View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Developmental Cell, Vol. 9, 351–363, September, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.06.010

Sec15, a Component of the Exocyst, Promotes Notch Signaling during the Asymmetric Division of *Drosophila* Sensory Organ Precursors

Hamed Jafar-Nejad,[1,2] Hillary K. Andrews,[3] Melih Acar,[3] Vafa Bayat,[1] Frederik Wirtz-Peitz,[4] Sunil Q. Mehta,[3] Juergen A. Knoblich,[4] and Hugo J. Bellen[1,2,3,*]

[1]Department of Molecular and Human Genetics
[2]Howard Hughes Medical Institute
[3]Program in Developmental Biology
Baylor College of Medicine
One Baylor Plaza
Houston, Texas 77030
[4]Institute of Molecular Biotechnology of the Austrian Academy of Sciences
Dr. Bohr-Gasse 3-5
1030 Vienna
Austria

Summary

Asymmetric division of sensory organ precursors (SOPs) in *Drosophila* generates different cell types of the mature sensory organ. In a genetic screen designed to identify novel players in this process, we have isolated a mutation in *Drosophila sec15*, which encodes a component of the exocyst, an evolutionarily conserved complex implicated in intracellular vesicle transport. *sec15*$^-$ sensory organs contain extra neurons at the expense of support cells, a phenotype consistent with loss of Notch signaling. A vesicular compartment containing Notch, Sanpodo, and endocytosed Delta accumulates in basal areas of mutant SOPs. Based on the dynamic traffic of Sec15, its colocalization with the recycling endosomal marker Rab11, and the aberrant distribution of Rab11 in *sec15* clones, we propose that a defect in Delta recycling causes cell fate transformation in *sec15*$^-$ sensory lineages. Our data indicate that Sec15 mediates a specific vesicle trafficking event to ensure proper neuronal fate specification in *Drosophila*.

Introduction

Asymmetric cell division is a conserved mechanism by which metazoan organisms acquire cells of different developmental fates from single progenitor cells (Betschinger and Knoblich, 2004). One of the best-established models to study this process is the generation of the mechanosensory bristles of the *Drosophila* notum, which form through a series of such asymmetric divisions. Each bristle develops from a single cell called the sensory organ precursor (SOP or pI), which is selected through Notch (N)-mediated lateral inhibition at ~8–12 hr after puparium formation (8–12 hr APF) (Hartenstein and Posakony, 1989). At ~15 hr APF, pI cells divide to create the anterior pIIb and the posterior pIIa cells. The pIIa divides to create the external cells of the sensory organ, the hair and socket cells. The pIIb divides twice to create the internal cells of the sensory organ, the neuron and sheath cells, and a glial cell that later migrates away from the cluster and undergoes apoptosis (Bardin et al., 2004; Gho et al., 1999). In order for these asymmetric divisions to produce the proper complement of cells in each sensory cluster, two events must occur: asymmetric inheritance of cell fate determinants like Numb and Neuralized (Neur) by only one of the two pI progeny, and intercellular communication between the pIIa and the pIIb via N signaling (Bardin et al., 2004; Le Borgne and Schweisguth, 2003b; Rhyu et al., 1994; Roegiers and Jan, 2004).

N signaling is an evolutionarily conserved mechanism for intercellular communication that is employed in numerous developmental processes, including fate determination of the pI progeny (Lai, 2004; Schweisguth, 2004). Loss of N signaling results in a pIIa to pIIb transformation. N undergoes a series of proteolytic cleavages before its active part, the N intracellular domain, is released from the membrane to enter the nucleus and activate transcription. Binding of N to its ligands Delta (Dl) or Serrate triggers the S2 cleavage (Lieber et al., 2002), which leaves an activated form of N tethered to the membrane. Finally, the S3 cleavage at an intramembranous site releases the N intracellular domain from the membrane (De Strooper et al., 1999; Hu et al., 2002).

Endocytosis and intracellular vesicle trafficking are emerging as key regulators of several developmental signaling pathways, including the N pathway (Di Fiore and De Camilli, 2001; Gonzalez-Gaitan, 2003; Le Borgne and Schweisguth, 2003a; Seto et al., 2002). Analysis of the mutant phenotype associated with *Drosophila dynamin (shibire)* has shown that endocytosis is required in both signal-sending and signal-receiving cells during N signaling (Seugnet et al., 1997). Also, both Numb and Neur are involved in endocytosis (Betschinger and Knoblich, 2004). Upon pI division, both proteins are segregated into the anterior pIIb cell (Knoblich et al., 1995; Le Borgne and Schweisguth, 2003b). Loss- and gain-of-function studies have suggested that Numb acts to antagonize N signaling in the pIIb cell (Rhyu et al., 1994; Uemura et al., 1989). Numb directly binds N and is linked to the endocytic machinery via binding to α-Adaptin (Berdnik et al., 2002; Guo et al., 1996). Numb also binds Sanpodo (Spdo), a four-pass transmembrane protein that binds N and is proposed to promote N signaling at the membrane of the signal-receiving cell (O'Connor-Giles and Skeath, 2003). Together, these observations have led to the model that by linking N or Spdo to the endocytic machinery, Numb mediates their removal from the pIIb membrane, preventing the reception of N signaling by this cell (Berdnik et al., 2002; O'Connor-Giles and Skeath, 2003; Roegiers and Jan, 2004).

Loss-of-function mutations in *neur* lead to a pIIa to pIIb transformation phenotype, opposite to the *numb* loss-of-function phenotype (Yeh et al., 2000). Neur is an E3 ubiquitin ligase that interacts with and monoubiquitinates Dl on its cytoplasmic domain (Lai et al., 2001;

*Correspondence: hbellen@bcm.tmc.edu

Pavlopoulos et al., 2001; Yeh et al., 2001). Unequal segregation of Neur into the pIIb cell promotes the endocytosis of Dl in this cell, a process shown to be required for the N signaling to occur properly between the pIIb and pIIa cells (Le Borgne and Schweisguth, 2003b; Parks et al., 2000). Although the fate of endocytosed Dl is not clear, recent reports have provided strong evidence that *Drosophila* Epsin, a cargo-selective endocytic adaptor protein encoded by *liquid facets*, promotes the internalization of monoubiquitinated Dl and is required for signaling in a nonautonomous manner (Overstreet et al., 2004; Tian et al., 2004; Wang and Struhl, 2004, 2005). It has been proposed that Epsin targets the endocytosed Dl to a specific recycling compartment, where Dl becomes competent to activate N (Wang and Struhl, 2004).

In a genetic screen designed to identify novel players in *Drosophila* sensory organ development, we have isolated a mutation causing a pIIa to pIIb transformation phenotype in *sec15*. Sec15 is a component of a multiprotein complex called the exocyst or Sec6/8 complex (Grindstaff et al., 1998; Hsu et al., 2004; Novick et al., 1980). Mutations in exocyst components were originally isolated in a yeast screen for secretion-defective mutants (Novick et al., 1980). Subsequent analysis of the exocyst complex in yeast and mammalian cell culture systems has indicated that it functions in intracellular vesicle transport (Grindstaff et al., 1998; TerBush and Novick, 1995). In yeast, the exocyst mediates the post-Golgi to membrane targeting of exocytic cargo via an interaction with the Rab GTPase Sec4p (Guo et al., 1999). In Madin-Darby canine kidney (MDCK) epithelial cells, the exocyst localizes to areas of cell-cell contact and is involved in basolateral delivery of vesicles (Grindstaff et al., 1998). However, none of the studies on the exocyst components have implicated these proteins in cell fate determination (Friedrich et al., 1997; Mehta et al., 2005; Murthy et al., 2003, 2005; Murthy and Schwarz, 2004). Our data suggest that Sec15 mediates highly specific intracellular trafficking events that promote N signaling and thereby ensure proper cell fate specification in *Drosophila* mechanosensory organs.

Results

Mutations in *sec15* Result in a pIIa to pIIb Transformation in *Drosophila* External Sensory Organ Lineages

To identify novel genes involved in specification of adult bristle lineages, we performed an F1 mitotic recombination screen (see Experimental Procedures). One of the complementation groups isolated in this screen, consisting of two homozygous lethal alleles, caused severe bristle loss in clones (Figure 1A). Although the majority of bristles fail to form in mutant clones, occasional mutant sensory organs are observed, as evidenced by the *yellow*$^{-}$ *Stubble*$^{+}$ (*y*$^{-}$ *Sb*$^{+}$) bristles (Figure 1B, stars). The bristle loss in this complementation group does not result from a failure in pI specification, as staining pupae at 12 hr APF for the pI marker Senseless (Jafar-Nejad et al., 2003; Nolo et al., 2000) shows that pIs form and divide in the mutant tissue (data not shown). Wild-type (wt) sensory clusters contain one sheath cell and one neuron at 24 hr APF (Figure 1C, left cluster; Berdnik et al., 2002). However, many mutant clusters contain 4–6 neurons and 0–2 sheath cells (Figure 1C, right cluster). Also, the majority of mutant sensory clusters do not contain a socket cell, as evidenced by lack of Su(H) staining (Figures 1D and 1E; Gho et al., 1996). Together, these data suggest a pIIa to pIIb fate transformation. Finally, double-staining for Cut and the neuronal marker ELAV or the sheath marker Prospero (Pros) shows that in the majority of mutant sensory clusters, all Cut^{+} cells stain with internal cell markers (Figures 1F–1G′). Quantification of the ratio of mutant clusters with multiple neurons and no sockets to those with one socket indicates that 71% of mutant sensory organs have external-to-internal fate transformation (n = 109 clusters) (Figures 1H and 1I).

