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Rab11 polarization of the *Drosophila* oocyte: a novel link between membrane trafficking, microtubule organization, and *oskar* mRNA localization and translation

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SUMMARY

The *Drosophila* embryonic body plan is specified by asymmetries that arise in the oocyte during oogenesis. These asymmetries are apparent in the subcellular distribution of key mRNAs and proteins and in the organization of the microtubule cytoskeleton. We present evidence that the *Drosophila* oocyte also contains important asymmetries in its membrane trafficking pathways. Specifically, we show that α-adaptin and Rab11, which function critically in the endocytic pathways of all previously examined animal cells, are localized to neighboring compartments at the posterior pole of stage 8-10 oocytes. Rab11 and α-adaptin localization occurs in the absence of a polarized microtubule cytoskeleton, i.e. in *grk* null mutants, but is later reinforced and/or refined by Osk, the localization of which is microtubule dependent. Analyses of germline clones of a *rab11* partial loss-of-function mutation reveal a requirement for Rab11 in endocytic recycling and in the organization of posterior membrane compartments. Such analyses also reveal a requirement for Rab11 in the organization of microtubule plus ends and *osk* mRNA localization and translation. We propose that microtubule plus ends and, possibly, translation factors for *osk* mRNA are anchored to posterior membrane compartments that are defined by Rab11-mediated trafficking and reinforced by Rab11-Osk interactions.

Key words: Oogenesis, Cell polarity, Membrane trafficking, Membrane recycling, *oskar*, mRNA localization, Microtubule plus ends, *Drosophila*, Rab11

INTRODUCTION

Owing to its large size, rich ultrastructure and amenability to genetic analysis, the *Drosophila* oocyte has been extensively studied as a model system for understanding mechanisms that generate and maintain cell polarity. The polarity of the oocyte has been best described in terms of the asymmetric distribution of mRNAs and proteins that play key instructive roles in patterning the future embryo (van Eeden and St Johnston, 1999). The posterior pole of the oocyte, for example, is defined by the accumulation of *osk* mRNA and protein (Osk), which specifies the formation of abdominal segments and pole cells in the embryo (Lehmann and Nüsslein-Volhard, 1986; Ephrussi and Lehmann, 1992; Smith et al., 1992). Oocyte polarity is also apparent in the organization of the microtubule cytoskeleton (Theurkauf et al., 1992). The localization patterns of β-gal fusion proteins linked to known motor domains indicate that the minus and plus ends of the microtubules of the mid stage oocyte are positioned along the anterior cortex and at the posterior pole, respectively (Clark et al., 1994; Clark et al., 1997). Pharmacological and genetic analyses show that such polarization is necessary for the subcellular localization of *osk* and other mRNAs (Pokrywka and Stephenson, 1995; Lane and Kalderon, 1994; Gonzäles-Reyes et al., 1995; Roth et al., 1995; Deng and Ruohola-Baker, 2000; Shulman et al., 2000). Indeed, several lines of experimentation support a model in which *osk* and other mRNAs associate with end-specific motor proteins and are unidirectionally transported on microtubule tracks to receptors that are anchored to the plasma membrane or cell cortex (Grünert and St Johnston, 1995; Brendza et al., 2000; Schnorrer et al., 2000).

To date, the best evidence that the plasma membrane of the oocyte and membrane trafficking pathways are polarized comes from the expression pattern of human transferrin receptor (Htr) in transgenic flies (Bretscher, 1996). During stages 8-10 [see Spradling (Spradling, 1993) for a complete description of the 14 stages of oogenesis], the posterior pole of the oocyte becomes enriched with Htr, both along the plasma membrane and in vesicles. Htr rapidly disappears from vesicles upon inhibition of endocytosis (Bretscher, 1996), indicating that it is actively internalized and recycled in *Drosophila* oocytes as it is in many other examined cells (Mukherjee et al., 1997). Together with the observation that Htr is restricted to the posterior plasma membrane, its active internalization and recycling strongly suggests that the membrane recycling pathway of the oocyte is polarized towards the posterior pole.

We now identify Rab11 and α-adaptin as endogenous markers of oocyte membrane polarity. These proteins are

localized to distinct compartments at the posterior end of the oocyte. Based on the reported roles of Rab11 and α-adaptin in other examined cells (Mukherjee et al., 1997; Novick and Zerial, 1997; Rodman and Wandinger-Ness, 2000) and on findings we present that Rab11 is required for the recycling of internalized transferrin, we propose that their expression patterns in mid-stage oocytes reflect polarized membrane recycling directed towards the posterior pole. Additional findings presented here indicate that Rab11-mediated receptor recycling plays a critical role in the polarization of the cytoplasm of the oocyte through its specification of membrane domains that organize microtubule plus ends and support *osk* mRNA translation and anchoring. Finally, we demonstrate the existence of a positive feedback loop whereby Osk amplifies its own synthesis and localization through its maintenance/enhancement of Rab11 localization.

MATERIALS AND METHODS

Fly stocks

The wild-type control stock was w^{1118}. The original *rab11* stock was w^{1118}; FRT^{82B}, $rab11^{P2148}$/*TM3, Sb* (kindly provided by Norbert Perrimon). The P element of the $rab11^{P2148}$ allele carries w^+ as a marker gene. For the *grk* null analysis, grk^{2B} *b cn bw*/CyO males were crossed to grk^{2E} *cn bw*/CyO females (both stocks kindly provided by T. Schüpbach) and CyO^+ (grk^{2B}/grk^{2E}) females were selected. The grk^{2E} and grk^{2B} alleles are RNA and protein nulls, respectively, and have been described by Thio et al. (Thio et al., 2000). The Osk protein null stock was *th st* kni^{ri-1} *roe* osk^6 p^p/TM3 *Sb Ser* and has been described by Kim-Ha et al. (Kim-Ha et al., 1991). The following transgenic stocks were also used: *y, w; KZ32 Kin:lacZ* (Clark et al., 1994) and *TauGFP24.1* (Micklem et al., 1997). Gratuitous marker genes and the *TM3, Sb* and *CyO* balancer chromosomes have been described by Lindsley and Zimm (Lindsley and Zimm, 1992).

Generation of *rab11* deletion mutants

The *rab11* deletion alleles were generated by imprecise excision. w^{1118}; FRT^{82B}, $rab11^{P2148}$/*TM3, Sb* females were mated to *TM2, ry, P[Δ2,3]/MKRS P[Δ2,3]* males. *w/Y;* FRT^{82B}, $rab11^{P2148}$/*MKRS[Δ2,3]* male progeny were mated to *w; TM3, Sb/TM6, Tb* females. Progeny with excised P elements were identified by searching vials for white-eyed flies with normal (Sb^+) bristles. These progeny were pair-mated to *TM6, Tb* flies to generate *w;* FRT^{82B}, $rab11^{(ex)}$/*TM6, Tb* stocks, where $rab11^{(ex)}$ indicates excision of w^+. Several of the resulting stocks failed to produce Tb^+ progeny, indicating the presence of a *rab11* mutation, which was subsequently verified by complementation tests with $rab11^{P2148}$. Two non-complementing mutations were shown by Southern blot analysis to carry small deletions. Physical maps of corresponding alleles ($rab11^{ex1}$ and $rab11^{ex2}$) are shown in Fig. 2.

Clonal analysis

GLCs of $rab11^{P2148}$, $rab11^{ex1}$ and $rab11^{ex2}$ were generated by mitotic recombination using the FRT/FLP-dominant female sterile technique (Chou and Perrimon, 1996; Perrimon et al., 1996). w^{1118}; FRT^{82B}, *rab11/TM3, Sb* females were mated to *FLP/Y; TM3, Sb/P[*ovo^{D1}*]* FRT^{82B} males. Progeny were reared until the second larval instar and then subjected to two 2 hours heat shocks at 37°C to induce FLP recombinase. Ovaries of Sb^+ females were dissected 3-5 days after eclosion. GLCs of $rab11^{P2148}$ were readily distinguished by the presence of mid- and late-stage egg chambers; ovo^{D1} is a dominant mutation that arrests egg chamber development before stage 6 (Perrimon et al., 1996). GLCs of $rab11^{ex1}$ and $rab11^{ex2}$ were identified by the presence of fused egg chambers (Fig. 2C); similarly fused egg chambers were not observed in ovo^{D1} control ovaries.

