Sec22 Regulates Endoplasmic Reticulum Morphology but Not Autophagy and Is Required for Eye Development in Drosophila

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Background: ER morphological changes are often observed in disease conditions, but the molecular mechanisms remain unclear. Results: Malfunction of Sec22 and its binding partners resulted in ER expansion and defects in fly eye development. Conclusion: Sec22 regulates ER morphology through regulating ER–Golgi trafficking but not autophagy. Significance: ER morphology changes under the disease conditions might be due to the defects of ER–Golgi trafficking.

The endoplasmic reticulum (ER) is a highly dynamic organelle that plays a critical role in many cellular processes. Abnormal ER morphology is associated with some human diseases, although little is known regarding how ER morphology is regulated. Using a forward genetic screen to identify genes that regulated ER morphology in Drosophila, we identified a mutant of Sec22, the orthologs of which in yeast, plants, and humans are required for ER to Golgi trafficking. However, the physiological function of Sec22 has not been previously investigated in animal development. A loss of Sec22 resulted in ER proliferation and expansion, enlargement of late endosomes, and abnormal Golgi morphology in mutant larvae fat body cells. However, starvation-induced autophagy was not affected by a loss of Sec22. Mosaic analysis of the eye revealed that Sec22 was required for photoreceptor morphogenesis. In Sec22 mutant photoreceptor cells, the ER was highly expanded and gradually lost normal morphology with aging. The rhodobinemes in mutants were small and sometimes fused with each other. The morphology of Sec22 mutant eyes resembled the eye morphology of flies with overexpressed eye (eyes closed), eye encodes for a Drosophila p47 protein that is required for membrane fusion. A loss of Syntaxin5 (Syx5), encoding for a t-SNARE on Golgi, also phenocopied the Sec22 mutant. Sec22 formed complexes with Syx5 and Eyc. Thus, we propose that appropriate trafficking between the ER and Golgi is required for maintaining ER morphology and for Drosophila eye morphogenesis.

The endoplasmic reticulum (ER) plays pivotal roles in many cellular processes. In addition to its important function in protein synthesis, modifications, and quality control, the ER is also critical for lipid synthesis, autophagy, and calcium homeostasis (1–3). The ER makes close contacts with other organelles, including mitochondria, the Golgi apparatus, endosomes, peroxisomes, and lysosomes. It exchanges lipids and proteins with these organelles to regulate their biogenesis and function (4, 5). Thus, the ER has diverse functions, and ER malfunctions can cause numerous human diseases, including diabetes and neurodegeneration (6).

The ER is a membrane network that consists of tubules and sheets that extend throughout a cell (7, 8). The ER is highly dynamic and constitutively undergoes rearrangements, which are critical for ER functions (9–11). ER morphological changes, including fragmentation and expansion, are often observed in cells that are subjected to pathological insults (6). However, the molecular mechanisms underlying these ER morphological changes remain unclear.

Using a forward genetic screen to identify genes that regulated ER morphology, we obtained a complementation group with remarkable ER proliferation and expansion. Molecular mapping located a lesion on Sec22, a gene encoding for a SNARE protein enriched on the ER (12, 13). SNARE proteins are a family of conserved proteins that mediate membrane fusion between vesicles derived from one subcellular compartment and their targeted membranes (14, 15). In the secretory pathway, proteins are transported by vesicle budding, docking, and fusion. Different SNAREs mediate the fusion step between various vesicles and their ultimate destinations. Vesicle membrane-localized v–SNAREs and targeting membrane-localized t–SNAREs form helix bundles to drive membrane fusion. Subsequently, these bundled SNAREs are dissociated and recycled by N-ethylmaleimide-sensitive factor (16).