Using a recombination-based mapping strategy (Zhai et al., 2003), we mapped the lethality of our alleles to cytological region 93B. We noticed that our alleles failed to complement the lethality of mutations in *Drosophila sec15*, which we have recently isolated in an independent *eyFLP* synaptic development screen (Mehta et al., 2005). Sequencing revealed that both alleles contain the same nonsense mutation in *sec15*, a stop codon at amino acid Q563 (Figure 1J). Hence, the two mutations are clonal in nature. The other two alleles isolated by Mehta et al. (2005) have earlier stop codons at N135 (*sec15*^{1}) and Q226 (*sec15*^{2}) (Figure 1J). We therefore named our allele *sec15*^{3}. *sec15*^{1} and *sec15*^{2} also showed loss of most, but not all, sensory organs in mutant clones (Figure 1K and data not shown). Quantification of *sec15*^{1} clones marked with cell fate markers at 24 hr APF showed a pIIa to pIIb transformation in 69% of the mutant sensory organs (n = 28 clusters), indicating that the three alleles have the same PNS phenotype. To ensure that the bristle phenotype associated with the *sec15*^{3} chromosome is due to the lesion in *sec15*, we overexpressed the *sec15* cDNA in mutant clones and observed a full rescue of the adult bristle phenotype (Figure 1L). In addition, a 5 kb genomic transgene containing *sec15* rescues the lethality of all *sec15* alleles. Together, these data demonstrate that a mutation in *sec15* underlies the peripheral nervous system (PNS) phenotype of the *sec15*^{3} clones.

sec15 and *spdo* Function in the Same Pathway to Specify pIIa versus pIIb Fates

Since the exocyst complex has been implicated in targeted vesicle transport and also in epithelial polarity (Grindstaff et al., 1998; Novick et al., 1980), we hypothesized that loss of *sec15* function might disrupt asymmetric localization and unequal inheritance of the cell fate determinants Numb and Neur. However, staining of pupae harboring mutant clones showed that both proteins are asymmetrically localized in the dividing pI cells and segregate properly into the pI progeny (see Figure S1 in the Supplemental Data available with this article online and data not shown). These data strongly suggest that a defect in the localization of Numb and Neur cannot account for the pIIa to pIIb transformation in *sec15* clones.

As the *sec15* mutant phenotype is in part similar to the *N* loss-of-function phenotype (Hartenstein and Po-

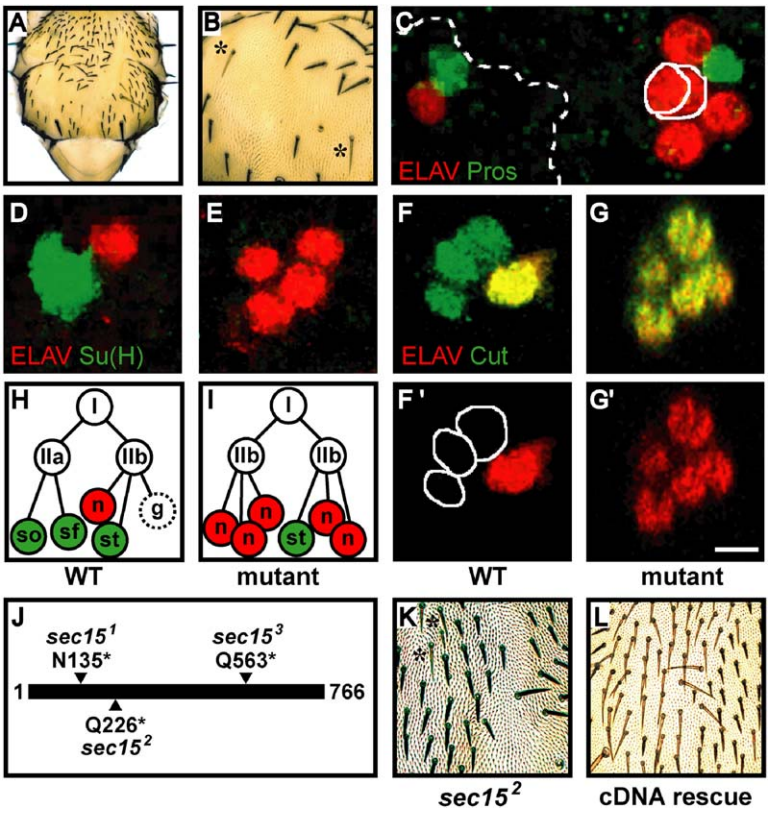

Figure 1. *sec15* Mutations Cause a pIIa to pIIb Transformation in the SOP Lineage

(A and B) Thorax of an adult fly with clones of a mutation isolated in a mosaic PNS screen. Stars in the close-up (B) show the occasional mutant bristles, which are y^- Sb^+.

(C) Part of the notum of a 24–26 hr APF pupa harboring a mutant clone stained for ELAV (red) and Pros (green). The homozygous mutant tissue is to the right of the dashed line. Note the extra neurons in the mutant clone.

(D and E) A wt (D) and a mutant (E) sensory cluster at 24–26 hr APF stained for ELAV (red) and Su(H) (green). Note the absence of a socket precursor in (E).

(F–G′) At 27–28 hr APF, all cells in a wt sensory cluster (F and F′) express Cut (green), but only the neuron expresses ELAV (red). However, in a mutant cluster (G and G′), all cells coexpress Cut (green) and ELAV (red), indicating the absence of shaft and socket precursors. The scale bar in (G′) is 5 μm and applies to all staining panels.

(H and I) Schematic of a typical sensory organ lineage in wt (H) and the mutant under study (I). In accordance with panels (C)–(G′), neuronal precursors are marked red. so, socket; sf, shaft; n, neuron; st, sheath; g, glia.

(J) Position of the stop codons in *sec15* alleles mapped on the full-length protein.

(K) Closeup of a $sec15^2$ clone with bristle loss and occasional y^- Sb^+ mutant bristles (stars).

(L) Expression of the wt *sec15* cDNA in a large marked clone in thorax fully rescues the bristle loss phenotype. The rescued bristles are y^- Sb^+, whereas the wt bristles are y^+ Sb^-.

sakony, 1990), we determined the epistatic relationship between *N* and *sec15*. We overexpressed a constitutively active membrane bound version of N that lacks the extracellular domain (N^{ECN}) in *sec15* clones using $sca^{109\text{-}68}$*-GAL4* (Jafar-Nejad et al., 2003; Struhl et al., 1993). N^{ECN} does not require ligand binding, but needs S3 cleavage to release the intracellular domain of N (Lai, 2004; Schweisguth, 2004). $sca^{109\text{-}68}$*-GAL4* drives N^{ECN} expression in pIs and their progeny, as well as a few surrounding epidermal cells, resulting in a pIIb to pIIa transformation and generation of extra socket cells in the majority of the sensory organs, although some sensory organs are lost, presumably due to a failure in pI formation (Figure 2A). *sec15* clones were generated using the *Ubx-FLP* transgene, which generates large clones in the thorax. In the 25 animals with *Ubx-FLP*-generated *sec15* clones and N^{ECN} overexpression that we examined, none exhibited regions of naked cuticle, which are evident in *sec15* clones (compare Figures 2B and 2C). The reversal of the pIIa to pIIb transformation phenotype of *sec15* by N^{ECN} indicates that N^{ECN} is epistatic to *sec15.* This places *sec15* upstream of the S3 cleavage of N.

One gene previously shown to be genetically downstream of *numb* and upstream of the S3 cleavage of N is *spdo* (Dye et al., 1998; O'Connor-Giles and Skeath, 2003; Skeath and Doe, 1998). Interestingly, *spdo* mutations result in pIIa to pIIb transformation in the embryonic PNS, similar to what we observe in the adult PNS of *sec15* clones. To establish whether *spdo* mutations cause a similar phenotype in the adult PNS, we generated marked *spdo* clones and observed that loss of *spdo* causes bald patches in the thorax (Figure 2D). Similar to *sec15* clones, some mutant y^- Sb^+ bristles form in *spdo* clones. Staining for cell fate markers shows a pIIa to pIIb transformation in about 47% of $spdo^-$ sensory clusters (n = 53 clusters).

As neither *sec15* nor *spdo* mutations cause a fully penetrant phenotype, we generated $sec15^-$ $spdo^-$ double mutant clones, arguing that if the two genes function in parallel pathways, loss of both genes should result in a more severe bristle loss. However, $sec15^-$ $spdo^-$ clones are indistinguishable from $sec15^-$ clones, as they exhibit loss of most, but not all, sensory organs (Figure 2E). These data indicate that *sec15* and *spdo* function in the same pathway during SOP cell fate determination.

Spdo Is Upregulated and Mislocalized in $sec15^-$ SOPs

Because *sec15* and *spdo* seem to function in the same pathway, we compared the subcellular distribution of Spdo in wt and $sec15^-$ sensory lineages using antibodies generated against the intracellular region of Spdo (O'Connor-Giles and Skeath, 2003). In wt pupal nota, Spdo is expressed only in pIs and their progeny (Figures 2F–2H). In pIs, Spdo is found in bright cytoplasmic vesicles. It also shows a diffuse low-level expression in

