thus, nearly 70% of affected *rab6*^{D23D} egg chambers displayed a mislocalization of Staufen that correlated with mispolarization of the MT network.

We next examined the distribution of Oskar protein in *rab6*^{D23D} egg chambers. In wild-type oocytes, Oskar protein is restricted to the posterior pole by a combination of *oskar* mRNA localization and localized translation (Gunkel et al., 1998; Kim-Ha et al., 1995; Markussen et al., 1995; Rongo et al., 1995). Consistent with the distribution of Staufen, in close to 30% of affected *rab6*^{D23D} egg chambers, *oskar* mRNA and Oskar protein formed a tight crescent, as occurred in wild type (Fig. 5E,F). In the remaining 70%, *oskar* mRNA was either partially or completely mislocalized and was detected away from the cortex (Fig. 5G′,H′). No ectopic Oskar protein was associated with the unlocalized mRNA in these egg chambers (Fig. 5G″,H″), indicating that the unlocalized *oskar* mRNA remained translationally repressed. Taken together, these results strongly suggest that the *oskar* mRNA mislocalization defect in *rab6*^{D23D} egg chambers results from a failure in mRNA localization, rather than in its anchoring.

Our analysis of egg-chamber organization suggests that Sec5 might be a downstream functional effector of Rab6 in that process. We therefore assessed whether the localization of *oskar* mRNA is normal in *sec5*^{E13} egg chambers. Whereas *rab6*^{D23D} oocytes displayed a mis-polarization of the MT cytoskeleton (Fig. 5C,D), *sec5*^{E13} oocytes did not (Murthy and Schwarz, 2004). Despite the disorganization of *sec5*^{E13} egg chambers, Staufen localization appeared in *sec5*^{E13} as it did in wild-type oocytes (Fig. 6B). Therefore, although both Sec5 and Rab6 are required for egg-chamber organization, Rab6 is specifically required for MT organization and *oskar* mRNA localization at mid-oogenesis.

Rab6 and BicD are in a complex and act together in *oskar* mRNA localization

In mammalian cells, BicD homologs localize to the TGN, where they interact with Rab6 and the Dynein-Dynactin complex, creating a link between Golgi vesicles and MT tracks and motors (Hoogenraad et al., 2001; Matanis et al., 2002). To test whether Rab6 and BicD are present in a complex in *Drosophila* ovaries, we performed co-immunoprecipitation experiments using ovarian extracts of transgenic flies expressing Myc-tagged Rab fusion proteins. In addition to flies expressing Myc-Rab6, we included flies expressing Myc-Rab7 as a control, because these two Rab proteins are 71.4% identical but perform different functions. As shown in Fig. 7A, BicD co-immunoprecipitates with Myc-Rab6 but not with Myc-Rab7. This demonstrates that Rab6 and BicD interact specifically in vivo.

We next tested whether the depletion of Rab6 might affect BicD protein function or localization in the egg chamber. At mid-oogenesis, BicD activity is required for cortical anchoring of the oocyte nucleus at the antero-dorsal corner of this cell (Swan and Suter, 1996). Although BicD localizes normally in *sec5*^{E13} oocytes, the nucleus is occasionally displaced (Murthy and Schwarz, 2004). By contrast, within *rab6*^{D23D} oocytes, no mislocalization of the nucleus was observed, and BicD was detected between the oocyte nucleus and the cortex, as it was in wild type (Fig. 7C′,D′).

