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Nak regulates Dlg basal localization in *Drosophila* salivary gland cells

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ABSTRACT

Protein trafficking is highly regulated in polarized cells. During development, how the trafficking of cell junctional proteins is regulated for cell specialization is largely unknown. In the maturation of *Drosophila* larval salivary glands (SGs), the Dlg protein is essential for septate junction formation. We show that Dlg was enriched in the apical membrane domain of proximal cells and localized basolaterally in distal mature cells. The transition of Dlg distribution was disrupted in *nak* mutants. Nak associated with the AP-2 subunit α-Ada and the AP-1 subunit AP-1γ. In SG cells disrupting AP-1 and AP-2 activities, Dlg was enriched in the apical membrane. Therefore, Nak regulates the transition of Dlg distribution likely through endocytosis of Dlg from the apical membrane domain and transcytosis of Dlg to the basolateral membrane domain during the maturation of SGs development.

Introduction

Most cells exhibit subdivisions in plasma membranes and underlying subcortical regions with specialized compositions and structures. Typical examples include the apical and basolateral domains of polarized epithelial cells and the axonal and somatodendritic domains of neurons that bear distinct and vital functions to the cells. During the establishment of the epithelial cell polarity, different cell junctions that function in cell–cell contact and tissue integrity are organized. Many junctional components also play essential roles in the establishment and the maintenance processes [1–3]. Depositions of junctional components and organization of specialized cellular domains rely on endocytosis, protein sorting, endosomal recycling, and polarized transportation processes [4,5]. Little is known for the reorganization of junctional proteins and the underlying mechanism regulating this process.

In clathrin-mediated trafficking processes, AP complexes recruit cargos, clathrin, and other accessory proteins to form clathrin-coated vesicles. The AP-2 complex is localized at the subcortical region of the plasma membrane and required for endocytosis. In mammalian cells, the AP-1A complex is ubiquitously expressed in all cell types and involved in trafficking between TGN and endosomes. The AP-1B complex is only present in polarized cells and required for sorting from the recycling endosomes [6–9]. In *Drosophila*, the only AP-1 complex might play dual roles performed by AP-1A and AP-1B in mammalian cells. In addition, the *Drosophila* AP-1 and AP-2 complexes share the β subunit, but differ in other subunits: the AP-1 complex includes γ, μ1, and σ1 subunits; the AP-2 complex includes α, μ2, and σ2 subunits [10].

Activities of the AP complexes are regulated by the Ark/Prk family of protein kinases which share sequence homology in the serine/threonine kinase domains [11]. In endocytosis, yeast Ark1p and Prk1p negatively regulate the formation of protein complexes that include the Eps-15 homolog Pan1p, Sla1p, and End3p [12,13]. Mammalian AAK1 and GAK/auxilin2 have multiple and partially redundant roles in clathrin-mediated trafficking processes, such as endocytosis, endosomal recycling, or lysosomal sorting [14–18]. AAK1, GAK, or both regulate these processes through phosphorylations of the μ subunits of AP-1 and AP-2 complexes by which facilitates the cargo binding efficiency [15,19–22]. Other mechanisms involve recruitment of adaptors through protein–protein interactions, phosphorylation of accessory proteins, and uncoating the clathrin-coated vesicles [16,18,23].

We show that the *Drosophila* Ark/Prk homolog Nak is involved in the polarization of SG cells. In *nak* mutants, basolateral membrane distribution of Dlg and apical-to-basal transition in SG cells were disrupted, and the growth of SG cells was severely retarded in *nak* mutants. We identified the interaction between Nak and the α subunit of the AP-2 complex and the γ subunit of AP-1 complex. Consistently, mutants for components of AP-1 or AP-2 complexes photocopied *nak* mutants, displayed similar Dlg distribution and cellular growth defects. Therefore, Nak regulates SGs

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development and the transition of Dlg likely through endocytosis and transcytosis.

Materials and methods

Drosophila strains. Wild type flies used in this study were w^{1118}. *Sgs3-GAL4*, $AP47^{EP1112}$, and $AP47^{SHE11}$ were from Bloomington *Drosophila* Stock Center. α-ada^{1} and α-ada^{3} were described previously [24]. The RNAi transgenic lines for knockdown of *AP-1*γ (#3275) and *AP-2*σ (#34148) were from Vienna *Drosophila* RNAi Center. Flies were maintained at standard food vials at 25 °C except for α-$ada^{1/3}$ that was raised at 22 °C.

Immunostaining. SGs were isolated from third instar larvae, and fixed and immunostained following standard procedures. Antibodies used were mouse α-Dlg (1:50, DSHB), α-aPKC (1:50, Santa Cruz), and α-Syx 1A (1:50, DSHB). TRITC-conjugated Phalloidin and Hoechst 33258 were used to mark the filamentous actin and nuclei, respectively. Fluorescence intensities of Dlg along the apical and basal membrane were measured by the Metamorph software and the *a*pical and *b*asal intensity per pixel were used to calculate for the A/B ratios.

