The Drosophila homolog of the Exo84 exocyst subunit promotes apical epithelial identity

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Summary
The polarized architecture of epithelial tissues involves a dynamic balance between apical and basolateral membrane domains. Here we show that epithelial polarity in the Drosophila embryo requires the exocyst complex subunit homolog Exo84. Exo84 activity is essential for the apical localization of the Crumbs transmembrane protein, a key determinant of epithelial apical identity. Adherens junction proteins become mislocalized at the cell surface in Exo84 mutants in a pattern characteristic of defects in apical, but not basolateral, components. Loss of Crumbs from the cell surface precedes the disruption of Bazooka and Armadillo localization in Exo84 mutants. Moreover, Exo84 mutants display defects in apical cuticle secretion that are similar to crumbs mutants and are suppressed by a reduction in the basolateral proteins Dlg and Lgl. In Exo84 mutants at advanced stages of epithelial degeneration, apical and adherens junction proteins accumulate in an expanded recycling endosome compartment. These results suggest that epithelial polarity in the Drosophila embryo is actively maintained by exocyst-dependent apical localization of the Crumbs transmembrane protein.

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Introduction
Epithelial cells in the Drosophila embryo generate molecularly distinct apical and basolateral surfaces that provide structural integrity to the developing embryo. Specialized cell surface domains are separated by intercellular adherens junctions that initiate as diffuse apicolateral accumulations and subsequently coalesce to form a discrete apical band called the zonula adherens (Tepass and Hartenstein, 1994). The spatial organization of mature adherens junctions is actively maintained by input from both apical and basolateral proteins (Tepass et al., 2001; Knust and Bossinger, 2002; Muller, 2003; Bilder, 2004). The Crumbs EGF-repeat transmembrane protein and its cytoplasmic binding partners Stardust and dPATJ localize to the apical cell surface and are required for epithelial structure and adherens junction morphology (Tepass et al., 1990; Grawe et al., 1996; Tepass, 1996; Bachmann et al., 2001; Nam and Choi, 2006). In addition, overexpression of Crumbs leads to a selective expansion of the apical cell surface, demonstrating that Crumbs is necessary and sufficient for apical identity (Wodarz et al., 1995; Pelliikka et al., 2002). The localization of mature adherens junctions also requires the basolateral PDZ-domain proteins Discs large (Dlg) and Scribble (Scrib) and the WD40-domain protein Lethal giant larvae (Lgl) (Woods et al., 1997; Bilder and Perrimon, 2000; Bilder et al., 2000). Epithelial defects caused by disruption of apical Crumbs activity can be rescued by a simultaneous reduction in the activity of basolateral proteins, indicating that apical and basolateral domains function in opposition to maintain epithelial polarity (Bilder et al., 2003; Tanentzapf and Tepass, 2003).

Misregulation of Crumbs activity can have severe effects on cell and tissue function and is associated with human retinal diseases (Wodarz et al., 1995; den Hollander et al., 1999; Lu and Bilder, 2005; Laprise et al., 2006). Multiple mechanisms contribute to Crumbs localization, stability and activity to precisely control its function. The basolateral proteins Dlg, Lgl and Scrib oppose Crumbs activity and restrict its localization in the Drosophila embryo (Bilder et al., 2003; Tanentzapf and Tepass, 2003), and the Yurt FERM-domain protein associates with the Crumbs cytoplasmic domain and negatively regulates Crumbs activity at the apicolateral cell surface (Laprise et al., 2006). Endocytosis of Crumbs protein is also required for tissue morphology, as mutations in the Avalanche syntaxin or the Rab5 GTPase lead to Crumbs accumulation and wing imaginal disc overgrowth (Lu and Bilder, 2005). In addition, a complex containing the Rich1 Cdc42 GAP protein and the angiomotin scaffolding protein associates with cytoplasmic binding partners of Crumbs and provides a potential link between the Crumbs complex and the endocytic machinery (Wells et al., 2006). However, the mechanisms that govern the delivery of Crumbs protein to the cell surface are not known.

