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Some, but not all, retromer components promote morphogenesis of *C. elegans* sensory compartments

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ABSTRACT

The endings of sensory receptor cells often lie within specialized compartments formed by glial cells. The main sensory organ of *Caenorhabditis elegans*, the amphid, provides a powerful setting for studying glial compartment morphogenesis. Our previous studies showed that amphid compartment size is controlled by opposing activities of the Nemo-like kinase LIT-1, which promotes compartment expansion, and the Patched-related protein DAF-6, which restricts compartment growth. From a genetic screen for mutations able to suppress the bloated sensory compartments of *daf-6* mutants, we identified an allele of the sorting nexin gene *snx-1*. SNX-1 protein is a component of the retromer, a protein complex that facilitates recycling of transmembrane proteins from the endosome to the Golgi network. We find that *snx-1* functions cell autonomously within glia to promote sensory compartment growth, and that SNX-1 protein is enriched near the surface of the sensory compartment. *snx-1* interacts genetically with *lit-1* and another regulator of compartment size, the Dispatched-related gene *che-14*. Mutations in *snx-3* and *vps-29*, also retromer genes, can suppress *daf-6* defects. Surprisingly, however, remaining retromer components seem not to be involved. Our results suggest that a novel assembly of retromer components is important for determining sensory compartment dimensions.

Introduction

Within sensory organs, glial cells often form compartments that surround and isolate sensory-cell receptive endings. In vertebrates, for example, modified Schwann cells wrap around mechanosensory nerve endings, retinal pigmented epithelial cells engulf the tips of photoreceptor cells, and sustentacular glial cells surround olfactory neurons (Burkitt et al., 1993; Ross et al., 1995). We have been using the main sensory organ of the nematode *Caenorhabditis elegans*, the amphid, as an experimentally approachable model to investigate sensory compartment morphogenesis (Oikonomou and Shaham, 2011). Each *C. elegans* has two bilaterally symmetric amphids located in the head, with each amphid consisting of twelve sensory neurons and two glial cells, the sheath and the socket (Fig. 1A) (Ward et al., 1975). The bipolar neurons extend an axon into the nerve ring, the main neuropil, as well as a dendrite that reaches the anterior tip of the animal. The two glial cells also extend anterior processes that parallel sensory neuron dendrite projections. At the tip, some of the dendrites perforate the sheath glia and enter a compartment that is referred to as the amphid sensory compartment or channel (Fig. 1B) (Perkins et al., 1986). The dendrites extend sensory cilia which traverse the length of the sensory compartment and, through an opening formed by the socket glia, sample the environment. The formation of the amphid sensory compartment may be associated with secretion of extracellular matrix material by the glia (Perens and Shaham, 2005; Perkins et al., 1986; Ward et al., 1975), and at least some matrix components are required for sensory neuron function (Bacaj et al., 2008).

daf-6, a gene required within amphid sheath glia for sensory compartment morphogenesis, encodes a Patched-related transmembrane protein that is also important for tubulogenesis in *C. elegans* (Perens and Shaham, 2005). Recently, we showed that DAF-6 acts to restrict sensory compartment growth during development, and that this activity is opposed by the Nemo-like kinase LIT-1, which promotes compartment expansion (Oikonomou et al., 2011). DAF-6 and LIT-1 both localize to the surface of the amphid sensory compartment.

Patched-related proteins such as DAF-6 have roles as both ligand-specific receptors and regulators of membrane trafficking (Kuwabara et al., 2000; Perens and Shaham, 2005; Zugasti et al., 2005). The retromer complex also has roles in the recycling of membrane proteins and in membrane dynamics. The retromer is thought to consist of two distinct subunits: a cargo-selection subunit consisting of VPS26, VPS29 and VPS35 proteins, and a membrane coating/bending subunit consisting of the sorting nexins SNX1/2 and SNX5/6 (Cullen, 2008). The cargo-selection complex identifies and binds cytoplasmic tails of transmembrane proteins that enter endosomes by endocytosis at the plasma membrane (Arighi et al., 2004; Vergés et al., 2004). The sorting nexins bind to endosomal membranes through a Phox-