S2 cell transfection, immunoprecipitation, and Western blots. Plasmids of p*WA-GAL4* and p*UAST-Myc-nak* or p*UAST-Myc-nakΔC* were mixed with Cellfectin (Invitrogen) and transfected into S2 cells. Two days after, the transfected cells were lysed for immunoprecipitation or Western blots. Antibodies used for immunoprecipitation were α-c-Myc antibody (A14, Santa Cruz). Primary antibodies used for immunoblots were α-Chc (1:250, Sigma), α-Eps15 (1:250, Covance Research), α-α-Ada (1:20,000, [24]), α-14-3-3ε [25], α-gamma Adaptin (1:1000, Abcam), and α-c-Myc (1:1000, A14). Secondary antibodies used were α-mouse-HRP or α-rabbit-HRP (1:5000, Jackson ImmunoResearch Laboratories).

Results

Dlg is mislocalized in the distal SG cells in nak mutants

Dlg is a founding member of the membrane-associated guanylate kinase (MAGUK) family proteins and is involved in the establishment of cell polarity, stabilization of septate junction, and inhibition of cell proliferation [26,27]. In distal part of SGs in *Drosophila* third instar larvae, Dlg localized at the subcortical region of the basal membrane (Fig. 1A). When viewing sections through distal SG cells, Dlg was found tightly associated with the basal membrane (Fig. 1A1). In the lateral membrane, Dlg expression was presented in an apicobasal gradient with diminishing concentration toward the apical end (arrowheads). In addition, cytoplasmic Dlg also formed a similar gradient (arrow) but with lower protein levels than the membrane-associated ones.

The polarized distribution of Dlg was altered in *nak* mutants, in which the SG cell growth was also hindered (Supplementary Fig. 1 and Table 1). Dlg localization in the basal membrane region was still observed; the protein levels, however, were reduced (Fig. 1B and B1). Dlg was enriched at the apical membrane region (arrow in Fig. 1B1) and the Dlg gradient along the lateral membrane domain was reversed with higher concentration toward the apical end (arrowhead). The gradient in the cytoplasm was no longer present. In addition, Dlg punctas were detected near the apical membrane (empty arrowhead). The fluorescence intensities of Dlg immunoreactivity in apical and basal membrane domains were measured, and the apical-to-basal ratio of Dlg immunoreactivity is expressed as the A/B ratio. In the WT and heterozygous $nak^{MR6/+}$ and $nak^{Del3/+}$ control cells, the A/B ratios are 55–65% (Fig. 1C). In $nak^{MR6/MR6}$ and $nak^{MR6/Del3}$ mutant cells (see Supplementary Fig. 2A for mutant description), however, the A/B ratios of Dlg immunoreactivity reached 3 and 2.4, respectively. These results