The targeting of transmembrane proteins to specific destinations at the cell surface is a widely used mechanism for establishing cell polarity (Hsu et al., 1999; Mostov et al., 2003; Schuck and Simons, 2004; Rodriguez-Boulan et al., 2005). The spatial specificity of vesicle trafficking is thought to occur at a late step in this process through the tethering of exocytic vesicles at defined membrane sites by the eight-subunit exocyst (or Sec6/8) complex (Lipschutz and Mostov, 2002; Whyte and
Exocyst components were originally identified based on their role in polarized secretion in *Saccharomyces cerevisiae* (Novick et al., 1980) and were subsequently shown to form a complex that is highly conserved from yeast to mammals (TerBush and Novick, 1995; TerBush et al., 1996; Kee et al., 1997; Grindstaff et al., 1998; Guo et al., 1999; Hsu et al., 1999). In multicellular organisms, exocyst components are required for multiple developmental processes including epithelial polarity (Grindstaff et al., 1998; Yeaman et al., 2001; Langevin et al., 2005), membrane integrity (Murthy and Schwarz, 2004; Beronja et al., 2005; Murthy et al., 2005), photoreceptor morphogenesis (Beronja et al., 2005), cell fate determination (Jafar-Nejad et al., 2005) and synapse formation (Mehta et al., 2005). These diverse functions demonstrate that polarized exocytosis is a fundamental mechanism for regulating cell morphology.

Here we provide evidence that the *Drosophila* homolog of the Exo84 exocyst complex subunit is essential for epithelial polarity and apical protein localization in the *Drosophila* embryo. In *Exo84* mutants, adherens junction proteins become mislocalized along the apical-basal axis in a manner reminiscent of cells lacking the Crumbs apical determinant. Loss of Crumbs from the apical surface is the earliest defect detected in *Exo84* mutants. *Exo84* mutants at advanced stages of epithelial degeneration display defects in trafficking apical and junctional proteins from the recycling endosome to the cell surface. These results demonstrate that the *Drosophila* homolog of the exocyst complex subunit Exo84 plays an essential role in epithelial polarity by regulating the localization of the Crumbs apical determinant.

**Results**

**Disrupted epithelial organization in onion rings mutant embryos**

The *onion rings*142-5 (*onr*) mutation was identified in a screen for male sterile mutants but generates viable and fertile females (Giansanti et al., 2004). When *onr*142-5 hemizygous females were mated to heterozygous males, 61% of the resulting embryos failed to hatch (*n*=200). To determine the cellular basis of this embryonic lethality, we examined embryos that were maternally and zygotically mutant for *onr*142-5 (referred to as *onr* mutant embryos). In wild-type embryos, epithelial cells are elongated along the apical-basal axis and organized into a columnar monolayer (Fig. 1A). In *onr* mutants, epithelial structure was established correctly but degenerated by stage 10, when epithelial cells displayed a rounded morphology and a multilayered organization (Fig. 1B,C). These results suggest that wild-type *onr* function is required for the maintenance of epithelial structure in the *Drosophila* embryo.

To determine whether a disruption of intercellular adherens junctions accompanies the epithelial defects in *onr* mutant embryos, we analyzed the distribution of the core adherens junction proteins DE-cadherin and Armadillo/β-catenin. In wild-type embryos, DE-cadherin and Armadillo localize to the apical margin of lateral cell interfaces, whereas Neurotactin and filamentous actin localize to basolateral surfaces (Fig. 1D,G). In stage 10 *onr* mutants, Armadillo and DE-cadherin failed to accumulate apically and were instead present in prominent aggregates at random locations along the apical-basal axis (Fig. 1E,H; 56% of *onr* embryos displayed aberrant Armadillo localization, *n*=50). In z-projections of optical

![Fig. 1](image-url). Disruption of epithelial structure and adherens junctions in onion rings mutant embryos. (A-C) Neurotactin localizes to basolateral cell surfaces in wild-type (A) and *onr* mutant embryos (B,C). At stage 6, *onr* mutants establish columnar epithelial morphology normally (B), whereas stage 10 *onr* mutants display a severe epithelial disruption (C) compared to the wild type (A). (D-I) At stage 10, Armadillo (green D-F) and DE-cadherin (green G-I) localize apically in the wild type (D,G) and in an *onr* mutant carrying the P[Exo84] transgene (I). *onr* mutants accumulate Armadillo (E,F) and DE-cadherin (H) at various positions along the apical-basal axis. Neurotactin (red D-F) and filamentous actin (F-actin red G-I) are enriched at basolateral surfaces in wild-type and *onr* mutant embryos. (F) A 5 μm projection of multiple optical sections stained for Armadillo (green), superimposed on a single 1 μm slice of the cell outline marker Neurotactin (red). Anterior, left; ventral, up. Bar, 20 μm.
sections encompassing a depth of one cell diameter (5 μm), each cell appeared to associate with approximately one large junctional aggregate (Fig. 1F). By contrast, Neurotactin and filamentous actin were correctly localized to basolateral surfaces (Fig. 1E,H). These results demonstrate that the apical localization of adherens junction proteins is disrupted in onr mutant embryos.