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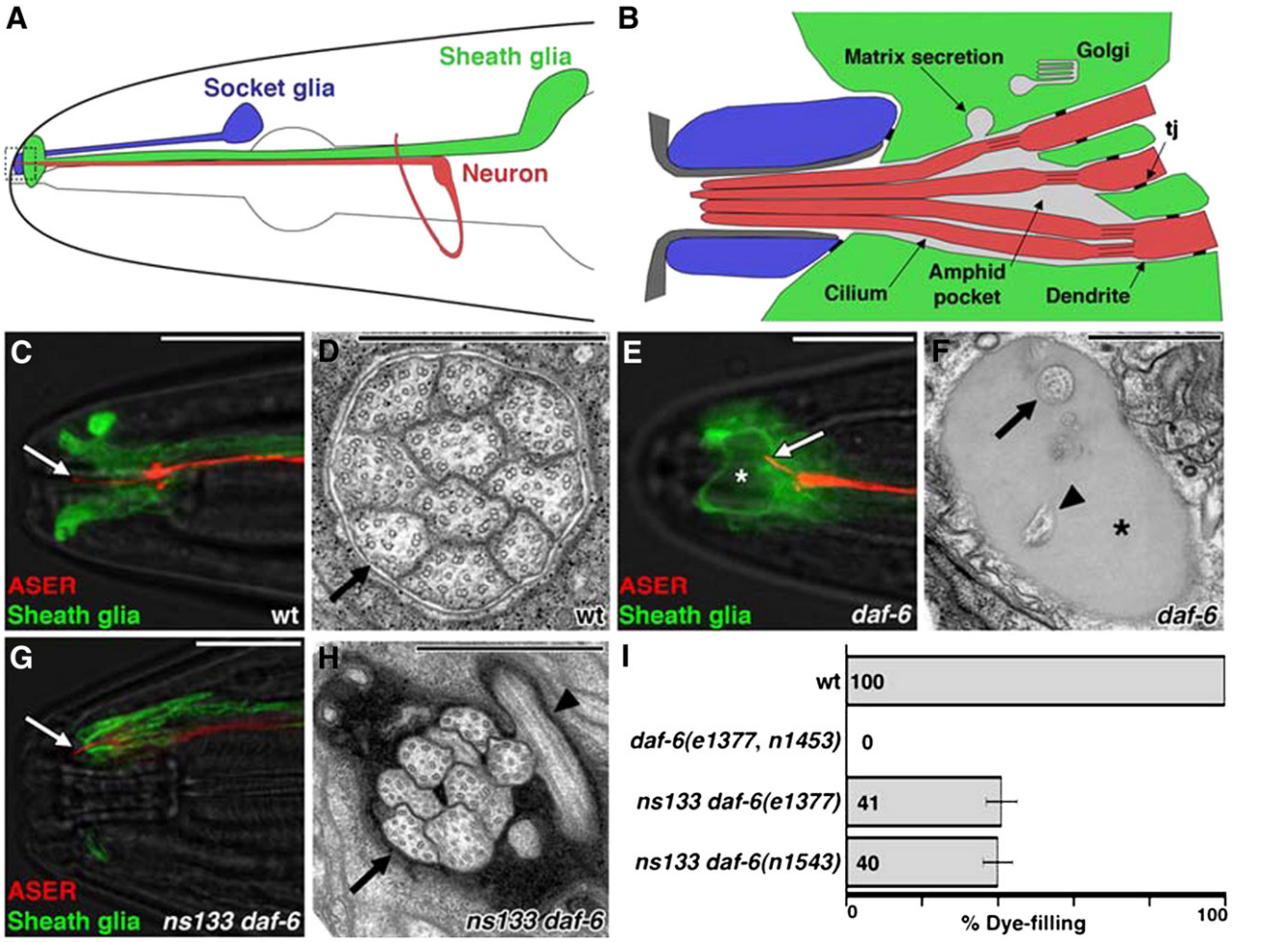

Fig. 1. *ns133* is a suppressor of *daf-6*. (A) Schematic of the *C. elegans* amphid. The head of the animal and the pharynx are in black. Only one of the 12 amphid neurons is depicted (red). The socket glia is in blue and the sheath glia in green. The outlined region is depicted in more detail in (B). Anterior is to the left. (B) Detail of the tip of the amphid. tj, tight junction. Anterior is to the left. Adapted from (Perkins et al., 1986). (C, E, G) The amphid sensory organs of wild-type (C), *daf-6*(*e1377*) (E), and *ns133 daf-6*(*e1377*) (G) adult animals visualized using fluorescence microscopy. The ASER amphid neuron is marked with mCherry (red; driven by the *gcy-5* promoter), while the sheath glia is marked with GFP (green; driven by the *T02B11.3* amphid sheath promoter). White arrows point to the sensory cilium of the ASER neuron. Asterisk in (E) marks the bloated sensory compartment. Anterior is to the left. White scale bars, 10 μm. (D, F, H) Electron micrographs of cross-sections through the amphid sensory compartment of wild-type (D), *daf-6*(*e1377*) (F), and *ns133 daf-6*(*e1377*) (H) adult animals. Black arrows point to cilia with normal orientation; black arrowheads point to bent cilia. Asterisk in (F) marks the bloated sensory compartment. Black scale bars, 1 μm. (I) Histogram depicting the suppression of daf-6 mutant defects by *snx-1*(*ns133*). Animals in which any subset of neurons absorbed the dye were scored as dye-filling. n > 100. Error bars, standard error of the mean (SEM).

homology (PX) phosphoinositide-binding domain, and may induce endosomal membrane bending and tubule formation through a carboxy-terminal Bin-Amphiphysin-Rsv (BAR) domain (see later discussion). Tubules bud off endosomes, are trafficked to the trans-Golgi network (TGN), and cargo proteins are then recycled back to the plasma membrane.

The sorting nexin SNX3 lacks a BAR domain, but also appears to be involved in retromer function. In yeast, the SNX3 homolog Grd19 interacts with both the SNX-BAR complex and the VPS complex to promote the recycling of the iron transporter complex Fet3p-Ftr1p (Strochlic et al., 2007). In *C. elegans*, efficient Wnt signaling depends on recycling by the retromer of Wntless, a transmembrane protein important for the secretion of Wnt ligands (Coudreuse et al., 2006; Pan et al., 2008; Yang et al., 2008). SNX-3 was recently shown to interact with the cargo-selection subunit of the retromer to promote recycling of Wntless, a process that does not appear to require the classic retromer components SNX1/2 and SNX5/6 (Harterink et al., 2011).

