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

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A symmetrical localization of transcripts coupled with localized translation constitutes an important mechanism widely deployed to regulate gene activity in a spatial manner. The conserved transmembrane protein Crumbs (Crb) is an important regulator of epithelial polarity. However, it remains unclear how Crb is targeted to the apical domain. Here, we show that the cytoplasmic dynein complex transports both Crb protein and transcripts to the apical domain of Drosophila melanogaster follicular cells (FCs). The crb 3’ untranslated region (UTR) is necessary and sufficient for the apical 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.

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
showed cytoplasmic localization (100%, Dhc64C 902

FCs are polarized along the A/B axis and form a single layer

for screen and mapping details). In the wild type (wt), the

FC A/B polarity (see Materials and methods and Fig. S1, avail-

abled at http://www.jcb.org/cgi/content/full/jcb.200707007/DC1).

We took advantage of a functional

transport is conserved between embryogenesis and oogenesis

blastoderm embryos, as it has been found that dynein-mediated

transport re-

quires an intact MT cytoskeleton,

survive the notion that the observed polarity defects seen in
dynein mutant and MT-depolymerized FCs are caused by defective dynein-

mediated transport.

The dynein 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 dynein mutant FCs (unpublished data), which is consistent with the notion that the observed polarity defects seen in dynein mutant and MT-depolymerized FCs are caused by defective dynein-mediated transport.

In the wt, crb mRNA is highly enriched on the apical domain. However, in the dynein 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 dynein-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 dynein-mediated transport.

Because of difficulties in studying how dynein transports crb mRNA in FCs, we examined crb mRNA localization in blastoderm embryos, as it has been found that dynein-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 intra-myct (this minigene contains the crb 3' untranslated region [UTR]; see Fig. S2 for a schematic presentation of the transcripts used in this study; Woda et al., 1995). Fluorescently labeled crb intra-myct 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 intra-myct (Dhc64C) and the dynactin component Glued 1L, 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 902 and Glued 1L 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 902 and Glued 1L mutants primarily affect apical localization of the Crb complex without markedly affecting localization of the 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 902 and Glued 1L mutant FCs (100%, n = 245; Fig. 2, A–C), whereas Sdt and Patj/Dict show cytoplasmic localization (100%, n = 156; see Fig. 4, A–F). The adherens junction (Admadiño; 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 902 and Glued 1L) or when p25 (another subunit of dynactin) function was compromised using double-stranded RNA–mediated knockdown (termed p25 RNAi; Fig. S2 and not depicted). Although recent data showed that dynein 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 dynein 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 902 mutant FCs (100%, n > 500; Fig. S1). Dynein and dynactin form a complex that mediates microtubule (MT)-based transport and both Dhc64C 902 and Glued 1L mutants showed virtually identical polarity defects. For simplicity, we show data mainly for Dhc64C 902.

Figure 1. Dynein 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 902 (B) and GluII (C) mutant FCs show altered morphology. wt FCs are monolayered (D), whereas Dhc64C 902 (E) and GluII (F) mutant FCs are multiple layered at the posterior end. Bars, 5 μm.
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<sup>intra-myc-wo</sup>, 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 β-galactosidase gene and found that this chimeric transcript localized apically (100%, n = 10; Fig. 3 H) when injected into the embryo, whereas control β-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<sup>intra-myc</sup> and crb<sup>intra-myc-wo</sup> minigenes and the subcellular localization of these transcripts was determined by RNA in situ. When ectopically expressed in wt FCs, crb<sup>intra-myc</sup> transcripts were predominantly found on the apical domain, whereas crb<sup>intra-myc-wo</sup> 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<sup>intra-myc</sup> transcripts and Crb<sup>intra-myc</sup> protein localized to the apical domain (100%, n > 200; Fig. 3, J and L). Interestingly, in wt FCs expressing crb<sup>intra-myc-wo</sup>, crb<sup>intra-myc-wo</sup> transcripts did not apically localize (Fig. 3 K), yet Crb<sup>intra-myc-wo</sup> 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<sup>intra-myc-wo</sup> 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,

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.