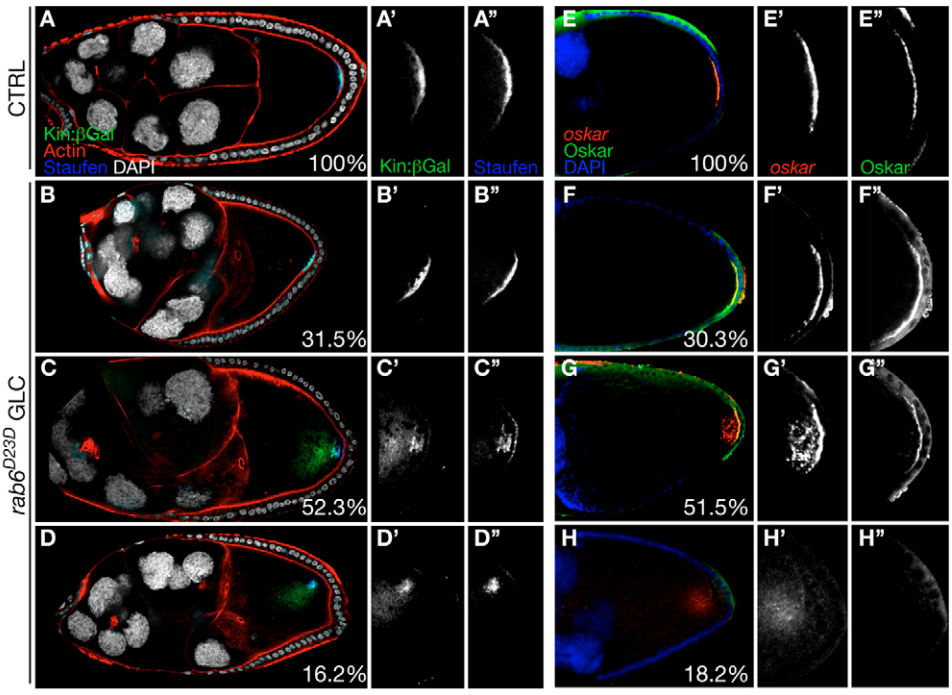

Fig. 5. *oskar* mRNA localization is affected at mid-oogenesis in *rab6*-null egg chambers. (**A-D**) Stage-9 egg chambers stained to reveal F-actin (phalloidin, red), Kin:β-gal (green), Staufen (blue) and DNA (DAPI, white). (**E-H**) Co-detection of *oskar* mRNA (red) and Oskar protein (green), and DNA (DAPI; blue). In wild type, Kin:β-gal and Staufen (A), and *oskar* mRNA and Oskar protein (E) colocalize in a sharp crescent at the oocyte posterior. All *rab6*^{D23D} egg chambers evaluated in this assay displayed a similarly affected actin cytoskeleton and the stereotypical pattern of eight- and four-nurse cell open syncytia (B-D). In nearly one third of these, localization of Kin:β-gal and Staufen (B), and of *oskar* mRNA and Oskar protein (F), appears normal. In the remaining affected *rab6*^{D23D} egg chambers, Kin:β-gal and Staufen/*oskar* are detected in blobs either partially (C,G) or completely (D,H) delocalized from the oocyte cortex. Oskar protein is detected at the posterior cortex only (G″), and not with unlocalized *oskar* mRNA (G″,H″).

