Dynein-mediated apical localization of crumbs transcripts is required for Crumbs activity in epithelial polarity

Zhouhua Li,1 Liwei Wang,1 Thomas S. Hays,3 and Yu Cai1,2
1Temasek Lifesciences laboratory and 2Department of Biological Science, National University of Singapore, Singapore 117604
3Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455

Introduction

Metazoan epithelial cells are polarized along the apicobasal (A/B) axis and this polarization is important for the formation and function of the epithelial structures they comprise (Tepass et al., 2001; Knust and Bossinger, 2002; Nelson, 2003; Macara, 2004; Shin et al., 2006). Along with this polarization, their plasma membranes are compartmentalized into several distinct domains with different protein complexes differentially localized along the A/B axis, including the Crumbs (Crb) complex (Crb, Stardust [Sdt], and Patj/Dlt), the PAR complex (PAR-3, PAR-6, and aPKC), and the Scribble complex (Discs large, Lethal giant larvae, and Scribble). Genetic studies in Drosophila melanogaster have revealed that these protein complexes function in a sequential yet interdependent manner to regulate the establishment, elaboration, and maintenance of cellular polarity (Tepass et al., 2001; Bilder et al., 2003; Tanentzapf and Tepass, 2003). The transmembrane protein Crb localizes on the apical domain and acts as an apical domain determinant by organizing a protein network that regulates A/B polarity (Tepass et al., 1990; Wodarz et al., 1995). However, it is unclear how Crb is targeted onto the apical domain.

There are at least two mechanisms for the asymmetrical deployment of proteins (Bashirullah et al., 1998). The first relies on the subcellular trafficking of proteins via the trans-Golgi network (Rodriguez-Boulan et al., 2005). Polarized deployment of the majority of transmembrane and secreted proteins is believed to be mediated by this mechanism (Nelson and Yeaman, 2001). The second mechanism involves polarized localization of transcripts before translation (St Johnston, 2005). These transcripts encode a variety of cytosolic proteins, ranging from transcription factors to cytoskeletal proteins. However, there are relatively few reported examples of localized transcripts encoding transmembrane proteins and the functional relevance of these localized transcripts remains to be investigated (Takizawa et al., 2000; Brittis et al., 2002; Juschke et al., 2004).

Results and discussion

To identify genes that regulate epithelial polarity, we performed a mosaic screen in D. melanogaster follicle cells (FCs) and identified mutants in D. melanogaster dynein heavy chain 64C localization of its transcript and this apical transcript localization is crucial for crb function. In crb mutant FCs, Crb protein derived from transgenes lacking the 3’ UTR does not effectively localize to the apical domain and does not effectively restore normal epithelial polarity. We propose that dynein-mediated messenger RNA transport coupled with a localized translation mechanism is involved in localizing Crb to the apical domain to mediate epithelial apicobasal polarity and that this mechanism might be widely used to regulate cellular polarity.
Figure 1. Dynsin function is required for FC A/B polarity. GFPnls are shown as green, DNA is shown as blue, and mutant clones are marked by the absence of GFP and apical up unless otherwise stated. (A) wt FCs display regular cuboidal morphology by rhodamine phalloidin staining (red). Dhc64C<sup>902</sup> and Glu<sup>ID</sup> FCs show altered morphology, wt FCs are monolayered (D), whereas Dhc64C<sup>902</sup> and Glu<sup>ID</sup> mutant FCs are multiple layered at the posterior end. Bars, 2 μm.

