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

J. Todd Blankenship[1],*, Margaret T. Fuller[2] and Jennifer A. Zallen[1]

[1]Developmental Biology Program, Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021, USA
[2]Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA
*Author for correspondence (e-mail: blankenj@mskcc.org)

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.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/120/17/3099/DC1

Key words: Epithelial polarity, Adherens junctions, Exocyst complex, Membrane trafficking, Apical-basal polarity

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; Pellikka 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

Munro, 2002). 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
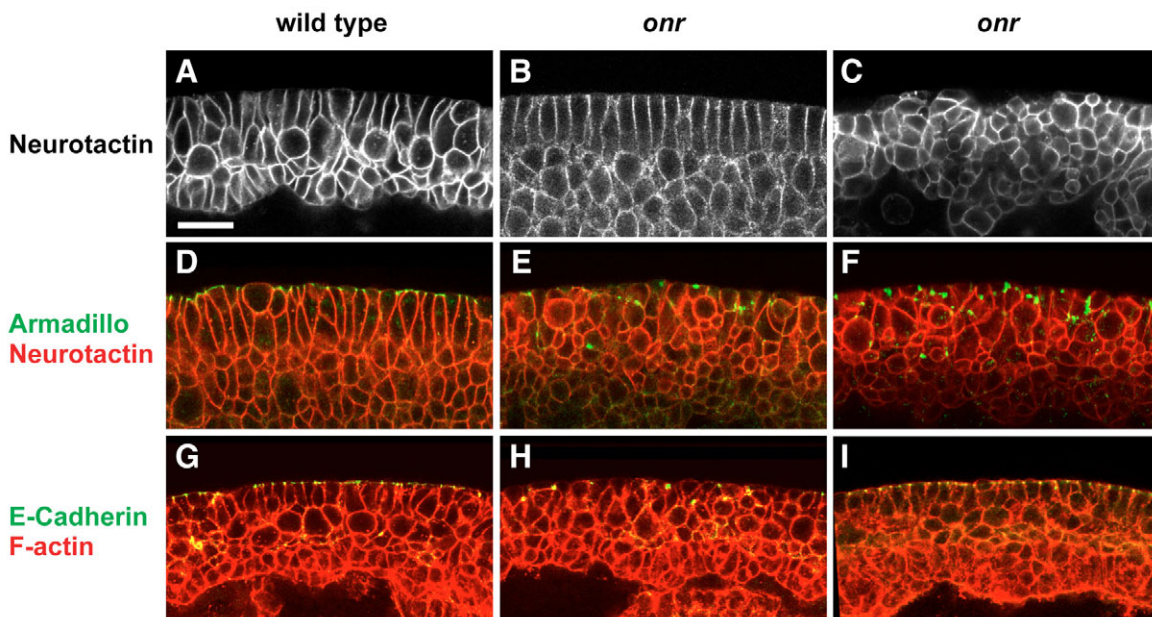

Fig. 1. Disruption of epithelial structure and adherens junctions in *onion rings* mutant embryos. (A-C) Neurotactin localizes to basolateral cell surfaces in wild-type (A) and *onr* mutant embryos (B,C). At stage 6, *onr* mutants establish columnar epithelial morphology normally (B), whereas stage 10 *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 *onr*[142-5] 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 *onr*[142-5] 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 *onr*[142-5] 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 *onr*[142-5] mutants may be due to hypomorphic *onr* function. Consistent with this possibility, the male sterile phenotypes of *onr*[142-5] hemizygotes were more severe than *onr*[142-5] homozygotes (data not shown). These results provide strong evidence that *onr*[142-5] represents a mutation in the *Exo84* gene, which we refer to as *Exo84*[onr].

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
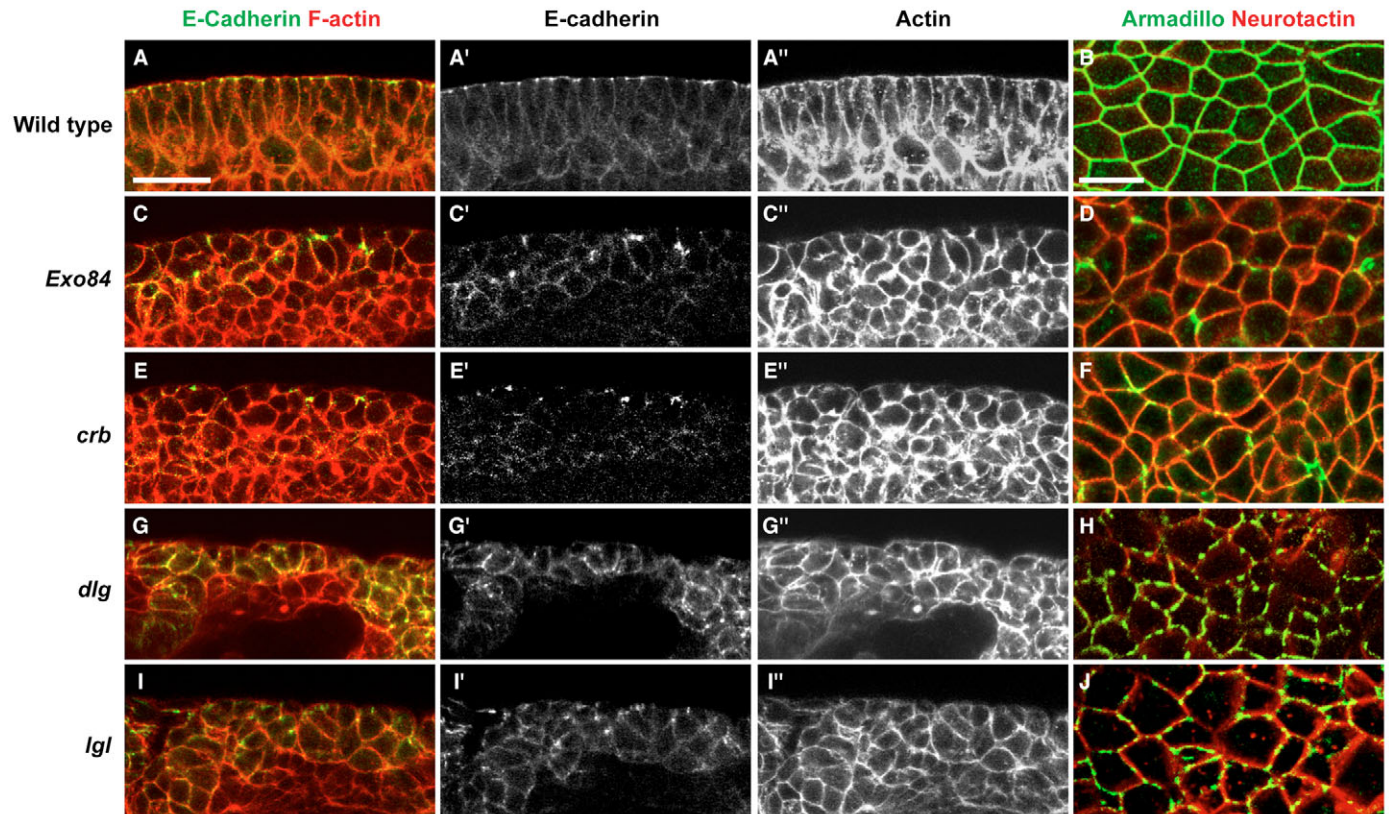

