Biochimica et Biophysica Acta 1823 (2012) 1720–1730

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Molecular remodeling mechanisms of the neural somatodendritic compartment

Fletcher B. Moore, James D. Baleja *

Department of Biochemistry, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111, USA

ARTICLE INFO

Article history:
Received 28 February 2012
Received in revised form 6 June 2012
Accepted 7 June 2012
Available online 15 June 2012

Keywords:
Neuronal plasticity
Eps15 homology domain
EH domain
Postsynaptic density
Endocytosis
Exocytosis

ABSTRACT

Neuronal cells use the process of vesicle trafficking to manipulate the populations of neurotransmitter receptors and other membrane proteins. Long term potentiation (LTP) is a long-lived increase in synaptic strength between neurons and increases postsynaptic dendritic spine size and the concentration of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate-type glutamate receptor (AMPAR) located in the postsynaptic density. AMPAR is removed from the cell surface via clathrin-mediated endocytosis. While the adaptor protein 2 (AP2) complex of endocytosis seems to have the components needed to allow temporal and spatial regulations of internalization, many accessory proteins are involved, such as epidermal growth factor receptor phosphorylation substrate 15 (Eps15). A sequence of repeats in the Eps15 protein is known as the Eps15 homology (EH) domain. It has affinity for asparagine–proline–phenylalanine (NPF) sequences that are contained within vesicle trafficking proteins such as epsin, Rab11 family interacting protein 2 (Rab11-FIP2), and Numb. After endocytosis, a pool of AMPAR is stored in the endosomal recycling compartment that can be transported to the dendritic spine surface upon stimulation during LTP for lateral diffusion into the postsynaptic density. Rab11 and the Eps15 homologue EHD1 are involved in receptor recycling. EHD family members are also involved in transcytosis of the neuronal cell adhesion molecule neuron-glia cell adhesion molecule (NgCAM) from the somatodendritic compartment to the axon. Neurons have a unique morphology comprising many projections of membrane that is constructed in part by the effects of the Eps15 homologue, intersectin. Morphogenesis in the somatodendritic compartment is becoming better understood, but there is still much exciting territory to explore, especially regarding the roles of various EH domain–NPF interactions in endocytic and recycling processes.

1. The control of plasma membrane composition in neurons

Precise control of plasma membrane composition through receptor trafficking is of importance for neural circuits because neurons have a specialized morphology that stores information which can be changed by the manipulation of receptor populations. Neurons transmit signals unidirectionally from one to another using small molecules called neurotransmitters at specialized points of contact called synapses. Neurons possess long processes which extend from the cell body, or soma (Fig. 1). They are polarized into two compartments. The axon of a neuron is a long process which transmits signal, and the dendritic arbor is a collection of processes which receives signal. The somatodendritic compartment comprises the soma and dendrite. Each dendrite possesses further protrusions of cell membrane called dendritic spines which are shaped like mushrooms with a wide head and a narrow neck. The membranes within these spines contain specialized areas of increased protein content which receive neurotransmitter called the postsynaptic density (PSD).

Synapses and neurons are classified according to their predominant transmitter: dopaminergic, glutaminergic, GABAergic, et cetera. There are a number of receptors that are sensitive to glutamate, the primary excitatory neurotransmitter. Glutamate receptors are divided into two categories depending on if they respond to glutamate by (1) directly allowing a flow of ions across the cell membrane (the ionotropic glutamate receptors, iGluRs), or (2) via a G protein second messenger (the metabotropic glutamate receptors, mGluRs). There are two iGluRs of particular importance: the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate-type glutamate receptor (AMPAR) and N-methyl D-aspartate receptor (NMDAR), which are named after compounds that act as exclusive agonists. Most NMDARs are heterodimers comprising two glycine-binding and two glutamate-binding subunits. They require glycine as a coagonist to glutamate, and in addition they are voltage-gated channels, that are sensitive to magnesium concentrations [1]. AMPARs are also tetramers, composed of different combinations of GluR1-4 subunits [2]. Each subunit is a transmembrane protein with a large extracellular domain, four transmembrane domains, and a cytoplasmic tail. The differences between the subunits are largely restricted to their cytoplasmic tails. GluR1 and GluR4 have relatively long tails, while GluR3 and GluR2 have shorter tails. These tails dictate binding partners and are sites of

* Corresponding author at: Department of Biochemistry, Tufts University School of Medicine, Boston, MA 02111, USA. Tel.: +1 617 636 6872; fax: +1 617 636 2409.
E-mail address: Jim.baleja@tufts.edu (J.D. Baleja).

0167-4889/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2012.06.006

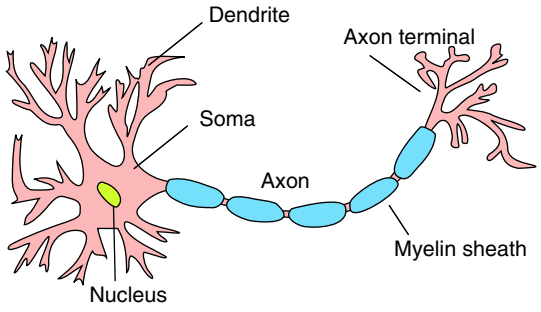

Fig. 1. Features of a generalized neuron. A neuron comprises the soma (cell body), dendrites, and axon. The long thin axon is covered by the myelin sheath. The soma gives rise to numerous dendrites, but only one axon. The dendrites receive synaptic signals from other neurons; signals to other neurons are transmitted by the axon.

regulation through phosphorylation. The concentration and number of AMPARs in the PSD will determine the sensitivity of the postsynaptic neuron to signal.

The PSD is composed of a high concentration of protein filaments and interconnected assemblies that provide a scaffold for directing the movement of glutamate receptors. The scaffold prevents the free diffusion of receptors by using molecular corrals or, more directly, by the binding of receptors to particular scaffold proteins. Glutamate receptors have a non-random arrangement with the PSD, reflecting its non-homogeneous organization; NMDARs are often located in the center, while AMPARs are peripheral [3]. Computational modeling suggests that receptor concentration and alignment with presynaptic neurotransmitter release sites play a significant role in synaptic strength in addition to absolute receptor numbers [4]. A study of the mobility of PSD-95, the major scaffolding protein of the PSD, revealed that while there is little movement of individual scaffolding proteins within subdomains of the PSD, the PSD overall is a flexible structure, stretching quickly in response to movements of the cytoskeleton. This flexibility could modulate receptor concentrations and consequently synaptic strength [5].