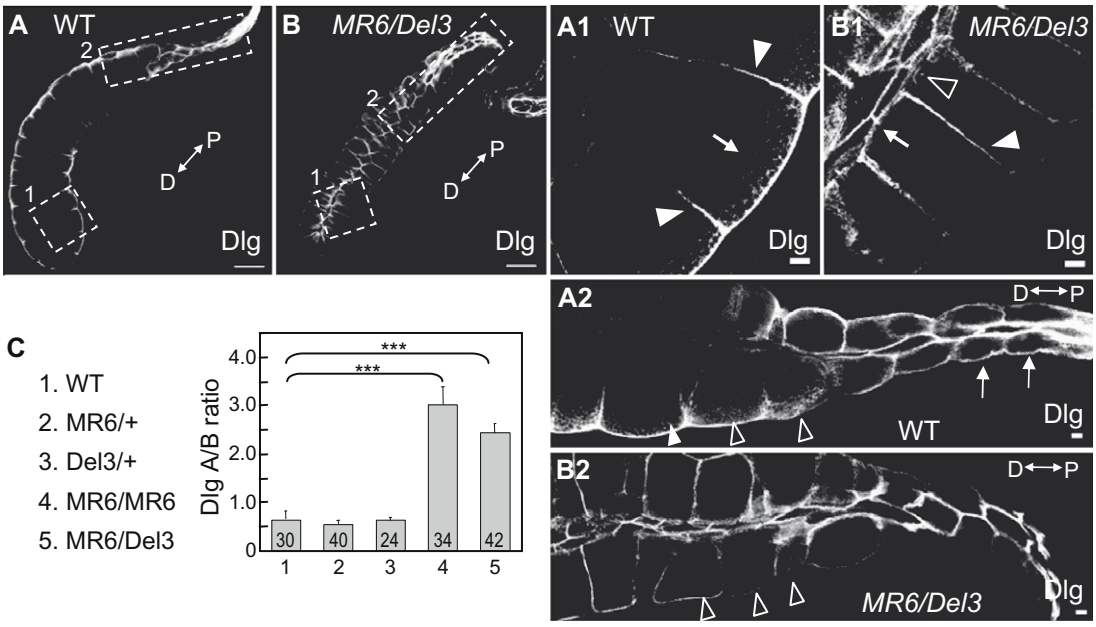

Fig. 1. Dlg mislocalization in *nak* mutant SG cells. Images for third instar larval SGs of WT (A) and $nak^{MR6/Del3}$ (B) stained for Dlg. In this and all following figures, SG images are oriented with proximal (P) to right and distal (D) to left (bi-headed arrows). (A1,B1) and (A2,B2) are enlarged images of regions (dashed outlined boxes, labeled 1 or 2) in (A,B), respectively. Apical direction is toward the left-top facing the lumen of SGs and basal direction is to the right-bottom. (A1) Dlg gradients in lateral membrane and basal cytoplasm are indicated by arrowheads and arrow, respectively. (B1) A arrow, apical Dlg; arrowhead, lateral Dlg; empty arrowhead, Dlg punctas. (A2) A arrows, proximal cells; empty arrowheads, transition zone cells; arrowhead, slightly distal cell. (B2) Empty arrowheads, transition zone cells. In these and all following figures, thin scale bars are 100 μm such in (A,B) and thick scale bars are 10 μm such as in (A1–B2). (C) A/B ratios of Dlg immunoreactivity in WT, $nak^{MR6/+}$, $nak^{Del3/+}$, $nak^{MR6/MR6}$, and $nak^{MR6/Del3}$ distal SG cells. In this and all following statistics, averages are mean ± SEMs; numbers of SG cells scored are indicated in the bars; and Student's *t* test is used for comparisons (significance: $^{***}p < 0.0001$; $^{**}p < 0.001$; and $^{*}p < 0.01$).

suggest that Nak is required for basolateral distribution of Dlg in SG cells. When Nak is inactivated, the Dlg protein is instead enriched at the apical side of SG cells.

The Dlg mislocalization in SG cells was phenocopied in *nak* knockdown by expressing the *nak-RNAi* transgene (see Supplementary Fig. S2B for knockdown efficiency) driven by the SG-specific driver *Sgs3-GAL4* (hereafter denoted as *Sgs3>nakRNAi*) [28] (Fig. 4B). Unlike the *nak* mutants, *Sgs3>nakRNAi* larvae had little defect in SG cell growth, as compared to the control GFP-expression cells (cf. Fig. 4A and B). Dlg distribution in control cells was similar to that in WT cells (cf. Figs. 4A1 and 1A1). However, in *Sgs3>nakRNAi* SG cells, Dlg was distributed in all circumferential membrane and highly enriched in the apical membrane (arrowhead in Fig. 4B1). The A/B ratio of Dlg is 0.65 in the control and reaches 3.4 in *Sgs3>nakRNAi* (Fig. 4E). Dlg punctas were also visible near the apical membrane in *Sgs3>nakRNAi* (empty arrowheads in Fig. 4B1), confirming the requirement of Nak for proper Dlg localization. This result also suggests that Dlg mislocalization is independent of the defects in SG cell growth and morphogenesis in the absence of Nak activity.

Apical-to-basal transition of Dlg is disrupted in nak mutant SG cells

We examined Dlg localization in the proximal region of SGs where the cell growth has just initiated. In the most proximal WT SG cells (arrows in Fig. 1A2), Dlg was distributed in all membrane regions but with higher concentration in the apical domain. Dlg was also detected in the cytoplasm. In cells located slightly distally (empty arrowheads), transition of Dlg localization was evident: a reduction of apical membrane signals, the formation of the apicobasal gradients in the lateral membrane and in the cytoplasm, and a prominent enrichment in the basal membrane. In the cell immediately distal to this transition zone, the pattern of Dlg distribution resembles that in distal cells (arrowhead).

The changes in Dlg distribution were examined in *nak* mutants. In $nak^{MR6/Del3}$ mutant cells in the transition zone (empty arrowheads in Fig. 1B2), Dlg distribution was enriched in the apical membrane and relatively even in all other membrane domains. Dlg gradients in the lateral membrane and the basal cytoplasmic region were not established. Indeed, cytoplasmic Dlg was almost undetectable. These results suggest that Nak is required for the Dlg redistribution in SG cells in the transition zone.

