Introduction

Vesicular trafficking between different membrane compartments in eukaryotic cells is essential for cell survival and function. To ensure precise membrane transport, elegant mechanisms have evolved to sort cargoes into the proper vesicles (reviewed by Bannykh et al., 1998; Pelham, 1999; Rothman and Wieland, 1996), which are then delivered to the target compartment (Bloom and Goldstein, 1998). Upon reaching their destination, vesicles will fuse to the target membrane, a process that requires the coordination of at least a dozen molecules, among which the SNAREs (SNAP receptors) are considered to assemble the core of the fusion machinery.

Originally identified as membrane-associated proteins essential for the presynaptic release of neurotransmitters, SNAREs comprise syntaxin, SNAP-25 and VAMP/synaptobrevin, each belonging to a protein family with increasing family members (reviewed by Chen and Scheller, 2001; Jahn and Sudhof, 1999; Rothman, 1994). Vesicle SNAREs can interact with cognate SNAREs on the target membrane. In the case of synaptic fusion, syntaxin 1 (on plasma membranes) and VAMP/synaptobrevin 2 (on synaptic vesicles) each contributes one helix, whereas SNAP-25 (on plasma membranes) contributes two helices to form a four-helix bundle. Lying in the core of the bundle are conserved layers of interacting amino-acid side chains, with the central layer being the most highly conserved (Sutton et al., 1998). Thus, on the basis of whether they provide a glutamine or arginine at the central layer, SNAREs can be classified as either Q- or R-SNAREs. Additional structural analysis suggests that a functional SNARE complex is most probably formed by coiled-coil interactions among three Q-SNAREs and one R-SNARE that are distributed on apposing membranes (Antonin et al., 2002; Fasshauer et al., 1998). Such complexes may be stable enough to survive mild SDS treatment and their disassembly requires the collaborative actions of the ATPase NSF (N-ethyl-maleimide-sensitive factor) and its ligand SNAP (soluble NSF associated protein) (Otto et al., 1997). These observations, together with the fact that the coiled-coil terminates at the C-terminal transmembrane domain of the SNAREs, have led to the hypothesis that the formation of the SNARE complex releases sufficient energy to bring the opposing membranes into close apposition and thereby promote fusion (Hughson, 1999). Strong support for this hypothesis comes from the observation that cognate Q- and R-SNAREs reconstituted separately on artificial liposomes were sufficient to mediate membrane fusion (Fukuda et al., 2000; Weber et al., 1998). However, recent evidence from a number of different systems has suggested that SNARE complex formation may not constitute the final step of membrane fusion (reviewed by Mayer, 1999; Mayer, 2001). At least in the case of yeast vacuolar fusion, additional proteins have been shown to act after SNARE complex formation (Peters et al., 1999; Peters and Mayer, 1998).

In spite of the debate on the exact role of SNAREs in the final stages of fusion, it has been generally accepted that the interaction of cognate SNAREs at least contributes to the specificity of membrane fusion and that most trafficking events require a different SNARE complex (Pelham, 2001; Rothman and Warren, 1994). This may explain why there are so many members of the SNARE super-family, with rather unique but sometimes overlapping distribution patterns along the Golgi apparatus. In addition, membranes containing dsyntaxin 16 become aggregated upon Brefeldin A treatment and are dispersed during meiosis. Inhibition of dsyntaxin 16 function by overexpression of truncated forms in cultured Schneider cells indicates that dsyntaxin 16 may selectively regulate Golgi dynamics.

Key words: Golgi, SNARE, Syntaxin, Membrane fusion, Drosophila
secretory and endocytic pathways. Unfortunately, studies on many individual mammalian SNAREs have yet to provide conclusive evidence for the functional pairing of cognate SNAREs. This is largely due to two reasons: the non-specific pairing of SNAREs under in vitro conditions (Tsui and Banfield, 2000; Yang et al., 1999) and the difficulty in generating mutant alleles in live animals or cultured tissues to address the issue in vivo. *Drosophila melanogaster*, by contrast, allows great flexibility in genetic manipulation while offering a similar level of complexity to mammals.