Cloning and molecular characterization of *rab11*

rab11 genomic DNA was cloned from w^{1118}; FRT^{82B}, $rab11^{P2148}$/*TM3, Sb* flies by plasmid rescue after cleavage of genomic DNA with *Xba*I and ligation under dilute conditions. The presence of the P element in recovered plasmids was verified by restriction mapping and partial DNA sequencing. Flanking chromosomal DNA was subcloned from positive clones and used to screen a *Drosophila* genomic DNA library (kindly provided by Richard Mann). Two overlapping genomic clones spanning ~22 kb were isolated. Partial DNA sequencing revealed that the P element was in the second intron of the *rab11* gene. A 6.3 kb *Asp*718I-*Xho*I genomic fragment was subsequently identified that restores viability and fertility to $rab11^{P2148}$ homozygotes. This fragment includes the entire *rab11* transcription unit and about 800 bp each of 5′ and 3′ flanking DNA (see Fig. 2A). Sequencing of the entire 6.3 kb rescuing fragment revealed excellent correspondence with previous cDNA sequencing (Sasamura et al., 1997; Satoh et al., 1997) and with the BDGP genomic sequence. Northern blot analyses were carried out as described by Frank et al. (Frank et al., 1994), using a partial *rab11* cDNA as a probe.

For the rescue experiment, the 6.3 kb genomic fragment was subcloned into the CaSpeR4 transformation vector (Pirrotta, 1988) and microinjected into w^{1118} flies according to standard procedures (Rubin and Spradling, 1982; Spradling and Rubin, 1982). Two independent transformed lines were established, one containing a single copy of the transgene and another containing two linked copies. Standard genetic crosses were used to introduce the transgenes into the w^{1118}; FRT^{82B}, $rab11^{P2148}$/*TM3, Sb* stock. Rescue was scored by the recovery of Sb^+ flies. Strong rescue was observed with two copies of the *rab11* transgene. Partial rescue was observed with a single copy of the transgene.

The Rab11-GFP construct was made within the context of the *rab11* rescuing fragment by substituting an *Eco*RI-*Bam*HI polylinker for the *rab11* translation stop codon. An *Eco*RI-*Bam*HI, GFP-containing fragment was subsequently inserted into the polylinker in-frame with Rab11. Transformation was as described above and resulted in several lines.

Generation of Rab11 antisera

The entire protein-coding region of *rab11* was amplified from a partially purified *rab11* cDNA clone using *Taq* DNA polymerase. The amplified sequence was fused to a 6× His tag and expressed in bacteria from the Pet14b expression vector (Novagen). The His-tagged protein was purified by passage through a Nickel-column and SDS-PAGE. Rats were immunized with 100 μg of gel-purified protein in Fruend's complete adjuvant and then given six booster shots consisting of 100 μg of fusion protein in Fruend's incomplete adjuvant at 2 week intervals. All of the data shown were obtained with crude sera.

Enzyme-linked in situ hybridization, immunolocalization and western blotting

Enzyme-linked in situ hybridization to whole-mount ovaries was carried out according to Tautz and Pfeifle (Tautz and Pfeifle, 1989) with modifications described by Cheung et al. (Cheung et al., 1992). Digoxigenin-labeled DNA probes were made by the random priming method (Feinberg and Vogelstein, 1983). The *grk* and *K10* probes were as described in Serano et al. (Serano et al., 1995). The *osk* probe was as described by Saunders and Cohen (Saunders and Cohen, 1999). The *bcd* probe corresponds to a 2368 nucleotide *Bam*HI-*Xba*I fragment, which contains the entire *bcd* transcription unit plus a small genomic region adjacent to the 3′ end of the transcript. Immunocytochemistry was carried out as described by Serano et al. (Serano et al., 1995), using primary antibodies at the following concentrations: Stau (1/5000), Vas and Osk (1/1000). Western blotting

was carried out according to standard procedures (Maniatis et al., 1982) using the Rab11 antisera at 1/2000 and a biotinylated secondary antibody-DAB detection scheme (Vector laboratories) according to procedures recommended by the manufacturer.

Immunofluorescence

Ovaries were fixed and incubated with antibodies as described above for enzyme-linked immunolocalization. Following incubation with secondary antibody, ovaries were washed in the dark for 4×15 minutes in TNBTT (Serano et al., 1995), 2×20 minutes in PBT (phosphate-buffered saline (PBS) with 0.1% Tween20), mounted in Pro-long antifade (Molecular probes), and visualized by confocal microscopy. Primary antibodies were used at the following concentrations: Rab11 (1/2000), D-clip190 (1/2000), α-adaptin (1/300), β-gal (1/700; ICN/Cappel) and Mab078 α-tubulin (1/10; Harlan Sera-lab). Secondary antibodies were FITC-conjugated AffiniPure donkey anti-rRabbit (Jackson Labs, used at 1/200) and Cy3-conjugated AffiniPure donkey anti-mouse (Jackson Labs, used at 1/100). For preservation of microtubules, ovaries were dissected at room temperature in Grace's Media (Gibco), and viewed under halocarbon oil 700 (Sigma; for live ovaries carrying a tau-GFP transgene) or put immediately into fix for immunofluorescence. For lectin labeling, ovaries were dissected in PBS, washed five times for 3 minutes, once for 10 minutes in PBT and twice for 5 minutes in lectin buffer (10 mM Hepes, pH 7.5, 0.15M NaCl), and incubated in the dark overnight at 4°C with fluorescein-*Datura stramonium* lectin and fluorescein-*Lycopersicon esculentum* (tomato) lectin (Vector labs) (20 μg/ml in lectin buffer) for labeling of the vitelline and plasma membrane (Bretscher, 1996). After incubation with lectins, ovaries were washed in three times of five minutes each in lectin buffer, then mounted and visualized as above.

Receptor recycling assay

Ovaries were dissected at room temperature in Grace's Media, incubated for 1 minute in Grace's Media containing 5 μg/ml Texas Red-conjugated human transferrin (Molecular Probes), washed in fresh Grace's Media for 20 minutes to allow for recycling, mounted in Grace's Media and visualized by confocal microscopy.

RESULTS

Identification of *rab11* mutants

We identified a *rab11* mutant by screening a collection of recessive zygotic lethal genes for roles in the establishment of oocyte polarity (see Materials and Methods). The *rab11* mutant corresponds to the P element insertion stock P2148 of the Berkeley Genome project. This was demonstrated by rescuing the zygotic lethality and oocyte polarity defects of P2148 flies by transformation with a 6.3 kb genomic DNA fragment that included *rab11*, but no other predicted gene sequences (Fig. 1A, and see Materials and Methods). As previously noted (Sasamura et al., 1997; Satoh et al., 1997), Rab11 is a highly conserved protein. It is 84% identical in amino acid sequence to human Rab11 and is 73% identical and 82% similar to yeast Rab11. Northern blot analyses showed that *Drosophila rab11* is transcribed throughout fly development (Fig. 1B). Studies presented below indicate that *Drosophila* Rab11, like its vertebrate and yeast counterparts, mediates endocytic recycling.

Because the P element is in an intron, we reasoned that the *rab11*^{P2148} allele might not be a null mutation. To identify the null phenotype, we carried out an imprecise P element excision experiment (see Materials and Methods). Two relatively small deletions were obtained, each removing all of the second exon, which includes the putative translation start codon (Fig. 1A). These mutations, like the parent *rab11*^{P2148} mutation, behave as recessive zygotic lethals, arresting development during mid-embryogenesis (Perrimon et al., 1996) (data not shown). Germline clones (GLCs) of the deletion alleles (*rab11*^{ex1} and *rab11*^{ex2}) were also analyzed. In contrast to the *rab11*^{P2148} GLCs, *rab11*^{ex1} and *rab11*^{ex2} GLCs arrested oogenesis before oocyte determination (Fig. 1C). These experiments indicate that *rab11*^{P2148} is not null, and that *rab11* gene function is required before (or for) oocyte determination as well as for oocyte polarization.

