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Previews

Snapin Snaps into the Dynein Complex for Late Endosome-Lysosome Trafficking and Autophagy

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Late endosome-lysosome trafficking plays a key role in regulating cell surface signaling and degradation of intracellular components by autophagy. New work by Cai and coworkers in this issue of *Neuron* provides evidence that snapin regulates the recruitment of late endosomes to the dynein motor complex for retrograde trafficking along microtubules and maturation of lysosomes.

Plasma membrane endocytosis regulates neuronal signaling processes by controlling the number of functional receptors and channels available at the cell surface. Endosomal cargoes are selected to take one of two major routes: returning to the plasma membrane in recycling endosomes, or passing through late endosomes before delivery to lysosomes for degradation. For example, the number of AMPA receptors at the postsynaptic site is thought to regulate synaptic plasticity, such as long-term potentiation and long-term depression (LTD). Endocytosed AMPA receptors are also recycled to the cell surface or degraded in lysosomes, depending on the stimulus conditions, such as activation of NMDA receptors, subunit composition, and phosphorylation status of AMPA receptor subunits (Hirling, 2009). In addition, certain cellular signaling events are thought to occur on endosome membranes. For example, nerve growth factor and its receptor trkA are endocytosed at nerve terminals and retrogradely transported along axons in vesicles positive for Rab7, a marker for late endosomes (von Zastrow and Sorkin, 2007). In addition to a role in the degradation of cell surface or exogenous proteins (i.e., heterophagy), lysosomes are also responsible for the degradation of intracellular components by the process of autophagy, and newly formed autophagosomes undergo a stepwise maturation by fusing with endosomes and lysosomes (Eskelinen, 2005). Finally, acid hydrolases, enzymes essential for degradation of target substrates in mature lysosomes, are also delivered to lysosomes from the *trans*-Golgi via endosomes (Saftig and Klumperman, 2009). Therefore, late endosome-lysosome trafficking plays a key role in coordinating the heterophagy and autophagy involved in critical neuronal signaling pathways and functions, such as synaptic plasticity and cell survival.

Lysosomes are predominantly localized in the soma near the microtubule-organizing center. To reach the soma, late endosomes and autophagosomes are thought to be transported by the motor protein dynein, which moves along microtubules toward their minus-end direction. Thus, for highly polarized cells such as neurons, transport of endosomes and autophagosomes from the cell periphery to the soma is a challenging task. Indeed, when dynein functions are disturbed, late endosomes were redistributed to the cell periphery (Burkhardt et al., 1997) and retrograde movement of autophagosomes were severely impaired in neuronal axons (Katsumata et al., 2010). In addition, mutations affecting the dynein machinery cause axonal degeneration and impair autophagic clearance of aggregate-prone proteins (Ravikumar et al., 2005). Similarly, the accumulation of autophagosomes and autolysosomes has been observed in axons during the early stages of many neurodegenerative diseases (Wong and Cuervo, 2010), including Alzheimer's disease, prion disease, Parkinson's disease, and Huntington's disease, and in neurons after hypoxia-ischemia and brain injury. Nevertheless, how the dynein complex is recruited to late endosomes and autophagosomes is not completely clear.

In this issue of *Neuron*, Cai et al. (2010) have shown that snapin regulates recruitment of late endosomes to the dynein complex by directly binding to dynein intermediate chain (DIC). Snapin was originally identified as a protein that binds to synaptosomal-associated protein 25 kDa (SNAP-25) and modulates neurosecretion by stabilizing the release-ready pool of dense-core and synaptic vesicles and facilitating synchronized release of synaptic vesicles (Pan et al., 2009). Cai et al. (2010) showed that immature lysosomes, which did not contain the mature form of cathepsin D, accumulated in *Snapin*-null neurons (Figure 1). In addition, retrograde transport of Rab7-positive late endosomes was significantly reduced in *snapin* null neuronal axons (Figure 1). As a result, degradation of endogenous epidermal growth factor (EGF) receptors after EGF-stimulated endocytosis was significantly retarded in *snapin* null neurons. Similarly, degradation of exogenous EGF receptor, dextran, or bovine serum albumin was impaired in mouse embryonic fibroblasts (MEFs) derived from *snapin* null mice. These phenotypes were rescued by reintroduction of wild-type snapin, but not a mutant snapin (snapin-L99K) that could not bind to DIC, into *snapin* null neurons. Furthermore, late autophagic vacuoles increased in *snapin* null neurons and MEFs by the reduced clearance of autolysosomes, reflecting the observed impairment of lysosomal functions (Figure 1). Interestingly, overexpression of wild-type snapin, but not snapin-L99K, in wild-type neurons resulted in increased formation of elongated, tubular lysosomes, suggesting that snapin-DIC interaction may facilitate fusion between late endosomes and lysosomes. Together, these results indicate

that snapin probably plays a crucial role in heterophagy and autophagy by regulating dynein-based trafficking of late endosomes to lysosomes and by facilitating maturation of lysosomes (Figure 1).