In an attempt to initiate studies on SNARE-mediated membrane trafficking in *Drosophila*, we screened a fly cDNA library for potential SNAREs using the yeast two-hybrid system. Using this approach, a novel syntaxin isoform that shows significant amino-acid sequence similarity to mammalian syntaxin 16 was identified. dSyx16 is ubiquitously expressed in *Drosophila* and appears to localize to the Golgi apparatus. Overexpression studies indicate that dSyx16 may selectively regulate Golgi dynamics in the fruity.

**Materials and Methods**

**Yeast two-hybrid screen**

A cDNA of *Drosophila* SNAP was generated by RT-PCR (see section 2.2) and confirmed by DNA sequencing. The cDNA was subcloned into the yeast two-hybrid vector pAS2 (Clontech), downstream of the GAL4 DNA-binding motif. The resulting construct was transferred into the yeast strain Y190 (Clontech), and the expression of the chimeric protein (or the bait) was examined by western blot analysis.

To screen for potential binding partners of dSNAP, a *Drosophila melanogaster* cDNA library (gift of J. Verdi, University of Western Ontario) constructed downstream of the GAL4 activating motif in the pACT2 vector was used to transform the Y190 strain that expressed the bait. Transformants were plated on HIS- agar plates and incubated with either H2O, 2 M KCl, 0.2 M Na2CO3 (pH 11-12), or GST (negative control) were then incubated with specific amounts of recombinant dSNAP (Mohtashami et al., 2001) in binding buffer (1/100 Tween 20, 5 mM EDTA, 100 mM NaCl, and 0.1% BSA) for 1 hour at 4°C. Following extensive washes with 50 mM HEPES (pH 7.5), 5 mM EDTA, 150 mM NaCl and 0.5% Triton X-100, proteins on the agarose beads were extracted with 2xSDS sample buffer and subjected to SDS-PAGE. Western blot analysis was performed with Anti-GST (1:1000) (K. Ross and W.S.T., unpublished) and anti-dSNAP [1:2000 (Mohtashami et al., 2001)].

**Oregon R** embryos at different developmental stages, third instar larvae, pupae, adults, adult heads, bodies, salivary glands and other imaginal discs dissected from third instar larvae were lysed using homogenization buffer (100 mM Tris, pH 6.8, 20% glycerol, 2% SDS and 5 mM EDTA). Following protein quantification with BCA reagents (Pierce), equal amounts of protein were subjected to 10% SDS-PAGE and western blot analysis using affinity-purified anti-dSyx16 antibody (1:800) that was raised against His-dSyx16 70 to 329. Immunoblotting and antigen detection was performed with Anti-GST (1:1000) (K. Ross and W.S.T., unpublished) and anti-dSNAP [1:2000 (Mohtashami et al., 2001)].

**Immunocytochemistry and transient expression in cultured cells**

Schneider cells (S2 cells) were grown on coverslips in Schneider’s *Drosophila* medium (Gibco) supplemented with 10% FBS overnight before they were treated with 1.5% DMSO alone (negative control) or 30 µg/ml of brefeldin A (Sigma) and 1.5% DMSO for 2 hours. Cells were then fixed with 4% paraformaldehyde in 100 mM Na3PO4 (pH 7.0) for 25 minutes. Following centrifugation for 10 minutes at 1200 g, the supernatant was centrifuged at 100,000 g for 1 hour to separate the soluble fraction and the membrane fraction. The membrane pellet was re-suspended and incubated with either H2O, 2 M KCl, 0.2 M Na2CO3 (pH 11-12), 4M urea, 2% Triton X-100 or 2% SDS for 1 hour at 4°C. After centrifugation, both the soluble and insoluble fractions were subjected to SDS-PAGE and western blot analysis.