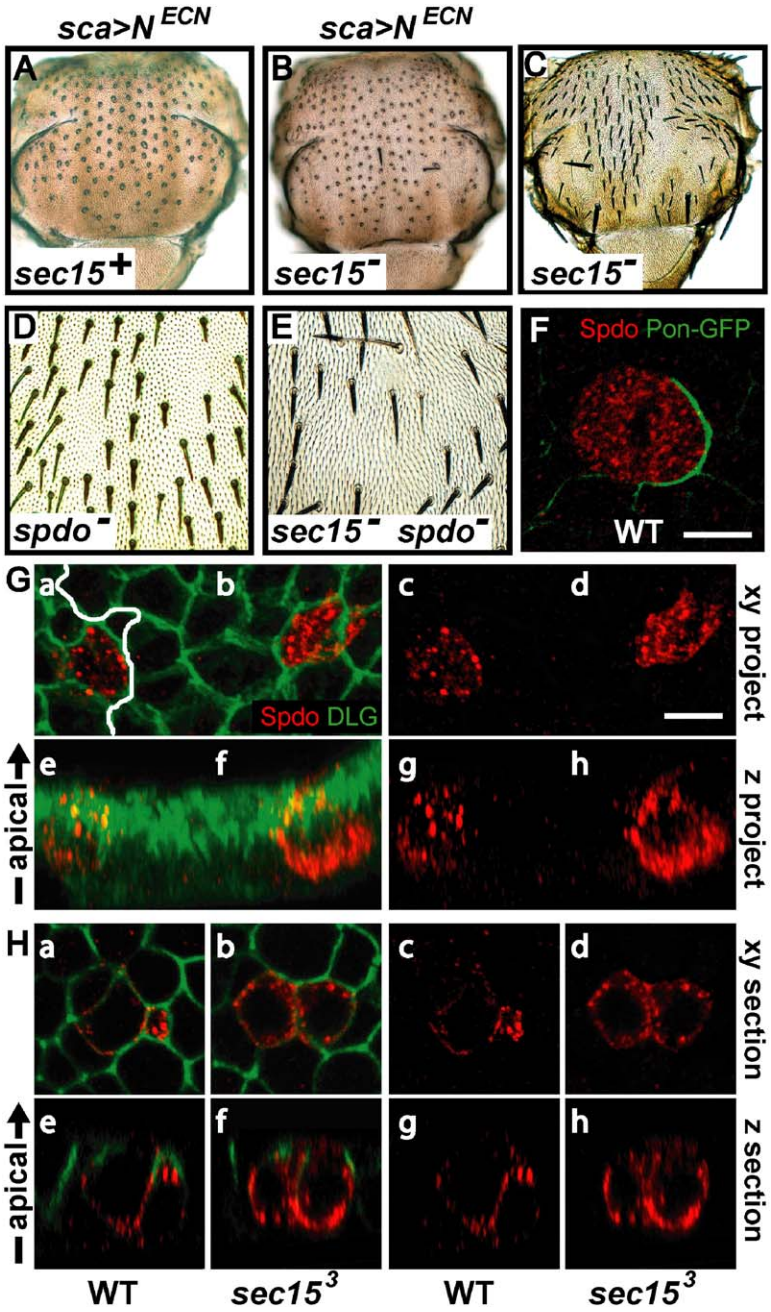

Figure 2. Spdo Is Upregulated and Mislocalized in *sec15* Clones

(A–C) *sec15* functions upstream of the N^{ECN} in the asymmetric division of the pI cell.

(A) Overexpression of the N^{ECN} in presumptive pI and its progeny using the $sca^{109\text{-}68}$-*GAL4* driver results in a multiple-socket phenotype in the majority of sensory organs.

(B) Thorax of a fly of the following genotype: *y w/y w Ubx-FLP;* $sca^{109\text{-}68}$*-GAL4/UAS-*N^{ECN}*; FRT82B pM* Sb^{63} y^{+} ry^{+}*/FRT82B* $sec15^{3}$.

(C) Note that we never observe a region of bristle loss comparable to those observed in *sec15* clones generated with the same driver at the same temperature.

(D) A $spdo^{G104}$ clone in the thorax. Note the bristle loss and also the presence of several y^{-} Sb^{+} mutant bristles.

(E) A $sec15^{3}$ $spdo^{G104}$ double mutant clone in thorax. Note the bristle loss and also the presence of occasional y^{-} Sb^{+} double mutant bristles.

(F) A single confocal section of a dividing pI of the *sca-GAL4 UAS-Pon::GFP* genotype. Note that Spdo (red) is uniformly distributed throughout the cell, compared to the anterior crescent of the Pon-GFP (green).

(G) Maximum projection views of xy (a–d) and z (e–h) sections of a wt (a, c, e, g) and a $sec15^{3}$ (b, d, f, h) pI cell stained for Spdo (red) and DLG (green). Apical is top in the z projections. The scale bar in (d) is 5 μm and applies to all panels in (G) and (H). Note the significant increase in Spdo staining, as well as its basal accumulation in the mutant pI.

(H) Single confocal xy (a–d) and z (e–h) sections of a wt (a, c, e, g) and a $sec15^{3}$ (b, d, f, h) sensory cluster at 2-cell stage stained for Spdo (red) and DLG (green). Anterior is to the right in all panels, apical is top in the z sections. Note that Spdo marks the membrane of the wt posterior pIIa cell but accumulates in a few cytoplasmic vesicles at the level of the septate junction in the anterior pIIb cell (a, c, e, g). Note the upregulation and mislocalization of Spdo in mutant pI progeny (b, d, f, h).

the cytoplasm, although a fraction seems to be associated with the plasma membrane (Figures 2F and 2G, a, c, e, g). The $Spdo^{+}$ vesicles are mainly found at or above the level of septate junctions, which are visualized by Discs large (DLG) staining (Figure 2G, e, g; Bilder et al., 2000). During mitotic division, Spdo is found in cytoplasmic puncta throughout the cell, with no antero-posterior or apico-basal preference (Figure 2F). After division, Spdo shows a differential distribution in pIIa and pIIb cells (Figure 2H, a, c, e, g). Similar to the pattern reported for the embryonic neuroblast progeny (O'Connor-Giles and Skeath, 2003), Spdo generally marks the membrane of pIIa, but in pIIb, Spdo is mainly found in cytoplasmic puncta at or above the DLG staining (Figure 2H, e, g). Only occasionally can we detect a cytoplasmic punctum in the pIIa or basally localized $Spdo^{+}$ puncta in pIIb (data not shown).

In *sec15* clones, there is a strong upregulation and mislocalization of Spdo (Figures 2G and 2H). In mutant pIs, many more $Spdo^{+}$ puncta are found (Figure 2G, b, d, f, h). Unlike wt pIs, the majority of these puncta are basally localized, in proximity to the membrane (Figure 2G, f, h). Upregulation of Spdo is also evident in the mutant pI progeny, both of which show many $Spdo^{+}$ puncta close to the membrane as well as an enhanced membrane staining (Figure 2H, b, d, f, h). Similar to the mutant pIs, much more Spdo accumulates at the basal side of the pIIa/pIIb than at the apical side (Figure 2H,

f, h). Quantification of the total intensity of Spdo staining in 3D shows that mutant pIIa/pIIb contain 12.1 ± 1.8 times more Spdo than wt cells (n = 6). We conclude that loss of *sec15* results in accumulation and aberrant subcellular localization of Spdo in pIs and their progeny.

As mentioned earlier, the loss-of-function phenotypes of *sec15* and *spdo* are similar. However, there is more Spdo in *sec15*$^{-}$ SOPs. To establish if increasing Spdo levels in sensory precursors causes a fate specification phenotype, we overexpressed *spdo* in pIs and their progeny using *sca*$^{109\text{-}68}$*-GAL4* and also in random heat shock-induced marked clones using the Flp-out technique (Struhl and Basler, 1993). In neither case did *spdo* overexpression cause any fate change in the pI progeny (Figures S2A and S2B). Hence, our data suggest that the fate change in *sec15* pI progeny does not result from elevated levels of Spdo and that a defect in Spdo trafficking mediated by the loss of *sec15* is involved in the pIIa to pIIb transformation.

Spdo Colocalizes with N and Dl in Wild-Type and *sec15*$^{-}$ SOPs

In the embryonic CNS, Spdo is reported to colocalize with N and Dl (O'Connor-Giles and Skeath, 2003). We therefore examined the distribution of Dl, Spdo, and N in wt and mutant SOPs. In wt pI and pIIb cells, 80%–90% of the large Spdo^{+} puncta are positive for N and Dl (Figures 3A, 3B, 3D, and 3E, n = 12 cells for each). Also, there is some degree of overlap between the membrane stainings of N and Spdo in the pIIa (Figure 3B, stars). Similarly, the basally located Spdo in mutant sensory precursors largely colocalizes with both N and Dl (Figures 3C, 3D, and 3F), indicating a similar increase in the level of intracellular N and Dl. Also, N and Spdo colocalize along the membranes of the mutant pI progeny (Figure 3C, stars). Hence, Spdo colocalizes with Dl and N in both wt and mutant sensory precursors.

As endocytosis and recycling of Dl has been implicated in signaling (Le Borgne and Schweisguth, 2003a, 2003b; Wang and Struhl, 2004), we explored if upon endocytosis, Dl can be found inside the cell together with Spdo. We performed an endocytosis assay for Dl using the method established by Le Borgne and Schweisguth (2003b) and found that in both wt and mutant pI progeny, Spdo sometimes colocalizes with the internalized Dl (Figures 3G and 3H). Since in this assay the Dl-α-Dl^{ECD} complexes were allowed to undergo endocytosis for only 15–20 min (see Experimental Procedures), we cannot assess what fraction of the upregulated Dl in *sec15*$^{-}$ cells is of endocytic origin. It is worth mentioning that unlike wt sensory precursors, where the internalized Dl shows a random distribution, in *sec15*$^{-}$ SOPs, more than half of the internalized Dl puncta accumulate basally, close to the plasma membrane (Figures 3G and 3H). We conclude that Spdo colocalizes with endocytosed Dl in wt and mutant SOPs.