(Dhc64C) and the dynactin component Gluded<sup>j50</sup>, which disrupt FC A/B polarity (see Materials and methods and Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200707007/DC1, for screen and mapping details). In the wild type (wt), the FCs are polarized along the A/B axis and form a single layer enclosing the developing germ cells (100%, n > 1,000; Fig. 1, A and D). However, Dhc64C<sup>902</sup> and Glu<sup>ID</sup> mutant FCs often became multiple layered when located at the posterior end of the follicle after stage six (98%, n = 125), which indicates a loss of A/B polarity (Fig. 1, B, C, E, and F). Examination of known polarized membrane markers showed that Dhc64C<sup>902</sup> and Glu<sup>ID</sup> mutants primarily affect apical localization of the Crb complex without markedly affecting the localization of other polarity complexes regardless of the position and developmental stage of the mutant clones induced. Crb localizes to the apical region in wt FCs (100%, n > 1,000) but is absent from the apical region in both Dhc64C<sup>902</sup> and Glu<sup>ID</sup> mutant FCs (100%, n = 245; Fig. 2, A–C), whereas Sdt and Patj/Dlt showed cytoplasmic localization (100%, n = 156; see Fig. 4, A–F). The adherens junction (Armadillo; 100%, n = 175), as well as the PAR complex (aPKC; 100%, n = 212) largely retained their normal localization, although their levels were somewhat reduced (Fig. 2, D–I); the lateral Scribble complex was slightly expanded into the apical domain (100%, n = 260; Fig. 2, J–L). Identical phenotypes were also observed in previously identified alleles (Dhc64C<sup>151</sup> and Glu<sup>ID</sup>) or when p25 (another subunit of dynactin) function was compromised using double-stranded RNA–mediated knockdown (termed p25<sup>RNAi</sup>; Fig. S2 and not depicted). Although recent data showed that dynsin activity is required for Bazooka (Baz, the fly homologue of Par3) localization in embryonic epithelia (Harris and Peifer, 2005), our data favor the model that dynsin functions primarily through the Crb complex to establish FC polarity. This may reflect the different requirement of these two systems. A wt Dhc64C transgene fully rescued the polarity defects in Dhc64C<sup>902</sup> mutant FCs (100%, n > 500; Fig. S1). Dynsin and dynactin form a complex that mediates microtubule (MT)-based transport and both Dhc64C<sup>902</sup> and Glu<sup>ID</sup> mutants showed virtually identical polarity defects. For simplicity, we show data mainly for Dhc64C<sup>902</sup>.

The dynsin complex transports cargos toward MT minus ends. To examine the effects of disrupting the MT cytoskeleton, we used colchicine to depolymerize MTs. Similar polarity defects were observed in these FCs with specific loss of Crb from their apical domains without markedly affecting other cell polarity complexes (100%, n > 200 for each marker; Fig. 2, M–P). Furthermore, the MT cytoskeleton is largely unaffected in dynsin mutant FCs (unpublished data), which is consistent with the notion that the observed polarity defects seen in dynsin mutant and MT-depolymerized FCs are caused by defective dynsin-mediated transport.

We next investigated how dynsin mediates the apical localization of Crb. Restricted mRNA localization coupled with local translation is widely used to generate cellular asymmetry. It has been reported that crb mRNA localizes apically in embryonic epithelia (Tepass et al., 1990). This prompted us to investigate whether dynsin functions via the localization of crb mRNA to localize Crb protein.

In the wt, crb mRNA is highly enriched on the apical domain. However, in the dynsin mutant, crb mRNA is no longer apically enriched (100%, n = 241; Fig. 3, A and B; and Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200707007/DC1). Consistent with the notion that dynsin-mediated transport requires an intact MT cytoskeleton, crb mRNA is mislocalized in colchicine-treated FCs (100%, n = 127; Fig. S3). Together, these data show that crb mRNA localizes to the apical domain via dynsin-mediated transport.