Rab11 accumulates at the posterior pole of the oocyte during mid-oogenesis and is required for its own localization

To gain insight into the role of Rab11 in the generation of oocyte polarity, we determined its subcellular distribution in

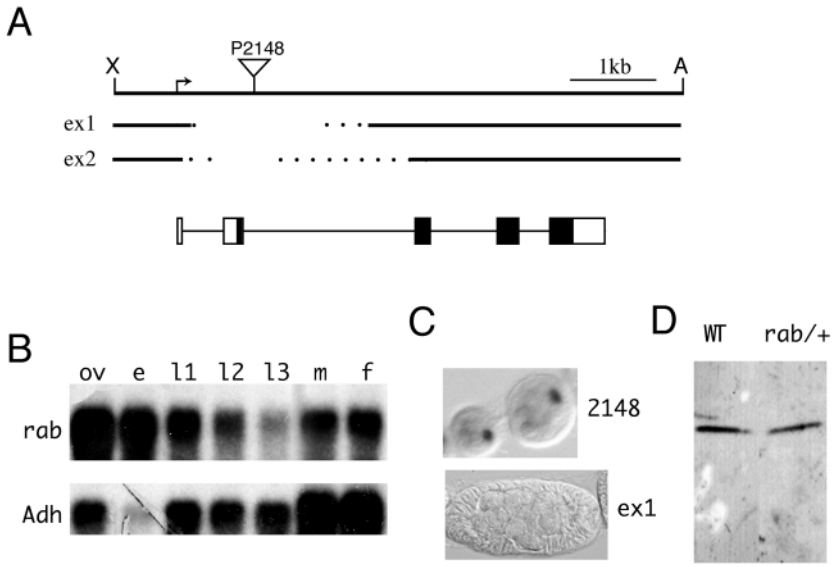

Fig. 1. *rab11* cloning and genetics. (A) Organization of the *rab11* locus. The top line shows the 6.3 kb genomic rescuing fragment, the position of the P element insertion and the transcription start site and direction (arrow). The structure of the *rab*^{ex1} and *rab*^{ex2} excision alleles is shown below the rescue fragment, where the broken lines indicate uncertainties in the locations of the breakpoints. The *rab11* transcription unit is shown at the bottom. Exons are depicted as rectangles, with the filled regions corresponding to protein coding segments. A, *Asp*718I; X, *Xho*I. (B) Developmental Northern blot showing *rab11* expression throughout the fly life cycle. The control blot in the bottom panel was probed for *Adh* mRNA. RNA was prepared from the following tissues and stages: Ov, adult female ovaries; e, 0-24 h embryos; L1-L3, first, second and third instar larvae, respectively; m, adult males; f, adult females. (C) In situ hybridization for *osk* mRNA in *rab11*^{P2148} and *rab*^{ex1} GLCs. The absence of *osk* transcripts in *rab*^{ex1} GLCs indicates that an oocyte is not determined. Similar results were obtained with *rab*^{ex2} GLCs. (D) Western Blot with Rab11 antisera. Equivalent amounts of total ovarian protein from wild-type (WT) and *rab11*^{P2148}/*rab*$^{+}$ flies (rab/+) were applied to each lane. A single major band of the expected size for Rab11 is detected in each lane.

developing oocytes by staining fixed ovaries with Rab11 antisera (see Materials and Methods). We also examined the distribution of green fluorescent protein (GFP)-tagged Rab11 in living tissues (see Materials and Methods). Both approaches revealed a similar Rab11 expression pattern.

Rab11 was abundant in stage 1-10 oocytes. Detection of the protein in later stage oocytes was problematic due to the deposition of the chorion and extrachorionic membranes. The protein was also expressed in follicle cells and nurse cells, but at reduced levels compared with oocytes. In stage 1-7 oocytes, Rab11 accumulated in a distinct perinuclear compartment and was abundantly distributed in a thick crescent along the lateral and posterior cortexes (Fig. 2A). In stage 8-10 oocytes, Rab11 continued to accumulate in the perinuclear compartment, but the thick cortical crescent was gradually replaced by a small cap of protein at the extreme posterior pole of the oocyte (Fig. 2B,F). Double label experiments showed near perfect colocalization of Rab11 with Osk in stage 9 and 10 oocytes (Fig. 2C).

In situ hybridization for Rab11 mRNA revealed no specific accumulation of the transcript at the posterior pole of the oocyte. Rather, the mRNA was uniformly dispersed throughout the oocyte through at least stage 9. Thus, in contrast to Osk, Rab11 localization would appear to be mediated by a protein-based localization machinery.

We also used the Rab11 antisera to stain *rab11*^{2148} GLCs. Protein was detected in stage 1-10 oocytes. Localization to the perinuclear compartment and to the lateral and posterior cortex of stage 1-7 oocytes was normal (Fig. 2D). However, no localization to the posterior pole was observed in stage 8-10 oocytes. Instead the protein was diffusely dispersed throughout the oocyte, with slight enrichment along the plasma membrane (Fig. 2E). To address the possibility that the *rab11*^{P2148} allele produces an altered form of the protein that is not competent for localization in stage 8-10 oocytes, we immunostained heterozygous (*rab11*^{P2148}/*rab11*$^{+}$) oocytes. Only normally localized protein was detected in these oocytes (data not shown), indicating that the *rab11*^{P2148} allele produces normal protein. Consistent with this idea, northern and western blots of *rab11*^{P2148}/*rab11*$^{+}$ ovaries revealed single RNA and protein products of the expected sizes (Fig. 1D and data not shown). We conclude from these data that *Rab11*^{P2148} GLCs produce abnormal amounts of otherwise normal protein and thus that Rab11 is required for its own localization.

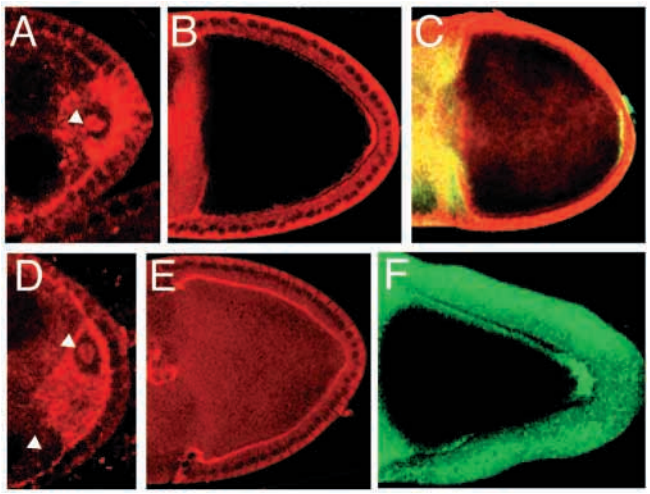

Fig. 2. Rab11 expression in wild-type oocytes and in *rab11*^{P2148} GLCs. (A-E) Confocal immunolocalization of Rab11 and Osk. (A-C) wild-type oocytes. (A) Stage 7 oocyte showing strong accumulation of Rab11 along the lateral and posterior cortex, and in a perinuclear compartment (arrowhead). In these and in all subsequent panels, anterior is towards the left. (B) Stage 10 oocyte showing specific accumulation of Rab11 at the posterior pole. (C) Early stage 10 oocyte doubly labeled for Rab11 (red) and Osk (green). The two proteins show near perfect colocalization at the posterior pole as evident by the mixed (yellow) fluorescent signal. (D-E) Confocal immunolocalization of Rab11 in *rab11*^{P2148} GLCs. (D) Late stage 7 oocyte showing normal accumulation of Rab11 in perinuclear compartment and along the cell cortex. (E) Early stage 10 oocyte showing diffuse accumulation of Rab11 throughout ooplasm with slight enrichment along cell cortex. (F) Rab11-GFP expression in a living wild-type stage 10 oocyte.

Rab11 is required for polarized endocytic recycling

To determine if *Drosophila* Rab11, like its vertebrate counterparts (Rodman and Wandinger-Ness, 2000; Prekeris et al., 2000), mediates endocytic recycling, we followed the recycling of transferrin receptor by monitoring the distribution of its ligand, iron-conjugated transferrin, in cultured ovaries (see Materials and Methods). The transferrin receptor is expressed on the plasma membranes of most cells, where it is actively internalized and recycled (Mukherjee et al., 1997). Upon ligand binding, the receptor-ligand complex is internalized and delivered to endosomes where iron atoms are released (Klausner et al., 1983). The transferrin-transferrin receptor complex is then recycled [often in a Rab11-dependent fashion (Ren et al., 1996)] to the plasma membrane, where transferrin dissociates from the receptor to scavenge more iron (Klausner et al., 1983). When added to cultured ovaries, transferrin-iron conjugates were rapidly transcytosed through the follicle cell epithelium and accumulated at the posterior pole of the oocyte and in the perivitelline compartment between the follicle cell epithelium and the oocyte (Fig. 3B). In wild-type ovaries, significantly more transferrin accumulated in the perivitelline space than in the oocyte (Fig. 3A,B). By contrast, in *rab11*^{P2148} GLCs, most of the transferrin accumulated in the oocyte (Fig. 3C). These patterns of accumulation indicate that wild-type oocytes and *rab11*^{P2148} GLCs both internalize transferrin, but that only wild-type oocytes are able to recycle it to the plasma membrane for release into the perivitelline compartment. To address the possibility that the altered distribution of transferrin in *rab11*^{P2148} GLCs reflects a general defect in membrane integrity rather than a specific defect in receptor recycling, we stained ovaries with fluorescein-conjugated lectins, which preferentially label the perivitelline and plasma membranes (Bretscher, 1996). No gaps or other indications of gross defects in the structure of either membrane in the *rab11*^{P2148} GLCs were observed (Fig. 3D-G). We conclude that *Drosophila rab11* is required for endocytic recycling of transferrin and, presumably, other molecules, to the posterior plasma membrane of the oocyte.