Here we demonstrate that mutations in the *snx-1* gene suppress the hyper-extended amphid sensory compartment defect of *daf-6* mutants. *snx-1* functions cell-autonomously within glia and interacts genetically with previously characterized components of the sensory compartment size-control machinery. SNX-1 protein localizes near the amphid compartment surface. Surprisingly, although both SNX-1 and SNX-3 are important for sensory compartment morphogenesis, neither SNX5/6, nor the main component of the cargo-selection subunit, VPS35, seem to be involved. Our results, therefore, suggest that a novel assembly of retromer components plays a role in sensory compartment formation.

Materials and methods

C. elegans strains

Strains were handled using standard methods (Brenner, 1974). All strains were maintained and scored at 20 °C unless otherwise indicated. The alleles used in this study were: *daf-6*(*e1377*, *n1543*) (Riddle et al., 1981 and Starich et al., 1995 respectively), *snx-1*(*ns133*) (described here), *snx-1*(*tm847*), *snx-3*(*tm1595*), *snx-6*(*tm3790*), *lst-4*(*tm2423*), *vps-26*(*tm1523*), *vps-29*(*tm1320*), *vps-35*(*hu68*) (Coudreuse et al., 2006), *lit-1*(*ns132*) (Oikonomou et al., 2011), *che-14*(*ok193*) (Michaux et al., 2000), *unc-3*(*e151*) (Prasad et al., 1998).

Extrachromosomal arrays

nsEx2766, *nsEx2767*, *nsEx2768*, *nsEx2750*, *nsEx2751*, *nsEx2752*, *nsEx2763*, *nsEx2764*, *nsEx2765* arrays contained plasmids *T02B11.3*pro::GFP, *gcy-5*pro::mCherry, and pEP51. *nsEx2373*, *nsEx2374*, *nsEx2375*, *nsEx2376* arrays contained the *snx-1* genomic region (from 2.1 kb upstream of start codon to 0.3 kb downstream of stop codon) and plasmid pMH135. *nsEx2558*, *nsEx2559*, *nsEx2560*, *nsEx2561* arrays contained linearized plasmid pGO35 and plasmid pMH135. *nsEx2566*, *nsEx2567*, *nsEx2568*, *nsEx2569* arrays contained plasmids pGO41 and pMH135. *nsEx2630*, *nsEx2631*, *nsEx2632* arrays contained plasmids pGO54 and pMH135. The *nsEx2619* array contained plasmids pGO38 and pMH135. The *mcEx178* array contained plasmids pML624 (*che-14*::gfp) and pRF4 (Michaux et al., 2000), and was a gift from Michel Labouesse.

Plasmid construction

All pGO constructs were made using pPD95.75 (Andrew Fire) as a backbone, unless otherwise noted. *T02B11.3*pro::GFP was a gift from Maya Tevlin (Wang et al., 2008). *gcy-5*pro::mCherry was based on (Yu et al., 1997). pGO35, *snx-1*pro::SNX-1::*snx-1* 3′ UTR: *snx-1*pro consists of 0.8 kb upstream of the *snx-1* start site (SphI/XmaI). *snx-1* 3′ UTR consists of a 0.6 kb region downstream of the *snx-1* stop codon. pGO38, *T02B11.3*pro::GFP::LIT-1. pGO41, *lin-26myo-2*pro::SNX-1::*snx-1* 3′ UTR: The e1 *lin-26* promoter fragment (Landmann et al., 2004) was fused to the *myo-2* minpro (Okkema et al., 1993) using SphI/XbaI. pGO54, *T02B11.3*pro::GFP::SNX-1::*snx-1* 3′ UTR. pRF4, *rol-6*(*su1006*) (Mello et al., 1991). pMH135, *pha-4*pro::GFP: a gift from Max Heiman (Heiman and Shaham, 2009). pEP51, *unc-122*pro::GFP.

Dye-filling assay

Animals were washed off NGM plates with M9 buffer, resuspended in a solution of 10 μg/mL of DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate)(Invitrogen D282), and rotated in the dark for 1.5 h at room temperature. Animals were then transferred to a fresh NGM plate, anaesthetized with 20 mM sodium azide, and observed using a dissecting microscope equipped with epifluorescence. Animals in which none of the amphid neurons filled with dye were scored as dye-filling defective (Dyf); all other animals were considered to dye-fill including cases where dye uptake was limited to a subset of the amphid neurons in either one or both amphids.

Fluorescence microscopy and image analysis

Images were acquired using a DeltaVision Image Restoration Microscope (Applied Precision) equipped with a 60X/NA 1.42 Plan Apo N oil immersion objective (Olympus) and a Photometrics CoolSnap camera (Roper Scientific). Acquisition and deconvolution of images were performed with Softworx (Applied Precision); analysis was performed with Softworx (Applied Precision) and ImageJ (NIH).

Transmission electron microscopy (EM)

Previously described conventional fixation methods (Perens and Shaham, 2005) as well as high-pressure freezing fixation (HPF) was used for adult animals. For HPF, samples were frozen using the Leica High Pressure Freezer EM-PACT2 (pressure of 18,000 bar, cooling rate of 20,000 °C/s). Freeze substitution was performed using the Leica EM AFS2 Automatic Freeze Substitution System (McDonald, 2007). Ultrathin serial sections (60 nm) were cut using a REICHERT Ultra-Cut-E ultramicrotome and collected on Pioloform-coated single-slot copper grids. EM images for every other section were acquired using an FEI Tecnai G2 Spirit BioTwin transmission electron microscope operating at 80 kV with a Gatan 4 K×4 K digital camera.