Sec22 family proteins are highly conserved from yeast to human. In yeast, there is only one Sec22 gene, and its functions partially overlap with those of another gene, ykt6 (13). Sec22p is involved in transport between the ER and Golgi compartments in both anterograde and retrograde directions in yeast (12). In addition, Sec22p is required for autophagosome biogenesis by regulating the transport of Atg9, an essential protein for autophagosome formation, to the phagophore assembly site.
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(17). In both yeast and plants, Sec22p/SEC22 regulates cesium accumulation, which indicates that Sec22 proteins play a role in vacuole function (18). A loss of SEC22 in plants also results in Golgi fragmentation and defects in gametophyte development (19). Studies of the rice fungus show that Sec22 is required for conidiogenesis, cell wall integrity, and host plant infection (20). Several Sec22 genes, SEC22A, SEC22B, and SEC22C, were also found in humans. In cultured mammalian cells, SEC22 proteins are also required for ER to Golgi trafficking (21, 22). However, knocking down SEC22B expression in cultured mammalian cells does not cause autophagy defects, presumably because of redundancy (23). It was recently shown that SEC22B also has a conserved fusogenic function in plasma membrane expansion (24).

All of these studies demonstrated that Sec22 was a key regulator of the secretory pathway. However, surprisingly, there have been no studies of the physiological functions of Sec22 in animal development and morphogenesis. In this study, we have found that Sec22 was an essential gene in flies. A loss of Sec22 resulted in morphogenesis defects in fly eyes. The ER was highly proliferated, and its morphology underwent very dramatic changes in Sec22 mutant flies. In contrast to yeast orthologs, Sec22 was not required for starvation-induced autophagy in *Drosophila*. Interestingly, a loss of Syntaxin 5 (Syx5), which encodes for a t-SNARE in Golgi, also phenocopied Sec22 mutants, which indicated that appropriate trafficking between the ER and Golgi was critical for ER morphology.

**EXPERIMENTAL PROCEDURES**

**Molecular Biology**—To generate a Sec22 genomic rescue construct, a 3.3-kb genomic fragment that contained the Sec22 gene was amplified using the primers, 5′-TGAGATCTGTCCAGGCAGGATGTCCTTCG-3′ and 5′-TACTCCGAGCCAGAGCTTACGACGACCAGACG-3′, using genomic DNA that was purified from FRT19A isogenized flies. To generate a Sec22 expression vector, we inserted the Sec22 cDNA into a pattB UAS-3HA vector. A 3XHA tag was fused to Sec22 at the N terminus. To generate a Flag-tagged Syntaxin 5 expression vector, we inserted the Flag sequence and Syntaxin 5 cDNA into the pattB UAS expression vector. The Flag tag was fused to the N terminus of the Syx5 protein. A Myc-Eye expression vector was generated by inserting full-length *eye* cDNA into the pattB UAS-Myc expression vector. Myc was fused to the N terminus of the Eyc protein.

**Fly Strains**—A Sec22 mutant was isolated from an ethane methylsulfonate screen as previously reported (25). The fly strains purchased from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN) were Df(1)ED6443, Df(1)BSC534, Dp(1;3)DC012, Dp(1;3)DC013, P[ey-FLP.N][2], P[EP] Syx5 EP2313P [neoFRT] 40A, P[UA5p-GFP.KDEL], and P[UA5p-RFP.KDEL]. Sec22 genomic rescue transgenic flies, UAS-3XHA-Sec22, UAS-Myc-Eyc, and UAS-Flag-Syx5 transgenic flies were generated by the Core Facility of *Drosophila* Resource and Technique (Institutes of Biochemistry and Cell Biology, Shanghai) using standard injection procedures. Syx5 RNAi fly strains were from the Tsinghua Fly Stock Center (Beijing, China).

cDNA Analysis of the Sec22 Mutant—Total RNA was isolated from 25 wild type larvae or larvae with homozygous mutation in Sec22 using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. cDNAs were synthesized using a first strand cDNA synthesis kit (Invitrogen) with oligo(dT) primers and used in PCRs with the Sec22-specific primers P1F (5′-ATGGCGCTGCTGACCATGATAG-3′) and P1R (5′-TTACAGAACCCAGAAATACATG-3′) or primers P2F (5′-GTTGTGCTGGCGGTTAATTACTCAC-3′) and P2R (5′-TTACAGAACCCAGAAATACATG-3′). The PCR products were sequenced.