Adjacent to the PSD is a separate endocytic zone (EZ) that is developed and maintained independent of synaptic activity. In this zone the clathrin-coated pits associated with endocytosis assemble and disassemble repeatedly. When the synapse is artificially stimulated or inhibited there is no effect on clathrin dispersal from the EZ relative to controls [6]. Because endocytosis does not occur within the PSD, the cargo proteins need to translocate out of the PSD to the EZ for internalization. Therefore, activity-dependent changes in receptor internalization are due to mobility of the receptor within the PSD.

2. Vesicle trafficking

Cells mobilize their membrane bound proteins via the process of vesicle trafficking whereby vesicles are formed and subsequently target their cargo proteins for degradation or return to the cell surface (Fig. 2). Vesicle trafficking is a constant and ongoing process, which results in a steady flow of proteins and lipids to and from the cell surface. Cells maintain and modulate the composition of the plasma membrane through regulation of this system via a number of molecular actors that sort and direct internalized membrane components through a variety of intracellular compartments before recycling back to the plasma membrane, and understanding the precise roles of these diverse actors is the goal of much current research.

Cells utilize different mechanisms for endocytosis: the more fully described clathrin-mediated endocytosis (CME), and the less understood clathrin-independent forms of endocytosis, such as caveolar endocytosis and the Arf6-associated pathway. The existence of a diverse set of mechanisms provides the cell with regulatory flexibility, as it allows the cell to independently modulate the internalization of different cargos simultaneously. Which mechanisms are available

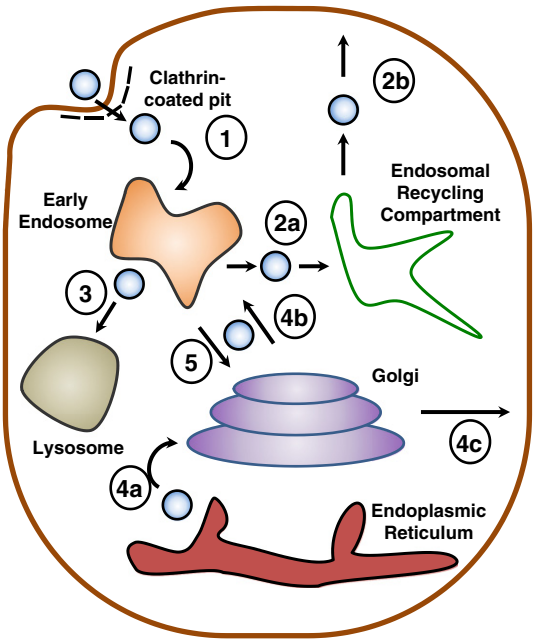

Fig. 2. The intracellular trafficking pathways of protein molecules, shown in blue, within a cell. Proteins that are internalized, often through clathrin-mediated endocytosis (Step 1), are subsequently trafficked through endocytic and recycling compartments back to the cell surface (Step 2) or degraded in the lysosome (Step 3). Proteins designated for secretion travel through the endoplasmic reticulum–Golgi secretory pathway (Step 4). Some proteins participate in retrograde transport back to the Golgi (Step 5).

for the endocytosis of a given cargo molecule depends on the capacity of that cargo to bind to pathway-specific molecules.

Regardless of the mechanism, most internalized cargos end up in a sorting/early endosome, one of three compartments within the endosomal system. The other two compartments are the late endosome/multivesicular body and the recycling endosome. From the early endosome, the cargo may proceed to the lysosome for degradation, passing through the late endosome. Alternatively, the cargo may travel to the trans-Golgi network or return to the cell surface. Recycling is complicated further in polarized cells by a specialized form of recycling called transcytosis. During the process of transcytosis, a specific cargo is internalized at one domain of the plasma membrane and transported to a different domain. An example discussed below is the transcytosis of L1/NgCAM, which is first deposited on the plasma membrane of the somatodendritic compartment of the neuron before undergoing transcytosis to the axon, its final destination [7].

After newly-formed early endosomes emerge from the plasma membrane via endocytosis, they fuse with each other and with existent early endosomes. Once formed the contents are rapidly acidified by vacuolar proton pumps. This acidification facilitates the separation of ligands from receptors, a requirement before sorting of receptors can occur. As a result of increasing acidification, the early endosome loses the ability to accept the fusion of new vesicles while acquiring the ability to interact with the lysosome [8].

Concurrently with acidification, tubules of membrane bud from the early endosome, starting off a process that recycles most of the lipid and protein components. Some material immediately returns to the plasma membrane by following a rapid recycling pathway. In a slower process, other cargo travels to an intermediate vesicle system called the endosomal recycling compartment (ERC) before returning to the cell surface. The ERC, formed from elongated membrane tubules that emerge from early endosomes, tends to be juxtanuclearly localized. A third subset of cargo is specifically designated for degradation by post-translational modifications such as ubiquitination [9]. Molecular machinery sorts these cargos into

intraluminal vesicles within the endosome, giving rise to multivesicular bodies.

The budding of recycling tubules, formation of intraluminal vesicles, and acidification of the endosome occur simultaneously as the endosome moves from a location near the cell surface toward the center of the cell. The degree of tubulation decreases while the number of intraluminal vesicles increases as the endosome ages and translocates. In this way the early endosome matures into the late endosome. The process ends when there is no longer any cargo to recycle and the endosome acquires the ability to fuse with the lysosome.

Identification of various endosomal compartments is necessary for observation and experimentation with the endosomal system. This task is challenging for two reasons: first, the marker proteins which might be used to classify an endosome are generally present on several different endosomes; and second, the endosomes themselves change over time during maturation. Nonetheless, identification is possible by examining the relative distribution or transit times of certain marker proteins in addition to the morphology of the vesicles. For example, early endosome antigen 1 (EEA1) and Rab5 are used as markers for the early endosome, whereas EHD1 and Rab11, along with a tubular morphology and high membrane to cytoplasmic ratio, designate the ERC.

The marker proteins used to identify endosomes are members of the large collection of proteins that associate with various endosomal compartments in order to regulate and maintain the system. The Rab family of small GTPases and the EH domain-containing proteins are prominent players. Thus far, a large network of associations between these and other proteins has been uncovered, but many of the details of the cellular trafficking system remain to be discovered. The EH domain-containing proteins (EH-proteins) are a particular focus of this review and we emphasize the emerging roles they play in endocytosis, recycling, and transcytosis in the somatodendritic compartment of neurons.

