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# Organelles and Trafficking Machinery for Postsynaptic Plasticity

**Matthew J. Kennedy** and **Michael D. Ehlers**
*Howard Hughes Medical Institute, Department of Neurobiology, Duke University Medical Center, Durham, North Carolina 27710; email: kennedy@neuro.duke.edu, ehlers@neuro.duke.edu*

## Abstract

Neurons are among the largest and most complex cells in the body. Their immense size and intricate geometry pose many unique cell-biological problems. How is dendritic architecture established and maintained? How do neurons traffic newly synthesized integral membrane proteins over such long distances to synapses? Functionally, protein trafficking to and from the postsynaptic membrane has emerged as a key mechanism underlying various forms of synaptic plasticity. Which organelles are involved in postsynaptic trafficking, and how do they integrate and respond to activity at individual synapses? Here we review what is currently known about long-range trafficking of newly synthesized postsynaptic proteins as well as the local rules that govern postsynaptic trafficking at individual synapses.

### Keywords

synapse; dendrite; spine; secretory pathway; Golgi; endocytosis; membrane trafficking

## INTRODUCTION

All cells face the challenge of trafficking integral membrane proteins, secreted factors, and lipids to the appropriate subcellular location in the right amounts at the right time. For neurons, this task is especially daunting given their immense size and complex architecture. Although membrane-trafficking organelles were observed in neurons more than a century ago (Golgi 1898), we are only now beginning to understand the mechanisms governing membrane transport to diverse neuronal functional domains and the role such transport plays in neuronal development, signaling, morphology, and plasticity. Many fundamental eukaryotic trafficking mechanisms are conserved in neurons, but neurons have evolved distinct modes of trafficking to accommodate their unique morphology.

Neurons are highly polarized cells, with one axon housing the molecular machinery necessary for action potential propagation and neurotransmitter release and several dendrites containing receptors and signaling components that respond to neurotransmitter. At most excitatory synapses in the brain, presynaptic terminals directly appose membranous dendritic protrusions called spines, which are located along the entire length of dendrites and harbor the postsynaptic density (PSD), a multiprotein complex responsible for anchoring neurotransmitter receptors near sites of neurotransmitter release (Sheng 2001). This layout requires that neurons traffic postsynaptic proteins over long distances, up to several hundred microns, through geometrically complex dendritic branches to satisfy the requirements of the most distal spines.

Once delivered to spines, many synaptic components are subject to a new set of local trafficking rules, which determine whether the components are inserted into or removed from the plasma membrane and whether they are recycled or degraded. In some cases, protein trafficking is coupled to neural activity. For example, high-frequency afferent stimulation often leads to neurotransmitter receptor addition to the postsynaptic membrane, whereas low-frequency stimulation triggers receptor removal (Carroll et al. 1999,Hayashi et al. 2000,Shi et al. 1999).

Given that the number and density of neurotransmitter receptors are critical determinants of synaptic strength, spine membrane protein trafficking has emerged as a key postsynaptic mechanism underlying various forms of synaptic plasticity, including long-term potentiation (LTP) and depression (LTD) (Bredt & Nicoll 2003,Malenka & Bear 2004). Because individual dendritic spines are decorated with only tens to hundreds of surface glutamate receptors, the addition or subtraction of just a few receptors to the spine surface can, in principle, alter neurotransmission (Matsuzaki et al. 2001,Momiyama et al. 2003,Tanaka et al.2005). Thus, the factors that govern postsynaptic receptor trafficking must be subject to stringent regulation. This regulation appears to have very precise spatial parameters considering that adjacent spines on a dendrite (often separated by only a few microns) can have strikingly different steady-state levels of synaptic proteins (Bagal et al. 2005,Matsuzaki et al. 2004).

How do neurons accurately sort and deliver newly synthesized integral membrane proteins from the cell body to dendritic spines? What factors determine the fate of cargo after it reaches the spine? How is neural activity coupled to the membrane trafficking machinery at dendritic spines? How are the unique properties and molecular profiles of individual spines established and maintained? Although many of these problems remain unsolved, recent work has begun unraveling the complex cell biology of neuronal membrane trafficking. Here we review what is currently known about the mechanisms and organelles involved in long-range trafficking of newly synthesized protein from the cell body to dendritic spines, as well as the local trafficking mechanisms and organelles that regulate the fate of molecules at individual excitatory synapses. Although local mRNA translation in dendrites plays an important role in localizing a number of postsynaptic proteins, we focus our review entirely on the trafficking events that take place following translation. For detailed reviews on local dendritic translation, see Steward & Schuman (2001) and Sutton & Schuman (2005).

## DENDRITIC ORGANELLES

### Endoplasmic Reticulum, Golgi, and the Neuronal Secretory Pathway

Much of what we know about eukaryotic protein trafficking is based on genetic experiments in *Saccharomyces cerevisiae* (Antonny & Schekman 2001,Lee et al. 2004a). Many of the general protein-trafficking principles established in this unicellular eukaryote also apply to neurons, including the pathway for secreting lipids and integral membrane proteins to the cell surface. The central organelles involved in the secretory pathway are the endoplasmic reticulum (ER), the Golgi apparatus, and the *trans*-Golgi network (TGN). Proteins destined for the plasma membrane enter the lumen of the ER as they are translated by ER-associated ribosomes, where they fold into their proper three-dimensional structure assisted by chaperone proteins such as BiP, calnexin, and calreticulin (Kleizen & Braakman 2004). Post-translational modifications, including N-glycosylation and disulfide bond formation, also occur in the ER. Modified cargo that is ready to advance to the next step in the secretory pathway is concentrated at specific exit sites and leaves the ER in COPII-coated vesicles. These vesicles merge with the Golgi apparatus, where further protein modifications, including glycosylation and proteolysis occur. Finally, cargo is sorted at the TGN for transport to other endomembrane compartments or to the plasma membrane. For detailed reviews of the secretory pathway in nonneuronal cells, see Hong (1998),Kuehn & Schekman (1997), and Lippincott-Schwartz et al. (2000).

In most eukaryotic cells, the ER extends throughout the cell, whereas the Golgi network is located near the cell nucleus and the microtubule organizing center (Levine & Rabouille 2005). In neurons, this arrangement is quite different (Horton & Ehlers 2004) (Figure 1). In dendrites isolated from the neuronal cell body, enzymatic activities associated with the Golgi network, such as protein glycosylation, persist (Torre & Steward 1996). This observation, along with the demonstration that mRNAs for several integral membrane proteins are translated and

secreted to the dendritic plasma membrane (Ju et al. 2004,Kacharmina et al. 2000), suggests that dendritic processes harbor all the necessary machinery for protein secretion. Indeed, electron microscopy studies have documented the presence of an extensive endomembrane network, including ER, that extends deepinto dendritic processes (Gardiol et al. 1999,Spacek & Harris 1997) (Figure 1). Protein markers for Golgi membranes, including α-mannosidase II, giantin, and Rab6, have been found in the dendrites of some neurons (Gardiol et al. 1999,Pierce et al. 2001,Spacek & Harris 1997). The dendritic localization of these organelles suggests that "satellite" protein secretion can occur at sites far from the nucleus. Investigators recently showed this by imaging live hippocampal neurons transfected with a temperature-sensitive variant of the vesicular stomatitis viral glycoprotein (VSVGtsO45), a transmembrane protein that is retained in the ER at 39.5°C but released when the temperature is reduced to 32°C (Bergmann 1989,Presley et al. 1997). Upon synchronous release from the ER, a fraction of pre-Golgi carriers harboring VSVGtsO45 merged with dendritic compartments positive for the Golgi markers galactosyl transfer ase and GM130, demonstrating that the Golgi network found in dendrites is functional and that membrane protein processing and secretion likely occur at sites distant from the cell body (Horton & Ehlers 2003a). At 20°C, a temperature at which vesicle budding from Golgi is blocked, newly released VSVGtsO45 frequently accumulated at Golgi outposts located at dendritic branch points (Figure 2) (Horton et al. 2005). Cargo destined for the distal ends of dendrites passes through several dendritic branch points on its journey, raising the question of what controls the flow of cargo to each branch? Golgi outposts situated at dendritic intersections engage in ongoing post-Golgi trafficking (Horton et al. 2005) and are properly positioned to regulate the identity or the amount of cargo that is trafficked to each branch.

Despite the presence of functional Golgi outposts in some hippocampal dendrites, not all dendrites possess detectable Golgi. Moreover, even in those dendrites containing Golgi outposts, most ER-to-Golgi carriers originating in the dendrite are trafficked all the way back to the somatic Golgi (Horton & Ehlers 2003a). Thus, dual modes of early secretory trafficking exist in dendrites. In fact, the major mode of ER-to-Golgi trafficking is directed long distances to the Golgi apparatus in the soma. This appears to be the exclusive mode of early secretory trafficking in those dendrites lacking Golgi outposts. A second, minor mode of ER-to-Golgi trafficking occurs locally in the dendrite, which may be specialized for localized control of dendritic secretion and dendritic membrane composition (Horton & Ehlers 2003a,2004). These experiments highlight important differences in the spatial organization of the secretory pathway between neurons and most other eukaryotic cells, whose Golgi compartments are generally confined to the perinuclear region.

In addition to their crucial roles in secreting integral membrane proteins, the ER and Golgi network are the primary sites of lipid biosynthesis. This is particularly important for neurons, which add an immense amount of plasma membrane during neurite outgrowth, allowing them to achieve surface areas up to 10,000 times greater than typical mammalian cells (Horton & Ehlers 2003b). Consistent with a requirement for membrane trafficking through the secretory pathway in dendrite growth, disrupting Golgi function with brefeldin A in developing hippocampal neurons resulted in neurons with fewer and shorter dendritic processes (Horton et al. 2005). Subjecting mature neurons to the same treatment caused a dramatic simplification of dendrites and a ∼30% loss in total dendrite length over 24 h, demonstrating that even after neuronal architecture is established, membrane flux through the secretory path-way continues and is required for maintaining dendritic size and geometry (Horton et al. 2005).

Where and how does dendritic membrane addition occur? The spatial organization of neuronal Golgi provides some clues. Somatic Golgi is nearly always oriented toward the apical dendrite (Figure 1A). As a result, the majority of post-Golgi carriers are directed to the apical dendrite,

which suggests that Golgi geometry may determine dendriticasymmetry. This idea is supported by the observation that somatic Golgi is asymmetrically distributed prior to specification of the apical dendrite. Furthermore, disrupting Golgi polarization by expressing GRASP65, a Golgi membrane protein required for cisternal stacking, disrupted Golgi polarization and blocked specification of the apical dendrite (Horton et al. 2005). In contrast with another recent study (de Anda et al. 2005), Horton et al. (2005) observed that somatic Golgi organization showed no relationship to axon position. Additionally, disrupting the secretory pathway by overexpressing a kinase-dead form of protein kinase D1, which prevents cargo budding from the TGN, resulted in cessation of dendritic growth, whereas axonal growth persisted for a period of days, indicating that a distinct pathway governs membrane addition to the growing axon (Horton et al. 2005).

## The Spine Apparatus

Whereas Golgi elements in dendrites appear to be localized to the dendritic shaft, the smooth endoplasmic reticulum (SER) extends through the neck of many spines (Figure 1),providing a direct conduit to the synapse. Although spine SER is thought to be mainly a reservoir for $Ca^{2+}$ (Verkhratsky 2002), it may also play a role in lipid/protein secretion to the spine surface. A specialized SER derivative called the spine apparatus is found in a large fraction of mature dendritic spines (Gray 1959,Gray & Guillery 1963,Spacek & Harris 1997). The presence of smooth vesicles near the tip of the spine apparatus raises the intriguing possibility that this organelle could supply membrane for spine growth and possibly traffic important synaptic proteins, such as AMPA and NMDA receptors, which have been localized to the spine apparatus (Nusser et al. 1998,Racca et al. 2000,Spacek & Harris 1997). The spine apparatus is absent in neurons from mice lacking synaptopodin, a protein of unknown function that normally localizes to the spine apparatus. These mice display deficits in LTP and spatial learning, demonstrating a potential link between the spine apparatus and the mechanisms of synaptic plasticity (Deller et al. 2003). Whether this underappreciated organelle plays any role in local spine trafficking or long-range (soma to spine) lipid/protein trafficking remains an open question.

## Dendritic Endosomes

Endosomes are intracellular, membrane bound structures that accept endocytic vesicles from the plasma membrane and sort newly internalized membrane proteins for degradation or transport back to the cell surface. The endosomal network is composed of early/sorting endosomes, recycling endosomes, and lysosomes. Newly internalized vesicles shed their clathrin coats before fusing with one another and with sorting endosomes, which have a tubular-vesicular morphology. Sorting endosomes mature into late endosomes ($t_{1/2}$ of ~8 min) as they become more acidic and acquire acid hydrolase activity (Maxfield & McGraw 2004). Before this occurs, molecules destined for reinsertion into the plasma membrane exit the sorting endosome on vesicles pinched off from small-diameter tubules and are either trafficked directly to the cell surface or to recycling endosomes (Dunn et al. 1989,Mayor et al. 1993). The remaining contents of late endosomes are degraded in lysosomes (Maxfield & McGraw 2004).

Internal membranous compartments resembling endosomal structures have been observed in dendrites. These include coated and uncoated vesicles, tubular structures, and multivesicular bodies (Figure 1) (Cooney et al. 2002). Approximately 70% of the endosome-like structures were situated within or at the base of dendritic spines. Approximately one in three spines were associated with these compartments, which suggests that multiple spines share the same endocytic organelles (Cooney et al. 2002). Clathrin-like coats are present at the tip of some tubular structures, which suggests that these structures are responsible for producing smaller

vesicles that may represent trafficking intermediates of the endosomal pathway (Cooney et al. 2002). The endosomal nature of these compartments was confirmed by serial electron microscopy of hippocampal slices incubated with extracellular gold-conjugated bovine serum albumin. Endocytosed gold particles were observed in coated pits, coated vesicles, large vesicles, and tubular compartments in dendrites (Cooney et al. 2002). Additionally, syntaxin 13, a molecule found primarily in early and recycling endosomes in nonneuronal cells, is found in dendritic tubular-vesicular structures, where it colocalizes with transferrin receptor, a protein known to be recycled through the endosomal pathway (Prekeris et al. 1999). These observations demonstrate the presence of a dendritic endosomal network and support a model for local protein recycling and degradation via endosomes near dendritic spines. We discuss in more detail the dendritic endosomal pathway as it pertains to postsynaptic protein trafficking in the sections entitled Post-Endocytic Sorting and Recycling Endosomes as AMPA Receptor Reservoirs.

### Dendritic Mitochondria

Dendrites also harbor mitochondria, which are located mainly in the dendritic shaft but are occasionally found associated with spines (Figure 1) (Adams & Jones 1982,Cameron et al. 1991a,Popov et al. 2005). A recent study demonstrated that mitochondria mobility in dendrites is controlled by synaptic activity. Synaptic stimulation decreased mitochondrial mobility and increased the association of mitochondria with dendritic spines (Li et al. 2004). Both global depolarization of neurons and local electrical stimulation caused mitochondria to translocate into spines hours after treatment. What is the functional significance of this phenomenon? Neural activity can cause synaptic remodeling through processes that require membrane fission, fusion, protein degradation, and local protein synthesis, all of which require energy. Because mitochondrial mobility is decreased near active synapses and increased in the absence of neural activity, mitochondria would be predicted to distribute near highly active, high ATP-utilizing, dendritic regions. Reducing the number of dendritic mitochondria by overexpressing a dominant-negative form of the dynamin-like GTPase Drp1, a protein involved in mitochondrial fission, decreased the number of synapses. However, increasing the number or activity of dendritic mitochondria by either overexpressing wild-type Drp1 or treating cells with creatine nearly doubled the number of synapses, which demonstrated that synapse formation or maintenance is normally limited by mitochondrial activity (Li et al. 2004). It remains to be determined whether mitochondrial ATP production, $Ca^{2+}$ buffering, or a different, unknown mitochondrial function limits synapse number.

## LONG-RANGE POSTSYNAPTIC TRAFFICKING

### Asymmetric Protein Trafficking

Polarized cells require sorting mechanisms to ensure the localization of membrane components to the appropriate cellular domain. In neurons, ionotropic glutamate receptors and other components of the postsynaptic density must be faithfully routed to dendrites, whereas factors responsible for neurotransmitter release and action potential propagation must be directed to the axon. Much of what we know about how this sorting takes place comes from studies in epithelial cell lines, which have apical/basolateral asymmetry (Yeaman et al. 1999). Several modes of polarized sorting have been described in epithelial cells, including selective delivery of cargo by post-Golgi carriers destined for specific cellular domains and nonspecific delivery to the plasma membrane followed by endocytosis and transport to the appropriate cellular domain via postendocytic carriers (Mostov et al. 2003,Rodriguez-Boulan et al. 2005,Tuma & Hubbard 2003).