Rab11 is required for organization of membrane compartments at the posterior pole of the oocyte

Given the evidence presented above that Rab11 is required for

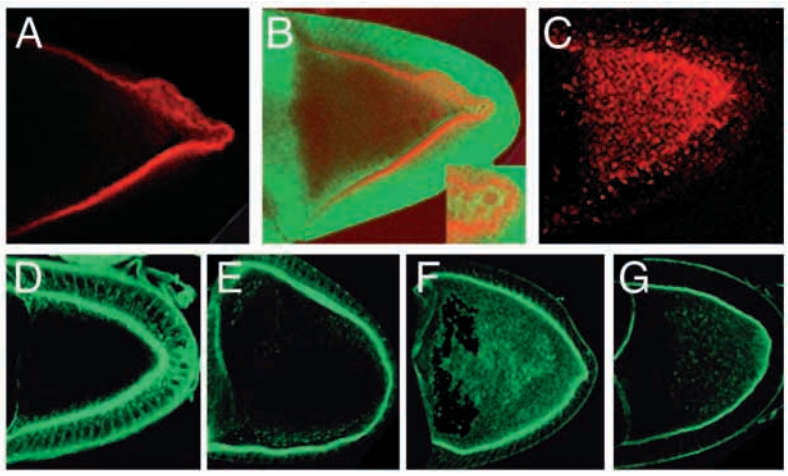

Fig. 3. Transferrin recycling in wild-type oocyte and in *rab11*^{P2148} GLCs. (A,B) A wild-type stage 9 oocyte cultured with Texas Red-transferrin for one minute followed by a 20 minute wash in media alone. (A) Red channel showing the accumulation of transferrin in the perivitelline compartment and in vesicles at posterior pole of the oocyte. (B) Merged image showing transferrin (red) and Rab11-GFP (green). Inset shows an enlarged view of posterior pole, where the accumulation of transferrin in intracellular vesicles is clearly evident. (C) *rab11*^{P2148} GLC cultured with transferrin as described above. Most of the transferrin is in the ooplasm, not in the perivitelline compartment. (D-G) FITC-Lectin labeling of plasma and vitelline membranes. (D-E) Plasma membrane labeling with *Lycopersicon esculentum* lectin. (F-G) Vitelline membrane labeling with *Datura stramonium* lectin. (D,F) Wild type. (E,G) *rab11*^{P2148} GLCs.

its own localization, we wondered if the protein is required to organize other endocytic proteins and/or compartments as has recently been reported for vertebrate Rab11 (Wilcke et al., 2000). To test this idea, we immunostained ovaries for α-adaptin, which in *Drosophila* and other animal cells is found at high levels along the plasma membrane at sites of endocytosis and is found in an intracellular compartment of unknown function (Dornan et al., 1997; Robinson, 1994). We found that α-adaptin is expressed in wild-type and *rab11*^{P2148} GLCs throughout oogenesis. In wild-type oocytes, the protein was enriched along the plasma membrane and was found in a distinct intracellular compartment (Fig. 4B-D). In stage 8-10 oocytes, the intracellular compartment was located at the posterior pole and had a tubulo-vesicular morphology indicative of active vesicle formation and/or fusion (Fig. 4B-D). Double-label experiments showed that the α-adaptin compartment is near, but distinct from, the Rab11 compartment (Fig. 4C,D). A wild-type α-adaptin pattern was seen in *rab11*^{P2148} GLCs through stage 7 (data not shown). However, in stage 8-10 mutant oocytes, α-adaptin was dispersed throughout the ooplasm and staining of the intracellular compartment was not observed (Fig. 4F,G). We conclude from these experiments that *rab11* is required for the organization of α-adaptin into a posterior intracellular compartment. Together with our evidence that Rab11 is required for its own localization, it seems likely that Rab11 has a general role in the organization of membrane compartments at the posterior pole of stage 8-10 oocytes.

Maximal localization of Rab11 to the posterior pole of the oocyte requires Osk

Persistent localization of *osk* mRNA to the posterior of the oocyte during late stages of oogenesis and early embryogenesis requires Osk, which begins to accumulate during stage 9 (Rongo et al., 1995; Kim-Ha et al., 1991). Osk is also required for the late-stage recruitment of *nanos* mRNA, Vasa protein and other components of the pole plasm, the determinant of abdominal patterning and pole cell formation in the embryo (Rongo et al., 1997). To determine if the localization of Rab11 to the posterior pole of the oocyte is Osk-dependent, we examined Rab11 expression in oocytes homozygous for *osk*^{6}, a protein null allele (Kim-Ha et al., 1991). We found that Rab11 accumulated at the posterior pole of *osk*^{6} oocytes, but at a reduced level compared with wild type; a two- to five-fold higher laser intensity setting was needed to image Rab11 at the posterior pole of *osk*^{6} oocytes than was needed to image the protein in wild-type oocytes. Osk was also required for normal α-adaptin localization. In this case, the requirement was

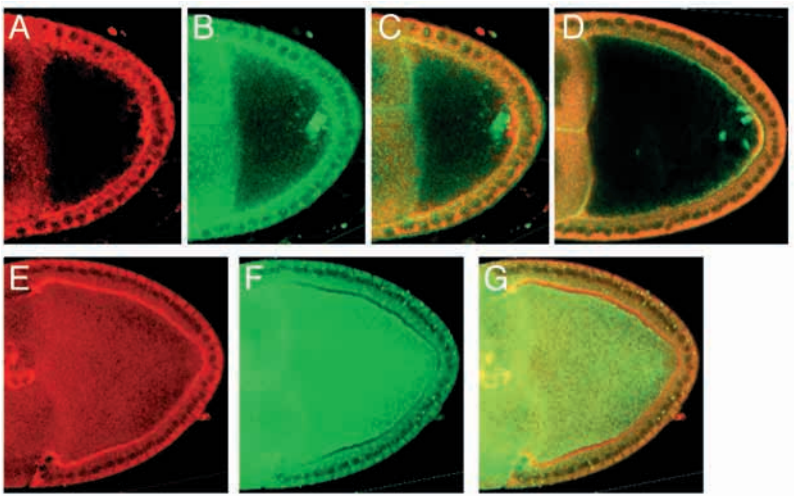

Fig. 4. Rab11-dependent localization of α-adaptin to the posterior pole of the oocyte. (A-C) Wild-type stage 9 oocyte doubly labeled for Rab11 (red) and α-adaptin (green). (C) Merged image. (D) Merged image of a stage 10 oocyte doubly labeled for Rab11 (red) and α-adaptin (green). In both oocytes, α-adaptin is abundant all along the plasma membrane and in a posterior compartment that is close to, but clearly distinct from, the Rab11 expression domain. (E-G) A late stage9/early stage 10 *rab11*^{P2148} GLC doubly labeled for Rab11 (red) and α-adaptin (green), where the merged image is shown in G. Neither protein is found at high levels at the posterior pole of the oocyte.

more qualitative than quantitative. Thus, while α-adaptin accumulated at the posterior pole of the *osk* null mutant, the protein was spread along the posterior membrane rather than concentrated into a distinct intracellular compartment (Fig. 5B,C). We conclude from these findings that maximal localization of Rab11 to the posterior pole of stage 9 and 10 oocytes requires Osk. While the nature of this requirement is not clear, the altered position of α-adaptin in *osk* null mutants suggests that Osk might itself have a role in the organization and/or stabilization of membrane compartments at the posterior pole of the oocyte.

Localization of Rab11 to the posterior pole of the oocyte does not require a polarized microtubule cytoskeleton

The localization of *osk* mRNA and other previously studied molecules to the posterior pole of stage 8 and older oocytes requires polarization of the microtubule cytoskeleton, whereby the minus and plus ends become concentrated along the anterior cortex and posterior pole, respectively (van Eeden and St Johnston, 1999). To determine if Rab11 localization has the same requirement, we examined its distribution in *grk* null mutants. Such mutants develop a bipolar microtubule cytoskeleton in which microtubule minus ends are concentrated at each pole of the cell and microtubule plus ends are concentrated at the cell center (Roth et al., 1995; Gonzäles-Reyes et al., 1995; Shulman et al., 2000). Remarkably, Rab11 accumulated exclusively at the posterior pole of *grk* null mutants (Fig. 5E,G,H). As expected, Stau and *osk* mRNA accumulated at the center of the *grk* null oocytes (Fig. 5F,G,I). The amount of Rab11 at the posterior pole of the *grk* nulls was less than that observed in wild-type oocytes. This reduction was not unexpected given the inability of the *grk* oocytes to accumulate normal amounts of Osk at the posterior pole. We conclude from these findings that Rab11 localization does not require normal polarization of the microtubule cytoskeleton.