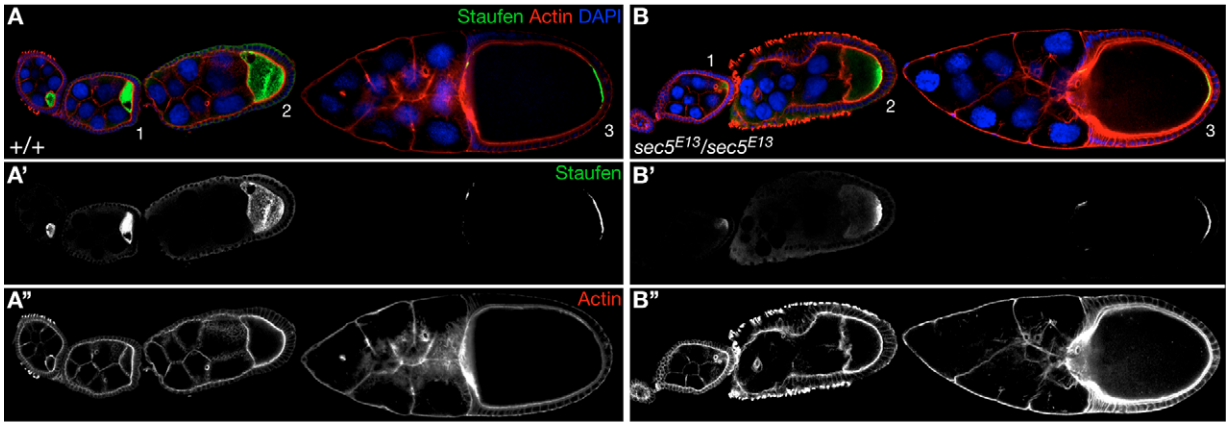

Fig. 6. Staufen localization is normal in *sec5^{E13}* egg chambers. Egg chambers stained for Staufen (green), F-actin (phalloidin, red) and DNA (DAPI, blue). (**A**) In wild-type egg chambers, Staufen concentrates in the oocyte during early oogenesis (egg-chamber 1). After stage 7 and repolarization of the oocyte MT cytoskeleton, Staufen is transiently detected in the middle of the oocyte (egg-chamber 2). From stage 9 onwards, Staufen forms a tight crescent at the posterior pole (egg-chamber 3). (**B**) In *sec5^{E13}* egg chambers, all steps of Staufen localization appear normal.

However, within *rab6^{D23D}* nurse cell syncytia, BicD protein was detected ectopically around the cluster of ring canals (Fig. 7D, arrowhead). This might reflect the abnormal distribution of Golgi material in *rab6^{D23D}* egg chambers. Thus, the functions of BicD in oocyte specification in early oogenesis and later, in the anchoring of the oocyte nucleus, do not appear to be affected by a lack of Rab6 and might therefore involve interactions with other proteins.

BicD, which has been shown to cooperate with Rab6 in dynactin-based motility in mammalian cultured cells (Hoogenraad et al., 2001; Hoogenraad et al., 2003; Matanis et al., 2002; Short et al., 2002), was originally identified in *Drosophila* for its role in embryonic patterning and in mRNA localization in the oocyte and embryo (Bullock and Ish-Horowicz, 2001; Oh and Steward, 2001; Suter and Steward, 1991). We therefore wished to test whether Rab6 might cooperate with BicD in *oskar* mRNA localization at mid-oogenesis. To this end, we examined Staufen localization at mid-oogenesis in various combinations of *rab6* and *BicD* mutant alleles. In addition to the genomic null allele *rab6^{D23D}*, we also made use of *rab6^{wrt}* and *BicD^{R5}*. As a control, we included *par-1^{9A}*, a mutant allele of *par-1*, whose product, the serine/threonine kinase Par-1, is a known regulator of the oocyte MT cytoskeleton, required for its reorganization at mid-oogenesis and for the concomitant localization of *oskar* mRNA (Shulman et al., 2000; Tomancak et al., 2000). Flies homozygous for the hypomorphic allele *rab6^{wrt}* are viable, fertile (Purcell and Artavanis-Tsakonas, 1999) and did not show any oogenesis phenotype (data not shown). Flies homozygous for the strong hypomorphic allele *par-1^{9A}* are viable, but 70% of the egg chambers show full or partial mislocalization of *oskar* mRNA in the middle of the oocyte at mid-oogenesis (Tomancak et al., 1998). *BicD^{R5}* is homozygous lethal and does not support oogenesis: *BicD^{R5}* egg chambers contain 16 nurse cells and no oocyte (Oh and Steward, 2001).

Staufen localization at mid-oogenesis appeared to be only very mildly affected, and to a similar degree, in *BicD^{R5}* heterozygous females, *par-1^{9A}* heterozygous females and in females bearing the strongest viable combination of *rab6* alleles (*rab6^{wrt}/rab6^{D23D}*) (Fig. 7B). An additive effect of the *rab6* and *par-1* mutations on the penetrance of Staufen mislocalization was observed in *rab6^{D23D},par-1^{9A}/rab6^{wrt}* double-mutant egg chambers (5.8%, Fig. 7B). By contrast, a synergistic effect of the simultaneous mutation of *rab6* and *BicD* was observed in *rab6^{D23D},BicD^{R5}/rab6^{wrt}* double-mutant egg chambers (20%, Fig. 7B). This synergy between the *rab6* and *BicD* mutant alleles in Staufen mislocalization, together with the presence of Rab6 and BicD proteins in a complex, suggests that they cooperate to promote *oskar* mRNA localization. They might act directly in this process, or might do so indirectly by affecting the organization of the oocyte MT and actin cytoskeletons.