At a basic level, neurons have two major sorting domains, the axon and the somatodendritic compartment, which are rough analogies of the apical and basolateral domains of polarized epithelial cells (Dotti & Simons 1990). Although subregions of these two domains clearly exist, many basic themes of apical/basolateral trafficking in epithelial cells seem to apply also to axon/dendrite trafficking in neurons (Dotti & Simons 1990,Horton & Ehlers 2003b). Madin-Darby canine kidney (MDCK) cells, which display apical/basolateral asymmetry, have served as an excellent model for elucidating how proteins destined for different cellular domains are sorted and delivered (Keller et al. 2001,Kreitzer et al. 2003). Studies in MDCK cells have demonstrated that polarized integral membrane proteins are assigned to distinct post-Golgi carriers destined for either the apical or basolateral domain (Keller & Simons 1997). The sorting of cargo into distinct carriers relies on intrinsic sequence determinants. Basolateral sorting sequences are generally located in the cytoplasmic tail, whereas apical sorting sequences have been discovered in the transmembrane domain (Keller & Simons 1997,Mellman 1996). Sorting into apicalcarriers can also be a result of posttranslational modifications, including N- or O-glycosylation or modification with glyco-sylphosphatidylinositol (Keller & Simons 1997,Lisanti et al. 1989). Are these apical/basolateral sorting signals universally recognized in all polarized cells? When expressed in neurons, several basolateral and apical proteins are sorted preferentially to the somatodendritic compartment and axon, respectively, including VSVG (basolateral/somatodendritic), LDL receptor (basolateral/somatodendritic), and influenza HA protein (apical/axonal) (Dotti & Simons 1990,Jareb & Banker 1998). However, recent experiments suggest that neurons do not recognize some dihydrophobic motifs, which direct cargo to the basolateral domain in MDCK cells (Silverman et al. 2005). Additionally, the sequence determinants that direct transferrin receptor to the basolateral domain in epithelial cells may only partially overlap with the somatodendritic targeting motif (West et al. 1997). Therefore, although many of the general principles of protein sorting established in epithelial cells apply to neurons, neurons have many of their own rules for establishing protein asymmetry (Winckler & Mellman 1999).

A more circuitous mode of polarized trafficking is observed in hepatocytes and enterocytes, where cargo destined for the apical plasma membrane is first exocytosed to the basolateral membrane and then endocytosed and transported to the apical membrane via intracellular vesicular carriers (Tuma & Hubbard 2003). This transcytotic mode of transport was originally discovered in capillaries, where researchers observed that circulating macromolecules could readily traverse the capillary endothelial cell layer to the interstitium of tissues (Pappenheimer et al. 1951). Much later, investigators discovered that not only extracellular factors, but also integral membrane proteins are transferred from one end of the cell to the other via transcytosis (Bartles et al. 1987).

This roundabout trafficking mechanism is also found in neurons. VAMP2 and NgCAM/L1, which localize to the presynaptic terminal and axon membrane, respectively, were observed to be initially delivered to the cell surface of the somatodendritic compartment (Wisco et al. 2003). Mutations that disrupt endocytosis of VAMP2 leave it stranded at the surface of the somatodendritic compartment, demonstrating that endocytosis is required for its proper localization. NgCAM is normally found on the plasma membrane of axons, yet intracellular vesicles carrying Ng-CAM are found in both dendrites and axons (Burack et al. 2000). Synchronous release of NgCAM from the ER revealed that NgCAM is also inserted into the plasma membrane of the somatodendritic compartment where it is internalized and trafficked to the axon (Wisco et al. 2003; but see also Sampo et al. 2003). A single tyrosine point mutation (Y33A) results in direct axonal trafficking of NgCAM without a layover at the surface of the somatodendritic compartment, which demonstrated that NgCAM can follow different routes to the same final destination (Wisco et al. 2003). The factors that determine the preferred route

remain unknown, but perhaps tyrosine 33 phosphorylation or some other posttranslational modification biases the route of NgCAM trafficking.

## Synapse Targeting

Once integral membrane proteins reach the plasma membrane, refinement of their localization is often necessary. For example, some components of the PSD are inserted into the somatic plasma membrane tens to hundreds of microns from synapses. How do newly synthesized integral membrane postsynaptic factors, such as neurotransmitter receptors, reach synapses? Before synapse formation, NMDA receptor clusters continually cycle between the cell surface and endosomal compartments at extrasynaptic sites, fluctuating between a mobile state and a paused state, which is associated with clathrin (Washbourne et al. 2004). These data demonstrate that NMDA receptors make their way to synapses either by diffusion on the surface of the cell or by intracellular vesicular "transport packets." Electron microscopy demonstrated that vesicles positive for NMDA receptors are often associated with microtubules, which serve as the substrate for the kinesin family of motor proteins (Washbourne et al. 2004). This observation is consistent with intracellular, kinesindriven, vesicular transport of NMDA receptors along the somatodendritic microtubule network (Setou et al. 2000,Washbourne et al. 2004). However, additional live cell-imaging results have demonstrated that synapses gradually acquire NMDA receptors, supporting a diffusional accumulation of NMDA receptors rather than delivery via discrete intracellular carriers (Bresler et al. 2004). Although a reconciliation of these results awaits more detailed studies, one possibility is that NMDA receptors are delivered intracellularly to the dendritic surface in the vicinity of synapses, and synaptic receptor clustering is mediated by diffusion.

Simple diffusion may be the synaptic targeting mechanism used by glycine receptors, which appear on the surface of the somatodendritic compartment at extrasynaptic sites after synchronous release from the ER (Rosenberg et al. 2001). Spatial analysis of surface glycine receptor puncta revealed that all receptor clusters were within 20 μm of the cell body 7 min after ER release, whereas receptor clusters were found as far as 80 μm from the cell body 60 min after release. These data suggest that receptors are exocytosed in the cell body and proximal dendritic regions before diffusing within the plasma membrane to distal dendritic regions, where they are eventually anchored at existing synaptic structures. Receptors diffusing to distal dendrites must encounter numerous synapses. How do receptors reach the most distal synaptic sites without being titrated by more proximal synapses? One model is that each synapse has only a defined number of slots for receptor molecules, and as proximal synapses are saturated, receptor clusters can proceed distally. At excitatory synapses, PSD-95 limits the number of synaptic AMPA receptors (Schnell et al. 2002); perhaps gephyrin plays a similar role in limiting the number of glycine receptors at inhibitory synapses (Sola et al. 2004). Recent experiments support a similar surface-diffusion model for AMPA receptor delivery to excitatory synapses. Using a photoreactive, irreversible AMPA receptor antagonist, Adesnik et al. (2005) observed that exchange of inactivated synaptic AMPA receptors occured only after several hours, a timescale much slower than previously thought. Although exchange of inactivated synaptic AMPA receptors took hours, inactivated receptors at the surface of the cell body were replaced after only several minutes, suggesting that under basal conditions, AMPA receptors are inserted into the plasma membrane of the cell body and could make their way to synapses by diffusion. Because diffusion to synapses hundreds of microns from the cell body is predicted to take many hours, this mechanism cannot account for the fast receptor insertion observed at synapses in response to potentiating stimuli, which takes place on the timescale of minutes (Shi et al. 1999). Perhaps the formation of synaptic "slots" for diffusing receptor, mobilization of a local intracellular store of receptors, or a combination of these possibilities sets the rate for synaptic incorporation of receptors in response to synapse-strengthening stimuli (Schnell et al.

2002,Park et al. 2004). If postsynaptic components are free to diffuse in the plasma membrane, what keeps them from entering the axon? Conversely, what keeps axonal components from spreading into the somatodendritic compartment? Proteins that stray from their appropriate domain could be retrieved by endocytosis. Alternatively, barriers between different cellular domains could prevent mislocalization of freely diffusing membrane-associated proteins. Membrane diffusion barriers have been observed in epithelial cells at tight junctions, where adjacent cells contact one another (van Meer & Simons 1988). This barrier prevents solutes from traversing the epithelial cell layer and blocks diffusion of membrane-associated proteins between apical and basolateral domains. To test if neurons have adopted this same strategy to separate their axonal and somatodendritic domains, Winckler et al. (1999) tested the lateral mobility of several different transmembrane proteins in various cellular domains, including the axon initial segment, which forms the boundary between the axon and the somatodendritic compartment. Antibody-conjugated beads were trapped and dragged along the cell surface using optical tweezers. The distance the bead could be dragged (i.e., its tractability) was used to assay the ability of a given transmembrane protein to diffuse laterally in a given environment. Beads with antibodies directed against several different transmembrane targets displayed reduced tractability in the axon initial segment, which suggested that a diffusion barrier exists in this region. In another study, (Kobayashi et al.1992) fused liposomes containing fluorescent lipids specifically to axons. Although the fluorescent lipids were free to diffuse in the axonal plasma membrane, no labeling of the soma or dendrites was observed, suggesting the presence of a diffusional barrier between the two compartments, which prevented lipid exchange. Another study tracked individual fluorophore-labeled phospholipid molecules in the plasma membrane of hippocampal neurons and found that mobility was restricted in the axon initial segment after 7-10 days in culture (Nakada et al. 2003). What forms the diffusion barrier? Electron micrographs reveal a specialized membrane cytoskeleton in the axon initial segment, which contains ankyrin and amphiphysin II (Butler et al. 1997,Kordeli et al. 1995,Peters et al. 1968,Winckler et al. 1999). Additionally, disrupting actin with latrunculin-B enhances membrane diffusion in the axon initial segment, demonstrating that it too plays a role in establishing the barrier between axon and soma (Winckler et al. 1999).

## Microtubule Transport

Intracellular transport mechanisms are required to deliver organelles and other cargo important for growth, function, and maintenance to axons and dendrites. To this end, neurons contain an elaborate network of microtubules radiating from the soma into dendritic and axonal processes. Axons contain microtubules with their plus ends pointed away from the cell body, whereas dendrites harbor microtubules in either orientation (Baas et al. 1988). The kinesin family of motor proteins travel along microtubule filaments, acting as intracellular couriers, shuttling soluble proteins, mRNA, and cellular organelles along the microtubule network (Vale & Fletterick 1997). Kinesin was originally discovered as the molecular motor responsible for fast axonal transport (Brady 1985,Vale et al. 1985). Subsequently, at least 45 members of the kinesin family have been discovered in mouse and human (Hirokawa & Takemura 2005,Miki et al. 2001). Some of these family members play transport roles in dendrites. For example, KIFC2 localizes to the somatodendritic compartment, where it plays a role in trafficking multivesicular body-like organelles (Saito et al. 1997). Kinesins also play a role in transporting postsynaptic molecules. KIF17 interacts with a PDZ domain of mLin-10, which is a component of a multiprotein complex including mLin-2, mLin-7, and NMDA receptor 2B (NR2B) (Setou et al. 2000). Vesicles immunopurified from brain with an antibody against KIF17 contained NR2B and were shuttled by KIF17 toward the positive ends of microtubules. Mutant KIF17 lacking its mLin10-binding domain retained motor activity but could not translocate NR2B-positive vesicles, indicating that mLin10 binding is essential for KIF17-dependent vesicle movement (Setou et al. 2000). Decreasing endogenous KIF17 expression in cultured

hippocampal neurons using antisense RNA resulted in decreased NR2B expression, whereas NR2A and NR2C, which do not formcomplexes with KIF17, were either upregulated (NR2A) or unaffected (NR2C) (Guillaud et al. 2003). In the same study, dominant-negative disruption of KIF17 decreased the number of NR2B-containing synapses but had no effect on overall synapse number, demonstrating that KIF17 plays a crucial and specific role in transporting NR2B to the postsynaptic membrane. A different kinesin, KIF1Bα, interacts with PSD95 and the synaptic scaffolding protein SAP90, which suggested that it too may play a role in trafficking constituents of the postsynapse (Mok et al. 2002).

The direction a particular kinesin travels along a microtubule can be dictated by the cargo it is carrying. The glutamate receptor interacting protein 1 (GRIP1), which binds GluR2, can direct KIF5 (also known as kinesin 1) to the somatodendritic domain, and a different kinesin-binding protein, JSAP1, routes KIF5 to axons (Setou et al. 2002), which demonstrated that accessory proteins can steer kinesins to specific cellular domains. Decreasing the level of GRIP1 by small interfering RNA (siRNA) caused a loss of dendrites that could be rescued by overexpression of EphB2, a receptor tyrosine kinase (RTK) that has been implicated in dendritic spine development and synaptic plasticity (Hoogenraad et al. 2005,Yamaguchi & Pasquale 2004). Specifically disrupting the GRIP1-KIF5 interaction impaired EphB2 trafficking to dendrites and inhibited dendritic growth, supporting a model in which GRIP1 acts as a kinesin adapter protein for dendritic trafficking (Hoogenraad et al. 2005,Setou et al. 2002).

## LOCAL POSTSYNAPTIC TRAFFICKING

### Dendritic Endocytosis

Endocytosis is a ubiquitous mechanism that allows cells to internalize external nutrients and trophic factors, subtract membrane from the cell surface, and regulate the level of specific cellsurface proteins. Rates of endocytosis vary from several milliseconds, in the case of membrane retrieval at the presynaptic terminal, to tens of minutes in the case of G protein-coupled receptor (GPCR) internalization after agonist stimulation, which indicates that many different endocytic mechanisms specific for different surface molecules and cellular domains exist (Sorkin & Von Zastrow 2002,Sudhof 2004).

Endocytosis can be found in four principal forms: clathrin independent, pinocytosis, phagocytosis, and caveolar. The most widely studied mechanism of endocytosis is mediated by clathrin, a triskelion composed of three heavy chains and three light chains, which forms lattice-like structures on the interior face of the plasma membrane (Kirchhausen 2000). Adaptor proteins, such as AP-2, bind endocytic target proteins and nucleate the formation of the clathrin lattice, which initiates membrane invagination and budding of clathrin-coated vesicles. The large GTPase dynamin is required for producing intracellular clathrin-coated vesicles by pinching off clathrin-coated invaginations (van der Bliek & Meyerowitz 1991,van der Bliek et al. 1993). Dynamin is also required for caveolar endocytosis but not for pinocytosis (Nichols & Lippincott-Schwartz 2001). All three dynamin family members (dynamins 1-3) are expressed in neurons. Dynamin 1 is critical for membrane retrieval at the presynaptic terminal following fusion of neuro transmitter vesicles, dynamin 2 is expressed ubiquitously and may play multiple roles in endocytosis and actin regulation, and dynamin 3 localizes to dendritic spine heads and may play a role in postsynaptic membrane trafficking (Gray et al. 2003,Urrutia et al. 1997)

An initial observation of dendritic endocytosis was made by immunostaining cerebellar Purkinje neurons for transferrin, albumin, and various immunoglobulins (Fishman et al. 1990). These factors, which are normally found in plasma, were also observed throughout the dendrites and soma of Purkinje cells. Subsequently, investigators showed that the transferrin

receptor, a transmembrane protein that localizes to the somatodendritic compartment, continually cycles back and forth between the dendritic cell surface and internal structures (Cameron et al. 1991b,Mundigl et al. 1993,West et al. 1997). More recently, endocytosis and postendocytic sorting of neurotransmitter receptors have emerged as critical mechanisms responsible for various forms of synaptic plasticity (Beattie et al. 2000;Carroll et al. 1999, 2001;Ehlers 2000;Lavezzari et al. 2004;Lee et al. 2004b;Lin et al. 2000;Luscher et al. 1999;Prybylowski et al. 2005;Roche et al. 2001;Scott et al. 2004).

**The spine endocytic zone—**Several postsynaptic components interact with endocytic factors, which suggests that endocytosis takes place near the PSD. For example, Homer and mGluR5 interact with dynamin 3 (Gray et al. 2003), and both the AMPA receptor subunit GluR2 and the NMDA receptor subunit NR2B interact with the AP-2 complex (Lavezzari et al. 2003,Lee et al. 2002,Roche et al. 2001). The association of postsynaptic receptors with the clathrin endocytic machinery is tightly regulated. The $Ca^{2+}$ binding protein hippocalcin interacts with the β2-adaptin subunit of the AP2 complex and forms a $Ca^{2+}$-dependent complex with GluR2 required for NMDA receptor-dependent LTD (Palmer et al. 2005). The interaction of NR2B with AP2 is negatively regulated by the binding of PSD-95 to an adjacent domain of NR2B (Roche et al. 2001) and via tyrosine phosphorylation by Fyn (Prybylowski et al. 2005). So, where exactly does clathrin-mediated endocytosis of postsynaptic receptors occur? When expressed in neurons, GFP-clathrin forms puncta throughout the neuron (Blanpied et al. 2002). In dendritic spines, clathrin puncta were found in close proximity to, but not overlapping, the PSD (Figure 3B). Clathrin assembles and disassembles repeatedly at the same site on the lateral spine membrane, suggesting the presence of stable endocytic zones on the spine surface near the PSD. Labeled transferrin was internalized at these sites, proving that these clathrin puncta marked bona fide endocytic sites (Blanpied et al. 2002). Electron microscopy revealed the presence of coated structures representing all phases of the endocytic process in dendritic spines (Petralia et al. 2003,Racz et al. 2004) and demonstrated that AP-2, clathrin, and dynamin localized to lateral domains of dendritic spines, with AP-2 closest to and dynamin furthest from the PSD (Racz et al. 2004) (Figure 3). In some cases, clathrin and AP-2 localized to the spine plasma membrane, even though no clathrin-coated invaginations or "pits" were apparent, which suggested that clathrin and AP-2 may be preorganized in spines for streamlined endocytosis (Racz et al. 2004). The presence of stable endocytic zones near the PSD in the spine head supports a model in which PSD components, such as AMPA receptors, are internalized locally, within spines, and do not traverse the spine neck for internalization in the dendritic shaft. Instead, these data suggest that synaptic components first dissociate from the PSD, bind readily available adaptor protein(s), and migrate to nearby endocytic sites, located in the spine head, where they are internalized (Figure 4). A spine endocytic zone could serve two important functions. First, endocytic zones near the PSD may simply provide efficient and fast internalization of nearby synaptic components, offering rapid regulation of factors displayed on the spine surface. Second, a spine endocytic zone could help maintain the molecular composition of a given spine by preventing diffusion of membrane components down the spine neck toward neighboring synapses. The presence of a continuously cycling endocytic zone adjacent to the PSD may sequester the components of a given synapse and may explain how individual spines maintain a unique and separate identity from neighboring spines.