Because of difficulties in studying how dynsin transports crb mRNA in FCs, we examined crb mRNA localization in blastoderm embryos, as it has been found that dynsin-mediated transport is conserved between embryogenesis and oogenesis (Karlin-Mcginness et al., 1996; Bullock and Ish-Horowicz, 2001). We took advantage of a functional crb minigene, crb<sup>intra-myc</sup> (this minigene contains the crb<sup>3</sup> untranslated region [UTR]; see Fig. S2 for a schematic presentation of the transcripts used in this study; Wodarz et al., 1995). Fluorescently labeled crb<sup>intra-myc</sup> transcripts rapidly localized to the apical domain after injection into the basal cytoplasm of embryos (100%, n = 13; Fig. 3 C). However, preinjection with the anti-Dhc64C antibody P1H4 (100%, n = 12) or colchicine (100%, n = 9) but not anti-Myc antibody (100%, n = 8) completely blocked apical localization of crb<sup>intra-myc</sup>.
transcripts (Fig. 3, D–F). These data demonstrate that dynein activity is required for the apical localization of in vitro labeled crb transcripts in embryos.

To test whether the crb 3’ UTR mediates its localization, we injected crb\textsuperscript{intra-\textit{myc}-\textit{wo}}, in which the crb 3’ UTR was replaced by the 3’ UTR of the SV40 large T antigen, into blastoderm embryos (Simmonds et al., 2001). These transcripts did not localize apically, which suggests that the crb 3’ UTR is required for its apical localization (100%, n = 9; Fig. 3 G). In a complementary experiment, we fused the crb 3’ UTR to an exogenous ß-galactosidase gene and found that this chimeric transcript localized apically (100%, n = 10; Fig. 3 H) when injected into the embryo, whereas control ß-galactosidase transcripts (lacZ-SV40 3’ UTR) did not (100%, n = 8; Fig. 3 I). We conclude that the crb 3’ UTR is necessary and sufficient for its apical localization in embryos. We next investigated whether the crb 3’ UTR also mediates its apical localization in FCs. We again used crb\textsuperscript{intra-\textit{myc}-\textit{wo}} and crb\textsuperscript{intra-\textit{myc}-\textit{wo}} minigenes and the subcellular localization of these transcripts was determined by RNA in situ. When ectopically expressed in wt FCs, crb\textsuperscript{intra-\textit{myc}} transcripts were predominantly found on the apical domain, whereas crb\textsuperscript{intra-\textit{myc}-\textit{wo}} transcripts were unlocalized (Fig. 3 J and K; and Fig. S3, compare with endogenous crb transcripts in Fig. 3 A). Together, these results demonstrate that the crb 3’ UTR mediates apical localization of crb transcripts in FCs.

Next, we investigated whether apical localization of crb transcripts is a prerequisite for Crb protein localization. Both transgenes are expressed at an equivalent level when driven by the same driver in both FCs and embryos, which suggests that the different 3’ UTRs do not affect protein expression levels (Fig. S2 F). In wt FCs, both crb\textsuperscript{intra-\textit{myc}} transcripts and Crb\textsuperscript{intra-\textit{myc}} protein localized to the apical domain (100%, n > 200; Fig. 3 J and L). Interestingly, in wt FCs expressing crb\textsuperscript{intra-\textit{myc}-\textit{wo}}, crb\textsuperscript{intra-\textit{myc}-\textit{wo}} transcripts did not apically localize (Fig. 3 K), yet Crb\textsuperscript{intra-\textit{myc}-\textit{wo}} protein remained localized to the apical domain in the great majority of FCs (95%, n = 73) in several independent transgenic lines (Fig. 3 M and not depicted). Only in ~5% (n = 73) of the FCs examined did Crb\textsuperscript{intra-\textit{myc}-\textit{wo}} protein show cytoplasmic localization (Fig. 3 N). These results suggest that apical localization of Crb protein can be independent of apical transcript localization and that dynein also transports Crb protein to the apical domain. It was found that endogenous oskar mRNA can direct the proper localization of exogenous transcripts derived from a transgene bearing the oskar 3’ UTR during D. melanogaster oogenesis (Hachet and Ephrussi, 2004). Thus, it is possible that endogenous crb transcripts/protein may have an impact on the behavior of these transgene products. Therefore, we examined the behavior of the transgene products in a crb mutant background where endogenous Crb protein is absent. Interestingly,

![Image](https://example.com/image.png)

