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Syntaxin 5 Is Required for Cytokinesis and Spermatid Differentiation in *Drosophila*

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Syntaxin 5 is a Golgi-localized SNARE protein that has been shown to be required for ER–Golgi traffic in yeast and Golgi reassembly following cell division in mammalian cells. Here, we describe the characterization of the *Drosophila* ortholog of syntaxin 5, dSyx5, and show that, like its mammalian and yeast counterparts, the protein is localized to the *Drosophila* Golgi and binds to α-SNAP. Null mutations in *dSyx5* are larval lethal and demonstrate impaired transport of a GFP-tagged membrane protein. A hypomorphic allele of *dSyx5* caused by insertion of an EP element results in impenetrant lethality, and escaping adult flies are male sterile. The male sterility results both from failure of germ cells to complete cytokinesis and from defects in spermatid elongation and maturation. Ectopic expression of *dSyx5* from the EP element can rescue the cytokinesis defect, but high levels of expression are required to restore maturation and fertility. Together, these results show that dSyx5 is required for the proper function of the Golgi apparatus and that an efficiently functioning Golgi apparatus is required for the steps leading to the completion of cytokinesis and formation of mature sperm.

Key Words: **meiosis; testis; Golgi; SNARE; cell division.**

INTRODUCTION

Transport of membranes and membrane proteins within the cell requires the interaction of SNARE proteins on the transport vesicle with their cognate SNARE partners on the target membrane, and this binding appears necessary to achieve membrane fusion. The SNARE proteins, so named because they form a stable complex that acts as the receptor for the soluble NSF attachment protein α-SNAP (hence SNAP Receptors; Sollner *et al.*, 1993), are composed of three protein families: VAMP, syntaxin, and SNAP-25. The formation of this strong coiled–coil complex is thought to draw the vesicle and target membranes into apposition, and in so doing may provide the energy needed to cause membrane fusion (Chen and Scheller, 2001).

Although the role of SNARE proteins in secretion is generally accepted, a large number of different SNARE protein isoforms have been identified that appear to have specific subcellular distributions. This has led to the idea that membrane fusion at each step in the secretory pathway may be mediated by the interactions of a unique set of SNAREs (Rothman and Warren, 1994). A recent comparison of the partially completed human and *Drosophila* genomes with those of *Caenorhabditis elegans* and *Saccharomyces cerevisiae* revealed that, while yeast, worms, and flies have roughly the same number of SNAREs, humans have significantly more SNARE proteins (Bock *et al.*, 2001). This suggests that mammals may have evolved unique forms of the SNAREs for specialized purposes. However, certain ancestral forms of the SNAREs, such as syntaxin 5 (also known as Sed5p), have orthologs that are present in all organisms.

Sed5 was first identified by Hardwick and Pelham (1992) as a multicopy suppressor of the lethal phenotype that arose from the lack of the yeast HDEL receptor ERD2. Their studies revealed that Sed5p participated in vesicular traffic between the ER and Golgi. The mammalian ortholog, called syntaxin 5, was found to have a cis-Golgi distribution (Bennett *et al.*, 1993) consistent with a role in this compartment. Similarly, the *Drosophila* ortholog, when expressed in mammalian cells, also localized to a perinuclear compartment (Banfield *et al.*, 1994). Subsequent studies revealed that overexpression of a truncated form of mamma-

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lian syntaxin 5 lacking the transmembrane domain (Dascher *et al.*, 1994) or microinjection of syntaxin 5-specific antibodies (Rowe *et al.*, 1998) blocked the transport of vesicular stomatitis virus glycoprotein in a pre-Golgi intermediate compartment. Together, these results suggested that syntaxin 5 was required for the fusion of the carrier vesicles at the *cis*-face of the Golgi complex. In addition, syntaxin 5 has been implicated in the reassembly of the Golgi apparatus from mitotic fragments following cell division (Rabouille *et al.*, 1998).

In single cell systems, there is clear evidence that Sed5/syntaxin 5 (Syx5) functions in the Golgi complex, but the overall function of Syx5 in the development of a multicellular organism is not known. Here, we have characterized the *Drosophila Syx5* (*dSyx5*) locus and show that, like its mammalian and yeast counterparts, dSyx5 is localized to the Golgi complex. As in mammals, dSyx5 binds to α-SNAP in both two-hybrid and *in vitro* assays. As well, *dSyx5* interacts genetically with *N*-ethylmaleimide sensitive factor (*NSF*). We have also characterized a null mutation within the *dSyx5* gene and find that the absence of dSyx5 protein causes lethality early during the first larval instar. Moreover, using the polarized epithelial cells in the embryonic salivary gland as a model, we find that null mutations display defects in apical transport. Interestingly, hypomorphic combinations of *dSyx5* mutations lead to male sterility, and we show that this is due to a failure in both cytokinesis and sperm maturation. Together, these results show that dSyx5 is required for normal membrane protein transport and development. Moreover, our results suggest that cytokinesis is dependent on a functional Golgi complex and that this process is particularly sensitive to levels of dSyx5.

MATERIALS AND METHODS

Fly Strains and Genetic Studies

Stocks were maintained at room temperature on standard cornmeal agar medium unless otherwise indicated. Visible markers and balancer chromosomes have been previously described (Lindsley and Zimm, 1992). EMS-induced alleles of *dSyx5* (*dSyx5*^{AA73}, *dSyx5*^{AR113}, *dSyx5*^{AE48}, and *dSyx5*^{AE73}) were kind gifts of Dr. T. Schüpbach (Princeton University). Lethal phase of the mutants was determined by visually monitoring the development of 100 embryos marked with a CyO-GFP balancer chromosome from 6-h collections of *dSyx5* embryos. Embryos of *Oregon R* were used as control.

dSyx5^{AR113} was recombined with *UAS-mCD8::GFP* (Bloomington Stock Center) or *actin-GAL4*. The recombinant *dSyx5*^{AR113}, *UAS-mCD8::GFP* line was used to establish *yw; dSyx5*^{AR113}, *UAS-mCD8::GFP/CyO*, y^+; *da-GAL4* by crossing to *yw; Sp/CyO*, y^+; *Ly/TM3, Ser, GFP* and *yw; Sp/CyO*, y^+; *da-Gal4. elav-GAL4, UAS-mCD8::GFP; dSyx5*^{AR113}*/CyO* was established from *elav-GAL4, UAS-mCD8::GFP*. The line *EP(2)2313* contains an insertion of a P-element coupled with GAL4 binding sites and a basal promoter (Rorth, 1996; Rorth *et al.*, 1998) in the 5′ regulatory region of *dSyx5* gene.

Cytokinesis defects of spermatocytes were evaluated in males (raised at 25°C or RT) bearing allele/EP combinations, the sterility of which was examined by crossing to virgin w^- females. *Oregon R* and *EP(2)2313* were used as wild-type controls. Percent viability in Table 1 was obtained by counting progeny from crosses of *yw; dSyx5*^{AR113}*/CyO*, y^+ or *yw; dSyx5*^{AR113}, *UAS-mCD8::GFP/CyO*, y^+; *da-GAL4* with *EP(2)2313*. At least 1000 flies were counted for each cross. Enhancement of wing margin phenotype was examined by crossing *yw; dSyx5*^{AR113}*/CyO*, y^+ to *UAS-dNSF2*$^{E/Q}$, *C96-GAL4/Tm3, Sb, Ser* (Stewart *et al.*, 2001). The wings of progeny that lacked marked balancer chromosomes were compared with *UAS-dNSF2*$^{E/Q}$, *C96-GAL4/+*.

Yeast Two-Hybrid Screen

cDNA of *Drosophila* α-SNAP (see below) was subcloned into the yeast two-hybrid vector pAS2 (Clontech), downstream of the Gal4 DNA binding motif. The resulting construct was transformed into the yeast strain Y190 (Clontech), and expression of the chimeric protein (or the bait) was examined by Western blotting analysis using anti-HA antibody as a probe.

To screen for potential binding partners of dα-SNAP, a *Drosophila melanogaster* ovary cDNA library (gift of Dr. 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 expresses the bait. Transformants were plated on yeast medium lacking His from which potential positive clones were selected for expression of a *HIS3* reporter gene. As a secondary screen, transformants with His^+ phenotype were tested for expression of a second reporter gene, *lacZ*, using a filter assay for β-galacosidase activity as recommended (Clontech). To eliminate false positives, candidate transformants were grown in medium with no selection for the bait vector. The transformants that lost the bait were tested for loss of *lacZ* expression.

Plasmid DNA corresponding to positive clones was isolated from yeast, transformed into *Escherichia coli* and then purified from *E. coli* for DNA sequencing.

Molecular Biology

Drosophila α-SNAP was cloned by RT-PCR. Total RNA of *Oregon R* was isolated by using Trizol reagent (Gibco). The first round of cDNA was made by AMV reverse transcriptase (Promega) using oligo(dT) as primer. Subsequent PCR was carried out by using primers: 5′-CATATGGGTGACAACGAACAGAAGGC and 5′-GTCGACTCGCAGATCGGGATCCTCG. The PCR product was subcloned into pBluescript SK^+ vector (Stratagene) for sequencing.

