Published in final edited form as:
Mol Cell Neurosci. 2011 December ; 48(4): 278–287. doi:10.1016/j.mcn.2011.06.013.

Mechanisms of polarized membrane trafficking in neurons – focusing in on endosomes

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Abstract

Neurons are polarized cells that have a complex and unique morphology: long processes (axons and dendrites) extending far from the cell body. In addition, the somatodendritic and axonal domains are further divided into specific subdomains, such as synapses (pre- and postsynaptic specializations), proximal and distal dendrites, axon initial segments, nodes of Ranvier, and axon growth cones. The striking asymmetry and complexity of neuronal cells is necessary for their function in receiving, processing and transferring electrical signals, with each domain playing a precise function in these processes. In order to establish and maintain distinct neuronal domains, mechanisms must exist for protein delivery to specific neuronal compartments, such that each compartment has the correct functional molecular composition. How polarized membrane domains are established and maintained is a long-standing question. Transmembrane proteins, such as receptors and adhesion molecules, can be transported to their proper membrane domains by several pathways. The biosynthetic secretory system delivers newly synthesized transmembrane proteins from the ER-Golgi via the trans-Golgi network (TGN) to the plasma membrane. In addition, the endosomal system is critically involved in many instances in ensuring proper (re)targeting of membrane components because it can internalize and degrade mislocalized proteins, or recycle proteins from one domain to another. The endosomal system is thus crucial for establishing and maintaining neuronal polarity. In this review, we focus mainly on the intracellular compartments that serve as sorting stations for polarized transport, with particular emphasis on the emerging roles of endosomes.

Keywords

axonal targeting; somatodendritic targeting; transcytosis; endocytosis; L1; NgCAM; NEEP21; Golgi outpost; microtubule motors; recycling endosomes; early endosomes; neuronal polarity; EHD; SNARE; rab proteins

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Polarized membrane trafficking in neurons

In neurons, the main polarized sorting decision is between axonal and somatodendritic domains. Membrane proteins could be sorted to axon vs. dendrites principally by at least three pathways (Figure 1):

Pathway 1) Membrane proteins are directly delivered in a polarized manner from the trans-Golgi network (TGN). This pathway is independent of endocytosis, but might involve transit through endosomes.

Pathway 2) Membrane proteins are delivered in a non-polarized fashion, followed by selective retrieval by endocytosis from the incorrect domain and degradation. Receptors present on the incorrect surface would therefore be removed whereas correctly localized receptors would be retained. Retention in the correct domain could entail not just lack of endocytosis, but also stabilization by cytoskeletal anchoring.

Pathway 3) Membrane proteins are initially sorted in the TGN and trafficked to one domain (axon or dendrite). Subsequently, the protein is endocytosed and resorted in endosomes. Polarized delivery to the final destination would then occur from endosomes. This pathway is called transcytosis (reviewed in Winckler and Mellman, 2010).

In polarized epithelial cells, all three pathways have been demonstrated for different proteins in different cell types. For instance, most apical and basolateral proteins are sorted in the TGN to their respective domains in Madin-Darby canine kidney cell (MDCK) (pathway 1), whereas in hepatocytes, most apical proteins reach the apical surface by transcytosis after initial delivery to the basolateral surface and subsequent resorting to the apical domain in endosomes (pathway 3). Some proteins (such as NaK-ATPase in MDCK cells) are enriched in their respective domain via a selective retention mechanism (pathway 2) (reviewed in Folsch, 2008). Interestingly, evidence is accumulating in epithelial cells, that even the TGN-sorting pathway (pathway 1) does not always traffic cargos directly to the apical or basolateral plasma membrane, but frequently proceeds via different endosomal subpopulations (Folsch et al., 2009). In these cases, though, cargo travels from the TGN to the endosome without prior appearance on the plasma membrane, whereas in transcytosis (pathway 3) cargo is delivered first to one plasma membrane domain (basolateral or somatodendritic), then retrieved into endosomes, and trafficked to the opposite domain (apical or axonal). The endosomal system therefore might also be involved in the secretory pathway from the TGN, although the extent of the interplay between secretory and endosomal pathways is not fully understood, especially in neurons. Since the steady-state distribution of a particular protein does not reveal its targeting pathway, more detailed experiments need to be carried out to determine which of the three pathways is followed by any given cargo protein. In order to distinguish between the different pathways, one needs to observe the trafficking kinetics and interfere with mechanisms that are known to be involved in specific trafficking routes, such as endocytosis. It then requires additional experimentation to distinguish the two endocytosis-dependent pathways (selective retrieval/retention (pathway 2) and transcytosis (pathway 3)) from each other.

Multiple classes of proteins are responsible for ensuring the specificity of the sorting, budding, transport, and fusion events during membrane transport, such as vesicle coat proteins, SNAREs, rab and Arf proteins, BAR domain proteins, motor proteins, etc. The exact identities and roles that these various proteins play in neuronal membrane trafficking are being investigated by many laboratories. Even though a large literature exists (too large to cover in this review), most of the specificity control in polarized targeting to axons and dendrites is still poorly understood. Furthermore, targeted protein delivery is supplemented by stabilization of membrane components in the membrane and creation of diffusion barriers

between domains for prevention of mixing of components. These other processes have been reviewed elsewhere, and we refer the reader to these excellent reviews (Arnold, 2009; Cognet et al., 2006; Kapitein and Hoogenraad, 2011; Newpher and Ehlers, 2008; Rasband, 2009; Salinas et al., 2008; Sann et al., 2009). Local protein synthesis also has the potential to ensure differential protein localization. However, this process is beyond the scope of this review. For recent reviews please see Andreassi and Riccio, 2009; Bassell and Warren, 2008; Lasiecka et al., 2009; Willis and Twiss, 2010. In this review, we will focus on the intracellular compartments that serve as sorting stations for polarized transport, with particular emphasis on the emerging roles of endosomes.

Axonal trafficking via transcytosis

Polarized MDCK cells use the TGN as a major sorting station for apical and basolateral cargos. In analogy with MDCK cells, it was assumed that neurons also use TGN-based sorting (pathway 1) for direct delivery of axonal and somatodendritic cargos to their respective domains. A different picture, though, emerged when the axonal trafficking of some proteins was investigated (reviewed in Lasiecka et al., 2009). The axonal cell adhesion molecule L1/NgCAM, for example, was dependent on endocytosis for axonal polarity, and additional evidence including study of transport kinetics to the surface and live imaging of endocytosed L1/NgCAM led to a model that L1/NgCAM used the indirect transcytosis route to reach the axon (pathway 3) (Wisco et al., 2003). The transcytotic pathway consists of initial delivery along the secretory pathway to the somatodendritic domain, subsequent endocytosis into somatodendritic endosomes, and transport through the endosomal pathway to the axonal domain. Both trafficking steps are signal-mediated and potentially regulated (see below). Transcytosis provides an axonal delivery pathway for other axonal membrane proteins, besides L1/NgCAM (Wisco et al., 2003), such as TrkA (Ascano et al., 2009). Both cannabinoid receptor CB1R (Leterrier et al., 2006), and Caspr2 (Bel et al., 2009) use endocytosis-dependent pathways to the axon, but the details of the pathway used (either pathway 2 or 3) have not been fully worked out.

The reasons why NgCAM transcytoses to the axon are not immediately apparent, and many questions remain regarding a possible biological function of this circuitous targeting route. For both CB1R (Leterrier et al., 2006) and TrkA (Ascano et al., 2009), ligand binding drives the transcytotic pathway. This observation raises the possibility that routing of axonal proteins can be subject to ligand-mediated signaling. It is thus possible that the transient appearance of L1/NgCAM on the somatodendritic surface serves a purpose, such as signaling in dendrites, binding of a ligand, or a guidance or feedback function that coordinates dendrite and axonal growth. It is also conceivable that transcytotic routing is regulated (Anderson et al., 2005; Schaefer et al., 2002) and can be turned on and off by the cell in a signal cascade-dependent manner. How changes in trafficking route would affect neuronal function is currently unknown.

Even though the signals for transcytotic targeting of L1/NgCAM have been mapped (see below), little is known about either the cellular machinery recognizing the signals in neurons or the endosomal compartments from which polarized sorting to axons takes place. Given the emerging role of endosomes in polarized sorting to the axon, we will introduce the reader in the following section to the insights gained from non-neuronal cells, and then discuss commonalities and differences between the endosomal system in neuronal and non-neuronal cells.

Endosomal trafficking in non-neuronal cells

Endocytosis is the process by which membrane proteins are removed from the cell surface by internalization (Jovic et al., 2010; Maxfield and McGraw, 2004b; Traub, 2009). It is

crucial for rapid and localized regulation of levels of proteins at the plasma membrane. After being endocytosed, transmembrane proteins can be either degraded or recycled back to the cell surface. Furthermore, the endosomal system is also important for intracellular signaling since internalized receptors continue to transmit signals from endosomes that can be distinct from those that immediately arise at the plasma membrane (Maxfield and McGraw, 2004a; Murphy et al., 2009). The endosomal system is most thoroughly studied in non-polarized cells. “Classical” endosomal system contains three major types of compartments: early endosomes (EE), recycling endosomes (RE) [also frequently referred to as ERC = endosomal recycling compartment], and late endosomes (LE)/lysosomes (Lys). Membrane proteins are first endocytosed into EEs. From there, they can either follow the degradative pathway to LE/Lys or recycle back to the plasma membrane either directly from EE or through the recycling endosome RE (Figure 2). Owing to the advancement in imaging techniques, especially high-resolution live cell imaging, it is now clear that the endosomal system is very dynamic and more complex than previously anticipated. In contrast to the long-held view that EEs are the initial cargo sorting station, live cell imaging demonstrated that various membrane receptors can be sorted prior to entering EEs. Cargo sorting starts already at the plasma membrane, at the level of clathrin-coated pit (CCP) formation, such that different cargo is accumulated in different CCPs. Endocytosed G protein-coupled receptors (GPCRs) accumulate in CCPs with prolonged surface residence time in comparison to transferrin receptors (TfR) which are internalized by short-lived CCPs (Puthenveedu and von Zastrow, 2006). As a consequence, distinct cargos are sorted into different types of early endosomes. Several cargos en route to degradation are preferentially targeted to dynamic EEs, whereas the recycling cargo transferrin is enriched in a larger, static population of EEs (Lakadamyali et al., 2006). The exact mechanisms and machinery responsible for these sorting events is not well understood.