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5'-CTATAGGTGGTACAGCAAGCAGAGGC and 5'-GTCGACTCGCAGATCGGGATCCTCG. The PCR product was subcloned into the pBluescript SK+ vector (Stratagene) for sequencing.

The partial cDNA of dSyx16, cloned via the yeast two-hybrid screen, was amplified by PCR using primers: 5'-CTATCGAGCTACTTGCG- and 5'-GAATTCATATGTC-.

In fractionation studies, *Oregon R* adults were homogenized in 50 mM HEPES (pH 7.5), 25% sucrose, 200 µM PMSF and 5 mM EDTA. Following centrifugation for 10 minutes at 1200 g, the supernatant was centrifuged at 100,000 g for 1 hour to separate the soluble fraction and the membrane fraction. The membrane pellet was re-suspended and incubated with either H2O, 2 M KCl, 0.2 M Na2CO3 (pH 11-12), 4M urea, 2% Triton X-100 or 2% SDS for 1 hour at 4°C. After centrifugation, both the soluble and insoluble fractions were subjected to SDS-PAGE and western blot analysis.

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Molecular biology

*Drosophila* SNAP was cloned by RT-PCR. Total RNA from *Oregon R* was isolated using Trizol reagent (Gibco). The first round of cDNA synthesis was achieved with AMV reverse transcriptase (Promega) using oligo(dT) as a primer. A subsequent PCR reaction was carried out using primers: 5'-GGAAGGATCTGCGATGCT- and 5'-CTCGAGCTACTTGCG-.

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Molecular Probes) for 1 hour, washed with PBT and then mounted and cleared with DAKO fluorescent mounting medium.

For transient expression, S2 cells on the coverslip were transiently transfected overnight using the CaCl₂ method with various pRmHa-3-myc-dSyx16 constructs, washed with PBS and re-incubated overnight in 1 mM CuSO₄ in Schneider’s Drosophila medium supplemented with 10% FBS medium. The cells were then fixed and co-stained with Rabbit anti-myc (1:100; Molecular Probes) and mouse monoclonal anti-P120, which recognizes a 120 kDa Golgi protein (Stanley et al., 1997).

To examine Golgi morphology, salivary glands of Oregon R were dissected from third instar larva, fixed in 4% paraformaldehyde and stained with anti-dSyx16 (1:400). Testes were prepared and immunostained as described previously (Hime et al., 1996). To visualize DNA, propidium iodide (5 mg/ml) was used during the secondary incubation. Images of salivary glands, S2 cells and testes were captured by a Zeiss LSM510 confocal microscope.

Fly stocks and genetic studies
Stocks were maintained at room temperature on standard cornmeal agar medium unless otherwise indicated. Visible markers and balancer Stocks were maintained at room temperature on standard cornmeal agar medium unless otherwise indicated. Visible markers and balancer chromosomes have been previously described (Lindsley, 1992). Transgenic flies UAS-dSyx16 and UAS-dSyx16 (1:400). Testes were prepared and immunostained as described previously (Hime et al., 1996). To visualize DNA, propidium iodide (5 mg/ml) was used during the secondary incubation. Images of salivary glands, S2 cells and testes were captured by a Zeiss LSM510 confocal microscope.

Results and Discussion
Cloning and sequence analysis of Drosophila syntaxin 16
SNAREs serve as receptors for SNAP. We therefore decided to search for novel Drosophila SNAREs using a yeast two-hybrid screen with Drosophila SNAP as bait. The cDNA of dSNAP was obtained by RT-PCR, inserted downstream of the GAL4 DNA-binding motif in the bait vector, and then used to screen a Drosophila ovary cDNA library. Since active membrane fusion events (e.g. cellularization) that take place at early stages of embryogenesis require numerous molecules maternally deposited during oogenesis, we expected that the ovary cDNA library would be an excellent pool for SNAREs. Indeed, after screening approximately 2 million clones, we identified, in addition to two known syntaxins (dSyx1 and dSyx5), one novel syntaxin family member. This clone (#396) showed significant sequence similarity to mammalian syntaxin 16 and was thought to carry the full-length cDNA of Drosophila syntaxin 16 at the time. By probing a P1 Drosophila high-density filter, we mapped the gene to 19E2-3 on the X chromosome. The subsequent completion of the Drosophila genome project (Adams et al., 2000) precisely located the gene to be at 19D1-2. The completion of the Drosophila genome project also allowed us to examine the genomic sequence of dSyx16 (GenBank Acc. # NT033768) and full-length cDNAs (GenBank Acc. # AI113714 and NM078696), all of which predict an additional 69 amino acids at the N-terminus of our original clone. The full-length dSyx16 cDNA thereby encodes 352 amino acids (Fig. 1).