Figure 2. Dynein is required for the apical localization of the Crb complex. Rhodamine phalloidin is shown in red and GFP\textsuperscript{nls} is shown in green. Crb (blue) localizes to the apical domain of wt FCs (A) but is lost from the apical domain in the dynein mutant and colchicine-treated FCs (B, C, and M). Arm (blue) localizes to the adherens junctions in wt FCs (D) and this localization is largely normal in dynein mutant and colchicine-treated FCs (E, F, and N). aPKC (blue) localizes to the apical region of wt FCs (G) and is largely unaffected in dynein mutant and colchicine-treated FCs (H, I, and O). Scrib (blue) localizes along the lateral domain of wt FCs (J) and is only slightly apically expanded in the dynein mutant cells (K, L, and P). Bars, 5 μm.
localization of the **crb** transcripts is required for effective **crb** function in epithelial polarity. Furthermore, these results suggest that a localized translational machinery near the apical domain may be involved in the generation of full Crb activity on the apical domain.

It has been found that, in embryonic epithelial cells, Crb binds Sdt and the two are mutually dependent for their localization and function (Tepass and Knust, 1993; Bachmann et al., 2001; Hong et al., 2001). Crb protein levels are markedly reduced in the **sdt** mutant and vice versa. Our data show that in dynein mutant FCs, Crb is undetectable by immunofluorescence, whereas Sdt is mainly cytoplasmic (Fig. 4, A–C). There are several possibilities for the inability to detect Crb in the dynein mutant. First, the apical localization and stability of Crb requires dynein activity. In the absence of dynein function, unlocalized Crb is not stable and is degraded. Second, the apical localization but not stability of Crb requires dynein activity. In the dynein mutant, Crb may be uniformly distributed in the cytoplasm and hence fall below the threshold of detection by immunofluorescence. To address these possibilities, we took advantage of MT-depolymerized although Crb**intra-myc** efficiently localized to the apical domain in the **crb** mutant (100%, n = 66), Crb**intra-myc-wo** showed largely cytoplasmic localization in 64.3% (n = 45) of the **crb** mutants (Fig. 3, O and P). Thus, endogenous wt **crb** product is required, directly or indirectly, for the apical localization of exogenous Crb**intra-myc-wo**, although the reasons for this remain unclear. These data suggest that, in **crb** mutant FCs, Crb**intra-myc-wo** protein, derived from transcripts that do not apically localize, is not effectively localized to the apical domain.

Thus far, we have shown that apical localization of **crb** mRNA contributes to the apical localization of Crb protein. Does this have any functional relevance? Consistent with previous reports that Crb**intra-myc** can rescue **crb** mutant defects in embryonic epithelial cells (Wodarz et al., 1995), apically localized Crb**intra-myc** recruits Sdt to the apical region and fully rescues the polarity defects in **crb** mutant FCs (100%, n = 55; Fig. 3 Q). However, in 60% (n = 20) of **crb** mutant FCs expressing crb**intra-myc-wo** Sdt remains in the cytoplasm and polarity defects are not rescued, which is consistent with the Crb**intra-myc-wo** localization in **crb** mutant FCs (Fig. 3 R). These data strongly indicate that apical localization of the **crb** transcripts is required for effective **crb** function in epithelial polarity. Furthermore, these results suggest that a localized translational machinery near the apical domain may be involved in the generation of full Crb activity on the apical domain.

It has been found that, in embryonic epithelial cells, Crb binds Sdt and the two are mutually dependent for their localization and function (Tepass and Knust, 1993; Bachmann et al., 2001; Hong et al., 2001). Crb protein levels are markedly reduced in the **sdt** mutant and vice versa. Our data show that in dynein mutant FCs, Crb is undetectable by immunofluorescence, whereas Sdt is mainly cytoplasmic (Fig. 4, A–C). There are several possibilities for the inability to detect Crb in the dynein mutant. First, the apical localization and stability of Crb requires dynein activity. In the absence of dynein function, unlocalized Crb is not stable and is degraded. Second, the apical localization but not stability of Crb requires dynein activity. In the dynein mutant, Crb may be uniformly distributed in the cytoplasm and hence fall below the threshold of detection by immunofluorescence. To address these possibilities, we took advantage of MT-depolymerized