To generate recombinant protein for binding assays, the cytoplasmic domain of dSyx5, or dSyx5Δ, was cloned by RT-PCR as described above, except that primers 5′-GAATTCCGGGATCCATGGCCGCACGCGATCGAACG and 5′-TCTAGACTCGAGCTAGATCATCAGCCAGCGATTTTTGG were used during the PCR. dSyx5Δ was then subcloned into pGEX-KG vector (Pharmacia) to generate GST-dSyx5Δ or pQE-30 vector (Qiagen) to generate His-dSyx5Δ.

RT-PCR was also used to examine the transcription of *dSyx5* activated through *EP(2)2313*. In this case, total RNA from adults of *EP(2)2313* or *yw; dSyx5*^{AR113}, *UAS-mCD8::GFP/CyO*, y^+; *da-GAL4/+* was extracted. Two sets of primers were used for PCR. One corresponds to the 5′ and 3′ ends of the coding sequence of dSyx5Δ (see above). This pair would detect all *dSyx5* transcripts since the primer sites are within both the wild type and EP-derived mRNAs. The other set includes the *3′* end primer for dSyx5Δ and p25

(5′-CGACGGGACCACCTTATGTTATTTC), which corresponds to the terminal sequence on the P-element and would detect only transcripts that derive from the EP.

To identify mutations in EMS-induced alleles, heterozygous adults were lysed in homogenization buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 25 mM NaCl, 200 μg/mL Proteinase K), which was then boiled for 1 min. Two independent PCRs were performed for each sample using primers 5′-GGAACCCGAACCAGAAATATTCG and 5′-GGCTTTTAATGGGCAACTGGCG, which flank the coding region of the *dSyx5* gene. PCR products were subcloned into pBluescript SK^+ vector and subjected to DNA sequencing. Sequence abnormality was reported only when it was observed in both PCR products.

The C terminus of the wild-type PCR product was used to replace that of dSyx5Δ to make a full-length *dSyx5* cDNA construct. For expressing dSyx5 in Schneider cells (S2 cells), pHmRa-3-myc was constructed by replacing the polycloning site of pRmHa-3 (gift of Dr. D. Williams, University of Toronto) with the polycloning site of pcDNA3.1-myc (Collins and W.S.T., unpublished observations), with a myc-*N* fragment at the 5′ end. dSyx5 was then coupled downstream to the myc-tag in this vector.

Binding Assays

BSJ72 expressing GST-dSyx5Δ fusion protein was lysed by French Press (Sim-Aminco). Fusion protein in the inclusion body was dissolved with 1% sarcosyl in PBS and subsequently treated with 2% Triton X-100 for 1 h at 4°C before it was coupled onto glutathione agarose beads (Sigma). A total of 200 ng of immobilized GST-dSyx5Δ or GST (negative control) was then incubated with a specific amount of recombinant dα-SNAP (Mohtashami *et al.*, 2001) in binding buffer (1× PBS, 0.05% Tween 20, 5 mM EDTA, 100 mM NaCl, 0.1% BSA, and 0.1% gelatin) for 1 h 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 2× SDS sample buffer and subjected to SDS–PAGE. Western blotting analysis was performed with anti-GST (1:1000) and anti-dα-SNAP (1:2000; Mohtashami *et al.*, 2001).

For pull-down experiments, frozen *Oregon R* adults (in liquid N_2) were vortexed and heads were collected through a metal sieve. The head collection was then homogenized in 50 mM Hepes, pH 7.5, 150 mM NaCl, 1 mM PMSF, 5 mM benzamidine, 2 μg/mL leupeptin, and 2 μg/mL pepstatin, solubilized with 1% Triton X-100 for 1 h at 4°C, and centrifuged at 25,000*g* for 20 min. Supernatant containing about 1 mg total protein was incubated with 10 μg of immobilized GST-dSyx5Δ fusion protein or GST for 2 h at 4°C in the presence of 0.1% BSA and 0.1% gelatin. Following extensive washes, proteins on the beads were extracted and subject to SDS–PAGE, with 3.5% of the lysate as control. Supernatant of 8C3 cell culture (1:20; ATCC) was used to detect dSyx1 in the Western blotting analysis.

Immunocytochemistry and Microscopy

For examination of apical transport in salivary glands, 20–24 h collections of embryos from *yw; dSyx5^{AR113}, UAS-mCD8::GFP/Cyo, y$^+$*; *da-GAL4* or *elav-GAL4, UAS-mCD8::GFP; dSyx5^{AR113}/Cyo* embryos were dechorionated with 50% bleach, fixed, and mounted in 70% glycerol containing 2% 1,4-diazabicyclo(2,2,2)-octane (Sigma).

For immunocytochemistry of *Drosophila* tissue culture cells, S2 cells were grown on coverslips in Schneider's complete medium (Gibco) supplemented with 10% FBS for 1 or 2 days before the cells were fixed with 4% paraformaldehyde in 100 mM Na_3PO_4, pH 7.0, for 20 min. Following fixation, the cells were then incubated with quench buffer (25 mM NH_4Cl, 25 mM glycine, 1× PBS) for 15 min and blocked overnight at 4°C with 2% BSA, 2% normal goat serum in PBT (PBS with 0.1% Triton X-100). Cells were then incubated for 2 h with rabbit anti-rat syntaxin 5 serum (1:1000; gift of Dr. M. Bennett) and mouse monoclonal anti-p120 (1:500; Calbiochem). Following washes with PBT, cells were incubated with Alexa 488-conjugated goat anti-rabbit (1:500; Molecular Probes) and Cy3-conjugated goat anti-mouse (1:500; Molecular Probes) for 1 h, washed with PBT, and then mounted and cleared with fluorescent mounting medium (DAKO). Alternatively, S2 cells on the coverslip were transfected overnight with pRaHm-3-myc-dSyx5, washed with PBS, and reincubated overnight in the Schneider's complete medium supplemented with 10% FBS medium. The cells were then fixed and costained with rabbit anti-myc (1:100; Molecular Probes), mouse monoclonal anti-p120, and the same secondary antibodies listed above. *In situ* hybridization was performed as described in White-Cooper *et al.* (1998).

Testes were dissected from adult males in testis buffer TB1 (15 mM K_2HPO_4, 15 mM KH_2PO_4, 80 mM KCl, 16 mM NaCl, 5 mM $MgCl_2$, 1% PEG8000), and transferred to a drop of TB1 on a slide, where the tip of the testis was cut open. The sample was then squashed with a coverslip and subject to phase contrast microscopy on a Nikon Optiphot 2 microscope. Images were obtained with a CCD camera. To examine Golgi morphology in the wild-type or mutant testes, samples were prepared and immunostained as described in Hime *et al.* (1996), except that anti-Lva (gift of John Sisson) was used at 1:1000 dilution (Sisson *et al.*, 2000). Images of salivary glands, S2 cells, and testes were captured by confocal microscopy on a Zeiss LSM510 confocal microscope.

RESULTS

Characterization of the Drosophila syntaxin 5 Gene

Fully understanding SNARE protein function will require analysis in a multicellular organism. For this reason, we have set out to characterize SNAREs in *D. melanogaster*. To search for novel SNARE proteins in *Drosophila*, we took advantage of the fact that members of the syntaxin family of SNARE proteins are able to bind directly to α-SNAP (McMahon and Sudhof, 1995). We therefore performed a two-hybrid screen of an ovarian cDNA library using *Drosophila* α-SNAP (dα-SNAP) as bait. In this screen, five independent, strongly positive clones were identified that were identical to the *Drosophila* homologue of syntaxin 5, *dSyx5*. The five lines shown beneath the protein model (Fig. 1C) represent the cDNAs isolated in this screen. All five were partial cDNAs and all contained sequences corresponding to the helical region of the protein (termed H3 in syntaxin family members) closest to the transmembrane domain. The H3 helical domain is involved in the formation of coiled–coil interactions with partner SNAREs and in mammalian syntaxin-1 is the region to which α-SNAP binds.

Sequence data obtained from these cDNA clones and clones available from the Berkeley *Drosophila* Genome Project were used to examine the organization of the *Drosophila syntaxin 5* (*dSyx5*) gene. The gene resides on chromosome 2, band 35E5, and this region has been fully sequenced. The *dSyx5* gene is quite compact with a single small intron of 68 nucleotides that splits the codon for amino acid 67 (Fig. 1A). The sequences we obtained were identical to those reported previously (Banfield *et al.*, 1994). *In situ* hybridization was used to analyze the expression pattern of *dSyx5* and revealed that *dSyx5* is broadly expressed throughout the developing embryo in virtually all tissues and is present at high levels in 1-h-old embryos, indicative of a significant maternal contribution (data not shown).