onr encodes the Drosophila homolog of the Exo84 exocyst complex subunit

The onr142-5 mutation was mapped to the 96F;97A interval on chromosome III in the region of the Exo84 gene (Giansanti et al., 2004). Exo84 encodes a protein with 27% identity to human and mouse Exo84 proteins and 18% identity to the S. cerevisiae Exo84 protein (supplementary material Fig. S1). Two lines of evidence indicate that onr142-5 is an allele of Exo84. First, a 4.5 kb genomic transgene containing the predicted Exo84 coding region, 1.5 kb of upstream promoter sequence, and 1 kb of downstream sequence fully rescued epithelial morphology in onr mutant embryos (Fig. 1I; 100% of Stage 10 progeny from onr hemizygous females bearing one copy of the rescuing transgene crossed to onr/+ males displayed wild-type epithelial morphology and DE-cadherin localization, n=56; compared with 44% without the transgene, n=50). The Exo84 genomic transgene also conferred partial rescue when supplied zygotically (82% of progeny from onr hemizygous females crossed to onr/+ males bearing the genomic transgene displayed wild-type epithelial morphology and DE-cadherin localization, n=50). Zygotic expression of the Exo84 genomic transgene allowed onr mutants to survive to adulthood (43% of adult progeny from onr hemizygous females crossed to onr/+ males bearing the genomic transgene were onr mutant, n=104). Moreover, a C→T mutation in the onr142-5 allele introduces a stop codon in the Exo84 coding region that is predicted to generate a truncated protein containing 581 of 672 amino acids. Although null mutations in other exocyst complex subunits cause developmental arrest in oogenesis (Murthy and Schwarz, 2004; Beronja et al., 2005; Murthy et al., 2005), the production of eggs in onr142-5 mutants may be due to hypomorphic onr function. Consistent with this possibility, the male sterile phenotypes of onr142-5 hemizygotes were more severe than onr142-5 homozygotes (data not shown). These results provide strong evidence that onr142-5 represents a mutation in the Exo84 gene, which we refer to as Exo84onr.

Distinct patterns of adherens junction localization in mutants defective for apical or basolateral proteins

To determine whether the mislocalization of adherens junction proteins in Exo84 mutant embryos results indirectly from a disruption of apical-basal polarity, we compared the
distribution of junctional proteins in Exo84 embryos with mutants defective for apical (crumbs) or basolateral (dlg and lgl) components. The DE-cadherin and Armadillo adherens junction proteins localize to the apical margin of contacting cell surfaces in wild-type embryos (Fig. 2A,B). In Exo84 and crumbs mutants, these proteins accumulated in isolated puncta at various locations along the basolateral membrane (Fig. 2C-F). Adherens junction proteins were correctly delivered to the plasma membrane during early embryonic stages in Exo84 and crumbs mutants, but subsequently became mislocalized at the cell surface (Fig. 2C-F). By contrast, in embryos maternally and zygotically defective for the basolateral Dlg or Lgl proteins, DE-cadherin and Armadillo were diffusely localized throughout the basolateral surface (Fig. 2G-J). The similar DE-cadherin and Armadillo distributions in Exo84 and crumbs mutants are consistent with a functional relationship between Exo84 and the Crumbs apical determinant.