Although endocytic zones have been observed in spine heads, it remains unclear what anchors these discrete functional domainsnear the PSD. Although endocytosis requires many different protein factors, a critical regulator of endocytosis is the phospholipid composition of the membrane itself. Various phosphorylated forms of phosphatidylinositol (PI) are binding partners for several protein domains and are asymmetrically distributed between different cellular organelles. The plasma membrane is rich in PI(4,5)P2, whereas early and recycling endosomes are rich in PI(3)P and PI(3,5)P2. These phosphovariants of PI create distinct lipid

environments that recruit various effector molecules and form discrete domains on the plasma membrane, which coordinate exoand endocytosis (Wenk & De Camilli 2004). Membranes rich in PI(4,5)P2 can recruit critical endocytic factors, such as AP-2, AP180, and dynamin, which suggested that lipid microdomains can nucleate endocytic domains. Perhaps the spine PI composition plays a role in defining the location of the postsynaptic endocytic zone. Cholesterol/sphingolipid microdomains (lipid rafts) are abundant in dendrites in which they are associated with AMPA receptors and additional postsynaptic proteins (Hering et al. 2003). Depletion of cholesterol/sphingolipid leads to a loss of AMPA receptors, spines, and synapses, supporting a requirement for synapse formation or maintenance (Hering et al. 2003). Beyond these initial studies, evidence for lipid microdomains in dendritic spines is sparse and deserves further investigation.

**Actin-associated proteins and spine endocytosis—**Although the dendritic shaft and axon harbor an extensive microtubule cytoskeleton, dendritic spines are rich in filamentous actin (F-actin). Actin plays a critical role in regulating spine morphology and synaptic function. In addition to acting as a structural scaffold for spines, actin may also play a direct role in membrane endocytosis by restricting the mobility of clathrin-coated pits and by acting as a guide or motile force for clathrin-coated vesicles upon their departure from the plasma membrane. Although there is an abundance of actin-regulatory proteins in spines, including various kinases, phosphatases, small GTPases, and their regulatory proteins, we focus our discussion on cortactin and myosin, two classes of proteins known to link the spine actin cytoskeleton to membrane trafficking directly. For detailed reviews of actin regulation in spines, see Carlisle & Kennedy (2005),Ethell & Pasquale (2005), and Oertner & Matus (2005).

The actin regulatory protein cortactin localizes to spine heads, where it is involved in the actin rearrangement that accompanies changes in spine morphology (Gray et al. 2005). Cortactin also plays a role in endocytosis because it binds dynamin and recruits Arp1/2 actin-polymerizing activity to sites of membrane internalization (McNiven et al. 2000,Weaver et al. 2001). Live imaging of Swiss 3T3 cells revealed that cortactin precisely clusters at sites of clathrin assembly just seconds prior to membrane scission, supporting its role in clathrin-dependent endocytosis (Merrifield et al. 2005). In neurons, cortactin associates with shank, which in turn forms a complex with NMDA receptors, guanylate kinase-associated protein (GKAP), and PSD-95. These interactions suggest that cortactin is directly linked to the PSD and could regulate endocytosis of PSD proteins (Naisbitt et al. 1999). Immunogold localization of cortactin supports this model by revealing two distinct pools of cortactin: a minor pool that lies near the cytoplasmic face of the PSD and a major pool that is situated a considerable distance (100-150 nm) from the PSD in the spine core (Racz & Weinberg 2004). Perhaps these pools have distinct functions; the minor PSD-associated pool may direct endocytosis of synaptic components, and the major pool may be important for actin based spine remodeling.

Another class of actin-associated proteins important for spine membrane trafficking is the myosin family of motor proteins. Myosins are multisubunit motor proteins that couple ATP hydrolysis to unidirectional movement of various cargos along actin filaments. Investigators have found in neurons myosins from several different families, I, II, V, and VI, although their individual roles are only beginning to emerge (Bridgman 2004). Myosin cargo ranges from individual proteins to cellular organelles. The smooth endoplasmic reticulum is absent from Purkinje cell dendritic spines in myosinVa-mutant *dilute* mice, pointing to a myosin-based mechanism for SER distribution in neurons (Takagishi et al. 1996). Myosin Va is abundant at the PSD where it binds to GKAP, a PSD component that binds to PSD-95, which suggested that myosin Va plays a role in transporting postsynaptic factors (Naisbitt et al. 2000). Myosin Va also interacts with kinesin and dynein light chain, both microtubule motor proteins, which

indicates that myosin Va could coordinate transport of postsynaptic molecules between the microtubule-rich dendritic shaft and spines, which are rich in actin (Brown et al. 2004,Naisbitt et al. 2000). Although myosin Va is abundant in the PSD, hippocampal synaptic function and plasticity are normal in myosinVa *dilute* mutant mice (Schnell & Nicoll 2001).

Zhang et al. (2005) recently showed myosin II regulatory light chain to be involved in dendritic spine formation downstream of Rac activation. Myosin II can also bind directly to NMDA receptor subunits and is sensitive to $Ca^{2+}$ through $Ca^{2+}$-calmodulin, which suggests it is important for $Ca^{2+}$-dependent remodeling of the PSD (Amparan et al. 2005). Myosin VI, which is highly expressed in brain and enriched at synapses, provides a direct molecular link between the actin cytoskeleton and AMPA receptor endocytosis (Osterweil et al. 2005). Myosin VI is unique among myosin family members in that it moves toward the minus or pointed end of actin filaments, which tend to point inward, away from the plasma membrane (Wells et al. 1999). Myosin VI plays a role in endocytosis and vesicle trafficking in nonneuronal cells and is thought to use its minusend-directed motor activity to pull endocytic vesicles away from the plasma membrane (Hasson 2003). Mice lacking myosin VI have fewer synapses in CA1 and shorter dendritic spines than do wild-type mice (Osterweil et al. 2005). Dominant-negative disruption of myosin VI in cultured hippocampal neurons impairs agonist-dependent internalization of AMPA receptors. Furthermore, myosin VI exists as a complex with AP-2 and AMPA receptors, but not NMDA receptors, which provides a direct and specific link between AMPA receptors and the endocytic machinery. A general endocytic marker (transferrin) was normally internalized in myosin VI null hippocampal neurons, emphasizing that myosin VI may play a very specialized role in receptor endocytosis at the dendritic spine (Osterweil et al. 2005). Whereas myosin VI regulates AMPA receptor endocytosis and removal from spines, myosin motors for receptor exocytosis in spines remain to be described.

**Activity dependent endocytosis at excitatory synapses—**Up- or down regulating the number of postsynaptic AMPA receptors leads to synapse potentiation or depression, respectively, making the regulation of receptor shuttling to and from the spine surface crucial for setting synaptic strength (Bredt & Nicoll 2003,Malenka & Bear 2004). Like many other types of cell-surface receptors, ionotropic glutamate receptors at excitatory synapses are endocytosed upon agonist binding (Carroll et al. 1999,Ehlers 2000). In the cases of GPCRs and RTKs, agonist-stimulated endocytosis desensitizes the cell toward the receptor ligand. In some cases, endocytosed receptors initiate activation of signal transduction molecules from internal endosomes (Howe et al. 2001,Lefkowitz & Shenoy 2005,Ye et al. 2003). Additionally, endocytosis plays a role in ultimately resensitizing receptor molecules. For example, after stimulation, many GPCRs are phosphorylated, bind arrestin, and are internalized, which uncouples them from their cognate G proteins. Following endocytosis, receptor molecules shed arrestin, unbind ligand, are dephosphorylated, and are recycled back to the membrane surface, fully resensitized (Pippig et al. 1995,Sibley et al. 1986,Yu et al. 1993).

AMPA receptors are also endocytosed in an activity dependent manner. In hippocampal cultures, AMPA receptors undergo endocytosis with a time constant of ~14 min in the presence of tetrodotoxin (TTX), a sodium channel blocker that inhibits spontaneous neural activity. Increasing excitatory neural activity by adding picrotoxin, which blocks inhibitory inputs, accelerates AMPA receptor endocytosis approximately three fold (Ehlers 2000). AMPA receptor internalization can be triggered either directly, by agonist (glutamate, AMPA) binding, or indirectly, by NMDA or insulin receptor activation (Beattie et al. 2000,Ehlers 2000,Lin et al. 2000). Although both direct and indirect stimulation lead to AMPA receptor internalization, the postendocytic fate of the receptor depends ultimately on the endocytic trigger. Directly stimulated AMPA receptor without simultaneous NMDA receptor activation is degraded, whereas NMDA stimulation alone or concurrent AMPA/NMDA stimulation leads to AMPA

receptor recycling (Ehlers 2000). Similar sorting events have been observed for kainate receptors and, for AMPA receptors, may be determined by the precise subunit composition of the receptor (Lee et al. 2004b,Martin & Henley 2004).

NMDA receptor-stimulated AMPA receptor endocytosis requires $Ca^{2+}$ influx through NMDA receptors. Recent experiments have uncovered several factors that may act as the sensors that link $Ca^{2+}$ influx to AMPA receptor endocytosis. The first potential $Ca^{2+}$ sensor to be discovered was calcineurin (PP2B), which binds $Ca^{2+}$ directly. Inhibiting calcineurin with FK506 blocked NMDA-triggered AMPA receptor internalization, demonstrating that a dephosphorylation event, perhaps dephosphorylation of the receptor itself, is required for its endocytosis (Beattie et al. 2000,Ehlers 2000). Other $Ca^{2+}$-sensing molecules may facilitate the interaction between AMPA receptors and endocytic machinery. The interaction between GluR2 and the clathrin adapter protein AP-2 is stimulated by $Ca^{2+}$-bound hippocalcin, which also binds to AP-2 (Palmer et al. 2005). Preventing the hippocalcin/AP-2 interaction with a truncated version of hippocalcin lacking its $Ca^{2+}$-binding domains disrupted LTD in hippocampal slices, providing functional evidence for hippocalcin's role in AMPA receptor endocytosis. Hippocalcin was not found in preparations of brain clathrin-coated vesicles, which suggested that its role is to initiate AMPA receptor binding to AP-2 and that it is not required to maintain this interaction. Recent experiments have demonstrated that the $Ca^{2+}$-binding protein PICK1 may also be important for $Ca^{2+}$-triggered AMPA receptor internalization (Hanley & Henley 2005). When bound to $Ca^{2+}$, PICK1 interacts with AMPA receptors to promote receptor endocytosis, whereas a dominant-negative version of PICK1 lacking its $Ca^{2+}$-binding domains blocked NMDA-triggered AMPA receptor endocytosis. In a different study, PICK1 interacted with activated PKC and competed with the AMPA receptor anchoring proteins ABP and GRIP for GluR2 binding. Once unbound from ABP/GRIP, the PICK1/GluR2 complex is directed to membrane invaginations via its BAR domain, perhaps for endocytosis (Lu & Ziff 2005).

The Rab family of GTPases contains members playing diverse roles in membrane trafficking, including AMPA receptor internalization. In several different cell types, Rab5 is involved in trafficking proteins on the plasma membrane to early endosomes via clathrin-mediated endocytosis (de Hoop et al. 1994,Kanaani et al. 2004,Mohrmann & van der Sluijs 1999). In neurons, Rab5 localizes to the perimeter of the PSD and is found in its activated (GTP-bound) state shortly after NMDA receptor stimulation (Brown et al. 2005). In the same study, overexpressing Rab5 in neurons did not affect the total level of AMPA receptor but selectively decreased the level of surface AMPA receptor on dendritic spines. Overexpressing Rab5 had no effect on the spine/shaft ratio of total (internal and surface) AMPA receptor, which suggested that receptors internalized by a Rab5-dependent mechanism remained in the spine, supporting a model where receptors are internalized at endocytic zones on the spine surface and maintained in a local endosomal pool (Blanpied et al. 2002,Brown et al. 2005).

Ubiquitination of activated receptors and receptor-binding proteins provides another possible trigger for activity dependent endocytosis. For example, activation of the β-adrenergic receptor leads to both its own ubiquitination as well as ubiquitination of β-arrestin, which recruits clathrin adapter proteins and is required for efficient internalization (Shenoy & Lefkowitz 2003,Shenoy et al. 2001). The *Caenorhabditis elegans* glutamate receptor-1 (GLR-1) is ubiquitinated in vivo, and mutations that disrupt its ubiquitination cause increased synaptic receptor accumulation. Overexpressing ubiquitin, a limiting factor at synapses, decreases the level of synaptic receptor. Mutations in the gene for the clathrin adapter protein AP180 suppress the effect of ubiquitin overexpression, providing a link between glutamate receptor ubiquitination and clathrin-dependent internalization (Burbea et al. 2002). In mammalian neurons, synaptic activity is linked to postsynaptic ubiquitination (Colledge et al. 2003,Ehlers 2003,Pak & Sheng 2003), which suggests that a similar mechanism could be important for

endocytosis of postsynaptic molecules on the spine surface, but little is known about the ubiquitin regulatory enzymes or their targets at mammalian synapses (Yi & Ehlers 2005).

Because neural activity stimulates the endocytosis of postsynaptic surface proteins, one might expect activity to influence the dynamics of clathrin assembly or disassembly within dendritic spines. This does not seem to be the case because spine clathrin dynamics and location of the endocytic zone are unaffected by activation of glutamate receptors, prolonged activity blockade, or electrical field stimulation (Blanpied et al. 2002). This apparent discrepancy can be resolved by a model in which zones adjacent to the PSD constitutively internalize spine cargo and, during activity, synaptic molecules destined for endocytosis first dissociate from the PSD and either diffuse or are escorted to these zones. This model predicts a constant stream of endocytic vesicles originating from the spine surface near the PSD that contains different numbers of synaptic molecules, depending on the level of activity at the synapse. In this way, the endocytic machinery is always poised to accept cargo, obviating the need for the potentially time-consuming assembly of all the necessary endocytic factors. Over longer time frames, activity may regulate the endocytic machinery by upregulating the coiled-coil spectrin-repeat protein CPG2, an activity-related gene product that localizes to the spine endocytic zone (Cottrell et al. 2004). Indeed, RNAi-mediated CPG2 knockdown increases the number of postsynaptic clathrin coated vesicles, disrupts the constitutive internalization of glutamate receptors, and inhibits the activity-induced internalization of synaptic AMPA receptors (Cottrell et al. 2004).

**Clathrin-independent endocytosis—**Although considerable evidence has demonstrated endocytosis via clathrin coats in postsynaptic plasticity, it is unclear what role, if any, clathrin-independent mechanisms play in trafficking spine membrane. Many experiments assaying receptor internalization have been based on dynamin disruption, which does not distinguish clathrin-dependent from clathrin-independent endocytosis. Nearly all molecules known to be internalized via clathrin-independent mechanisms are found in lipid microdomains enriched in cholesterol, glycosphingolipids, sphingomyelin, and long-chain unsaturated phospholipids known as lipid rafts (Nichols 2003). The presence of lipid microdomains has been documented in neurons, and several key PSD proteins associate with lipid rafts (Hering et al. 2003,Wong & Schlichter 2004). However, disruption of lipid rafts by cholesterol depletion actually increases the rate of AMPA receptor endocytosis (Hering et al. 2003), which suggests that dendritic lipid microdomains actually stabilize AMPA receptors at the spine surface. There is evidence for clathrin-independent endocytosis of the postsynaptic metabotropic glutamate receptor, mGluR5, which is efficiently internalized in hippocampal neurons even in the presence of a dominant-negative mutant of Eps15, an AP-2-associated protein (Fourgeaud et al. 2003). However, the mechanisms governing clathrin-independent internalization at the postsynaptic membrane and its role in synapse regulation remain obscure and deserve further investigation.

## Post-Endocytic Sorting

Following endocytosis, membrane-associated proteins can either be recycled back to the cell surface (local recycling), routed to a different membrane domain (transcytosis), or directed to intracellular lysosomes where they are degraded (Figure 4, Table 1). The first stop for newly budded vesicles leaving the cell surface is the early endosome. The early endosome serves as a sorting station for newly endocytosed proteins in much the same way that the TGN serves as a sorting station for newly synthesized membrane-associated proteins. The fate of newly internalized surface molecules depends on their identity and partitioning into membrane microdomains within the early endosome (Maxfield & McGraw 2004). Much of this partitioning is controlled by monoubiquitination of endocytic cargo. Monoubiquitinated cargo

associates with endosomal clathrin and Hrs and is subsequently routed to late endosomes, where association with the ESCRT complex directs cargo for intralumenal budding into multivesicular bodies and subsequent degradation by fusion with lysosomes (Hicke & Dunn 2003,Raiborg et al. 2003). In the absence of such sorting, membrane cargo in early endosomes partitions in tubular extensions that bud off for trafficking back to the plasma membrane or to a distinct recycling endosome compartment (Maxfield & McGraw 2004).