Nak regulates polarized distribution of proteins with specificity

We then examined whether Nak similarly regulates other basally localized proteins. The aPKC protein was also localized to the basal membrane and formed a basal cytoplasmic gradient in distal cells (Fig. 2A and arrowhead in Fig. 2A1). In addition, weak nuclear and apical membrane signals of aPKC were also present. In the proximal SG cells, the majority of aPKC was enriched at the apical membrane (data not shown), suggesting that aPKC also undergoes an apical-to-basal transition during SG development. In $nak^{MR6/Del3}$ and $nak^{MR6/MR6}$ mutant cells, association of aPKC with the basal membrane was still maintained (Fig. 2B1 and data not shown). The basal cytoplasmic gradient was also evident, although not as steep as in WT SG cells (empty arrowhead in Fig. 2B1). The apical membrane and the nuclear distributions were indistinguishable from those in WT cells. Therefore, unlike Dlg, the majority of aPKC protein remains localized in the basal side in *nak* mutant SG cells.

Altered Dlg distribution in *nak* mutants could be caused by a disruption of the apical membrane structure or function, resulting in the failure of Dlg transition. The distribution of the apically localized protein, the t-SNARE component Syntaxin 1A (Syx1A) was located at the apical membrane along the entire proximal–distal axis of SGs (Fig. 2C1). Although Dlg distribution was altered in *Sgs3>nakRNAi*, the apical localization of Syx1A was completely normal (Fig. 2D1), suggesting that the apical membrane structure is largely unaffected in *nak* mutant SG cells. Therefore, Nak specifically regulates the redistribution of Dlg during SG cell development.

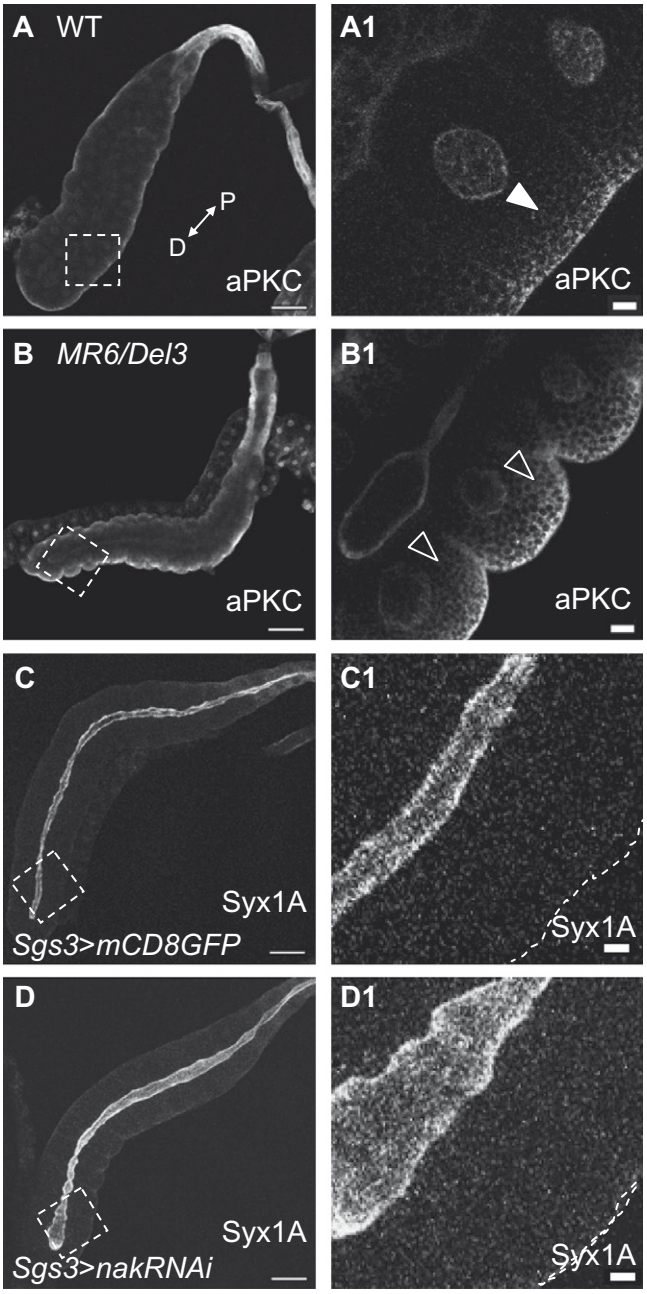

Fig. 2. Localization of aPKC and Syx1A in WT and *nak* mutant SG cells. aPKC staining in WT (A) and $nak^{MR6/Del3}$ (B) SG cells. (A1,B1) are enlarged images for distal SG cells in boxed regions in (A,B), respectively. Arrowhead and empty arrowheads mark basal cytoplasmic aPKC gradients in (A1) and (B1), respectively. Syx1A staining in *Sgs3>mCD8GFP* (C) and *Sgs3>nakRNAi* (D) SG cells. (C1,D1) are enlarged images for distal SG cells in boxed regions in (C,D), respectively.