3. Eps15 and clathrin-mediated endocytosis

The EH domain was originally described as a sequence of three repeats at the N terminus of the Eps15 protein, which was discovered during a cDNA screen for phosphorylation substrates of the tyrosine kinase epidermal growth factor receptor (EGFR) [10]. Searches for similar sequences found a number of proteins in yeast and *Caenorhabditis elegans*. The search also revealed a second highly homologous human protein which was designated Eps15-related protein (Eps15R, aka Eps15L1) [11]. The screening of expression libraries using the EH domain demonstrated a preference for partners containing an asparagine–proline–phenylalanine (NPF) motif [12].

The Eps15 and Eps15R proteins are characterized by three regions: three EH domain repeats near the N terminus, a coiled–coiled region in the center, and DPF (aspartic acid–proline–phenylalanine) repeats at the C terminus. The coiled–coiled region is used for homodimerization and heterodimerization with intersectin, another EH domain-containing protein [13]. Most Eps15 within the cell exists in macromolecular complexes, as shown by size exclusion column chromatography using cell lysates [14].

The first structure of an EH domain was determined for the central EH domain of Eps15 using NMR methods [15]. The fold comprises two EF hand motifs (helix–loop–helix structures) connected by a short antiparallel β sheet. EF hand motifs are the most common calcium-binding motifs found in proteins [16]. However, the ability of an EH domain to bind calcium is not conserved. When present, as in the central EH domain of Eps15, the calcium ion appears to be structural as it is bound with high affinity and thus is not sensitive to physiological changes in calcium concentrations [11]. Nearby, the hydrophobic binding pocket for NPF is formed from Leu155, Leu165, and Trp169; if either of the latter two residues are mutated, NPF cannot bind [17].

The EH domain's affinity for NPF sequences suggests a role in intracellular trafficking and organization as several NPF-containing proteins are well-established as endocytic adaptors [12]. Other work places Eps15 and Eps15R at the site of CME and demonstrates association of these proteins with AP2, a CME component [18]. The importance of Eps15 and Eps15R in the CME of the EGFR is highlighted by experiments with dominant negative mutants which block endocytosis altogether, showing the Eps15 proteins to be essential components [19]. Eps15 dominant negative mutants are commonly used to downregulate CME.

All cells utilize CME, and CME is the internalization pathway for several well-studied receptors. The uptake of essential nutrients such as iron by the transferrin receptor or cholesterol by the low density lipoprotein (LDL) receptor is clathrin-mediated, as well as endocytosis of the EGFR, which is involved in mitogenic signaling. The shared trait of these transmembrane receptors is their clustering into clathrin-coated pits in anticipation of endocytosis, either constitutively, as in the case of the LDL receptor [20], or after binding ligand, as with EGFR. These pits are characterized by an inward dimpling of the plasma membrane and a clathrin-coated cytosolic face. Clathrin-coated pits do not assemble randomly throughout the cell, but often occur at specific locations, constrained by the actin cytoskeleton by scaffolding proteins [21].

Clathrin itself is a cytosolic protein possessing a triskelion geometry, which facilitates the deformation of membrane into a sphere when it is recruited and polymerized [22]. Cells utilize clathrin not only in endocytosis but also in other vesicle-forming processes within the cell such as secretion from the trans-Golgi network. It is formed from three heavy chain peptides, each with three light chain peptides. Clathrin chains spontaneously assemble in vitro, but adaptor proteins (AP) are required to nucleate assembly in vivo [23]. As the name implies, adaptor proteins provide intermolecular connections between clathrin, lipid, receptor, and other accessory proteins.

There are several different adaptor proteins. AP2 is primarily involved in CME. AP2, like the other adaptor proteins (AP1, AP3, and AP4), is a heterotetramer composed of two large subunits and two smaller subunits. For AP2, the larger subunits are α and β2 and the smaller subunits are μ2, and σ2. The α subunit is used to target AP2 to the plasma membrane [24]. The μ2 subunit interacts with the cytoplasmic component of transmembrane receptors [25], and the β2 subunit interacts with clathrin [26].

While AP2 seems to have all of the components necessary for initiating CME, many other important accessory proteins are involved. Initialization of the clathrin-coated pits on the membrane begins with FCHo proteins. FCHo proteins bind to the plasma membrane and recruit Eps15, Eps15R, intersectin-1, and intersectin-2 [27]. FCHo proteins have no ability to bind clathrin or AP2, yet they are localized to the plasma membrane and their expression is proportional to clathrin-coated pit formation. The DPF-rich C terminus of the Eps15 proteins binds to the α-adaptin subunit of AP [28], and Eps15 is constitutively associated with AP2 in the cytoplasm. Although Eps15 is associated with AP2 during the formation of clathrin-coated pits, the ratio of Eps15 to AP2 at the plasma membrane is not one to one because as clathrin polymerization proceeds, Eps15 is released from AP2 [29]. Instead Eps15 is restricted to the perimeter of the nascent pit [30]. Together, these results suggest a model in which FCHo binds to the plasma membrane, recruits Eps15, which in turn recruits AP2. AP2 then facilitates the formation of clathrin polymers and the anchoring of cargo receptors through its β2 and μ2 subunits, respectively, while Eps15 is released for further cycles of protein recruitment.

The model is complicated by the fact that not all cargo that undergoes CME is recognized by AP2 and instead relies on post-translational modifications. G-protein coupled receptors tend to be recognized by beta-arrestins after undergoing phosphorylation [31]. Ubiquitination is also used as a signal for endocytosis of membrane

bound proteins. Specific ubiquitin ligases mediate ubiquitination of target proteins, and proteins with ubiquitin interacting motifs (UIM) are then able to bind. The specificity of the ligases allows ubiquitination based endocytosis to be precisely controlled. Eps15 and Eps15R contain UIMs, which provide an alternate link between the cargo and the remainder of the clathrin machinery [32].