As an example of differential postendocytic sorting, activated epidermal growth factor receptors are generally trafficked to lysosomes and degraded following endocytosis, whereas transferrin receptors are sorted to specialized recycling endosomes and sent back to the cell surface (Dautry-Varsat et al. 1983,Wiley & Burke 2001). The fate of internalized surface molecules can also be governed by extrinsic factors. In neurons, AMPA receptors internalized in response to AMPA stimulation are trafficked to dendritic lysosomes and degraded. However, AMPA receptors internalized in response to NMDA receptor-mediated $Ca^{2+}$ influx are sorted into recycling endosomes and reinserted in a PKA-dependent manner (Ehlers 2000). In the case of NMDA receptors, NR2A and NR2B subunits contain sorting motifs that direct internalized receptors along a degradative and recycling pathway, respectively (Lavezzari et al. 2004). In addition, both NR1 and NR2 subunits contain conserved membrane proximal endocytic motifs that direct internalized receptors for degradation in a manner that is suppressed by C terminal recycling motifs present in NR2B (Scott et al. 2004). Such studies indicate that postendocytic fate is not a simple matter of identity, but can also be regulated by intracellular signaling and by specific subunit composition of individual receptors.

The molecular mechanisms in neurons that determine whether internalized receptors are degraded or recycled back to the dendritic surface are only beginning to emerge. The neuron-enriched endosomal protein of 21 kDa (Neep21) localizes to early endosomes, where it plays a role in sorting surface receptors to recycling endosomes (Steiner et al. 2002). In COS-7 cells, overexpressing Neep21 causes internalized neurotensin receptor-1, which is normally degraded, to be recycled to the cell surface (Debaigt et al. 2004). In neurons, Neep21 acts in a similar way to regulate AMPA receptor recycling. Loss of Neep21 function decreases recycling of GluR1 and GluR2 following endocytosis (Steiner et al. 2002). This effect is mediated, at least for GluR2, by an activity dependent interaction between Neep21 and GRIP1. Disrupting this interaction specifically causes GluR2, but not GluR1 or transferrin receptor, to accumulate in early endosomes and lysosomes (Steiner et al. 2005). Disrupting Neep21 function in hippocampal slices impairs synaptic potentiation, presumably because AMPA receptor recycling to the spine surface is impaired (Alberi et al. 2005). These data support a model in which Neep21 acts as a sorting factor on early endosomes that directs AMPA receptors to recycling endosomes. The activity dependence of the Neep21/GRIP1/GluR2 complex suggests that the fraction of internalized receptor that is sorted for recycling depends on the level of neurotransmission at a given synapse and that this sorting step may play a critical role in defining the amount of receptor available for activity dependent synaptic potentiation.

## Recycling Endosomes as AMPA Receptor Reservoirs

Intracellular endosomal compartments can serve as reservoirs for molecules that are rapidly shuttled to the cell surface in response to various physiological stimuli. In the case of glutamatergic synapses, selectively blocking transport from recycling endosomes by expressing inhibitory mutants of Rab11a, Rme1/EHD1, or syntaxin-13 reduces surface AMPA receptors (Figure 5). Blocking recycling endosome transport not only decreases the basal level of surface AMPA receptor, but also disrupts the NMDA receptor-dependent delivery of AMPA receptors to the dendritic surface, a process that is critical for long term potentiation (LTP). Indeed, inhibition of recycling endosome transport in postsynaptic CA1 pyramidal neurons in

hippocampal slice preparations rapidly abolishes LTP at Schaffer collateral inputs without affecting synaptic NMDA currents or presynaptic properties (Park et al. 2004). These data support a model in which recycling endosomes act as intracellular storehouses that supply the AMPA receptors required for potentiating synapses during experience-dependent plasticity (Park et al. 2004). Given the ongoing recycling of AMPA receptors under basal conditions (Ehlers 2000), recycling endosomes likely function as a kinetic trap in which activity-dependent tuning of recycling kinetics controls the steady-state abundance of postsynaptic receptors in an online fashion. The molecular cues and endosome-associated machinery that mobilizes this intracellular pool of AMPA receptors in response to activity remain unknown.

Intracellular pools of glutamate receptors may not only act as storehouses for strengthening synaptic transmission, but also act as buffers for maintaining synaptic strength at a specific set point. The abundance of postsynaptic glutamate receptors can be scaled up or down in response to prolonged changes in neuronal activity over hours to days (Davis & Bezprozvanny 2001,Perez-Otano & Ehlers 2005,Turrigiano & Nelson 2004). Chronically blocking or stimulating excitatory neurotransmission in spinal, cortical, and hippocampal neurons increases or decreases the number of surface AMPA receptors and NMDA receptors, respectively (Mu et al. 2003,O'Brien et al. 1998,Rao & Craig 1997,Thiagarajan et al. 2005,Turrigiano et al. 1998). This compensatory or homeostatic plasticity is thought to adjust synaptic strength globally to maintain neurons within an optimal firing frequency range (Burrone & Murthy 2003,Turrigiano & Nelson 2004). Although the receptor trafficking events underlying this form of synaptic plasticity remain mostly obscure, AMPA receptor half life in spinal neurons is extended from 18 to 32 h when neurotransmission is chronically blocked (O'Brien et al. 1998). This experiment demonstrates a link between long-term changes in synaptic activity and AMPA receptor sorting and supports a model in which AMPA receptors are recycled to the synapse to offset decreased synaptic input or are trafficked to lysosomes and degraded to compensate for increased synaptic input (Ehlers 2000,O'Brien et al. 1998).

## Dendritic Exocytosis

Exocytosis requires that intracellular vesicles fuse with the plasma membrane to form a continuous lipid bilayer. Upon exocytosis, components inside lipid vesicles are released to the extracellular environment, and membrane ssociated proteins linked to the vesicle are transferred to the plasma membrane. Membrane fusion is not a spontaneous process but requires the concerted actions of many different factors first to tether a vesicle to the membrane, to prime it for release, and finally to fuse the distinct lipid bilayers into a contiguous membrane (Chen & Scheller 2001,Jahn et al. 2003). The conserved soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) family of proteins mediate this process in eukaryotic cells. Vesicle-bound SNARES (v-SNARES) form a complex with target membrane-associated SNARES (t-SNARES) through coiled-coil motifs in a process facilitated by molecular complexes assembled by members of the Rab family of GTPases. Studies on presynaptic neurotransmitter release, in which depolarization is coupled to exocytosis through $Ca^{2+}$ entry into the presynaptic terminal, have established many of the principles governing regulated fusion of intracellular vesicles with the plasma membrane. Many details of this mechanism have been worked out, including the identity of the SNARE proteins that form the core vesicle tethering complex, the role of synaptotagmin as a sensor that couples $Ca^{2+}$ influx to vesicle fusion, and the spatial organization of vesicle release (An & Zenisek 2004,Sudhof 2004).

In addition to presynaptic terminals, regulated exocytosis has also been observed near the postsynaptic membrane. A first indication came from experiments demonstrating that dendrites loaded with the styryl dye FM1-43 unload the dye in response to neural activity through a

process that requires $Ca^{2+}$ entry (Maletic-Savatic & Malinow 1998). Activity dependent unloading of FM1-43 also requires the activity of CaMKII, providing a link between exocytosis and the molecules involved in synaptic plasticity (Maletic-Savatic et al. 1998). Additional evidence for this link comes from the observations that brefeldin A, which disrupts the secretory pathway, impairs synaptic potentiation in hippocampal slices, as does postsynaptic loading of the membrane fusion inhibitors, N-ethylmaleimide and botulinum toxin (Broutman & Baudry 2001,Lledo et al. 1998). More direct evidence for the link between exocytosis and synaptic plasticity came from the observation that AMPA receptors are rapidly inserted into the postsynaptic plasma membrane in response to stimuli that trigger synapse potentiation (Ehlers 2000,Hayashi et al. 2000,Passafaro et al. 2001,Shi et al. 1999). However, the mechanisms of postsynaptic exocytosis remain unclear. For AMPA receptors, the sites of rapid accumulation following exocytosis depend on subunit composition; newly inserted GluR2 receptors appear closer to synapses than do recently inserted GluR1 receptors (Passafaro et al. 2001). Tomita et al. (2003) have characterized several proteins that facilitate AMPA receptor surface expression, including stargazin and other members of the transmembrane AMPA receptor regulatory protein (TARP) family. A recent study by Yoshihara et al. (2005) demonstrated that synaptotagmin 4 serves as a postsynaptic $Ca^{2+}$ sensor for membrane fusion of vesicles containing retrograde signals that enhance presynaptic neurotransmitter release and trigger synaptic growth of larval *Drosophila* neuromuscular synapses. Despite these initial clues, many questions remain. Which specific molecules tether postsynaptic vesicles to the plasma membrane? Which molecules link postsynaptic exocytosis to neural activity? Does exocytosis occur directly in dendritic spines? In the following section, we focus on the emerging cellular mechanisms of postsynaptic exocytosis, including several potential links between neural activity and postsynaptic exocytosis.

**Rab proteins and exocytosis—**The complexity of the intracellular membrane system requires mechanisms that ensure selective delivery of vesicle carriers to appropriate acceptor membranes. Members of the large Rab family of GTPases regulate trafficking specificity by assembling specific molecular complexes on membrane microdomains and facilitating interactions between protein complexes on donor and acceptor membranes (Mohrmann & van der Sluijs 1999). Although the mechanism for this process remains somewhat unclear, vesicle-associated Rab in its GTP-bound state probably mediates binding of vesicle-bound factors with proteins on the target membrane. When the appropriate combination of vesicle and target membrane proteins is realized, vesicle-associated Rab hydrolyzes its bound GTP, locking the vesicle in place for subsequent fusion. For example, at the presynaptic terminal, Rab3 on neurotransmitter vesicles may mediate vesicle priming through its interaction with RIM1, a presynaptic active zone protein (Dulubova et al. 2005,Wang et al. 1997).

Rab proteins also play a role in trafficking vesicles for postsynaptic exocytosis. Rab11 is a key component of recycling endosomes and was the first small GTPase discovered to be involved in trafficking through this compartment (Ullrich et al. 1996). In neurons, a dominant-negative version of Rab11a impairs NMDA receptor-dependent insertion of AMPA receptors into the plasma membrane, which indicates that one source of newly inserted synaptic protein is from recycling endosomes (Figure 5) (Park et al. 2004). Rab8, which is involved in trafficking cargo from the TGN to the cell surface, also seems to be involved in trafficking postsynaptic proteins to the spine surface because blocking Rab8 function disrupts delivery of postsynaptic AMPA receptors to the plasma membrane (Gerges et al. 2004). Disrupting postsynaptic Rab8 and Rab11 prevents activity-induced insertion of synaptic molecules into the plasma membrane, but it remains unclear if these postsynaptic Rab proteins are themselves regulated by neural activity. In the case of Rab5, which mediates endocytic trafficking from the plasma membrane to early endosomes, LTD-inducing stimuli promote GDP-GTP exchange and thereby activate Rab5, leading to internalization of AMPA receptors (Brown et al. 2005). Many factors

modulate Rab activity, including guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), making them attractive candidates for coupling neural activity to postsynaptic exocytosis.

**The exocyst—**Exactly where postsynaptic membrane proteins are first delivered to the cell surface remains an open question. In the case of inhibitory glycine receptors on spinal neurons, initial membrane insertion occurs on the soma (Rosenberg et al. 2001). In contrast, for AMPA receptors on hippocampal neurons, exocytosis of exogenously expressed receptors can occur at the dendritic plasma membrane near synapses (Passafaro et al. 2001). Recent experiments demonstrate that synaptic receptors recycle to and from the cell surface (Ehlers 2000,Luscher et al. 1999,Park et al. 2004,Shi et al. 1999), suggesting the presence of local sites of exocytosis within or near dendritic spines. Live imaging of neuro transmitter vesicle fusion at presynaptic terminals of retinal bipolar neurons revealed that exocytosis can occur repeatedly at the same site (Zenisek et al. 2000). Could there be similar exocytic zones on the other side of the synapse where postsynaptic components are preferentially delivered to the cell surface?

One candidate for directing exocytosis to distinct cellular domains is the exocyst, a large multiprotein complex consisting of eight members (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo 70, and Exo84) (Hsu et al. 2004,Lipschutz & Mostov 2002). In yeast, where the exocyst complex was first discovered, mutations in exocyst components block polar secretion and cause accumulation of intracellular secretory vesicles (Novick et al. 1980,Salminen & Novick 1989). Many of the components of the complex reside on the plasma membrane, which suggests that the exocyst complex determines the sites of vesicle fusion. Mutants of *Drosophila* Sec5, Sec6, and Sec15 have revealed a role for the exocyst complex in neurons (Mehta et al. 2005;Murthy et al. 2003,2005). Mutations in Sec5 and Sec6 disrupt insertion of membrane proteins into the plasma membrane and neurite out-growth in cultured cells (Murthy et al. 2003,2005). Exocytosis of neurotransmitter vesicles at the neuromuscular junction was normal in *sec*5 mutants, which demonstrated that not all forms of exocytosis depend on exocyst function (Murthy et al. 2003). Mutants in a different member of the exocyst complex, Sec15, display normal neurite outgrowth but defective axon targeting. Trafficking of the cell adhesion molecules fasciclin II and chaoptin, which are normally delivered to the cell surface, was disrupted in *sec*15 mutants. However, N-cadherin and flamingo trafficking was normal, which demonstrated that delivery of some, but not all, proteins to the plasma membrane depends on Sec15 function (Mehta et al. 2005). These findings imply that components of the exocyst complex may play distinct roles in trafficking various cell surface proteins in different cell types.

Components of the exocyst complex are also found in the mammalian nervous system (Hsu et al. 1996). In the developing rat brain, Sec6/8 is present in layers with ongoing synaptogenesis (Hazuka et al. 1999). In cultured hippocampal neurons, the exocyst is located at sites of membrane addition, including neurites, filopodia, and growth cones (Hazuka et al. 1999,Vega & Hsu 2001). A role for the exocyst in trafficking postsynaptic proteins in mammalian neurons was recently discovered. Specifically, the synapse-associated protein-102 (SAP102), a member of the MAGUK family of multivalent PDZ scaffolds, associates with the PDZ-binding domain of Sec8, and this interaction regulates NMDA receptor trafficking to the cell surface (Sans et al. 2003). In COS cells, the interaction between Sec8, SAP102, and unassembled NR2 subunits occurs in the ER, which suggests that components of the exocyst can associate with cargo early in the secretory pathway and may play a role in guiding secretory vesicles to sites of exocytosis, marked by other members of the exocyst complex. However, precisely where postsynaptic components are delivered to the cell surface and whether the exocyst complex directs vesicles to specific postsynaptic exocytic zones remain open questions.

**Membrane lipid composition and exocytosis—**In addition to protein factors, the lipid composition of plasma and vesicular membranes is also a critical determinant of exocytosis. The active zone of the presynaptic terminal, where neurotransmitter vesicles fuse with the plasma membrane, is rich in PI(4,5)P2, whereas intracellular neurotransmitter vesicles are rich in PI(4)P (Wenk & De Camilli 2004). Some proteins associated with neurotransmitter vesicles, including synaptotagmin and rabphilin, contain PI(4,5)P2-binding domains, which indicates that these factors direct synaptic vesicles to the PI(4,5)P2-rich active zone for release. Activezone proteins such as piccolo and Rim, which tether vesicles to the plasma membrane, also contain PI(4,5)P2-binding motifs, which indicates that membrane phospholipid composition plays an important role in establishing the spatial parameters of presynaptic vesicle fusion (Garner et al. 2000).

Recent evidence points to a role for the lipid composition of the postsynaptic membrane in regulating neurotransmitter receptor exocytosis. An inhibitor of phosphatidylinositol 3-kinase (PI3K), which converts PI(4,5)P2 to PI(3,4,5)P3, disrupted glycine-stimulated synaptic potentiation, but not NMDA-triggered synaptic depression, in cultured hippocampal neurons (Man et al. 2003). Loading neurons with PI3K increased the amplitude of mEPSCs by increasing the surface level of GluR2, which suggested that PI3K exerts its effect on synaptic potentiation by facilitating the exocytosis of neurotransmitter receptor. Endogenous PI3K is concentrated at synapses, where it interacts with AMPA receptors and is activated by NMDA receptor stimulation (Man et al. 2003). Furthermore, PI3K is known to be stimulated directly by $Ca^{2+}$-calmodulin and indirectly by CaMKII activation of Ras (Chen et al. 1998), providing a possible mechanism linking activity-induced $Ca^{2+}$ influx to changes in the postsynaptic plasma membrane lipid composition. However, because of limitations in tracking membrane phospholipid components at specific subcellular domains, we still know very little about the relationship between the various phosphoisoforms of PI and vesicle trafficking in dendritic spines.