Nak associates with α-Ada, AP-1γ, Chc, and Eps15

The Nak polypeptide contains motifs that are suggested to interact with α-Ada, clathrin heavy chain (Chc), and Eps15 (Fig. 3A). We then examined their association with Nak by immu-

noprecipitating Myc-tagged Nak that was expressed in S2 cells. From the immunocomplex, α-Ada, Chc, and Eps15 were identified (line 4 in Fig. 3B, left panel). The control protein 14-3-3ε was not detected. Those endocytic motifs are spanning the C-terminal region to the kinase domain. In the immunocomplex co-precipitated with Myc-tagged NakΔC (a.a. 1–317) that truncates the C-terminal fragment of Nak, α-Ada, Chc, and Eps15 were not identified (line 3 in Fig. 3B, left panel), suggesting that Nak associates with these endocytic components through the C-terminal region.

Several AP-1γ-interacting motifs were predicted in the Nak polypeptide, with one located in the N-terminal kinase domain and others distributed in the C-terminal region (arrows and predicted motifs in Fig. 3A). Similarly assay was performed to test the association between Nak and AP-1γ in S2 cells. AP-1γ indeed co-precipitated with the Myc-Nak immunocomplex (line 4 in Fig. 3B, right panel), but not with the C-terminal truncated Myc-Nak-ΔC immunocomplex (lane 3 in Fig. 3B, right panel), indicating that the C-terminal region of Nak that includes eleven putative AP-1γ-interacting motifs is required for the interaction with AP-1γ.

Requirement of AP-1 and AP-2 in Dlg basal localization in SG cells

The associations of Nak with α-Ada and AP-1γ prompted us to study whether Dlg basal localization in SG cells depends on AP-2 and AP-1 complexes. In the transheterozygous mutants for the α subunit of AP-2 complex, *α-ada*$^{1/3}$, and for the μ1 subunit of AP-1 complex, *AP47*$^{SHE11/EP1112}$, the survival larvae allowed us to examine the Dlg localizations in mutant SG cells. Although the cell growth and morphology were severely affected in both mutants (Supplemental Table 1), Dlg localizations were strongly biased toward the apical membrane, similar to what was observed in *nak* mutants (Supplementary Fig. 3).

Due to growth and morphological defects in *α-ada* and *AP47* mutants, we depleted *AP-2σ* or *AP-1γ* expression by respective RNAi transgenes driven by *Sgs3-GAL4*. In both cases, the morphology and the size of SG cells were similar to the control (cf. Fig. 4A, C, and D). However, the Dlg localization was altered. Dlg was detected in all circumferential membrane with enrichments in the apical membranes (Fig. 4C1 and D1). The A/B ratio reached about 2 in *Sgs3>AP-2σRNAi* and 5 in *Sgs3>AP-1γRNAi* SG cells, respectively. In addition, Dlg gradients in the basal cytoplasm were not visible. With the analyses of two different subunits in each adaptor complexes, our results strongly suggest that the AP-2 and AP-1 complexes are required for the Dlg basal localization in SG cells.

Discussion

In this study, we described the re-localization of the Dlg protein during *Drosophila* SG specialization. From our analyses, Dlg underwent a transition from the apical to the basal membrane domain. In addition, we found that Nak and components of AP-1 or AP-2 complexes are required for the Dlg transition. Two possible models