Another layer of complexity was recently observed in sorting within EEs. It was previously shown that recycling cargo sorts to the tubular subdomain of early endosome and later to tubular REs, whereas degradative cargo remains in the vacuolar portion of EEs (Mayor et al., 1993). It was also shown that within REs different cargos are transported in distinct carriers with different transport kinetics (Lampson et al., 2001). Now, live cell imaging demonstrated that tubular microdomains on endosomes are heterogeneous, with dynamic tubules sorting constitutively recycling transferrin and more stable tubules sorting sequence-dependent recycling β2 adrenergic receptor (Puthenveedu et al., 2010). Generally, there are more flavors to each type of endosomal compartment than we anticipated. Furthermore, certain cargos also traffic retrogradely from REs to the TGN (Ghosh et al., 1998; Mallard et al., 1998), and REs are on the anterograde, biosynthetic route from the TGN to the plasma membrane of some cargos in epithelial cells (Ang et al., 2004; Folsch et al., 2009). There is clearly a close and dynamic relationship between REs and the TGN (Schmidt and Haucke, 2007), making the distinction between secretory and endosomal pathways more difficult. This complexity is presumably crucial for precise sorting of various receptors depending on their destinations.

Along with the segregation of cargos to different endosomal compartments, there is also segregation of endosomal regulators (Miaczynska and Zerial, 2002; Stenmark, 2009), which are crucial for cargo trafficking and the maintenance of specific endosomal compartments. These regulators belong to multiple different families of proteins such as: 1) small GFP-ases (rabs) and their interactors such as EEA1 and APPL, 2) adaptor proteins (AP-1, -2, -3, -4), that recognize cargo sorting motifs and recruit coat proteins important for vesicle formation 3) SNARE proteins involved in vesicle fusion, 4) exocyst complex involved in exocytosis, 5) EHD proteins proposed to be involved in tubulation and fission processes, 6) BAR domain-containing proteins which cause membrane bending, 7) sorting nexins containing a PX domain for membrane binding, among others. Some of these regulators are

transmembrane proteins (such as most of the SNAREs), but others like rabs, EHDs, EEA1 and APPL, can be recruited in a dynamic and regulated fashion to the endosomal membrane from the cytosol. EEs are defined by the presence of a subset of endosomal regulators, in particular EEA1 and rab5. REs associate with rab11, and LEs with rab7.

How endosomal identity is maintained in the face of continuous membrane and cargo flux, and how transport between specific endosomal populations is regulated is under intense investigation, particularly in non-neuronal cells. It is now well-established that individual endosomes frequently consist of heterogeneous subdomains which segregate laterally within a single endosome. For instance, EEs have distinct rab5- and rab4- subdomains, REs have rab4- and rab11-subdomains (Miaczynska and Zerial, 2002; Sonnichsen et al., 2000), and LEs have rab7- and rab9-subdomains (Barbero et al., 2002). These mosaic subdomains might correspond to regions of cargo fusion into an endosome, subsequent cargo sorting into distinct domains, and ultimate budding of new endosomal transport carriers containing a subset of the total membrane cargos contained in the endosome. In addition, there is striking evidence that early compartments can mature into later compartments by shedding early regulators and gaining later regulators. This has been beautifully demonstrated by a recent paper from the De Camilli laboratory. Live cell imaging of endosomal compartments labeled with different endosomal regulators showed that pre-early endosomal compartments (APPL-positive) convert to early endosomes (EEA1-positive) (Zoncu et al., 2009), by shedding APPL and recruiting EEA1 to the same pre-existing endosome. Subsequently, early endosomes (rab5-positive) can mature into late endosomes (rab7-positive) (Rink et al., 2005) by shedding rab5 and recruiting rab7. Little is known about how the conversion of one compartment to another (and its concomitant switch in associated regulators) is achieved. For the switch from the APPL-positive preEE to the EEA1-positive EE, rab5 activity and accumulation of a different phosphoinositide species, PI-3P, are required (Zoncu et al., 2009). How rab5 converts to a rab7-positive LE is not known, but rab activity and phosphoinositides likely play a role here as well.

Neuronal specializations of the secretory and endosomal system

Even in non-polarized cells, many questions as to the regulation of endosomal trafficking are still unanswered. In polarized cells, even less is known, but it is clear that an additional level of complexity is added since recycling can occur to different domains of the plasma membrane, such as apical vs. basolateral domains in epithelial cells or axonal vs. somatodendritic domains in neurons. Our knowledge of the neuronal endosomal system and neuronal polarized trafficking is still limited and much remains to be done to unravel the complexities of polarized membrane traffic in neurons. The neuronal soma is roughly the size of an epithelial cell, but neurons have also long axons and dendrites that extend far from the cell body. Bi-directional transport (retrograde and anterograde) of various secretory and endosomal compartments occurs along both axons and dendrites. For example, syntaxin13-positive endosomal compartments traffic bidirectionally along both dendrites and axons (Prekeris et al., 1999). Anterograde transport in axons has been shown for multiple biosynthetic cargos, including VAMP2, and βAPP. The carriers for these cargos are presumed to be derived from the TGN (Nakata and Hirokawa, 2003). Trafficking of endosomes along the axon occurs primarily in the retrograde direction towards the cell soma. Retrograde axonal transport has received particular attention since it is crucial for neurotrophic signaling and neuronal survival (reviewed in (Howe and Mobley, 2004; Ibanez, 2007). Endosomal trafficking along the axon in the anterograde direction is less well established, but was observed for endosomes containing L1/NgCAM axonal adhesion molecule (Yap et al., 2008b), Trk receptors (Ascano et al., 2009), and integrins (Eva et al., 2010), as well as endosomal regulators, such as syntaxin13 (Prekeris et al., 1999) and rab 11 (Ascano et al., 2009). Vesicular transport in dendrites is also bi-directional and occurs

presumably for both TGN-derived as well as endosomally-derived carriers. For instance, endosomes containing the endosomal regulator EHD1 or rab11 traffic bi-directionally along dendrites (Lasiecka et al., 2010).

Besides the long-range trafficking of proteins to the correct locations using endosomal trafficking, there is also constant local recycling and degradation of proteins within specific domains (such as synapses or axon growth cones). These local endosomal pathways dynamically regulate the number and availability of plasma membrane receptors and adhesion molecules. Such local recycling is well understood for adhesion molecules during growth cone advance in axonal tips where endocytosis occurs in the center portion of the growth cone and endosomes recycle adhesion receptors back to the advancing edge of the growth cone (Kamiguchi and Lemmon, 2000; Long and Lemmon, 2000). In dendrites, local recycling has been demonstrated especially well for pre- and post-synaptic sites (reviewed in Dittman and Ryan, 2009; Kennedy and Ehlers, 2006; Schweizer and Ryan, 2006). At the post-synaptic site, live imaging has shown the insertion of glutamate receptors from endosomes into spine heads. In fact, this local recycling of glutamate receptors contributes to early phases of LTP (Kennedy and Ehlers, 2006). Overall, the large size and spatial specializations present in neurons necessitate a specialized endosomal system which acts locally as well as long distance and is responsive to extracellular events, such as growth factors, guidance cues, neurotrophins, and electrical activity. Endocytosis and endosomal trafficking therefore not only play important roles in long-range polarized trafficking, but also support a plethora of neuronal functions, such as axon and dendrite outgrowth and pathfinding, neurotrophic signaling for neuronal survival, synaptic plasticity, and synaptic transmission.

How is the neuronal endomembrane system built to handle the diverse demands of many cargos in far-flung places? In neurons, the secretory as well as the endosomal system are different in axons and dendrites. The secretory pathway in neurons contains not only the canonical perinuclear Golgi and TGN compartments, but in addition specialized Golgi compartments (called “Golgi outposts”) within dendritic arbors (Hanus and Ehlers, 2008; Horton and Ehlers, 2004; Horton et al., 2005; Pierce et al., 2001). The somatic Golgi in neurons is frequently quite extensive and extends far into the major apical dendrite of pyramidal neurons in vivo and in culture. This polarized placement of the Golgi is important for proper elaboration of dendrites and neuronal polarity (Horton et al., 2005; Matsuki et al., 2010). Golgi outposts are completely discontinuous from the somatic Golgi and are frequently localized at dendritic branch points. They might ensure local distribution of dendritic proteins (Horton et al., 2005; Ye et al., 2007). Consequently, dendrite branching and growth, but not axonal outgrowth, relies on the function of Golgi outposts in Drosophila (Ye et al., 2007). Which endogenous cargos might use the Golgi outposts is still largely unknown, but NMDA receptors have been reported to bypass the somatic Golgi (Jeyifous et al., 2009). Axons are largely devoid of secretory pathway compartments, but a few publications have reported the presence of markers for secretory compartments, such as Golgi and TGN, in axons (Hengst and Jaffrey, 2007; Lee and Hollenbeck, 2003; Merianda et al., 2009; Willis et al., 2005; Yao et al., 2006). The exact roles and cargos using these “axonal outposts” remain to be established.