Sec15 Is Associated with a Vesicular Compartment that Exhibits Dynamic Trafficking in Both pIIa and pIIb Cells

To follow the distribution and traffic of Sec15 in SOPs, we expressed a GFP-tagged version of Sec15 in SOPs and performed live imaging during and after division. Expression of the GFP-tagged Sec15 is able to rescue the bristle phenotype in *sec15* mitotic clones, suggesting normal trafficking. Also, its overexpression using *sca*$^{109\text{-}68}$*-GAL4* or the Flp-out technique does not result in any SOP fate transformation (data not shown). A 2D movie in which the depth of structures is coded by a spectrum of colors reveals that Sec15 resides in a dynamic vesicular compartment that mainly traffics between apical and subapical regions of pI and its progeny (Movie S1). Shortly after the end of cytokinesis, a vesicular structure forms subapically in both pI progeny and quickly moves toward apical regions as the cell finishes mitotic division. A movie generated from projections of a similar experiment performed on a pI cell coexpressing Pon-RFP and GFP-Sec15 also shows the dynamic and symmetrical traffic of Sec15 in both pIIa and pIIb (Movie S2). To better visualize the dynamics of Sec15 traffic in SOPs, we generated 3D movies from the mitotic division of pIs expressing GFP-Sec15 and Histone-RFP, which marks the nucleus (Movie S3). Figures 4A–4E present snapshots of this 3D movie. Sec15 accumulates in rather large vesicular structures at the anterior/apical part of the pI during interphase (Figure 4A). As the cell enters mitotic division, the Sec15^{+} compartment becomes dispersed around the cell (Figure 4B). Shortly after division, vesicles containing Sec15 accumulate near the nucleus in both pIIa and pIIb (Figure 4C). In a few minutes, the Sec15^{+} perinuclear compartments begin to move toward the apical tips of pIIa and pIIb cells (Figures 4D and 4E). A fraction of the apical Sec15 compartment seems to continue moving back and forth between apical and subapical regions, suggesting a continued cross-talk between the two compartments (Movies S1 and S3). We also performed staining using an α-Sec15 antibody (Mehta et al., 2005) and observed a similar apical enrichment in interphase sensory precursors (Figure 4F). The dynamic trafficking of Sec15 between perinuclear and apical compartments suggests that Sec15 is involved in the apical trafficking of Spdo/Dl/N^{+} vesicles.

Apical-Basal Polarity Is Not Disrupted in *sec15* Clones

Traffic of Sec15 toward the apical areas of the SOPs and aberrant accumulation of Spdo/Dl/N at the basal side of the mutant SOPs suggests a possible defect in the apical-basal polarity in the absence of Sec15 function. To address this question, we stained pupal nota containing *sec15*^{3} clones with two adherens junction markers Armadillo (Arm) and E-Cadherin (E-Cad), which stain the apical cortex of the epidermal and sensory cells (Le Borgne et al., 2002; Tepass et al., 2000). As shown in Figures 4G and 4H, both proteins are localized normally in the apical region of mutant cells, suggesting that the polarity is established and that general apical protein transport is not impaired. In agreement with these observations, the apical staining of N is not reduced in *sec15*^{3} mutant cells (Figure 4I). Finally, the apical stalks of the pIIb cells (Le Borgne et al., 2002) are formed normally in *sec15* mutants, suggesting normal apical membrane trafficking (Figures 4J and 4K). Therefore, *sec15*^{3} does not cause a defect in the general apical transport machinery of the cells.

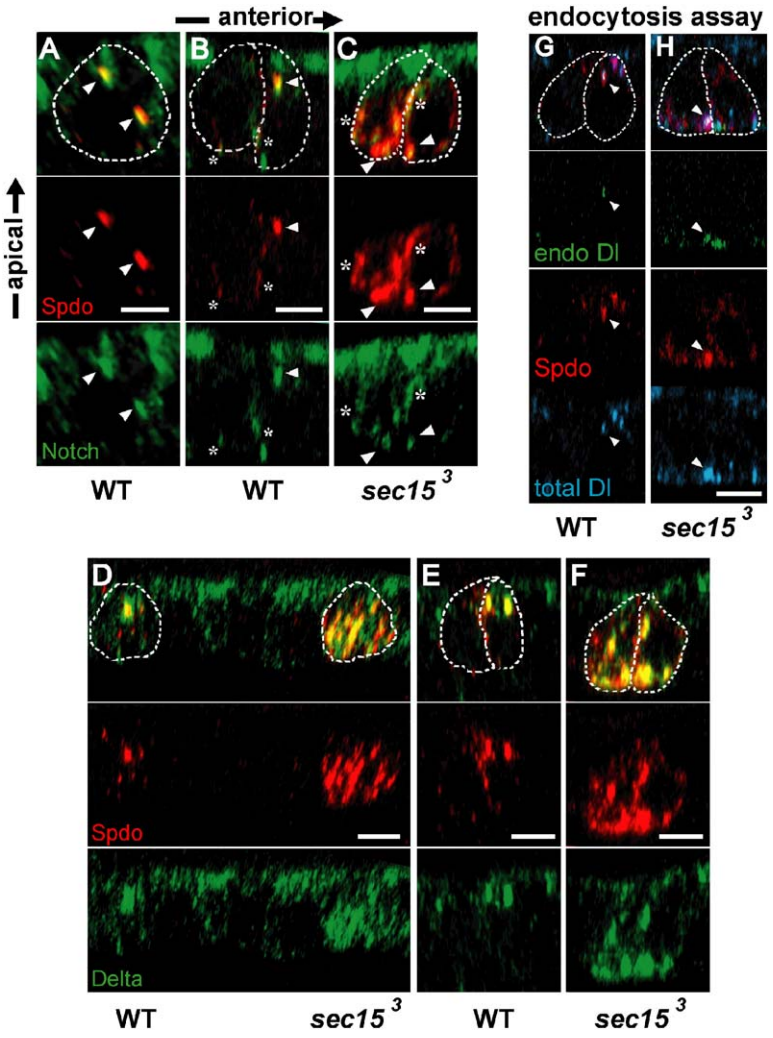

Figure 3. Spdo, N, and Dl Colocalize in Wild-Type and *sec15*^{3} SOPs

All panels are single z sections. Anterior is to the right, apical is top in all panels. Spdo is red in all panels; N (A–C) and Dl (D–F) are green. All scale bars are 5 μm.

(A) N and Spdo colocalize in cytoplasmic vesicles (arrows) in a wt pI cell.

(B) N and Spdo colocalize in a cytoplasmic vesicle (arrowhead) in the apical area of the anterior pIIb and also in smaller puncta (stars) that seem to be along the cell membrane.

(C) N and Spdo colocalize in cytoplasmic vesicles (arrowheads) and along the membranes (stars) of both cells of a *sec15*^{3} sensory cluster. Note the basal accumulation of proteins in mutant cells.

(D) Dl and Spdo colocalize in a large cytoplasmic vesicle in a wt pI cell (left side) as well as in multiple basally located vesicles in a mutant pI cell (right side).

(E and F) Dl and Spdo colocalize in two cytoplasmic vesicles of the pIIb cell of a wt sensory cluster (E) as well as in multiple vesicles in both cells of a *sec15*^{3} sensory cluster.

(G and H) Single z sections of a wt (G) and a *sec15*^{3} (H) 2-cell stage sensory cluster after an endocytosis assay. Internalized Dl-α-Dl^{ECD} complexes are evidenced by the colocalization of the α-Dl^{ECD} (green) with guinea pig α-Dl (blue). Spdo (red) colocalizes with endocytosed Dl in wt (arrowhead in [G]) and in *sec15*^{3} (arrowhead in [H]) SOPs.

The Spdo^{+} Vesicles Are Often Associated with Golgi

So far, our data suggest that Sec15 is involved in the transport of a specific vesicular compartment toward apical areas of the cell. We argue that since the Spdo/Dl/N^{+} vesicles accumulate in the basal parts of *sec15*$^{-}$ SOPs, identification of the nature of these vesicles might provide insight into the type of cargo in whose transport Sec15 is involved. Staining with an antibody raised against the Golgi-associated protein Lava lamp (Lva) (Sisson et al., 2000) shows that in both wt and mutant sensory precursors, many Spdo^{+} vesicles are closely associated with the Golgi apparatus, suggesting a biosynthetic nature (Figures 5A and 5B, arrowheads). Note that as the two sections shown in Figures 5A and 5B are at different levels in the cells, they give the impression that there is more Lva staining in the mutant pI. However, examining >15 stainings, each with several wt and mutant SOPs, indicates no aberration in Golgi staining intensity, morphology, or distribution in *sec15* clones (data not shown). Indeed, quantification of the total Lva staining in 3D did not indicate a significant difference between *sec15*$^{-}$ and wt cells. Association of Spdo/Dl/N^{+} vesicles with a Golgi marker suggests that Spdo transport is somehow blocked after exit from the *trans*-Golgi network (TGN). If Sec15 is involved in transport from the TGN, we expect to see some association between Sec15 and the Golgi, similar to previous reports for other exocyst components (Prigent et al., 2003; Xu et al., 2005; Yeaman et al., 2001). Indeed, staining pupal nota of the *neur-GAL4 UAS-GFP::sec15* genotype—which is also used in our live imaging experiments—for Lva shows some association between the Lva^{+} puncta and GFP-Sec15 vesicles (Figure 5C, arrowheads). These data are compatible with a role for Sec15 in transport of Spdo^{+} vesicles from the TGN.