Figure 2. Dynein is required for the apical localization of the Crb complex. Rhodamine phalloidin is shown in red and GFPxls is shown in green. Crb (blue) localizes to the apical domain of wt FCs (A) but is lost from the 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 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
solic 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 intra-myc largely co-localizes with Sdt on the apical domain (Fig. 4 J). In addition, an anti-Myc antibody can bring down Sdt in coimmunoprecipitation 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 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 intra-myc largely co-localizes with Sdt on the apical domain (Fig. 4 J). In addition, an anti-Myc antibody can bring down Sdt in coimmunoprecipitation
(Co-IP) experiments (Fig. 4 M, lane 2). These suggest that in the wt, Crb\textsuperscript{a/myc} forms a complex with Sdt. In the \textit{crb} mutant, however, Crb\textsuperscript{a/myc} apically localized, it localized with Sdt. However, when Crb\textsuperscript{a/myc-wo} was found in the cytosol, it did not localize well with Sdt (unpublished data), which suggests that these two molecules may not form a complex when not localized. To test this possibility, we again took advantage of colchicine-treated FCs. When ectopically expressed in colchicine-treated FCs, Crb\textsuperscript{a/myc} does not bring down Sdt in Co-IP experiments, which suggests that these proteins do not form a complex when both are cytoplasmic (Fig. 4 M). Similarly, an anti-Crb antibody could bring down Sdt in wt FCs but not p25\textsuperscript{RNAI} FCs, confirming that colchicine-treated FCs actually reflect loss of dynein activity and not other MT-based activity (Fig. S2). Collectively, these data suggest that Crb and Sdt form a complex on the apical cortex and that this complex is stabilized, directly or indirectly, by dynein activity.

When expressed in wt FCs (in the presence of wt \textit{crb}), Crb\textsuperscript{a/myc} localizes apically and forms a complex with Sdt. However, in dynein mutant FCs (in the presence of wt \textit{crb}), Crb\textsuperscript{a/myc} is localized to the cytoplasm and does not form a complex with Sdt (Figs. 4 and S2). Our data suggest that in the wt, an endogenous \textit{crb} product is required, directly or indirectly, for the apical localization of exogenous Crb\textsuperscript{a/myc} protein. It is possible that apically localized endogenous Crb could be involved in this process via its requirement for A/B polarity, which in turn directs exogenous Crb\textsuperscript{a/myc} apical localization and subsequently allows the formation of the Crb–Sdt complex. In the dynein mutant, although endogenous Crb is still present (as confirmed by Western blotting), it is not localized apically. As a result, A/B polarity is not properly established. Consequently, exogenous Crb does not form a complex with cytoplasmic Sdt when in the cytosol.

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 \textit{crb} 3’ UTR is necessary and sufficient for the apical localization of \textit{crb} mRNA. The apical localization of \textit{crb} mRNA appears to be crucial for \textit{crb} function. Crb\textsuperscript{a/myc} produces transcripts and protein that apically localize and can fully rescue the polarity defects associated with \textit{crb} mutant FCs. In contrast, Crb\textsuperscript{a/myc-wo} produces transcripts and proteins that do not fully localize apically and show reduced ability to rescue the polarity defects of \textit{crb} mutant FCs. 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 EphA2 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 \textit{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: \textit{y\textsuperscript{w\textasciitilde118}, Dhc64C\textsuperscript{P25}, Dhc64C\textsuperscript{EH}}, \textit{Dhc64C\textsuperscript{902}}, \textit{Dhc64C\textsuperscript{419}}, \textit{Glued\textsuperscript{1}}, \textit{Df(3L)10H}, \textit{p(Dhc64C,ac)\textsuperscript{sdh} (a gift of E. Knust, Max Planck Institute of Molecular Biology and Genetics, Dresden, Germany), crb\textsuperscript{1620}}, \textit{UAS-crbc\textsuperscript{myc-wo}}, \textit{p(Dhc64C,ac)\textsuperscript{sdh} (a gift of E. Knust, Max Planck Institute of Molecular Biology and Genetics, Dresden, Germany)}, \textit{crb\textsuperscript{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.
(Invitrogen), and gel was recovered using standard kits (GE Healthcare). The fragments were sequenced using a set of sequencing primers. Sequencing of the Dhc64Ciso^2 mutant identified a premature stop codon at Trp^1^72 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^124 mutant (Glue124) , 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 cbriso^asmg^ transgene stocks