![Figure 3. Apical **crb** transcript localization is required for Crb activity.](image-url)
Figure 4. Crb and Sdt interact on the apical cortex. Sdt (red) localizes on the apical cortex in wt FCs (A). Sdt localization in newly induced (B) and aged dynein mutant clones [C]. Patj/Dlt (red) localizes on the apical domain in wt FCs (D) and gradually localizes into the cytoplasm in the dynein mutant (E, a mutant clone shortly after induction; F, an aged mutant clone). Sdt gradually delocalizes in crb mutant cells (G, early clone; H and I, aged clone). Sdt (red) largely colocalizes with Crb\textsuperscript{intra-myc} (green) on the apical domain in wt FCs (J). Both Sdt and Crb\textsuperscript{intra-myc} become cytoplasmic and do not colocalize in colchicine-treated FCs, which mimic dynein mutant FCs (K). Equal protein loadings of total lysates from wt (left lane) and colchicine-treated ovary (right lane) were probed with an anti-Crb antibody (L); loading control was probed with an anti-\textit{\gamma}H\textsubscript{9251} -tubulin antibody (L'). Crb\textsuperscript{intra-myc} and Sdt only form a complex when both localize on the apical cortex (M). Western blot is probed with Sdt. (lane 1) wt (crb\textsuperscript{intra-myc} expressed in wt background) input (10%). (lane 2) Anti-Myc immunoprecipitation from wt sample. (lane 3) Anti-Flag immunoprecipitation (negative control) from wt sample. (lane 4) Anti-Myc immunoprecipitation from a colchicine-treated sample. (lane 5) Anti-Flag immunoprecipitation (control) from a colchicine-treated sample. Bars, 5 \(\mu\)m.

FCs that mimic the effects of dynein mutants (Fig. 2, M–P). Interestingly, although no protein can be detected in these FCs by immunostaining, normal levels of Crb protein are detectable by Western blotting (Fig. 4 L), which is consistent with the notion that the apical localization but not stability of Crb requires dynein activity. Furthermore, in the \textit{crb} mutant, Sdt also displays cytosolic localization (Fig. 4, G–I), suggesting that apical localization but not stability of Sdt protein requires Crb activity.

We next examined whether Crb and Sdt form a complex when in the cytosol. When expressed in wt FCs, Crb\textsuperscript{intra-myc} largely colocalizes with Sdt on the apical domain (Fig. 4 J). In addition, an anti-Myc antibody can bring down Sdt in coimmunoprecipitation
transcripts are translated by specialized subcortical domains. Ist2p is normally secreted via the normal secretory pathway but rather suggests that the localized mRNA at the plasma membrane of daughter but not mother cells. This asymmetrical delivery of Ist2p does not require the apical domain? One precedent comes from the study of the budding yeast membrane protein Ist2p (Juschke et al., 2004). When ectopically expressed in colchicine-treated FCs, Ist2p does not bring down Sdt in Co-IP experiments, which suggests that these proteins do not form a complex when both are cytoplasmic. Similar to an anti-Crb antibody could bring down Sdt in wt FCs but not p25 

Our data suggest that dynein transports both Crb protein and mRNA to the apical domain, where Crb protein interacts with Sdt to form an apical complex. The crb 3' UTR is necessary and sufficient for the apical localization of crb mRNA. The apical localization of crb mRNA appears to be crucial for crb function. crb mRNA produces transcripts and protein that apically localize and can fully rescue the polarity defects associated with crb mutants. In contrast, crb mRNA produces transcripts and proteins that do not fully localize apically and show reduced ability to rescue the polarity defects of crb mutants. Based on these observations, we propose that localized transcripts coupled with a local translation mechanism contribute to the apical localization of Crb and its ability to mediate epithelial polarity.