The neuronal endosomal system, similarly, differs in several respects from that of fibroblasts. For example EEA1, a canonical early endosomal regulator in non-neuronal cells, is localized only to the somatodendritic domain in neurons but is absent from axons (Wilson et al., 2000). Since EEA1 is thought to provide an essential function in endosome-endosome fusion, its absence from axonal early endosomes in growth cones and axon terminals raises the question of what might be a somatodendritic-specific function of EEA1 and what protein(s) might substitute for EEA1 function in the axon. The morphology of recycling

endosomes also differs from that in non-neuronal cells. Whereas in non-neuronal cells recycling endosomes are clustered tightly near the nucleus in close proximity of the TGN, in neurons recycling endosomes, labeled with transferrin or rab11, are spread almost evenly throughout soma, dendrites and axons (Ascano et al., 2009; Park et al., 2006; Prekeris et al., 1999; Thompson et al., 2007; Wang et al., 2008). Such a spread-out distribution likely serves the diverse spatial demands of the neuron.

Neuronal-specific functions of endosomal regulators

Several classes of endosomal regulators are required for proper endosomal trafficking. These regulators, many of them well-studied in fibroblasts, also play important roles in neurons. EHD1/Rme1, as well as the recycling endosome regulators rab11 and syntaxin13, were shown to be important for local AMPA receptor recycling at postsynaptic sites (Park et al., 2004) and in transcytotic trafficking of L1/NgCAM (Lasiecka et al., 2010; and our unpublished data). Rab11 is also important for anterograde axonal trafficking of Trk in endosomes in sympathetic neurons (Ascano et al., 2009). Other rabs, such as rab5 and rab7, are important in regulating endosomal trafficking at postsynaptic sites (Brown et al., 2005), for retrograde trafficking along the axon (Deinhardt et al., 2006), and for the migration of newborn neurons in the neocortex (Kawauchi et al., 2010).

Interestingly, many membrane trafficking regulators are highly enriched in the brain, or even expressed in a brain-specific fashion. For instance, the neuronal early endosome protein NEEP21 (originally identified as Neural Specific Gene 1, Nsg1) is expressed primarily in neurons and is found in an early endosomal population largely distinct from EEA1-positive endosomes. NEEP21 interacts with the SNARE protein syntaxin13 and localizes to rab4-positive but rab5 negative early endosomes (Steiner et al., 2002). NEEP21-positive endosomes accumulate endocytosed L1/NgCAM adhesion molecules, as well as AMPA receptors (Steiner et al., 2005; Steiner et al., 2002; Yap et al., 2008b) and are involved in trafficking of multiple cargos, including transferrin, L1/NgCAM, and AMPAR (Alberi et al., 2005; Debaigt et al., 2004; Steiner et al., 2005; Steiner et al., 2002; Yap et al., 2008b). NEEP21 also binds to GRIP1, an interaction important for GluR2 trafficking (Steiner et al., 2005). Recently, NEEP21 was shown to interact with and affect proteolytic processing of βAPP (Norstrom et al., 2010). The precise mode of NEEP21 action and the role of its interaction with syntaxin13 are still unknown.

Another known neuronal-specific protein is GRASP-1 (GRIP-associated protein-1). GRASP-1 is a neuron-specific effector of Rab4 and an important component of the molecular machinery that coordinates recycling endosome maturation in dendrites. GRASP-1 is also necessary for AMPAR recycling, maintenance of spine morphology, and synaptic plasticity (Hoogenraad et al., 2010). The endocytosis protein dynamin also exists in neuronal-specific and ubiquitously-espressed isoforms. Dynamin 1 and 3 are neuron-specific whereas dynamin2 is also expressed in non-neuronal cells. Elegant work by the De Camilli lab has uncovered that dynamin 1 (and possible also dynamin 3) support a distinct endocytic mechanism from dynamin 2. Dynamin 1, in particular, is required for maintaining endocytosis of synaptic vesicle components during prolonged stimulation (Robinson, 2007; Ferguson, 2007). It will be important to elucidate how these neuronal-specific components of various organelles modify the canonical machinery to achieve neuron-specific functions.

Besides the presence of neuronal-specific endosomal regulators, many canonical endosomal regulators found in non-neuronal cells have specialized functions in neurons. In non-neuronal cells members of the EHD family, EHD1–EHD4, regulate trafficking through early and recycling endosomes (Grant and Caplan, 2008). EHD1 (also called Rme1) has also been suggested to regulate endocytosis of IGF1 receptor (Rotem-Yehudar et al., 2001). The exact

molecular functions of EHD1 are still under investigation. EHD1 associates with pre-existing tubules in fibroblasts (Sharma et al., 2009), but tubular EHD1-containing compartments are rarely seen in neurons (Yap et al., 2010). In addition, EHD2 has been shown to support tubulation of artificial liposomes, suggesting the model that EHD proteins may function in a manner similar to dynamin family proteins in causing tubulation and subsequent fission of vesicular carriers (Daumke et al., 2007). Since EHD proteins interact with multiple trafficking regulators via their C-terminal EH domains (Naslavsky and Caplan, 2011), they likely regulate and coordinate recruitment and activation of other effectors classes, such as rabs. Interestingly, in neurons, EHD4 (also called pincher) (Shao et al., 2002) is involved in endocytosis, rather than (or in addition to) recycling (Sharma et al., 2008): TrkA-mediated NGF-dependent survival signaling depends on EHD4/pincher-mediated endocytosis (Valdez et al., 2005). Recently Nogo-A, a repulsive cue for axon growth cones, was also shown to be endocytosed on an EHD4/pincher pathway (Joset et al., 2010). Furthermore, EHD4 (likely as a hetero-dimer with EHD1) mediates a specialized internalization pathway utilized by L1/NgCAM in neurons. This pathway is cargo-specific (not utilized by transferrin or AMPARs) (Park et al., 2004) and cell type-specific (not involved in the internalization of L1/NgCAM in fibroblasts) (Yap et al., 2010; Winckler and Yap, 2011).

In addition to a role in endocytosis, EHD1 also has a role in endosomal transport in neurons. EHD1 associates dynamically with endosomes early in the endosomal pathway, prior to the recruitment of EEA1 to early endosomes. In this respect, neurons might differ from fibroblasts in which EHD1 function has been associated most frequently with recycling endosomes, although roles in early endosomes are also becoming apparent (Cai et al., 2011; Jovic et al., 2010). EHD1 remains associated with endosomes after EEA1 dissociates, indicating that EHD1 is a rather long-lived component of endosomes. Downregulation of EHD1 using small hairpin plasmids leads to accumulation of endocytosed NgCAM in EEA1-positive endosomes and delayed accumulation in NEEP21-endosomes, suggesting a role for EHD1 in transport from EEA1-positive to NEEP21-positive early endosomes (Lasiecka et al., 2010). If EHD1 also plays a role in later transport steps in neurons for cargo leaving recycling endosomes remains to be determined.

Signals and machinery for targeting to the somatodendritic domain

Much of the work discussed so far has focused on the compartments involved in polarized sorting and the machinery regulating compartmental progression. A lot of work has also focused on identifying signals in the polarized cargo molecules themselves that would be responsible for recruiting polarized targeting machinery. Targeting of proteins to different domains is governed by specific sorting motifs within the protein, which encode protein localization. There is no universally used axonal or dendritic motif, and only few strong consensus sequences have emerged in the studies carried out to date. Similarly to basolateral sorting in epithelial cells (Folsch, 2008), the sorting of cargo towards the somatodendritic domain depends on intrinsic signals contained within the cytoplasmic domain of the cargo protein (Craig and Banker, 1994; Dotti and Simons, 1990; Winckler and Mellman, 1999; Yap and Winckler, 2009). In particular, two sequence motifs have been identified as dendritic targeting signals: a tyrosine-based motif, and a dileucine-based motif. In epithelial cells, tyrosine-based motifs interact with the μ subunit of AP adaptors (AP1-4), whereas di-leucine motifs interact with γ/σ1 of AP1 or δ/σ3 of AP4 (reviewed in Folsch, 2008). Studies showed that these two motifs in some membrane proteins confer both dendritic targeting in neurons and basolateral targeting in polarized epithelial cells (Jareb and Banker, 1998). In contrast, in other proteins, basolateral and somatodendritic tyrosine-based targeting motifs map to different domains (Francesconi and Duvoisin, 2002; West et al., 1997). Similarly, di-leucine motifs, which act as basolateral targeting motifs, are not universally active as

somatodendritic targeting signals (Silverman et al., 2005). Novel motifs associated with dendritic targeting have also been identified (Francesconi and Duvoisin, 2002; Ruberti and Dotti, 2000). However, these motifs are not conserved in other dendritically targeted membrane proteins. Therefore, mapping of somatodendritic targeting signals needs to be carried out for each protein individually.

It is widely assumed that these targeting signals ensure accurate TGN-based sorting (Figure 1: pathway 1), such that the signals are required for either cargo enrichment and/or inclusion into somatodendritically-directed vesicles. This assumption is based on the finding in epithelial cells that basolateral targeting signals mediate interaction with clathrin adaptors (Folsch, 2008). In reality, the site of action for most of the currently characterized somatodendritic sorting signals has not been directly demonstrated. Specific roles for AP adaptors in somatodendritic sorting have only been proposed for AP-4. Knockout of the β subunit of AP-4 results in mis-targeting of AMPA receptors to axons, along with TARPs, δ2 glutamate receptors, and LDLR. NMDA receptors, on the other hand, are not affected in their localization to the somatodendritic domain, even though they can bind to AP-4 directly (Lavezzari et al., 2004). AMPA receptors do not directly bind to AP-4, but rather associate with the AP-4 complex indirectly via TARPs. Surprisingly, the mis-targeted AMPA receptors do not accumulate on the axonal surface, but rather in autophagosomes in the axon (Matsuda et al., 2008). Endocytosis might also be important for somatodendritic targeting of some receptors. For example, DNER, a transmembrane Notch ligand, was shown to require endocytosis for accurate somatodendritic targeting (Kurisu et al., 2010). It remains to be determined if other somatodendritic proteins might also show impaired ability to localize to the somatodendritic domain when endocytosis is impaired.