Results

Mutations in the sorting nexin gene snx-1 suppress the developmental defects of daf-6 mutants

The sheath glial cell of the amphid sensory organ forms a compartment that envelops the ciliated endings of some amphid sensory neurons (Figs. 1A–D). Within this compartment, the tightly bundled cilia are arranged in a characteristic 3:4:3 dorso-ventral pattern (Fig. 1D) (Ward et al., 1975). We previously demonstrated that the gene *daf-6*, encoding a Patched-related protein, is required for formation of this glial compartment (Perens and Shaham, 2005). In *daf-6* mutants, the sensory compartment is grossly malformed: excess matrix material accumulates within a bloated compartment, the ciliary bundle is disorganized, and the cilia are not exposed to the environment (Figs. 1E and F). Recently, we demonstrated that *daf-6* regulates the morphogenesis of the amphid sensory compartment by restricting its expansion (Oikonomou et al., 2011). Based on this discovery, we reasoned that mutations in genes that promote sensory compartment expansion would suppress the *daf-6* over-expansion defects. Indeed, from a *daf-6* genetic suppression screen, we identified a key role for the Nemo-like kinase LIT-1 in sensory compartment expansion (Oikonomou et al., 2011). In the same screen we isolated another suppressor of *daf-6* with the designated allele number *ns133*.

To examine the extent to which *ns133* suppresses the amphid morphogenesis defects of *daf-6*, we generated *ns133 daf-6*(*e1377*) double mutant animals in which the sheath glia and ASER amphid neuron are marked with green fluorescent protein (GFP) and mCherry, respectively. In these animals, unlike *daf-6* single mutants, the ASER cilium projects through a seemingly normal sensory compartment (compare Figs. 1E and G). We further assessed the morphology of the sensory compartment in *ns133 daf-6*(*e1377*) mutants using electron microscopy (EM). We found that the sensory compartment defects associated with *daf-6* lesions are generally corrected (n = 3, compare Figs. 1F and H). Sensory compartment size is much smaller than in *daf-6* mutants, and the cilia are bundled together and are exposed to the environment. Nonetheless, some defects in amphid channel structure persist: cilia are not arranged in the strict 3:4:3 pattern, and at least some cilia are bent (arrowhead in Fig. 1H).

To assess more quantitatively the suppression of *daf-6* mutant defects by *ns133*, we used a dye-filling assay that measures access of sensory neurons to the environment (Hedgecock et al., 1985). In this assay, animals are incubated in a solution of fluorescent lipophilic dye (see Materials and methods). Wild-type animals readily take up dye through their exposed amphid sensory cilia, while *daf-6* animals fail to do so, presumably because of defects in sensory compartment morphology (Fig. 1I) (Perkins et al., 1986; Starich et al., 1995). *ns133 daf-6*(*e1377*) animals display a gradient of dye-filling defects, with some animals being indistinguishable from wild-type, some taking up the dye in only a subset of amphid neurons, and others failing to dye-fill at all, similar to *daf-6* mutants. In line with our previous studies (Oikonomou et al., 2011), animals were considered to dye-fill when any number of neurons absorbed dye. We found that *ns133 daf-6*(*e1377*) double mutants were able to take up dye in at least one amphid in 41% of animals we observed. Importantly, dye filling was also restored to animals carrying a different allele of *daf-6*, suggesting that *ns133* likely bypasses the function of *daf-6* (Fig. 1I). The dye-filling status of *ns133 daf-6*(*e1377*) amphids correlates with suppression of *daf-6* defects seen by EM. In 3/3 animals in which only one amphid accumulated dye, only one suppressed sensory compartment was seen by EM.

To identify the gene defective in *ns133* mutants, we first mapped the mutation to the left arm of chromosome X using standard methods (Wicks et al., 2001). We next prepared genomic DNA from

ns133 daf-6 animals, and used whole genome sequencing, followed by single-nucleotide polymorphism (SNP) and deletion analysis using the galign software (Shaham, 2009) to identify candidate lesions. Among these, we found a deletion of 97 bp at the end of the fourth exon of the gene *snx-1*, which encodes the *C. elegans* homolog of sorting nexin 1 (Fig. 2A).

Three observations suggest that this *snx-1* lesion is the relevant defect in *ns133* mutants. First, while about 40% of *ns133 daf-6* (*e1377*) animals are able to take up dye in amphid sensory neurons, only 4% of *ns133 daf-6*(*e1377*) mutants carrying an extrachromosomal array containing the *snx-1* genomic region take up dye. Thus, wild-type *snx-1* restores the *daf-6* dye-filling defect to *ns133 daf-6* double mutants (Fig. 2B). Second, a transgene in which 0.8 kb upstream of the *snx-1* start codon drives expression of the *snx-1* cDNA can also rescue the suppression (Fig. 2B). Third, a different allele, *snx*-1 (*tm847*), containing a large deletion in *snx-1* (Fig. 2A) can also suppress *daf-6* dye-filling defects to a similar extent as *ns133*. Together, these results demonstrate that *ns133* is an allele of *snx-1*.

snx-1 acts within glia and localizes to the amphid sensory compartment

We previously demonstrated that *daf-6* activity is required within glia and during amphid morphogenesis to promote sensory compartment formation (Perens and Shaham, 2005). We therefore wondered whether the same might be true for *snx-1*. To test this hypothesis, we constructed *snx-1*(*ns133*) *daf-6*(*e1377*) double mutant animals containing a transgene in which a *lin-26* promoter fragment drives expression of the *snx-1* cDNA within the amphid glia at the time of amphid sensory compartment formation (Landmann et al., 2004). We found that only 14% of these animals are able to take up dye, compared with 41% of animals that do not carry the transgene (Fig. 3A). Importantly, expression of the *snx-1* cDNA using the glia-specific promoter of the gene *T02B11.3*, which is expressed later in development (Wang et al., 2008) and after amphid morphogenesis takes place, had no effect (Fig. 3A). These observations suggest that *snx-1* acts within glia, at the time of amphid formation.