How molecular motors are specifically targeted to their cargos is a major problem that remains to be elucidated in cell biology. Several adaptor proteins and the regulatory molecules are involved in this process. For example, adaptor protein complex-4 (AP-4) mediates the selective trafficking of AMPA receptors to the somatodendritic domain; genetic disruption of AP-4 results in the mislocalization of AMPA receptors, which accumulate in autophagosomes in the swollen axons (Matsuda et al., 2008). Many studies indicate that dynactin helps to link dynein to its cargo (Kardon and Vale, 2009). Spectrin βIII, which binds to acidic phospholipids on axonal vesicles, has previously been shown to interact with the ARP1 filament of dynactin, and the Rab7 effector Rab-interacting lysosomal protein (RILP) is suggested to be involved in this step (Kardon and Vale, 2009). In contrast, Cai et al. (2010) propose that snapin links dynein motors to late endosomes by directly binding to DIC. Indeed, binding between the dynein heavy chain and dynactin was not affected in *snapin*-null neurons. Therefore, the identification of a snapin/DIC complex provides new insights on the mechanisms responsible for late endosome-lysosome trafficking in neurons. Since activation of the autophagic pathway is protective against Huntington's disease and related conditions (Wong and Cuervo, 2010), snapin could also be a potential therapeutic target in the regulation of autophagy.

There are several issues that remain to be solved. First, it is unclear how the spectrin/dynactin-based cargo recognition process is related to the snapin/DIC-based pathway. This question is related to mechanisms by which snapin recognizes late endosomes that are ready for transporting along microtubules and releases them at appropriate sites. Second, the exact mechanisms whereby snapin facilitates late endosome-lysosome fusion are unclear. Snapin may simply use dynein motors to move late endosomes to perinuclear locations where lysosomes are concentrated and facilitate fusion frequency (Figure 1), but it may also recruit other proteins necessary for fusion. Alternatively, the transport defects could be secondary to a fusion step required for cargo loading and transport. Third, it would be interesting to know whether the retrograde trafficking of autophagosomes and their fusion competency are also regulated by snapin. If so, reduced autophagic activities in *snapin*-null neurons may also be affected by these processes. Fourth, it is unclear whether and how snapin regulates retrograde transport of late endosomes in dendrites. Unlike in axons, microtubules in dendrites bind microtubule-associated protein 2 and have mixed polarity (Conde and Cáceres, 2009). When overexpressed in neurons, snapin was previously shown to reduce the number of primary dendrites by binding to cypin and slowing cypin-promoted microtubule assembly (Chen et al., 2005). This phenotype might also be explained by enhanced late endosomal trafficking if snapin similarly modulates late endosomal trafficking in dendrites. In addition, AMPA receptor trafficking during LTD might be affected in *snapin*-null neurons.