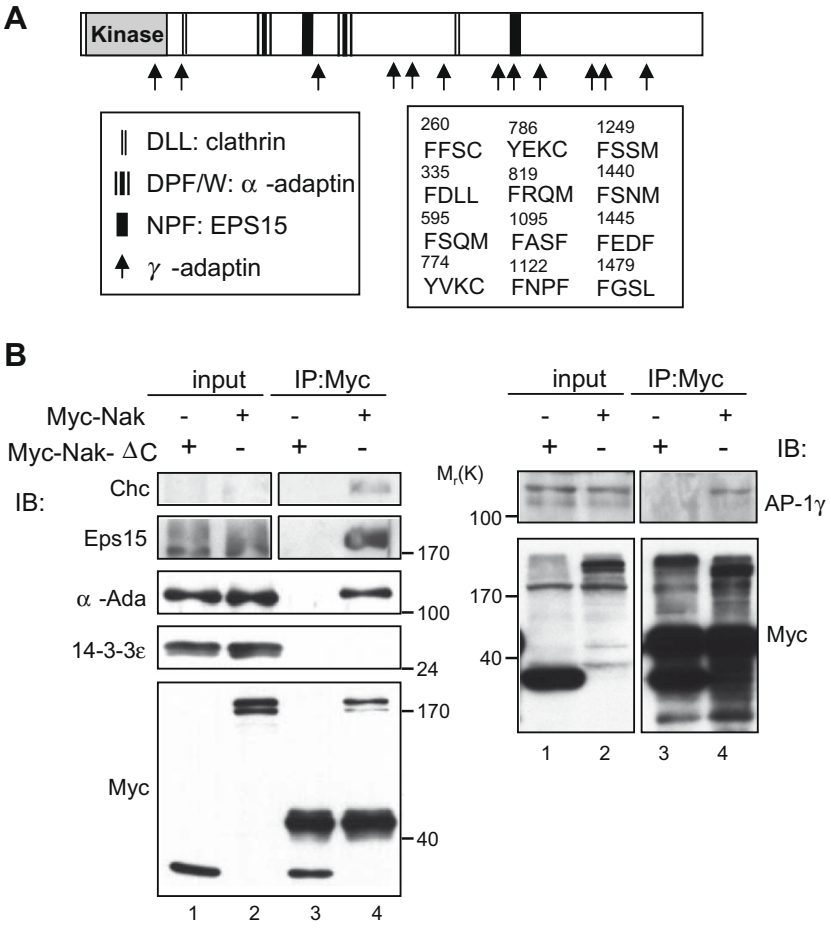

Fig. 3. Nak interacts with α-Ada, AP-1γ, Chc, and Eps15. (A) Schematic diagram for the Nak polypeptide indicating the kinase domain and endocytic motifs interacting with Chc (DLL), α-Ada (DPF/W), and EPS15 (NPF). Arrows indicate the positions of AP-1γ-interacting motifs and their sequences are shown. (B) S2 cells expressed with Myc-Nak or Myc-NakΔC were lysed and performed immunoprecipitation. The immunocomplexes were analyzed through Western blots with antibodies against Chc, Eps15, α-Ada, or 14-3-3ε (left panel), AP-1γ (right panel), or c-Myc (lower in both panels). Input controls are 1/15 of the lysates used for immunoprecipitation.