To investigate the subcellular localization of SNX-1 protein within glia, we examined expression of a GFP::SNX-1 fusion protein in this cell. We found this protein enriched in the area surrounding the amphid sensory compartment (Figs. 3B and S1). This localization is similar to that described for other known regulators of sensory compartment morphogenesis, including DAF-6, CHE-14, and LIT-1 (Perens and Shaham, 2005; Michaux et al., 2000; Oikonomou et al., 2011).

Some, but not all, retromer components participate in sensory compartment formation

SNX-1 protein could be involved in sensory compartment morphogenesis as part of the retromer complex, or may have retromer-independent roles. To distinguish between these possibilities, we examined the effects of mutations in other retromer components on amphid compartment formation.

SNX-6 protein is the *C. elegans* homolog of SNX5/6, which partners with SNX1/2 to form the membrane bending complex of the retromer (Fig. 4A). We investigated whether mutation of the *snx-6* gene could suppress the *daf-6*(*n1543*) defects, as do mutations in *snx-1*. Surprisingly, we found that *snx-6*(*tm3790*); *daf-6*(*n1543*) double mutants failed to take up dye, just like *daf-6*(*n1543*) single mutants (Fig. 4B), suggesting that SNX-6 may not be involved in sensory compartment morphogenesis. We also found that VPS35, the retromer component believed to interact directly with transmembrane cargo proteins (McGough and Cullen, 2011) (Fig. 4A), does not seem to play a role in sensory compartment formation, since the *vps-35*(*hu68*) allele does not suppress the dye-filling defects of *daf-6* mutants (Fig. 4B). Similarly, a mutation in *vps-26*, which encodes the *C. elegans* VPS26 protein, also fails to suppress *daf-6* (Fig. 4B). Furthermore, although *lit-1* mutants, which have reduced compartment size, have dye-filling defects (Oikonomou et al., 2011), neither *snx-6*, *vps-35*, nor *vps-26* single mutants have defects in amphid dye uptake.

Nonetheless, as shown in Fig. 4B, a mutation in *vps-29*, encoding another cargo-selection subunit of the retromer, does suppress the dye-filling defects of *daf-6* mutants. This observation suggests that components of the cargo-selection complex of the retromer have separable functions. The *snx-3* gene, which encodes a sorting nexin that lacks the membrane bending BAR domain, has been recently shown to interact with the cargo-selection subunit of the retromer to direct the recycling of Wntless from the endosomes back to the plasma

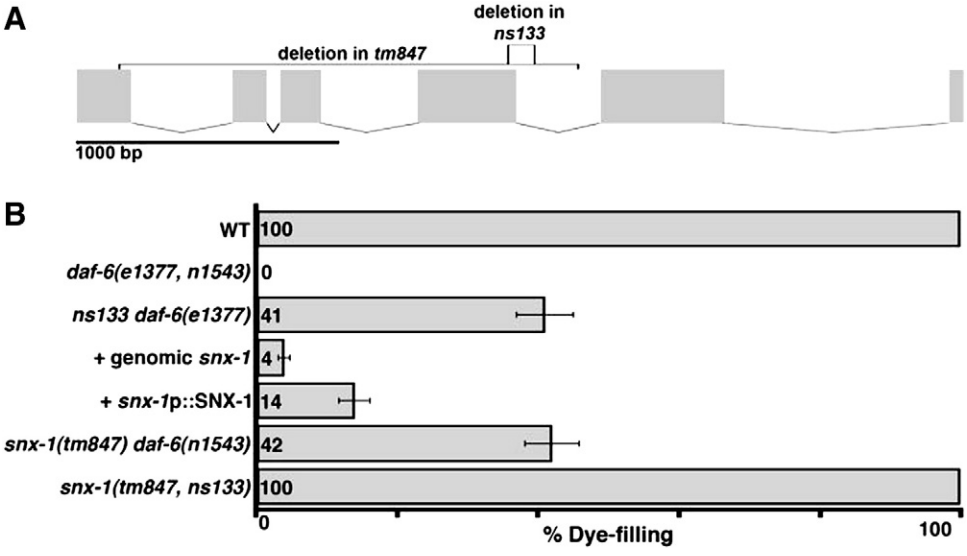

Fig. 2. *ns133* is an allele of the sorting nexin gene *snx-1*. (A) The architecture of the *snx-1* genomic locus. Gray boxes represent exons, black lines introns. The regions deleted in the alleles *tm847* and *ns133* are marked. (B) Dye-filling assay for indicated genotypes ($n \geq 100$). *daf-6*(*n1543*) is marked with *unc-3*(*e151*). *unc-3*(*e151*) does not affect dye filling (data not shown). Animals in which any subset of neurons absorbed the dye were scored as dye-filling. Error bars, SEM.