To confirm the significance of the two-hybrid analysis, we generated a GST fusion protein with a *dSyx5* cDNA lacking the transmembrane domain (dSyx5Δ) and examined the interaction of dSyx5Δ with increasing amounts of recombinant His_6-dα-SNAP. Western blots of eluted protein were probed with anti-GST to confirm the presence of GST-dSyx5Δ on the glutathione–agarose beads, and with anti-dα-SNAP to measure dα-SNAP binding. Under nonsaturating binding conditions, dα-SNAP bound in a dose-dependent manner, but did not bind to GST alone (Fig. 1D). dα-SNAP from adult fly homogenates was also able to bind to immobilized recombinant dSyx5Δ, while a control protein, *Drosophila* syntaxin 1, did not (Fig. 1E). These results establish that dSyx5 acts as a dα-SNAP receptor and define the H3 domain as the likely region for this interaction.

Another predicted function of a SNARE protein is its ability to interact with other members of the SNARE pathway. We previously described the phenotype that results from the overexpression of a dominant-negative form of *Drosophila* NSF2 at the developing wing margin (Stewart *et al.*, 2001). To determine whether *dSyx5* interacts genetically with *dNSF2*, we introduced a single copy of a null mutation in *dSyx5* into the flies expressing dominant-negative dNSF2 along the wing margin and saw a significant enhancement of wing notching (data not shown). This enhancement was as strong as any of the other known interactors identified previously (Stewart *et al.*, 2001) and provides further evidence that dSyx5 functions as a SNARE *in vivo*.

dSyx5 Localizes to the Golgi Complex in Drosophila Cells

In mammals, syntaxin 5 has been localized to a perinuclear compartment likely to be the *cis* portion of the Golgi complex (Bennett *et al.*, 1993). To determine whether Syx5 in *Drosophila* behaved the same as its mammalian ortholog, we first determined whether antibodies against rat syntaxin 5 would recognize *Drosophila* Syx5. Figure 2A shows that this antibody recognized an IPTG-inducible band that represented the GST-dSyx5 fusion. This antibody was then used for immunocytochemistry on Schneider S2 cells. Anti-syntaxin 5 antibodies revealed a punctate, perinuclear pattern of staining (Fig. 2B) that overlaps extensively with the Golgi marker p120 (Figs. 2C and 2D). To further confirm this colocalization, the full-length cDNA was subcloned into the pRmHa-3 expression vector in frame with an N-terminal myc epitope. This was transfected into S2 cells, and the cells were costained with antibodies against myc (Fig. 2E) and p120 (Fig. 2F). Again, significant overlap of the myc and p120 signals was seen (Fig. 2G). Hence, the *Drosophila* Syx5 protein, like its mammalian ortholog, predominantly resides in the Golgi complex.

Flies Lacking Functional dSyx5 Are Lethal during Early Larval Stages and Have Impaired Protein Transport

The *dSyx5* gene maps near *cornichon* and extensive EMS mutagenic analysis of the locus by Ashburner *et al.* (1990) had identified several lethal complementation groups that appeared to be in the vicinity of the *dSyx5* gene. Roth and Schüpbach expanded this screen and determined that complementation group *l(2)35Ff* was likely to correspond to the *Drosophila* gene homologous to *Sed5/Syx5* (T. Schüpbach, personal communication). To determine whether any of the members of this complementation group contained mutations in *dSyx5*, we used RT-PCR to amplify the *dSyx5* cDNAs from this group with four alleles ($dSyx5^{AR113}$, $dSyx5^{AE48}$, $dSyx5^{AA73}$, and $dSyx5^{AE73}$). Sequence analysis revealed that the line $dSyx5^{AR113}$ contained an amber mutation at glutamine residue 153 (Fig. 1). This mutation would create a truncated N-terminal peptide that should not interact with the SNARE proteins or α-SNAP and would therefore be expected to be functionally null. Analysis of the homozygous or transheterozygous combinations of the complementation group resulted in lethality during the first larval instar. This indicates that, although the molecular basis for the other mutations is not known, all are likely to be null or severe hypomorphic alleles.

As dSyx5 is implicated in the traffic of membranes and membrane proteins through the Golgi complex, we monitored exocytosis of an exogenous fluorescent transgene in *Oregon R* (*Ore-R*) and *dSyx5* mutant flies. For this purpose, we used UAS-mCD8-GFP flies generated by Lee and Luo (1999). mCD8-GFP is a protein fusion between the murine lymphocyte receptor CD8 and EGFP. It accumulates at the plasma membrane of most cells (Lee and Luo, 1999), and in epithelial cells, is found to accumulate at the apical membranes. To express the mCD8-GFP construct broadly, we introduced the UAS-mCD8-GFP and a *GAL4* gene driven by the *daughterless* promoter (*da-GAL4*) into the $dSyx5^{AR113}$ or wild type backgrounds. In the embryonic salivary gland of heterozygous $dSyx5^{AR113}/CyO$ flies, mCD8-GFP can be seen to accumulate on the apical surface during embryogenesis (Fig. 3B). In contrast, flies homozygous for the *dSyx5* mutation had little if any mCD8-GFP on the salivary gland apical surface and instead appeared to accumulate fluores-

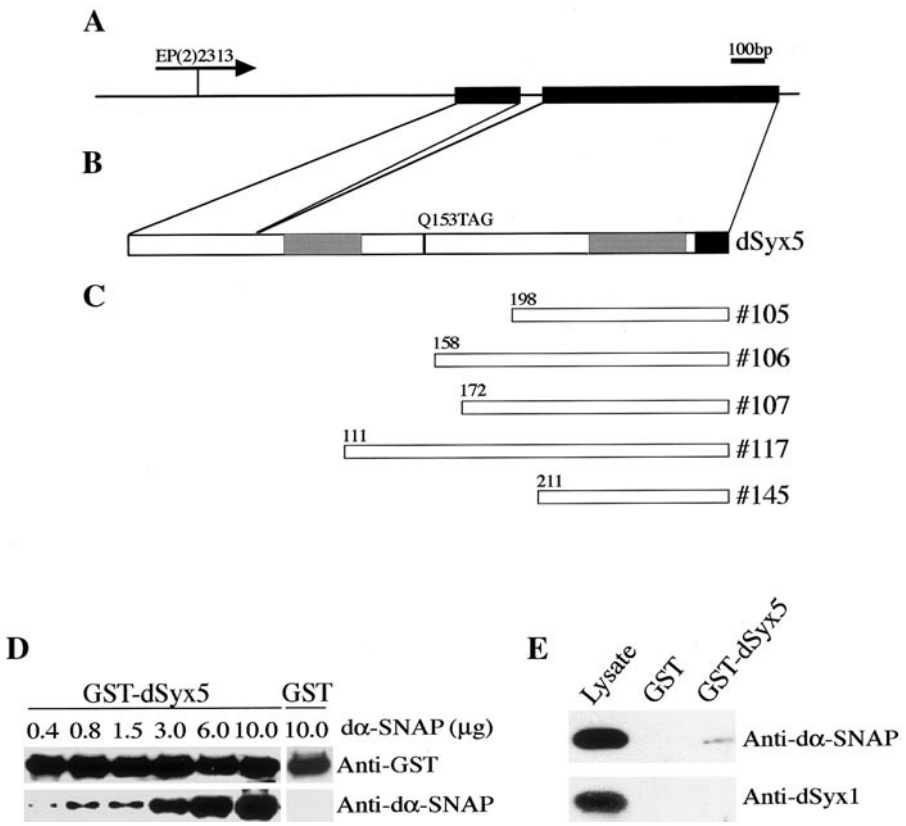

FIG. 1. *Drosophila* Syx5 binds dα-SNAP in a yeast two-hybrid screenand *in vitro*. (A) The genomic structure of *dSyx5* is summarized on the top of the figure. The coding sequences of *dSyx5* are illustrated as black boxes (untranslated regions are not shown). *EP(2)2313* is inserted 46 bp upstream from the longest *dSyx5* cDNAs deposited in Flybase. (B) The *dSyx5* gene encodes a 310-amino-acid polypeptide schematically represented in the middle of the figure. The gray boxes represent the coiled–coil motifs (predicted by software COILS version 2.1). The black box at the C terminus represents the transmembrane fragment. The black bar in the middle of the Syx5 polypeptide indicates the position of the mutation in line $dSyx5^{AR113}$. (C) Two-hybrid clones identified in the screen. The open bars below the polypeptide chain represent the portion of the *dSyx5* isolated in the positive clones obtained from the yeast two-hybrid screen. Number of the first amino acid found in each clone is indicated to the left. (D) Recombinant dα-SNAP binds directly to GST-Syx5 immobilized on glutathione beads. Increasing amounts of dα-SNAP were incubated with the beads; then, following extensive washes, proteins on the beads were eluted with SDS-loading buffer and subject to SDS–PAGE followed by Western blotting. Blots were probed with anti-dα-SNAP to monitor binding and with anti-GST to confirm equivalent levels of GST-dSyx5 on the beads. (E) dα-SNAP from fly head lysate binds to GST-Syx5 immobilized on glutathione beads. Lysates were incubated with beads loaded with GST or GST-dSyx5, extensively washed, then electrophoresed and blotted as above. Blots were probed with anti-dα-SNAP to monitor binding, and with anti-syntaxin 1 as a control for nonspecific binding to the beads. The lysate lane reflects 5% of the input material.

cence throughout the cytoplasm (Fig. 3A). Hence, *dSyx5* function appears necessary for proper transport through the secretory pathway.