Apical proteins are mislocalized before the disruption of adherens junctions in Exo84 mutants

The epithelial defects in Exo84 and crumbs mutant embryos suggest that Exo84 may contribute to specification of the epithelial apical domain. Consistent with this possibility, we found that apical protein localization is selectively disrupted in Exo84 mutants. In wild-type embryos, Bazooka, Crumbs, atypical protein kinase C (aPKC) and dPATJ localize to the apical margins of lateral cell surfaces (Fig. 3A-D) (Tepass, 1996; Bhat et al., 1999; Wodarz et al., 1999; Wodarz et al.,

![Image of localization of apical proteins in Exo84 mutant embryos](image.png)
2000), in the vicinity of adherens junctions by confocal microscopy (Fig. 3K). By contrast, in Exo84 mutants, Bazooka, Crumbs, aPKC and dPATJ accumulated in large aggregates at ectopic locations along the apical-basal axis (Fig. 3E-H). Apical protein aggregates in Exo84 and crumbs mutants also frequently contained the junctional protein Armadillo (Fig. 3L,O,P). By contrast, Discs large often localized correctly to lateral surfaces in Exo84 mutant embryos, despite Bazooka mislocalization and the moderate loss of columnar structure in these embryos (Fig. 3I).

We found that Crumbs mislocalization is an early step in the loss of epithelial polarity in Exo84 mutants, before the disruption of adherens junction localization. In stage 9 Exo84 mutant embryos at an early stage of epithelial breakdown, cells with intact apical domains containing Crumbs, Bazooka, and Armadillo were juxtaposed with areas of disrupted epithelial morphology (Fig. 4B). Loss of Crumbs from the apical surface was observed in cells that retained apical Bazooka and Armadillo localization (Fig. 4B). In other areas of the epithelial layer, cells were identified that were depleted for Crumbs and Bazooka but maintained apicolateral Armadillo localization.

Cells with apical Crumbs protein in the absence of Bazooka or Armadillo were not observed. By contrast, disruption of adherens junctions in armadillo or shotgun/DE-cadherin mutant embryos produced a distinct phenotype in which cells were either wild type in appearance or simultaneously defective for the localization of all three proteins (Fig. 4C,D). dPATJ mislocalization did not obviously precede loss of Crumbs in our observations (data not shown). These results indicate that junctional defects in Exo84 mutants first become evident after the loss of Crumbs from the cell surface and may initially occur as a consequence of Crumbs mislocalization.

**Exo84 promotes apical identity and opposes basolateral determination by Dlg and Lgl**

Epithelial organization and apical-basal polarity in the *Drosophila* embryo are actively maintained by a mutual antagonism between apical and basolateral determinants (Bilder et al., 2003; Tanentzapf and Tepass, 2003). When the activity of apical proteins is defective, a compensatory reduction in basolateral protein activity can partially restore epithelial structure. We found that Exo84 mutant embryos

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**Fig. 4.** Crumbs mislocalization precedes the loss of Bazooka and Armadillo in Exo84 mutants. (A-D) Crumbs (green), Bazooka (blue) and Armadillo (red) in stage 9 wild-type (A), Exo84 (B), arm (C) and shg (D) mutant embryos. (A) In the wild type, Crumbs, Bazooka and Armadillo localize apically. (B) At the onset of epithelial disruption in Exo84 mutants, Armadillo localizes apically whereas Crumbs is absent from large areas. The degree of apical Bazooka localization is intermediate. (C,D) In arm and shg mutants, Crumbs and Bazooka are absent in regions lacking Armadillo. Anterior, left; ventral, down. Bar, 10 μm.
displayed a temperature-sensitive defect in apical cuticle formation – an established assay for embryonic epithelial formation. *Exo84* mutants exhibited strong defects at 25°C (79% of embryos displayed moderate or severely disrupted cuticles, Fig. 5B,J) and a milder phenotype at 20°C (81% of embryos displayed a largely intact cuticle, Fig. 5F,J). Reducing the dosage of the exocyst complex subunits *sec5* and *sec6* enhanced the cuticle defects of *Exo84* mutant embryos raised at 20°C, resulting in a substantial reduction in the amount of cuticle formed (Fig. 5J). Genetic interactions often indicate that two genes affect a common process, suggesting that Exo84 may function with other subunits of the exocyst complex in the embryonic epithelium. Moreover, a weak *crumbs* allele significantly enhanced the mild phenotypes of *Exo84* mutant embryos raised at 20°C, and embryos simultaneously mutant for *Exo84* and a strong *crumbs* allele displayed defects similar to *crumbs* single mutants (Fig. 5J). By contrast, reducing *dlg* and *lgl* function partially suppressed cuticle defects in *Exo84* embryos at 25°C (Fig. 5J). Consistent with these results, Crumbs overexpression in moderately affected *Exo84* embryos at 22°C increased the fraction of wild-type cuticles, whereas Lgl overexpression enhanced the *Exo84* mutant defects (Fig. 5J). However, Crumbs overexpression was less effective in rescuing *Exo84* phenotypes at 25°C (Fig. 5J), indicating that a low level of *Exo84* activity is required for Crumbs function. These results indicate that Exo84 promotes apical epithelial identity in the *Drosophila* embryo in a process that requires the Crumbs apical determinant and is opposed by the basolateral Dlg and Lgl proteins.