In general, transmembrane proteins are cotranslationally inserted into the ER and trafficked via the exocytic pathway to the plasma membrane. How might the transmembrane Crb protein be translated and inserted into the plasma membrane near the apical domain? One precedent comes from the study of the budding yeast membrane protein Ist2p (Juschke et al., 2004). Ist2 mRNA is asymmetrically localized to the cortex of daughter cells and a localized transcript is required for the accumulation of Ist2p at the plasma membrane of daughter but not mother cells. This asymmetrical delivery of Ist2p does not require the normal secretory pathway but rather suggests that the localized Ist2 transcripts are translated by specialized subcortical domains of the ER (small daughter cells contain only cortical and not perinuclear ER) and trafficked via a novel pathway linking these specialized ER domains with the plasma membrane. More generally, during vertebrate axon growth, transcripts encoding an Eph2A receptor are locally translated and their protein products can be exported to the cell surface (Brittis et al., 2002). Our observations suggest that transcripts of the transmembrane protein Crb may be locally translated near the apical domain and that this mechanism contributes to epithelial A/B polarity. As the crb 3' UTR is highly conserved through evolution (unpublished data), together with the conserved function of MT-based dynein activity, our results suggest that this directional transport of mRNA plus local translation may be a widely used mechanism to generate epithelial polarity.

Materials and methods

Fly genetics

Stocks were raised on standard cornmeal-agar medium at 25°C. Information about strains used in this study is described in the text or in FlyBase. The following strains are used: y1 w1118, Dhc64C 4-19, Dhc64C 419, Glued, Df(3L)10H, and pDHc64C (a gift of E. Knust, Max Planck Institute of Molecular Biology and Genetics, Dresden, Germany), crb 1L, crb 3L, crb 2L, crb 3L, crb 2L, and crb 3L. Rescue experiments were performed in pDHc64C (+)/+, Dhc64C 4-19/1L flies. Mutant clones were generated by the FLP-FRT technique (Xu and Rubin, 1993). Clones were induced by heat shock during third instar larvae for 2 h on two consecutive days. Adult flies were dissected 3–5 d after eclosion.

Ethyl methanesulfonate (EMS) mutagenesis screen

An FLP-FRT–mediated mosaic screen was performed as follows. hs-FLP; FRT79D-ubi-GFP virgin flies were crossed with EMS-mutagenized males carrying an FRT79D chromosome (Slack et al., 2006). The progeny were grown at room temperature until third instar larvae, which were then subjected to heat shock to induce mitotic clones (Xu and Rubin, 1993). Adult progeny were aged for 4–6 d before dissection and stained with rhodamine phalloidin and DNA dye (To-Pro 3; Invitrogen) to visualize cell morphology. In this screen, two EMS lines (902 and 1L) were recovered that showed cell morphological change and formed multiple layers when mutant clones were generated in the posterior end of follicle, which implies a loss of cell polarity. Both 902 and 1L homozygous progeny died at first instar larvae. Genetic mapping for these two EMS mutations was then performed by using a 3L deficiency kit (Bloomington Drosophila Stock Center). Three deficiency lines including BL3686, BL3640, and BL3640 were mapped to 1L. These lethal mutations were recombined onto an FRT79D chromosome and clones were generated to examine the phenotype in follicular cells. Only Dhc64C 4-19 and Glued showed identical phenotypes observed in 902 and 1L. Furthermore, Df(3L)10H, a small deficiency line that deletes the Dhc64C genomic region, failed to complement 902. The introduction of a copy of transgene Dhc64C 4-19 into 902 not only reverted the lethality of 902 when in transheterozygous to Df(3L)10H but also fully rescued the FC mutant phenotype (Fig. 5). Thus, we conclude that 902 is a new Dhc64C allele and that 1L is a Glued allele; we referred to them as Dhc64C 4-19 and Glued, respectively. To identify the molecular lesion of these mutations, we sequenced the mutant chromosome loci. Homozygous mutant embryos were collected and genomic DNA was extracted according to standard protocol. 2-kb walking of the genomic region with 1-kb overlapping was performed for the mutants and amplified using amplification primers with HiFi Taq polymerase.