## Balance Between Exocytosis and Endocytosis

Mature neurons retain their overall size and architecture for years, a remarkable feat considering that the proteins and lipids that make up the cell are being continually degraded and replenished. The morphological stability of neurons indicates that the overall balance between endo and exocytosis of lipids and cell-surface proteins must be tightly coupled to prevent overall changes in cell size and shape. However, close observation of dendritic spines has revealed that they are highly dynamic structures, growing and shrinking over time scales of seconds to minutes. Spine size correlates with AMPA receptor abundance and synaptic strength (Matsuzaki et al. 2001,Takumi et al. 1999). LTP-inducing stimuli trigger an increase in spine size, whereas LTD-inducing stimuli cause spines to shrink or even to disappear altogether (Lang et al. 2004,Matsuzaki et al. 2004,Nagerl et al. 2004,Ostroff et al. 2002,Zhou et al. 2004). Actin rearrangement is crucial for spine dynamics (Carlisle & Kennedy 2005,Ethell & Pasquale 2005,Oertner & Matus 2005), but intuitively, the growth and shrinkage of spines must also require membrane redistribution. Where does the membrane used for spine growth come from? The dendritic shaft, and in some cases, the spine head itself, houses an endomembrane network of ER, Golgi, endosomal compartments, and vesiculotubular organelles, which suggests that a local internal store of membrane could be the source (Cooney et al. 2002,Gardiol et al. 1999,Spacek & Harris 1997). Recent experiments demonstrating that exocytosis of cargo from recycling endosomes is increased by LTP-inducing stimuli suggest that spine growth could be a result of an increased exocytosis rate of endosome derived vesicles containing needed membrane or spine-promoting molecular material (Park et al. 2004). Because clathrin coat dynamics, and thus likely endocytosis itself, at spine endocytic zones is largely unaffected by neuronal activity (Blanpied et al. 2002), the ratio of the rates of

exocytosis/endocytosis probably increases upon synaptic potentiation, which could, in principle, result in a net addition of membrane to the spine head. In this regard, the rates of AMPA receptor endocytosis and recycling are exquisitely coupled under basal conditions or upon longer-term alterations in neuronal activity (Ehlers 2000), suggesting that any relative change in spine endocytosis or exocytosis is compensated for within several minutes to hours. Alternative to a requirement for exocytotic membrane delivery for spine growth, membrane may simply be recruited from lateral domains of the dendritic shaft. Indeed, membrane proteins can rapidly diffuse to and from postsynaptic regions (Borgdorff & Choquet 2002,Groc et al. 2004), and such diffusion coupled with internal actin rearrangements could account for spine membrane expansion. Resolving these possibilities remains a fundamentally important question for spine architecture.

Dendritic spines rarely exceed ~0.4 $\mu m^3$ in volume (Harris et al. 1992), raising the question, what sets the upper limit of spine size? One possible answer is that the available intracellular stores of membrane in proximity to the spine becomes depleted as spines grow. Another factor may be an intrinsic property of the spine itself. As a spine grows, the number of NMDA receptors only weakly correlates with spine size (Petralia et al. 1999,Racca et al. 2000,Takumi et al. 1999). Thus, for a given stimulus, intraspine $Ca^{2+}$ will be diluted in larger, more voluminous spines, compared with smaller, more compact spines. Additionally, $Ca^{2+}$ conductance through the spine neck may be greater in larger spines, resulting in a more rapid diffusion of $Ca^{2+}$ out the spine following NMDA receptor activation (Noguchi et al. 2005). Therefore, one possibility is that $Ca^{2+}$ may be quickly diluted in large, potentiated spines to a level below the threshold required to enhance membrane exocytosis or expand the spine actin network. Such an intrinsic geometric negative feedback mechanism could play a role in preventing runaway spine growth following synapse potentiation and set an upper limit for spine size. In addition to promoting spine growth, activity-dependent stimuli can reduce diffusional coupling between spines and the dendritic shaft (Bloodgood & Sabatini 2005), suggesting a feedback limitation on their ability to deliver membrane or spine growth-promoting material. In any case, tight coordination between membrane trafficking, calcium signaling, and actin dynamics is almost certainly crucial for controlling spine size and molecular composition.

## UNRESOLVED QUESTIONS AND FUTURE DIRECTIONS

The discovery that membrane trafficking underlies various forms of synaptic plasticity was a fundamental advance that has provided a focal point for experiments addressing the mechanisms of learning and memory and now raises numerous questions. Many core issues of synapse and neural circuit plasticity are boiling down to cell biology, where the regulation of multiprotein complexes and internal organelles provides the requisite integration of diverse signals and the requisite output for coordinating functional and structural plasticity. Although much recent work has focused on neurotransmitter receptor trafficking to and from the postsynaptic membrane, there is scant information regarding how other synaptic proteins, which may be equally important, are trafficked to and from synapses. For example, the level of postsynaptic proteins including PSD-95 and CamKII at the synapse determines the number of slots available for receptor insertion, which suggests that PSD size is a limiting factor that can govern changes in synaptic strength (Ehrlich & Malinow 2004,Lisman 2003,Schnell et al. 2002,Stein et al. 2003). Which components, besides glutamate receptors, are cycled to the membrane or added to the PSD in response to LTP-inducing stimulation? Which sensors couple neural activity to synaptic membrane trafficking? What controls the formation, location, and transport of dendritic organelles themselves? Although synaptic plasticity is an intriguing phenomenon, individual synapses can also display remarkable stability, persisting for months, years, or perhaps a life dtime (Zuo et al. 2005). How do neurons preserve individual spine

morphology and molecular content while the cell is continuously rebuilding itself? How are changes in synaptic strength confined and maintained at specific synapses? The answers to these questions are crucial steps for developing a true cell-biological understanding of synapse function in the context of learning, memory, and disease.

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## Glossary

PSD, postsynaptic density; LTP, long-term potentiation; LTD, long-term depression; ER, endoplasmic reticulum; TGN, trans-Golgi network; VSVG, vesicular stomatitis viral glycoprotein; Golgi outpost, discrete Golgi membrane located mainly in the apical dendrite that receives a fraction of newly released cargo from the dendritic ER; SER, smooth endoplasmic reticulum; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; NMDA, N-methyl D-aspartate; Apical domain, plasma membrane of polarized epithelial cells that faces the external environment (lumen); often characterized by microvilli and cilia; Basolateral domain, lateral and basal plasma membranes of polarized epithelial cells that face adjacent cells and the substratum; Transcytosis, intracellular movement of membrane, generated by endocytosis from one cellular domain to another; Diffusion barrier, restricts movement of membrane proteins, lipids and in some cases, soluble factors from one cellular domain to another; Spine endocytic zone, region of repeated clathrin assembly adjacent to, but not overlapping the PSD in the spine head; PI, phosphatidylinositol; Lipid raft, membrane microdomain rich in cholesterol and glycolipids that recruits a subset of membrane-associated proteins; Ubiquitin, a polypeptide that is covalently linked to proteins and targets them for degradation by the ubiquitin proteasome system.

## LITERATURE CITED

Adams I, Jones DG. Quantitative ultrastructural changes in rat cortical synapses during early-, mid- and late-adulthood. Brain Res 1982;239:349–63. [PubMed: 7093695]

Adesnik H, Nicoll RA, England PM. Photoinactivation of native AMPA receptors reveals their real-time trafficking. Neuron 2005;48:977–85. [PubMed: 16364901]

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;29:313–19. [PubMed: 15911354]

Amparan D, Avram D, Thomas CG, Lindahl MG, Yang J, et al. Direct interaction of myosin regulatory light chain with the NMDA receptor. J Neurochem 2005;92:349–61. [PubMed: 15663482]

An S, Zenisek D. Regulation of exocytosis in neurons and neuroendocrine cells. Curr Opin Neurobiol 2004;14:522–30. [PubMed: 15464884]

Antonny B, Schekman R. ER export: public transportation by the COPII coach. Curr Opin Cell Biol 2001;13:438–43. [PubMed: 11454450]

Baas PW, Deitch JS, Black MM, Banker GA. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. Proc Natl Acad Sci USA 1988;85:8335–39. [PubMed: 3054884]

Bagal AA, Kao JP, Tang CM, Thompson SM. Long-term potentiation of exogenous glutamate responses at single dendritic spines. Proc Natl Acad Sci USA 2005;102:14434–39. [PubMed: 16186507]

Bartles JR, Feracci HM, Stieger B, Hubbard AL. Biogenesis of the rat hepatocyte plasma membrane in vivo: comparison of the pathways taken by apical and basolateral proteins using subcellular fractionation. J Cell Biol 1987;105:1241–51. [PubMed: 3654750]

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, et al. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat Neurosci 2000;3:1291–300. [PubMed: 11100150]

Bergmann JE. Using temperature-sensitive mutants of VSV to study membrane protein biogenesis. Methods Cell Biol 1989;32:85–110. [PubMed: 2558277]

Blanpied TA, Scott DB, Ehlers MD. Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. Neuron 2002;36:435–49. [PubMed: 12408846]

Bloodgood BL, Sabatini BL. Neuronal activity regulates diffusion across the neck of dendritic spines. Science 2005;310:866–69. [PubMed: 16272125]

Borgdorff AJ, Choquet D. Regulation of AMPA receptor lateral movements. Nature 2002;417:649–53. [PubMed: 12050666]

Brady ST. A novel brain ATPase with properties expected for the fast axonal transport motor. Nature 1985;317:73–75. [PubMed: 2412134]

Bredt DS, Nicoll RA. AMPA receptor trafficking at excitatory synapses. Neuron 2003;40:361–79. [PubMed: 14556714]

Bresler T, Shapira M, Boeckers T, Dresbach T, Futter M, et al. Postsynaptic density assembly is fundamentally different from presynaptic active zone assembly. J Neurosci 2004;24:1507–20. [PubMed: 14960624]

Bridgman PC. Myosin-dependent transport in neurons. J Neurobiol 2004;58:164–74. [PubMed: 14704949]

Broutman G, Baudry M. Involvement of the secretory pathway for AMPA receptors in NMDA-induced potentiation in hippocampus. J Neurosci 2001;21:27–34. [PubMed: 11150316]

Brown JR, Stafford P, Langford GM. Short-range axonal/dendritic transport by myosin-V: a model for vesicle delivery to the synapse. J Neurobiol 2004;58:175–88. [PubMed: 14704950]

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]

Burack MA, Silverman MA, Banker G. The role of selective transport in neuronal protein sorting. Neuron 2000;26:465–72. [PubMed: 10839364]

Burbea M, Dreier L, Dittman JS, Grunwald ME, Kaplan JM. Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in C. elegans. Neuron 2002;35:107–20. [PubMed: 12123612]

Burrone J, Murthy VN. Synaptic gain control and homeostasis. Curr Opin Neurobiol 2003;13:560–67. [PubMed: 14630218]

Butler MH, David C, Ochoa GC, Freyberg Z, Daniell L, et al. Amphiphysin II (SH3P9; BIN1), a member of the amphiphysin/Rvs family, is concentrated in the cortical cytomatrix of axon initial segments and nodes of ranvier in brain and around T tubules in skeletal muscle. J Cell Biol 1997;137:1355–67. [PubMed: 9182667]

Cameron HA, Kaliszewski CK, Greer CA. Organization of mitochondria in olfactory bulb granule cell dendritic spines. Synapse 1991a;8:107–18. [PubMed: 1715612]

Cameron PL, Sudhof TC, Jahn R, De Camilli P. Colocalization of synaptophysin with transferrin receptors: implications for synaptic vesicle biogenesis. J Cell Biol 1991b;115:151–64. [PubMed: 1918133]

Carlisle HJ, Kennedy MB. Spine architecture and synaptic plasticity. Trends Neurosci 2005;28:182–87. [PubMed: 15808352]

Carroll RC, Beattie EC, von Zastrow M, Malenka RC. Role of AMPA receptor endocytosis in synaptic plasticity. Nat Rev Neurosci 2001;2:315–24. [PubMed: 11331915]

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC. Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci 1999;2:454–60. [PubMed: 10321250]

Chen HJ, Rojas-Soto M, Oguni A, Kennedy MB. A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. Neuron 1998;20:895–904. [PubMed: 9620694]

Chen YA, Scheller RH. SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol 2001;2:98–106. [PubMed: 11252968]

Colledge M, Snyder EM, Crozier RA, Soderling JA, Jin Y, et al. Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. Neuron 2003;40:595–607. [PubMed: 14642282]

Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. J Neurosci 2002;22:2215–24. [PubMed: 11896161]

Cottrell JR, Borok E, Horvath TL, Nedivi E. CPG2: a brain- and synapse-specific protein that regulates the endocytosis of glutamate receptors. Neuron 2004;44:677–90. [PubMed: 15541315]

Dautry-Varsat A, Ciechanover A, Lodish HF. pH and the recycling of transferrin during receptor-mediated endocytosis. Proc Natl Acad Sci USA 1983;80:2258–62. [PubMed: 6300903]

Davis GW, Bezprozvanny I. Maintaining the stability of neural function: a homeostatic hypothesis. Annu Rev Physiol 2001;63:847–69. [PubMed: 11181978]

de Anda FC, Pollarolo G, Da Silva JS, Camoletto PG, Feiguin F, Dotti CG. Centrosome localization determines neuronal polarity. Nature 2005;436:704–8. [PubMed: 16079847]

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;279:35687–91. [PubMed: 15187090]

de Hoop MJ, Huber LA, Stenmark H, Williamson E, Zerial M, et al. The involvement of the small GTP-binding protein Rab5a in neuronal endocytosis. Neuron 1994;13:11–22. [PubMed: 8043272]

Deller T, Korte M, Chabanis S, Drakew A, Schwegler H, et al. Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. Proc Natl Acad Sci USA 2003;100:10494–99. [PubMed: 12928494]

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]

Dulubova I, Lou X, Lu J, Huryeva I, Alam A, et al. A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? EMBO J 2005;24:2839–50. [PubMed: 16052212]

Dunn KW, McGraw TE, Maxfield FR. Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. J Cell Biol 1989;109:3303–14. [PubMed: 2600137]

Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron 2000;28:511–25. [PubMed: 11144360]

Ehlers MD. Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. Nat Neurosci 2003;6:231–42. [PubMed: 12577062]

Ehrlich I, Malinow R. Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. J Neurosci 2004;24:916–27. [PubMed: 14749436]

Ethell IM, Pasquale EB. Molecular mechanisms of dendritic spine development and remodeling. Prog Neurobiol 2005;75:161–205. [PubMed: 15882774]

Fishman PS, Farrand DA, Kristt DA. Internalization of plasma proteins by cerebellar Purkinje cells. J Neurol Sci 1990;100:43–49. [PubMed: 1708409]

Fourgeaud L, Bessis AS, Rossignol F, Pin JP, Olivo-Marin JC, Hemar A. The metabotropic glutamate receptor mGluR5 is endocytosed by a clathrin-independent path-way. J Biol Chem 2003;278:12222–30. [PubMed: 12529370]

Gardiol A, Racca C, Triller A. Dendritic and postsynaptic protein synthetic machinery. J Neurosci 1999;19:168–79. [PubMed: 9870948]

Garner CC, Kindler S, Gundelfinger ED. Molecular determinants of presynaptic active zones. Curr Opin Neurobiol 2000;10:321–27. [PubMed: 10851173]

Gerges NZ, Backos DS, Esteban JA. Local control of AMPA receptor trafficking at the postsynaptic terminal by a small GTPase of the Rab family. J Biol Chem 2004;279:43870–78. [PubMed: 15297461]

Golgi C. Intorno alla struttura delle cellule nervose. Bollettino della Società Medico-Chirurgica di Pavia 1898;13:3–16.

Gray EG. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. J Anat 1959;93:420–33. [PubMed: 13829103]

Gray EG, Guillery RW. A note on the dendritic spine apparatus. J Anat 1963;97:389–92. [PubMed: 14047356]

Gray NW, Fourgeaud L, Huang B, Chen J, Cao H, et al. Dynamin 3 is a component of the postsynapse, where it interacts with mGluR5 and Homer. Curr Biol 2003;13:510–15. [PubMed: 12646135]

Gray NW, Kruchten AE, Chen J, McNiven MA. A dynamin-3 spliced variant modulates the actin/cortactin-dependent morphogenesis of dendritic spines. J Cell Sci 2005;118:1279–90. [PubMed: 15741233]

Groc L, Heine M, Cognet L, Brickley K, Stephenson FA, et al. Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nat Neu-rosci 2004;7:695–96.