Furthermore, polarized membrane traffic is a multi-step pathway and correct sorting at the TGN is only one of multiple steps that need to be accomplished correctly to ensure polarized targeting. After inclusion into a somatodendritically-directed vesicle, the vesicle needs to be targeted to dendrites rather than the axon, suggesting important roles for motor proteins in polarized membrane traffic. Kinesins are one of the major motor proteins and much work is being done on their roles in membrane trafficking in neurons (Hirokawa et al., 2010; Kapitein and Hoogenraad, 2011). For instance, KIF17 is important in mediating the correct somatodendritic targeting of Kv4.2 (Chu et al., 2006). In addition to kinesins, the minus-end directed motor dynein also plays a role in directing traffic to the dendrites (Kapitein et al., 2010). Interestingly, neuron-specific dynein subunits might contribute to cargo selectivity, as has been shown for retrograde transport of TrkB in axons (Ha et al., 2008). Intriguingly, actin-based myosin motors have recently emerged as playing unexpected and important roles in polarized trafficking (Lewis et al., 2009). It is currently not clear if tyrosine- or dileucine-based targeting signals mediate (direct or indirect) interactions with motor proteins or if distinct sets of motifs are needed to mediate those interactions. In the case of Kv4.2, though, it has been shown that the di-leucine motif is not responsible for binding KIF17 (Chu et al., 2006), suggesting that somatodendritic targeting is regulated by multiple signals and binding partners.

Signals and machinery for targeting to the axonal domain

Axonal targeting signals are diverse, and the mechanisms of their action are not yet well understood. Several axonal sorting signals map to the extracellular domain (Biermann et al., 2010; Sampo et al., 2003), reminiscent of some extracellular apical signals operating in epithelial cells. Other axonal signals are found in the cytoplasmic domain, but show no conserved consensus sequences with each other. These diverse signals might operate at distinct steps on the pathways to the axon, using one or multiple cellular mechanisms.

One open question is how many distinct axonally-targeted vesicles are generated at the TGN or in endosomes. Do all axonal proteins travel together in one kind of axonally-directed vesicle? It appears that multiple classes of axonally-directed vesicles are generated: Two classes of presumably TGN-derived carriers that carry distinct axonal cargos were observed in one study (Kaether et al., 2000); at the same time multiple axonal cargos can share the same vesicle (Nakata and Hirokawa, 2003). Live imaging of A-VSV-G-GFP (an apical/axonal version of VSV-G) and amyloid precursor protein (APP) showed that they were directly transported to the axon after a biosynthetic transport block was released (Nakata and Hirokawa, 2003). These cargos therefore appear to use a direct pathway from the TGN to the axon. Whether they briefly stopped over at a local endosome on their way into the axon cannot be easily determined from the experiments and remains a possibility.

For a number of axonal proteins axonal targeting motifs mapped to sequences that were shown to be endocytosis motifs (Fache et al., 2004; Garrido et al., 2003; Sampo et al., 2003; Xu et al., 2006). These axonal proteins likely travel on an indirect trafficking pathway through initial non-polarized delivery and subsequent selective removal/retention route (mechanism 2). Proteins on that pathway (such as voltage-gated sodium channels) are first inserted into both somatodendritic and axonal domains. Subsequently, the missorted dendritic pool is endocytosed and presumably degraded, whereas the properly inserted axonal pool is retained and stabilized on the axonal surface. Axonal accumulation of this class of axonal proteins is therefore dependent on somatodendritic endocytosis (reviewed in Winckler and Mellman, 2010). The identity of the endocytic pathway and which adaptors might be involved is not yet known. Somewhat surprisingly, this type of endocytosis-dependent axonal trafficking is currently the most widely documented mode of axonal accumulation. Work on more axonal receptors is needed to determine how many axonal proteins travel on an endocytosis-independent vs. an endocytosis-dependent pathway.

The molecular machinery specialized for axonal targeting is still incompletely understood. Binding partners for axonal targeting signals have only been identified in a small number of cases. For instance, potassium channels in the shaker class require the T1 tetramerization domain for axonal targeting (Gu et al., 2003). Two different molecular pathways have been proposed to be mediated by T1 interactions. The first one is based on binding of T1 domains to the auxiliary cytosolic Kvβ subunit and indirect binding to kinesins, the second one is based on direct binding to kinesins. Binding of T1 domains to Kvβ was shown to be required for ER exit and axonal targeting of the Kv shaker channels. Kvβ mediates binding to the kinesin motor KIF3 indirectly via the microtubule tip complex protein EB1, thereby regulating entry into the axon (Gu et al., 2006). Interestingly, the association of Kvβ with EB1 is regulated by phosphorylation downstream of cdk kinases, thereby affecting the efficieny of surface delivery of Kv1 channels (Vacher et al., 2011). T1, alternatively, can also bind directly to the KIF5B motor for transport into axons (Rivera et al., 2007).

How a particular kinesin motor selectively transports cargo vesicles into axons or dendrites is under intense investigation. Many interesting studies have been reported in the last couple of years, but much remains to be clarified. Some of the motor domains preferentially bind to and translocate on axonal microtubules (Konishi and Setou, 2009), and posttranslational modifications of tubulins might be distinct in axons and dendrites. Such differences in posttranslational modifications as well as in the identity and abundance of microtubule-associate proteins (MAPs) likely contribute to privileged entry of certain kinesins into axons. Furthermore, a specialized substructure at the beginning of the axon, the axon initial segment, has been proposed to regulate entry of axonal kinesins into the axon (Song et al., 2009). The exact mechanism for such a “filter” at the axon initial segment is still not known, but it requires ankyrinG and intact microfilaments. In addition to kinesins, myosins play unexpected roles in axonal trafficking. In particular, myosinVI promotes axonal targeting of

several proteins, including L1/NgCAM (Lewis et al., 2011). Strikingly, the effect of myosinVI can be linked to aiding somatodendritic endocytosis (pathways 2 and/or 3) as well as having effects on direct trafficking to the axon along the biosynthetic pathway (pathway 1). The exact mechanisms and sites of action for myosinVI await future studies.

Some of the molecular machinery for anterograde transport of Trk receptors is also being discovered. CRMP2, collapsin response mediator protein-2, is involved in the anterograde transport of vesicles containing TrkB (Arimura et al., 2009b). Slp1, a CRMP-2 interacting molecule, binds directly to the cytoplasmic tail of TrkB. Slp1 is also an effector of Rab27. Slp1 can therefore link TrkB and Rab27B to CRMP2. Since CRMP2 interacts with kinesin light chain, it could directly link the TrkB/Slp1/Rab27B complex to kinesin 1 for anterograde axonal transport (Arimura et al., 2009a). The molecular machinery for how Rabs recognize specific vesicles for targeting is therefore starting to be unraveled. In addition to rabs and rab effectors, sortilin has been implicated in mediating anterograde transport of Trk receptors to the axon (Vaegter et al., 2011)

Signals and machinery for transcytotic routing of NgCAM

The signals required for transcytotic trafficking are best understood for L1/NgCAM. L1/NgCAM contains a specific tyrosine-based motif (YRSLE) in the cytoplasmic tail that mediates sorting from the TGN to the somatodendritic domain (Yap et al., 2008a). TGN-based sorting thus contributes to L1/NgCAM polarity. This motif presumably binds AP adaptors, but the identity of the AP adaptor in neurons is not known. In addition to the YRSLE somatodendritic signal, an axonal signal maps to a 15 amino acid glycine- and serine-rich stretch in the cytoplasmic tail of L1/NgCAM. Both signals are required for the sequential regulated routing via the multi-step transcytotic pathway (Yap et al., 2008a). Surprisingly, L1/NgCAM contains a second sufficient axonal targeting signal in the extracellular domain (Sampo et al., 2003). It is not clear why L1/NgCAM contains two sufficient axonal signals, but the presence of the second signal improves axonal targeting. It is also unknown which cellular machinery recognizes and executes either the cytoplasmic or the extracellular axonal signals.

Interestingly, NgCAM also accumulates on the apical domain in MDCK cells and reaches its apical destination via transcytosis, on a pathway apparently very similar to the one described in neurons (Anderson et al., 2005). The same tyrosine-based motif found to be important for the initial somatodendritic targeting in neurons also mediates the initial basolateral sorting of NgCAM in MDCK cells. In MDCK cells, transcytotic trafficking of NgCAM is regulated by phosphorylation (Anderson et al., 2005). The epithelial-specific adaptor AP-1B recognizes the YRSLE signal and mediates the initial basolateral sorting of newly-synthesized NgCAM. The YRSLE motif is also recognized by the endocytosis clathrin adaptor AP-2 (Kamiguchi et al., 1998), which mediates the subsequent endocytosis of NgCAM into basolateral endosomes. The YRSLE motif is then subject to phosphorylation, likely by a src family kinase (Schaefer et al., 2002). This phosphorylation inactivates the basolateral signal and inhibits AP-1B binding. NgCAM with a phosphorylated YRSLE motif is therefore not recycled back to the basolateral domain, but rather transcytosed to the apical domain based on apical sorting signals. The apical signals are only active when the YRSLE basolateral signal is inactivated by phosphorylation. In agreement with this mechanism, NgCAM carrying a point mutation in the YRSLE motif (NgCAM Y33A) is unable to undergo transcytosis in neurons and travels to the axon on a direct, endocytosis-independent pathway instead (Wisco et al., 2003). The molecular basis for the sequential execution of the somatodendritic and axonal signals in NgCAM are not known. Cryptic apical signals that only become active after inactivation or removal of a basolateral signal are also prevalent in MDCK cells (Rodriguez-Boulan and Musch, 2005).

A similar mechanism involving spatial regulation of adaptor binding via phosphorylation thus likely also regulates NgCAM transcytosis in neurons, with an adaptor other than AP-1B likely mediating the initial somatodendritic sorting.