Once invagination of the plasma membrane has proceeded to a sufficient degree by the action of clathrin polymerization, the coated pit can separate from the plasma membrane into an independent clathrin-coated vesicle. As an unorganized disruption of the energetically favorable bilayer state of the membrane would likely result in leakage of cell or vesicle contents, this fission process is directed by a large GTPase called dynamin. Dynamin has five domains: an N-terminal GTPase, an α-helical middle domain, a GTPase effector domain (GED), a lipid-binding pleckstrin homology domain (PH), and a C-terminal proline–arginine domain (PRD). The PRD is responsible for targeting dynamin to the clathrin-coated pits by interacting with SH3 domain-containing proteins [33]. In a process first suggested by electron micrographs of *Drosophila* dynamin with a temperature sensitive mutation, dynamin is localized to the pits, and oligomerizes into a collar around the neck of the incipient endocytic vesicle [34]. Then, through GTPase-dependent conformational changes, dynamin exerts differential stress on the inner and outer lipid layers of the membrane bilayer that promotes the formation of a hemifission state, a necessary intermediate for non-leaky fission which stochastically reverts or proceeds to full fission [35]. Following membrane fission, the clathrin coat is shed from the liberated vesicle in an energy-dependent process involving Hsc70 and auxilin [36].

In addition to CME, there are other endocytic mechanisms that do not utilize clathrin for internalization of proteins that do not have the protein sequences required to recruit the CME machinery. These clathrin-independent endocytic pathways may involve a different coat protein, as in the case of caveolar endocytosis, and they do not necessarily require dynamin for fission. A proposed classification scheme is to separate them according to dependence on dynamin and on certain small GTPases: ADP ribosylation factor 6 (Arf6), RhoA, or Cdc42 [37]. Despite internalization differences, the fate of these internalized membrane components is largely similar to those internalized by CME: after separating from the plasma membrane into disparate vesicles, most cargo is delivered to the EEA1- and Rab5-labeled early endosome.

4. Molecular switches in vesicle trafficking

Regulation of processes in the cell often occurs through molecular switches, and vesicular trafficking is no exception. Many trafficking events are mediated by members of the Ras superfamily of GTPases, which includes the Arf and Rab families. Some members of these families have been mentioned above as endosomal compartment markers. Ras GTPases are small proteins that bind GDP and GTP. When bound to GTP, these proteins are in an active state, whereas when bound to GDP they are inactive. With the change of a phosphate group in the nucleotide, the GTPase undergoes a conformational change, which alters its binding capacity by exposing, creating, or hiding domains. Bound GTP is hydrolyzed to GDP by GTPase activating proteins (GAPs), and GDP is exchanged for GTP by guanine nucleotide exchange factors (GEFs). Ras family members bind to a variety of effector proteins: adaptors, phosphatases, motors, et cetera. Some of these effectors are of interest because they in turn bind to EH-proteins.

Ral is one such GTPase involved in molecular trafficking. The two Ral genes, RalA and RalB, bind phospholipase D, phospholipase C-δ1, and calmodulin in a nucleotide-independent manner. Ral also binds two effectors which connect to both endocytosis and exocytosis: (1) Ral-binding protein 1 (RalBP1, also termed RLIP76 and RIP1 reflecting its independent discovery by three different groups [38–40]) and (2) the exocyst complex through its Sec5 and Exo84 subunits. RalA and RalB share approximately 85% sequence identity and their overall structure is the same. Nonetheless, functional differences between the two have been found. For example, in MDCK cells only RalA upregulates the targeting of E-cadherin to the basolateral membrane [41].

Ral has been linked to endocytosis of several receptors—notably, EGFR and transferrin. This association is likely through the effector RalBP1 because RalBP1 binds to several proteins known to be involved in CME. RalBP1 has been shown to bind the EH-proteins Reps1 (RalBP1 associated Eps15 homology protein) and Reps2/POB1 (partner of RalBP1) from a yeast two hybrid screen of mouse cDNA. Both Reps1 and Reps2 contain an EH domain, a proline rich region, a coiled coil region, and a RalBP1 interacting domain, and both are phosphorylated by EGFR and interact with Eps15 [42,43]. RalBP1's N terminal region also binds AP2 and epsin, another endocytic protein [44].

Other endosomal regulatory proteins are cell specific. For example, Neuron Enriched Endosomal Protein of 21 kDa (NEEP21) is found in the somatodendritic compartment of neurons. NEEP21 colocalizes with Rab4 and internalizes transferrin on the early endosome as well as plays a role in receptor sorting and recycling. Modulating NEEP21's expression influences the recycling of transferrin and GluR1 [45]. In addition, downregulation of NEEP21 leads to the missorting of L1/NgCAM (a protein that undergoes transcytosis) to the lysosome [46].

Many other GTPases are involved in the endosomal trafficking system. Rab11, in particular, is involved in receptor recycling through the ERC. A family of proteins that interact with Rab11 designated Rab11 family interacting proteins (Rab11-FIPs) has been discovered. Rab11-FIP2 connects Rab11 activity with vesicle motility by association with Myosin Vb [47], and Rab11-FIP3 connects Rab11 to the positioning of the ERC within the cell [48]. MyoVb belongs to the class V type of actin-based myosin motors, which has been implicated in vesicular trafficking. Rab11-FIP5 facilitates the sorting of a cargo from the early endosome to the ERC [49]. Arf6, the regulator associated with some forms of clathrin-independent endocytosis, also works in tandem with Rab11, at least in the case of integrin β1 recycling [50].

In addition to its roles in recycling, Rab11-FIP2 represents another regulator that is connected to the EH domain. Rab11-FIP2 possesses three NPF motifs; the second motif interacts with the EH domain present at the C-terminus of two members of a group of C-terminal EH-proteins comprising four mammalian Eps15 homology domain proteins (EHDs), named EHD1, EHD2, EHD3, and EHD4 [51]. Each EHD has a single EH domain at the C terminus and adds to the network of N-terminal EH-proteins which play roles in endocytosis [52,53]. Even the initial discovery of EHD1 in 1999 suggested a role in receptor-mediated endocytosis because of the EH domain and cellular localization to vesicular structures [52]. EHD1 localizes to the ERC. EHD1 and Rme-1, the *C. elegans* homolog to EHD1, colocalize with transferrin receptor, and dominant negative mutants slowed its recycling [54]. In HeLa cells, EHD recruitment to the ERC is mediated by the Rab8a effector MICAL-L1 [55]. Moreover, EHD1 is involved in clathrin-independent receptor recycling as well. Mutations were shown to disrupt clathrin-independent recycling of MHC-I via the Arf6 pathway, while overexpression led to increased recycling in HeLa cells [56].