Fig. 2. Disruption of apical or basolateral polarity leads to distinct effects on adherens junction localization. (A,C,E,G,I) DE-cadherin (green) and F-actin (red) in the wild type (A) and *Exo84* (C), *crumbs* (E), *dlg* (G) and *lgl* (I) mutant embryos at stage 10. (B,D,F,H,J) Armadillo (green) and Neurotactin (red) in the wild type (B) and *Exo84* (D), *crb* (F), *dlg* (H) and *lgl* (J) mutant embryos at stage 10. (A,B) In wild-type embryos, DE-cadherin (green A) and Armadillo (green B) localize to the apical edge of lateral interfaces and form a continuous circumferential band. (C-F) In *Exo84* (C,D) and *crumbs* (E,F) mutants, DE-cadherin (green C,E) and Armadillo (green D,F) form large isolated puncta at various locations along the apical-basal axis. (G-J) In *dlg* (G,H) and *lgl* (I,J) mutants, DE-cadherin (green G,I) and Armadillo (green H,J) are diffusely distributed along the cell cortex. Anterior left, ventral up. Bars, 20 µm (A), 5 µm (B).

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.,
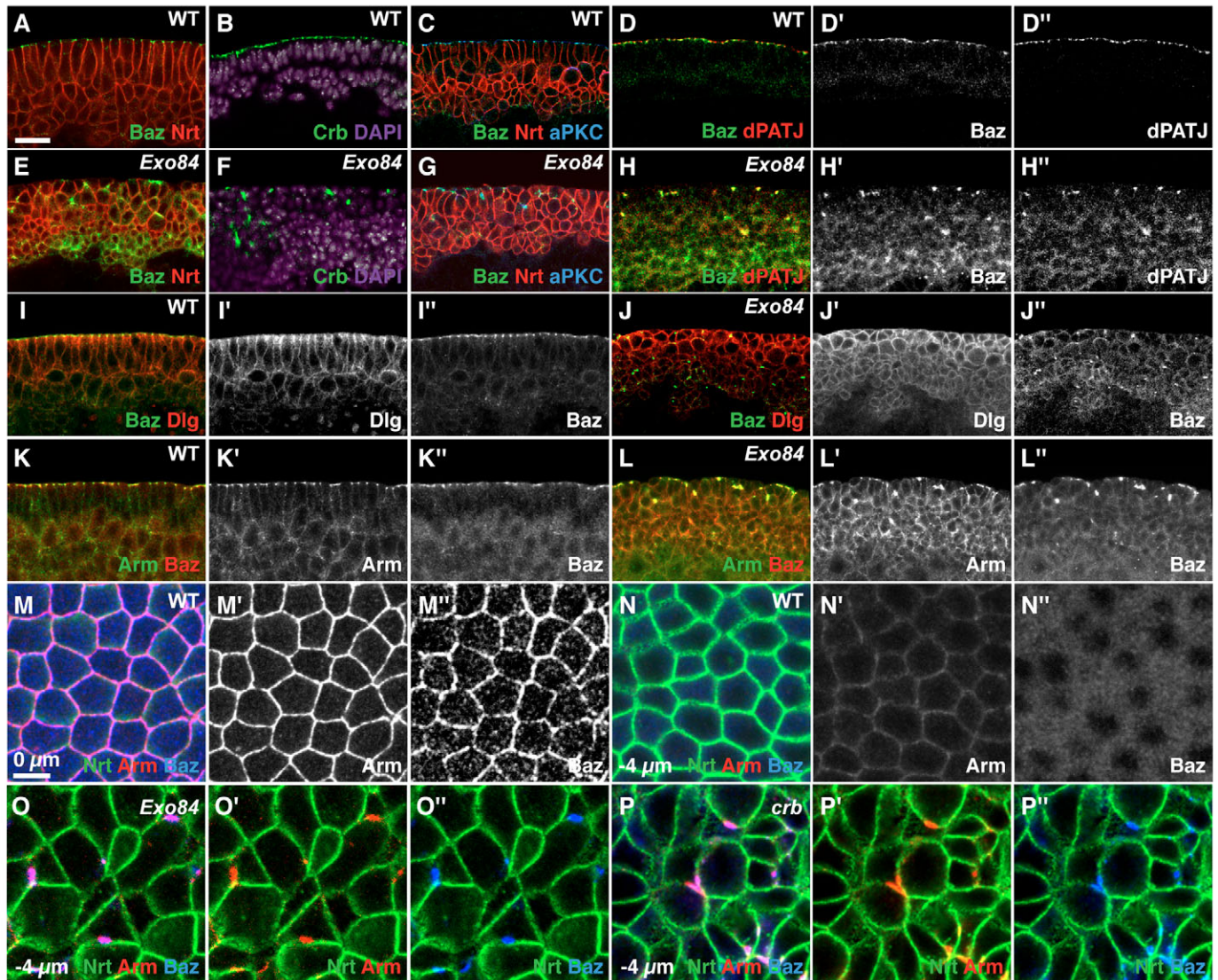

Fig. 3. Mislocalization of apical proteins in *Exo84* mutant embryos. (A-J) Bazooka (green A,C-E,G-J), Crumbs (green B,F), aPKC (blue C,G) and dPATJ (red D,H) localize to the apical margin of lateral cell surfaces in the wild type (A-D,I), but form ectopic aggregates in *Exo84* mutants (E-H,J). Dlg (red I,J) is excluded from the apical surface in the wild type (I). (J) *Exo84* mutants display disrupted Bazooka localization in all cells pictured, whereas apical Dlg exclusion is often maintained. Neurotactin (red A,C,E,G) delineates cell outlines and DAPI (purple B,F) labels nuclei. (K,L) Armadillo (green K,L) and Bazooka (red K,L) colocalize in wild-type adherens junctions (K) and in basolateral aggregates in *Exo84* mutants (L). (M-P) In the wild type (M,N), Armadillo (red) and Bazooka (blue) localize to adherens junctions (M) and are not detected basally (N, 4 μm below M). In *Exo84* (O) and *crumbs* mutants (P), Armadillo (red) and Bazooka (blue) are mislocalized along the apical-basal axis. All embryos are stage 10. Anterior, left; ventral, down. Bar, 20 μm (A); 5 μm (K).