**Accumulation of intermediate vesicular compartments in *Exo84* mutants**

The exocyst complex is implicated in the delivery of vesicles to the plasma membrane from the recycling endosome and Golgi compartments (Grindstaff et al., 1998; Beronja et al., 2005; Langevin et al., 2005), and mutations in exocyst proteins have been shown to disrupt recycling endosome morphology (Jafar-Nejad et al., 2005; Langevin et al., 2005). To investigate whether the vesicular trafficking machinery is disrupted in *Exo84* mutants, we examined the distribution of Golgi, early endosomal and late endosomal compartments. The Golgi protein Lava lamp was present in vesicles throughout the cytoplasm in wild-type (Fig. 6B) and *Exo84* mutant embryos (Fig. 6C), suggesting that Golgi structure is unaffected. However, we found that the localization of the recycling endosome protein Rab11 was substantially disrupted in *Exo84* mutants. In wild-type embryos, Rab11 was present in numerous small puncta, predominantly enriched in the apical cytoplasm (Fig. 6D). In *Exo84* mutants, Rab11-positive vesicles were aberrantly distributed in large irregularly shaped aggregates that appeared to consist of multiple small vesicles (Fig. 6E). These aggregates are not likely to represent a fusion of multiple endosomal compartments, because early endosomes visualized with Rab5-GFP and late endosomes labeled with antibodies to Hrs were maintained as separate compartments with distinct morphologies (Fig. 6H,J). Both early and late endosomes showed a tendency to form small aggregates in *Exo84* mutant embryos, with Rab5 maintaining a more diffuse distribution than Hrs (Fig. 6H,J).

The mislocalization of Rab11 in *Exo84* mutants is consistent with a requirement for *Exo84* in vesicle trafficking from recycling endosomes to the plasma membrane. In particular, loss of the transmembrane Crumbs protein from the apical cell surface in *Exo84* mutant embryos suggests a specific role for *Exo84* in trafficking Crumbs or a protein required for Crumbs localization. Consistent with this possibility, in severely affected *Exo84* mutant embryos at late stage 10, Crumbs was detected in large aggregates in basolateral locations within the cell (Fig. 7C'). Bazooka and Armadillo colocalized with Crumbs in these aggregates (Fig. 7C'',C'''), which were also positive for DE-cadherin (Fig. 7D). By contrast, the basolateral protein Dlg did not localize to cytoplasmic aggregates in *Exo84* mutants (data not shown). In *shotgun*; *Exo84* double mutant embryos in which DE-cadherin and Armadillo proteins were nearly undetectable, cytoplasmic aggregates of Crumbs and Bazooka were still present (Fig. 7K,M). These results suggest that the mislocalization of apical proteins in *Exo84* mutant embryos is independent of junctional protein localization. Crumbs, Bazooka and Armadillo colocalized with aggregates of Rab11-positive recycling endosomes (Fig. 7E,I), indicating that mislocalized proteins in *Exo84* mutants are present in a Rab11-positive vesicular compartment. Therefore, in contrast to the relatively specific mislocalization of Crumbs in *Exo84* mutant embryos at stage 9, by late stage 10 *Exo84* mutants display defects in the delivery of apical and adherens junction proteins to the cell surface.