Taken together, our findings indicate that *Drosophila* Rab6 exerts dual functions during oogenesis. First, Rab6 mediates membrane trafficking that is important for growth and organization of the egg chamber, possibly acting upstream of exocyst component Sec5 in this process. Second, Rab6, in conjunction with BicD but independently of Sec5, has a novel and specific role in the polarization of the oocyte MT cytoskeleton and in *oskar* mRNA localization.

DISCUSSION

We have shown that a lack of Rab6, a small GTPase of the Rab family, known in mammals to promote trafficking at the level of the Golgi apparatus, leads to a progressive loss of cystocyte plasma membranes and to the formation of open syncytia in *Drosophila* egg chambers. Rab6 is also required for polarization of the oocyte MT cytoskeleton, and for concomitant *oskar* mRNA localization at mid-oogenesis.

During polarized exocytosis, secretory vesicles emerging from the TGN are targeted via molecular motors and cytoskeletal tracks to the plasma membrane, where they are tethered. Subsequently, their fusion with the plasma membrane permits the secretion of the vesicle contents, as well as the incorporation of vesicular lipids and proteins into the plasma membrane, allowing membrane growth and the establishment of specific domains. The exocyst complex plays a crucial role in the incorporation of particular membranes and membrane proteins at specific sites or in active domains of the plasma membrane (reviewed in Hsu et al., 2004; Lipschutz and Mostov, 2002). Consistent with this, *Drosophila sec5* mutant egg chambers display mislocalization of other exocyst components, cytoplasmic clusters of actin and a loss of plasma membranes (Murthy et al., 2005; Murthy and Schwarz, 2004). Thus, Sec5 protein is at the core of the exocyst complex in *Drosophila*, as is the case in yeast and in mammals (Guo et al., 1999; Matern et al., 2001).

Both *sec5* null (*sec5^{E10}*) and strongly affected *rab6^{D23D}* egg chambers display actin and general organization defects, and arrest development during early oogenesis (Murthy and Schwarz, 2004)