Some Spdo^{+} Vesicles Colocalize with Endosomal Markers

It has been shown that the Dl/N^{+} vesicles in the pIIa/pIIb cells often colocalize with the endosomal marker Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) (Le Borgne and Schweisguth, 2003b; Lloyd et al., 2002). Therefore, in parallel to exploring the association of Spdo with a Golgi marker, we performed

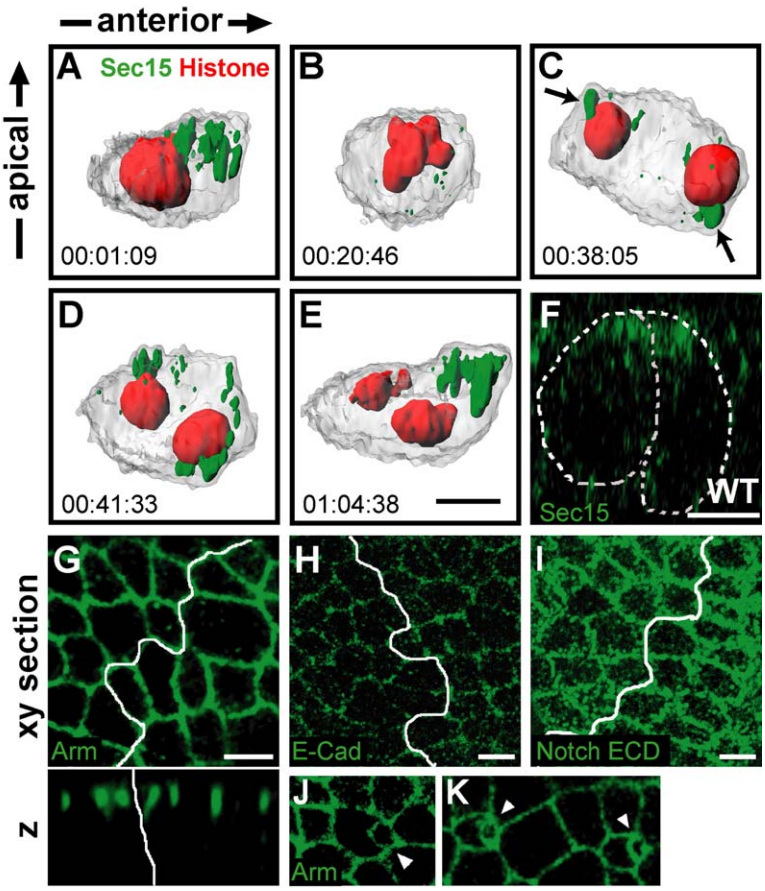

Figure 4. Apical-Basal Polarity Is Preserved in *sec15*^{3} Mutant Cells

(A–E) Snapshots of a live imaging experiment performed during and after the division of a pI cell expressing Histone-RFP (red) and GFP-Sec15 (green) under the control of *neur-GAL4*. The scale bar is 5 μm. Note that the perinuclear compartments (arrows in [C]) quickly travel toward the apical side of the cell (D). The start time was arbitrarily set during the interphase.

(F) A single z section of a wt sensory cluster at the 2-cell stage, stained with α-Sec15 antibody. Note the apical enrichment of Sec15. Anterior is to the right.

(G–I) The apical staining for Arm (G), E-Cad (H), and the extracellular domain (ECD) of N (I) are not affected in *sec15*^{3} tissue. The white lines show the clonal boundary, with mutant tissue to the right in all three panels. Note that Arm staining is restricted to the apical regions of the wt and mutant cells (lower section of [G]).

(J and K) Apical sections of wt (J) and *sec15*^{3} (K) 2-cell stage sensory clusters stained for Arm. Arrowheads: pIIb apical stalks.

colocalization studies for Spdo and the endosomal markers HRS and Rab5 (Lloyd et al., 2002; Wucherpfennig et al., 2003). We find that 40%–50% of the large Spdo^{+} puncta colocalize with HRS and Rab5 in both wt and *sec15*$^{-}$ SOPs (Figures 5D–5G, arrowheads), indicating the endosomal nature of this compartment. Note that Rab5 is somewhat enriched in the apical regions of wt and more so in the mutant SOPs (Figure 5G, star) but shows little colocalization apically with Spdo/N/Dl^{+} vesicles in the mutant cells, as anticipated from the predominantly basal localization of these vesicles.

Loss of *sec15* Function Results in Upregulation and Abnormal Distribution of the Recycling Endosomal Marker Rab11

It has recently been proposed that in order to become activated, Dl needs to be targeted to a specific endocytic recycling compartment (Wang and Struhl, 2004). The small GTPase protein Rab11 is associated with perinuclear recycling endosomes (Dollar et al., 2002; Pelissier et al., 2003; Ullrich et al., 1996). The asymmetric distribution of the Rab11^{+} recycling endosomal compartment in pI progeny has led to the proposal that by mediating the recycling of Dl in the pIIb but not in pIIa, Rab11 regulates pIIa/pIIb fate specification (Emery et al., 2005). Moreover, it has recently been reported that in mammalian cells, Sec15 binds Rab11 in a GTP-dependent manner and colocalizes with Rab11 to the perinuclear region (Zhang et al., 2004). In agreement with these observations, we find extensive colocalization between Rab11 and GFP-Sec15 in pI and its progeny (Figures 6A and 6B). Moreover, pupae harboring *sec15*^{3} clones show a strong upregulation of Rab11 (4.56 ± 0.47 times wt) at the apical side of all mutant cells in the thorax, except for cells that have recently divided (Figure 6C). Although there is occasional colocalization of Spdo and Rab11 in subapical regions of both wt (Figure 6D) and mutant (Figure 6E) sensory precursors, the aberrant Rab11^{+} compartment at the apical regions of mutant SOPs rarely colocalizes with Spdo. Altogether, these data suggest that intracellular trafficking of Spdo/N/Dl is abnormal in *sec15* clones due to a defect in the Rab11^{+} recycling endosomal compartment.

Discussion

The various cell types that form an adult sensory organ in *Drosophila* are generated via asymmetric divisions of a pI and its progeny. Differential activation of the N signaling pathway between the two daughter cells of each division ensures that each sensory organ acquires the proper complement of cell types necessary to function. Here we report that Sec15, a component of the evolutionarily conserved exocyst complex, is required for proper cell fate specification of the pI progeny. Our

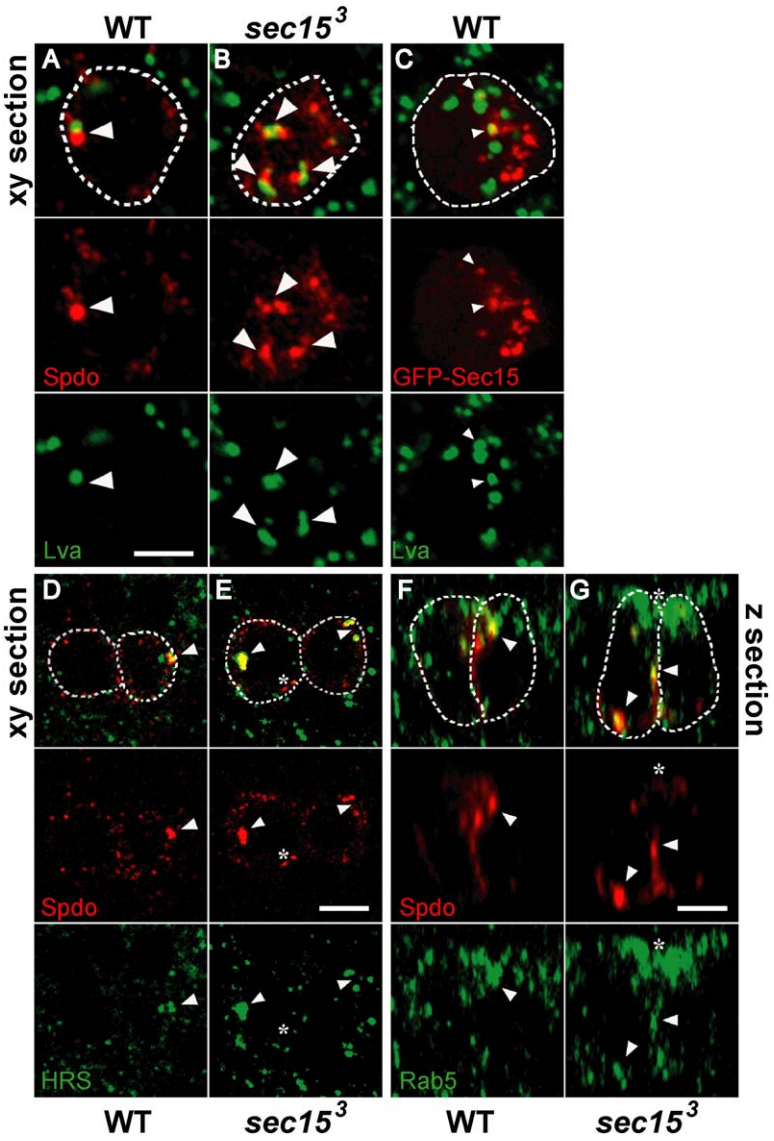

Figure 5. Spdo+ Vesicles Are Associated with the Golgi and Colocalize with Endosomal Markers in Both Wild-Type and *sec15*^{3} SOPs

(A and B) Single xy sections of a wt (A) and a *sec15*^{3} (B) pI cell stained for Spdo (red) and the Golgi marker Lva (green). The Spdo vesicles are often associated with the Golgi in wt and mutant cells (arrowhead). The scale bar is 5 μm in this and all other panels.

(C) Single xy section of a wt pI cell stained for Lva (green). GFP-Sec15 (red) is driven by *neur-GAL4.*

(D and E) Single xy sections of a wt (D) and a *sec15*^{3} (E) sensory cluster at 2-cell stage stained for Spdo (red) and HRS (green). Some of the Spdo+ vesicles colocalize with HRS in wt (arrowhead in [D]) and mutant (arrowheads in [E]) SOPs. Star in (E) shows two Spdo+ vesicles that do not colocalize with HRS. Anterior is to the right in (D)–(G).

(F and G) Single z sections of a wt (F) and a *sec15*^{3} (G) 2-cell sensory cluster stained for Spdo (red) and Rab5 (green). Some of the Spdo+ vesicles colocalize with Rab5 in wt (arrowhead in [F]) and mutant (arrowheads in [G]) SOPs. Note the apical enrichment of Rab5 in mutant compared to the wt SOPs (star in [G]).

studies on *sec15* mutations in the eye did not reveal any fate change in the photoreceptors (Mehta et al., 2005) . Loss-of-function mutations in three other exocyst components have been reported previously: *sec5* and *sec6* in flies and *sec8* in mice (Beronja et al., 2005; Friedrich et al., 1997; Murthy et al., 2003, 2005; Murthy and Schwarz, 2004). *sec8* mutant mice die at day E7.5, before the development of specific neuronal populations can be studied (Friedrich et al., 1997). Also, *sec5* and *sec6* mutations are cell lethal in the *Drosophila* eye (Beronja et al., 2005; Mehta et al., 2005; Murthy et al., 2003). Therefore, this report is to our knowledge the first to identify a role for an exocyst component in cell fate determination. At this point, we cannot predict if *sec5* and *sec6* also play a role in neuronal cell fate specification. However, given the data obtained from studies of the fly eye, we favor the hypothesis that components of the exocyst may form more than a single functional unit and/or have subunit-specific roles (Mehta et al., 2005; Wiederkehr et al., 2004).