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,J).

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
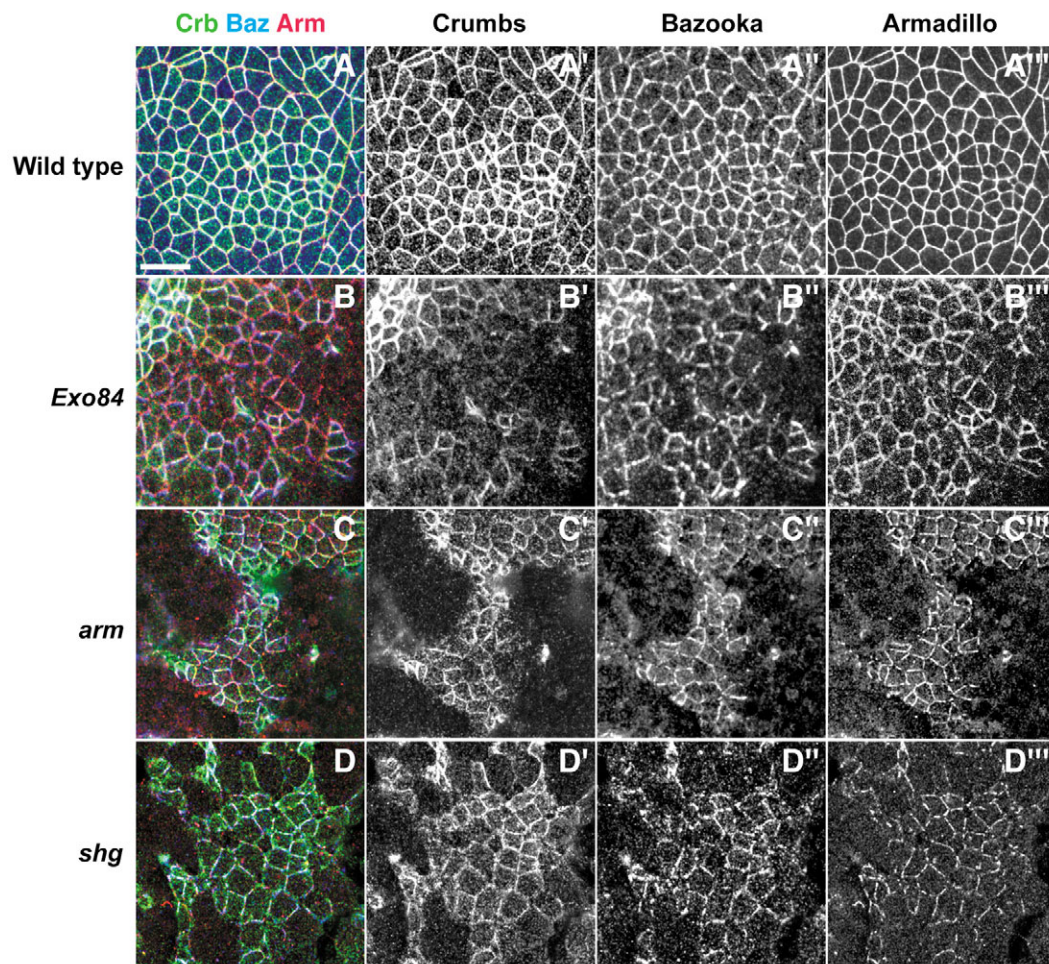