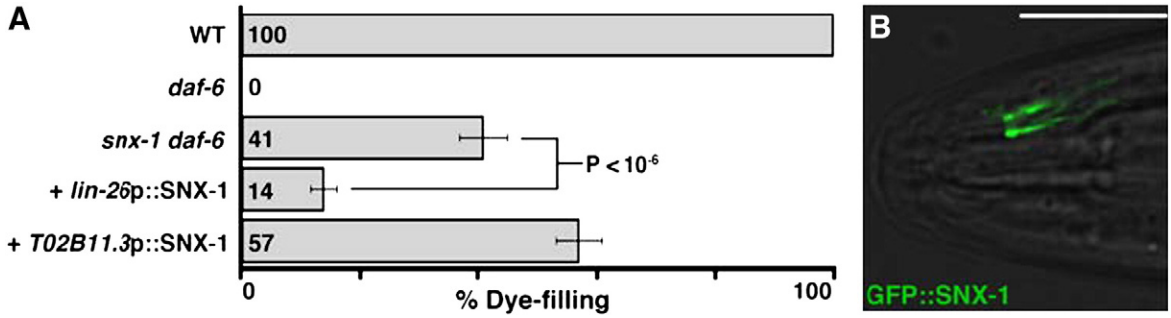

Fig. 3. *snx-1* acts within glia to regulate sensory compartment morphogenesis. (A) Dye-filling assay for indicated genotypes (n ≥ 100). The alleles used are: *daf-6*(*e1377*) and *snx-1* (*ns133*). Animals in which any subset of neurons absorbed the dye were scored as dye-filling. Error bars, SEM. (B) A GFP::SNX-1 fusion protein localizes to the amphid sensory compartment (compare to the diffuse localization of GFP in Fig. 1C). Expression was driven by the glia-specific promoter *T02B11.3* (Wang et al., 2008). Anterior is to the left. Scale bar, 10 μm. See Fig. S1 for a Z-axis projection of the whole sheath cell.

membrane (Harterink et al., 2011). We found that *snx-3*(*tm1595*); *daf-6*(*n1543*) animals were able to dye-fill 23% of the time (Fig. 4B). We previously showed that the Wnt signaling pathway is not involved in amphid morphogenesis (Oikonomou et al., 2011), suggesting that *snx-3* likely acts in a Wnt-independent context to control sensory compartment formation.

Finally, we previously showed that mutations in the Wiskott–Aldrich syndrome protein (WASP) can also suppress mutations in *daf-6*, and that WASP can physically bind to LIT-1/Nemo-like kinase. *lst-4* encodes the *C. elegans* homolog of the SNX9 sorting nexin, and contains a WASP-interacting domain. We therefore tested whether *lst-4* might be involved in amphid morphogenesis. We found, however,

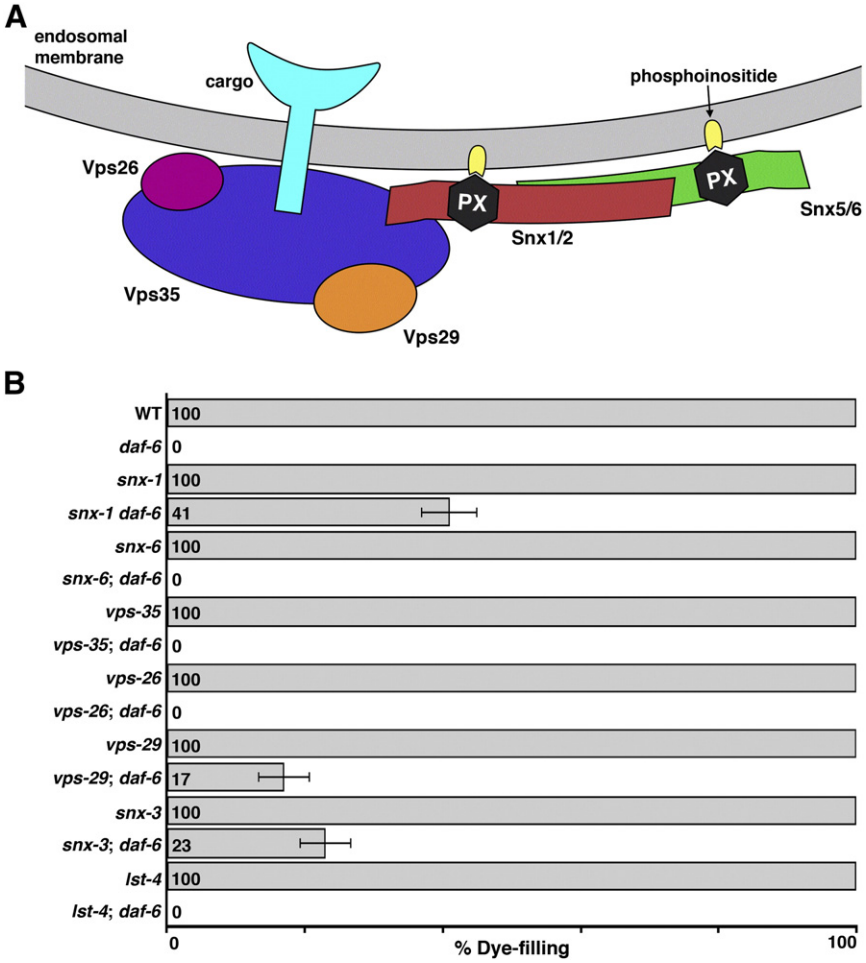

Fig. 4. Mutations of retromer components suppress the developmental defects of *daf-6* mutants. (A) Schematic of the retromer complex. The VPS complex is thought to act as the cargo recognition module, while the SNX heterodimer acts as a membrane association and bending module. Adapted from (Seaman, 2005). (B) Dye-filling assay for indicated genotypes (n ≥ 100). The alleles used are: *daf-6*(*n1543*), *snx-1*(*ns133*), *snx-6*(*tm3790*), *vps-29*(*tm1320*), *vps-35*(*hu68*), *snx-3*(*tm1595*), and *lst-4*(*tm2423*). Animals in which any subset of neurons absorbed the dye were scored as dye-filling. Error bars, SEM.