N and C terminal EH-proteins bind to different proteins even though both EH domains target NPF sequences. Unlike N terminal EH proteins, the binding partners of EHDs possess acidic residues near their NPF sequence at the +1, +2, or +3 location. It has been shown that the EH domain of EHD1 has a positive surface electrostatic potential. This contrasts with the N terminal EH domains found in proteins such as Eps15 and intersectin, which have a negative potential [57]. The NMR solution of EHD1 in complex with MICAL-L1 showed that the glutamate residues adjacent to the NPF sequence were well-positioned to form salt bridges to particular lysines on EHD1 [58].

A mechanism by which EHDs associate with membranes has been proposed from the seminal electron microscopy (EM) and X-ray structures of mouse EHD2 [59]. EHDs form into dimers with a curved surface which inserts into the membrane perpendicular to the direction of tubule membrane curvature. The dimers then further oligomerize around the membrane. The crystal structure of the dimer shows a nucleotide-binding region that is very similar in three-dimensional structure to the nucleotide-binding region in dynamin [59]. A particular region is called the G-region due to its similarity to canonical GTP-binding sequences, although unlike dynamin, EHD2 binds ATP instead of GTP [60]. The binding to ATP is required for oligomerization [61,62]. Oligomerization, moreover, is required for association with endosomes. With EHD inserted into the membrane, its curvature and ATP hydrolysis likely destabilize the lipid bilayer, suggesting a role facilitating membrane fission or fusion.

Despite structural similarities, the roles of the four EHDs are not exactly the same. Rab11-FIP2 binds to only EHD1 and EHD3, for example. Both individually serve to relocalize Rab11-FIP2 from vesicular structures to tubular structures when expressed. For each, oligomerization and the EH–NPF interaction were required for this effect. Even so, RNAi knockdown of EHD1 and EHD3 had differential effects on Rab11-FIP2 localization and transferrin recycling. EHD1 knockdown resulted in transferrin and Rab11-FIP2 accumulation in the ERC, whereas during EHD3 knockdown, the block was “up-stream” and transferrin and Rab11-FIP2 failed to reach the ERC. Transferrin recycling was accelerated, probably due to shuttling into the fast recycling route, while Rab11-FIP2 instead accumulated in the early endosome. These data suggest that EHD3 plays some role in sorting from the early endosome to the recycling endosome consistent with EHD3's endogenous colocalization with EEA1. Transient heterooligomerization of different EHDs may thus facilitate the transport of a cargo through the various endosomal compartments [51].

The final EHD, EHD4, also called Pincher, plays a role in the exit of a cargo from the early endosome as well. Evidence includes the colocalization of EHD4 with EEA1, Rab5, Arf6, and internalized transferrin [63]. Moreover, knockdown of EHD4 results in the accumulation of transferrin on enlarged early endosomes, like EHD3. Enlargement of the early endosome is likely due to EHD4's interaction with Rab5, as knockdown of EHD4 increased not only the size of the early endosome but also the accumulation of GTP bound Rab5 on the early endosome. Even though EHD4 and EHD1 each predominantly localize to different compartments, they form complexes with each other in vivo and knockdown of one significantly affected the localization of the other. EHD4 loss eliminates EHD1-labeled tubules, and a decrease in EHD1 expression results in EHD4 relocalization to the perinuclear endocytic recycling region.

5. AMPA receptor recycling

Long-term potentiation (LTP) and long-term depression (LTD) are the names given to long-lived increases or decreases, respectively, in synaptic strength induced by neurotransmission between neurons. The change in synaptic strength is believed to be the fundamental mechanism behind learning and memory. In glutamatergic synapses potentiation entails a change in postsynaptic dendritic spine size as well as a change in AMPAR concentration and number via a constellation of molecular responses including the trigger for both LTP and LTD, NMDAR activation (Fig. 3). In addition to stimulation-induced calcium ion influx, NMDAR activates a number of signaling cascades depending on whether the stimulation received is high or low frequency. These cascades in turn effect LTP or LTD. High frequency stimulation of NMDAR induces LTP via CaMKII, protein kinase A (PKA) and protein kinase C (PKC), which phosphorylate AMPARs. Low frequency stimulation activates pathways that dephosphorylate AMPARs.

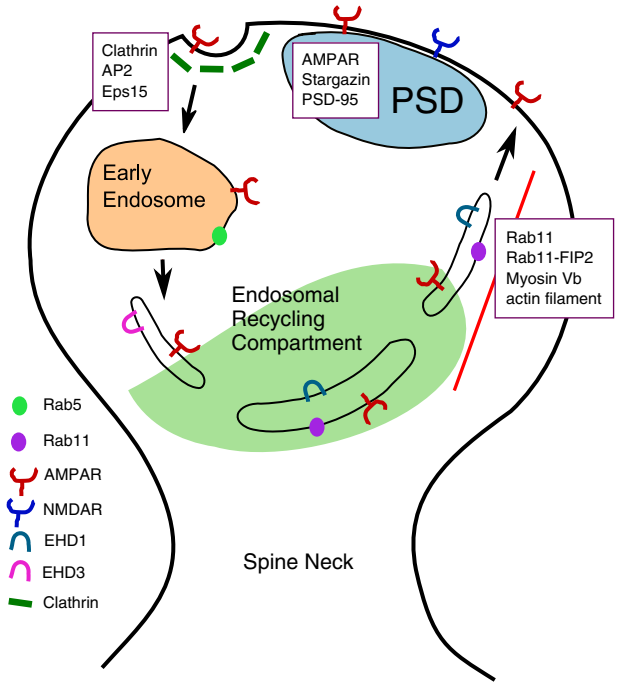

Fig. 3. AMPAR recycling in the dendritic spine. Various regulators and scaffolding proteins govern the localization of AMPAR to the postsynaptic density (PSD), the early endosome, or the endosomal recycling compartment.

The AMPAR concentration is largely controlled by local regulation within the spine and not the amount of AMPAR synthesized [64]. To understand the mechanics underlying the tight association between EZs and the PSD, a search was focused on proteins relatively increased at the subset of PSDs (10–15% of total PSDs) that are associated with adjacent EZs [6], [65]. A reasonable candidate important for the association was dynamin-3, which was already connected with endocytosis and found to bind to Homer, a PSD scaffolding protein [66]. Binding to Homer was decreased by inhibiting dynamin-3's ability to oligomerize, or by downregulating dynamin-3; concomitantly the percentage of PSDs without an EZ increased, confirming the role of dynamin-3 in maintaining adjacent EZs [65]. The Ehlers group also found that, paradoxically, PSDs lacking EZs had 50% less AMPAR. They hypothesized that the EZ helps maintain synaptic strength by preventing loss of AMPAR from the PSD that would occur by local diffusion by capturing them for recycling to the cell surface [6].