Live imaging of dividing pI cells indicates that Sec15 is associated with a vesicular compartment that traffics between apical and subapical areas. In *sec15*$^-$ SOPs, we observe an expanded compartment that contains Spdo, N, and Dl (Figures 2 and 3). Unlike wt pI and pIIb cells, in which Spdo/N/Dl+ vesicles tend to reside at or above the level of septate junctions, in mutant SOPs these puncta accumulate at the basal side of the cell. Together, these observations suggest that Sec15 is involved in vesicle trafficking to the apical parts of the cell. The defect in the apical trafficking of proteins does not seem to be a general one, as localization of E-Cad and Arm at the adherens junction is not disrupted in mutant tissue. Therefore, our data link a specific vesicle trafficking event to a developmental decision made by sensory precursor cells.

Our genetic experiments and immunohistochemical stainings strongly suggest that Sec15 and Spdo function in the same pathway in sensory cell fate determination process (Figure 2). It has been proposed, based

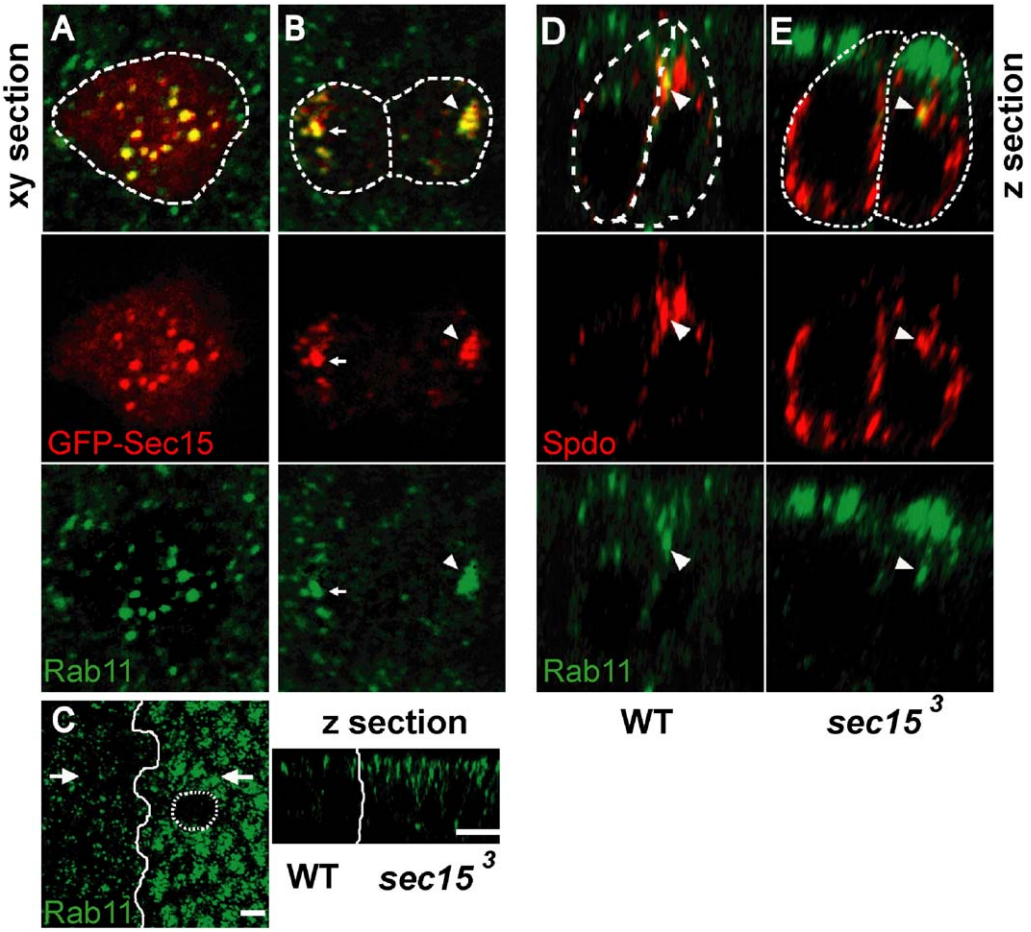

Figure 6. *sec15* Mutations Cause a Defect in the Recycling Endosomal Compartment

(A and B) GFP-Sec15 (red) and Rab11 (green) colocalize in pI (A) and pIIa/pIIb (B). Note that in perinuclear areas of both pIIa and pIIb, the two proteins colocalize, although there is more perinuclear Rab11 in pIIb (arrowhead) compared to the pIIa (arrow).

(C) Rab11 is upregulated and apically enriched in *sec15*^{3} cells. Staining of a 17–18 hr APF pupal notum harboring a *sec15*^{3} clone for Rab11 is shown. The mutant area is to the right of the white line. The z section is the region between the two arrows. The dashed circle shows a dividing cell in which Rab11 is not detected apically.

(D and E) There is little colocalization between Spdo (red) and Rab11 (green) in the apical region of mutant SOPs, although, similar to the wt pIIb, mutant pI progeny occasionally show colocalization of Rab11 and Spdo below the apical areas (arrows in [D] and [E]).

on studies performed on the asymmetric divisions of *Drosophila* embryonic neuroblasts, that Spdo promotes N signaling at the membrane of the signal-receiving cell. In contrast, Numb and α-Adaptin in the signal-sending cell might promote endocytosis of Spdo and its removal from the membrane, thereby preventing the reception of signal by this cell (O'Connor-Giles and Skeath, 2003). The subcellular distribution of Spdo in pIIa and pIIb cells is similar to its localization in embryonic neuroblast progeny, suggesting that this model might also apply to adult bristle formation. Notably, however, we observe Spdo at or close to the membrane of both pI progeny in *sec15* clones (Figure 2H). Therefore, while we cannot rule out the proposed role for Spdo in promoting N signaling at the membrane of the signal-receiving cell, our data suggest a role for Spdo in Dl recycling in the signal-sending cell. It should be noted, though, that these two models are not mutually exclusive. Presence of a significantly higher number of vesicles containing both Dl and N in pIIb compared to the pIIa in wt sensory precursors has been implicated in the ability of the pIIb cell to send the Dl signal (Le Borgne and Schweisguth, 2003b). Colocalization of Spdo with Dl in a significant fraction of these vesicles suggests that a defect in Spdo/Dl trafficking in pIIb contributes to the *sec15* loss-of-function phenotype.

Presence of endocytosed Dl in vesicles that accumulate in *sec15* clones implicates these vesicles in the endocytic traffic of Dl. This notion is further supported by the observation that in both wt and *sec15*$^{-}$ SOPs, the Spdo/Dl/N puncta show a significant colocalization with the endosomal markers Rab5 and HRS. It has recently been proposed that in order to signal, Dl needs to traffic through a specific endocytic compartment, which will lead to recycling of the "active" Dl to the membrane of the signal-sending cell (Wang and Struhl, 2004). A defect in Dl recycling is further suggested by the aberrant accumulation of the recycling endosomal

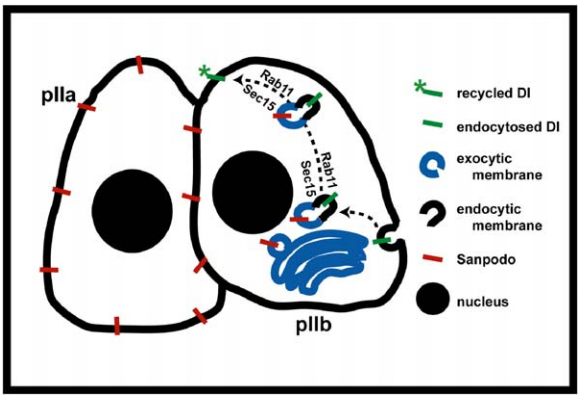

Figure 7. A Model for Sec15 Function

An intrinsic difference between the endocytic traffic of Dl in pIIa and pIIb allows the pIIb cell to employ the Sec15-Rab11 machinery differentially from the pIIa cell and thereby assume the role of signal-sending cell.

marker Rab11 in *sec15* clones. The Rab11$^+$ endosomal compartment is thought to be a central trafficking intermediate in both exocytic and endocytic pathways and is shown to control the traffic of cargo from the perinuclear recycling endosomal compartment to the membrane (Dollar et al., 2002; Pelissier et al., 2003; Satoh et al., 2005; Ullrich et al., 1996). Interestingly, Zhang et al. (2004) have shown that Sec15 meets the criteria of being an effector for Rab11 in mammalian cell lines: Sec15 physically binds Rab11 in a GTP-dependent manner; Sec15 colocalizes with Rab11 in the perinuclear region of the cells; Sec15 labels structures containing an endocytosed protein in immuno-EM experiments. Similarly, *Drosophila* Sec15 and Rab11 interact physically (Wu et al., 2005) and show a high level of colocalization in SOPs (Figure 6). Altogether, our data are compatible with a model in which Sec15 regulates the traffic of a subset of endocytosed Dl to the membrane of the pIIb cell via a Rab11$^+$ recycling endosomal compartment (Figure 7). Sec15 traffics symmetrically in pIIa and pIIb. Therefore, we propose that an intrinsic difference between the endocytic traffic of Dl in pIIa and pIIb allows the pIIb cell to employ the Sec15-Rab11 machinery differentially from the pIIa cell and thereby assume the role of signal-sending cell. The most likely mechanisms for the proposed intrinsic difference are unequal segregation of Neur into the pIIb, which promotes Dl endocytosis in this cell (Le Borgne and Schweisguth, 2003b), and asymmetric distribution of the Rab11$^+$ recycling endosomes in the pIIb versus pIIa, which is thought to specifically mediate Dl recycling in the pIIb (Emery et al., 2005).