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(Invitrogen), and gel was recovered using standard kits (GE Healthcare). The fragments were sequenced using a set of sequencing primers. Sequencing of the Dhc64C^iso2 mutant identified a premature stop codon at Trp^123, causing a truncated product before the DHC_N2 domain and deleting all four ATPase domains. Furthermore, no signaling could be detected using an anti-Dhc64C antibody that recognizes the N-terminal region of Dhc64C polypeptide in immunofluorescent staining, which suggests that this is a protein null allele. There is also a premature stop codon identified in Glu^21 mutant (Glu^122 stop), which lies in the second coiled-coil domain implicated for protein–protein interactions (Fig. S1).

MT drug treatment
To depolymerize the MT cytoskeleton, flies were starved at 25°C for 2.5 h and fed with 200 μg/ml colchicines for 24 h before dissection.

Generation of crb^iso2-iso3 transgene stocks

Embryos after standard transgene protocol to generate crb^iso2-iso3 transgene stock. Three independent lines were selected for further analysis. Hs-flp; AyGal4-uas-lacZ was used to drive the expression of transgenes in FCs. Note added in proof.

Capped fluorescent RNA synthesis and injection

Embryos were injected with 200 ng/μl of labeled RNA. To depolymerize the MT cytoskeleton, 100 μg/ml colchicine (Sigma-Aldrich) was injected 10 min before RNA injection. mAb P1H4 or anti-Myc antibodies were injected 10 min after RNA injection. Injected embryos were imaged in vivo.

Immunocytochemistry

Ovaries were fixed according to standard protocols. Anti-Crb (Cq4, 1:50), mouse anti-Flag (1:2,000; Santa Cruz Biotechnology, Inc.), rabbit anti-anti-Flag (1:1,000; Bio- Rad), rabbit anti-β-tubulin (1:1,000; Sigma-Aldrich), mouse anti-Dhc64C (1:100), and rabbit and mouse anti–α-galactosidase-β (Invitrogen). Fluorescently conjugated goat anti–mouse and rabbit secondary antibodies and rhodamine phalloidin were used (Invitrogen).

Microscopy

Samples were mounted in Vectashield mounting medium (Vector Laboratories). Images were collected using a microscope (Axioplan 2) with an upright confocal system (LSM510 META; both from Carl Zeiss, Inc.) at room temperature. The objective lens was used a Plan NEOFI LAR 40x 1.3 oil and the imaging software used was Zeiss LSM510 (both from Carl Zeiss, Inc.). The confocal images were extracted with LSM510 browser software (Carl Zeiss, Inc.) and then processed in Photoshop 7.0.1 (Adobe). Scale bars are indicated in each individual image.

Immunoprecipitation

Ovaries with corresponding genotype were dissected in S2 culture medium (Invitrogen) and collected in PBS buffer. Protein extract and CoIP experiments were performed according to standard protocols and probed with corresponding antibody (Fig. 4, L and M) and detected with an ECL kit (Thermo Fisher Scientific).

Online supplemental material

Fig. S1 shows molecular lesions of Dhc64C^iso2 and Glu^21 and the rescue of apical Crb localization in the Dhc64C^iso2 mutant by a Dhc64C transgene. Fig. S2 shows phenotypic analyses of p25^iso1/FCs, schematic presentation of the transcripts used in this study, and that Crb forms a complex with Sdt on the apical domain. Fig. S3 shows that apical localization of crb transcripts depends on dynin function in FCs. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200707007/DC1.

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Note added in proof. A complementary study examining the contributions of Dynen and Sdt mRNA localization to A/B polarity regulation has been performed (Home-Badovinac, S., and D. Bilder. 2007. PLoS Genet. doi:10.1371/journal.pgen.0040008).

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