that *lst-4*(*tm2423*) animals dye-fill normally, and that *lst-4*(*tm2423*); *daf-6*(*n1543*) double mutants are completely dye-filling defective (Fig. 4B). Thus, if there is a role for *lst-4* in amphid morphogenesis, our current assays do not allow us to detect it.

Taken together, our results suggest that the classic retromer configuration is unlikely to be involved in sensory compartment morphogenesis, however, a modified retromer may participate in formation of this structure.

snx-1 genetically interacts with components of the sensory compartment size-determination system

The observation that *snx-1* mutations suppress the compartment over-expansion defects of *daf-6* mutants raises the possibility that *snx-1* may normally be involved in compartment expansion in a similar way to *lit-1* (Oikonomou et al., 2011). To test this idea, we examined *snx-1*(*ns133*) single mutants for defects in dye-filling. We found that unlike *lit-1* single mutants, *snx-1*(*ns133*) animals can dye-fill normally (100% dye-filling, Fig. 2B). In addition, the sensory compartments of these mutants appear normal in EM sections (n = 3, data not shown). These results raise the possibility that *snx-1* might function redundantly with *lit-1* or other compartment expansion genes to promote compartment expansion, and that defects in *snx-1* are compensated for by these other genes.

To test this idea, we assessed genetic interactions between *snx-1* and *lit-1*. We found that although *lit-1*(*ns132*); *daf-6*(*e1377*) animals dye-fill at about the same rate as *snx-1*(*ns133*) *daf-6*(*e1377*) mutants (~40% dye uptake), *lit-1*(*ns132*); *snx-1*(*ns133*) *daf-6*(*e1377*) triple mutants exhibit 86% dye filling (Fig. 5). This striking result suggests that the extensive defects in amphid morphogenesis that result from loss of *daf-6* can be nearly entirely compensated for by removing the activities of both *lit-1* and *snx-1*. This strong synergistic effect suggests that *snx-1* may indeed function redundantly with *lit-1* to promote sensory compartment expansion. *che-14*/Dispatched is required for apical secretion by the sheath glia, and defects in this gene also result in sensory compartment morphogenesis abnormalities (Michaux et al., 2000). Unlike *daf-6* defects, *che-14* mutant defects are enhanced by mutations in *lit-1* (Fig. 5; Oikonomou et al., 2011). Consistent with parallel activities of *lit-1* and *snx-1*, we found that an *snx-1* mutation also strongly enhances the dye-filling defects of *che-14*(*ok193*) mutants (Fig. 5).

Given the role of the retromer in the regulation of protein trafficking, we wondered whether mutation of *snx-1* might suppress *daf-6* lesions by impinging upon the ability of LIT-1 or CHE-14 to localize to the amphid sensory compartment. We therefore investigated the localization of GFP::LIT-1 and CHE-14::GFP protein fusions in *snx-1* mutants (Fig. S2). In both cases, the fusion proteins localized as previously described for wild-type animals (Michaux et al., 2000; Oikonomou et al., 2011), consistent with parallel, redundant roles for SNX-1 and LIT-1 in amphid sensory compartment formation.

Discussion

The Patched-related gene *daf-6* acts to restrict the size of the amphid sensory compartment (Oikonomou et al., 2011). To delineate the mechanism that guides compartment expansion, we performed a screen for suppressors of *daf-6* mutants and identified a mutant allele of the retromer sorting nexin gene *snx-1*. Mutations in *snx-1* suppress the morphological defects of *daf-6* mutants by a number of criteria. Gross morphological structure is restored, as demonstrated by fluorescence imaging; higher resolution structures are partially restored, as found in our EM studies; and at least some functional capacity of sensory neurons is restored, as these are now able to access the environment and fill with dye. Furthermore, wild-type animals encountering high temperature, overcrowding, or starvation enter an alternative developmental stage termed dauer. Dauer formation is dependent on amphid sensory neuron function (Bargmann et al., 1993). While *daf-6* mutants are strongly dauer defective (Riddle et al., 1981), *snx-1*; *daf-6* double mutants are dauer competent. Indeed, their ability to enter the dauer stage was the basis for their initial isolation (Oikonomou et al., 2011). In addition, we demonstrated that *snx-1* genetically interacts with known components of the sensory compartment expansion machinery, including the Nemo-like kinase *lit-1* and the Dispatched homolog *che-14*. These observations suggest that *snx-1*, a component of the retromer complex, acts to drive sensory compartment growth.