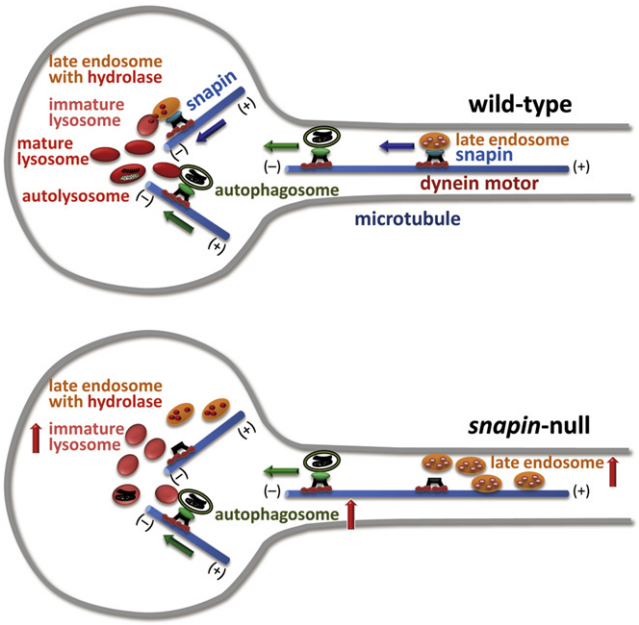

Figure 1. Model for Snapin-Regulated Trafficking of Late Endosomes

In wild-type neurons (top), snapin links dynein motors to the late endosomes and facilitates their retrograde trafficking along microtubules toward the minus (–) end. The same pathway is used to transport acid-hydrolase-containing late endosomes, which will be fused with immature lysosomes to form mature lysosomes. Mature lysosomes are necessary to form autolysosomes. In *snapin*-null neurons (bottom), late endosomes cannot be recruited to dynein motors, resulting in accumulation of standstill late endosomes in axons. Reduced trafficking of late endosomes also results in accumulation of immature lysosomes and reduction of autolysosomes.

Finally, snapin is known to bind to at least a dozen other proteins, such as cypin, SNAP-25, syntaxin 8, and dysbindin, which are all related to a variety of membrane trafficking and fusion systems. Importantly, Cai et al. (2010) showed that a mutant snapin, which cannot bind to DIC but can presumably still bind to other partners, played a dominant-negative role in retrograde transport of lysosomes, indicating that binding to DIC is essential for the function of snapin in regulating late endosomal trafficking. Nevertheless, it is not completely clear whether and how other associated proteins are involved in the recruitment of snapin to late endosomes and their fusion with lysosomes. Furthermore, snapin is also reported as a component of biogenesis of lysosome-related organelles complex-1 (BLOC-1), which is required for the biogenesis of specialized lysosome-related organelles, such as melanosomes and platelet-dense granules (Starcevic and Dell'Angelica, 2004). Although mutation in BLOC-1 gene results in Hermansky-Pudlak syndrome (HPS)-like specific phenotypes characterized by hypopigmentation and platelet storage pool deficiency, *snapin*-null mice die just after birth and cultured neurons display axonal swelling and reduced survival in vitro, indicating that phenotypes of *snapin*-null mice cannot be explained by the defective BLOC-1.

Nevertheless, the role of snapin in regulating late endosomal trafficking and lysosomal fusion could be related to the function of BLOC-1 and novel forms of HPS. The perinatal lethality of *snapin*-null mice, which may be related to such a multivalent role of snapin in coordinating several membrane transport/fusion systems, has hampered elucidation of the specific roles of snapin in vivo. Therefore, it would be important to clarify the physiological and pathological role of snapin in regulating the late endosome-lysosome pathway in vivo by using snapin-L99K knockin mice.

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VGLUTs—Potential Targets for the Treatment of Seizures?

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Vesicular glutamate transporters (VGLUTs) load glutamate into synaptic vesicles. In this issue of *Neuron*, Juge et al. report that ketone bodies compete with chloride-dependent activation of VGLUTs, leading to suppression of glutamate release and seizures. These findings provide a surprising explanation for the efficacy of the ketogenic diet in controlling epilepsy.

The functioning of the mammalian CNS depends on a delicate balance between excitatory and inhibitory synaptic activities. Glutamate is the major excitatory neurotransmitter in the brain, and its release is tightly regulated. At a given synapse, the amount of glutamate released per action potential depends on factors such as past firing patterns, activity of neighboring synapses, retrograde signaling, and second-messenger cascades. Most commonly, the number of synaptic vesicles undergoing exocytosis is regulated, and a vast amount of studies have addressed this mechanism of synaptic plasticity. However, it is becoming apparent that transmitter output may also be controlled by changing the transmitter content of synaptic vesicles (for review see Ahnert-Hilger et al., 2003; Edwards, 2007).

Loading of synaptic vesicles with glutamate is mediated by a small family of vesicular glutamate transporters termed VGLUTs1–3 (Edwards, 2007). Transport is driven by a proton electrochemical gradient across the vesicle membrane, which is generated by a vacuolar ATPase (Edwards, 2007). In contrast to the well-characterized Na^+-dependent glutamate transporters in the plasma membrane, our knowledge about VGLUTs is lagging behind because VGLUTs can only be studied in vesicles that contain an active proton pump, greatly limiting experimental flexibility. To deter researchers even further, VGLUTs expressed in nonneuronal cells display low activities, and once incorporated in the plasma membrane they "moonlight" as Na^+-dependent transporters for inorganic phosphate (Aihara et al., 2000; Ni et al., 1994).

Since the early days of measuring vesicular glutamate uptake (Disbrow et al., 1982; Naito and Ueda, 1985), it is known that uptake depends on chloride ions at millimolar concentrations. However, it has not been easy to distinguish