Hypomorphic dSyx5 Flies Exhibit Male Sterility

In addition to the EMS alleles of *dSyx5*, we also obtained an EP line (*EP(2)2313*) from the original screen by Rorth (1996) that resulted from an insertion into the *dSyx5* promoter region (position shown in the top line of Fig. 1A). EP elements are modified P transposable elements that contain a GAL4 binding site and a weak promoter, allowing the directional expression of adjacent genes (Rorth, 1996). *EP(2)2313* is 803 bp upstream of the *dSyx5* ATG and only 46 bp upstream of the longest cDNA in the dbEST database. In addition, *EP(2)2313* is in the correct orientation to permit overexpression by GAL4. The location of *EP(2)2313* also suggested that it might be hypomorphic for *dSyx5*. *EP(2)2313* is homozygous lethal, but this lethality is likely due to second-site mutations or the effect of the EP on adjacent genes, as it is only semilethal in combination with the *dSyx5* mutant allele $dSyx5^{AR113}$ (Table 1). When *da-GAL4* is introduced into the $EP(2)2313/dSyx5^{AR113}$ background, expression of *dSyx5* is increased and the semilethality is fully rescued (see below). Similar results were obtained by using *da-GAL4* to rescue another allele of

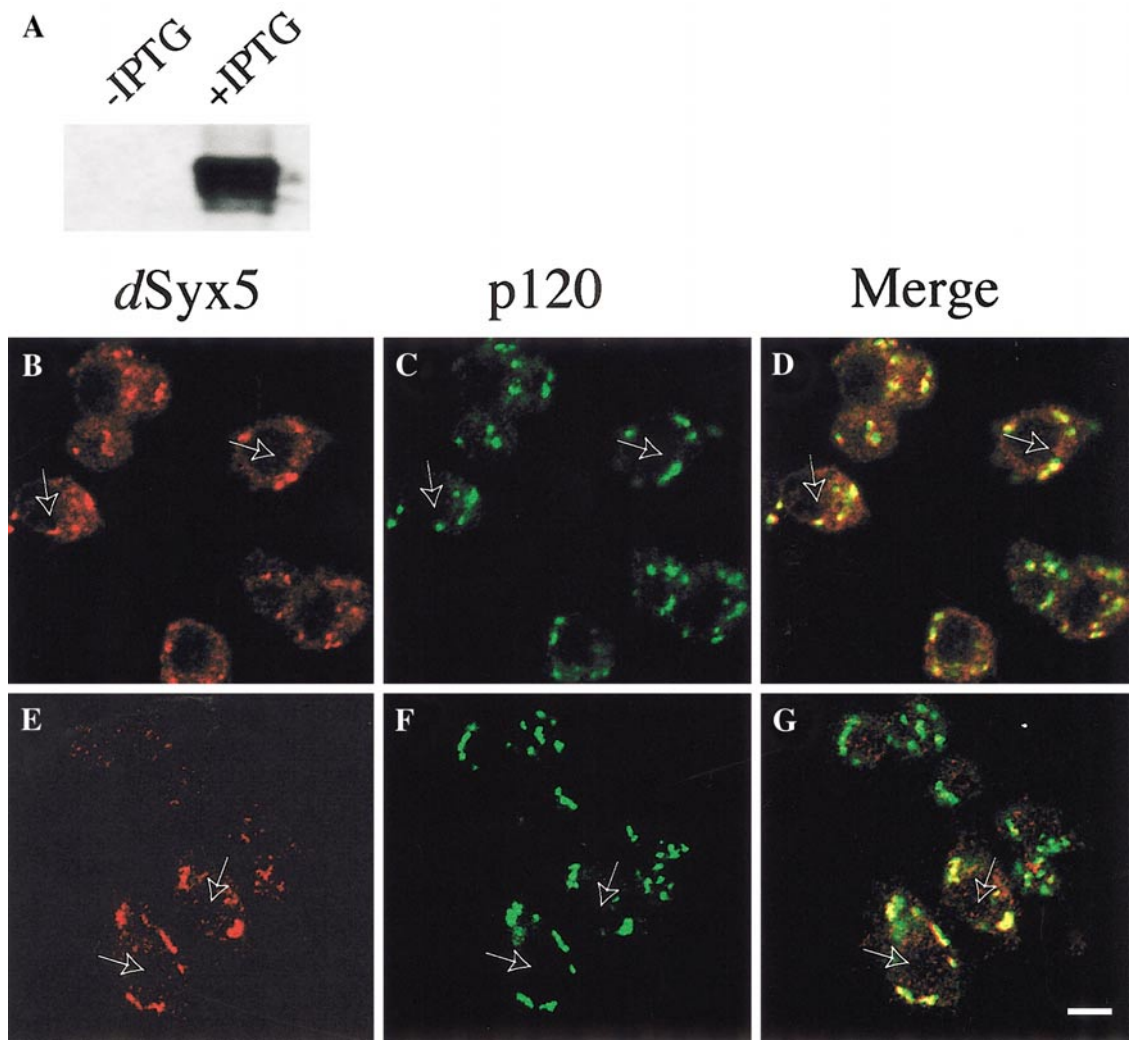

FIG. 2. dSyx5 is localized on the *Golgi* apparatus. (A) Antibody specific for rat syntaxin 5 recognizes recombinant dSyx5. Bacteria containing pGEX-dSyx5 were grown in the presence or absence of IPTG to induce dSyx5 expression, then electrophoresed, blotted, and probed with anti-rat syntaxin 5. Bands were only detected upon induction. (B–G) dSyx5 colocalizes with the Golgi marker p120. In (B–D), S2 cells were stained with crude anti-rSyx5 serum (B) and anti-p120 (C), a *Drosophila* Golgi marker. Yellow spots indicate overlapping location in merged image (D). Arrows indicate nuclei of representative cells. In (E–G), S2 cells were first transfected with myc-tagged dSyx5 and then stained with anti-myc (E) and anti-p120 (F). A merged image revealing yellow overlapping spots is shown in (G). Transfected cells are visible in (E), while all cells are visible in (F). Scale bar in (G), 5 μM.

dSyx5, $dSyx5^{AE48}$ as a transheterozygote with *EP(2)2313*. In contrast, *da-GAL4* cannot rescue the lethality of the *EP(2)2313* homozygotes, indicating that their lethality is not due to lack of dSyx5. Hence, it appears that *EP(2)2313* is hypomorphic for *dSyx5*.

The incomplete penetrance of the lethal phenotype exhibited by *EP(2)2313/*$dSyx5^{AR113}$ permitted analysis of the adult escapers. Whereas the females appeared normal, the males were sterile when crossed to female escapers or *Ore-R* females, suggesting that *dSyx5* mutant flies had a defect in some aspect of spermatogenesis. The process of spermatogenesis in *Drosophila* has been the subject of extensive analysis and mutations have been identified that affect many stages in sperm development (for review see Fuller, 1993). During spermatogenesis in *Drosophila*, germ cells undergo four mitotic divisions and two meiotic divisions, each with incomplete cytokinesis. These divisions lead to the formation of syncytial cysts of 64 spermatids connected to each other by 63 ring canals. Each testis contains a number of cysts at different stages in maturation. Within each syncytium, the spermatids form bundles that elongate the length of the testis, but then must acquire

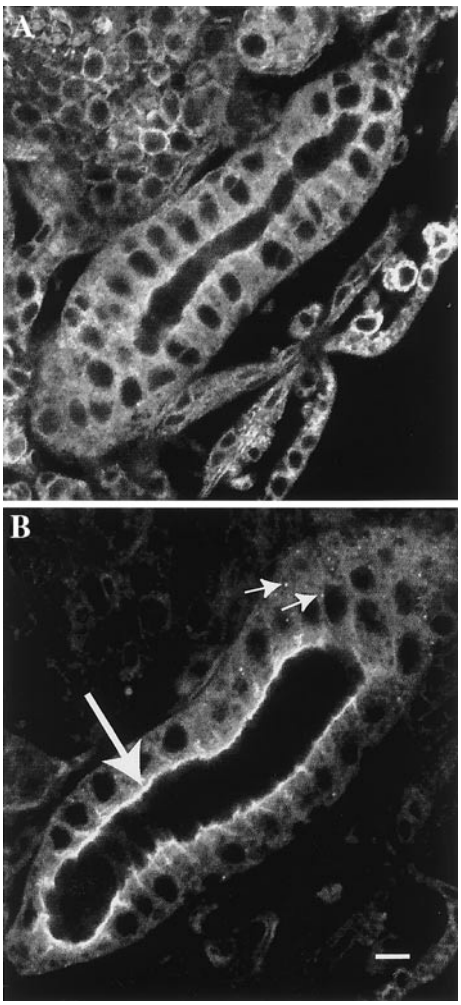

FIG. 3. Apical localization of mCD8-GFP was significantly decreased in *dSyx5* mutant. Embryos from *yw;mCD8GFPdSyx5*^{AR113}/*CyO,y*$^{+}$*;da-GAL4* flies were collected 20–24 h after deposition and subject to confocal microscopy. Shown in (A) is the salivary gland of a *dSyx5*^{AR113} homozygote, whereas in (B) is that of a heterozygote. The large white arrow points to the apical membrane region. Small arrow heads point to punctate green fluorescent signals observed only in heterozygotes. Similar results were observed in over 50 embryos analyzed. Similar results were also observed by using *elav-GAL4;mCD8GFP; dSyx5*^{AR113}*/CyO* (data not shown). Scale bar, 5 μM.

their own cell membranes in a process called individualization. Individualization occurs when a fiber-rich structure called the investment cone surrounds each elongated spermatid and progresses toward the caudal end of the spermatids, excluding all of the organelles into a so-called waste bag and encasing each spermatid in its own membrane.