In contrast to the severe defects in recycling endosome morphology in *Exo84* mutant embryos, recycling endosomes in *crumbs* mutants were wild type in appearance and did not aggregate near Bazooka puncta at the plasma membrane (Fig. 6F). These results suggest that the early, surface-associated accumulations of junctional proteins in *Exo84* and *crumbs* mutants may be qualitatively distinct from the later, cytoplasmic aggregates that are specific to *Exo84* and colocalize with the recycling endosome. In *Exo84* mutants at early stages of degeneration, junctional proteins may become mislocalized at the surface of the cell as a consequence of disrupted Crumbs localization. However, the aggregation of junctional proteins in the recycling endosome in later-stage *Exo84* mutant embryos is not recapitulated in *crumbs* mutants. These results suggest that the defects in recycling endosome morphology in *Exo84* mutants do not represent a secondary consequence of the disruption of Crumbs localization or defects in apical-basal polarity.

**Discussion**

The multiprotein exocyst complex plays a conserved role in the delivery of subcellular vesicles and their transmembrane cargo proteins to precise locations at the surface of polarized cells (Hsu et al., 1999; Lipschutz and Mostov, 2002; Whyte and Munro, 2002; Rodriguez-Boulan et al., 2004). Here we demonstrate that epithelial polarity in the *Drosophila* embryo is actively maintained by the Exo84-dependent localization of the Crumbs transmembrane protein to the apical surface. *Exo84* mutants display an aberrant distribution of junctional proteins that resembles the phenotype of *crumbs* mutants, and depletion of Crumbs from the apical surface is the earliest defect detected in *Exo84* mutants. In addition, the onset of epithelial disruption at stage 9 in *Exo84* mutants is comparable with the timing of the *crumbs* mutant defects, and the Crumbs protein still aggregates in *Exo84* embryos with greatly reduced E-cadherin. *Exo84* is likely to function as part of the exocyst
Fig. 5. Exo84 genetically interacts with apical and basolateral components. (A-F) Wild-type cuticle at the end of embryogenesis (A). In Exo84 at 25°C (B) and crumbs (C) small scraps of cuticle form, whereas dlg (D) and lgl (E) produce a continuous, malformed cuticle. Exo84 mutants at 20°C exhibit a weak defect in cuticle formation resulting in small ventral holes (F). (G-I) In strongly defective embryos, little cuticle is present (G). In moderately defective embryos, defects range from large ventral holes (H) to embryos with patches of cuticle. A weak classification indicates small ventral holes (I). (J) Cuticle defects in combinations of Exo84 with mutations in epithelial polarity genes. Exo84 embryos at 20°C that carry mutations in crumbs, sec5 or sec6 exhibit a stronger defect in cuticle integrity than Exo84 alone. By contrast, a decrease in the dosage of the basolateral determinants dlg or lgl partially restored cuticle formation in Exo84 mutants at 25°C. Overexpression of crumbs in Exo84 embryos allowed partial cuticle formation, whereas overexpression of lgl in Exo84 enhanced the cuticular defects. Defects in Exo84 mutants at 25°C were not suppressed by crumbs overexpression. For mutant combinations of Exo84 and crumbs, all Exo84 mutant embryos received half the maternal dosage of crumbs and one half were predicted to be homozygous for crumbs. For mutant combinations of Exo84 and sec5, sec6, dlg or lgl, all embryos received half the maternal dosage of sec5, sec6, lgl or dlg and one quarter were predicted to be homozygous for sec5, sec6, lgl or dlg. For experiments in which crumbs or lgl were overexpressed in an Exo84 background, one quarter of the embryos were predicted to receive both the Gal4 driver and the UAS transgene (see Materials and Methods). Percentages represent the fraction of embryos that did not hatch. Anterior, left; ventral, down. Bar, 100 μm.
Fig. 6. The recycling endosome compartment is disrupted in \textit{Exo84} mutants. (A) Schematic of results depicting the distribution of Golgi (Lva), early endosome (Rab5), late endosome (Hrs) and recycling endosome (Rab11) compartments in cross section in wild-type and \textit{Exo84} mutant embryos. (B-J) Localization of Lva (red B,C), Rab11 (green D-F), Rab5-GFP (green G,H), Hrs (green I,J), Armadillo (green B,C) and Bazooka (blue B,C,G-J, red D-F) in stage 10 wild-type (B,D,G,I), \textit{Exo84} (C,E,H,J) and \textit{crumbs} mutant (F) embryos. (B,C) The Golgi compartment (Lva, red) appears normal in size and distribution in the wild type (B) and \textit{Exo84} mutant (C). (D-F) Recycling endosomes (Rab11, green) are diffusely distributed in the apical cytoplasm in wild type (D), but form large aggregates in \textit{Exo84} mutants (E). Despite disruption of epithelial polarity and Bazooka localization (red) in \textit{crumbs} mutant (F), recycling endosomes are comparable in appearance to the wild type (D). (G-J) Early endosomes (Rab5-GFP, green G,H) and late endosomes (Hrs, green I,J) are distinct compartments from recycling endosomes (Rab11, red) in the wild type (G,I) and \textit{Exo84} mutant (H,J). Bazooka aggregates (blue) colocalize with recycling endosome aggregates. Anterior, left; ventral, down. Bar, 10 \textmu m (B); 5 \textmu m (G,I).
Epithelial polarity requires Exo84 complex in the *Drosophila* embryo, in light of the genetic interactions we observe between Exo84 and the Sec5 and Sec6 exocyst subunits and the common defects in recycling endosome morphology caused by exocyst disruption in multiple cellular contexts (Jafar-Nejad et al., 2005; Langevin et al., 2005). These results suggest a role for exocyst-dependent membrane trafficking in the maintenance of apical epithelial identity in the *Drosophila* embryo.