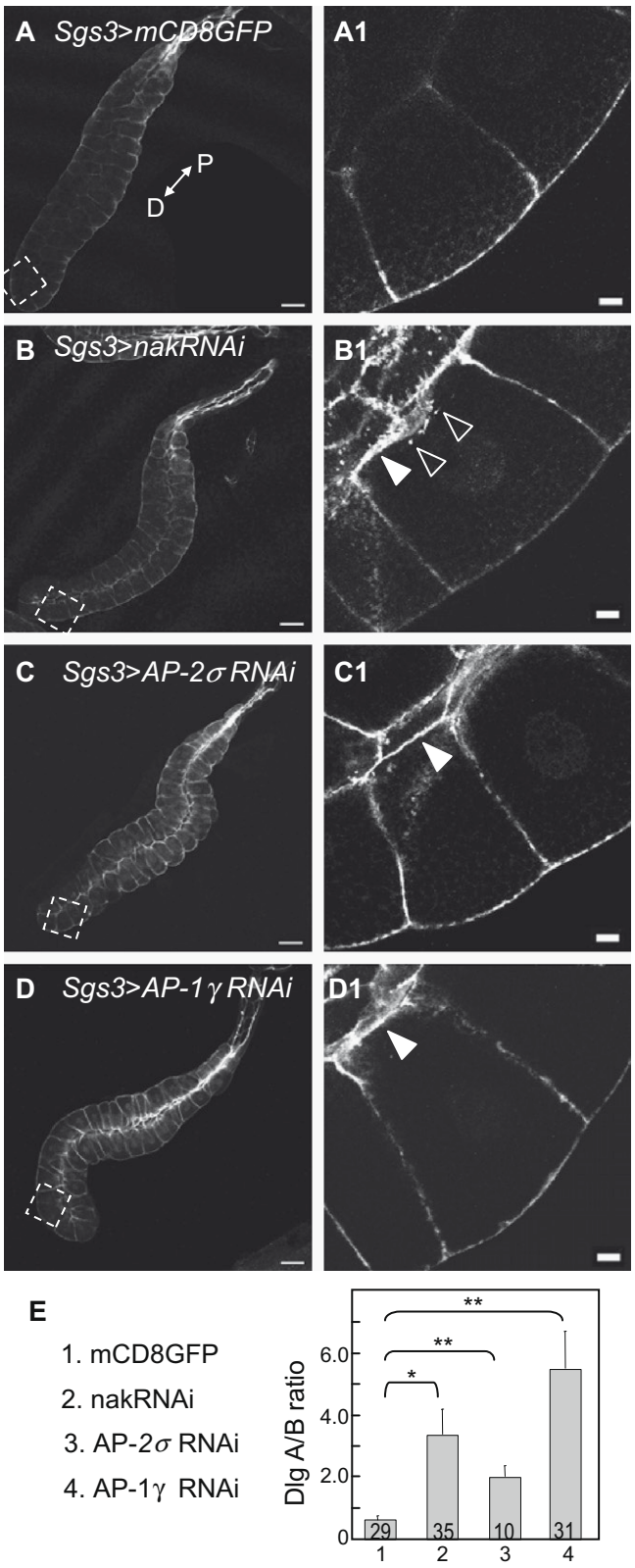

Fig. 4. Dlg mislocalization in *Sgs3-GAL4* driven RNAi-knockdown SG cells. (A–D) SGs carrying genotypes *Sgs3-GAL4*; *UAS-mCD8GFP* (A), *Sgs3-GAL4*; *UAS-nakRNAi* (B), *Sgs3-GAL4*; *UAS-AP-2σRNAi* (C), and *Sgs3-GAL4*; *UAS-AP-1γRNAi* (D) were stained for Dlg and boxed areas were enlarged for (A1–D1). A arrowheads in (B1–D1) indicate apical Dlg and empty arrowheads in (B1) indicate apical Dlg punctas. (E) Quantifications for the A/B ratios of Dlg immunoreactivities in SG cells for genotypes in (A–D).

could explain the transition of Dlg re-localization in SG cells. First, in the proximal SG cells, newly synthesized Dlg is transported to and maintained in the apical membrane. In the distal cells, however, the newly synthesized Dlg is directed toward the basal membrane. Apically deposited Dlg has to be depleted in the transition zone cells, which could be mediated through the endocytic and lysosomal degradative pathway. An alternatively model is that newly synthesized Dlg is targeted to the apical membrane in all SG cells. However, the apical membrane-associated Dlg is re-routed to the basolateral membrane domains, starting in the transition zone cells and continuing in distal cells, via endocytosis and transcytosis. This model could explicitly explain the Dlg mislocalization in the mutants we have analyzed. Due to defects in endocytosis in the AP-2 complex mutants, the Dlg protein is retained in the apical membrane. In AP-1 mutants such as the $AP47^{SHE11/EP1112}$ transheterozygotes, large Dlg-enriched tubular structures were observed. Similar structures have been reported as specific stages, types, or structures of endosomes [29–31]. Therefore, in the absence of AP-1 activity, failure in vesicle budding from TGN or endosomes may lead to the disruption of targeting Dlg to the basal membrane and Dlg retaining in these organelles. Enrichment of Dlg in apical membrane might be an inadvertently result caused by abnormal intracellular transportation or a pool of Dlg being recycled back to the apical membrane through an AP-1-independent route.

In *nak* mutant SG cells, only the transition of Dlg is defected, but not other polarized proteins, such as aPKC, Syx1A, and Arm (Fig. 2 and data not shown). In the severe *AP47* and *α-ada* mutants when Dlg was retained at the apical membrane, the ruffled apical membrane might result from the enriched Dlg protein, leading to excess membrane addition. Such phenotype has been described when Dlg was overexpressed during embryonic cellularization [32]. Therefore, the Dlg transition is potentially accounted for the establishment of new septate junctions or basolateral membrane addition during SG cell maturation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.02.139.

References

[1] U. Tepass, G. Tanentzapf, R. Ward, R. Fehon, Epithelial cell polarity and cell junctions in Drosophila, Annu. Rev. Genet. 35 (2001) 747–784.
[2] E. Knust, O. Bossinger, Composition and formation of intercellular junctions in epithelial cells, Science 298 (2002) 1955–1959.
[3] M. Georgiou, E. Marinari, J. Burden, B. Baum, Cdc42, Par6, and aPKC regulate Arp2/3-mediated endocytosis to control local adherens junction stability, Curr. Biol. 18 (2008) 1631–1638.
[4] C. D'Souza-Schorey, Disassembling adherens junctions: breaking up is hard to do, Trends Cell Biol. 15 (2005) 19–26.
[5] A. Leibfried, Y. Bellaiche, Functions of endosomal trafficking in Drosophila epithelial cells, Curr. Opin. Cell Biol. 19 (2007) 446–452.
[6] H. Folsch, M. Pypaert, S. Maday, L. Pelletier, I. Mellman, The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains, J. Cell Biol. 163 (2003) 351–362.
[7] Y. Gan, T.E. McGraw, E. Rodriguez-Boulan, The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane, Nat. Cell Biol. 4 (2002) 605–609.