**Immunostaining for Fly Fat Bodies**—Dissected fly fat bodies were 3.7% formaldehyde in PBS for 20 min and then washed three times with PBST (PBS + 0.1% Triton X-100). Tissues were incubated with a primary antibody (rabbit anti-HA used at 1:1000; CST) overnight, extensively washed, and then incubated with a secondary antibody (Alexa 488-conjugated used at 1:500; Invitrogen) at room temperature for 1 h. After extensive washing, samples were mounted in vector shield and observed by a confocal microscope.

**Phalloidin and Immunostaining of the Photoreceptor Cells**—For the late pupal stage flies, the dissected brain-eye complexes were fixed in PBS with 4% formaldehyde for 20 min. For the adult flies, heads were fixed in PBS with 4% formaldehyde for 1 h upon removal of the proboscis. Then the photoreceptor cells were dissected and fixed for 15 min. After the fixation, the tissues were washed three times with PBST (PBS + 0.4% Triton X-100) and stained with primary antibody mouse anti-Rh1 (1:50; Developmental Studies Hybridoma Bank) overnight at 4 °C. After extensive washing with PBST, secondary antibody (1:500, Invitrogen) and Alexa 488-labeled phalloidin (1:1000, Invitrogen) were incubated with the tissues for 2 h at room temperature. Samples were washed with PBST four times and mounted in vector shield and observed by a confocal microscope.

**Cell Culture and Immunoprecipitation**—S2 cells were cultured at room temperature and transfected using Opti-PEI. For co-immunoprecipitation, cells were harvested at 48 h after transfection and lysed with lysis buffer. Cell lysates were incubated with beads (anti-HA, anti-Myc, or anti-Flag) at 4 °C for 4 h in lysis buffer. After extensive washing, the immunoprecipitation products were analyzed by Western blot using anti-HA (1:1,000), anti-Myc (1:1,000), or anti-FLAG (1:1,000), followed by an HRP-conjugated secondary antibody (1:5,000).

**Transmission Electron Microscopy**—For electron microscopic observations of photoreceptors, fly heads were dissected and fixed in 4% paraformaldehyde and 1% glutaraldehyde at 4 °C overnight. Then the samples were post-fixed in 2% OsO4 for 2 h, followed by dehydration in ethanol and propylene oxide. Samples were embedded in Embed-812 resin (Electron Microscope Scienes). TEM images were acquired using a transmission electron microscope equipped with a digital camera.

**Confocal Microscopy**—All mounted samples were examined and imaged with a confocal microscope LSM710 (Carl Zeiss) outfitted with a Plan-Apochromat 63× 1.4NA oil immersion objective lens (Carl Zeiss). The data were acquired using Zen 2010 Software and processed with Photoshop CS (Adobe).
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ERM1 Mutants Correspond to Sec22 Mutants—Because the ERM1 phenotypes were interesting, we performed duplication and deficiency mapping. ERM1 was rescued by an X chromosome duplication that covered a region from 1A1 to 2C1. Several overlapping deficiencies that covered this region were used to further narrow down the mutant lesion. Df(1)BSC534 did but the overlapping Df(1)ED6433 did not rescue the lethality of ERM1, which indicated that the mutation was localized in the region deleted in Df(1)ED6433 but not in Df(1)BSC534. To further pinpoint the mutant region, small duplications that covered the deleted region in the deficiencies were used for a complementation test. ERM1 was rescued by two overlapping small duplications: Dp(1;3) DC012 and Dp(1;3) DC013, which narrowed the putative gene down to nine genes (Fig. 2A). Sequencing revealed point mutations in Sec22 (Fig. 2B). A splicing acceptor G to A mutation at the end of the third intron was detected in ERM1. To analyze the transcripts in this mutant, we isolated mRNA from the mutant larvae, made cDNAs, and sequenced the cDNAs with two pair of primers. Two different cDNAs were identified: Sec22 mu1 and Sec22 mu2 (Fig. 2C). An alternative splicing acceptor site was adopted by Sec22 mu1, which resulted in a deletion of part of the fourth exon. Wild type Sec22 encodes for a SNARE protein that contains a longin domain and a synaptobrevin domain (Fig. 2D). The frameshift caused by the deletion in Sec22 mu1 resulted in the mistranslation of the most part of the synaptobrevin domain. Sec22 mu2 had an unspliced intron and that led to a premature stop codon in the synaptobrevin domain.