Fig. 4. Crumbs mislocalization precedes the loss of Bazooka and Armadillo in *Exo84* mutants. (A-D) Crumbs (green), Bazooka (blue) and Armadillo (red) in stage 9 wild-type (A), *Exo84* (B), *arm* (C) and *shg* (D) mutant embryos. (A) In the wild type, Crumbs, Bazooka and Armadillo localize apically. (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
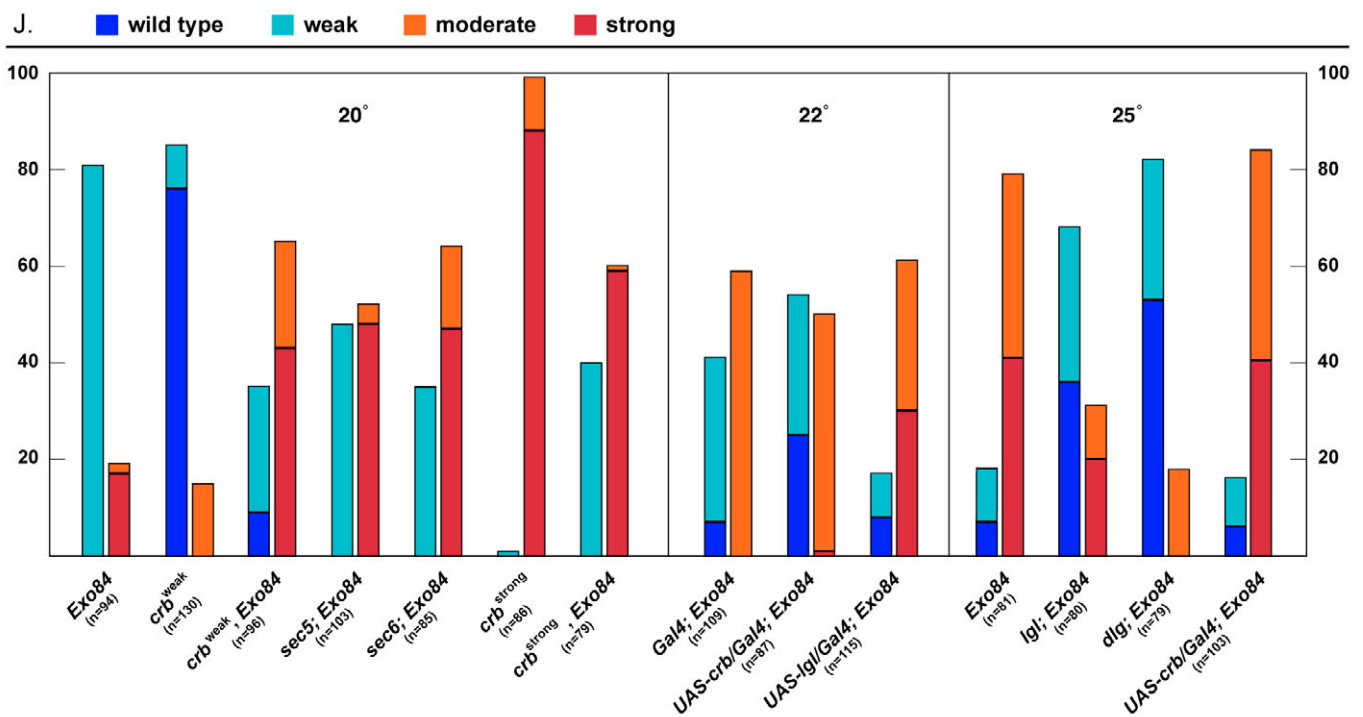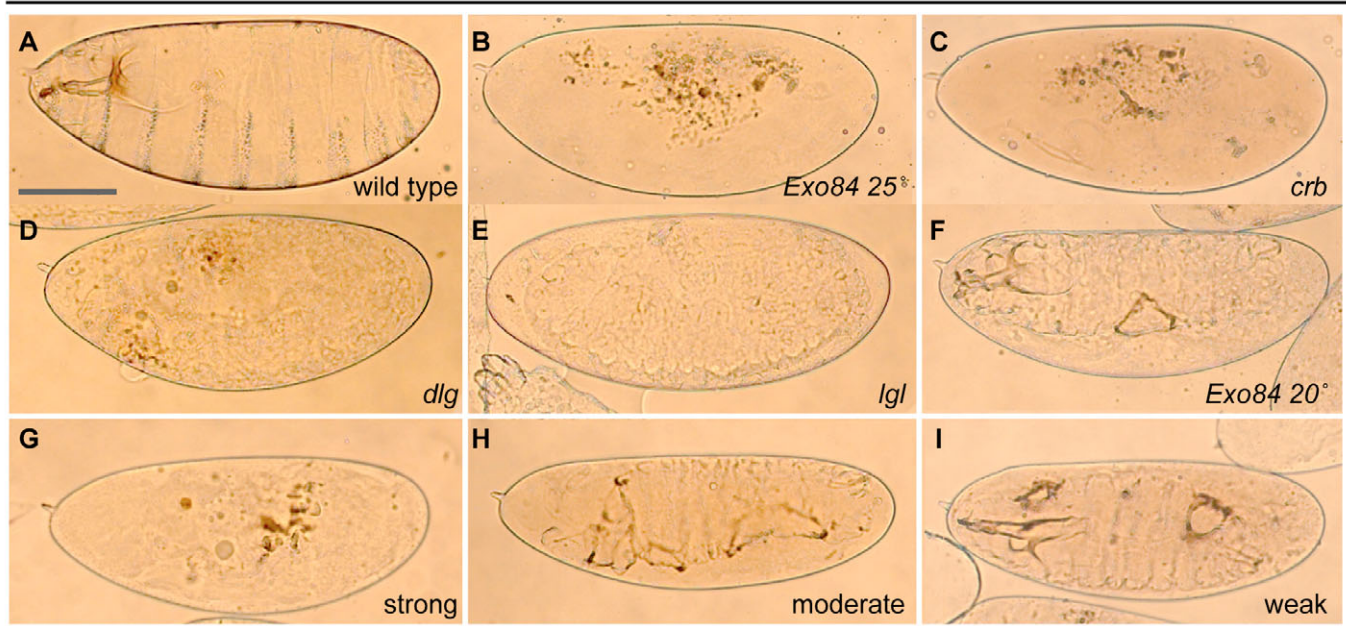
Fig. 5. *Exo84* genetically interacts with apical and basolateral components. (A-F) Wild-type cuticle at the end of embryogenesis (A). In *Exo84* at 25°C (B) and *crumbs* (C) small scraps of cuticle form, whereas *dlg* (D) and *lgl* (E) produce a continuous, malformed cuticle. *Exo84* mutants at 20°C 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.
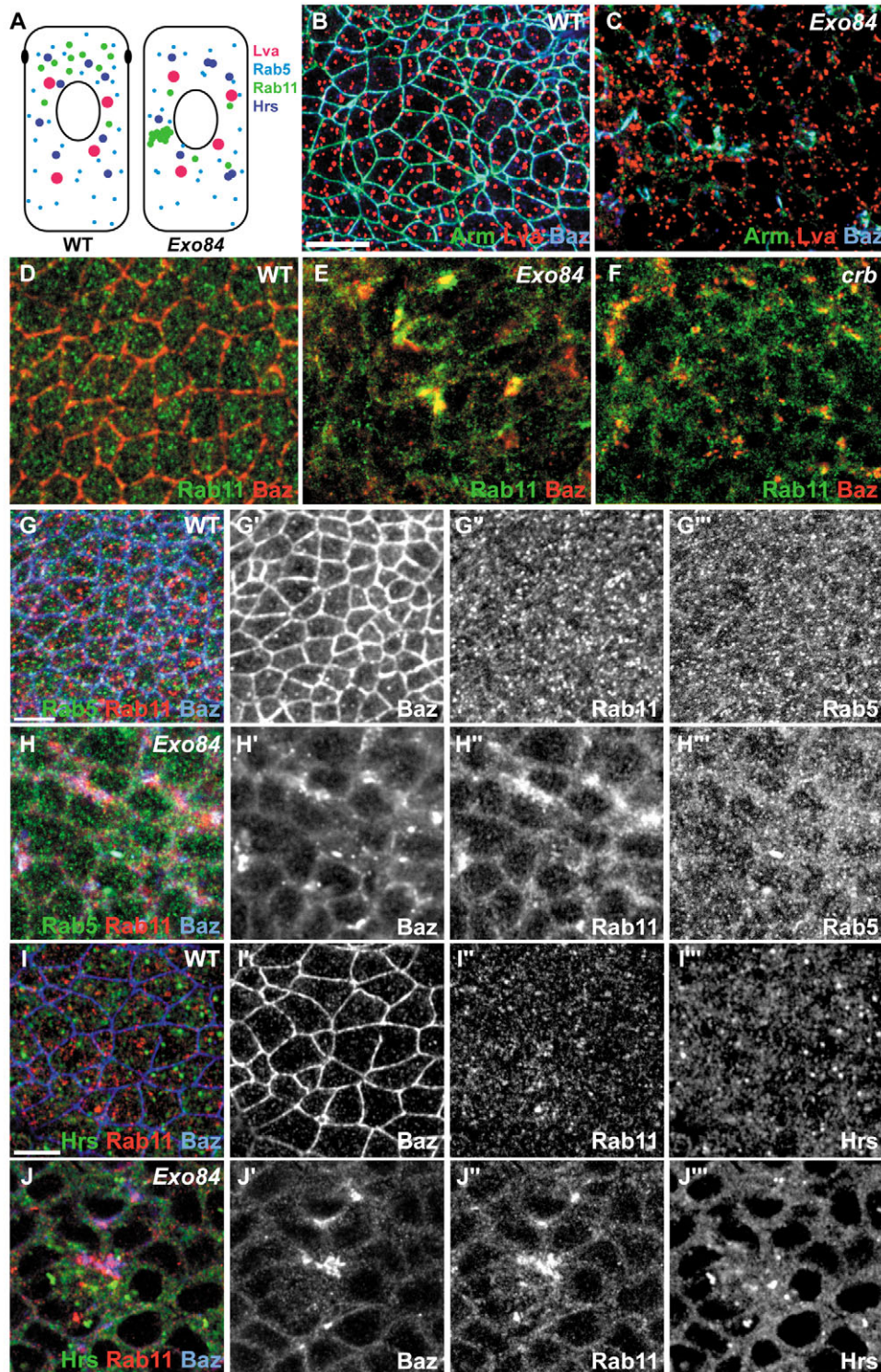
Fig. 6. The recycling endosome compartment is disrupted in *Exo84* mutants. (A) Schematic of results depicting the distribution of Golgi (Lva), early endosome (Rab5), late endosome (Hrs) and recycling endosome (Rab11) compartments in cross section in wild-type and *Exo84* mutant embryos. (B-J) Localization 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), *Exo84* (C,E,H,J) and *crumbs* mutant (F) embryos. (B,C) The Golgi compartment (Lva, red) appears normal in size and distribution in the wild type (B) and *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 *Exo84* mutants (E). Despite disruption of epithelial polarity and Bazooka localization (red) in *crumbs* mutants (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 *Exo84* mutant (H,J). Bazooka aggregates (blue) colocalize with recycling endosome aggregates. Anterior, left; ventral, down. Bar, 10 μm (B); 5 μm (G,I).