Initial examination of the testes of the *dSyx5* mutant flies revealed that they had no motile sperm and appeared to be defective in spermatid elongation (Fig. 4). In contrast to wild type testes, *dSyx5* mutant testes contained predominantly large, oval cysts and few elongated bundles. *In situ* hybridization was used to examine when during spermatogenesis *dSyx5* is expressed. Figure 4C demonstrates that *dSyx5* is expressed in the primary spermatocytes (arrow), but expression does not persist past the meiosis stage and is essentially absent from the elongating bundles.

Examination of the Golgi marker Lava lamp (Lva) (Sisson *et al.*, 2000) revealed that, in those cysts that did undergo elongation, the Lva staining appeared diffuse with a mixture of large and small punctate structures (Fig. 5C). In contrast, wild type flies displayed exclusively large punctate structures (Fig. 5A). These results indicate that spermatogenesis may be a developmental process that is particularly sensitive to the function of dSyx5.

dSyx5 Is Required for Cytokinesis

Closer analysis of the spermatids revealed that more than 80% of those from the *dSyx5* mutant flies had abnormally large mitochondrial derivatives that were associated with multiple nuclei. As mitochondria fuse together following meiosis, the presence of a large mitochondrial derivative surrounded by multiple nuclei is consistent with cells having failed to complete meiotic cytokinesis (Fuller, 1993). It is important to note that membrane fusion often occurs during the preparation of these cells for microscopy, causing nuclei to become colocalized. However, the presence of the a single large mitochondrial derivative surrounded by nuclei is diagnostic of failed cytokinesis events, whereas multiple mitochondria and nuclei of the same size within a cell have arisen as an artifact in the unfixed squashed preparations (for example, see Brill *et al.*, 2000). As shown in Fig. 6B, typical mutant spermatids had an enlarged mitochondrial derivative associated with two or more nuclei. Quantification of the cytokinesis failure obtained from examining a large number of cysts revealed that, of a total of 1361 mutant nuclei counted, the vast majority were present in multinucleated cells (Table 1). In contrast, most cysts from control flies had mitochondrial derivatives and nuclei of approximately the same size and fewer than 0.5% of cysts contained multiple nuclei (Fig. 6A). Spermatids with 2 nuclei likely failed a single meiotic division while those with four probably failed to divide during both meioses. Occasionally, some spermatids were observed that had 8 nuclei, suggestive of an additional failure at an earlier mitotic division step. In addition, some spermatids had numbers of nuclei that could occur if a multinucleated parental cell divided unequally (*i.e.*, 3, 6, or 10 nuclei). These phenotypes are similar to those observed in spermatids from several male meiosis mutants including *four wheel drive* (*fwd*) (Brill *et al.*, 2000). *fwd* encodes a phosphatidylinositol 4-kinase that is required for completion of cytokinesis, particularly during the meiotic divisions (Brill *et al.*, 2000), and the similarity of the two phenotypes suggests that *dSyx5* is also required for this process.

If the defects in cytokinesis were due to the lack of dSyx5, then it should be possible to rescue this by increasing *dSyx5*

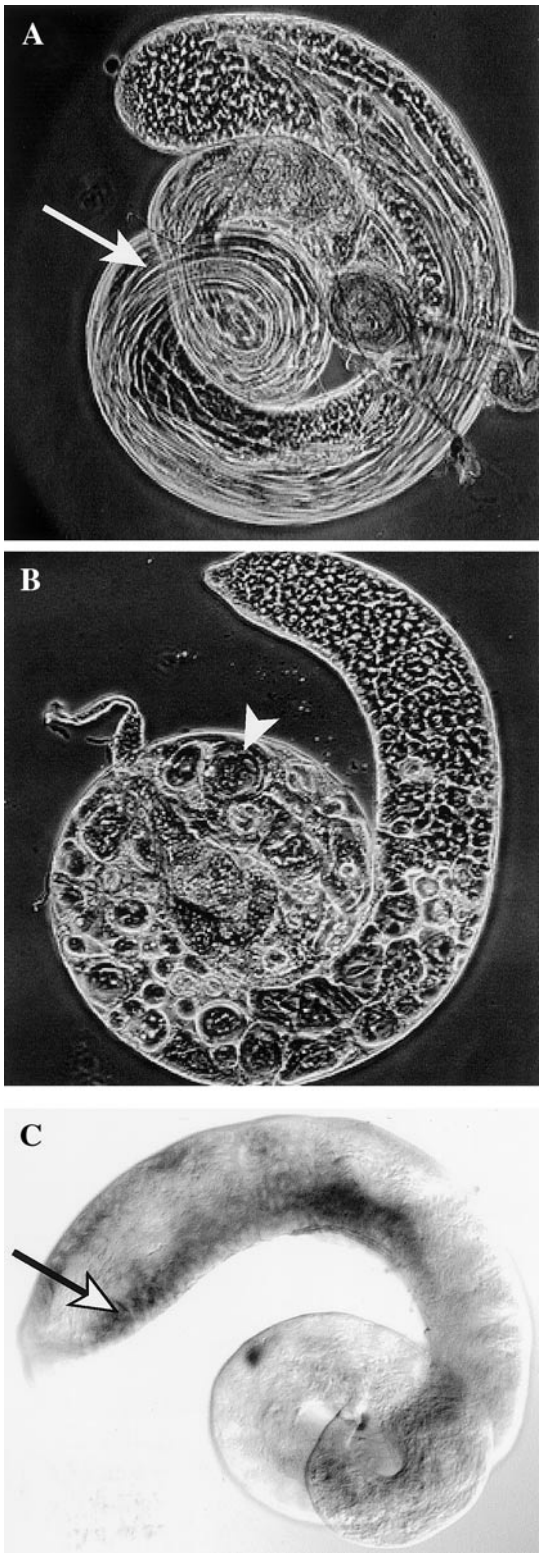

FIG. 4. *dSyx5* hypomorph shows a global defect in testis development. Low-magnification image of wild-type testis (A) reveals abundant elongated bundles (arrow). However, in the mutant testis (B), there is an accumulation of oval cysts (arrowhead). (C) *In situ* hybridization reveals that *dSyx5* is predominantly expressed in primary spermatocytes (arrow) but does not persist into elongated spermatids.

expression. We therefore introduced *da-GAL4* into the *EP(2)2313/dSyx5*^{AR113} background to drive *dSyx5* expression from *EP(2)2313* chromosome. We first determined whether the EP line could lead to elevated *dSyx5* expression in the presence of the *GAL4* driver. To measure this, we generated a pair of PCR primers, one from the EP element and one from the 3′ end of the *dSyx5* gene (P25 + Syx5C), to measure transcripts generated from the EP. The EP primer would be included in transcripts that arose from the EP element when activated by GAL4 and would measure only those transcripts. As a control, we used primers from *dSyx5* from the 5′ and 3′ ends of the *dSyx5* coding sequence (Syx5NC). These would detect all *dSyx5* transcripts, as the primer sites are within both the wild type and EP-derived mRNAs. Indeed, using RT-PCR, we could detect fusion transcripts from the EP only when the *da-GAL4* driver was present, but not in the control flies (Fig. 6D). Cysts from the *da-GAL4; EP(2)2313/dSyx5*^{AR113} flies that overexpress dSyx5 revealed significant rescue of the multinucleated phenotype (Fig. 6C). Occasional cells with two nuclei were observed, but quantification of these data (Table 1) clearly revealed a nearly complete rescue of cytokinesis by *dSyx5* expression. Only 3.9% of the spermatids counted contained multiple nuclei (compared with 80% without rescue), and viability was completely restored.

dSyx5 Is Required for Spermatid Bundle Formation and Maturation

In addition to a failure in cytokinesis, *EP(2)2313/dSyx5*^{AR113} testes also exhibit an accumulation of unusual ovoid spermatid cysts (Fig. 4). Expression of *dSyx5* from the EP chromosome with *da-GAL4* rescued both the cytokinesis and these bundle elongation defects (Table 1; not shown). Interestingly, few motile sperm were observed and the flies remained infertile. This suggested that either insufficient levels of dSyx5 were reached to achieve complete rescue, or other unrelated causes were responsible for the late stage maturation defects. In support of the former idea, introducing the stronger *actin-GAL4* driver into the *EP(3)2313/dSyx5*^{AR113} background achieved complete rescue of the spermatid maturation defect, resulting in fertile flies with motile sperm (Table 1). Thus, strong expression of dSyx5 from the EP was sufficient to rescue all spermatogenesis defects associated with mutations in *dSyx5*.