To confirm that ERM1 mutants indeed corresponded to Sec22 mutants, a genomic fragment that contained only the Sec22 gene was introduced into these mutants (Fig. 2A). The genomic rescue construct not only fully rescued the lethality of ERM1 to adulthood but also rescued the ER morphology defects in the mutant clones (Fig. 2E). It indicated that Sec22 was the gene affected in this mutant.

Sec22 Is Not Required for Autophagy in Drosophila—A recent study with yeast showed that Sec22 is required for autophagosome formation. Because the ER is degraded in the autophagosome through “ER-phagy” (27), the accumulated morphologically abnormal ER in Sec22 mutants could be due to an autophagy malfunction.

To test whether Sec22 was required for autophagy in flies, we examined different autophagy markers in Sec22 mutant cells after their starvation. We labeled autophagy vacuoles in fat bodies by expressing different markers driven by a fat body Gal4 driver, Cg-Gal4 (28). We negatively labeled the Sec22 mutant clones with RFP in third instar larvae fat bodies and then examined the different autophagy markers after starvation for 4 h. Autophagy initiation requires type III PI3K activity, which can be monitored based on the intracellular distribution of FYVE-GFP. Without PI3K activity, FYVE-GFP signals are dif-
fusely distributed inside cells. If PI3K is activated, then FYVE-GFP exhibits punctate staining (29). In Sec22 mutant cells, FYVE-GFP exhibited a punctate distribution that was similar to that in control cells (Fig. 3, A and A’), which indicated that PI3K activity was intact.

Atg8 is an ubiquitin-like protein that is required for autophagosome formation and autophagy progression (30). Without starvation, Atg8 signals are diffusely distributed inside cells. Upon starvation, Atg8 forms punctate structures and labels autophagosomes. Once autophagosomes fuse with lysosomes, Atg8 is degraded inside autolysosomes. In Sec22 mutant clones, Atg8-GFP punctate structures were formed, and these patterns were comparable with those in controls (Fig. 3, B and B’), which indicated that Sec22 was not required for autophagosome formation.

To monitor autophagosome and lysosome fusion, Atg8-GFP RFP was used as a marker. GFP signals but not RFP signals are quenched inside acidic autolysosomes; thus, autophagosomes will appear yellow, and autolysosomes will appear red (31). If there are fusion defects between autophagosomes and...
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FIGURE 3. Sec22 is not required for starvation-induced autophagy. No obvious autophagy defects could be detected in Sec22 mutant clones (absence of RFP: red in A, A’, C, C’, D, and D’). A and A’, FYVE-GFP indicated that PI3K activity, whose activity is required for autophagy initiation, was intact in Sec22 mutant cells. B and B’, upon starvation, Atg8-GFP formed punctate structures inside fat body cells. Both control and Sec22 clones had similar responses to starvation. C and C’, MARCM clones of control and Sec22 mutant cells showed the Atg8-GFP-RFP signals for monitoring autophagosome and lysosome fusion. There was no difference between control and Sec22 cells. D and D’, distribution of p62, an autophagy substrate, in Sec22 mutant cells was similar to that in control cells.