Outlook

Besides axons and dendrites, neurons also have other specialized domains, such as proximal and distal dendrites, axon growth cones, synapses (pre-synaptic and postsynaptic specializations), axon initial segment and nodes of Ranvier. Each domain contains specific sets of proteins and plays distinct role in neuronal physiology. The assembly and maintenance of these distinct domains almost certainly rely on regulated trafficking from biosynthetic and endosomal compartments. The details of how the composition of these distinct domains are assembled and dynamically regulated are still being worked out. Current advances in live cell imaging and super-resolution microscopy will enable the study of trafficking in cultured neuronal cells in great detail. In the future, as the in vivo imaging techniques evolve, we might be able to study trafficking in neuronal cells in the context of brain development as well as in disease states in vivo. We could observe trafficking involved in developmental processes or specialized functions of different brain regions and different neuronal cell types. Furthermore, as more and more connections between dysfunction of the endomembrane system and neurodegenerative diseases surface, studying neuronal transport will gain additional clinical relevance.

Acknowledgments

We thank Dr. Chan Choo Yap for critical reading of the manuscript and insightful suggestions. Work in the Winckler laboratory is supported by NIH (R01GM086913) and the American Heart Association (10GRNT3770063).

References

Alberi S, Boda B, Steiner P, Nikonenko I, Hirling H, Muller D. The endosomal protein NEEP21 regulates AMPA receptor-mediated synaptic transmission and plasticity in the hippocampus. Mol Cell Neurosci. 2005; 29:313–319. [PubMed: 15911354]

Anderson E, Maday S, Sfakianos J, Hull M, Winckler B, Sheff D, Folsch H, Mellman I. Transcytosis of NgCAM in epithelial cells reflects differential signal recognition on the endocytic and secretory pathways. J Cell Biol. 2005; 170:595–605. [PubMed: 16087710]

Andreassi C, Riccio A. To localize or not to localize: mRNA fate is in 3'UTR ends. Trends Cell Biol. 2009; 19:465–474. [PubMed: 19716303]

Ang AL, Taguchi T, Francis S, Folsch H, Murrells LJ, Pypaert M, Warren G, Mellman I. Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. J Cell Biol. 2004; 167:531–543. [PubMed: 15534004]

Arimura N, Kimura T, Nakamuta S, Taya S, Funahashi Y, Hattori A, Shimada A, Menager C, Kawabata S, Fujii K, Iwamatsu A, Segal RA, Fukuda M, Kaibuchi K. Anterograde transport of TrkB in axons is mediated by direct interaction with Slp1 and Rab27. Dev Cell. 2009; 16:675–686. [PubMed: 19460344]

Arnold DB. Actin and microtubule-based cytoskeletal cues direct polarized targeting of proteins in neurons. Sci Signal. 2009; 2:pe49. [PubMed: 19671926]

Ascano M, Richmond A, Borden P, Kuruvilla R. Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. J Neurosci. 2009; 29:11674–11685. [PubMed: 19759314]

Barbero P, Bittova L, Pfeffer SR. Visualization of Rab9-mediated vesicle transport from endosomes to the trans-Golgi in living cells. J Cell Biol. 2002; 156:511–518. [PubMed: 11827983]

Bassell GJ, Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron. 2008; 60:201–214. [PubMed: 18957214]

Bel C, Oguievetskaia K, Pitaval C, Goutebroze L, Faivre-Sarrailh C. Axonal targeting of Caspr2 in hippocampal neurons via selective somatodendritic endocytosis. J Cell Sci. 2009; 122:3403–3413. [PubMed: 19706678]

Biermann B, Ivankova-Susankova K, Bradaia A, Abdel Aziz S, Besseyrias V, Kapfhammer JP, Missler M, Gassmann M, Bettler B. The Sushi domains of GABAB receptors function as axonal targeting signals. J Neurosci. 2010; 30:1385–1394. [PubMed: 20107064]

Brown TC, Tran IC, Backos DS, Esteban JA. NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. Neuron. 2005; 45:81–94. [PubMed: 15629704]

Cai B, Katafiasz D, Horejsi V, Naslavsky N. Pre-sorting endosomal transport of the GPI-anchored protein, CD59, is regulated by EHD1. Traffic. 2011; 12:102–120. [PubMed: 20961375]

Chu PJ, Rivera JF, Arnold DB. A role for Kif17 in transport of Kv4.2. J Biol Chem. 2006; 281:365–373. [PubMed: 16257958]

Cognet L, Groc L, Lounis B, Choquet D. Multiple routes for glutamate receptor trafficking: surface diffusion and membrane traffic cooperate to bring receptors to synapses. Sci STKE. 2006; 2006:pe13. [PubMed: 16552090]

Craig AM, Banker G. Neuronal polarity. Ann Rev Neurosci. 1994; 17:267–310. [PubMed: 8210176]

Daumke O, Lundmark R, Vallis Y, Martens S, Butler PJ, McMahon HT. Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. Nature. 2007; 449:923–927. [PubMed: 17914359]

Debaigt C, Hirling H, Steiner P, Vincent JP, Mazella J. Crucial role of neuron-enriched endosomal protein of 21 kDa in sorting between degradation and recycling of internalized G-protein-coupled receptors. J Biol Chem. 2004; 279:35687–35691. [PubMed: 15187090]

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G. Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Neuron. 2006; 52:293–305. [PubMed: 17046692]

Dittman J, Ryan TA. Molecular circuitry of endocytosis at nerve terminals. Ann Rev Cell Dev Biol. 2009; 25:133–160. [PubMed: 19575674]

Dotti CG, Simons K. Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. Cell. 1990; 62:63–72. [PubMed: 2163770]

Eva R, Dassie E, Caswell PT, Dick G, ffrench-Constant C, Norman JC, Fawcett JW. Rab11 and its effector Rab coupling protein contribute to the trafficking of beta 1 integrins during axon growth in adult dorsal root ganglion neurons and PC12 cells. J Neurosci. 2010; 30:11654–11669. [PubMed: 20810886]

Fache MP, Moussif A, Fernandes F, Giraud P, Garrido JJ, Dargent B. Endocytotic elimination and domain-selective tethering constitute a potential mechanism of protein segregation at the axonal initial segment. J Cell Biol. 2004; 166:571–578. [PubMed: 15302857]

Ferguson SM, Brasnjo G, Hayashi M, Wölfel M, Collesi C, Giovedi S, Raimondi A, Gong LW, Ariel P, Paradise S, O'toole E, Flavell R, Cremona O, Miesenböck G, Ryan TA, De Camilli P. A selective activity-dependent requirement for dynamin 1 in synaptic vesicle endocytosis. Science. 2007; 316:570–574. [PubMed: 17463283]

Folsch H. Regulation of membrane trafficking in polarized epithelial cells. Curr Opin Cell Biol. 2008; 20:208–213. [PubMed: 18282697]

Folsch H, Mattila PE, Weisz OA. Taking the Scenic Route: Biosynthetic Traffic to the Plasma Membrane in Polarized Epithelial Cells. Traffic. 2009; 10:972–981. [PubMed: 19453969]

Francesconi A, Duvoisin RM. Alternative splicing unmasks dendritic and axonal targeting signals in metabotropic glutamate receptor 1. J Neurosci. 2002; 22:2196–2205. [PubMed: 11896159]

Garrido JJ, Giraud P, Carlier E, Fernandes F, Moussif A, Fache MP, Debanne D, Dargent B. A targeting motif involved in sodium channel clustering at the axonal initial segment. Science. 2003; 300:2091–2094. [PubMed: 12829783]

Ghosh RN, Mallet WG, Soe TT, McGraw TE, Maxfield FR. An endocytosed TGN38 chimeric protein is delivered to the TGN after trafficking through the endocytic recycling compartment in CHO cells. J Cell Biol. 1998; 142:923–936. [PubMed: 9722606]

Grant BD, Caplan S. Mechanisms of EHD/RME-1 Protein Function in Endocytic Transport. Traffic. 2008; 9:2043–2052. [PubMed: 18801062]

Gu C, Jan YN, Jan LY. A conserved domain in axonal targeting of Kv1 (Shaker) voltage-gated potassium channels. Science. 2003; 301:646–649. [PubMed: 12893943]

Gu C, Zhou W, Puthenveedu MA, Xu M, Jan YN, Jan LY. The microtubule plus-end tracking protein EB1 is required for Kv1 voltage-gated K+ channel axonal targeting. Neuron. 2006; 52:803–816. [PubMed: 17145502]

Ha J, Lo KW, Myers KR, Carr TM, Humsi MK, Rasoul BA, Segal RA, Pfister KK. A neuron-specific cytoplasmic dynein isoform preferentially transports TrkB signaling endosomes. J Cell Biol. 2008; 181:1027–1039. [PubMed: 18559670]

Hanus C, Ehlers MD. Secretory outposts for the local processing of membrane cargo in neuronal dendrites. Traffic. 2008; 9:1437–1445. [PubMed: 18532987]

Hengst U, Jaffrey SR. Function and translational regulation of mRNA in developing axons. Semin Cell Dev Biol. 2007; 18:209–215. [PubMed: 17478114]

Hirokawa N, Niwa S, Tanaka Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. Neuron. 2010; 68:610–638. [PubMed: 21092854]

Hoogenraad CC, Popa I, Futai K, Sanchez-Martinez E, Wulf PS, van Vlijmen T, Dortland BR, Oorschot V, Govers R, Monti M, Heck AJ, Sheng M, Klumperman J, Rehmann H, Jaarsma D, Kapitein LC, van der Sluijs P. Neuron specific Rab4 effector GRASP-1 coordinates membrane specialization and maturation of recycling endosomes. PLoS Biol. 2010; 8:e1000283. [PubMed: 20098723]

Horton AC, Ehlers MD. Secretory trafficking in neuronal dendrites. Nat Cell Biol. 2004; 6:585–591. [PubMed: 15232591]

Horton AC, Racz B, Monson EE, Lin AL, Weinberg RJ, Ehlers MD. Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. Neuron. 2005; 48:757–771. [PubMed: 16337914]