In contrast to the relatively specific mislocalization of Crumbs in stage 9 Exo84 mutant embryos, by late stage 10 these embryos display defects in the delivery of multiple proteins to the cell surface. Epithelial polarity and the distribution of apical and junctional proteins are established correctly in Exo84 mutants (Fig. 8A), either because these processes occur independently of Exo84 or because of residual Exo84 activity in this mutant background. The earliest defect observed in Exo84 mutants is a loss of Crumbs from the apical surface during epithelial maturation (Fig. 8B). As a likely consequence of the loss of cell-surface Crumbs localization, adherens junction proteins become mislocalized to varying positions along the basolateral cell membrane (Fig. 8B). Mutant embryos at later stages display a cytoplasmic accumulation of apical and adherens junction proteins in an expanded Rab11 recycling endosome compartment (Fig. 8C),

Fig. 7. Aggregation of polarity proteins in Exo84 mutant embryos. (A) Schematic depicting the distribution of Crumbs (Crb), Bazooka (Baz), Armadillo (Arm), and Rab11 in cross section in wild-type and Exo84 mutant embryos. In Exo84 mutants, these proteins colocalize in large aggregates. (B,C) Stage 10 Exo84 mutant embryos stained for Crumbs (green), Armadillo (red) and Bazooka (blue). In severely disrupted cells in which Crumbs, Armadillo and Bazooka proteins are absent from the apical surface (panel B, lower right), ectopic aggregates of these proteins occur basolaterally (C, shown 2 µm below B). Note that cells adjacent to the affected region lack apical Crumbs but maintain junctional Armadillo (B). (D) Aggregation of DE-cadherin (green) and Bazooka (red) in Exo84 mutant embryos, shown 4 µm below the apical surface. (E) Colocalization of Crumbs (red) with recycling endosome aggregates (Rab11, green) in Exo84 embryos. (F-I) An Exo84 embryo imaged at three positions along the apical-basal axis. In regions where Bazooka (blue) and Armadillo (red) are absent from the apical surface (F), large accumulations of Bazooka and Armadillo colocalized with recycling endosomes in z-planes 2 µm (G) and 4 µm (H) below the apical plane in (F). (I) An enlarged view of the boxed area in G. (J-M) In the wild type (J,L) and shg;Exo84 (K,M), aggregation of Crumbs (red L,M) and Bazooka (blue) occurs despite strongly reduced levels of Armadillo (green) and E-cadherin (red J,K). Anterior, left; ventral, down. Bars, 10 µm.
consistent with a defect in vesicular transport to the cell surface. The failure to deliver junctional proteins to the cell surface is unlikely to result from a defect in Crumbs localization, because the cytoplasmic accumulation of junctional proteins does not occur in crumbs mutants. These results indicate that disruption of exocyst-dependent membrane trafficking ultimately results in the failure to deliver both apical and junctional proteins from the recycling endosome to the cell surface. The mislocalization of apical and junctional proteins in Exo84 mutant embryos is associated with a loss of columnar morphology, demonstrating that Exo84 activity is essential for epithelial organization.