Interestingly, Syx5 overexpression in fat body cells also led to ER expansion similar to the ER morphology in the Syx5 RNAi tissues, suggesting Syx5 overexpression has dominant negative effects (Fig. 4E). In yeast, ER-Golgi transport requires four SNARE proteins, Sec22p, Bo1p, Bet1p, and Sed5p (encoded by yeast orthologs of Sec22 and Syx5), to form complexes. The dominant negative effects of Syx5 overexpression probably is due to the extra Syx5 competing with the endogenous Syx5 to form ineffective complexes with other endogenous SNARE proteins, such as Sec22 or the proteins encoded by the orthologs of Bo1p or Bet1p. Strikingly, when we co-expressed Sec22 and Syx5, the ER punctuate structures in the fat body cells were much larger than those in the cells with Syx5 overexpression alone (Fig. 4D–F). Because Sec22 overexpression did not have ER morphology defects, the enhancement of the Syx5 overexpression phenotypes suggested that Sec22 might form a complex with Syx5, and together they were a better competitor than Syx5 alone to titrate out of other SNARE proteins. These data implied that Sec22 and Syx5 might function in a same pathway to regulate ER morphology.
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Sec22 Is Required for Eye Morphogenesis—The dramatic ER morphological changes in the Sec22 mutant cells suggested that Sec22 played a critical role in regulating ER function. However, the physiological function of Sec22 has not been previously investigated in animals. We found that a Sec22 hemizygous mutant cannot survive to the adulthood. To investigate whether Sec22 was required for nerve system development and neuron homeostasis, we generated Sec22 mutant clones in the eye using the eyFLP system. In wild type control eyes, round, tightly packed, and oval-shaped rhabdomeres were in a trapezoid arrangement. In Sec22 mutant eyes, irregularly shaped rhabdomeres were small and sometimes fused with each other (Fig. 5, A, a, C, and c). Inside photoreceptor cells, the ER was highly expanded with numerous stacks and gradually lost its normal morphology with aging (Fig. 5, A–D and a–d). Occasionally, lipid droplets could be detected in the cytoplasm, another hallmark of ER malfunction (Fig. 5, A–D and a–d).

It has been reported that mistrafficking of Rh1 (Rhodopsin 1) leads to retinal developmental defects and degeneration (34, 35). Rh1 is a light sensor that presents in the R1–R6 photoreceptor cells. During fly photoreceptor cell development, Rh1 is synthesized and matured in the ER and transports to the rhabdomeres through the Golgi. The ER defects are likely to cause mistrafficking of Rh1 (34, 35). We therefore tested whether there is Rh1 trafficking defects in the Sec22 mutants. We made Sec22 mutant clones in the fly eyes and stained the tissue with anti-Rh1 antibody and phalloidin to examine the distribution Rh1 in the photoreceptor cells at the late pupae (84 h after puparium formation) and adult stages. There was a massive accumulation of Rh1 in the cytoplasm (Fig. 5E), which might explain the degeneration phenotypes we observed in Sec22 mutant eyes.

Syx5 Mutants, Sec22 Mutants, and Eyc Overexpression Animals All Have Similar ER Phenotypes—Studies of Syx5 functions in the fat body cells suggested that it might form a complex with Sec22 to regulate ER morphology. We then tested whether Syx5 mutants also had phenotypes similar to those of Sec22 mutants in the eyes. Indeed, the rhabdomeres were small and irregularly shaped in the Syx5 mutants. The photoreceptor cells were also packed with overproliferated ER and lipid droplets (Fig. 6, A and a).