Our data suggest that at least some of the Spdo/N/Dl-containing vesicles that accumulate in the basal areas of *sec15*$^-$ SOPs are of a mixed exo-endocytic nature. This is not unprecedented, as traffic from the TGN to an endosomal compartment has previously been documented (Ang et al., 2004; Folsch et al., 2003). Accordingly, it has been proposed that some exocytic cargo might pass through the recycling endosome on its way from the TGN to the plasma membrane. Recently, it has been shown that upon exit from the Golgi apparatus, newly synthesized E-Cad fuses with a Rab11$^+$ recycling endosomal compartment before it reaches the plasma membrane (Lock and Stow, 2005). It is interesting to note that members of the exocyst complex have been shown to localize to both the TGN and recycling endosomes in polarizing epithelial cells (Folsch et al., 2003; Prigent et al., 2003; Yeaman et al., 2001). Although the recycling endosome has been proposed as an intermediate to transfer the exocytic cargo to the plasma membrane, it is possible that passing through these vesicles somehow enhances the signaling ability of internalized Dl. In other words, presence of Spdo might be part of the specific environment that Wang and Struhl (2004) propose Dl needs to traffic through. Although Dl endocytosis and recycling are also implicated in N signaling during lateral inhibition (Wang and Struhl, 2004), we do not observe lateral inhibition defects in *sec15* clones. We propose that the link to Spdo results in the specificity of the *sec15* phenotype to the asymmetric divisions, as loss of *spdo* does not affect lateral inhibition either.

In summary, our data indicate that one component of the highly conserved exocyst complex affects the asymmetric division of the sensory precursors in the *Drosophila* PNS through specific vesicle trafficking events. Components of the exocyst complex are conserved from yeast to human (Kee et al., 1997), and several reports have shown parallels between the contribution of asymmetric divisions to *Drosophila* and vertebrate neurogenesis (Betschinger and Knoblich, 2004; Das et al., 2003; Haydar et al., 2003; Shen et al., 2002; Zhong et al., 1996). Therefore, it is conceivable that Sec15, and perhaps other members of the exocyst complex, are involved in neural cell fate determination in other species.

Experimental Procedures

Fly Strains and Genetics

The following strains were used: *y w, y w; actin-FRT-y$^+$-FRT-GAL4 UAS-GFP^{nls}/CyO* (Bloomington Stock Center), *y w; UAS-FLP; FRT82B Sb^1/TM6, Tb^1, y w hs-FLP; actin-FRT-y$^+$-FRT-GAL4 UAS-GFP^{nls}/CyO, y w; UAS-FLP; C684-GAL4 FRT82B sec15^3/TM6B, y w; C684-GAL4 FRT82B spdo^{G104}/TM6B, y w; C684-GAL4 FRT82B sec15^3 spdo^{G104}/TM6B, y$^+$, w; UAS-GFP::sec15/CyO, w; UAS-GFP::sec15 neur-GAL4/TM3, Sb^1; y w Ubx-FLP; FRT82B pM Sb^{63} ry$^+$ y$^+$/TM6B* (this study), *y w; FRT82B sec15^1/TM3, Sb^1, y w; FRT82B sec15^2/TM3, Sb^1, y w; UAS-HA-sec15$^{2-4}$/CyO* (Mehta et al., 2005), *w; UAS-spdo, spdo^{G104}/TM3, Sb^1* (O'Connor-Giles and Skeath, 2003), *C684-GAL4* (Manseau et al., 1997), *y w; FRT82B pM Sb^{63} ry$^+$ y$^+$/TM3, Ser* (C. Doe), *FRT82B ubi-GFP^{nls} M(3)/TM6B, y$^+$, y w hs-FLP; FRT82B ubi-GFP, y w; sca$^{109-68}$-GAL4, y w;sca-GAL4 UAS-Pon::GFP/CyO* (Y.N. Jan), and *y w hsFLP; UAS-N^{ECN}/CyO; MKRS/TM2* (G. Struhl). *sec15^3* mutant clones in pupae were identified by the absence of GFP^{nls} from the *FRT82B ubi-GFP^{nls} M(3)* chromosome. For details of the genetic screen and crossing schemes see the Supplemental Experimental Procedures.

Immunostainings and Endocytosis Assay

Dissections, stainings, and the endocytosis assay were performed as described previously (Berdnik et al., 2002; Le Borgne and Schweisguth, 2003b). Primary antibodies utilized in this study are guinea pig α-Sec15 1:2000 (Mehta et al., 2005), mouse α-Elav 1:200 (9F8A9; DSHB), rat α-Elav 1:500 (7E8A10; DSHB), rat α-Su(H) 1:2000 (Gho et al., 1996), rabbit α-Pros 1:1000 (Y.N. Jan), mouse α-Cut 1:500 (2B10; DSHB), rabbit α-Numb 1:1000 (Rhyu et al., 1994), rabbit α-Baz 1:1000 (Wodarz et al., 1999), rat α-DE-Cad

1:1000 (DCAD2; DSHB), rabbit α-Pins 1:1000, rabbit α-Neur 1:600 (Lai et al., 2001), mouse α-Dlg 1:1000 (K. Cho), rat α-Rab11 1:1000 (Dollar et al., 2002), rabbit α-Rab5 1:200 (Wucherpfennig et al., 2003), guinea pig α-HRS 1:600 (Lloyd et al., 2002), rabbit α-HRP 1:1000 (Y.N. Jan), guinea-pig α-Lva 1:1000 (Sisson et al., 2000), rabbit α-Spdo 1:1000 and rat α-Spdo 1:200 (O'Connor-Giles and Skeath, 2003), guinea-pig α-Dl 1:3000 (M. Muskavitch), mouse α-Dl^{ECD} 1:1000 (C54.9B; DSHB), mouse α-N^{intra} 1:1000 (C17.9C6; DSHB), mouse α-N^{ECD} 1:100 (C458.2H; DSHB), rat α-Alpha-Tubulin 1:200 (Serotec), rabbit α-Myc 1:200 (Santa Cruz), and mouse α-Arm 1:100 (N2 7A1; DSHB). Alexa488-conjugated (Molecular Probes), and Cy2-, Cy3-, and Cy5-conjugated (Jackson ImmunoResearch Laboratories) secondary antibodies were used at 1:250–1:500.

Time-Lapse Microscopy, Image Acquisition and Processing, and Molecular Biology

See the Supplemental Experimental Procedures.

Supplemental Data

Supplemental Data include two figures, three movies, and Supplemental Experimental Procedures and can be found with this article online at http://www.developmentalcell.com/cgi/content/full/9/3/351/DC1/.

Acknowledgments

We are grateful to S. Artavanis-Tsakonas, K. Cho, R. Cohen, C. Doe, M. Gonzalez-Gaitan, Y.-N. Jan, E. Lai, L. Manseau, M. Muskavitch, F. Schweisguth, J. Sisson, J. Skeath, G. Struhl, A. Wodarz, the Bloomington Stock Center, and the Developmental Studies Hybridoma Bank for reagents. We thank N. Giagtzoglou, P.R. Hiesinger, P. Patel, A. Rajan, E. Seto, J. Skeath, and A.-C. Tien for comments on the manuscript, R. Atkinson and P.R. Hiesinger for advice on imaging, H.L. Pan and M. Zaheer for help with the screen, and members of the Bellen lab, especially K.L. Schulze, for their support. H.J.-N. is supported by HHMI and the NIH Medical Genetics Research Fellowship Program grant T32-GMO7526. S.Q.M. is supported by NIH grant EY07001. V.B. and S.Q.M. are members of the Medical Scientist Training Program. H.J.B. is an HHMI Investigator. Work in J.A.K.'s lab is supported by Boehringer Ingelheim, the Austrian Academy of Sciences, and the FWF. F.W.-P. is supported by the Boehringer Ingelheim Fonds.

Received: March 19, 2005
Revised: June 13, 2005
Accepted: June 30, 2005
Published online: September 1, 2005

References

Ang, A.L., Taguchi, T., Francis, S., Folsch, H., Murrells, L.J., Pypaert, M., Warren, G., and Mellman, I. (2004). Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. J. Cell Biol. *167*, 531–543.

Bardin, A.J., Le Borgne, R., and Schweisguth, F. (2004). Asymmetric localization and function of cell-fate determinants: a fly's view. Curr. Opin. Neurobiol. *14*, 6–14.

Berdnik, D., Torok, T., Gonzalez-Gaitan, M., and Knoblich, J.A. (2002). The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in *Drosophila*. Dev. Cell *3*, 221–231.

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., and Knoblich, J.A. (2004). Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. Curr. Biol. *14*, R674–R685.

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

Das, T., Payer, B., Cayouette, M., and Harris, W.A. (2003). In vivo time-lapse imaging of cell divisions during neurogenesis in the developing zebrafish retina. Neuron *37*, 597–609.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J.S., Schroeter, E.H., Schrijvers, V., Wolfe, M.S., Ray, W.J., et al. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. Nature *398*, 518–522.

Di Fiore, P.P., and De Camilli, P. (2001). Endocytosis and signaling. an inseparable partnership. Cell *106*, 1–4.

Dollar, G., Struckhoff, E., Michaud, J., and Cohen, R.S. (2002). Rab11 polarization of the *Drosophila* oocyte: a novel link between membrane trafficking, microtubule organization, and *oskar* mRNA localization and translation. Development *129*, 517–526.

Dye, C.A., Lee, J.K., Atkinson, R.C., Brewster, R., Han, P.L., and Bellen, H.J. (1998). The *Drosophila sanpodo* gene controls sibling cell fate and encodes a tropomodulin homolog, an actin/tropomyosin-associated protein. Development *125*, 1845–1856.