One way NMDAR activation influences AMPAR receptor concentrations within the PSD to modulate AMPAR affinity for PSD scaffolding proteins. AMPAR can laterally diffuse into and out of the PSD and move around within the PSD. NMDAR numbers and lateral diffusion rates on the other hand are not significantly affected by synaptic activity or spine morphology, consistent with AMPAR's role as the major source of increased postsynaptic sensitivity [67,68]. PSD-95 interacts with AMPAR indirectly through members of the TARP family, such as stargazin. TARP members bind to PSD-95 by its PDZ domains and bind to all four subunits of AMPAR. Experiments that disrupt the interaction have a strong influence on lateral diffusion [69]. Within the PSD are populations of mobile and immobile AMPARs, and the number of the mobile ones changes in response to synaptic transmission [70]. Active synapses are better at trapping AMPARs in the PSD. Calcium influx, which occurs during the opening of ion channels, diminishes lateral diffusion of GluR2 by immobilization [71]. In addition, spine morphology plays a role in lateral diffusion. Experiments using membrane-targeted GFP showed that its diffusion was diminished at the spine neck even though it had no specific interactions with proteins of the PSD [72]. The decrease may be due to geometry or the cytoskeletal arrangement. PSD proteins might also be restricted in this way.

Geometry also plays an important role in the stability of the PSD itself. Larger PSDs retain PSD-95 for a longer period of time. PSD-95 has a half-life within PSDs on the order of minutes. As PSDs share a common pool of PSD-95 via lateral diffusion, simple diffusion suggests that smaller PSDs would acquire PSD-95 at the expense of larger PSDs which would have greater efflux. However, the increased probability of binding to another protein within a larger PSD instead decreases the turn-over time of PSD-95 within the PSD [73], and is synergistic with the spine growth that occurs during LTP.

NMDAR affects not only the lateral diffusion of AMPAR, but also its endocytosis. AMPAR undergoes both constitutive endocytosis and activity modulated endocytosis. In both cases, AMPAR endocytosis is clathrin-dependent, as indicated by extensive colocalization with Eps15 during internalization [74], and dynamin-dependent. Yet different causes of AMPAR endocytosis utilize different signaling pathways that interact with the cytoplasmic tails of the GluR subunits of AMPAR [75]. This is made apparent by experiments that independently disrupt these pathways. For example, peptides that disrupt the AP2–GluR2 interaction also disrupt NMDA-dependent LTD, but had no effect on AMPA-induced endocytosis of AMPAR [76].

Ubiquitination-induced endocytosis of AMPAR is another signaling pathway for internalization that is stimulus-dependent. The ligase Nedd4 (a neural precursor cell-expressed developmentally down-regulated gene 4 family ligase) ubiquitinates a C-terminal lysine of the GluR1 subunit of AMPAR, which causes its endocytosis in response to activation. Knockdown of Nedd4-1 inhibited AMPA-activated internalization, but did not affect NMDA-activated internalization, which still occurred at a rate similar to control cells. This result indicates that separate mechanisms exist for these two causes of AMPAR endocytosis [77]. Though not yet certain in AMPAR endocytosis, evidence from the internalization of other cargos would suggest that ubiquitin-signaled endocytosis is likely to involve Eps15.

Though different mechanisms are known to signal for the endocytosis of AMPAR, the sequence of events from receptor activation to CME in each of these mechanisms is only beginning to be revealed. In the case of LTD-induced endocytosis of AMPAR, recent work has uncovered more of the process [78]. As mentioned above, LTD is associated with the phosphatase activity initiated by NMDAR activation. A series of experiments with embryonic rat hippocampal neurons demonstrated that RalA mediates endocytosis through interaction with RalBP1 in response to NMDAR stimulation. Pull-down assays showed RalA is activated during NMDA treatment. Once activated, RalA binds RalBP1 and translocates it to the dendritic spine. RalBP1 has also been discovered to bind PSD-95, involving it in AMPA receptor endocytosis. NMDAR activity causes the dephosphorylation of RalBP1, which facilitates its binding to PSD-95. RalBP1 is also important for the stability of Reps2 and forms a tight complex with it in vivo, such that Reps2 can be used as an inhibitor of RalBP1. While NMDA influences the binding of RalBP1 to PSD-95, it does not affect RalBP1's interaction with Reps2. Knockdown of RalA and RalBP1 diminished NMDA-induced AMPAR endocytosis. Moreover, transfection of constitutively active RalA and RalBP1 resulted in decreased surface AMPAR and occluded the effect of NMDA treatment. Together, these results demonstrate the role of RalA and RalBP1 in LTD-mediated AMPAR endocytosis. Further details remain speculative. Because AMPAR is bound to PSD-95 via TARP, the binding of PSD-95 by RalBP1 may bring the AMPAR in close proximity to the endocytic proteins Reps2, Eps15, AP2, and epsin, which then may lead to AMPAR endocytosis [78].

In addition to the well-regulated endocytosis of AMPARs during potentiation, the recycling of AMPAR is also affected. Neurons differ from other cells in that in addition to a perinuclear endosomal recycling compartment, they also have additional, smaller ERCs located at the base of dendritic spines [79]. During LTP, these ERCs move from the base of the spine into the neck. This process facilitates exocytosis-driven growth of the spine [80]. Spine morphology changes in response to LTP by increasing in size [81], thus requiring the addition of membrane. Results from experiments that block recycling from the ERC to the plasma membrane using Rab11 and EHD1 mutants indicate that the source of membrane is the local ERCs [80]. Exocytosis was found to be proportional to spine growth by simultaneously measuring transfected TfR-pHluorin fluorescence intensity and spine surface area.