Howe CL, Mobley WC. Signaling endosome hypothesis: A cellular mechanism for long distance communication. J Neurobiol. 2004; 58:207–216. [PubMed: 14704953]

Ibanez CF. Message in a bottle: long-range retrograde signaling in the nervous system. Trends Cell Biol. 2007; 17:519–528. [PubMed: 18029183]

Jareb M, Banker G. The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. Neuron. 1998; 20:855–867. [PubMed: 9620691]

Jeyifous O, Waites CL, Specht CG, Fujisawa S, Schubert M, Lin EI, Marshall J, Aoki C, de Silva T, Montgomery JM, Garner CC, Green WN. SAP97 and CASK mediate sorting of NMDA receptors through a previously unknown secretory pathway. Nat Neurosci. 2009; 12:1011–1019. [PubMed: 19620977]

Joset A, Dodd DA, Halegoua S, Schwab ME. Pincher-generated Nogo-A endosomes mediate growth cone collapse and retrograde signaling. J Cell Biol. 2010; 188:271–285. [PubMed: 20083601]

Jovic M, Sharma M, Rahajeng J, Caplan S. The early endosome: a busy sorting station for proteins at the crossroads. Histol Histopathol. 2010; 25:99–112. [PubMed: 19924646]

Kaether C, Skehel P, Dotti CG. Axonal membrane proteins are transported in distinct carriers: a two-color video microscopy study in cultured hippocampal neurons. Mol Biol Cell. 2000; 11:1213–1224. [PubMed: 10749925]

Kamiguchi H, Lemmon V. Recycling of the cell adhesion molecule L1 in axonal growth cones. J Neurosci. 2000; 20:3676–3686. [PubMed: 10804209]

Kamiguchi H, Long KE, Pendergast M, Schaefer AW, Rapoport I, Kirchhausen T, Lemmon V. The neural cell adhesion molecule L1 interacts with the AP-2 adaptor and is endocytosed via the clathrin-mediated pathway. J Neurosci. 1998; 18:5311–5321. [PubMed: 9651214]

Kapitein LC, Hoogenraad CC. Which way to go? Cytoskeletal organization and polarized transport in neurons. Mol Cell Neurosci. 2011; 46:9–20. [PubMed: 20817096]

Kapitein LC, Schlager MA, Kuijpers M, Wulf PS, van Spronsen M, MacKintosh FC, Hoogenraad CC. Mixed microtubules steer dynein-driven cargo transport into dendrites. Curr Biol. 2010; 20:290–299. [PubMed: 20137950]

Kawauchi T, Sekine K, Shikanai M, Chihama K, Tomita K, Kubo K, Nakajima K, Nabeshima Y, Hoshino M. Rab GTPases-dependent endocytic pathways regulate neuronal migration and maturation through N-cadherin trafficking. Neuron. 2010; 67:588–602. [PubMed: 20797536]

Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci. 2006; 29:325–362. [PubMed: 16776589]

Konishi Y, Setou M. Tubulin tyrosination navigates the kinesin-1 motor domain to axons. Nat Neurosci. 2009; 12:559–567. [PubMed: 19377471]

Kurisu J, Fukuda T, Yokoyama S, Hirano T, Kengaku M. Polarized targeting of DNER into dendritic plasma membrane in hippocampal neurons depends on endocytosis. J Neurochem. 2010; 113:1598–1610. [PubMed: 20367751]

Lakadamyali M, Rust MJ, Zhuang X. Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes. Cell. 2006; 124:997–1009. [PubMed: 16530046]

Lampson MA, Schmoranzer J, Zeigerer A, Simon SM, McGraw TE. Insulin-regulated release from the endosomal recycling compartment is regulated by budding of specialized vesicles. Mol Biol Cell. 2001; 12:3489–3501. [PubMed: 11694583]

Lasiecka ZM, Yap CC, Caplan S, Winckler B. Neuronal early endosomes require EHD1 for L1/NgCAM trafficking. J Neurosci. 2010; 30:16485–16497. [PubMed: 21147988]

Lasiecka ZM, Yap CC, Vakulenko M, Winckler B. Compartmentalizing the neuronal plasma membrane from axon initial segments to synapses. Int Rev Cell Mol Biol. 2009; 272:303–389. [PubMed: 19121821]

Lavezzari G, McCallum J, Dewey CM, Roche KW. Subunit-specific regulation of NMDA receptor endocytosis. J Neurosci. 2004; 24:6383–6391. [PubMed: 15254094]

Lee SK, Hollenbeck PJ. Organization and translation of mRNA in sympathetic axons. J Cell Sci. 2003; 116:4467–4478. [PubMed: 13130093]

Leterrier C, Laine J, Darmon M, Boudin H, Rossier J, Lenkei Z. Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. J Neurosci. 2006; 26:3141–3153. [PubMed: 16554465]

Lewis TL Jr, Mao T, Svoboda K, Arnold DB. Myosin-dependent targeting of transmembrane proteins to neuronal dendrites. Nat Neurosci. 2009; 12:568–576. [PubMed: 19377470]

Lewis TL, Mao T, Arnold DB. A Role for Myosin VI in the Localization of Axonal Proteins. PLoS biology. 2011; 9:e1001021. [PubMed: 21390300]

Long KE, Lemmon V. Dynamic regulation of cell adhesion molecules during axon outgrowth. J Neurobiol. 2000; 44:230–245. [PubMed: 10934325]

Mallard F, Antony C, Tenza D, Salamero J, Goud B, Johannes L. Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of shiga toxin B-fragment transport. J Cell Biol. 1998; 143:973–990. [PubMed: 9817755]

Matsuda S, Miura E, Matsuda K, Kakegawa W, Kohda K, Watanabe M, Yuzaki M. Accumulation of AMPA receptors in autophagosomes in neuronal axons lacking adaptor protein AP-4. Neuron. 2008; 57:730–745. [PubMed: 18341993]

Matsuki T, Matthews RT, Cooper JA, van der Brug MP, Cookson MR, Hardy JA, Olson EC, Howell BW. Reelin and stk25 have opposing roles in neuronal polarization and dendritic Golgi deployment. Cell. 2010; 143:826–836. [PubMed: 21111240]

Maxfield FR, McGraw TE. Endocytic recycling. Nat Rev Mol Cell Biol. 2004a; 5:121–132. [PubMed: 15040445]

Mayor S, Presley JF, Maxfield FR. Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. J Cell Biol. 1993; 121:1257–1269. [PubMed: 8509447]

Merianda TT, Lin AC, Lam JS, Vuppalanchi D, Willis DE, Karin N, Holt CE, Twiss JL. A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins. Mol Cell Neurosci. 2009; 40:128–142. [PubMed: 19022387]

Miaczynska M, Zerial M. Mosaic organization of the endocytic pathway. Exp Cell Res. 2002; 272:8–14. [PubMed: 11740860]

Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, Bunnett NW. Endosomes: a legitimate platform for the signaling train. Proc Natl Acad Sci USA. 2009; 106:17615–17622. [PubMed: 19822761]

Nakata T, Hirokawa N. Microtubules provide directional cues for polarized axonal transport through interaction with kinesin motor head. J Cell Biol. 2003; 162:1045–1055. [PubMed: 12975348]

Naslavsky N, Caplan S. EHD proteins: key conductors of endocytic transport. Trends Cell Biol. 2011; 21:122–131. [PubMed: 21067929]

Newpher TM, Ehlers MD. Glutamate receptor dynamics in dendritic microdomains. Neuron. 2008; 58:472–497. [PubMed: 18498731]

Norstrom EM, Zhang C, Tanzi R, Sisodia SS. Identification of NEEP21 as a ss-amyloid precursor protein-interacting protein in vivo that modulates amyloidogenic processing in vitro. J Neurosci. 2010; 30:15677–15685. [PubMed: 21084623]

Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. Science. 2004; 305:1972–1975. [PubMed: 15448273]

Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM, Ehlers MD. Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. Neuron. 2006; 52:817–830. [PubMed: 17145503]

Pierce JP, Mayer T, McCarthy JB. Evidence for a satellite secretory pathway in neuronal dendritic spines. Curr Biol. 2001; 11:351–355. [PubMed: 11267872]

Prekeris R, Foletti DL, Scheller RH. Dynamics of tubulovesicular recycling endosomes in hippocampal neurons. J Neurosci. 1999; 19:10324–10337. [PubMed: 10575030]

Puthenveedu MA, Lauffer B, Temkin P, Vistein R, Carlton P, Thorn K, Taunton J, Weiner OD, Parton RG, von Zastrow M. Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. Cell. 2010; 143:761–773. [PubMed: 21111236]

Puthenveedu MA, von Zastrow M. Cargo regulates clathrin-coated pit dynamics. Cell. 2006; 127:113–124. [PubMed: 17018281]

Rasband MN. Converging on the origins of axonal ion channel clustering. PLoS Genet. 2009; 5:e1000340. [PubMed: 19148277]

Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. Cell. 2005; 122:735–749. [PubMed: 16143105]

Rivera J, Chu PJ, Lewis TL Jr, Arnold DB. The role of Kif5B in axonal localization of Kv1 K(+) channels. Eur J Neurosci. 2007; 25:136–146. [PubMed: 17241275]

Robinson PJ. Neuroscience. How to fill a synapse. Science. 2007; 316:551–553. [PubMed: 17463275]

Rodriguez-Boulan E, Musch A. Protein sorting in the Golgi complex: shifting paradigms. Biochim Biophys Acta. 2005; 1744:455–464. [PubMed: 15927284]

Rotem-Yehudar R, Galperin E, Horowitz M. Association of insulin-like growth factor 1 receptor with EHD1 and SNAP29. J Biol Chem. 2001; 276:33054–33060. [PubMed: 11423532]

Ruberti F, Dotti CG. Involvement of the proximal C terminus of the AMPA receptor subunit GluR1 in dendritic sorting. J Neurosci. 2000; 20:RC78. [PubMed: 10807936]