Rab11 is required for the sharp focusing of microtubule plus ends onto the posterior pole of stage 8-10 oocytes

Given recent findings in other systems that microtubule ends may be organized through attachment to specific membrane compartments (Schuyler and Pellman, 2001), we investigated the organization of the microtubule cytoskeleton in *rab11*^{P2148} GLCs. Tubulin immunolocalization experiments revealed normal organization of microtubule minus ends in *rab11*^{P2148} GLCs. The highest levels of tubulin staining occurred at the posterior end of the oocyte during stages 1-6 (data not shown) and at the anterior cortex during stages 7-10 (Fig. 6A,D). Similar labeling patterns were observed in non-fixed oocytes following expression of a GFP-tagged version of the microtubule associated Tau protein (Fig. 6B,E) (Micklem et al., 2000). Additional evidence that *rab11* is not required for the organization of microtubule minus ends comes from observations that *bicoid* and *K10* mRNA are correctly localized to the anterior cortex of *rab11* oocytes and that *gurken* (*grk*) mRNA is correctly localized to the dorsal anterior corner of *rab11* oocytes (Fig. 6C,F, respectively). Similar results have also recently been reported (Janovics et al., 2001).

To determine whether *rab11* has a role in microtubule plus end organization, we expressed a kinesin-*lacZ* fusion gene in *rab11*^{P2148} GLCs. The kinesin part of this protein possesses plus end motor activity and localizes to the posterior pole of wild-type oocytes from late stage 8 through early stage 10

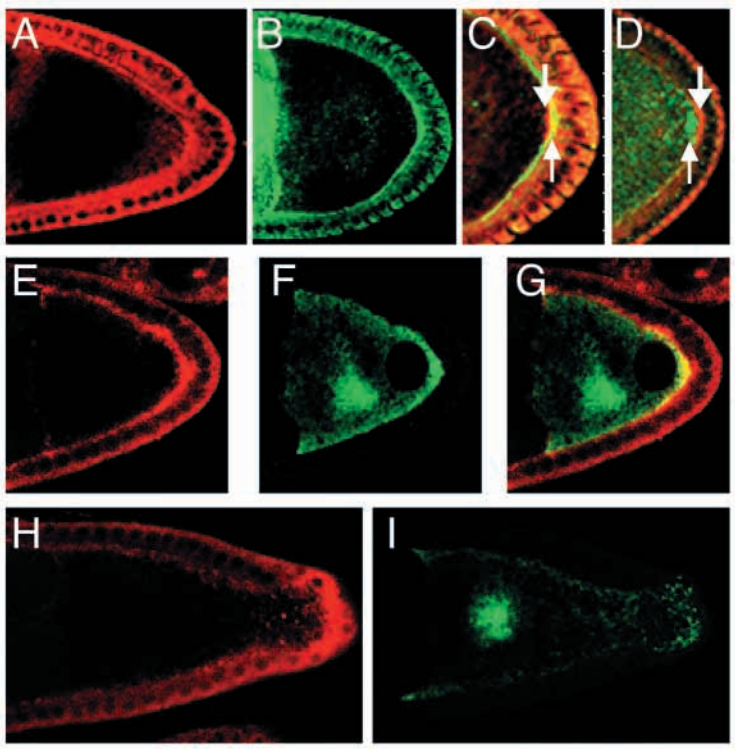

Fig. 5. The localization of Rab11 and α-adaptin to the posterior pole of the oocyte is reinforced by Osk, but does not require a polarized microtubule cytoskeleton. Confocal immunolocalization of Rab11 (red) and α-adaptin in stage 9 *osk* protein null (A-C) and wild-type (D) oocytes. Rab11 is localized to the posterior pole of *osk* protein null oocytes (A), but at a reduced level compared with that in wild type (see text). Normal or near normal amounts of α-adaptin accumulate at the posterior pole of *osk* protein null oocytes (B), but the protein is distinctly more cortical than in wild-type oocytes. Note, for example, that while α-adaptin (upward pointing arrows) is anterior to Rab11 (downward pointing arrows) in wild-type oocytes (D), it is posterior to Rab11 in the *osk* protein null oocyte (C). (E-I) Confocal immunolocalization of Rab11 (red) and Stau (green) in *grk* null oocytes. (E-G) Stage 9 oocyte showing specific accumulation of Rab11 at the posterior pole of the oocyte (E) and Stauffen protein at the cell center and posterior pole (F). (G) Merged image. (H,I) A rare stage 10 *grk* null oocyte in which Stauffen protein is found exclusively or almost exclusively at the cell center (I), consistent with complete depolarization of the microtubule cytoskeleton of the oocyte . Even though no microtubule polarity is evident in this oocyte, Rab11 accumulates specifically at the posterior pole of the oocyte (H). Although it is difficult to rule out the Rab11 signal in H is from neighboring (posterior) follicle cells, this possibility seems unlikely given that no specific accumulation of Rab11 is seen in posterior follicle cells of other *grk* nulls (see E). Moreover, anterior and posterior follicle cells are thought to adopt identical (i.e. anterior) fates in *grk* mutants (Roth et al., 1995; Gonzäles-Reyes et al., 1995). The α-adaptin expression pattern in *grk* null oocytes resembles that seen in the *osk* protein null oocytes (data not shown).

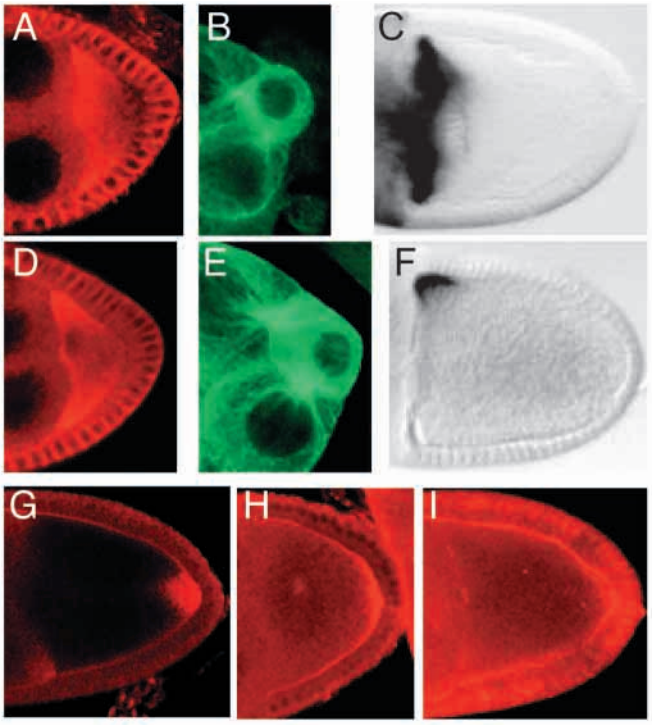

Fig. 6. Microtubule organization in wild-type oocytes and in *rab11*^{P2148} GLCs. (A-F) Organization of microtubule minus ends in wild-type oocytes and in *rab11*^{P2148} GLCs. Expression of tau-GFP in living stage 7 oocytes from wild-type (B) and *rab11*^{P2148} GLC-bearing flies (E). Intense fluorescence at anterior cortex and the absence of fluorescence at the posterior pole indicate normal reorganization of the microtubule cytoskeleton in both oocytes, resulting in a concentration of microtubule minus ends at the anterior cortex. Immunolocalization of α-tubulin in wild-type stage 8 oocytes (A) and in stage 8 *rab11*^{P2148} GLCs (D). A distinct anterior-posterior gradient of microtubule density is seen in both oocytes, again indicative of normal microtubule minus end organization. In situ hybridization for *bicoid* (C) and *gurken* (F) transcripts in stage 10 *rab11*^{P2148} GLCs. Both patterns are indistinguishable from that seen in wild-type controls (Saunders and Cohen, 1999; St Johnston, 1995). (G-I) Organization of microtubule plus ends in wild-type oocytes and in *rab11*^{P2148} GLCs. Immunolocalization of Kin:β-gal fusion protein in wild-type stage 9 oocytes (G) and in early (H) and late (I) stage 9 *rab11*^{P2148} GLCs. The tight fluorescence at the posterior tip of the wild-type oocyte indicates sharp focusing of microtubule plus ends. Conversely, the expanded fluorescence along the lateral and posterior cortexes of *rab11*^{P2148} GLCs indicates poor focusing of microtubule plus ends.