The photoreceptor morphogenesis defects and ER accumulation in Sec22 mutants were reminiscent of a mutant called eyc (eyes closed) (36). eyc encodes for a fly p47 protein that forms a complex with p97 ATPase to regulate membrane fusion (37). Eyc overexpression during the early pupal stage resulted in fused rhabdomeres. Eyc misexpression at a later stage resulted in ER proliferation in the photoreceptor cells (Fig. 6, B and b) (36). The trafficking of Rh1 is defective in the fly eyes with Eyc overexpressed (36). All of these phenotypes were very similar to what we observed in Sec22 mutant eyes. To test whether Sec22 and Eyc interacts with each other, we performed IP experiments with cultured fly S2 cells. Indeed, Sec22 bound to Eyc (Fig. 6, C and D). We wondered whether Sec22, Syx5, and Eyc function in a same pathway to regulate ER morphology and photoreceptor cell morphogenesis. We knocked Syx5 known or overexpressed Eyc in the Sec22 mutant background using MARCM technology and analyzed the ER morphology in the fat body cells. Both Syx5 RNAi and Eyc overexpression in the Sec22 mutant cells led to cell growth defects. The cells became very small, which hindered the ER morphology analysis (Fig. 6, E–J). The synergistic effects of regulating cell size suggested that these proteins may have paralleled functions in cell growth. However, whether they work together in regulating ER morphology is still an open question.

Because both Eyc and Syx5 interact with Sec22, we hypothesized that Eyc might compete with Syx5 to bind Sec22. However, we did not observe any decrease of the interaction between Sec22 and Syx5 when Eyc was overexpressed (Fig. 6K).
We also did not observe any alleviation of ER morphology defects in Eyc overexpression cells when Sec22 was overexpressed. Therefore, Eyc overexpression phenotypes were not due to competing Sec22 with Syx5 (Fig. 6, L–O).

**DISCUSSION**

Losses of Sec22 and Syx5 Result in ER Expansion—The ER is highly dynamic, and its morphology is tightly regulated (10, 11). Abnormal ER morphology is associated with hereditary spastic paraplegias and related disorders (38). In this study, we found that both Sec22 and Syx5 mutants had an overproliferated ER and expanded ER lumens. Sec22 is primarily localized on the ER, and Syx5 is found on the Golgi. The interaction between Sec22 and Syx5 mediates the fusion of ER-derived vesicles to Golgi cysts. The similar ER phenotypes between Sec22 and Syx5 mutations suggested that defective ER-Golgi trafficking resulted in ER proliferation and morphology defects.

ER proliferation has been found in cells that were exposed to multiple pathological insults; however, the molecular mechanisms involved are less well understood (6). It would be interesting to test whether the trafficking route between the ER and Golgi is blocked in these disease conditions. It is still not clear how these trafficking defects result in ER proliferation and morphology defects. ER stress could result in ER proliferation. Thus, it needs to be determined whether these Sec22 mutants have ER stress responses.

Eyc, p97, and ER Dynamics—SNARE proteins can assemble into unproductive cis-SNARE complexes that need to be disso-
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References