Guillaud L, Setou M, Hirokawa N. KIF17 dynamics and regulation of NR2B trafficking in hippocampal neurons. J Neurosci 2003;23:131–40. [PubMed: 12514209]

Hanley JG, Henley JM. PICK1 is a calcium-sensor for NMDA-induced AMPA receptor trafficking. EMBO J 2005;24:3266–78. [PubMed: 16138078]

Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci 1992;12:2685–705. [PubMed: 1613552]

Hasson T. Myosin VI: two distinct roles in endocytosis. J Cell Sci 2003;116:3453–61. [PubMed: 12893809]

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 2000;287:2262–67. [PubMed: 10731148]

Hazuka CD, Foletti DL, Hsu SC, Kee Y, Hopf FW, Scheller RH. The sec6/8 complex is located at neurite outgrowth and axonal synapse-assembly domains. J Neurosci 1999;19:1324–34. [PubMed: 9952410]

Hering H, Lin CC, Sheng M. Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J Neurosci 2003;23:3262–71. [PubMed: 12716933]

Hicke L, Dunn R. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu Rev Cell Dev Biol 2003;19:141–72. [PubMed: 14570567]

Hirokawa N, Takemura R. Molecular motors and mechanisms of directional transport in neurons. Nat Rev Neurosci 2005;6:201–14. [PubMed: 15711600]

Hong W. Protein transport from the endoplasmic reticulum to the Golgi apparatus. J Cell Sci 1998;111 (Pt 19):2831–39. [PubMed: 9730976]

Hoogenraad CC, Milstein AD, Ethell IM, Henkemeyer M, Sheng M. GRIP1 controls dendrite morphogenesis by regulating EphB receptor trafficking. Nat Neurosci 2005;8:906–15. [PubMed: 15965473]

Horton AC, Ehlers MD. Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. J Neurosci 2003a;23:6188–99. [PubMed: 12867502]

Horton AC, Ehlers MD. Neuronal polarity and trafficking. Neuron 2003b;40:277–95. [PubMed: 14556709]

Horton AC, Ehlers MD. Secretory trafficking in neuronal dendrites. Nat Cell Biol 2004;6:585–91. [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;48:757–71. [PubMed: 16337914]

Howe CL, Valletta JS, Rusnak AS, Mobley WC. NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway. Neuron 2001;32:801–14. [PubMed: 11738027]

Hsu SC, TerBush D, Abraham M, Guo W. The exocyst complex in polarized exocytosis. Int Rev Cytol 2004;233:243–65. [PubMed: 15037366]

Hsu SC, Ting AE, Hazuka CD, Davanger S, Kenny JW, et al. The mammalian brain rsec6/8 complex. Neuron 1996;17:1209–19. [PubMed: 8982167]

Jahn R, Lang T, Sudhof TC. Membrane fusion. Cell 2003;112:519–33. [PubMed: 12600315]

Jareb M, Banker G. The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. Neuron 1998;20:855–67. [PubMed: 9620691]

Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, et al. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nat Neurosci 2004;7:244–53. [PubMed: 14770185]

Kacharmina JE, Job C, Crino P, Eberwine J. Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. Proc Natl Acad Sci USA 2000;97:11545–50. [PubMed: 11027353]

Kanaani J, Diacovo MJ, El-Husseini Ael D, Bredt DS, Baekkeskov S. Palmitoylation controls trafficking of GAD65 from Golgi membranes to axon-specific endosomes and a Rab5a-dependent pathway to presynaptic clusters. J Cell Sci 2004;117:2001–13. [PubMed: 15039456]

Keller P, Simons K. Post-golgi biosynthetic trafficking. J Cell Sci 1997;110(Pt 24):3001–9. [PubMed: 9365270]

Keller P, Toomre D, Diaz E, White J, Simons K. Multicolour imaging of post-golgi sorting and trafficking in live cells. Nat Cell Biol 2001;3:140–49. [PubMed: 11175746]

Kirchhausen T. Clathrin. Annu Rev Biochem 2000;69:699–727. [PubMed: 10966473]

Kleizen B, Braakman I. Protein folding and quality control in the endoplasmic reticulum. Curr Opin Cell Biol 2004;16:343–49. [PubMed: 15261665]

Kobayashi T, Storrie B, Simons K, Dotti CG. A functional barrier to movement of lipids in polarized neurons. Nature 1992;359:647–50. [PubMed: 1406997]

Kordeli E, Lambert S, Bennett V. AnkyrinG. A new ankyrin gene with neural-specific iso-forms localized at the axonal initial segment and node of Ranvier. J Biol Chem 1995;270:2352–59. [PubMed: 7836469]

Kreitzer G, Schmoranzer J, Low SH, Li X, Gan Y, et al. Three-dimensional analysis of post-golgi carrier exocytosis in epithelial cells. Nat Cell Biol 2003;5:126–36. [PubMed: 12545172]

Kuehn MJ, Schekman R. COPII and secretory cargo capture into transport vesicles. Curr Opin Cell Biol 1997;9:477–83. [PubMed: 9261052]

Lang C, Barco A, Zablow L, Kandel ER, Siegelbaum SA, Zakharenko SS. Transient expansion of synaptically connected dendritic spines upon induction of hippocampal long-term potentiation. Proc Natl Acad Sci USA 2004;101:16665–70. [PubMed: 15542587]

Lavezzari G, McCallum J, Dewey CM, Roche KW. Subunit-specific regulation of NMDA receptor endocytosis. J Neurosci 2004;24:6383–91. [PubMed: 15254094]

Lavezzari G, McCallum J, Lee R, Roche KW. Differential binding of the AP-2 adaptor complex and PSD-95 to the C-terminus of the NMDA receptor subunit NR2B regulates surface expression. Neuropharmacology 2003;45:729–37. [PubMed: 14529712]

Lee MC, Miller EA, Goldberg J, Orci L, Schekman R. Bi-directional protein transport between the ER and golgi. Annu Rev Cell Dev Biol 2004a;20:87–123. [PubMed: 15473836]

Lee SH, Liu L, Wang YT, Sheng M. Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 2002;36:661–74. [PubMed: 12441055]

Lee SH, Simonetta A, Sheng M. Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. Neuron 2004b;43:221–36. [PubMed: 15260958]

Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science 2005;308:512–17. [PubMed: 15845844]

Levine T, Rabouille C. Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Curr Opin Cell Biol 2005;17:362–68. [PubMed: 15975783]

Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell 2004;119:873–87. [PubMed: 15607982]

Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, et al. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nat Neurosci 2000;3:1282–90. [PubMed: 11100149]

Lippincott-Schwartz J, Roberts TH, Hirschberg K. Secretory protein trafficking and organelle dynamics in living cells. Annu Rev Cell Dev Biol 2000;16:557–89. [PubMed: 11031247]

Lipschutz JH, Mostov KE. Exocytosis: the many masters of the exocyst. Curr Biol 2002;12:R212–14. [PubMed: 11909549]

Lisanti MP, Caras IW, Davitz MA, Rodriguez-Boulan E. A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. J Cell Biol 1989;109:2145–56. [PubMed: 2478564]

Lisman J. Long-term potentiation: outstanding questions and attempted synthesis. Philos Trans R Soc London B Biol Sci 2003;358:829–42. [PubMed: 12740130]

Lledo PM, Zhang X, Sudhof TC, Malenka RC, Nicoll RA. Postsynaptic membrane fusion and long-term potentiation. Science 1998;279:399–403. [PubMed: 9430593]

Lu W, Ziff EB. PICK1 interacts with ABP/GRIP to regulate AMPA receptor trafficking. Neuron 2005;47:407–21. [PubMed: 16055064]

Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, et al. Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron 1999;24:649–58. [PubMed: 10595516]

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004;44:5–21. [PubMed: 15450156]

Maletic-Savatic M, Koothan T, Malinow R. Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part II: mediation by calcium/calmodulin-dependent protein kinase II. J Neurosci 1998;18:6814–21. [PubMed: 9712652]

Maletic-Savatic M, Malinow R. Calcium-evoked dendritic exocytosis in cultured hip-pocampal neurons. Part I: trans-golgi network-derived organelles undergo regulated exocytosis. J Neurosci 1998;18:6803–13. [PubMed: 9712651]

Man HY, Wang Q, Lu WY, Ju W, Ahmadian G, et al. Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. Neuron 2003;38:611–24. [PubMed: 12765612]

Martin S, Henley JM. Activity-dependent endocytic sorting of kainate receptors to recycling or degradation pathways. EMBO J 2004;23:4749–59. [PubMed: 15549132]

Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci 2001;4:1086–92. [PubMed: 11687814]

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. Nature 2004;429:761–66. [PubMed: 15190253]

Maxfield FR, McGraw TE. Endocytic recycling. Nat Rev Mol Cell Biol 2004;5:121–32. [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;121:1257–69. [PubMed: 8509447]

McNiven MA, Kim L, Krueger EW, Orth JD, Cao H, Wong TW. Regulated interactions between dynamin and the actin-binding protein cortactin modulate cell shape. J Cell Biol 2000;151:187–98. [PubMed: 11018064]

Mehta SQ, Hiesinger PR, Beronja S, Zhai RG, Schulze KL, et al. Mutations in Drosophila sec15 reveal a function in neuronal targeting for a subset of exocyst components. Neuron 2005;46:219–32. [PubMed: 15848801]

Mellman I. Endocytosis and molecular sorting. Annu Rev Cell Dev Biol 1996;12:575–25. [PubMed: 8970738]

Merrifield CJ, Perrais D, Zenisek D. Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. Cell 2005;121:593–606. [PubMed: 15907472]

Miki H, Setou M, Kaneshiro K, Hirokawa N. All kinesin superfamily protein, KIF, genes in mouse and human. Proc Natl Acad Sci USA 2001;98:7004–11. [PubMed: 11416179]

Mohrmann K, van der Sluijs P. Regulation of membrane transport through the endocytic pathway by rabGTPases. Mol Membr Biol 1999;16:81–87. [PubMed: 10332741]

Mok H, Shin H, Kim S, Lee JR, Yoon J, Kim E. Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins. J Neurosci 2002;22:5253–58. [PubMed: 12097473]

Momiyama A, Silver RA, Hausser M, Notomi T, Wu Y, et al. The density of AMPA receptors activated by a transmitter quantum at the climbing fiber-Purkinje cell synapse in immature rats. J Physiol 2003;549:75–92. [PubMed: 12665613]

Mostov K, Su T, ter Beest M. Polarized epithelial membrane traffic: conservation and plasticity. Nat Cell Biol 2003;5:287–93. [PubMed: 12669082]

Mu Y, Otsuka T, Horton AC, Scott DB, Ehlers MD. Activity-dependent mRNA splicing controls ER export and synaptic delivery of NMDA receptors. Neuron 2003;40:581–94. [PubMed: 14642281]

Mundigl O, Matteoli M, Daniell L, Thomas-Reetz A, Metcalf A, et al. Synaptic vesicle proteins and early endosomes in cultured hippocampal neurons: differential effects of Brefeldin A in axon and dendrites. J Cell Biol 1993;122:1207–21. [PubMed: 8376458]

Murthy M, Garza D, Scheller RH, Schwarz TL. Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists. Neuron 2003;37:433–47. [PubMed: 12575951]

Murthy M, Ranjan R, Denef N, Higashi ME, Schupbach T, Schwarz TL. Sec6 mutations and the Drosophila exocyst complex. J Cell Sci 2005;118:1139–50. [PubMed: 15728258]

Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron 2004;44:759–67. [PubMed: 15572108]

Naisbitt S, Kim E, Tu JC, Xiao B, Sala C, et al. Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. Neuron 1999;23:569–82. [PubMed: 10433268]

Naisbitt S, Valtschanoff J, Allison DW, Sala C, Kim E, et al. Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of myosin-V and dynein. J Neurosci 2000;20:4524–34. [PubMed: 10844022]

Nakada C, Ritchie K, Oba Y, Nakamura M, Hotta Y, et al. Accumulation of anchored proteins forms membrane diffusion barriers during neuronal polarization. Nat Cell Biol 2003;5:626–32. [PubMed: 12819789]

Nichols B. Caveosomes and endocytosis of lipid rafts. J Cell Sci 2003;116:4707–14. [PubMed: 14600257]

Nichols BJ, Lippincott-Schwartz J. Endocytosis without clathrin coats. Trends Cell Biol 2001;11:406–12. [PubMed: 11567873]

Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H. Spine-neck geometry determines NMDA receptor-dependent Ca2+ signaling in dendrites. Neuron 2005;46:609–22. [PubMed: 15944129]

Novick P, Field C, Schekman R. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 1980;21:205–15. [PubMed: 6996832]

Nusser Z, Lujan R, Laube G, Roberts JD, Molnar E, Somogyi P. Cell type and path-way dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron 1998;21:545–59. [PubMed: 9768841]

O'Brien RJ, Kamboj S, Ehlers MD, Rosen KR, Fischbach GD, Huganir RL. Activity-dependent modulation of synaptic AMPA receptor accumulation. Neuron 1998;21:1067–78. [PubMed: 9856462]

Oertner TG, Matus A. Calcium regulation of actin dynamics in dendritic spines. Cell Calcium 2005;37:477–82. [PubMed: 15820396]

Osterweil E, Wells DG, Mooseker MS. A role for myosin VI in postsynaptic structure and glutamate receptor endocytosis. J Cell Biol 2005;168:329–38. [PubMed: 15657400]

Ostroff LE, Fiala JC, Allwardt B, Harris KM. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. Neuron 2002;35:535–45. [PubMed: 12165474]

Pak DT, Sheng M. Targeted protein degradation and synapse remodeling by an inducible protein kinase. Science 2003;302:1368–73. [PubMed: 14576440]

Palmer CL, Lim W, Hastie PG, Toward M, Korolchuk VI, et al. Hippocalcin functions as a calcium sensor in hippocampal LTD. Neuron 2005;47:487–94. [PubMed: 16102532]

Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. Am J Physiol 1951;167:13–46. [PubMed: 14885465]

Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. Science 2004;305:1972–75. [PubMed: 15448273]

Passafaro M, Piech V, Sheng M. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 2001;4:917–26. [PubMed: 11528423]

Perez-Otano I, Ehlers MD. Homeostatic plasticity and NMDA receptor trafficking. Trends Neurosci 2005;28:229–38. [PubMed: 15866197]

Peters A, Proskauer CC, Kaiserman-Abramof IR. The small pyramidal neuron of the rat cerebral cortex. The axon hillock and initial segment. J Cell Biol 1968;39:604–19. [PubMed: 5699934]

Petralia RS, Esteban JA, Wang YX, Partridge JG, Zhao HM, et al. Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. Nat Neurosci 1999;2:31–36. [PubMed: 10195177]

Petralia RS, Wang YX, Wenthold RJ. Internalization at glutamatergic synapses during development. Eur J Neurosci 2003;18:3207–17. [PubMed: 14686895]

Pierce JP, Mayer T, McCarthy JB. Evidence for a satellite secretory pathway in neuronal dendritic spines. Curr Biol 2001;11:351–55. [PubMed: 11267872]

Pippig S, Andexinger S, Lohse MJ. Sequestration and recycling of beta 2-adrenergic receptors permit receptor resensitization. Mol Pharmacol 1995;47:666–76. [PubMed: 7723728]

Popov V, Medvedev NI, Davies HA, Stewart MG. Mitochondria form a filamentous reticular network in hippocampal dendrites but are present as discrete bodies in axons: a three-dimensional ultrastructural study. J Comp Neurol 2005;492:50–65. [PubMed: 16175555]

Prekeris R, Foletti DL, Scheller RH. Dynamics of tubulovesicular recycling endosomes in hippocampal neurons. J Neurosci 1999;19:10324–37. [PubMed: 10575030]

Presley JF, Cole NB, Schroer TA, Hirschberg K, Zaal KJ, Lippincott-Schwartz J. ER-to-golgi transport visualized in living cells. Nature 1997;389:81–85. [PubMed: 9288971]

Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ. The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron 2005;47:845–57. [PubMed: 16157279]

Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P. NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J Neurosci 2000;20:2512–22. [PubMed: 10729331]

Racz B, Blanpied TA, Ehlers MD, Weinberg RJ. Lateral organization of endocytic machinery in dendritic spines. Nat Neurosci 2004;7:917–18. [PubMed: 15322548]

Racz B, Weinberg RJ. The subcellular organization of cortactin in hippocampus. J Neurosci 2004;24:10310–17. [PubMed: 15548644]

Raiborg C, Rusten TE, Stenmark H. Protein sorting into multivesicular endosomes. Curr Opin Cell Biol 2003;15:446–55. [PubMed: 12892785]

Rao A, Craig AM. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. Neuron 1997;19:801–12. [PubMed: 9354327]

Roche KW, Standley S, McCallum J, Dune Ly C, Ehlers MD, Wenthold RJ. Molecular determinants of NMDA receptor internalization. Nat Neurosci 2001;4:794–802. [PubMed: 11477425]

Rodriguez-Boulan E, Kreitzer G, Musch A. Organization of vesicular trafficking in epithelia. Nat Rev Mol Cell Biol 2005;6:233–47. [PubMed: 15738988]

Rosenberg M, Meier J, Triller A, Vannier C. Dynamics of glycine receptor insertion in the neuronal plasma membrane. J Neurosci 2001;21:5036–44. [PubMed: 11438579]

Saito N, Okada Y, Noda Y, Kinoshita Y, Kondo S, Hirokawa N. KIFC2 is a novel neuron-specific C-terminal type kinesin superfamily motor for dendritic transport of multivesicular body-like organelles. Neuron 1997;18:425–38. [PubMed: 9115736]

Salminen A, Novick PJ. The Sec15 protein responds to the function of the GTP binding protein, Sec4, to control vesicular traffic in yeast. J Cell Biol 1989;109:1023–36. [PubMed: 2504727]

Sampo B, Kaech S, Kunz S, Banker G. Two distinct mechanisms target membrane proteins to the axonal surface. Neuron 2003;37:611–24. [PubMed: 12597859]

Sans N, Prybylowski K, Petralia RS, Chang K, Wang YX, et al. NMDA receptor trafficking through an interaction between PDZ proteins and the exocyst complex. Nat Cell Biol 2003;5:520–30. [PubMed: 12738960]

Schnell E, Nicoll RA. Hippocampal synaptic transmission and plasticity are preserved in myosin Va mutant mice. J Neurophysiol 2001;85:1498–501. [PubMed: 11287473]

Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc Natl Acad Sci USA 2002;99:13902–7. [PubMed: 12359873]

Scott DB, Michailidis I, Mu Y, Logothetis D, Ehlers MD. Endocytosis and degradative sorting of NMDA receptors by conserved membrane-proximal signals. J Neurosci 2004;24:7096–09. [PubMed: 15306643]

Setou M, Nakagawa T, Seog DH, Hirokawa N. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science 2000;288:1796–802. [PubMed: 10846156]

Setou M, Seog DH, Tanaka Y, Kanai Y, Takei Y, et al. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. Nature 2002;417:83–87. [PubMed: 11986669]

Sheng M. Molecular organization of the postsynaptic specialization. Proc Natl Acad Sci USA 2001;98:7058–61. [PubMed: 11416187]

Shenoy SK, Lefkowitz RJ. Trafficking patterns of beta-arrestin and G protein-coupled receptors determined by the kinetics of beta-arrestin deubiquitination. J Biol Chem 2003;278:14498–506. [PubMed: 12574160]

Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science 2001;294:1307–13. [PubMed: 11588219]

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, et al. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 1999;284:1811–16. [PubMed: 10364548]

Sibley DR, Strasser RH, Benovic JL, Daniel K, Lefkowitz RJ. Phosphorylation/dephosphorylation of the beta-adrenergic receptor regulates its functional coupling to adenylate cyclase and subcellular distribution. Proc Natl Acad Sci USA 1986;83:9408–12. [PubMed: 3025843]

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;29:173–80. [PubMed: 15911342]

Sola M, Bavro VN, Timmins J, Franz T, Ricard-Blum S, et al. Structural basis of dynamic glycine receptor clustering by gephyrin. EMBO J 2004;23:2510–19. [PubMed: 15201864]

Sorkin A, Von Zastrow M. Signal transduction and endocytosis: close encounters of many kinds. Nat Rev Mol Cell Biol 2002;3:600–14. [PubMed: 12154371]

Spacek J, Harris KM. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. J Neurosci 1997;17:190–203. [PubMed: 8987748]

Stein V, House DR, Bredt DS, Nicoll RA. Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. J Neu-rosci 2003;23:5503–6.