DISCUSSION

Syntaxin 5 is a Golgi-localized SNARE protein required for transport of membrane proteins from the ER to the

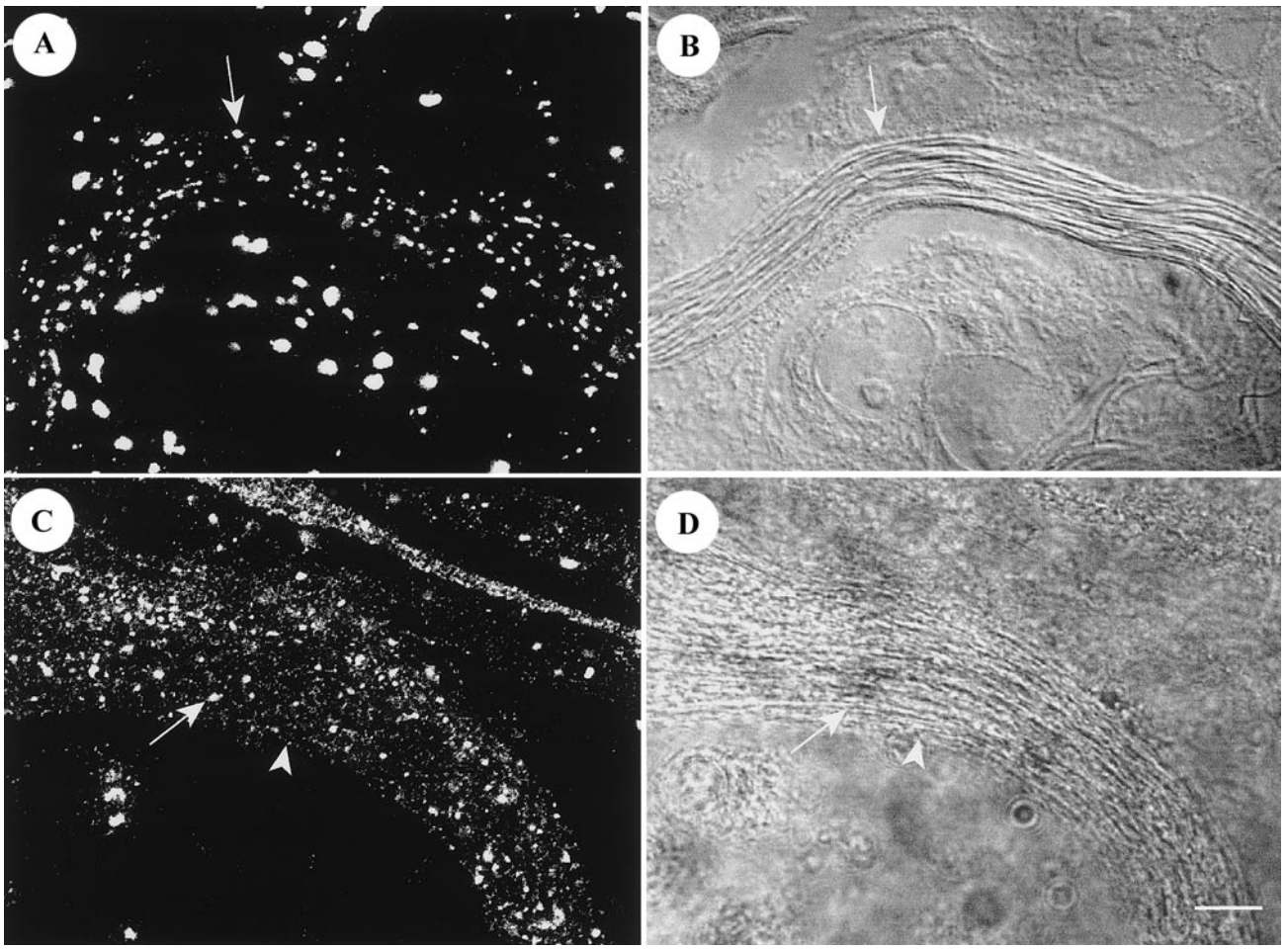

FIG. 5. *Golgi* is more dispersed in the elongated bundle of *dSyx5* hypomorph. Wild-type (A, B) and mutant (C, D) testes were fixed and immunostained with anti-Lva antibody (A, C). Punctate flurorescent signals from the wild-type testis are uniformly distributed (A, arrow). Although *Golgi* signals of similar size are also present in elongated mutant cysts (C, arrow), a much more dispersed staining pattern is observed (arrowhead). Scale bar, 10 μm.

Golgi (Dascher *et al.*, 1994) and for reassembly of the Golgi complex following mitosis (Rabouille *et al.*, 1998). Here, we have shown that the *Drosophila* ortholog of this protein, dSyx5, behaves like its mammalian and yeast counterparts in these respects. dSyx5 is localized to the Golgi complex, binds directly to dα-SNAP, and interacts genetically with *dNSF2*. Null mutations in *dSyx5* cause an accumulation of membrane proteins in intracellular compartments and are larval lethal, providing evidence for a required role for membrane traffic during early stages of development. This suggests that dSyx5 is important for transport of membrane proteins and, further, that blockade of the ER-Golgi traffic by dSyx5 deficiency would likely result in the accumulation of secretory proteins in ER-derived transport vesicles. Moreover, the early larval lethality observed likely arose due to depletion of the maternally supplied dSyx5, leading to the arrest of a variety of signaling pathways and physiological controls that require membrane protein synthesis and transport. Unexpectedly, however, these studies have also revealed a role for dSyx5 function in animal cell cytokinesis and spermatid differentiation.

By analyzing the phenotype of hypomorphic alleles of *dSyx5*, we have found that the developmental steps most sensitive to the proper function of dSyx5 are the processes of cytokinesis and sperm maturation within the male germline. The *EP(2)2313/dSyx5*^{AR113} hypomorphs together with flies expressing increasing levels of dSyx5 from the EP chromosome (driven by *da-GAL4* and *act-GAL4*) represent, in effect, an allelic series that demonstrates a requirement for dSyx5 at multiple stages of spermatogenesis (see Table 1). *EP(2)2313/dSyx5*^{AR113} flies are defective in cytokinesis, the formation of elongated spermatid bundles, and sperm individualization. Expression of *dSyx5* under *da-GAL4* control rescues both cytokinesis and bundle elongation, yet

TABLE 1

dSyx5 Alleles Show Cytokinesis and Spermatogenesis Failures during Male Meiosis

Developmental stage:		Mitotic/meiotic cytokinesis									Elongat.	Individ.	Fertility
		Number of cells with the indicated ratio of haploid spermatid nuclei per mitochondrial derivative											
Genotype	Percent adult viability	1:1	2:1	3:1	4:1	6:1	8:1	10:1	Total nuclei	% defective	Elong. bund.	Motile sperm	Male fertility
AR113/EP	7.6	225	289	26	95	11	3	1	1361	80.2	No	No	sterile
AR113/EP	126	1480	30	1	1	0	0	0	1540	3.9	Yes	No	sterile
da-GAL4/+ act-GAL4; AR113/EP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Yes	Yes	fertile
EP/CyO	100	1247	3	0	0	0	0	0	1253	0.5	Yes	Yes	fertile

Note. Cells were counted in live squash preparations. Stages of sperm maturation are listed above (meiosis, elongation, individualization, fertility). Because cell membranes are often disrupted by squashing, the number of cells reported is actually the number of mitochondrial derivatives examined. *Percentage defective* describes the percentage of spermatid nuclei derived from one or more defective meiotic and/or mitotic divisions. *Percentage viability* measures the percentage of the expected number of test class progeny.

mature individualized sperm fail to form. Expression of *dSyx5* at the highest level (from *act-GAL4*) results in the production of motile sperm. These results imply that different levels of dSyx5-dependent secretion are required for at least three distinct processes in this tissue: meiotic cytokinesis, outgrowth of membranes to form elongated spermatid cysts, and individualization of sperm. Interestingly, *in situ* hybridization studies revealed that *dSyx5* expression is highest in primary spermatocytes, suggesting that the proteins are produced at sufficient levels to persist throughout individualization.

Spermatid bundle elongation and individualization represent two aspects of male germ cell development that are accompanied by extensive plasma membrane remodeling. During the elongation of spermatids, a specialized part of the germ cell cytoplasm called the fusome passes through the intercellular bridges, or ring canals, which are composed of the actin-binding proteins anillin and the septins (Hime *et al.*, 1996). The fusome consists of highly branched membranous structures, probably contiguous with ER (Tates, 1971), and is marked by the presence of membrane skeletal proteins α-spectrin and adducin (Hime *et al.*, 1996). It is possible that the fusome may provide membrane for bundle elongation. Alternatively, a source for this membrane may be the many Lva-positive vesicles present along the elongating spermatid tails that are disrupted in *dSyx5* mutants (Fig. 5). As septins play roles in secretion (Beites *et al.*, 1999), perhaps they coordinate fusion of these Golgi-derived vesicles at the site of membrane growth during bundle formation.