Like human syntaxin 16, Drosophila syntaxin 16 carries at the C-terminus a 21 amino-acid-long hydrophobic motif, which probably serves as a transmembrane domain that anchors the protein to the membrane. Adjacent to the C-terminal hydrophobic motif is a predicted helical domain of about 60 amino acids with the potential to form a coiled-coil structure (Fig. 1). This motif is conserved within the syntaxin family (Weimbs et al., 1997) and apparently mediates the interaction of syntaxin with many of its binding partners (i.e., SNAP, VAMP, SNAP25 etc.). In fact, Drosophila and human Syx16 share more than 60% amino-acid identity along this domain, although the overall identity is approximately 35%.

dSyx16 interacts with SNAP and NSF
Two independent approaches were undertaken to test whether dSyx16 indeed functions as a SNARE. A biochemical approach was first used to examine whether dSyx16 and dSNAP interact in vitro. Recombinant GST-dSyx16 fusion protein was attached to glutathione beads and then incubated with purified dSNAP. Following extensive washes, proteins on the beads were eluted and subjected to western blot analysis. As shown in Fig. 2, GST-dSyx16 binds to dSNAP in a concentration-dependent fashion, although an equivalent amount of GST retains no dSNAP. Hence, recombinant dSNAP binds directly to dSyx16.

A second approach to examine the role of dSyx16 involved a genetic approach in vivo. Each unique SNARE complex would at one stage be disassembled by the actions of SNAP...

![Fig. 1. Alignment of dSyx16 with hSyx16. The sequences are numbered on the right. Identical amino acids are shaded black. Conserved amino acids are shaded gray. A potential transmembrane domain at the C-terminal end is underlined. The star below the residue Q indicates the central residue of the predicted coiled-coil. The heptad repeats are numbered above the sequence.](Image)
and NSF so that freed SNAREs can participate in subsequent rounds of fusion events. Blocking NSF function would block the disassembly of the SNARE complexes and thereby interfere with membrane trafficking. For example, overexpression of a dominant-negative form of NSF at the wing margin resulted in a notch-wing phenotype in adult flies, presumably by inhibiting secretion/signalling during wing development ([Stewart et al., 2001]; Fig. 3B).