(Clark et al., 1994; Clark et al., 1997). In contrast to wild-type controls (Fig. 6G), Kin:β-gal was distributed over a broad portion of the plasma membrane of the oocyte in *rab11*^{P2148} GLCs, with only slight enrichment at the posterior pole (Fig. 6H,I). Interestingly, the amount of enrichment at the posterior pole decreased over time (e.g. compare the early stage 9 pattern of Fig. 6H with the late stage 9 pattern of Fig. 6I) at the expense of increased deposition of Kin-β-gal in more lateral regions of the cell cortex. We conclude from these findings that Rab11 is required to establish and maintain the sharp focus of microtubule plus ends onto the posterior pole of the oocyte.

Rab11 is required for efficient transport of *osk* mRNA to the posterior pole of the oocyte and for its subsequent translation

To investigate further the role of Rab11 in the organization of the posterior pole of the oocyte, we examined the distribution of *osk* mRNA and protein in *rab11*^{P2148} GLCs. In wild-type oocytes, *osk* mRNA is transported to the posterior pole during stages 8 and 9 (Kim-Ha, 1991; Karlin-McGinness et al., 1996; Brendza et al., 2000), coincident with the polarization of the microtubule cytoskeleton. During transport, and in the initial hours following transport, *osk* mRNA is seen as a large ball (Fig. 7A). By the end of stage 9, the ball resolves into a thin cap along the posterior cortex, which persists through the end of oogenesis (Kim-Ha et al., 1991) (Fig. 7B). The nature of the transition from the ball to the cap is not clear, but coincides with the activation of *osk* translation (Rongo et al., 1995) (Fig. 7C). The cap structure is much more resistant to disruption with colchicine than is the ball (Pokrywka and Stephenson, 1995) (G. D., E. S., J. M. and R. S. C., unpublished) and appears, then, to represent the binding of the mRNA to an anchor, which might be Osk (Rongo et al., 1997). We found that *rab11*^{P2148} GLCs were defective in the transport of *osk* mRNA to the posterior pole, and in its subsequent translation and anchoring. The transport defect was temporal in nature. Thus, while most *osk* transcripts reached the posterior pole of wild-type oocytes during stage 8 (Fig. 7A), only a small fraction of transcripts reached the posterior pole of *rab11*

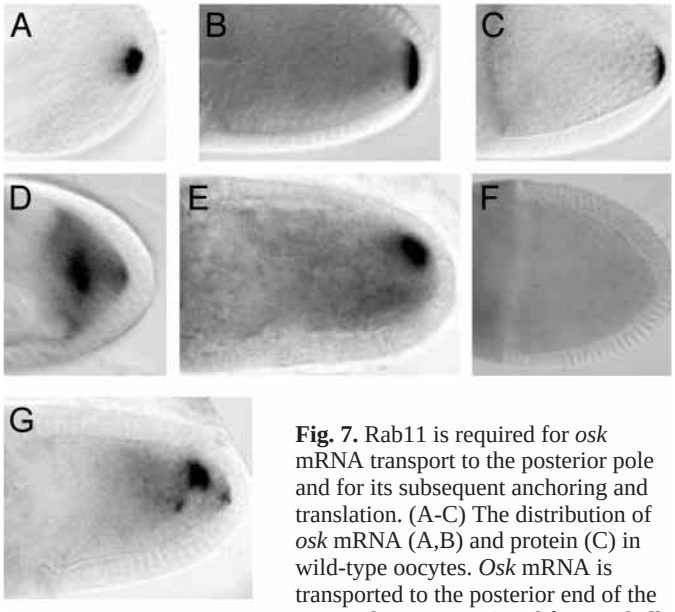

Fig. 7. Rab11 is required for *osk* mRNA transport to the posterior pole and for its subsequent anchoring and translation. (A-C) The distribution of *osk* mRNA (A,B) and protein (C) in wild-type oocytes. *Osk* mRNA is transported to the posterior end of the oocyte during stage 8 and forms a ball-shaped complex (A). During stage 9, the ball resolved into a cap-shaped structure (B) that persists through the completion of oogenesis. Osk (C) is first detected coincident with the *osk* mRNA ball to cap transition. (D-G) The distribution of *osk* mRNA (D,E,G) and protein (F) in *rab11*^{P2148} GLCs. *osk* mRNA is slow to accumulate at the posterior pole of *rab11*^{P2148} GLCs. A mass of *osk* mRNA is often seen at the center of stage 8 *rab11*^{P2148} GLCs (D), possibly reflecting stalled transport. Ultimately, *osk* mRNA reaches the posterior pole of *rab11*^{P2148} GLCs, but the mRNA remains as a ball (E), or often breaks up into several smaller balls (G). Consistent with a defect in anchoring, the *osk* mRNA is not translated as evident by the absence of detectable Osk (F).

oocytes during stage 8 (Fig. 7D). Delayed transport to the posterior pole of the oocyte has also recently been reported by Jancovics et al. (Jancovics et al., 2000). Typically, the lagging transcripts were aggregated into a mass near the center of the cell, possibly representing stalled transport at an intermediate step (Fig. 7D). Although most *osk* transcripts eventually reached the posterior pole of *rab11* oocytes (Fig. 7E) two observations suggest that they are never anchored. First, no Osk was ever detected in *rab11* oocytes (Fig. 7F). Second, the *osk* transcripts of *rab11*^{P2148} GLCs never formed the characteristic cap at the posterior pole, but instead remained as a ball (Fig. 7E). Moreover, during late stages of oogenesis (e.g. when microtubules are bundled along the entire egg cortex), the ball of *osk* mRNA appeared to drift away from the posterior pole and was often fragmented into several smaller balls (see Fig. 7G). We conclude from these studies that Rab11 is required for the efficient transport of *osk* mRNA to the posterior pole of the oocyte and for its subsequent translation and anchoring.

Given the apparent absence of Osk in *rab11*^{P2148} GLCs, it is likely that the resulting embryos would lack pole cells and have defects in the formation of abdominal segments (Lehmann and Nüsslein-Volhard, 1986; Ephrussi and Lehmann, 1992; Smith et al., 1992; van Eeden et al., 2001). We have not, however, been able to test this idea directly as the eggs recovered from *rab11*^{P2148} GLCs are fragile and die before or shortly after fertilization (Perrimon et al., 1996) (G. D., E. S., J. M. and R. S. C., unpublished). The basis for the fragile egg phenotype is not known but may reflect secondary requirements for Rab11 in eggshell formation and/or yolk uptake.

DISCUSSION

We have shown that Rab11 and α-adaptin are localized to the posterior pole of mid-stage oocytes and that such localization does not require a polarized microtubule cytoskeleton or *grk* signaling. A reduction of *rab11* activity in the oocyte alters the subcellular distribution of Rab11, α-adaptin and internalized transferrin. These alterations indicate that Rab11 is required for endocytic recycling and to organize posterior membrane compartments. A reduction of *rab11* activity in the oocyte also causes defects in Kin:β-gal localization, *osk* mRNA transport, and *osk* mRNA translation and anchoring. These latter defects suggest that the posterior membrane compartments established by Rab11 organize microtubule plus ends and, possibly, the translation factors and/or anchors for *osk* mRNA.

The *Drosophila* oocyte plasma membrane has a unique posterior domain defined by *rab11*

The expression pattern of Htr in transgenic flies shows clearly that the oocyte establishes a posterior plasma membrane domain (PMD) (Bretscher, 1996). Further evidence for such a domain comes from our finding that Rab11 localizes to the posterior pole of wild-type oocytes. Interestingly, the PMD is established independently of microtubule polarity or *grk* signaling, as Rab11 is localized normally in *grk* null mutants. Given the rapid rate at which Htr is internalized and recycled (Bretscher, 1996; Hanover et al., 1984), it is likely that the maintenance, if not also the initial specification, of the PMD requires polarized endocytic recycling directed towards the posterior pole. The data we present indicate that Rab11 is responsible for such recycling: Rab11 is localized to the posterior pole of the oocyte and is required for the recycling of internalized transferrin (the ligand for transferrin receptor) to the plasma membrane of cultured oocytes. Independent evidence that Rab11 mediates polarized endocytic recycling comes from studies with vertebrates, where Rab11 recycles internalized molecules to the apical surface of polarized epithelial cells (Prekeris et al., 2000; Wang et al., 2000).

What polarizes endocytic recycling to the posterior pole of the oocyte? The polarization of the endocytic pathways of other cells is triggered by Rho GTPase family members (i.e. Rho, Cdc42 and Rac) (Kroschewski et al., 1999; Ellis and Mellor, 2000; Garrett et al., 2000; Garred et al., 2001), which are activated at specific regions of the cell cortex by a variety of intrinsic and extrinsic cues (Drubin and Nelson, 1996). Rho GTPases have also been strongly implicated in the polarization of exocytosis (Adamo et al., 1999; Guo et al., 1999; Guo et al., 2001). Specifically, they have been shown to recruit the 'exocyst' to specific sites of the plasma membrane. The exocyst is a conserved complex of proteins to which vesicles of the secretory pathway fuse (Grindstaff et al., 1998; Adamo et al., 1999; Guo et al., 2001). Thus, through local activation of Rho GTPases, secretory vesicles are targeted to specific regions of the plasma membrane. By analogy, the Rho GTPases could localize Rab11 and polarize receptor recycling through local recruitment of an exocyst-like complex for Rab11-containing vesicles. Because *Drosophila* Rho GTPases are required for progression through early oogenesis (Genova et al., 2000; Murphy and Montell, 1996), the analysis of their role in Rab11 localization and other aspects of oocyte polarization must await the identification of conditional mutants.