1. Friedman, J. R., and Voeltz, G. K. (2011) The ER in 3D: a multifunctional dynamic membrane network. Trends Cell Biol. 21, 709–717
2. Ellgaard, L., and Helenius, A. (2003) Quality control in the endoplasmic reticulum. Nat. Rev. Mol. Cell Biol. 4, 181–191
3. Lamb, C. A., Yoshimori, T., and Tooze, S. A. (2013) The autophagosome: origins unknown, biogenesis complex. Nat. Rev. Mol. Cell Biol. 14, 759–774
4. Rowland, A. A., and Voeltz, G. K. (2012) Endoplasmic reticulum-mitochondria contacts: function of the junction. Nat. Rev. Mol. Cell Biol. 13, 607–625
5. Brandizzi, F., and Barlowe, C. (2013) Organization of the ER-Golgi interface for membrane traffic control. Nat. Rev. Mol. Cell Biol. 14, 382–392
6. Hetz, C., and Mollereau, B. (2014) Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat. Rev. Neurosci. 15, 233–249
7. Voeltz, G. K., and Prinz, W. A. (2007) Sheets, ribbons and tubules: how organelles get their shape. Nat. Rev. Mol. Cell Biol. 8, 258–264
8. Mattaj, I. W. (2004) Sorting out the nuclear envelope from the endoplasmic reticulum. Nat. Rev. Mol. Cell Biol. 5, 65–69
9. Vedrenne, C., and Hauri, H. P. (2006) Morphogenesis of the endoplasmic reticulum: beyond active membrane expansion. Traffic 7, 659–646
10. Chen, S., Novick, P., and Ferro-Novick, S. (2013) ER structure and function. Curr. Opin. Cell Biol. 25, 428–433
11. Lin, S., Sun, S., and Hu, J. (2012) Molecular basis for sculpting the endoplasmic reticulum membrane. Int. J. Biochem. Cell Biol. 44, 1436–1443
12. Lewis, M. J., Rayner, J. C., and Pelham, H. R. (1997) A novel SNARE complex implicated in vesicle fusion with the endoplasmic reticulum. EMBO J. 16, 3017–3024
13. Liu, Y., and Barlowe, C. (2002) Analysis of Sec22p in endoplasmic reticulum/Golgi transport reveals cellular redundancy in SNARE protein function. Mol. Biol. Cell 13, 3314–3324
14. Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H., and Rothman, J. E. (1993) A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418
15. Söllner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J. E. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324
16. Rizo, J., and Südhof, T. C. (2012) The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices: guilty as charged? Annu. Rev. Cell Dev. Biol. 28, 279–308
17. Nair, U., Jotwani, A., Gammoh, N., Richerson, D., Yen, W. L., Griffin, J., Nag, S., Wang, K., Moss, T., Baba, M., McNew, J. A., Jiang, X., Reggiori, F., Melia, T. J., and Klionsky, D. J. (2011) SNARE proteins are required for macroautophagy. Cell 146, 290–302
18. Dräxl, S., Müller, J., Li, W. B., Michalke, B., Scherb, H., Hense, B. A., Tschiersch, J., Kanner, U., and Schäffer, A. R. (2013) Caesium accumulation in yeast and plants is selectively repressed by loss of the SNARE Sec22p/SEC22. Nat. Commun. 4, 2092
19. El-Kasmi, F., Pacher, T., Strompen, G., Sierhof, Y. D., Müller, L. M., Koncz, C., Mayer, U., and Jürgens, G. (2011) Arabidopsis SNARE protein SEC22 is essential for gametophyte development and maintenance of Golgi-stack integrity. Plant J. 66, 268–279
20. Song, W., Dou, X., Qi, Z., Wang, Q., Zhang, X., Zhang, H., Guo, M., Dong, S., Zhang, Z., Wang, P., and Zheng, X. (2010) R-SNARE homolog MoSec22 is required for conidiosgenesis, cell wall integrity, and pathogenesis of Magnaporthe oryzae. PLoS One 5, e13193
21. Paek, I., Orci, L., Ravazzola, M., Erdjument-Bromage, H., Amherdt, M., Tempst, P., Söllner, T. H., and Rothman, J. E. (1997) ERS-24, a mammalian v-SNARE implicated in vesicle traffic between the ER and the Golgi. J. Cell Biol. 137, 1017–1028
22. Hay, J. C., Chao, D. S., Kuo, C. S., and Scheller, R. H. (1997) Protein interactions regulating vesicle transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Cell 89, 149–158
23. Moreau, K., Ravikumar, B., Renna, M., Puri, C., and Rubinsztein, D. C. (2011) Autophagosome precursor maturation requires homotypic fusion. Cell 146, 303–317