Separation of syncytial spermatids into individual sperm is achieved by the action of an individualization complex (IC) composed of F-actin-rich investment cones that form around the nuclei of elongated spermatids and traverse the length of the spermatid bundles (Fabrizio *et al.*, 1998). This process invests each cell with its own plasma membrane and simultaneously strips away excess cytoplasmic material not needed by mature sperm. The discarded material is deposited in so-called waste bags (Tokuyasu *et al.*, 1972) that contain, among other components, investment cones, ring canals, and fusome material (J.A.B., unpublished observations). Proper IC function requires a large number of genes (Fabrizio *et al.*, 1998), including *jaguar,* which encodes an unconventional myosin (Fabrizio *et al.*, 1998; Hicks *et al.*, 1999). ICs formed and progressed at least part way down the bundles of *da-GAL4; EP(2)2313/dSyx5*^{AR113} spermatids. However, few waste bags were observed and no mature sperm formed (H.X. and J.A.B., unpublished observations; Table 1). Flies expressing higher levels of dSyx5 (from the *act-GAL4* driver) had motile sperm and were fertile. dSyx5 is thus the first secretory protein shown to play a role in the elaborate membrane remodeling events required to produce individual sperm.

While a requirement for membrane addition during animal cell cytokinesis is only an emerging concept, it has long been known that cytokinesis in plants requires membrane traffic for the formation of a membrane plate, called the phragmoplast, to separate the daughter cells (for review see Verma, 2001). Unlike animal cells in which fission is mediated in part by an actomyosin-based constrictive ring, the rigid cell wall precludes the action of constriction to divide the cytoplasm. In *Arabidopsis,* two proteins called KNOLLE and KEULE interact to promote vesicle fusion during cytokinesis (Assaad *et al.*, 2001; Lauber *et al.*, 1997; Waizenegger *et al.*, 2000). KNOLLE is a cytokinesis-specific syntaxin homolog, and KEULE is related to the syntaxin-binding protein Sec1, suggesting that KNOLLE proteins may serve as the target SNARE proteins during membrane addition. Interestingly, centrifugal growth of the phragmoplast plate is thought to occur by the addition of mem-

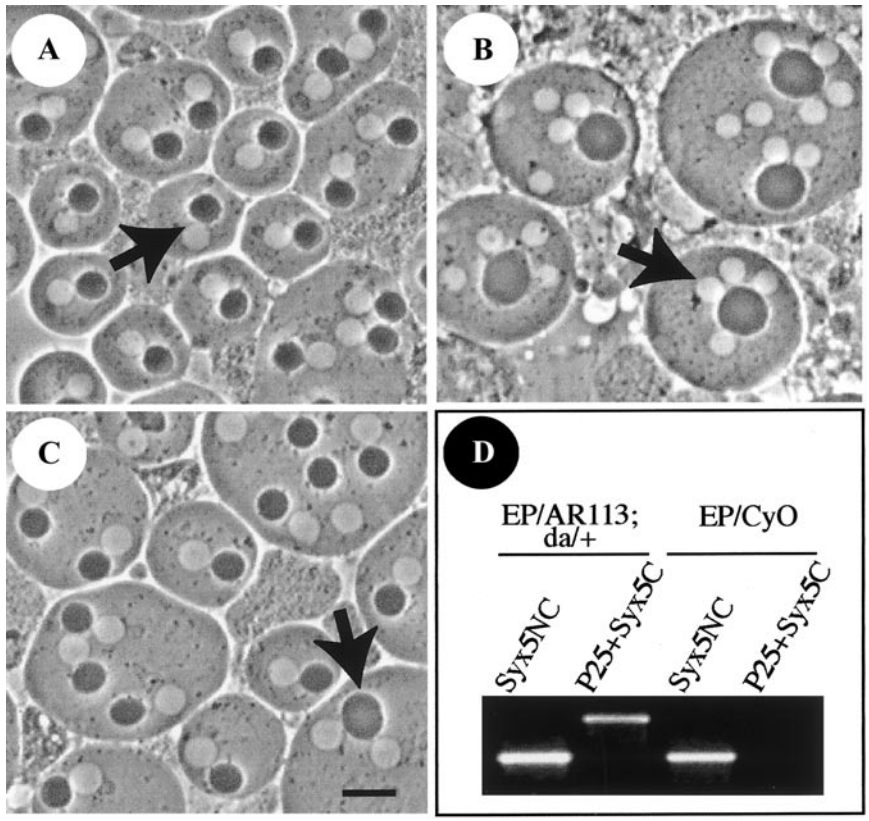

FIG. 6. *dSyx5* hypomorph demonstrates *fwd* phenotype. Phase-contrast light micrographs of onion stage spermatids in unfixed squashed preparations are shown in (A–C). Note in wild-type testis (A), one mitochondria derivative is associated with one nucleus (arrow). It is important to note that membrane fusion often occurs during the preparation of these cells for microscopy, causing nuclei to become colocalized. However, the presence of the a single large mitochondrial derivative surrounded by nuclei indicates failed cytokinesis events, whereas multiple mitochondria and nuclei of the same size within a cell have arisen as a preparation artifact. (B) In *dSyx5* mutant (*dSyx5*^{AR113}*/EP(2)2313*), large mitochondrial derivative aggregates were often attached to two, four, or multiple nuclei. However, this phenotype was largely rescued when *da-Gal4* was introduced (C), although mitochondrial derivative attached by two nuclei were occasionally observed (arrow). Scale bar, 10 μM. (D) RT-PCR was performed to confirm that *da-Gal4* did drive *dSyx5* expression. RNA was derived from either *EP(2)2313/dSyx5*^{AR113} bearing the *da-GAL4* (left two lanes) or *EP(2)2313* balanced over CyO and lacking a GAL4 driver as a negative control. Primer pairs used are indicated above each lane. Syx5N and Syx5C (Syx5NC) amplify the coding sequence portion of the *dSyx5* transcript, while P25 and Syx5C amplify from the 5′ end of transcripts originating in the EP element to the 3′ end of the coding sequence.

branes derived from the Golgi complex, since the fungal metabolite brefeldin A inhibits it, apparently by eliminating the supply of vesicles from the Golgi complex (Yasuhara and Shibaoka, 2000).

To date, the best evidence of a role for membrane addition during the division of animal cells has been the demonstration that plasma membrane syntaxin proteins in sea urchin and *C. elegans* are required for this process. In sea urchins, introduction of Botulinum C1 neurotoxin, a protease that cleaves certain mammalian syntaxins, blocked cytokinesis, although the specific target for this toxin was not identified (Conner and Wessel, 1999). In *C. elegans,* double-strand RNA inhibition (dsRNAi) was used to test the function of syntaxin isoforms, and it was found that inhibition of syntaxin 4, the worm ortholog of mammalian syntaxin 1, led to multinucleated cells (Jantsch-Plunger and Glotzer, 1999). Interestingly, of the eight syntaxins present in *C. elegans,* dsRNAi injections of only two led to embryonic lethality—syntaxin 4 and syntaxin 3 (the *C. elegans* ortholog of dSyx5). However, the latter had a complex and pleiotropic phenotype and it was not analyzed for its effect on cytokinesis. Our data clearly implicate syntaxin 5 as playing an important role in cytokinesis. Taken together, these results suggest that SNARE-mediated membrane addition is required for cytokinesis in animal cells and support the notion that syntaxin proteins play an important role as target SNAREs for this new membrane.

The source of the membranes involved in cytokinesis, however, has been less clear. As indicated above, phragmo-

plast formation occurs through the fusion of vesicles derived from the Golgi apparatus. Similarly, cellularization in *Drosophila* is also achieved through vesicles from the Golgi (Sisson *et al.*, 2000). Recent studies in *C. elegans* have shown that incubation of embryos in brefeldin A led to an inhibition of the completion of cytokinesis. In this case, the cleavage furrow ingressed properly, but stalled and finally regressed (Skop *et al.*, 2001). Interestingly, using FM1-43 labeling methods, the authors were able to demonstrate the accumulation of vesicles near the cleavage furrow in normal embryos, but showed an absence of such vesicles following brefeldin A treatment, suggesting that they may be derived from the Golgi. Finally, the recent discovery that a kinesin-like protein (Rab6-KIFL) that binds Rab6, a Golgi-localized Rab protein necessary for intra-Golgi transport (Hill *et al.*, 2000), is localized to the narrow bridge linking dividing HeLa cells during late telophase, has implicated Golgi membranes in mammalian cell division. Our data now provide clear evidence for a well-characterized Golgi protein, syntaxin 5, in this process.