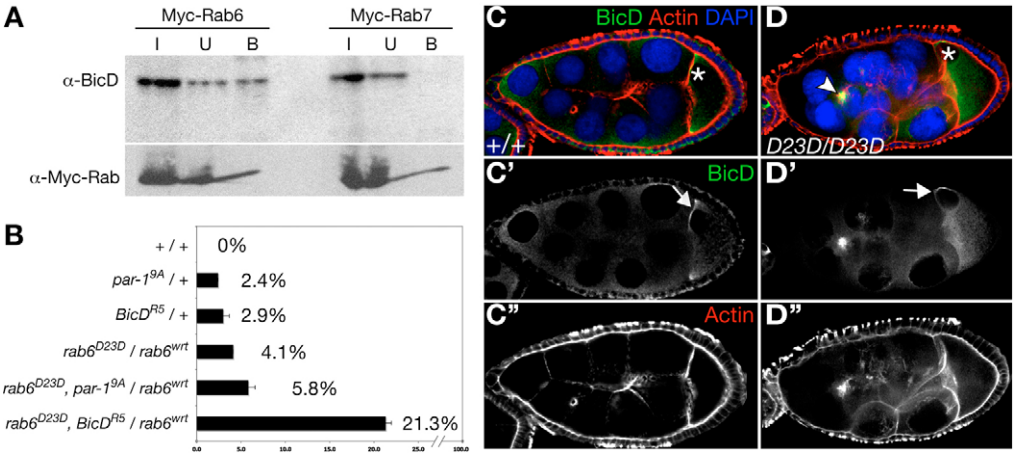

Fig. 7. Rab6 and BicD are in a complex and act together in Staufen localization at mid-oogenesis. (**A**) BicD co-immunoprecipitates specifically with Rab6 in ovarian extracts. Ovarian extracts of transgenic flies expressing Myc-tagged Rab6 or Rab7 were immunoprecipitated using a monoclonal anti-Myc antibody. Western blots were probed using anti-BicD or anti-Myc antibodies. (**B**) *BicD* and *rab6* interact genetically in Staufen localization. The graph shows the percentage of stage-9 and -10 egg chambers of different genotypes displaying mislocalized Staufen. An average of 215 egg chambers per genotype were counted. Compared with *rab6* single mutants, the proportion of egg chambers displaying defects in Staufen localization increases additively in *rab6*, *par-1* double mutants, and synergistically in *rab6*, *BicD* double mutants. (**C**,**D**) Egg chambers stained for BicD (green), F-actin (phalloidin, red) and DNA (DAPI, blue). In wild-type egg chambers (C), after anterior migration of the oocyte nucleus (asterisk), BicD is detected between the oocyte cortex and the nucleus (C′, arrow). In $rab6^{D23D}$ egg chambers (D), BicD appears to be associated with the oocyte nucleus (D, asterisk), as in wild-type (D′, arrow), and ectopically aggregates with ring canal clusters in nurse cell syncytia (D, arrowhead). I, input; U, unbound; B, bound.

(our observations). Similarly, *sec5* hypomorphic ($sec5^{E13}$) and $rab6^{D23D}$ egg chambers that develop past stage 7 display phenotypes ranging from wild type to a loss of nurse cell cortical actin and the concomitant presence of ring canal clusters in the nurse cell cytoplasm (Murthy and Schwarz, 2004) (this study). The striking parallel between the *rab6* and *sec5* phenotypes, together with our finding that a loss of Rab6 affects Sec5 localization, suggests that the varying degrees of membrane loss observed in $rab6^{D23D}$ egg chambers reflects the relative reduction of exocyst-complex function in the egg chamber. Thus, during *Drosophila* oogenesis, Rab6 promotes Sec5 localization and therefore appears to be important for exocyst-complex organization and function. However, consequent to loss of *rab6* function, we observed a striking difference between nurse cells and oocyte in the severity of plasma membrane collapse and Sec5 mislocalization. We hypothesize that the oocyte acts as a major source of membrane in $rab6^{D23D}$ egg chambers and/or that multiple exocytic pathways cooperate within the germline cyst to promote cyst development.

The oocyte as major source of membrane in $rab6^{D23D}$ egg chambers

Differences in membrane content between the oocyte and the nurse cells, as well as between the individual nurse cells, are observed as early as the germarium stage in wild-type egg chambers. The fusome, a membranous Spectrin-rich structure derived from the spectrosome, which itself is a precursor organelle present in the germline stem cells, grows asymmetrically through the ring canals during the divisions of the germline cyst, linking each cystocyte (Lin and Spradling, 1995; Lin et al., 1994). It is thought that the oocyte is the four-ring-canal cell that retains the greater part of fusome during the first division (de Cuevas and Spradling, 1998; Huynh and St Johnston, 2004). Furthermore, a *Drosophila* Balbiani body has recently been discovered, which, together with the fusome, organizes the specific enrichment of organelles in the oocyte throughout oogenesis (Cox and Spradling, 2003). It is therefore possible that, in *rab6* clones, in which the fusome appears normal (data not shown), such a mechanism of enrichment of organelles in the oocyte concomitantly ensures that the concentration in the oocyte of any perduring Rab6 protein, thus privileging the growth of the plasma membrane of the oocyte over that of the nurse cells. Supporting this notion is the observation that GFP-tagged Rab6 expressed in the germline is enriched in the oocyte from the early stages of oogenesis (germarium region 2) onwards (see Fig. S2 in the supplementary material). Together, the combined actions of a residual Rab6-dependent and of additional Rab6-independent pathways might also permit most $rab6^{D23D}$ oocytes to maintain sufficient vesicular trafficking to develop past stage 7.

The stereotypic organization of affected $rab6^{D23D}$ egg chambers at mid-oogenesis is striking (see Fig. S4A in the supplementary material). The oocyte is connected to open syncytia via its four ring canals, suggesting that the membranes linking nurse cells and oocyte are the most resistant (see Fig. S4B in the supplementary material). Furthermore, the growth of the remaining membranes indicates that additional vesicular material is delivered and incorporated into these plasma membranes. This suggests that, in these $rab6^{D23D}$ egg chambers, sustained vesicle trafficking in the oocyte causes new membrane addition to the oocyte plasma membrane. We hypothesize that, due to the continuity of the plasma membrane defining the cyst, the oocyte acts as a source of membrane that spreads by lateral diffusion throughout the plasma membrane of the cyst, allowing its growth.