[8] L.M. Traub, G. Apodaca, AP-1B: polarized sorting at the endosome, Nat. Cell Biol. 5 (2003) 1045–1047.
[9] A. Icking, M. Amaddii, M. Ruonala, S. Honing, R. Tikkanen, Polarized transport of Alzheimer amyloid precursor protein is mediated by adaptor protein complex AP1-1B, Traffic 8 (2007) 285–296.
[10] M. Boehm, J.S. Bonifacino, Adaptins: the final recount, Mol. Biol. Cell 12 (2001) 2907–2920.
[11] E. Smythe, K.R. Ayscough, The Ark1/Prk1 family of protein kinases. Regulators of endocytosis and the actin skeleton, EMBO Rep. 4 (2003) 246–251.
[12] G. Zeng, M. Cai, Regulation of the actin cytoskeleton organization in yeast by a novel serine/threonine kinase Prk1p, J. Cell Biol. 144 (1999) 71–82.
[13] G. Zeng, X. Yu, M. Cai, Regulation of yeast actin cytoskeleton-regulatory complex Pan1p/Sla1p/End3p by serine/threonine kinase Prk1p, Mol. Biol. Cell 12 (2001) 3759–3772.
[14] S.D. Conner, S.L. Schmid, Differential requirements for AP-2 in clathrin-mediated endocytosis, J. Cell Biol. 162 (2003) 773–779.
[15] D.M. Henderson, S.D. Conner, A novel AAK1 splice variant functions at multiple steps of the endocytic pathway, Mol. Biol. Cell 18 (2007) 2698–2706.
[16] C.X. Zhang, A.E. Engqvist-Goldstein, S. Carreno, D.J. Owen, E. Smythe, D.G. Drubin, Multiple roles for cyclin G-associated kinase in clathrin-mediated sorting events, Traffic 6 (2005) 1103–1113.
[17] S. Kametaka, K. Moriyama, P.V. Burgos, E. Eisenberg, L.E. Greene, R. Mattera, J.S. Bonifacino, Canonical interaction of cyclin G associated kinase with adaptor protein 1 regulates lysosomal enzyme sorting, Mol. Biol. Cell 18 (2007) 2991–3001.
[18] D.W. Lee, X. Zhao, F. Zhang, E. Eisenberg, L.E. Greene, Depletion of GAK/auxilin 2 inhibits receptor-mediated endocytosis and recruitment of both clathrin and clathrin adaptors, J. Cell Sci. 118 (2005) 4311–4321.
[19] A. Umeda, A. Meyerholz, E. Ungewickell, Identification of the universal cofactor (auxilin 2) in clathrin coat dissociation, Eur. J. Cell Biol. 79 (2000) 336–342.
[20] D. Ricotta, S.D. Conner, S.L. Schmid, K. von Figura, S. Honing, Phosphorylation of the AP2 mu subunit by AAK1 mediates high affinity binding to membrane protein sorting signals, J. Cell Biol. 156 (2002) 791–795.
[21] S.D. Conner, S.L. Schmid, Identification of an adaptor-associated kinase, AAK1, as a regulator of clathrin-mediated endocytosis, J. Cell Biol. 156 (2002) 921–929.
[22] P. Ghosh, S. Kornfeld, AP-1 binding to sorting signals and release from clathrin-coated vesicles is regulated by phosphorylation, J. Cell Biol. 160 (2003) 699–708.
[23] E.B. Sorensen, S.D. Conner, AAK1 regulates Numb function at an early step in clathrin-mediated endocytosis, Traffic 9 (2008) 1791–1800.
[24] M. Gonzalez-Gaitan, H. Jackle, Role of Drosophila alpha-adaptin in presynaptic vesicle recycling, Cell 88 (1997) 767–776.
[25] A.C. Tien, H.Y. Hsei, C.T. Chien, Dynamic expression and cellular localization of the Drosophila 14-3-3epsilon during embryonic development, Mech. Dev. 81 (1999) 209–212.
[26] D.F. Woods, P.J. Bryant, The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions, Cell 66 (1991) 451–464.
[27] D.F. Woods, C. Hough, D. Peel, G. Callaini, P.J. Bryant, Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia, J. Cell Biol. 134 (1996) 1469–1482.
[28] I.F. Zhimulev, E.S. Belyaeva, V.F. Semeshin, V.V. Shloma, I.V. Makunin, E.I. Volkova, Overexpression of the SuUR gene induces reversible modifications at pericentric, telomeric and intercalary heterochromatin of *Drosophila melanogaster* polytene chromosomes, J. Cell Sci. 116 (2003) 169–176.
[29] D. Hernandez-Deviez, A. Mackay-Sim, J.M. Wilson, A role for ARF6 and ARNO in the regulation of endosomal dynamics in neurons, Traffic 8 (2007) 1750–1764.
[30] J.G. Lock, L.A. Hammond, F. Houghton, P.A. Gleeson, J.L. Stow, E-cadherin transport from the trans-Golgi network in tubulovesicular carriers is selectively regulated by golgin-97, Traffic 6 (2005) 1142–1156.
[31] C.J. Traer et al., SNX4 coordinates endosomal sorting of TfnR with dynein-mediated transport into the endocytic recycling compartment, Nat. Cell Biol. 9 (2007) 1370–1380.
[32] O.K. Lee, K.K. Frese, J.S. James, D. Chadda, Z.H. Chen, R.T. Javier, K.O. Cho, Discs-large and strabismus are functionally linked to plasma membrane formation, Nat. Cell Biol. 5 (2003) 987–993.