24. Petkovic, M., Jemaiel, A., Daste, F., Specht, C. G., Izeddin, I., Vorkel, D., Brandizzi, F., and Barlowe, C. (2013) Organization of the ER-Golgi interface for membrane traffic control. Nat. Rev. Mol. Cell Biol. 14, 382–392
25. Zhang, K., Li, Z., Jaiswal, M., Bayat, V., Xiong, B., Sandoval, H., Chargin, W. L., David, G., Haueter, C., Yamamoto, S., Graham, B. H., and Bellen, H. J. (2013) The C8ORF38 homologue Sicily is a cytosolic chaperone for a mitochondrial complex I subunit. J. Cell Biol. 200, 807–820
26. Ye, B., Zhang, Y., Song, W., Younger, S. H., Jan, L. Y., and Jan, Y. N. (2007) Growing dendrites and axons differ in their reliance on the secretory
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pathway. Cell 130, 717–729
27. Bernales, S., Schuck, S., and Walter, P. (2007) ER-phagy: selective autophagy of the endoplasmic reticulum. Autophagy 3, 285–287
28. Juhasz, G., and Neufeld, T. P. (2008) Experimental control and characterization of autophagy in Drosophila. Methods Mol. Biol. 445, 125–133
29. Juhasz, G., Hill, J. H., Yan, Y., Sass, M., Baehrecke, E. H., Backer, J. M., and Neufeld, T. P. (2008) The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila. J. Cell Biol. 181, 655–666
30. Scott, R. C., Schuldiner, O., and Neufeld, T. P. (2004) Role and regulation of starvation-induced autophagy in the Drosophila fat body. Dev. Cell 7, 167–178
31. Mizushima, N., Yoshimori, T., and Levine, B. (2010) Methods in mammalian autophagy research. Cell 140, 313–326
32. Pircs, K., Nagy, P., Varga, A., Venkei, Z., Erdi, B., Hegedus, K., and Juhasz, G. (2012) Advantages and limitations of different p62-based assays for estimating autophagic activity in Drosophila. PLoS One 7, e44214
33. Sacher, M., Stone, S., and Ferro-Novick, S. (1997) The synaptobrevin-related domains of Bos1p and Sec22p bind to the syntaxin-like region of Sed5p. J. Biol. Chem. 272, 17134–17138
34. Colley, N. J., Cassill, J. A., Baker, E. K., and Zuker, C. S. (1995) Defective intracellular transport is the molecular basis of rhodopsin-dependent dominant retinal degeneration. Proc. Natl. Acad. Sci. U.S.A. 92, 3070–3074
35. Kumar, J. P., and Ready, D. F. (1995) Rhodopsin plays an essential structural role in Drosophila photoreceptor development. Development 121, 4359–4370
36. Sang, T. K., and Ready, D. F. (2002) Eyes closed, a Drosophila p47 homolog, is essential for photoreceptor morphogenesis. Development 129, 143–154
37. Uchiyama, K., and Kondo, H. (2005) p97/p47-Mediated biogenesis of Golgi and ER. J. Biochem. 137, 115–119
38. Fink, J. K. (2013) Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 126, 307–328
39. Block, M. R., Glick, B. S., Wilcox, C. A., Wieland, F. T., and Rothman, J. E. (1988) Purification of an N-ethylmaleimide-sensitive protein catalyzing vesicular transport. Proc. Natl. Acad. Sci. U.S.A. 85, 7852–7856
40. Acharya, U., Jacobs, R., Peters, J. M., Watson, N., Farquhar, M. G., and Malhotra, V. (1995) The formation of Golgi stacks from vesiculated Golgi membranes requires two distinct fusion events. Cell 82, 895–904
41. Latterich, M., Fröhlich, K. U., and Schekman, R. (1995) Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. Cell 82, 885–893
42. Rabouille, C., Levine, T. P., Peters, J. M., and Warren, G. (1995) An NSF-like ATPase, p97, and NSF mediate cisternal regrowth from mitotic Golgi fragments. Cell 82, 905–914
43. Edwardson, J. M. (1998) Membrane fusion: all done with SNAREpins? Curr. Biol. 8, R390–R393
44. Mellman, I. (1995) Enigma variations: protein mediators of membrane fusion. Cell 82, 869–872
45. Levine, B., and Kroemer, G. (2008) Autophagy in the pathogenesis of disease. Cell 132, 27–42
46. Moreau, K., and Rubinsztein, D. C. (2012) The plasma membrane as a control center for autophagy. Autophagy 8, 861–863
47. Bernard, A., and Klionsky, D. J. (2013) Autophagosome formation: tracing the source. Dev. Cell 25, 116–117