The role of Rab11 in microtubule plus end organization and *osk* mRNA transport

The Kin:β-gal expression studies indicate that microtubule plus ends are not sharply focused onto the posterior pole of the oocyte in *rab11*^{P2148} mutant oocytes. The simplest interpretation of this finding is that microtubule plus ends are attached to the PMD, or to a neighboring membrane domain whose identity is established and/or maintained by Rab11. In wild-type oocytes, this domain is tightly defined such that Kin:β-gal is concentrated at the posterior tip of the oocyte, while in *rab11*^{P2148} mutant oocytes, the domain is poorly defined and the Kin:β-gal expression pattern is expanded. The slight enrichment of Kin:β-gal at the posterior tip of *rab11*^{P2148} oocytes could reflect partial Rab11 activity and/or the polarizing activities of membrane trafficking pathways that may not rely on Rab11 (e.g. the secretory pathway) (Rodman and Wandinger-Ness, 2000), which targets newly synthesized molecules from the Golgi to the plasma membrane. Recent studies have identified two types of protein-protein interactions (CLIP-CLASP and APC-EB1) responsible for the stable association of microtubule plus ends with membranes (Akhmanova et al., 2000; Nakamura et al., 2001; Lu et al., 2001; Schuyler and Pellman, 2001). While the CLIP, CLASP, APC and EB1 protein families are all well-represented in the *Drosophila* genome, their role in the establishment of oocyte polarity has not yet been investigated.

Apart from Rab11, the only protein known to play a specific

role in microtubule plus end organization in *Drosophila* oocytes is Par-1, a kinase, whose suspected targets include the microtubule associated protein Tau (Shulman et al., 2000). In strong *par-1* mutants, microtubule plus ends, as revealed by Kin:β-gal expression patterns, are not enriched at the posterior pole of the oocyte, but instead are concentrated tightly as a dot at the center of the cell. In weak *par-1* mutants, a small amount of Kin:β-gal is also found at the posterior pole. This small amount of Kin:β-gal is always tightly localized to the cell tip, suggesting that Par-1 is not required for the specification of the PMD, but rather only for the efficient movement of already focused microtubule plus ends from the cell center to the PMD. Consistent with the idea that microtubule plus ends initially focus to a sharp point at the center of the cell and then move to the posterior pole, Kin:β-gal and *oskar* mRNA show transient concentration at the center of the cell in wild-type oocytes (Clark et al., 1994; Clark et al., 1997) (G. D., E. S., J. M. and R. S. C., unpublished). How Par-1 might promote the movement of microtubule plus ends from the cell center to the posterior pole is not clear. One possibility is that it promotes attachment of microtubule plus ends to a structure that is then moved to the posterior pole. Alternatively, Par-1 might stimulate a burst of microtubule growth, forcing growth toward the posterior end of the cell.

The observation that *osk* mRNA transport to the posterior pole is delayed in *rab11*^{P2148} mutant oocytes suggests that Rab11 might also have a role in the movement of microtubule plus ends from the cell center to the posterior pole, and therefore, that such movement is membrane dependent. For example, microtubule plus ends could become attached to membrane compartments or vesicles at the cell center, and the vesicles may then be targeted to the posterior pole in a Rab11-dependent manner. Because *osk* mRNA arrives at the posterior pole as a fairly well-defined ball in *rab11*^{P2148} oocytes, Rab11 does not appear to be required for focusing microtubule plus ends at the cell center, but rather only for their timely movement and attachment to the posterior membrane domain.

The role of Rab11 and microtubule plus ends in *osk* mRNA anchoring and translation

Although most *osk* transcripts are eventually transported to the posterior pole in *rab11*^{P2148} oocytes, they are not translated. As Osk is required to anchor *oskar* mRNA at the posterior pole (Rongo et al., 1995), the lack of *oskar* translation in *rab11*^{P2148} GLCs could explain the inability of the *oskar* mRNA ball to resolve into the thin posterior crescent. The nature of the *osk* translation block in *rab11*^{P2148} oocytes is not clear. One possibility is that key *osk* translation factors are localized to the posterior membrane domain established by Rab11. In *rab11*^{P2148} oocytes, this domain may be too poorly defined to support assembly of such factors into an active translation complex.

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REFERENCES

Adamo, J. E., Rossi, G. and Brennwald, P. (1999). The Rho GTPase Rho3 has a direct role in exocytosis that is distinct from its role in actin polarity. *Mol. Biol. Cell* **12**, 4121-4133.

Akhmanova, A., Hoogenraad, C. C., Drabek, K., Stepanova, T. Dortland, B., Verlerk, T., Vermeulen, W., Burgering, B. M., De Zeeuw, C. I., Drosveld, F. and Galjart, N. (2000). CLASPS are CLIP-115 and CLIP-170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. *Cell* **104**, 923-935.

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.

Bretscher, M. S. (1996). Expression and changing distribution of the human transferrin receptor in developing *Drosophila* oocytes and embryos. *J. Cell Sci.* **109**, 3113-3119.

Cheung, H.-K., Serano, T. L. and Cohen, R. S. (1992). Evidence for a highly selective RNA transport system and its role in establishing the dorsoventral axis of the *Drosophila* egg. *Development* **114**, 653-661.

Chou, T. B. and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in Drosophila melanogaster. *Genetics* **144**, 1673-1678.

Clark, I., Giniger, E., Ruohola-Baker, H., Jan, L. Y. and Jan, Y. N. (1994). Transient posterior localization of a Kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* **4**, 289-300.

Clark, I., 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.

Deng, W.-M. and Ruohola-Baker, H. (2000). Laminin A is required for follicle cell-oocyte signaling that leads to establishment of the anterior-posterior axis in *Drosophila*. *Curr. Biol.* **10**, 683-686.

Dornan, S., Jackson, A. P. and Gay, N. J. (1997). α-adaptin, a marker for endocytosis, is expressed in complex patterns during *Drosophila* development. *Mol. Biol. Cell* **8**, 1391-1403.

Drubin, D. G. and Nelson, W. J. (1996). Origins of cell polarity. *Cell* **84**, 335-344.

Ellis, S. and Mellor, H. (2000). Regulation of endocytic traffic by rho family GTPases. *Trends Cell Biol.* **10**, 85-88.

Ephrussi, A. and Lehmann, R. (1992). Induction of germ cell formation by oskar. *Nature* **358**, 387-392.

Feinberg, A. P. and Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6-13.

Frank, L. H., Cheung, H. K. and Cohen, R. S. (1992). Identification and characterization of Drosophila female germ line transcriptional control elements. *Development* **114**, 481-491.

Garred, O., Rodal, S. K., van Deurs, B. and Sandvig, K. (2001). Reconstitution of clathrin-independent endocytosis at the apical domain of permeabilized MDCK II cells: requirement for a Rho-family GTPase. *Traffic* **2**, 26-36.

Garrett, W. S., Chen, L. M., Kroschewski, R., Ebersold, M., Turley, S., Trombetta, S., Galan, J. E. and Mellman, I. (2000). Developmental control of endocytosis in dendritic cells by Cdc42. *Cell* **102**, 325-334.

Genova, J. L., Jong, S., Camp, J. T. and Fehon, R. G. (2000). Functional analysis of *Cdc42* in actin filament assembly, epithelial morphogenesis, and cell signaling during Drosophila development. *Dev. Biol.* **221**, 181-194.

Gonzäles-Reyes, A., Elliot H. and St Johnston, D. (1995). Polarisation of both major body axes in *Drosophila* by *grk*-torpedo signaling. *Nature* **375**, 654-658.

Grindstaff, K. K., Yeaman, C., Anandasabapathy, N., Hsu, S.-H., Rodriguez-Boulan, E., Scheller, R. H. and Nelson, W. J. (1998). Sec6/8complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* **93**, 731-740.

Grünert, S. and St Johnston, D. (1996). RNA localization and the development of asymmetry during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **6**, 395-402.

Guo, W., Roth, D., Walch-Solimena, C. and Novick, P. (1999). The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* **18**, 1071-1080.

Guo, W., Tamanoi, F. and Novick, P. (2001). Spatial regulation of the exocyst complex by Rho1 GTPase. *Nat. Cell Biol.* **4**, 353-360.