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),
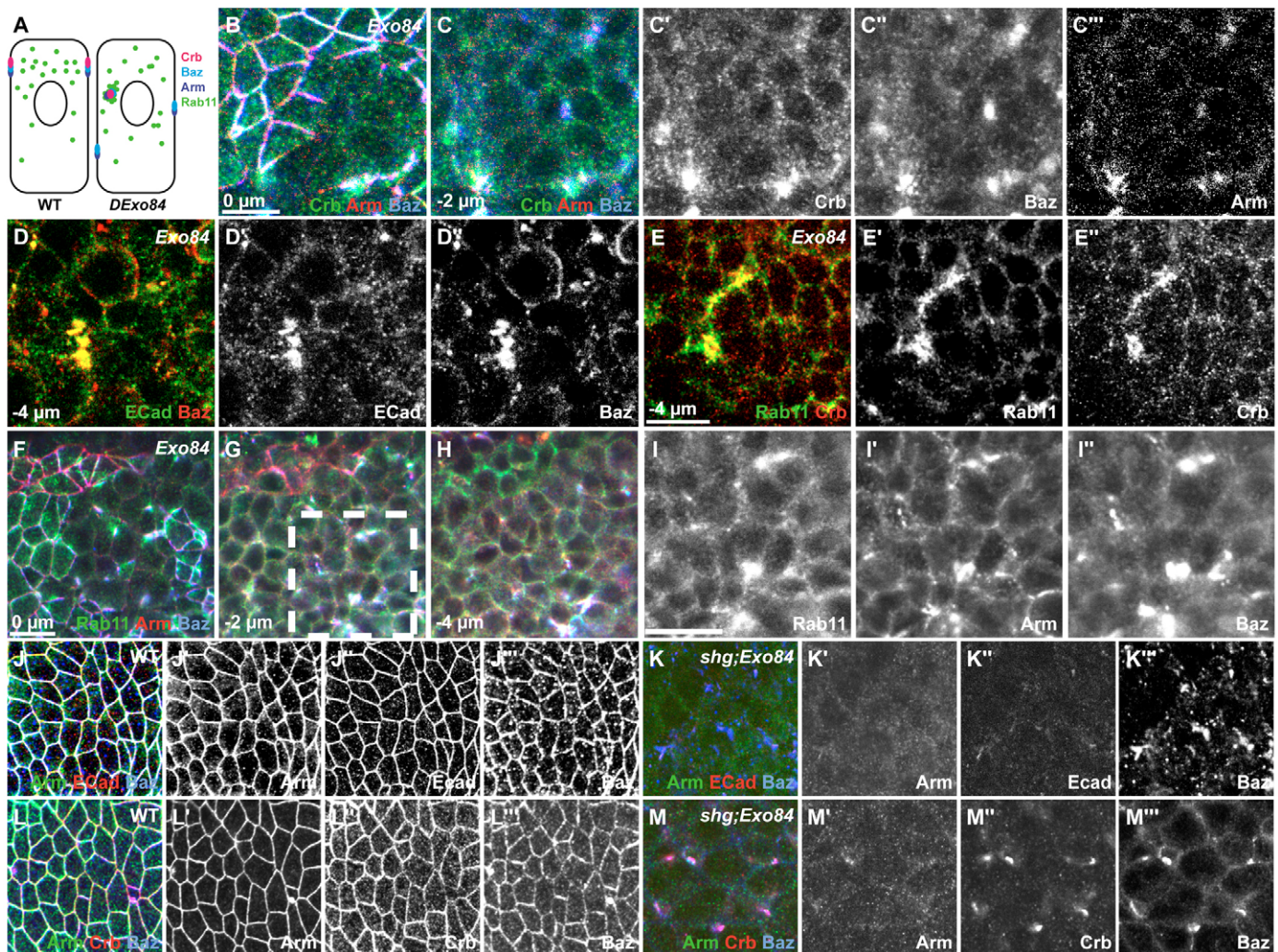