Salinas S, Bilsland LG, Schiavo G. Molecular landmarks along the axonal route: axonal transport in health and disease. Curr Opin Cell Biol. 2008; 20:445–453. [PubMed: 18495455]

Sampo B, Kaech S, Kunz S, Banker G. Two distinct mechanisms target membrane proteins to the axonal surface. Neuron. 2003; 37:611–624. [PubMed: 12597859]

Sann S, Wang Z, Brown H, Jin Y. Roles of endosomal trafficking in neurite outgrowth and guidance. Trends Cell Biol. 2009; 19:317–324. [PubMed: 19540123]

Schaefer AW, Kamei Y, Kamiguchi H, Wong EV, Rapoport I, Kirchhausen T, Beach CM, Landreth G, Lemmon SK, Lemmon V. L1 endocytosis is controlled by a phosphorylation-dephosphorylation cycle stimulated by outside-in signaling by L1. J Cell Biol. 2002; 24:1223–1232. [PubMed: 12082080]

Schmidt MR, Haucke V. Recycling endosomes in neuronal membrane traffic. Biol Cell. 2007; 99:333–342. [PubMed: 17504239]

Schweizer FE, Ryan TA. The synaptic vesicle: cycle of exocytosis and endocytosis. Curr Opin Neurobiol. 2006; 16:298–304. [PubMed: 16707259]

Shao Y, Akmentin W, Toledo-Aral JJ, Rosenbaum J, Valdez G, Cabot JB, Hilbush BS, Halegoua S. Pincher, a pinocytic chaperone for nerve growth factor/TrkA signaling endosomes. J Cell Biol. 2002; 157:679–691. [PubMed: 12011113]

Sharma M, Giridharan SS, Rahajeng J, Naslavsky N, Caplan S. MICALL1 links EHD1 to tubular recycling endosomes and regulates receptor recycling. Mol Biol Cell. 2009; 20:5181–5194. [PubMed: 19864458]

Sharma M, Naslavsky N, Caplan S. A role for EHD4 in the regulation of early endosomal transport. Traffic. 2008; 9:995–1018. [PubMed: 18331452]

Silverman MA, Peck R, Glover G, He C, Carlin C, Banker G. Motifs that mediate dendritic targeting in hippocampal neurons: a comparison with basolateral targeting signals. Mol Cell Neurosci. 2005; 29:173–180. [PubMed: 15911342]

Song AH, Wang D, Chen G, Li Y, Luo J, Duan S, Poo MM. A selective filter for cytoplasmic transport at the axon initial segment. Cell. 2009; 136:1148–1160. [PubMed: 19268344]

Sonnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M. Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. J Cell Biol. 2000; 149:901–914. [PubMed: 10811830]

Steiner P, Alberi S, Kulangara K, Yersin A, Sarria JC, Regulier E, Kasas S, Dietler G, Muller D, Catsicas S, Hirling H. Interactions between NEEP21, GRIP1 and GluR2 regulate sorting and recycling of the glutamate receptor subunit GluR2. EMBO J. 2005; 24:2873–2884. [PubMed: 16037816]

Steiner P, Sarria JC, Glauser L, Magnin S, Catsicas S, Hirling H. Modulation of receptor cycling by neuron-enriched endosomal protein of 21 kD. J Cell Biol. 2002; 157:1197–1209. [PubMed: 12070131]

Stenmark H. Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol. 2009; 10:513–525. [PubMed: 19603039]

Thompson A, Nessler R, Wisco D, Anderson E, Winckler B, Sheff D. Recycling Endosomes of Polarized Epithelial Cells Actively Sort Apical and Basolateral Cargos into Separate Subdomains. Mol Biol Cell. 2007; 18:2687–2697. [PubMed: 17494872]

Traub LM. Tickets to ride: selecting cargo for clathrin-regulated internalization. Nat Rev Mol Cell Biol. 2009; 10:583–596. [PubMed: 19696796]

Vacher H, Yang JW, Cerda O, Autillo-Touati A, Dargent B, Trimmer JS. Cdk-mediated phosphorylation of the Kvβ2 auxiliary subunit regulates Kv1 channel axonal targeting. J Cell Biol. 2011; 192:813–824. [PubMed: 21357749]

Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, Kjolby M, Richner M, Erdmann B, Nyengaard JR, Tessarollo L, Lewin GR, Willnow TE, Chao MV, Nykjaer A. Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. Nat Neurosci. 2011; 14:54–61. [PubMed: 21102451]

Valdez G, Akmentin W, Philippidou P, Kuruvilla R, Ginty DD, Halegoua S. Pincher-mediated macroendocytosis underlies retrograde signaling by neurotrophin receptors. J Neurosci. 2005; 25:5236–5247. [PubMed: 15917464]

Wang Z, Edwards JG, Riley N, Provance DW Jr, Karcher R, Li XD, Davison IG, Ikebe M, Mercer JA, Kauer JA, Ehlers MD. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. Cell. 2008; 135:535–548. [PubMed: 18984164]

West AE, Neve RL, Buckle yKM. Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. J Neurosci. 1997; 17:6038–6047. [PubMed: 9236215]

Willis D, Li KW, Zheng JQ, Chang JH, Smit A, Kelly T, Merianda TT, Sylvester J, van Minnen J, Twiss JL. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J Neurosci. 2005; 25:778–791. [PubMed: 15673657]

Willis DE, Twiss JL. Regulation of protein levels in subcellular domains through mRNA transport and localized translation. Mol Cell Proteomics. 2010; 9:952–962. [PubMed: 20167945]

Wilson JM, de Hoop M, Zorzi N, Toh BH, Dotti CG, Parton RG. EEA1, a tethering protein of the early sorting endosome, shows a polarized distribution in hippocampal neurons, epithelial cells, and fibroblasts. Mol Biol Cell. 2000; 11:2657–2671. [PubMed: 10930461]

Winckler B, Mellman I. Neuronal polarity: controlling the sorting and diffusion of membrane components. Neuron. 1999; 23:637–640. [PubMed: 10482229]

Winckler B, Mellman I. Trafficking guidance receptors. Cold Spring Harb Perspect Biol. 2010; 2:a001826. [PubMed: 20504966]

Winckler B, Yap CC. Endocytosis and Endosomes at the Crossroads of Regulating Trafficking of Axon Outgrowth-Modifying Receptors. Traffic. 2011 Apr 28.

Wisco D, Anderson ED, Chang MC, Norden C, Boiko T, Folsch H, Winckler B. Uncovering multiple axonal targeting pathways in hippocampal neurons. J Cell Biol. 2003; 162:1317–1328. [PubMed: 14517209]

Xu J, Zhu Y, Heinemann SF. Identification of sequence motifs that target neuronal nicotinic receptors to dendrites and axons. J Neurosci. 2006; 26:9780–9793. [PubMed: 16988049]

Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ. An essential role for beta-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. Nat Neurosci. 2006; 9:1265–1273. [PubMed: 16980965]

Yap CC, Lasiecka ZM, Caplan S, Winckler B. Alterations of EHD1/EHD4 protein levels interfere with L1/NgCAM endocytosis in neurons and disrupt axonal targeting. J Neurosci. 2010; 30:6646–6657. [PubMed: 20463227]

Yap CC, Nokes RL, Wisco D, Anderson ED, Fölsch H, Winckler B. Pathway selection to the axon depends on multiple targeting signals in NgCAM. J Cell Sci. 2008a; 121:1514–1525. [PubMed: 18411247]

Yap, CC.; Winckler, B. Encyclopedia of Neuroscience. Vol. Vol. 10. Oxford Academic Press; 2009. Vesicular sorting to axons and dendrites; p. 115-120.

Yap CC, Wisco D, Kujala P, Lasiecka ZM, Cannon JT, Chang MC, Hirling H, Klumperman J, Winckler B. The somatodendritic endosomal regulator NEEP21 facilitates axonal targeting of L1/NgCAM. J Cell Biol. 2008b; 180:827–842. [PubMed: 18299352]

Ye B, Zhang Y, Song W, Younger SH, Jan LY, Jan YN. Growing dendrites and axons differ in their reliance on the secretory pathway. Cell. 2007; 30:717–729. [PubMed: 17719548]

Zoncu R, Perera RM, Balkin DM, Pirruccello M, Toomre D, De Camilli P. A phosphoinositide switch controls the maturation and signaling properties of APPL endosomes. Cell. 2009; 136:1110–1121. [PubMed: 19303853]