Hanover, J. A., Willingham, M. C. and Psatan, I. (1984). Kinetics of

transferrin and epidermal growth factor through clathrin-coated membranes. *Cell* **39**, 283-293.

Jankovics, F., Sinka, R. and Erdélyi, M. (2001). An interaction type of genetic screen reveals a role of the rab11 gene in *oskar* mRNA localization in the developing Drosophila melanogaster oocyte. *Genetics* **158**, 1177-1188.

Karlin-McGinness, M., Serano, T. L. and Cohen, R. S. (1996). A comparative analysis of the kinetics and dynamics of *K10*, *bicoid*, and *oskar* mRNA localization in the *Drosophila* oocyte. *Dev. Genet.* **19**, 238-248.

Kim-Ha, J., Smith, J. L. and Macdonald, P. M. (1991). *oskar* mRNA is localized to the posterior pole of the Drosophila oocyte. *Cell* **66**, 23-35.

Klausner, R. D., Ashwell, G., Renswoude, J. V., Harford, J. B. and Bridges, K. R. (1983). Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. *Proc. Natl. Acad. Sci. USA* **89**, 7437-7441.

Kroschewski, R., Hall, A. and Mellman, I. (1999). Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nat. Cell Biol.* **1**, 8-13.

Lane, M. E. and Kalderon, D. (1994). RNA localization along the anteroposterior axis of the Drosophila oocyte requires PKA-mediated signal transduction to direct normal microtubule organization. *Genes Dev.* **15**, 2986-2995.

Lehmann, R. and Nüsslein-Volhard, C. (1986). Abdominal segmentation, pole cell formation and embryonic polarity require the localized activity of oskar, a maternal gene in Drosophila. *Cell* **47**, 141-152.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. New York: Academic Press.

Lu, B., Roegiers, F., Jan, L. Y. and Jan, Y. N. (2001). Adherens junctions inhibit asymmetric division in the Drosophila epithelium. *Nature* **409**, 522-525.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning; A Laboratory Manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Micklem, D. R., Adams, J., Grünert, S. and St Johnston, D. (2000). Distinct roles of two conserved Staufen domains in *oskar* mRNA localization and translation. *EMBO J.* **19**, 1366-1377.

Mukherjee, S., Ghosh, R. N. and Maxfield, F. R. (1997). Endocytosis. *Physiol. Rev.* **77**, 759-803.

Murphy, A. M. and Montell, D. J. (1996). Cell type-specific roles for Cdc42, Rac, and RhoL in *Drosophila* oogenesis. *J. Cell Biol.* **133**, 617-630.

Nakamura, M., Zhou, X. Z. and Lu, K. P. (2001). Critical role for the EB1 and APC interaction in the regulation of microtubule polymerization. *Curr. Biol.* **11**, 1062-1067.

Novick, P. and Zerial, M. (1997). The diversity of Rab proteins in vesicle transport. *Curr. Opin. Cell Biol.* **9**, 496-504.

Perrimon, N., Lanjuin, A., Arnold, C. and Noll, E. (1996). Zygotic lethal mutations with maternal effect phenotypes in Drosophila melanogaster. II. Loci on the second and third chromosomes identified by P-element induced mutations. *Genetics* **144**, 1681-1692.

Pirrotta, V. (1988). Vectors for P-mediated transformation in *Drosophila*. In *Vectors*: *A Survey of Molecular Cloning Vectors and Their Uses* (ed. R. L. Rodriguez and D. T. Denhardt), pp. 437-456. Boston, MA: Butterworths.

Pokrywka, N. J. and Stephenson, E. C. (1995). Microtubules are a general component of mRNA localization systems in Drosophila oocytes. *Dev. Biol.* **167**, 363-370.

Prekeris, R., Klumerman, J. and Scheller, R. H. (2000). A Rab11/Rip11 protein complex regulates apical membrane trafficking via recycling endosomes. *Mol. Cell* **6**, 1437-1448.

Ren, M., Xu, G., Zeng, J., De Lemos-Chiarandini, C., Adesnik, M. and Sabatini, D. D. (1996). Hydrolysis of GTP on rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes. *Proc. Natl. Acad. Sci. USA* **95**, 6187-6192.

Robinson, M. S. (1994). The role of clathrin, adaptors and dynamin in endocytosis. *Curr. Opin. Cell Biol.* **6**, 538-544.

Rodman, J. S. and Wandinger-Ness A. (2000). Rab GTPases coordinate endocytosis. *J. Cell Sci.* **113**, 183-192.

Rongo, C. G., Gavis, E. R. and Lehmann, R. (1995). Localization of oskar RNA regulates oskar translation and requires Oskar protein. *Development* **121**, 2737-2746.

Rongo, C., Broihier, H. T., Moore, L., Van Doren, M., Forbes, A. and Lehmann, R. (1997). Germ plasm assembly and germ cell migration in Drosophila. *Cold Spring Harbor Symp. Quant. Biol.* **62**, 1-11.

Roth, S., Neuman-Silberberg, F. S., Barcelo, G. and Schüpbach, 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.

Rubin, G. M. and Spradling, A. C. (1982). Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**, 348-353.

Sasamura, T., Kobayashi, T., Kojima, S., Qadota, H., Ohya, Y., Masai, I. and Hotta, Y. (1997). Molecular cloning and characterization of Drosophila genes encoding small GTPases of the rab and rho families. *Mol. Gen. Genet.* **254**, 486-494.

Satoh, A. K., Tokunaga, F. and Ozaki, K. (1997). Rab proteins of Drosophila melanogaster: novel members of the Rab-protein family. *FEBS Lett.* **404**, 65-69.

Saunders, C. and Cohen, R. S. (1999). The role of oocyte transcription, the 5′UTR, and translation repression and derepression in Drosophila *gurken* mRNA and protein localization. *Mol. Cell* **3**, 43-54.

Schnorrer, F., Bohmann, K. and Nusslein-Volhard, C. (2000). The molecular motor dynein is involved in targeting Swallow and *bicoid* RNA to the anterior pole of Drosophila oocytes. *Nat. Cell Biol.* **2**, 185-190.

Schuyler, S. C. and Pellman, D. (2001). Microtubule 'plus-end-tracking proteins': the end is just the beginning. *Cell* **105**, 421-424.

Serano, T. L., Karlin-McGinness, M. and Cohen, R. S. (1995). The role of *fs(1)K10* in the localization of the mRNA of the TGFα- homolog *grk* within the *Drosophila* oocyte. *Mech. Dev.* **51**, 183-192.

Shulman, J. M., Benton, R. and St Johnston, D. (2000). The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs *oskar* mRNA localization to the posterior pole. *Cell* **101**, 377-388.

Smith, J. L., Wilson, J. E. and MacDonald, P. M. (1992). Overexpression of oskar directs ectopic activation of nanos and presumptive pole cell formation in Drosophila embryos. *Cell* **70**, 849-859.

Spradling, A. C. (1993). Developmental genetics of oogenesis. In *The Development of* Drosophila melanogaster (eds. M. Bate and A. Martinez Arias), pp. 1-70. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Spradling, A. C. and Rubin, G. M. (1982). Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* **218**, 341-347.

St Johnston, D. (1995). The intracellular localization of messenger RNAs. *Cell* **81**, 161-170.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Thio, G. L., Ray, R. P., Barcelo, G. and Schupbach, T. (2000). Localization of gurken RNA in Drosophila oogenesis requires elements in the 5′ and 3′ regions of the transcript. *Dev. Biol.* **221**, 435-446.

Theurkauf, W. E., Smiley, S., Wong, M. L. and Alberts, B. M. (1992). Reorganization of the cytoskeleton during Drosophila oogenesis: implication for axis specification and intercellular transport. *Development* **115**, 923-936.

van Eeden, F. and St Johnston, D. (1999). The polarisation of the anterior-posterior and dorsal-ventral axes during *Drosophila* oogenesis. *Curr. Opin. Gen. Dev.* **9**, 396-404.

van Eeden, F. J., Palacios, I. M., Petronczki, M., Weston, M. J. and St Johnston, D. (2001). Barentsz is essential for the posterior localization of oskar mRNA and colocalizes with it to the posterior pole. *J. Cell Biol.* **6**, 511-523.

Wang, X., Kumar, R., Navarre, J., Casanova, J. E. and Goldenring, J. R. (2000). Regulation of vesicle trafficking in MDCK cells by Rab11a and Rab25. *J. Biol. Chem.* **275**, 29138-29146.

Wilcke, M., Johannes, L., Galli, T., Mayau, V., Goud, B. and Salamero, J. (2000). Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the trans-Golgi network. *J. Cell Biol.* **151**, 1207-1220.