The subunit composition of the tetrameric AMPAR determines its behavior. In the adult hippocampus the receptors tend to arrange into two populations: one composed of GluR1 and GluR2 subunits, and another composed of GluR2 and GluR3 subunits [82]. The behavior of the GluR1 subunit takes precedence over GluR2, which in turn takes precedence over GluR3 and GluR4. Thus, a receptor composed of GluR1 and GluR2 will behave in a GluR1 fashion and a GluR2/GluR3 combination will behave like GluR2. Shi et al. found that GluR2/GluR3 receptors are delivered to the synapse in an activity-independent fashion and replace existing AMPARs, including GluR1/GluR2 receptors, in a manner which does not result in a change in synaptic strength or the activation of silent synapses. This process was found to be dependent on the GluR2 subunit's cytoplasmic tail's interaction with the PDZ domain. GluR1/GluR2 receptors, on the other hand, are added to synapses during LTP in a fashion that results in a new, more sensitive steady state equilibrium and is consistent with a model in which additional scaffolding materials are added to the synapse to mediate LTP [2].

Once internalized via endocytosis, AMPAR, like other cargos, may proceed to the lysosome for degradation or to the recycling endosome for return to the plasma membrane. Regardless of the trigger for internalization, AMPAR receptors first travel to the early endosome. Then, if the synapse is under conditions of potentiation, recycling is preferred, whereas if the cell is undergoing LTD the degradative pathway is upregulated. This sorting depends on the relative glutamatergic stimulation of NMDAR and AMPAR. NMDAR activation leads to phosphatase-dependent endocytosis of AMPAR receptors which results in rapid recycling. AMPAR activation leads to phosphatase-independent endocytosis, which targets the AMPAR to the lysosome for degradation [83].

These endosomal recycling compartments also maintain a pool of AMPARs which is transported to the dendritic spine surface via exocytosis during LTP for lateral diffusion into the PSD. This process is driven by the molecular motor myosin Vb (MyoVb), which is sensitive to the calcium influx that occurs upon activation of NMDAR during LTP-inducing stimuli [84]. Vesicles do not float unrestrained through the cytoplasm. Instead they are directed along the cytoskeleton by molecular motors. Long range trafficking occurs along microtubules, whereas short transport distances are crossed using actin specific motors. MyoVb in particular has been shown to interact with the recycling endosome through Rab11-FIP2 [47]. Class V myosins have an N-terminal motor domain, a calmodulin-binding domain and a C-terminal globular tail domain (GTD). During calmodulin binding, the GTD is exposed, allowing the molecule to function as a calcium sensitive switch. In the case of MyoVb, the GTD interacts with Rab11-FIP2.

Experiments disrupting or enhancing the interaction between MyoVb and Rab11-FIP2 interaction demonstrate MyoVb's role in recruitment of the recycling endosome to the dendritic spine and in exocytosis-driven LTP via spine growth and AMPAR trafficking [84]. Normally, MyoVb is localized within the spine but does not colocalize with the ERC. However, upon glycine administration (which activates NMDAR causing LTP), MyoVb relocalizes to the ERC. Two mutants of MyoVb, one with tighter binding to Rab11-FIP2 and one with diminished binding capacity, were used to show correlation of MyoVb–Rab11-FIP2 binding to recycling endosome trafficking. RNAi knockdown of MyoVb eliminated LTP-induced exocytosis. Exocytosis could then be rescued using a Rab11-FIP2 binding MyoVb mutant, but not a MyoVb mutant with reduced Rab11-FIP2 binding. These experiments highlight the importance of the Rab11-FIP2 interaction with MyoVb during LTP.

Rab11-FIP2 and MyoVb are not the only factors so far discovered to be essential to exocytosis of AMPAR for LTP. Two other proteins which interact with Rab11-FIP2 are also necessary: EHD1 and Rab11. Initially, EHD1 and Rab11 were separately shown to control the exit of material from the recycling endosome to the plasma membrane [85]. This overlapping functionality suggested a link between them. However, no direct interaction was discovered. Instead EHD is connected to Rab11 through Rab11-FIP2. Using a mutant, dominant negative EHD1 and a mutant constitutively inactive Rab11a, Rab11a and EHD1 were separately shown to be vital to AMPAR recycling and LTP in hippocampal neurons. Moreover, accelerated AMPAR recycling was demonstrated to be the mechanism by which LTP occurs. When the Rab11a or EHD1 mutants were employed, the surface level of AMPAR was diminished and intracellular levels increased proportionally. They had no effect on NMDAR levels. Under conditions which produce LTP in control cells (glycine application or high frequency stimulation) the mutants prevented LTP. To rule out increased synthesis of AMPAR as a source of increased synaptic strength, an experiment was designed to inhibit protein synthesis during LTP-inducing conditions [86]. Surface levels of AMPAR increased even during inhibited protein synthesis. Interestingly, recycling of transferrin was also accelerated during LTP. EHD has also been demonstrated to be required for synaptic vesicle formation and dynamin function in lamprey [87]. All together, these results show that the source of AMPAR for LTP is the pool maintained in the local ERC [86].

6. Transcytosis

Transcytosis is another process in the somatodendritic compartment that has been shown to involve protein adaptors of molecular trafficking. Because neurons are unique polarized cells, they have developed specialized endocytic routes for the targeting of proteins to axonal and somatodendritic compartments. In several cases, this targeting occurs via transcytosis. The protein in question is first exocytosed to the somatodendritic compartment; then at some point afterward undergoes endocytosis and movement to the axonal compartment for exocytosis on that surface. Examples of such proteins include neuron–glia cell adhesion molecule (NgCAM), the chick homologue of L1 (L1/NgCAM) [7], tropomyosin related kinase (Trk) receptors which respond to neurotrophins [88], and contactin-associated protein 2 (Caspr2) [89].

L1 is of interest because a point mutation in it is implicated in X-linked hydrocephalus [90]. L1/NgCAM is a transmembrane protein that is involved in signaling for neurite growth [91]. Its cytoplasmic tail binds to AP2, and it is internalized by CME [92]. Somatodendritic targeting of L1/NgCAM from the trans-Golgi network is mediated by a YRSLE motif in the cytoplasmic tail, and axonal targeting is mediated by glycine and serine rich area also in the cytoplasmic tail, along with an extracellular domain [93,94].