cbriso^asmg^ was amplified from a cbriso^mouse^ fly and cloned into a pUAST vector. This construct was microinjected into FRT82B-crb^1A22^/TM006 embryos after standard transgene protocol to generate cbriso^mouse^ transgene stock. Three independent lines were selected for further analysis. Hs-flp; AyGal-vas-laZ was used to express the expression of transgenes in FCs.

In situ hybridization

crb template was amplified using Crb-5 (ATTACGCCCAAGGAGACG) and Crb-3 (CTAAATATGGCCCTCTCCGC) primers. Probes were digoxi- genin labeled according to the manufacturer’s instructions (Roche). In situ hybridization was performed as described previously using an HRP-conjugated anti-digoxigenin antibody (Roche) and detected with a fluorescein tyramide signal amplification system (PerkinElmer, Wilkie et al., 1999; Vananzo and Ephrussi, 2002).

Capped RNA synthesis and injection

cbriso^Transgenic^ and cbriso^intra^ were amplified from transgenic flies (Wodarz et al., 1995) using crb-5 (GGGAATTGGGAATTCCCCCCCCCA) and cbriso^Transgenic^ (AGATCTTGACTGCTCTCC). The crb 3’UTR was amplified using CGG- AAATCTTGGATTTTGTTGACAGCCGACG and cbriso^intra^ and cloned into pCS2+. Alexa 543 UTP (Invitrogen) was used to synthesize labeled transcripts. RNAs typically contained 1 fluorochrome per 250 nucleotides for Alexa 543 – Glutathione S-transferase. Alexa 543 – UTP (Invitrogen) was used to synthesize RNAs typically contained 1 fluorochrome per 250 nucleotides for Alexa 543 – UTP (Invitrogen).

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 PHA or anti-Myc antibodies were injected 10 min before RNA injection. Injected embryos were imaged in vivo.

Immunocytochemistry

Ovaries were fixed according to standard protocols. Anti-Crb (Cq4, 1:50), anti-Arm (N2 7A1, 1:50), Developmental Studies Hybridoma Bank, rabbit anti-PKC (1:1,000), mouse anti-Flag (1:1,000; Santa Cruz Biotechnology, Inc.), rabbit anti-Phos/DIL (1:1,000; Bhagat et al., 1999), rabbit anti-Baz (1:1,000; a gift of L. M. Kibler, University of Michigan) and rabbit anti-GFP (1:1,000; Sigma-Aldrich), rabbit anti-Dhc64C (1:1,000; H. J. Bellen, 1999), and rabbit and mouse anti–α-galactosidase (Invitrogen). Fluorescently conjugated goat anti-mouse and rabbit secondary antibodies and rhodamine phallolidin 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 used was a Plan APO/UL 40x 1.3 oil and the imaging software used was Zeiss LSM510 (both from Carl Zeiss, Inc.). The confocal images were exported 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 experi- ments were performed according to standard protocols and probed with corresponding antibody (Fig. 4, I and M) and detected with an ECL kit (Thermo Fisher Scientific).

Online supplemental material

Fig. S1 shows molecular lesions of Dhc64Ciso^2 and Glue^124 and the rescue of apical Crb localization in the Dhc64Ciso^2 mutant by a Dhc64C transgene. Fig. S2 shows phenotypic analyses of p25^TM^ 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 dynein 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 Dynein 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.0040020).

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