Why would the Golgi complex represent an ideal source of membranes to carry out the task of building a membrane plate between dividing cells? Firstly, Golgi-derived vesicles can fuse with other Golgi complex compartments, a type of homotypic fusion necessary to generate a membrane *de novo*. Indeed, during mitosis the Golgi complex is dissociated into mitotic vesicles that partition between the daughter cells and subsequently reassemble into new Golgi complexes. Interestingly, Syx5 is important in this reassembly, as antibodies against syntaxin 5 block reassembly *in vitro* (Rabouille *et al.*, 1998). Secondly, since the Golgi membranes must be partitioned between the two cells, their movement must be linked to the cell cycle. In fact, Rab6-KIFL may be responsible for partitioning the Golgi into the daughter cells and to the midbody between the daughter cells in telophase. Thirdly, the Golgi complex is itself a plate-like structure and the formation of a sheet of membranes may be ideally achieved with vesicles of the lipid composition that makes up the flat stacks of the Golgi complex. The homotypic fusion of Golgi-derived vesicles at the narrow, constricted midbody region could contribute to furrow ingression, or form a sheetlike stucture akin to the phragmoplast that would sever the two cells and resolve cytokinesis. Future studies will be aimed at determining how cytokinesis is regulated and which other membrane trafficking proteins are involved.

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Note added in proof. During the course of these studies, a screen for mutations in spermatogenesis led to the discovery of *four way stop* (*fws*) the *Drosophila* ortholog of cog5, a protein that appears to act as a molecular tether to enhance Golgi transport *in vitro*. Remarkably, null mutations in *fws* give a virtually identical phenotype to the hypomorphic mutation in syntaxin 5 (R. M. Farkas, M. G. Giansanti, M. Gatti, and M. T. Fuller. The *Drosophila* Cog5 homolog is required for cytokinesis, polarized cell growth, and assembly of specialized Golgi architecture during spermatogenesis. *Mol. Biol. Cell*, in press), further supporting the requirement of efficient Golgi function for spermatocyte mitosis and maturation.

REFERENCES

Ashburner, M., Thompson, P., Roote, J., Lasko, P. F., Grau, Y., el Messal, M., Roth, S., and Simpson, P. (1990). The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VII. Characterization of the region around the snail and cactus loci. *Genetics* **126,** 679–694.

Assaad, F. F., Huet, Y., Mayer, U., and Jurgens, G. (2001). The cytokinesis gene KEULE encodes a Sec 1 protein that binds the syntaxin KNOLLE. *J. Cell Biol.* **152,** 531–543.

Banfield, D., Lewis, M., Rabouille, C., Warren, G., and Pelham, H. (1994). Localization of Sed5, a putative vesicle targeting molecule, to the cis-Golgi network involves both its transmembrane and cytoplasmic domains. *J. Cell Biol.* **127,** 357–371.

Beites, C. L., Xie, H., Bowser, R., and Trimble, W. S. (1999). The septin CDCrel-I binds syntaxin and inhibits exocytosis. *Nat. Neurosci.* **2,** 434–439.

Bennett, M., Garcia-Arraras, J., Elferink, L., Peterson, K., Fleming, A., Hazuka, C., and Scheller, R. (1993). The syntaxin family of vesicular transport receptors. *Cell* **74,** 863–873.

Bock, J. B., Matern, H. T., Peden, A. A., and Scheller, R. H. (2001). A genomic perspective on membrane compartment organization. *Nature* **409,** 839–841.

Brill, J. A., Hime, G. R., Scharer-Schuksz, M., and Fuller, M. T. (2000). A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. *Development* **127,** 3855–3864.

Chen, Y. A., and Scheller, R. H. (2001). SNARE-mediated membrane fusion. *Nat. Rev. Mol. Cell. Biol.* **2,** 98–106.

Conner, S., and Wessel, G. (1999). Syntaxin is required for cell division. *Mol. Biol. Cell* **10,** 2735–2743.

Dascher, C., Matteson, J., and Balch, W. (1994). Syntaxin 5 regulates endoplasmic reticulum to Golgi transport. *J. Biol. Chem.* **269,** 29363–29366.

Fabrizio, J. J., Hime, G., Lemmon, S. K., and Bazinet, C. (1998). Genetic dissection of sperm individualization in *Drosophila melanogaster. Development* **125,** 1833–1843.

Fuller, M. (1993). Spermatogenesis. In "The Development of *Drosophila melanogaster*" (M. Bate and A. Arias, Eds.), pp. 71–147. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Hardwick, K., and Pelham, H. (1992). SED5 encodes a 39-kD integral membrane protein required for vesicular transport between the ER and the Golgi complex. *J. Cell Biol.* **119,** 513–521.

Hicks, J. L., Deng, W. M., Rogat, A. D., Miller, K. G., and Bownes, M. (1999). Class VI unconventional myosin is required for spermatogenesis in Drosophila. *Mol. Biol. Cell* **10,** 4341–4353.

Hill, E., Clarke, M., and Barr, F. A. (2000). The Rab6-binding kinesin, Rab6-KIFL, is required for cytokinesis. *EMBO J.* **19,** 5711–5719.

Hime, G. R., Brill, J. A., and Fuller, M. T. (1996). Assembly of ring canals in the male germ line from structural components of the contractile ring. *J. Cell Sci.* **109,** 2779–2788.

Jantsch-Plunger, V., and Glotzer, M. (1999). Depletion of syntaxins in the early Caenorhabditis elegans embryo reveals a role for membrane fusion events in cytokinesis. *Curr. Biol.* **9,** 738–745.

Lauber, M. H., Waizenegger, I., Steinmann, T., Schwarz, H., Mayer, U., Hwang, I., Lukowitz, W., and Jurgens, G. (1997). The Arabidopsis KNOLLE protein is a cytokinesis-specific syntaxin. *J. Cell Biol.* **139,** 1485–1493.

Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible neurotechnique cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22,** 451–461.

Lindsley, D., and Zimm, G. (1992). "The Genome of *Drosophila melanogaster*." Academic Press, San Diego.

McMahon, H., and Sudhof, T. (1995). Synaptic core complex of synaptobrevin, syntaxin, and SNAP25 forms high affinity alpha-SNAP binding site. *J. Biol. Chem.* **270,** 2213–2217.

Mohtashami, M., Stewart, B. A., Boulianne, G. L., and Trimble, W. S. (2001). Analysis of the mutant Drosophila N-ethylmaleimide sensitive fusion-I protein in comatose reveals molecular correlates of the behavioural paralysis. *J. Neurochem.* **77,** 1407–1417.

Rabouille, C., Kondo, H., Newman, R., Hui, N., Freemont, P., and Warren, G. (1998). Syntaxin 5 is a common component of the NSF- and p97-mediated reassembly pathways of Golgi cisternae from mitotic Golgi fragments in vitro. *Cell* **92,** 603–610.

Rorth, P. (1996). A modular misexpression screen in Drosophila detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93,** 12418–12422.

Rorth, P., Szabo, K., Bailey, A., Laverty, T., Rehm, J., Rubin, G. M., Weigmann, K., Milan, M., Benes, V., Ansorge, W., and Cohen, S. M. (1998). Systematic gain-of-function genetics in Drosophila. *Development* **125,** 1049–1057.

Rothman, J., and Warren, G. (1994). Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. *Curr. Biol.* **4,** 220–233.

Rowe, T., Dascher, C., Bannykh, S., Plutner, H., and Balch, W. E. (1998). Role of vesicle-associated syntaxin 5 in the assembly of pre-Golgi intermediates. *Science* **279,** 696–700.

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.

Skop, A. R., Bergmann, D., Mohler, W. A., and White, J. G. (2001). Completion of cytokinesis in *C. elegans* requires a brefeldin A-sensitive membrane accumulation at the cleavage furrow apex. *Curr. Biol.* **11,** 735–746.

Sollner, T., Whiteheart, S., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J. (1993). SNAP receptors implicated in vesicle targeting and fusion [see comments]. *Nature* **362,** 318–324.

Stewart, B. A., Mohtashami, M., Zhou, L., Trimble, W. S., and Boulianne, G. L. (2001). SNARE-dependent signaling at the Drosophila wing margin. *Dev. Biol.* **234,** 13–23.

Tates, A. D. (1971). "Cytodifferentiation during Spermatogenesis in *Drosophila melanogaster*: An Electron Microscope Study." Rijksuniversiteit, Leiden.

Tokuyasu, K. T., Peacock, J. W., and Hardy, R. W. (1972). Dynamics of spermiogenesis in *Drosophila melanogaster*. I. Individualization process. *Z. Zellforsch. Mikrosk. Anat.* **124,** 479–506.

Verma, D. P. (2001). Cytokinesis and building of the cell plate in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52,** 751–784.

Waizenegger, I., Lukowitz, W., Assaad, F., Schwarz, H., Jurgens, G., and Mayer, U. (2000). The Arabidopsis KNOLLE and KEULE genes interact to promote vesicle fusion during cytokinesis. *Curr. Biol.* **10,** 1371–1374.

White-Cooper, H., Schafer, M. A., Alphey, L. S., and Fuller, M. T. (1998). Transcriptional and post-transcriptional control mechanisms coordinate the onset of spermatid differentiation with meiosis I in Drosophila. *Development* **125,** 125–134.

Yasuhara, H., and Shibaoka, H. (2000). Inhibition of cell-plate formation by brefeldin A inhibited the depolymerization of microtubules in the central region of the phragmoplast. *Plant Cell Physiol.* **41,** 300–310.

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