Multiple exocytic pathways within the germline cyst

It appears that Rab6-independent exocytic pathways also contribute to the delivery of vesicular material to the plasma membrane in the *Drosophila* egg chamber. Indeed, Syx1A is detected on the remaining plasma membrane of both *rab6*-null (Fig. 4) and *sec5* egg chambers (Murthy and Schwarz, 2004; Sommer et al., 2005), supporting the existence of a Rab6- and

Sec5-independent exocytic pathway mediating protein export. This selective loss of Sec5 from nurse cell membranes in *rab6* open syncytia, together with the known functions of the exocyst, suggest a simple explanation (depicted in Fig. S4 in the supplementary material) for the defects caused by a lack of Rab6 function in oogenesis. We hypothesize that Rab6-dependent and -independent pathways might differ qualitatively in the proteins whose traffic they mediate, or quantitatively in their relative contributions to the delivery of the same cargo between nurse cells and oocyte. These differences may account for the observed differential requirement for Rab6 in the localization of Sec5 in nurse cell, versus oocyte, plasma membranes (see Fig. S4C,C′ in the supplementary material).

Oocyte polarization and vesicular trafficking

Our analysis has revealed two separate functions of Rab6: one is a general role in the organization and growth of the egg chamber, and the other is its specialized role in MT cytoskeleton polarization and *oskar* mRNA localization (Fig. 5). This second function appears specific to Rab6 because, in *sec5* mutant egg chambers, Staufen localization is normal (Fig. 6) and the MT cytoskeleton is correctly organized (Murthy and Schwarz, 2004). Only *oskar* mRNA, and not Oskar protein, is ectopically detected in $rab6^{D23D}$ egg chambers (Fig. 5). This suggests an impairment of *oskar* mRNA localization, rather than a defect in its anchoring, in which case Oskar protein would be detected with the detached RNA (Vanzo and Ephrussi, 2002). Defects in *oskar* mRNA localization, which relies on MT polarity, could be due to a failure in the focusing of the MT cytoskeleton that is observed in *rab6* egg chambers (Fig. 5).

In *Drosophila* and mammalian cells, BicD is known to regulate MT organization (Oh and Steward, 2001; Fumoto et al., 2006; Claussen and Suter, 2005). At mid-oogenesis, Rab6 and BicD cooperation could direct MT organization and/or promote the vesicular transport necessary for oocyte polarization and *oskar* mRNA localization. Given the implication of membrane trafficking in the asymmetric localization of mRNAs (reviewed in Cohen, 2005), it also possible that polarized membrane transport along the oocyte MT network directs *oskar* mRNA to the posterior of the oocyte, by hitch-hiking along trafficking vesicles.

In MDCK cells, definition of apical and basolateral plasma membrane domains is required during polarization for the arrangement of MT along an apical-basal axis (Bacallao et al., 1989). Vesicular trafficking is crucial to establish, specify and maintain these membrane domains (reviewed in van der Wouden et al., 2003; van Ijzendoorn et al., 2003). By analogy, at stage 7, the polarizing signal from the posterior follicular cells to the *Drosophila* oocyte that causes repolarization of the MT cytoskeleton might do so by inducing the definition of anterior-lateral and posterior membrane domains. It is therefore possible that, in $rab6^{D23D}$ oocytes, as in epithelia, defects in vesicular trafficking and TGN sorting underlie the observed defects in MT-network organization. Consistent with this idea, a mispolarized MT cytoskeleton is also observed in oocytes lacking Rab11 (Dollar et al., 2002; Hoekstra et al., 2004; Jankovics et al., 2001). Thus, vesicular trafficking and the specification of membrane domains may be required for repolarization of the MT network and for the localization of molecular determinants in the *Drosophila* oocyte at mid-oogenesis.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/134/7/1419/DC1

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