Fig. 7. Aggregation of polarity proteins in *Exo84* mutant embryos. (A) Schematic depicting the distribution of Crumbs (Crb), Bazooka (Baz), Armadillo (Arm), and Rab11 in cross section in wild-type and *Exo84* mutant embryos. In *Exo84* mutants, these proteins colocalize in large aggregates. (B,C) Stage 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.
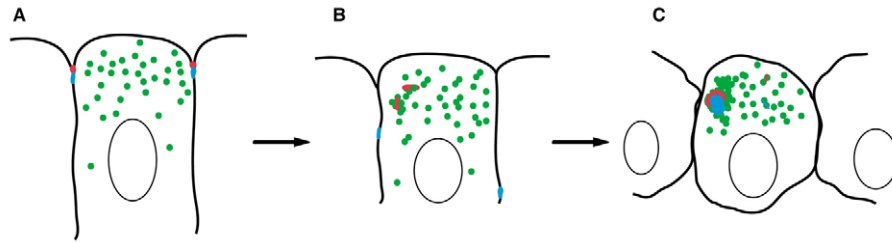
Fig. 8. A model for exocyst function in epithelial polarity of the *Drosophila* embryo. Epithelial polarity is established correctly in *Exo84* mutant embryos (A). Crumbs (red) and adherens junction proteins (blue) localize to the apicolateral cell surface, and recycling endosomes (green) are distributed throughout the apical cytoplasm. (B) Failure to traffic Crumbs to the apical surface, first apparent at stage 9, is accompanied by a loss of apical identity and a mislocalization of adherens junction proteins along the cell surface in a manner that resembles *crumbs* mutants. (C) At later stages, a defect in trafficking from recycling endosomes to the cell surface causes apical and adherens junction proteins to accumulate in an enlarged recycling endosome compartment.

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

exocyst complex in different cellular contexts. Alternatively, DE-cadherin and Crumbs may be delivered to the cell surface in an exocyst-dependent fashion in multiple cell types, but undergo different rates of turnover. For example, Crumbs may be dynamically trafficked in the embryo but stably maintained at the surface of pupal epithelial cells. Differential effects on specific target proteins are not atypical of exocyst function, because loss of Sec6 activity in *Drosophila* photoreceptor cells disrupts the localization of the rhabdomere proteins Chaoptin and Rhodopsin1, whereas the apical localization of Crumbs and DE-cadherin occurs normally (Beronja et al., 2005). The *Drosophila* embryonic epithelium undergoes pronounced changes in structure and organization during development that rely on a balance between apical and basolateral surface domains. A requirement for exocyst-dependent Crumbs trafficking during this process may facilitate the dynamic remodeling of epithelial polarity during morphogenesis.

Materials and Methods

Fly stocks and genetics

Fly stocks were maintained by standard procedures. Oregon R was the wild-type stock. $Exo84^{onr}$ mutant embryos were the progeny of $onr^{142-5}/Df(3R)Espl3$ females crossed to $onr^{142-5}/TM3,hb-lacZ$ males; mutant embryos were genotyped by the absence of lacZ expression. Alleles used were onr^{142-5} (Giansanti et al., 2004), shg^{G119}, shg^{k03401}, dlg^{m32}, $sec5^{E13}$, lgl^4, $crb^{S010409}$, crb^1, arm^{O43A01}, lgl^4, dlg^{m52}, UAS-crb^{wt} (Wodarz et al., 1995), UAS-lgl (Betschinger et al., 2003) and Rab5-GFP (Wucherpfennig et al., 2003). Germline clones were generated by the FLP-DFS system (Chou and Perrimon, 1996). Embryos were scored at 25°C, except as noted in Fig. 5. The following crosses were conducted (females listed first):

(1) $onr^{142-5}/Df(3R)Espl3 \times onr^{142-5}/TM3,hb-lacZ$;
(2) $onr^{142-5}/Df(3R)Espl3 \times P[Exo84]$; $onr^{142-5}/TM3,hb-lacZ$;
(3) $P[Exo84]/+$; $onr^{142-5}/Df(3R)Espl3 \times P[Exo84]$; $onr^{142-5}/TM3,hb-lacZ$;
(4) dlg^{m32}, FRT101/ovoD, FRT 101; $hsFLP38/+ \times$ Oregon R;
(5) $hsFLP22/+$; lgl^4, FRT40A/ovoD, FRT40A $\times lgl^4$, FRT40A/CyO;
(6) $crb^{S010409}$, $onr^{142-5}/Df(3R)Espl3 \times crb^{S010409}$, $onr^{142-5}/TM3,hb-lacZ$;
(7) crb^1, $onr^{142-5}/Df(3R)Espl3 \times crb^1$, $onr^{142-5}/TM3,hb-lacZ$;
(8) $dlg^{m32}/+$; $onr^{142-5}/Df(3R)Espl3 \times onr^{142-5}/TM3,hb-lacZ$;
(9) $lgl^4/+$; $onr^{142-5}/Df(3R)Espl3 \times lgl^4/CyO$; $onr^{142-5}/TM3,hb-lacZ$;
(10) $sec5^{E13}/+$; $onr^{142-5}/Df(3R)Espl3 \times sec5^{E13}/CyO$; $onr^{142-5}/TM3,hb-lacZ$;
(11) $sec6^{KG08199}/+$; $onr^{142-5}/Df(3R)Espl3 \times sec6^{KG08199}/CyO$; $onr^{142-5}/TM3,hb-lacZ$;
(12) $shg^{k03401}/+$; $onr^{142-5}/Df(3R)Espl3 \times shg^{k03401}/CyO$; $onr^{142-5}/TM3,hb-lacZ$;
(13) ubi-$gal4/+$; $onr^{142-5}/Df(3R)Espl3 \times onr^{142-5}/TM3,hb-lacZ$;
(14) ubi-$gal4/+$; $onr^{142-5}/Df(3R)Espl3 \times UAS$-$crb/CyO$; $onr^{142-5}/TM3,hb-lacZ$;
(15) ubi-$gal4/+$; $onr^{142-5}/Df(3R)Espl3 \times UAS$-$lgl/CyO$; $onr^{142-5}/TM3,hb-lacZ$.

Transgenic rescue experiments

A 4.5 kb *Eco*RI-*Nco*I genomic fragment was subcloned from BACR13F13 into pCasper4. *Exo84* was the only complete predicted open reading frame in this genomic fragment. Transgenic stocks expressing this transgene were crossed to onr^{142-5} and assayed for rescue of embryonic lethality and epithelial disruption.