**Apical and basolateral proteins direct adherens junction localization through different mechanisms**

A precise balance between apical and basolateral determination is essential for epithelial integrity and the placement of the zonula adherens in the Drosophila embryo (Bilder et al., 2003; Tanentzapf and Tepass, 2003). Here we demonstrate that this balance is actively maintained by Exo84-dependent localization of the Crumbs transmembrane protein to the apical cell surface. Loss of apical or basolateral identity leads to distinct patterns of junctional protein distribution, suggesting that the apical and basal limits of the zonula adherens are defined by different mechanisms. In crumbs mutants, DE-cadherin and Armadillo are restricted to focused puncta at varying locations at the cell surface. By contrast, in embryos defective for the basolateral proteins Dlg and Lgl, junctional proteins are dispersed along the plasma membrane rather than aggregating at a single site (Bilder et al., 2000) (this study). A basolateral expansion of the apical Crumbs domain has also been reported in dlg and lgl mutants (Bilder et al., 2000; Tanentzapf and Tepass, 2003). These results suggest that basolateral proteins create a nonpermissive barrier to adherens junction expansion, whereas apical proteins may play a positive role in recruiting or stabilizing junctions at the apical cell surface. Consistent with this possibility, the apical Crumbs domain is closely apposed to the zonula adherens (Tepass, 1996), and we found that Bazooka and Armadillo colocalize at the cell surface and in the cytoplasm of Exo84 mutant embryos. Exocyst-dependent trafficking of Crumbs to the apical surface may reinforce the apical epithelial domain and stabilize the apicolateral localization of the zonula adherens.

**Exo84 is required for membrane trafficking from the recycling endosome to the cell surface**

We show here that the recycling endosome is the primary vesicular compartment affected in embryos mutant for the exocyst subunit homolog Exo84, while Golgi, early endosomal and late endosomal compartments remain largely intact. Exocyst proteins are required for recycling endosome morphology in several epithelial and sensory cell types (Jafar-Nejad et al., 2005; Langevin et al., 2005) and the Rab11 recycling endosome protein can associate directly with the exocyst subunits Sec5 and Sec15 (Zhang et al., 2004; Beronja et al., 2005; Jafar-Nejad et al., 2005; Langevin et al., 2005; Wu et al., 2005). We found that Rab11 vesicles in maturing embryonic epithelia are enriched in the apical cytoplasm, where they preferentially accumulate in the plane of the adherens junctions. Conversely, a basal expansion of recycling endosomes during cellularization correlates with a basal bias in membrane addition (Lecuit and Wieschaus, 2000; Pelissier et al., 2003). These results suggest that there is a spatial correlation between the sites of recycling endosome accumulation and the surface destinations of proteins trafficked through recycling endosomes. The redistribution of the recycling endosome compartment to the apical cytoplasm accompanies the transition from basolateral to apical membrane insertion and may reflect the onset of a critical requirement for Crumbs activity during epithelial maturation.

**The exocyst is required for distinct properties of epithelial organization**

The requirement for Exo84 in apical protein localization in the Drosophila embryo is distinct from exocyst functions in other epithelia, in which exocyst components are required for the localization of basolateral or junctional proteins (Grindstaff et al., 1998; Yeaman et al., 2001; Langevin et al., 2005). Our results indicate that Exo84 is also required for delivery of DE-cadherin to the cell surface in the embryo, consistent with the demonstrated roles for Sec5, Sec6 and Sec15 in DE-cadherin trafficking in the pupal epithelium (Langevin et al., 2005). However, although the mislocalization of the apical Crumbs protein is a primary defect of Exo84 mutant embryos, exocyst mutations do not appreciably affect Crumbs localization in pupal epithelial and photoreceptor cells (Beronja et al., 2005; Langevin et al., 2005). These results are consistent with a model in which distinct cargo proteins are trafficked by the
Epithelial polarity requires Exo84

Agoled embryos (~48 hours after egg laying) were dechorionated in 50% bleach, mounted in Hoyer’s lactate acid and baked overnight at 60°C. Phase-contrast images were obtained on a Zeiss AxioImager microscope with a PlanApo 10×/0.3NA Plan objective and an AxioCam MRc camera using AxioVision software.

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