Steiner P, Alberi S, Kulangara K, Yersin A, Sarria JC, et al. Interactions between NEEP21, GRIP1 and GluR2 regulate sorting and recycling of the glutamate receptor subunit GluR2. EMBO J 2005;24:2873–84. [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;157:1197–209. [PubMed: 12070131]

Steward O, Schuman EM. Protein synthesis at synaptic sites on dendrites. Annu Rev Neurosci 2001;24:299–325. [PubMed: 11283313]

Sudhof TC. The synaptic vesicle cycle. Annu Rev Neurosci 2004;27:509–47. [PubMed: 15217342]

Sutton MA, Schuman EM. Local translational control in dendrites and its role in long-term synaptic plasticity. J Neurobiol 2005;64:116–31. [PubMed: 15883999]

Takagishi Y, Oda S, Hayasaka S, Dekker-Ohno K, Shikata T, et al. The dilute-lethal (dl) gene attacks a Ca2+ store in the dendritic spine of Purkinje cells in mice. Neurosci Lett 1996;215:169–72. [PubMed: 8899740]

Takumi Y, Ramirez-Leon V, Laake P, Rinvik E, Ottersen OP. Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat Neurosci 1999;2:618–24. [PubMed: 10409387]

Tanaka J, Matsuzaki M, Tarusawa E, Momiyama A, Molnar E, et al. Number and density of AMPA receptors in single synapses in immature cerebellum. J Neurosci 2005;25:799–807. [PubMed: 15673659]

Thiagarajan TC, Lindskog M, Tsien RW. Adaptation to synaptic inactivity in hippocamal neurons. Neuron 2005;47:725–37. [PubMed: 16129401]

Tomita S, Chen L, Kawasaki Y, Petralia RS, Wenthold RJ, et al. Functional studies and distribution define a family of transmembrane AMPA receptor regulatory proteins. J Cell Biol 2003;161:805–16. [PubMed: 12771129]

Torre ER, Steward O. Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. J Neurosci 1996;16:5967–78. [PubMed: 8815879]

Tuma PL, Hubbard AL. Transcytosis: crossing cellular barriers. Physiol Rev 2003;83:871–932. [PubMed: 12843411]

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB. Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 1998;391:892–96. [PubMed: 9495341]

Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 2004;5:97–107. [PubMed: 14735113]

Ullrich O, Reinsch S, Urbe S, Zerial M, Parton RG. Rab11 regulates recycling through the pericentriolar recycling endosome. J Cell Biol 1996;135:913–24. [PubMed: 8922376]

Urrutia R, Henley JR, Cook T, McNiven MA. The dynamins: redundant or distinct functions for an expanding family of related GTPases? Proc Natl Acad Sci USA 1997;94:377–84. [PubMed: 9012790]

Vale RD, Fletterick RJ. The design plan of kinesin motors. Annu Rev Cell Dev Biol 1997;13:745–77. [PubMed: 9442886]

Vale RD, Reese TS, Sheetz MP. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 1985;42:39–50. [PubMed: 3926325]

van der Bliek AM, Meyerowitz EM. Dynamin-like protein encoded by the Drosophila shibire gene associated with vesicular traffic. Nature 1991;351:411–14. [PubMed: 1674590]

van der Bliek AM, Redelmeier TE, Damke H, Tisdale EJ, Meyerowitz EM, Schmid SL. Mutations in human dynamin block an intermediate stage in coated vesicle formation. J Cell Biol 1993;122:553–63. [PubMed: 8101525]

van Meer G, Simons K. Lipid polarity and sorting in epithelial cells. J Cell Biochem 1988;36:51–58. [PubMed: 3277985]

Vega IE, Hsu SC. The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. J Neurosci 2001;21:3839–48. [PubMed: 11356872]

Verkhratsky A. The endoplasmic reticulum and neuronal calcium signaling. Cell Calcium 2002;32:393–404. [PubMed: 12543098]

Wang Y, Okamoto M, Schmitz F, Hofmann K, Sudhof TC. Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. Nature 1997;388:593–98. [PubMed: 9252191]

Washbourne P, Liu XB, Jones EG, McAllister AK. Cycling of NMDA receptors during trafficking in neurons before synapse formation. J Neurosci 2004;24:8253–64. [PubMed: 15385609]

Weaver AM, Karginov AV, Kinley AW, Weed SA, Li Y, et al. Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. Curr Biol 2001;11:370–74. [PubMed: 11267876]

Wells AL, Lin AW, Chen LQ, Safer D, Cain SM, et al. Myosin VI is an actin-based motor that moves backwards. Nature 1999;401:505–8. [PubMed: 10519557]

Wenk MR, De Camilli P. Protein-lipid interactions and phosphoinositide metabolism in membrane traffic: insights from vesicle recycling in nerve terminals. Proc Natl Acad Sci USA 2004;101:8262–69. [PubMed: 15146067]

West AE, Neve RL, Buckley KM. Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. J Neurosci 1997;17:6038–47. [PubMed: 9236215]

Wiley HS, Burke PM. Regulation of receptor tyrosine kinase signaling by endocytic trafficking. Traffic 2001;2:12–18. [PubMed: 11208164]

Winckler B, Forscher P, Mellman I. A diffusion barrier maintains distribution of membrane proteins in polarized neurons. Nature 1999;397:698–701. [PubMed: 10067893]

Winckler B, Mellman I. Neuronal polarity: controlling the sorting and diffusion of membrane components. Neuron 1999;23:637–40. [PubMed: 10482229]

Wisco D, Anderson ED, Chang MC, Norden C, Boiko T, et al. Uncovering multiple axonal targeting pathways in hippocampal neurons. J Cell Biol 2003;162:1317–28. [PubMed: 14517209]

Wong W, Schlichter LC. Differential recruitment of Kv1.4 and Kv4.2 to lipid rafts by PSD-95. J Biol Chem 2004;279:444–52. [PubMed: 14559911]

Yamaguchi Y, Pasquale EB. Eph receptors in the adult brain. Curr Opin Neurobiol 2004;14:288–96. [PubMed: 15194108]

Ye H, Kuruvilla R, Zweifel LS, Ginty DD. Evidence in support of signaling endosome-based retrograde survival of sympathetic neurons. Neuron 2003;39:57–68. [PubMed: 12848932]

Yeaman C, Grindstaff KK, Nelson WJ. New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol Rev 1999;79:73–98. [PubMed: 9922368]

Yi JJ, Ehlers MD. Ubiquitin and protein turnover in synapse function. Neuron 2005;47:629–32. [PubMed: 16129392]

Yoshihara M, Adolfsen B, Galle KT, Littleton JT. Retrograde signaling by Syt 4 induces presynaptic release and synapse-specific growth. Science 2005;310:858–63. [PubMed: 16272123]

Yu SS, Lefkowitz RJ, Hausdorff WP. Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. J Biol Chem 1993;268:337–41. [PubMed: 8380158]

Zenisek D, Steyer JA, Almers W. Transport, capture and exocytosis of single synaptic vesicles at active zones. Nature 2000;406:849–54. [PubMed: 10972279]

Zhang H, Webb DJ, Asmussen H, Niu S, Horwitz AF. A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. J Neurosci 2005;25:3379–88. [PubMed: 15800193]

Zhou Q, Homma KJ, Poo MM. Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 2004;44:749–57. [PubMed: 15572107]

Zuo Y, Lin A, Chang P, Gan WB. Development of long-term dendritic spine stability in diverse regions of cerebral cortex. Neuron 2005;46:181–89. [PubMed: 15848798]

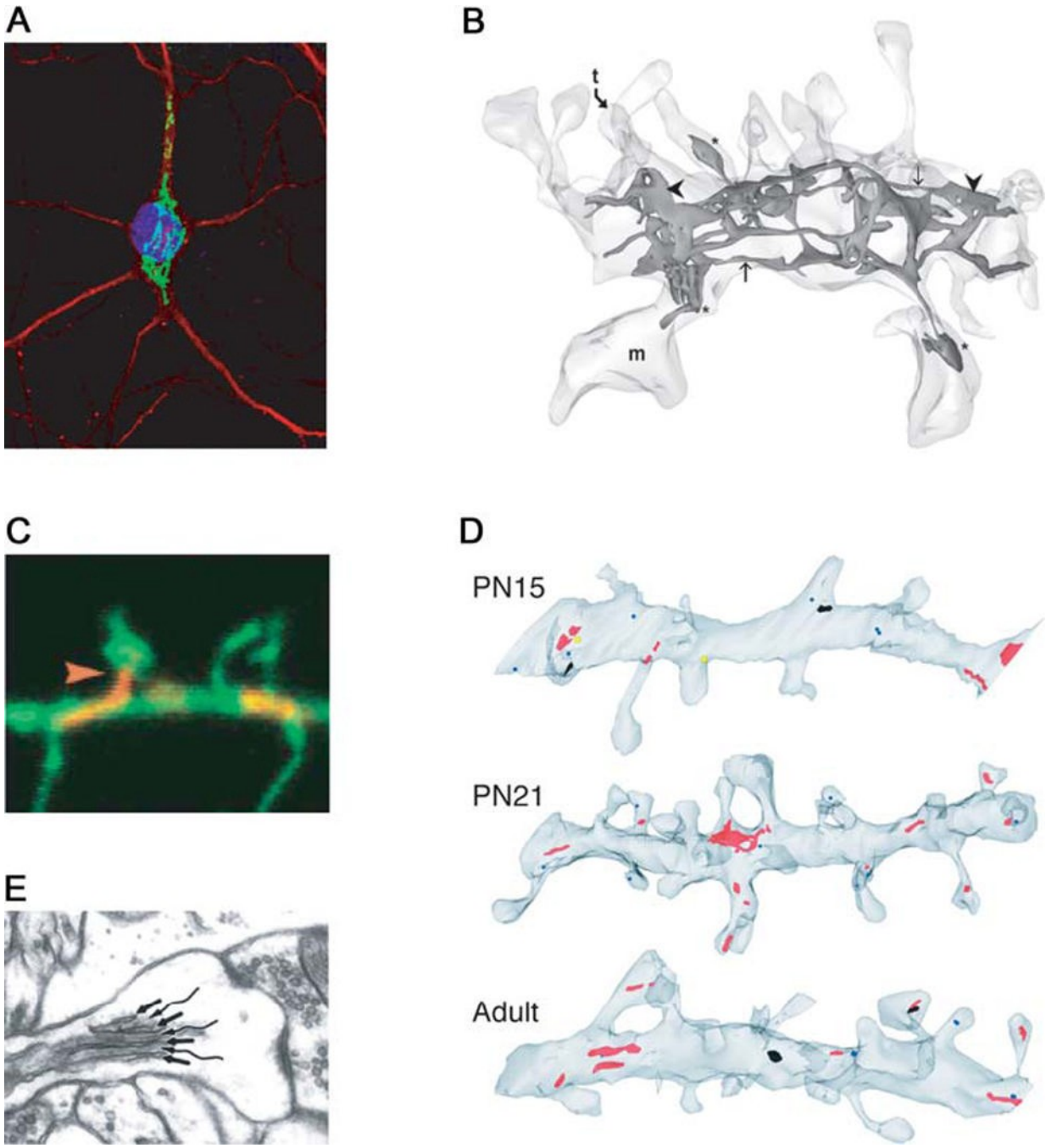


**Figure 1.**

Dendritic organelles. (*A*) Hippocampal neuron stained for MAP2 (*red*), DAPI (*blue*), and the Golgi marker GM130 (*green*). Adapted from Horton et al. (2005); reproduced with permission from Elsevier, copyright 2005. (*B*) Three-dimensional reconstruction of serial electron micrographs showing the distribution of SER (*dark gray*) in dendrites and spines. Large flat compartments (*arrowheads*) are linked by thin extensions (*thin arrows*). Several spines are invaginated by SER (*asterisks*). Adapted from Cooney et al. (2002); reprinted with permission from the Society for Neuroscience, copyright 2002 and deg; (*C*) Cultured hippocampal neurons expressing the mitochondrial targeting sequence of cytochrome oxidase fused to DsRed2. Dendritic mitochondria (*red*) are present in the dendritic shaft where they occasionally

associate with dendritic spines (*arrows*). Adapted from Li et al. (2004); reproduced with permission from Elsevier, copyright 2004. (*D*) Three-dimensional reconstruction of serial electron micrographs showing the distribution of dendritic endosomes in hippocampal neurons from adult rats (*bottom*) and rats at postnatal day 15 (*top*), postnatal day 21 (*middle*). Endocytic vesicles (*yellow*), endosomes (*red*), small vesicles (*blue*), and amorphous vesicles (*black*) are distributed throughout dendrites. Adapted from Cooney et al. (2002); reprinted with permission from the Society for Neuroscience, copyright 2002; (*E*) Electron micrograph of the spine apparatus showing lamination of smooth ER (*straight arrows*) between regions of high electron density (*wavy arrows*). Adapted from Spacek & Harris (1997); reproduced with permission from the Society for Neuroscience, copyright 1997.