Immunohistochemistry

Unless otherwise specified, embryos were fixed by heat-methanol fixation (Muller and Wieschaus, 1996). Embryos stained with antibodies to Dlg and β-galactosidase were fixed for 25 minutes in 3.7% formaldehyde/PBS:heptane and devitellinized in heptane:methanol. Embryos stained for F-actin and DE-cadherin were fixed for 1 hour in 3.7% formaldehyde/0.1M sodium phosphate buffer:heptane and manually devitellinized.

The following antibodies were used: rabbit anti-Arm [1:200 (Riggleman et al., 1990)], rabbit anti-Baz [1:1000 (Wodarz et al., 1999)], guinea pig anti-Baz [1:500, made by JAZ as described in Wodarz et al. (Wodarz et al., 1999)], mouse anti-Crb (1:1, Developmental Studies Hybridoma Bank, DSHB), mouse anti-Dlg (1:5, DSHB), mouse anti-β-Galactosidase (1:5, DSHB), mouse anti-Neurotactin (1:200, DSHB), rat anti-DEcadherin [1:100, DSHB (Oda et al., 1994)], rabbit anti-Rab11 [1:500 (Satoh et al., 2005)], rabbit anti-dPATJ [1:1000 (Bhat et al., 1999)], guinea pig anti-Hrs [1:500 (Lloyd et al., 2002)], rabbit anti-Lva [1:1000 (Sisson et al., 2000)] and rabbit anti-β-Galactosidase (1:1000, Cappell). F-actin was visualized with Alexa Fluor 488-labelled phalloidin (1:1000). Secondary antibodies were conjugated to Alexa Fluor-488, -568 and -647 (Molecular Probes). Embryos were mounted in ProLong Gold with DAPI and imaged on a Zeiss LSM510 META confocal with a PlanNeo 40×/1.3NA objective. Images represent 1 μm optical sections and z-stacks were acquired at 0.5 μm steps. Figures were assembled in Adobe Photoshop and Illustrator.

Cuticle preparation

Aged embryos (~48 hours after egg laying) were dechorionated in 50% bleach, mounted in Hoyer's:Lactic acid and baked overnight at 60°C. Phase-contrast images were obtained on a Zeiss AxioImager microscope with a PlanApo 10×/0.3NA Ph1 objective and an AxioCam MRC camera using AxioVision software.

References

Bachmann, A., Schneider, M., Theilenberg, E., Grawe, F. and Knust, E. (2001). Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity. *Nature* **414**, 638-643.

Beronja, S., Laprise, P., Papoulas, O., Pellikka, M., Sisson, J. and Tepass, U. (2005). Essential function of Drosophila Sec6 in apical exocytosis of epithelial photoreceptor cells. *J. Cell Biol.* **169**, 635-646.

Betschinger, J., Mechtler, K. and Knoblich, J. A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature* **422**, 326-330.

Bhat, M. A., Izaddoost, S., Lu, Y., Cho, K. O., Choi, K. W. and Bellen, H. J. (1999). Discs Lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. *Cell* **96**, 833-845.

Bilder, D. (2004). Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. *Genes Dev.* **18**, 1909-1925.

Bilder, D. and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676-680.

Bilder, D., Li, M. and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* **289**, 113-116.

Bilder, D., Schober, M. and Perrimon, N. (2003). Integrated activity of PDZ protein complexes regulates epithelial polarity. *Nat. Cell Biol.* **5**, 53-58.

Chou, T. B. and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in Drosophila melanogaster. *Genetics* **144**, 1673-1679.

den Hollander, A. I., ten Brink, J. B., de Kok, Y. J., van Soest, S., van den Born, L. I., van Driel, M. A., van de Pol, D. J., Payne, A. M., Bhattacharya, S. S., Kellner, U. et al. (1999). Mutations in a human homologue of Drosophila crumbs cause retinitis pigmentosa (RP12). *Nat. Genet.* **23**, 217-221.

Giansanti, M. G., Farkas, R. M., Bonaccorsi, S., Lindsley, D. L., Wakimoto, B. T., Fuller, M. T. and Gatti, M. (2004). Genetic dissection of meiotic cytokinesis in Drosophila males. *Mol. Biol. Cell* **15**, 2509-2522.

Grawe, F., Wodarz, A., Lee, B., Knust, E. and Skaer, H. (1996). The Drosophila genes *crumbs* and *stardust* are involved in the biogenesis of adherens junctions. *Development* **122**, 951-959.

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

Guo, W., Grant, A. and Novick, P. (1999). Exo84p is an exocyst protein essential for secretion. *J. Biol. Chem.* **274**, 23558-23564.

Hsu, S. C., Hazuka, C. D., Foletti, D. L. and Scheller, R. H. (1999). Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. *Trends Cell Biol.* **9**, 150-153.

Jafar-Nejad, H., Andrews, H. K., Acar, M., Bayat, V., Wirtz-Peitz, F., Mehta, S. Q., Knoblich, J. A. and Bellen, H. J. (2005). Sec15, a component of the exocyst, promotes notch signaling during the asymmetric division of Drosophila sensory organ precursors. *Dev. Cell* **9**, 351-363.

Kee, Y., Yoo, J. S., Hazuka, C. D., Peterson, K. E., Hsu, S. C. and Scheller, R. H. (1997). Subunit structure of the mammalian exocyst complex. *Proc. Natl. Acad. Sci. USA* **94**, 14438-14443.

Knust, E. and Bossinger, O. (2002). Composition and formation of intercellular junctions in epithelial cells. *Science* **298**, 1955-1959.

Langevin, J., Morgan, M. J., Sibarita, J. B., Aresta, S., Murthy, M., Schwarz, T., Camonis, J. and Bellaiche, Y. (2005). Drosophila exocyst components Sec5, Sec6, and Sec15 regulate DE-cadherin trafficking from recycling endosomes to the plasma membrane. *Dev. Cell* **9**, 355-376.

Laprise, P., Beronja, S., Silva-Gagliardi, N. F., Pellikka, M., Jensen, A. M., McGlade, C. J. and Tepass, U. (2006). The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. *Dev. Cell* **11**, 363-374.

Lecuit, T. and Wieschaus, E. (2000). Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the Drosophila embryo. *J. Cell Biol.* **150**, 849-860.

Lipschutz, J. H. and Mostov, K. E. (2002). Exocytosis: the many masters of the exocyst. *Curr. Biol.* **12**, 212-214.

Lloyd, T. E., Atkinson, R., Wu, M. N., Zhou, Y., Pennetta, G. and Bellen, H. J. (2002). Hrs regulates endosome membrane invagination and tyrosine kinase receptor signaling in Drosophila. *Cell* **108**, 261-269.