To genetically address whether dSyx16 interacts with NSF, we took advantage of the observations that overexpression of a syntaxin (with or without its transmembrane motif) can specifically interfere with the membrane trafficking step this molecule is responsible for (Dascher and Balch, 1996; Hatsuzawa et al., 2000; Low et al., 1998; Mallard et al., 2002; Nagamatsu et al., 1996; Nakamura et al., 2000; Wu et al., 1998). Two dSyx16 transgenic flies bearing either amino acids 70 to 329 (UAS-dSyx16<sub>70 to 329</sub>) or 70 to 352 (UAS-dSyx16<sub>70 to 352</sub>) under the control of the GAL4 upstream activating sequence were created. Overexpression of the dSyx16 protein fragments in the wing margin was accomplished by crossing the UAS lines with C96-GAL4, which expresses GAL4 protein in the wing margin during wing development ([Stewart et al., 2001]), ectopic expression of the dominant-negative form of NSF2 under the control of C96-GAL4 gave rise to mild notches on the wing margin (compare Fig. 3B with 3A), which were enhanced by specific alleles of dsyntaxin 1 ([Stewart et al., 2001]). Overexpression of the soluble dSyx16 (amino acid 70 to 329) did not appear to modify the notch-wing phenotype caused by dominant-negative NSF2 (compare Fig. 3C and 3B). However, overexpression of dSyx16<sub>70 to 352</sub> significantly enhanced the notch-wing phenotype (compare Fig. 3D and 3B). Since the two isoforms were expressed at a similar level (both isoforms can be distinguished from the wild-type on western blot; data not shown), it is unlikely that the expression levels are responsible for the differential effect on wing margin development. One conceivable explanation is that unlike dSyx16<sub>70 to 329</sub>, which is dispersed in the cytosol, dSyx16<sub>70 to 352</sub> is delivered to its designated location where its overexpression may sequester other molecules needed for fusion. Interestingly, overexpression of dSyx16<sub>70 to 352</sub> by itself does not lead to any noticeable defects in the wing margin or elsewhere (data not shown), indicating that the dominant-negative effect derived from GAL4-driven overexpression of dSyx16 is not as prominent as that of NSF2. Nevertheless, both biochemical and genetic studies argue that the dSyx16 is a functional component of the SNARE complex.

Temporal and spatial localization of dSyx16
To determine the temporal distribution of dSyx16, embryos from Oregon R were collected every three hours after embryo deposition (AED) and allowed to develop for up to 24 hours. Embryos were then lysed and equal amounts of total protein were separated by SDS-PAGE and then immunoblotted with affinity-purified anti-dSyx16, which was raised in rabbits against His-dSyx16<sub>70 to 329</sub>. This antibody recognized a 44 kDa band from fly lysates, slightly above the predicted molecular mass of the polypeptide (40 kDa). The band disappeared if the antibody was pre-incubated with recombinant dSyx16 (data not shown), indicating that the antibody is specific. Using this antibody, we were able to detect a similar level of dSyx16 in all embryonic collections (Fig. 4A), suggesting a role for dSyx16 during embryogenesis. To examine the distribution of dSyx16 in late developmental stages, third instar larvae, pupae, adults and imaginal discs were collected and subjected to western blot analysis. As shown in Fig. 4B, dSyx16 was expressed at all stages examined and appeared to be more abundant in the adult head than the adult body. In addition, dSyx16 was abundantly expressed in imaginal discs and other tissues including CNS and salivary gland, where active membrane trafficking is required during development. That

![Fig. 2](image_url)

**Fig. 2.** dSyx16 binds dSNAP in a concentration-dependent fashion. From lane 1 to 5, immobilized GST-dSyx16 or GST were incubated with 10, 4, 2, 0 and 10 μg of recombinant dSNAP, respectively. Proteins on the glutathione beads were then eluted and subjected to SDS-PAGE and western blot analysis.

![Fig. 3](image_url)

**Fig. 3.** dSyx16 interacts genetically with NSF2 during wing margin development. Wings from wild-type fly (A) and flies overexpressing dominant-negative NSF2 alone (B) or together with soluble dSyx16<sub>70 to 329</sub> (C) or together with dSyx16<sub>70 to 352</sub> (D) are shown. Several independent transgenic lines inserted with either the soluble dSyx16 or dSyx16<sub>70 to 352</sub> were tested and the phenotypes shown in C and D have been consistently observed.
Characterization of *Drosophila* syntaxin 16

Fact that dSyx16 is ubiquitously expressed throughout the life cycle of *Drosophila* is consistent with its potential role as a Golgi SNARE, which has been suggested by studies on its mammalian homologue Syx16 (Simonsen et al., 1998; Tang et al., 1998).