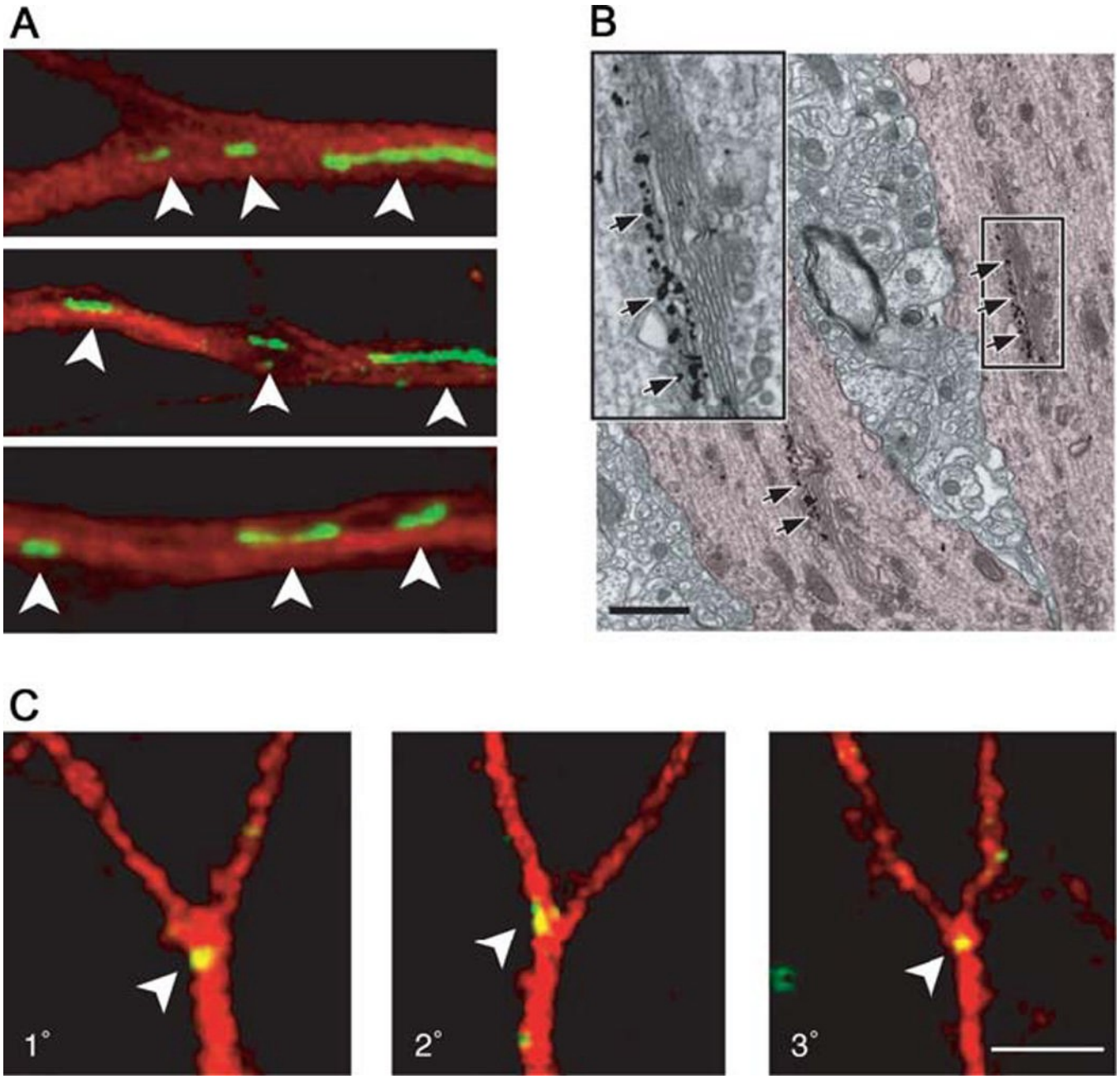


**Figure 2.**
Dendritic Golgi outposts. (*A*) Labeling with GM130 (*green*) demonstrates the presence of local Golgi outposts (*arrowheads*) in dendritic shafts from three different cultured hippocampal neurons. (*B*) Immunogold labeling for GM130 in adult rat hippocampus demonstrates the presence of Golgi in the apical dendrite of a CA1 pyramidal neuron in vivo. Scale bar, 1 μm. (*C*) Golgi outposts at dendritic branch points. VSVGtsO45 (*green*) accumulates at primary (1°), secondary (2°), and tertiary (3°) dendritic branch points following release from the ER at 20°C. Scale bar, 5 μm. Adapted from Horton et al. (2005);reprinted with permission from Elsevier, copyright 2005.

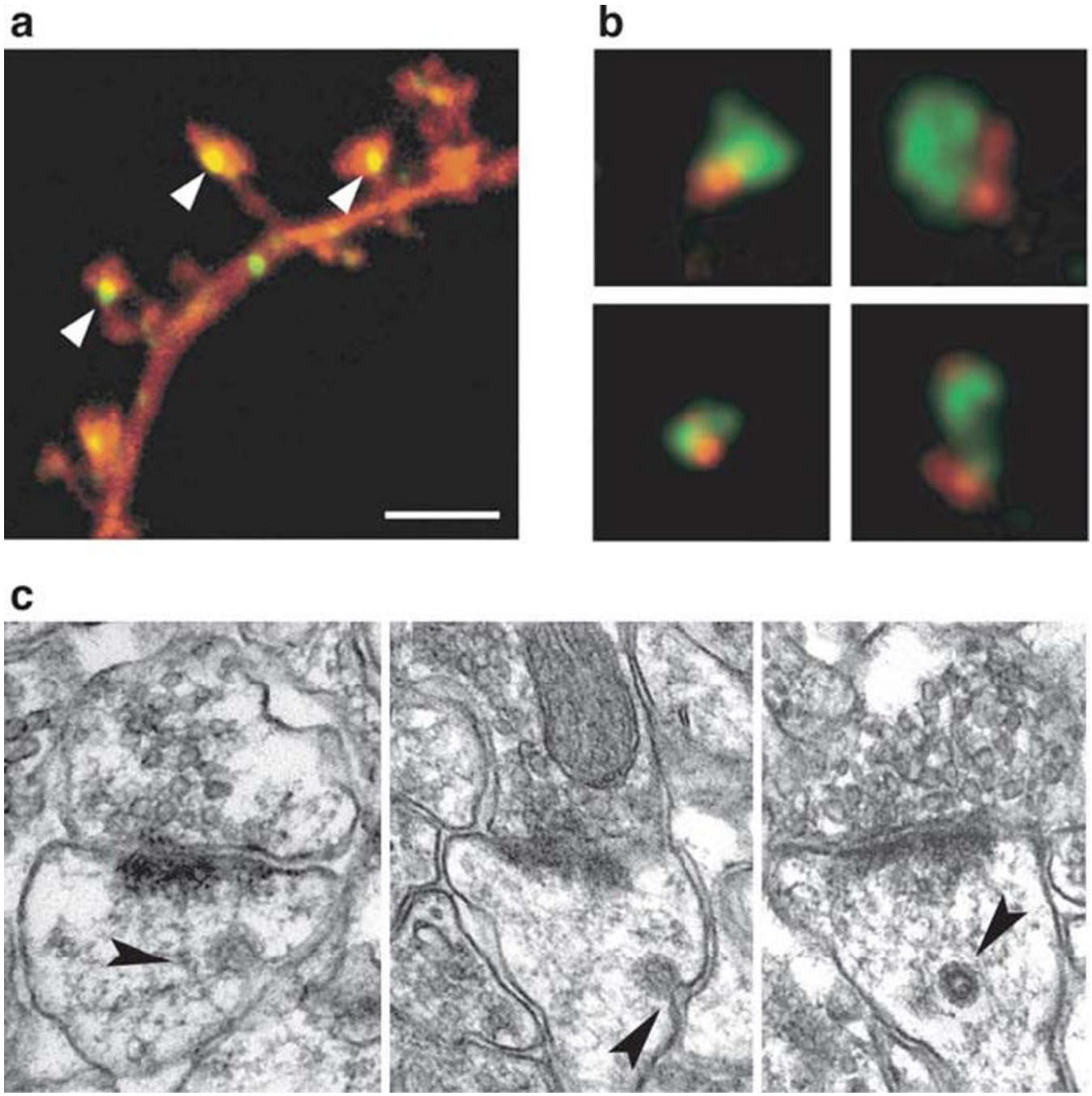


**Figure 3.**
The spine endocytic zone. (*A*) Clathrin-YFP (*yellow*) localizes to dendritic spines. Scale bar, 2 μm. (*B*) Clathrin-DsRed (*red*) lies adjacent to, but not overlapping, PSD95-GFP (*green*) in the spine head. Four different spines are shown. Scale bar, 1 μm. Adapted from Blanpied et al. (2002); reprinted with permission from Elsevier, copyright 2002; (*C*) Electron micrographs showing the different stages of endocytosis in different dendritic spines: clathrin-coated invagination prior to scission (*left panel*), a coated invagination (*middle panel*), coated vesicle postscission (*right panel*). Adapted from Racz et al. (2004); reprinted with permission from Macmillan Publishers Ltd., copyright 2004.

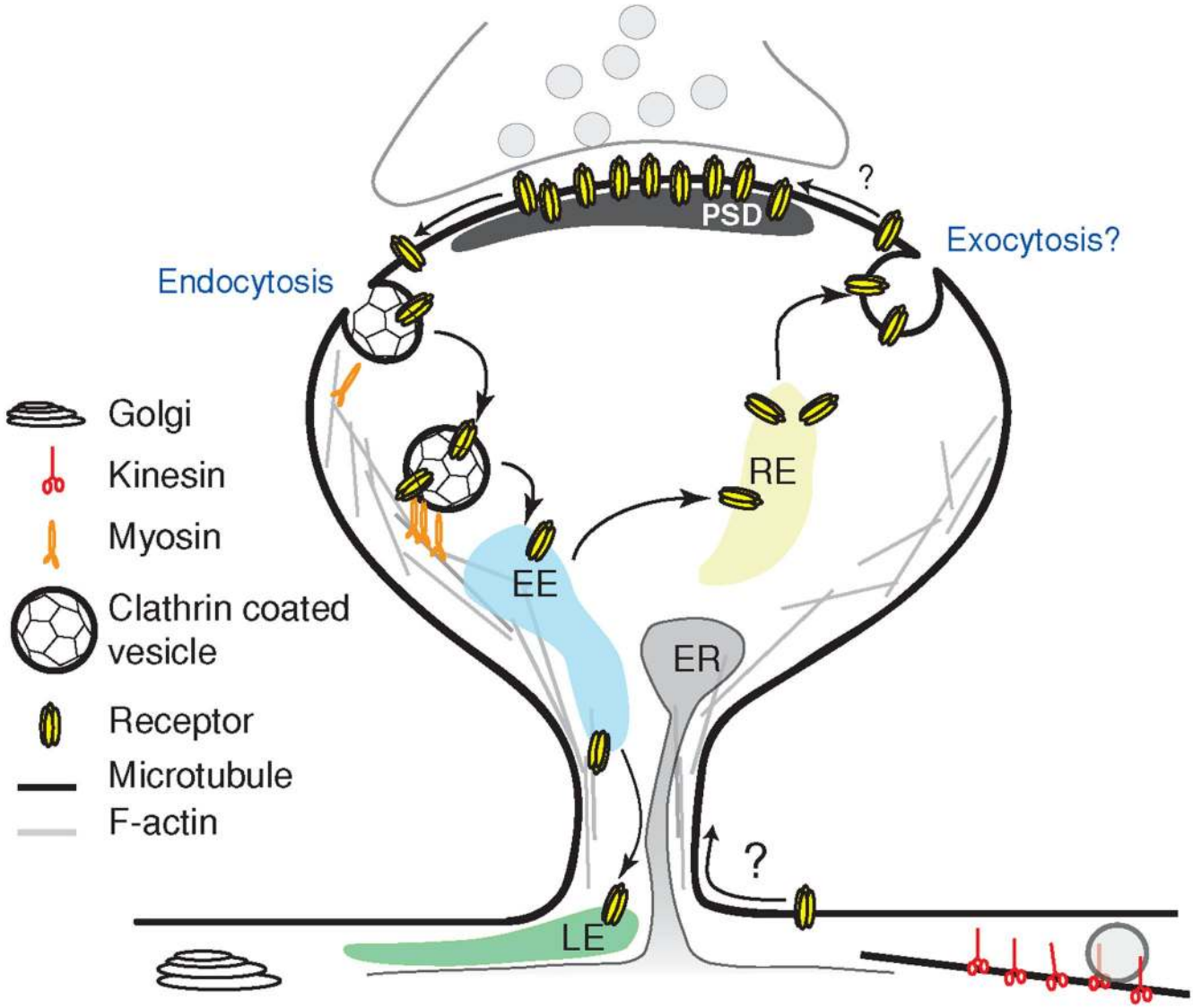


**Figure 4.**

Model for local postsynaptic trafficking. Components of the synapse either diffuse from the synapse or are actively transported to endocytic zones surrounding the PSD, where they are internalized and trafficked to early endosomes (EE) and sorted either to late endosomes (LE) for degradation or to recycling endosomes (RE) for return to the spine surface. Cargo destined for the synapse is exocytosed to the cell surface at an unknown location, perhaps at the plasma membrane of the spine head. Alternatively, synaptic components could be exocytosed to the surface of the dendritic shaft and diffused through the spine neck to the synapse. See Table 1 for a partial list of molecules known to be involved in dendritic trafficking.

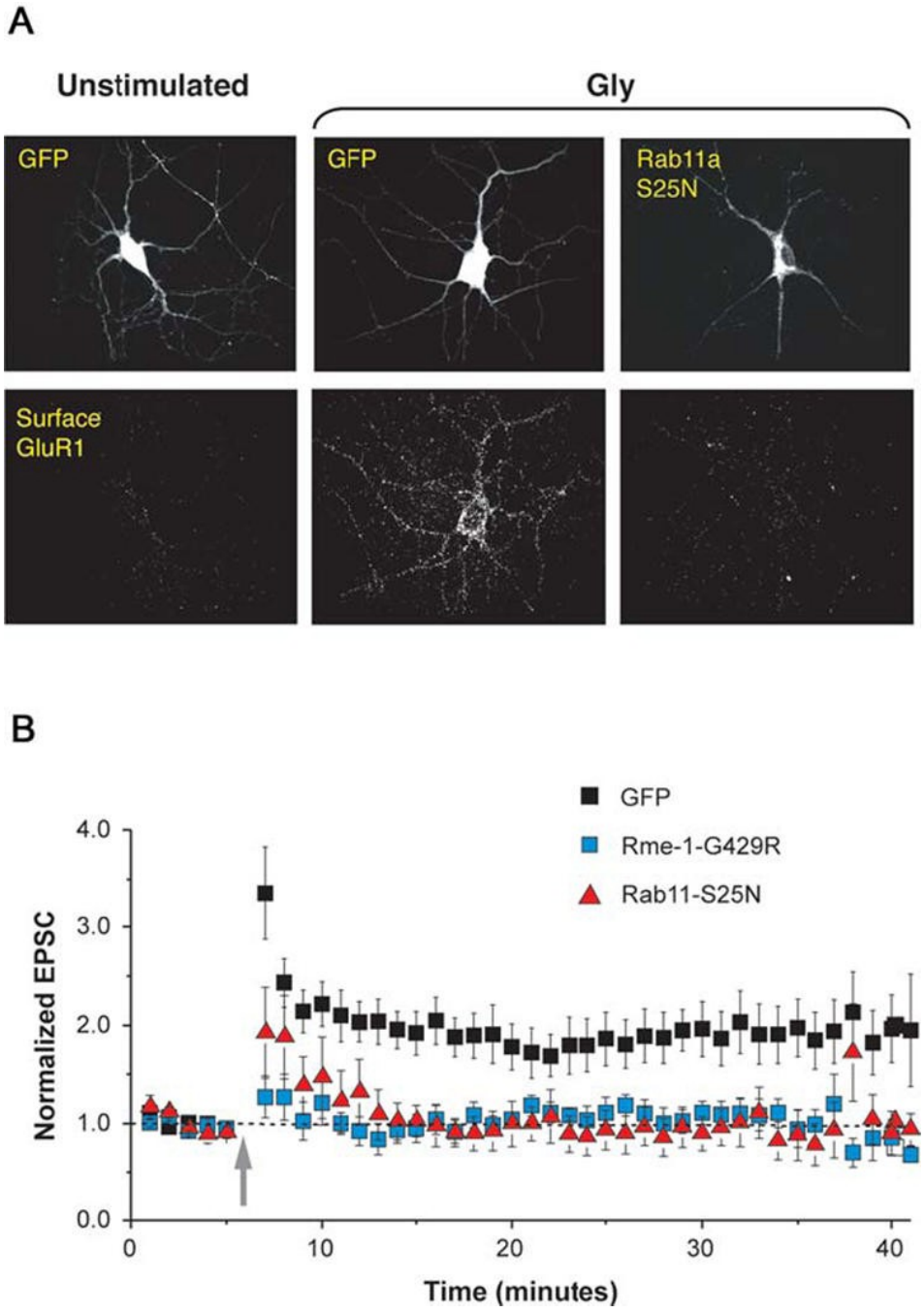


**Figure 5.**
Recycling endosomes act as AMPA receptor reservoirs. (*A*) HA-tagged GluR1 is trafficked to the cell surface of cultured hippocampal neurons in response to glycine stimulation (*left and middle panels*). Glycine-stimulated surface expression of GluR1 is impaired in neurons expressing a dominant-negative mutant of Rab11a (S25N) (*right panels*). Cells were transfected with GFP for visualization (*upper panels*). (*B*) Blocking transport through recycling endosomes with dominant-negative forms of Rme1 (G429R) and Rab11a (S25N) abolishes LTP. EPSCs were measured by whole-cell patch clamp from CA1 pyramidal neurons transfected with Rme1 (G429R), Rab11a (S25N), or GFP before and after high-frequency stimulus (100 Hz, four 1-s trains).

**Table 1**

A partial list of molecular components involved in dendritic spine trafficking

| Process | Molecules | References |
|---|---|---|
| Endocytosis | Clathrin | Blanpied et al. 2002,Racz et al. 2004 |
| | MyosinVI | Osterweil et al. 2005 |
| | AP-2 | Lee et al. 2002 |
| | Dynamin3 | Gray et al. 2003 |
| | Rab5 | Brown et al. 2005 |
| | cortactin | Racz & Weinberg 2004 |
| | hippocalcin | Palmer et al. 2005 |
| | CPG2 | Cottrell et al. 2004 |
| | Pick1 | Hanley & Henley 2005,Lu & Ziff 2005 |
| Postendocytic sorting | NEEP21 | Alberi et al. 2005; Steiner et al. 2002, 2005 |
| Exocytosis | Rab11a | Park et al. 2004 |
| | Rab8 | Gerges et al. 2004 |
| | sec6/8 complex | Sans et al. 2003 |