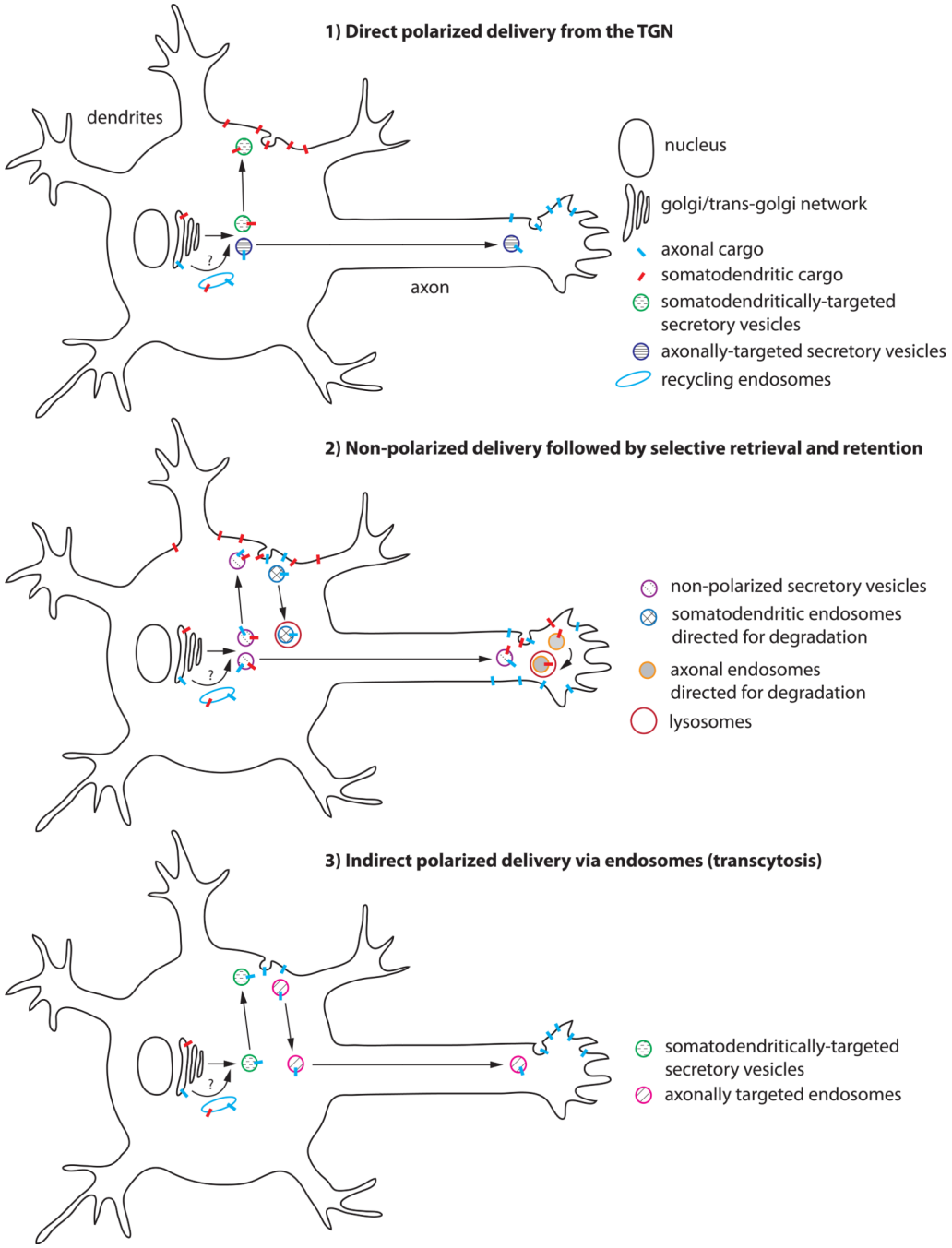

Figure 1. Model of polarized trafficking in neurons

Membrane proteins can be sorted to axon vs. dendrites by three major pathways: 1) direct polarized delivery from the trans-golgi network (TGN), 2) non-polarized delivery followed by selective retrieval and retention, 3) indirect polarized delivery via endosomes (transcytosis). In the first pathway (1) axonal and somatodendritic proteins are sorted in TGN into axonally- and somatodendritically-targeted secretory vesicles. Those vesicles are transported and fuse with axonal and somatodendritic membranes, respectively. Some evidence, coming from epithelial cells studies, suggests that cargo, which exit TGN might enter endosomes without prior appearance on the plasma membrane, which would implicate the involvement of endosomes in secretory pathway. In the second pathway (2) axonal and

somatodendritic cargo exits TGN into secretory vesicles, which can fuse with both somatodendritic and axonal membranes. After this initial non-polarized insertion, proper polarized distribution of proteins is achieved by subsequent endocytic removal of missorted proteins (presumably for degradation) and retention of the properly targeted proteins at the plasma membrane. In the third pathway (3) proteins coming out of TGN are sorted into somatodendritically-targeted secretory vesicles, and then inserted into somatodendritic membrane and subsequently endocytosed into axonally-targeted endosomal compartments, which finally fuse with axonal membrane.

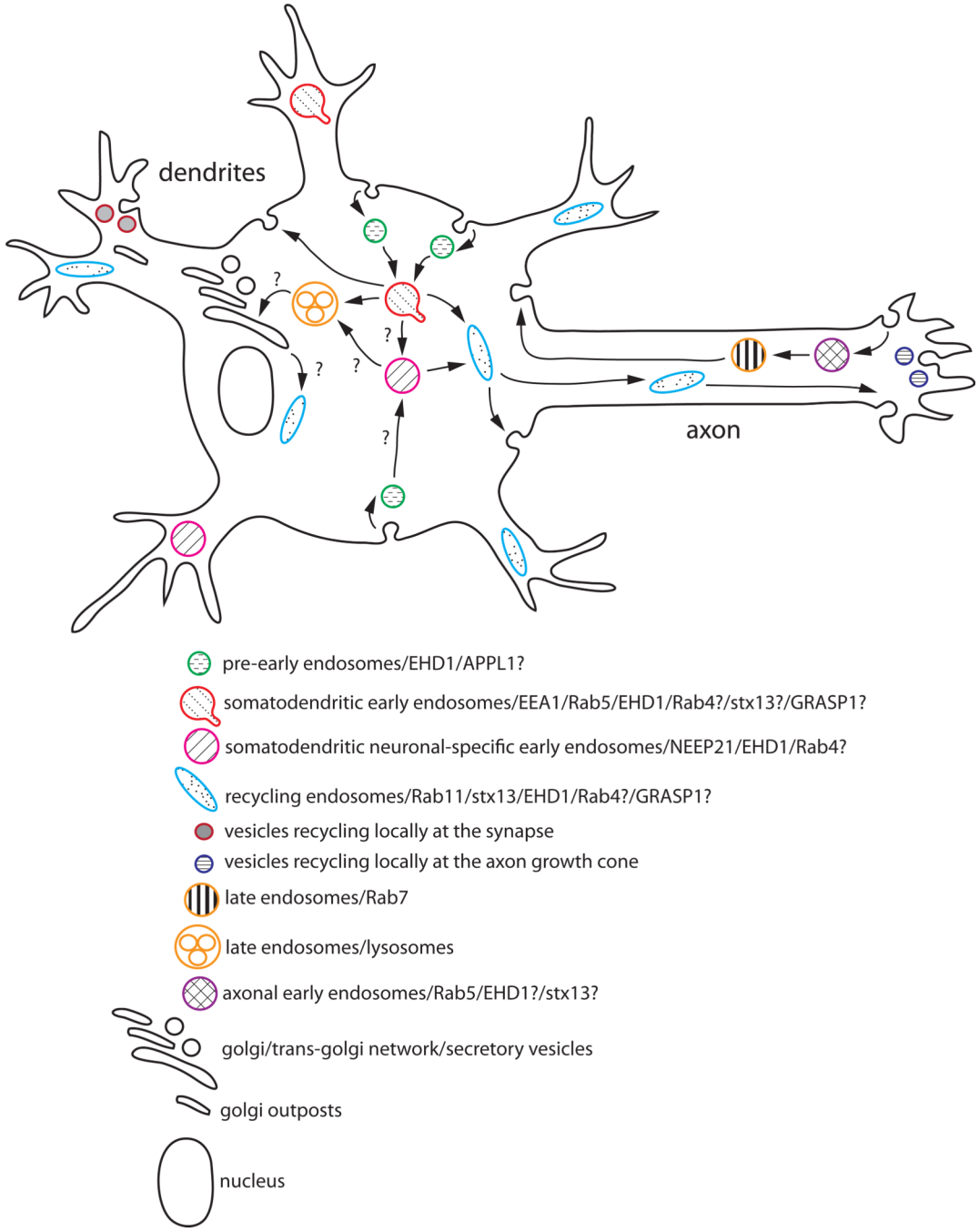

Figure 2. Model of neuronal endomembrane system

Neurons contain an extensive somatic Golgi clustered near the nucleus which frequently extends substantially into the major apical dendrite. In addition to the somatic Golgi, distinct Golgi outposts can be found in more distal portions of major dendrites, especially at dendritic branch points. At the plasma membrane different membrane proteins are endocytosed into different populations of pre-early endosomal compartments (pre-EEs). Some of the pre-EEs contain EHD1 (shown in neurons) and/or APPL1 (not shown in neurons). Pre-EE mature and/or fuse into canonical early endosomal compartments containing such endosomal regulators as: EEA1, rab5, syntaxin13, and EHD1. Within those EEs different proteins might be sorted into different subdomains. At this point some proteins

might be 1) directly recycled back to the plasma membrane or 2) directed to specialized recycling compartment (RE) that function in polarized sorting and recycling, or 3) targeted to late endosomes and subsequently to lysosomes where they are being degraded. In neurons there is also an additional early endosomal compartment containing neuron-enriched endosomal protein 21 (NEEP21). This compartment does not colocalize with rab5 and EEA1 but colocalizes with rab4 (in PC12 cells) and to certain extent with EHD1. Some cargoes (AMPAR and transferrin) reach the NEEP21-compartment after the canonical EE compartment and before the RE compartment on their recycling pathway to the somatodendritic plasma membrane. It is not known whether cargo can be endocytosed directly to NEEP21-positive EE, bypassing canonical EE. Furthermore the NEEP21-positive compartment is important on the L1/NgCAM transcytotic pathway to the axon. Another neuronal-specific endosomal regulator, GRASP1, is localized to rab4-positive early recycling endosomes mainly in the somatodendritic domain. Both NEEP21-positive and EEA1-positive compartments are present in somatodendritic domain but not in axon. Proteins that are endocytosed in the axon (as shown for neurotrophins) accumulate in the rab5-positive axonal early endosomes, which likely mature into rab7 late endosomal compartments, which travel retrogradely along the axon towards the cell soma. Recycling endosomal compartments positive for rab11, syntaxin13, and EHD1 are localized to both axons and dendrites and can travel bidirectionally along those processes presumably transporting cargo. Besides the endosomal compartments that are involved in protein sorting and long-range trafficking, there are endosomal compartments important for local protein recycling at the axon growth cone and later at the pre-and post-synaptic sites. Finally, work done in non-neuronal cells uncovered substantial cross-talk between secretory and endosomal pathways. Certain proteins can be transported from late endosomes back to TGN and at the same time proteins exiting TGN can enter endosomal compartments without being inserted into the plasma membrane.