Amino-acid sequence analysis (Fig. 1) indicates that dSyx16 may associate with membranes through its C-terminal hydrophobic domain. To confirm this, adult fly lysates were separated into soluble and membrane fractions by centrifugation. Subsequent SDS-PAGE and western blot analysis showed that although dSyx16 was predominant in the crude membrane fraction, a small portion was present in the soluble fraction (Fig. 4C). This is probably because of the fact that dSyx16 carries only two amino acids following the potential transmembrane domain. Proteins with similar secondary structure are likely to be deposited into the cytoplasm upon synthesis, since their membrane insertion is not coupled to translation but requires alternative mechanisms (Kim et al., 1999). To further determine whether dSyx16 is an integral membrane protein, the membrane fraction was treated with KCl, Na2CO3 (high pH), urea, Triton X-100 and SDS respectively. KCl and Na2CO3 did not solubilize dSyx16, suggesting that dSyx16 does not bind loosely to the membrane through ionic or hydrophobic interaction (Fig. 4C). Urea, which disrupts hydrogen bonds, was able to extract a small fraction of dSyx16, a phenomenon also observed with human syntaxin 18 (Hatsuzawa et al., 2000). The fact that most dSyx16 remained urea-insoluble excludes hydrogen bonding as a significant force that associates dSyx16 with membrane. Meanwhile, like many mammalian syntaxins (Wong et al., 1998), dSyx16 was soluble in SDS and partially soluble with Triton X-100. Taken together, our data suggest that dSyx16 is probably an integral membrane protein and its partial insolubility in Triton X-100 suggests that it may associate with cytoskeletal elements (Beites et al., 1999).

We then went on to determine the subcellular localization of dSyx16 in salivary gland cells. We chose salivary glands because our developmental western (Fig. 4B) showed that dSyx16 was abundant in salivary gland cells, which are much larger than cells from other tissues. We observed a punctate intracellular staining pattern in duct cells (Fig. 5), as well as punctate staining amongst granules in secretory cells (data not shown). It is evident that in duct cells the distribution pattern of dSyx16 overlaps with that of p120, a widely used *Drosophila* Golgi marker, although from time to time, very small puncta were found to be positive for anti-dSyx16 but not anti-p120. It is not known whether these fine punctate structures are simply staining artefacts or specific to duct cells. Similarly, in cultured Schneider (S2) cells, the staining pattern of dSyx16 matches that of p120, although the two do not overlap completely (Fig. 6E). Because p120 colocalizes with β-cop (Stanley et al., 1997), a cis-Golgi protein that shuttles between cis-Golgi and ER, we speculate that dSyx16 may be localized to a compartment adjacent to the cis-Golgi.

Human syntaxin 16 has been reported to localize on either the cis-Golgi (Simonsen et al., 1998) or the trans-Golgi network (TGN) (Mallard et al., 2002). Very recently, a possible role for hSyx16 in early/recycling endosomes-to-TGN transport has been reported (Mallard et al., 2002). In an attempt to further clarify the localization of dSyx16, we treated S2 cells with brefeldin A (BFA), a fungal metabolite that disrupts ER-to-Golgi trafficking. In mammalian systems, this drug causes Golgi markers to redistribute to the ER (Sciaky et al., 1997) and TGN markers to aggregate around the microtubule organization center. As shown in Fig. 6D, dSyx16 formed
aggregates that associated frequently with ring structures that only became evident upon BFA treatment (compare Fig. 6C with D). However, to our surprise, a similar effect on p120 was also observed (compare Fig. 6A with B). The two aggregates have distinct morphologies but maintain partial colocalization in most cells (Fig. 6F). Therefore, our data support the notion that dSyx16 is a Golgi SNARE localized in a cisterna adjacent to cis-Golgi that may be the counterpart of the TGN in mammalian cells.