Lu, H. and Bilder, D. (2005). Endocytic control of epithelial polarity and proliferation in Drosophila. *Nat. Cell Biol.* **7**, 1232-1239.

Mehta, S. Q., Hiesinger, P. R., Beronja, S., Zhai, R. G., Schulze, K. L., Verstreken, P., Cao, Y., Zhou, Y., Tepass, U., Crair, M. C. et al. (2005). Mutations in Drosophila sec15 reveal a function in neuronal targeting for a subset of exocyst components. *Neuron* **46**, 219-232.

Mostov, K., Su, T. and ter Beest, M. (2003). Polarized epithelial membrane traffic: conservation and plasticity. *Nat. Cell Biol.* **5**, 287-293.

Muller, H. A. (2003). Epithelial polarity in flies: more than just crumbs. *Dev. Cell* **4**, 1-3.

Muller, H. A. and Wieschaus, E. (1996). armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in Drosophila. *J. Cell Biol.* **134**, 149-163.

Murthy, M. and Schwarz, T. L. (2004). The exocyst component Sec5 is required for membrane traffic and polarity in the Drosophila ovary. *Development* **131**, 377-388.

Murthy, M., Ranjan, R., Denef, N., Higashi, M. E., Schupbach, T. and Schwarz, T. L. (2005). Sec6 mutations and the Drosophila exocyst complex. *J. Cell Sci.* **118**, 1139-1150.

Nam, S. C. and Choi, K. W. (2006). Domain-specific early and late function of Dpatj in Drosophila photoreceptor cells. *Dev. Dyn.* **235**, 1501-1507.

Novick, P., Field, C. and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21**, 205-215.

Oda, H., Uemura, T., Harada, Y., Iwai, Y. and Takeichi, M. (1994). A Drosophila homolog of cadherin associated with armadillo and essential for embryonic cell-cell adhesion. *Dev. Biol.* **165**, 716-726.

Pelissier, A., Chauvin, J. P. and Lecuit, T. (2003). Trafficking through Rab11 endosomes is required for cellularization during Drosophila embryogenesis. *Curr. Biol.* **13**, 1848-1857.

Pellikka, M., Tanentzapf, G., Pinto, M., Smith, C., McGlade, C. J., Ready, D. F. and Tepass, U. (2002). Crumbs, the Drosophila homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature* **416**, 143-149.

Riggleman, B., Schedl, P. and Wieschaus, E. (1990). Spatial expression of the Drosophila segment polarity gene armadillo is post-transcriptionally regulated by wingless. *Cell* **63**, 549-560.

Rodriguez-Boulan, E., Musch, A. and Le Bivic, A. (2004). Epithelial trafficking: new routes to familiar places. *Curr. Opin. Cell Biol.* **16**, 436-442.

Rodriguez-Boulan, E., Kreitzer, G. and Musch, A. (2005). Organization of vesicular trafficking in epithelia. *Nat. Rev. Mol. Cell Biol.* **6**, 233-247.

Satoh, A. K., O'Tousa, J. E., Ozaki, K. and Ready, D. F. (2005). Rab11 mediates post-Golgi trafficking of rhodopsin to the photosensitive apical membrane of Drosophila photoreceptors. *Development* **132**, 1487-1497.

Schuck, S. and Simons, K. (2004). Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* **117**, 5955-5964.

Sisson, J. C., Field, C., Ventura, R., Royou, A. and Sullivan, W. (2000). Lava lamp, a novel peripheral golgi protein, is required for Drosophila melanogaster cellularization. *J. Cell Biol.* **151**, 905-918.

Tanentzapf, G. and Tepass, U. (2003). Interactions between the crumbs, lethal giant larvae and bazooka pathways in epithelial polarization. *Nat. Cell Biol.* **5**, 46-52.

Tepass, U. (1996). Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of Drosophila. *Dev. Biol.* **177**, 217-225.

Tepass, U. and Hartenstein, V. (1994). The development of cellular junctions in the Drosophila embryo. *Dev. Biol.* **161**, 563-596.

Tepass, U., Theres, C. and Knust, E. (1990). *crumbs* encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. *Cell* **61**, 787-799.

Tepass, U., Tanentzapf, G., Ward, R. and Fehon, R. (2001). Epithelial cell polarity and cell junctions in Drosophila. *Annu. Rev. Genet.* **35**, 747-784.

TerBush, D. R. and Novick, P. (1995). Sec6, Sec8, and Sec15 are components of a multisubunit complex which localizes to small bud tips in Saccharomyces cerevisiae. *J. Cell Biol.* **130**, 299-312.

TerBush, D. R., Maurice, T., Roth, D. and Novick, P. (1996). The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. *EMBO J.* **15**, 6483-6494.

Wells, C. D., Fawcett, J. P., Traweger, A., Yamanaka, Y., Goudreault, M., Elder, K., Kulkarni, S., Gish, G., Virag, C., Lim, C. et al. (2006). Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. *Cell* **125**, 535-548.

Whyte, J. R. and Munro, S. (2002). Vesicle tethering complexes in membrane traffic. *J. Cell Sci.* **115**, 2627-2637.

Wodarz, A., Hinz, U., Engelbert, M. and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila. *Cell* **82**, 67-76.

Wodarz, A., Ramrath, A., Kuchinke, U. and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in Drosophila neuroblasts. *Nature* **402**, 544-547.

Wodarz, A., Ramrath, A., Grimm, A. and Knust, E. (2000). Drosophila atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150**, 1361-1374.

Woods, D. F., Wu, J. W. and Bryant, P. J. (1997). Localization of proteins to the apico-lateral junctions of Drosophila epithelia. *Dev. Genet.* **20**, 111-118.

Wu, S., Mehta, S. Q., Pichaud, F., Bellen, H. J. and Quiocho, F. A. (2005). Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. *Nat. Struct. Mol. Biol.* **12**, 879-885.

Wucherpfennig, T., Wilsch-Brauninger, M. and Gonzalez-Gaitan, M. (2003). Role of Drosophila Rab5 during endosomal trafficking at the synapse and evoked neurotransmitter release. *J. Cell Biol.* **161**, 609-624.

Yeaman, C., Grindstaff, K. K., Wright, J. R. and Nelson, W. J. (2001). Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. *J. Cell Biol.* **155**, 593-604.

Zhang, X. M., Ellis, S., Sriratana, A., Mitchell, C. A. and Rowe, T. (2004). Sec15 is an effector for the Rab11 GTPase in mammalian cells. *J. Biol. Chem.* **279**, 43027-43034.