Emery, G., Hutterer, A., Berdnik, D., Mayer, B., Wirtz-Peitz, F., Gonzalez Gaitan, M., and Knoblich, J.A. (2005). Asymmetric Rab11 endosomes reglate Delta recycling and specify cell fate in the *Drosophila* nervous system. Cell *122*, in press. Published online September 1, 2005. 10.1016/j.cell.2005.08.017

Folsch, H., Pypaert, M., Maday, S., Pelletier, L., and Mellman, I. (2003). The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains. J. Cell Biol. *163*, 351–362.

Friedrich, G.A., Hildebrand, J.D., and Soriano, P. (1997). The secretory protein Sec8 is required for paraxial mesoderm formation in the mouse. Dev. Biol. *192*, 364–374.

Gho, M., Lecourtois, M., Geraud, G., Posakony, J.W., and Schweisguth, F. (1996). Subcellular localization of Suppressor of Hairless in *Drosophila* sense organ cells during Notch signalling. Development *122*, 1673–1682.

Gho, M., Bellaiche, Y., and Schweisguth, F. (1999). Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development *126*, 3573–3584.

Gonzalez-Gaitan, M. (2003). Endocytic trafficking during *Drosophila* development. Mech. Dev. *120*, 1265–1282.

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, M., Jan, L.Y., and Jan, Y.N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. Neuron *17*, 27–41.

Guo, W., Roth, D., Walch-Solimena, C., and Novick, P. (1999). The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. EMBO J. *18*, 1071–1080.

Hartenstein, V., and Posakony, J.W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development *107*, 389–405.

Hartenstein, V., and Posakony, J.W. (1990). A dual function of the Notch gene in *Drosophila* sensillum development. Dev. Biol. *142*, 13–30.

Haydar, T.F., Ang, E., Jr., and Rakic, P. (2003). Mitotic spindle rotation and mode of cell division in the developing telencephalon. Proc. Natl. Acad. Sci. USA *100*, 2890–2895.

Hsu, S.C., TerBush, D., Abraham, M., and Guo, W. (2004). The exocyst complex in polarized exocytosis. Int. Rev. Cytol. *233*, 243–265.

Hu, Y., Ye, Y., and Fortini, M.E. (2002). Nicastrin is required for gamma-secretase cleavage of the *Drosophila* Notch receptor. Dev. Cell *2*, 69–78.

Jafar-Nejad, H., Acar, M., Nolo, R., Lacin, H., Pan, H., Parkhurst, S.M., and Bellen, H.J. (2003). Senseless acts as a binary switch during sensory organ precursor selection. Genes Dev. *17*, 2966–2978.

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.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. Nature *377*, 624–627.

Lai, E.C. (2004). Notch signaling: control of cell communication and cell fate. Development *131*, 965–973.

Lai, E.C., Deblandre, G.A., Kintner, C., and Rubin, G.M. (2001). *Drosophila* neuralized is a ubiquitin ligase that promotes the internalization and degradation of delta. Dev. Cell *1*, 783–794.

Le Borgne, R., and Schweisguth, F. (2003a). Notch signaling: endocytosis makes delta signal better. Curr. Biol. *13*, R273–R275.

Le Borgne, R., and Schweisguth, F. (2003b). Unequal segregation of Neuralized biases Notch activation during asymmetric cell division. Dev. Cell *5*, 139–148.

Le Borgne, R., Bellaiche, Y., and Schweisguth, F. (2002). *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr. Biol. *12*, 95–104.

Lieber, T., Kidd, S., and Young, M.W. (2002). kuzbanian-mediated cleavage of *Drosophila* Notch. Genes Dev. *16*, 209–221.

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.

Lock, J.G., and Stow, J.L. (2005). Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. Mol. Biol. Cell *16*, 1744–1755.

Manseau, L., Baradaran, A., Brower, D., Budhu, A., Elefant, F., Phan, H., Philp, A.V., Yang, M., Glover, D., Kaiser, K., et al. (1997). GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of *Drosophila*. Dev. Dyn. *209*, 310–322.

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., and Bellen, H.J. (2005). Mutations in *Drosophila sec15* reveal a function in neuronal targeting for a subset of exocyst components. Neuron *46*, 219–232.

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., Garza, D., Scheller, R.H., and Schwarz, T.L. (2003). Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists. Neuron *37*, 433–447.

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.

Nolo, R., Abbott, L.A., and Bellen, H.J. (2000). Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in *Drosophila*. Cell *102*, 349–362.

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.

O'Connor-Giles, K.M., and Skeath, J.B. (2003). Numb inhibits membrane localization of Sanpodo, a four-pass transmembrane protein, to promote asymmetric divisions in *Drosophila*. Dev. Cell *5*, 231–243.

Overstreet, E., Fitch, E., and Fischer, J.A. (2004). Fat facets and Liquid facets promote Delta endocytosis and Delta signaling in the signaling cells. Development *131*, 5355–5366.

Parks, A.L., Klueg, K.M., Stout, J.R., and Muskavitch, M.A. (2000). Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. Development *127*, 1373–1385.

Pavlopoulos, E., Pitsouli, C., Klueg, K.M., Muskavitch, M.A., Moschonas, N.K., and Delidakis, C. (2001). *neuralized* encodes a peripheral membrane protein involved in delta signaling and endocytosis. Dev. Cell *1*, 807–816.

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.

Prigent, M., Dubois, T., Raposo, G., Derrien, V., Tenza, D., Rosse, C., Camonis, J., and Chavrier, P. (2003). ARF6 controls post-endocytic recycling through its downstream exocyst complex effector. J. Cell Biol. *163*, 1111–1121.

Rhyu, M.S., Jan, L.Y., and Jan, Y.N. (1994). Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell *76*, 477–491.

Roegiers, F., and Jan, Y.N. (2004). Asymmetric cell division. Curr. Opin. Cell Biol. *16*, 195–205.

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.

Schweisguth, F. (2004). Notch signaling activity. Curr. Biol. *14*, R129–R138.

Seto, E.S., Bellen, H.J., and Lloyd, T.E. (2002). When cell biology meets development: endocytic regulation of signaling pathways. Genes Dev. *16*, 1314–1336.

Seugnet, L., Simpson, P., and Haenlin, M. (1997). Requirement for dynamin during Notch signaling in *Drosophila* neurogenesis. Dev. Biol. *192*, 585–598.

Shen, Q., Zhong, W., Jan, Y.N., and Temple, S. (2002). Asymmetric Numb distribution is critical for asymmetric cell division of mouse cerebral cortical stem cells and neuroblasts. Development *129*, 4843–4853.

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.

Skeath, J.B., and Doe, C.Q. (1998). Sanpodo and Notch act in opposition to Numb to distinguish sibling neuron fates in the *Drosophila* CNS. Development *125*, 1857–1865.

Struhl, G., and Basler, K. (1993). Organizing activity of wingless protein in *Drosophila*. Cell *72*, 527–540.

Struhl, G., Fitzgerald, K., and Greenwald, I. (1993). Intrinsic activity of the Lin-12 and Notch intracellular domains in vivo. Cell *74*, 331–345.

Tepass, U., Truong, K., Godt, D., Ikura, M., and Peifer, M. (2000). Cadherins in embryonic and neural morphogenesis. Nat. Rev. Mol. Cell Biol. *1*, 91–100.

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.

Tian, X., Hansen, D., Schedl, T., and Skeath, J.B. (2004). Epsin potentiates Notch pathway activity in *Drosophila* and *C. elegans*. Development *131*, 5807–5815.

Uemura, T., Shepherd, S., Ackerman, L., Jan, L.Y., and Jan, Y.N. (1989). *numb*, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos. Cell *58*, 349–360.

Ullrich, O., Reinsch, S., Urbe, S., Zerial, M., and Parton, R.G. (1996). Rab11 regulates recycling through the pericentriolar recycling endosome. J. Cell Biol. *135*, 913–924.

Wang, W., and Struhl, G. (2004). *Drosophila* Epsin mediates a select endocytic pathway that DSL ligands must enter to activate Notch. Development *131*, 5367–5380.

Wang, W., and Struhl, G. (2005). Distinct roles for Mind bomb, Neuralized and Epsin in mediating DSL endocytosis and signaling in *Drosophila*. Development *132*, 2883–2894.

Wiederkehr, A., De Craene, J.O., Ferro-Novick, S., and Novick, P. (2004). Functional specialization within a vesicle tethering complex: bypass of a subset of exocyst deletion mutants by Sec1p or Sec4p. J. Cell Biol. *167*, 875–887.

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.

Wu, S., Mehta, S.Q., Pichaud, F., Bellen, H.J., and Quiocho, F.A. (2005). The exocyst Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. Nat. Struct. Mol. Biol. *402*, 544–547. in press.

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.

Xu, K.F., Shen, X., Li, H., Pacheco-Rodriguez, G., Moss, J., and Vaughan, M. (2005). Interaction of BIG2, a brefeldin A-inhibited guanine nucleotide-exchange protein, with exocyst protein Exo70. Proc. Natl. Acad. Sci. USA *102*, 2784–2789.

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.

Yeh, E., Zhou, L., Rudzik, N., and Boulianne, G.L. (2000). Neuralized functions cell autonomously to regulate *Drosophila* sense organ development. EMBO J. *19*, 4827–4837.

Yeh, E., Dermer, M., Commisso, C., Zhou, L., McGlade, C.J., and Boulianne, G.L. (2001). Neuralized functions as an E3 ubiquitin ligase during *Drosophila* development. Curr. Biol. *11*, 1675–1679.

Zhai, R.G., Hiesinger, P.R., Koh, T.W., Verstreken, P., Schulze, K.L., Cao, Y., Jafar-Nejad, H., Norga, K.K., Pan, H., Bayat, V., et al. (2003). Mapping *Drosophila* mutations with molecularly defined *P* element insertions. Proc. Natl. Acad. Sci. USA *100*, 10860–10865.

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.

Zhong, W., Feder, J.N., Jiang, M.M., Jan, L.Y., and Jan, Y.N. (1996). Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron *17*, 43–53.