**Overexpression of dSyx16 affects Golgi dynamics**

To study the role(s) of dSyx16, we chose to use the overexpression approach. As mentioned earlier, overexpression of a syntaxin may inhibit the specific membrane fusion step this syntaxin is assigned to without interfering with other trafficking events. Studies on yeast (Banfield et al., 1994), fruitfly (Wu et al., 1998) and cultured mammalian cells (Dascher and Balch, 1996; Hatazawa et al., 2000; Low et al., 1998; Mallard et al., 2002; Nakamura et al., 2000) have demonstrated that the inhibitory effect can be obtained with either the wild-type or the cytosolic form. However, we did not observe any significant phenotype when we ectopically expressed dSyx16 in a variety of Drosophila tissues. This is probably due to the relatively low overexpression level permitted by the UAS-GAL4 system. Therefore, we went on to transiently express dSyx16 in cultured S2 cells. By placing dSyx16 under the control of the metallothionein promoter, we expected to see a significant increase in dSyx16 level upon copper induction. Three different forms of dSyx16 were used to transfect S2 cells, full-length dSyx16 to 352, dSyx16 to 329 and dSyx16 to 352. After transfection, cells were induced overnight with 1 mM CuSO4 before they were fixed and then stained with anti-myc (to detect transfected cells), anti-p120 or anti-lava. In cells with relatively low expression levels, myc-dSyx16 maintained partial colocalization with p120 in the Golgi (data not shown). Overexpression of either dSyx16 or dSyx16 to 352 caused the dispersal of p120 in more than 60% of the cells, whereas overexpression of the soluble form had no apparent effect on the Golgi marker (Fig. 8C,F). This suggests that the first 69 amino-acid residues have little to do with the negative effect caused by overexpression and that the transmembrane domain is important for the phenotype. We also noticed that when dSyx16 to 352 was overexpressed, it was
Fig. 7. dSyx16 distribution during cell division in *Drosophila* testis. Testes from *Oregan R* were dissected and stained with anti-dSyx16 (A,E), anti-p120 (B) or propidium iodide (F). A-D are interphase cells with intact nuclei arrows. dSyx16 is localized in distinct puncta (arrowhead). E-H are anaphase cells with dSyx16 much more dispersed (open arrowhead). Occasionally, larger puncta can be observed (open arrows), but they are not comparable with those in interphase cells. Scale bars, 10 μM.

Fig. 8. Overexpression of dSyx16 in S2 cells. S2 cells transfected with myc-dSyx1670 to 329 (A-C) or myc-dSyx1670 to 352 (D-I) were fixed and co-stained with anti-myc (red channel) and anti-p120 (green channel, B,C,E,F) or anti-lva (green channel, H,I) antibodies. The arrows point to transfected cells. Open arrowheads point to non-transfected cells. Scale bar, 10 μM.
no longer localized in large peri-nuclear puncta. Instead, it was dispersed in numerous fine punctate structures throughout the cytoplasm. Interestingly, although overexpressing dSyx16\textsuperscript{1600} to 352 might affect the localization of the Golgi marker p120 or even itself, it did not appear to affect the distribution of lavalamp (Fig. 81), another protein known to be localized to the Golgi (Sisson et al., 2000). Two possible scenarios could account for this observation. First, the Golgi apparatus may still be intact upon dSyx16 overexpression. Thus, the overexpression experiments did not simply disrupt the entire secretory pathway but rather had an inhibitory effect on the dynamics of specific Golgi proteins such as p120. However, since lavalamp is a peripheral membrane protein associated with microtubules, we cannot rule out the possibility that anti-lva could decorate Golgi remnants even after Golgi membranes had been recycled. Future studies will be aimed at addressing these issues.

In yeast and mammals, syntaxin 5 has been shown to function in ER-to-Golgi trafficking. In mammals, syntaxin 16 and syntaxin 6 are thought to be localized to the late Golgi compartments and have recently been shown to receive retrograde transport from the endosomes. Our overexpression studies provided evidence that Drosophila syntaxin16 is likely to be involved in Golgi dynamics but have not precisely defined the role of this protein, because blocking traffic at either side of the Golgi can potentially disturb the distribution of Golgi proteins. Future work is warranted to address this issue as well as the functional relationship between dSyx16 and its partners.

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