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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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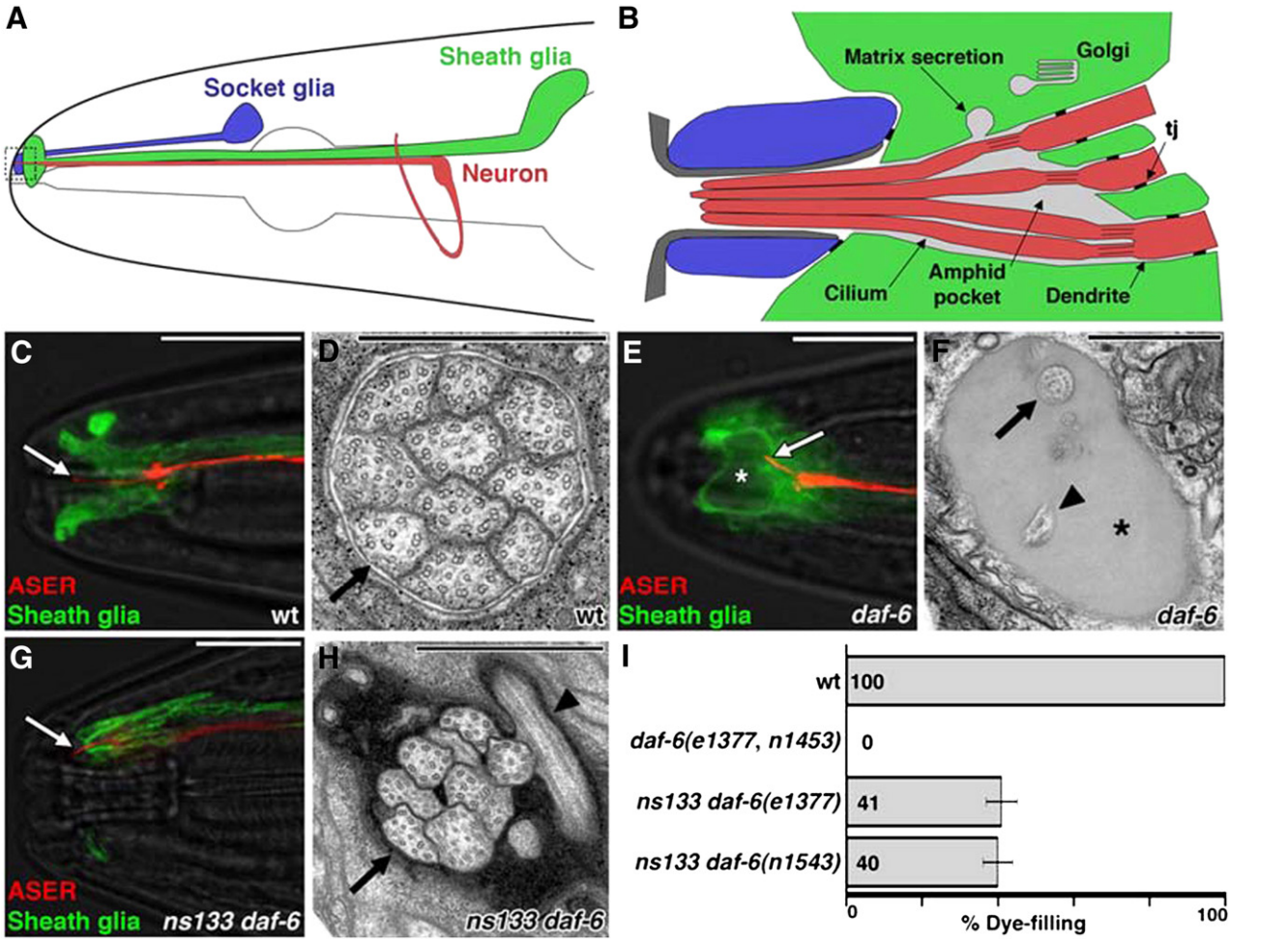

Fig. 1. *ns133* is a suppressor of *daf-6*. (A) Schematic of the *C. elegans* amphid. The head of the animal and the pharynx are in black. Only one of the 12 amphid neurons is depicted (red). The socket glia is in blue and the sheath glia in green. The outlined region is depicted in more detail in (B). 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).