The retromer is thought to recycle transmembrane proteins from endosomes to Golgi through the coordinated action of its two subunits: a cargo-selection subunit comprised of VPS26, VPS29 and VPS35, and a membrane coating/bending subunit comprised of a heterodimer of the SNX-BAR proteins SNX1/2 and SNX5/6 (Cullen, 2008). In the case of Wntless, a protein required for efficient secretion of the Wnt morphogen, SNX3 seems to replace the SNX-BAR heterodimer as the main recycling sorting nexin (Harterink et al., 2011). The studies presented here expand our understanding of sensory compartment morphogenesis and suggest the involvement of a novel combination of retromer components in sensory compartment morphogenesis (Fig. 6). Mutations in the genes *snx-1* and *snx-3*, encoding the *C. elegans* homologs of SNX1/2 and SNX3 respectively, are able to suppress the developmental defects of *daf-6* animals, whereas a mutation in the SNX5/6 homolog, *snx-6*, does not. Furthermore, while a role for the cargo-selection subunit of the retromer is

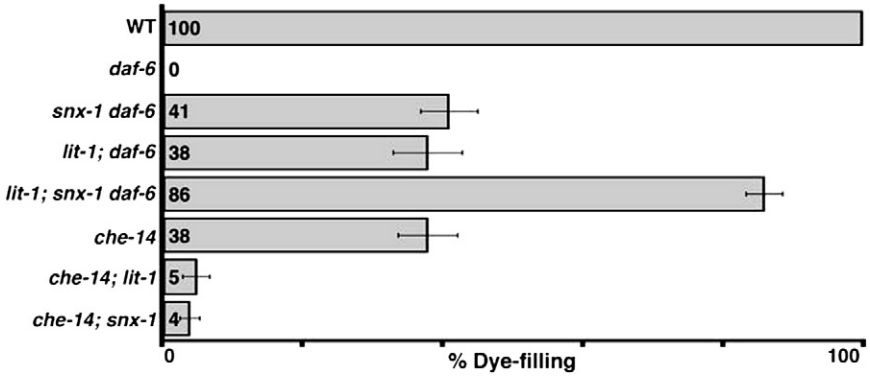

Fig. 5. *snx-1* interacts with components of the sensory compartment expansion module. Dye-filling assay for indicated genotypes (n ≥ 100). The alleles used are: *daf-6*(*n1543*), *snx-1* (*ns133*), *lit-1*(*ns132*), and *che-14*(*ok193*). Animals in which any subset of neurons absorbed the dye were scored as dye-filling. Error bars, SEM.

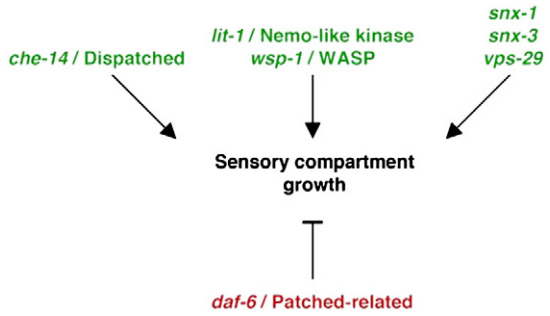

Fig. 6. A model for the regulation of sensory compartment growth. *daf-6* acts to inhibit compartment expansion (Oikonomou and Shaham, 2011), while *che-14* (Michaux et al., 2000), *lit-1*, *wsp-1* (Oikonomou and Shaham, 2011), and components of the retromer (this study), act in the opposite direction, to promote sensory compartment expansion.

suggested by the ability of *vsp-29*; *daf*-6 double mutants to take up dye, the two other components of the cargo-selection module, VPS26 and VPS35, do not appear to be involved in sensory compartment morphogenesis. This functional separation of the VPS proteins is particularly surprising, as previous studies have demonstrated that stability of the cargo-selection subunit depends on the presence of all of its components (Arighi et al., 2004; Vergés et al., 2004). Thus, similar to Wntless, alternate subcomplexes of the retromer may be specifically tasked with sensory compartment expansion. A more nuanced relationship between SNX1 and other retromer components is also suggested by the finding that SNX1 can directly interact with cargo molecules such as the protease-activated receptor-1 (PAR1) (Wang et al., 2002), a G protein-coupled receptor for thrombin, as well as a variety of other GPCRs (Heydorn et al., 2004). In the particular case of the GPCR $P2Y_1$ (an adenosine diphosphate receptor found on platelets), SNX1 mediated recycling was recently shown to be independent of retromer components (Nisar et al., 2010).

An important question concerning the involvement of retromer components in sensory compartment morphogenesis is the identity of the relevant cargo. One possible hypothesis is that the novel combination of retromer components we identified is responsible for the retrieval of components that directly function to promote sensory compartment expansion. Two sets of observations suggest that none of the known components of this machinery are retromer targets. First, the localization of both LIT-1 and CHE-14 to the amphid sensory compartment is independent of *snx-1*. Second, a putative null mutation of *snx-1* strongly enhances the suppression of *daf-6* defects by *lit-1* lesions, suggesting that SNX-1 and LIT-1 function independently in sensory compartment formation. Alternatively, or perhaps concurrently, the retromer may directly or indirectly be involved in adding membrane to the surface of the amphid sensory compartment to promote its growth, independent of specific cargo. This model is consistent with our finding that the main cargo-selector protein, VPS35, does not seem to play a role in compartment formation. Identifying additional genes that regulate amphid compartment shape will allow us to distinguish between these possibilities and further our understanding of the process and mechanism that governs sensory compartment morphogenesis.

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