A series of recent experiments investigating L1/NgCAM trafficking in hippocampal neurons has suggested novel cell and cargo specific roles for EHD1 and EHD4. The first set demonstrated that dimerization of EHD1 and EHD4 is required for proper axonal targeting of L1/NgCAM [95]. Using the same dominant negative EHD1 used to inhibit AMPAR exocytosis during LTP, Yap et al. showed that L1/NgCAM axonal localization was negatively affected. In addition, overexpression of wild type EHD1 or EHD4 as well as shRNA knockdown of EHD1 similarly affected L1/NgCAM targeting. Overexpression of wild type EHD3 or an inactive form of EHD4, however, did not negatively affect targeting. To explain the unusual result that wild type upregulation and dominant negative knockdown of the same protein have similar functional consequences, Yap et al. proposed a requirement for a proper balance between EHD1 and EHD4 [95]. To test this hypothesis, they simultaneously overexpressed both wild type EHD1 and EHD4 and noted that L1/NgCAM targeting was consistent with controls. They also coexpressed wild type EHD1 and a mutant form of EHD4 incapable of dimerization, which disrupted L1/NgCAM axonal targeting. Notably, wild type expression of EHD1 did not affect transferrin recycling. These results support a model in which EHD1–EHD4 dimerization is required for the cargo specific trafficking of L1/NgCAM [95].

The second set of experiments involving L1/NgCAM and EHD1 proposed EHD1's role in recycling is possibly different in neurons than has been previously described in other cell lines [95]. EHD1, transferrin, Rab11, EEA1, and L1 localization were examined using immunofluorescent staining and live imaging. EHD1-labeled endosomes were mostly vesicular and not tubular. Moreover, EHD1 more often colocalized with EEA1 than Rab11. This contrasts previous colocalization studies which place EHD1 along with Rab11 at the ERC. The results were otherwise consistent with the established model of endosomal maturation involving transiently associated membrane bound regulators [96].

7. Intersectin and spine morphogenesis

Another unique aspect of neurons is their morphology which consists of many projections of membrane. As the neuron matures, it must increase its membrane surface area many fold, which requires a large degree of membrane component synthesis and trafficking. This process occurs as in other cells through the endoplasmic reticulum–Golgi secretory pathway. Once established, the morphology is then continually maintained for months to years. Forming and maintaining such a complicated structure involve regulated membrane trafficking via the endosomal system and the actin cytoskeleton.

An EH protein of interest in this process is the EH-protein intersectin that, as mentioned previously, is involved in nucleation of CME along with Eps15 [27]. The upregulation of Intersectin-1 (Itsn1) in individuals with Down syndrome has been suggested to contribute to the pathogenesis of both Down syndrome and Alzheimer's disease [97,98]. There are two human genes: ITSN1 and ITSN2 which each have two isoforms, a long and a short form, that arise from alternate splicing [99]. The short form has two N-terminal EH domains, a coiled coil region and five SH3 domains. The long form contains three additional C-terminal domains: a Dbl homology domain (DH), a PH domain, and a C2 domain. The EH domain of intersectin binds epsin family members, and intersectin's SH3 associates it with dynamin, which helps regulate dynamin's localization to clathrin-coated pits [13]. Expression of the two isoforms is not uniform. The long form (intersectin-l) is mostly expressed in mammalian neurons, whereas the short form is expressed in other cells [100]. In a study of rat hippocampal neurons, intersectin-l was localized to the somatodendritic compartment and colocalized with clathrin and AP2. Knockdown did not impair synaptic vesicle recycling, but disrupted transferrin recycling and spine maturation [101].

The association of intersectin with the GTPase Cdc42 provides further evidence of its role in dendritic spine formation. Intersectin activates Cdc42 through its DH domain, which acts as a GEF. Cdc42 is a member of the Rho family of small GTPases. Activation of Cdc42 induces formation of filopodia, small projections of membrane-containing polymerized actin [102]. In neurons, filopodia also serve as the precursor to dendritic spines. In addition, dominant negative mutants of Cdc42 inhibited spine morphogenesis [103], possibly through a PI3K–C2b–AKT pathway [104]. Cdc42 nucleates actin branching through WASp and the Arp2/3 complex by binding to WASp. Binding to Cdc42 undoes WASp's autoinhibition, which allows it to bind to the Arp2/3 complex [105]. The WASp–Arp2/3 complex in turn nucleates actin branching [102]. Intersectin also binds to neuronal enriched homologue of WASp (N-WASP). This N-WASP–intersectin interaction was found to stabilize the interaction between intersectin and Cdc42 and stimulate intersectin's GEF activity toward Cdc42. Since increased Cdc42 activity affects actin polymerization through N-WASP, N-WASP in conjunction with intersectin stimulates its own activity [106]. In this way, intersectin plays a role in filopodia formation by participating in actin assembly (Fig. 4).

EphB2 tyrosine kinase also stimulates intersectin's GEF activity [103]. EphB2 is a member of the Eph family of tyrosine kinase receptors which has been subdivided into two classes: A and B. These receptors are involved in cell–cell interactions, as their ligands, ephrins, are also membrane-bound. A-ephrins are GPI anchored, while B-ephrins are transmembrane proteins. When ephrin binds an Eph receptor, signaling pathways are activated in both cells. EphB2 receptors localize in the postsynaptic region through interactions with PDZ-containing proteins [107]. In murine hippocampal neurons, EphB2 was found to coimmunoprecipitate with intersectin-l, and double-labeled immunofluorescence experiments colocalized EphB2, intersectin-l, and N-WASP in spines. EphB2 was shown to synergistically amplify the GEF activity of intersectin-l along with N-WASP. Together, these results suggest a mechanism whereby signaling of EphB2 induces local spine growth through actin polymerization by way of the N-WASP–Arp2/3 complex [103].

Another avenue that indicates intersectin plays a role in spine maturation is its interaction with Numb. Numb has been associated with neurite growth, endocytosis, and the determination of cell fate [108,109]. Numb was first discovered as a mutant which disrupted *Drosophila* sensory neuron differentiation [109], that is similar to the defects observed with loss-of-function intersectin mutants [110,111]. In order for a cell to create two daughter cells with different differentiation programs, proteins are asymmetrically distributed during mitosis. Alternatively, external signals may be applied differently to daughter cells. Numb acts as a cell fate determinant both through its asymmetric distribution [112] and by inhibiting Notch signaling, an external regulator [113]. Dominant negative mutants of Numb also disrupt endocytosis, and Numb binds to AP2 and Eps15, further suggesting a role in endocytosis [108]. Numb possesses a C-terminal NPF and a DPF motif, allowing for interaction with EH-proteins, and the CME protein α-adaptin, respectively.

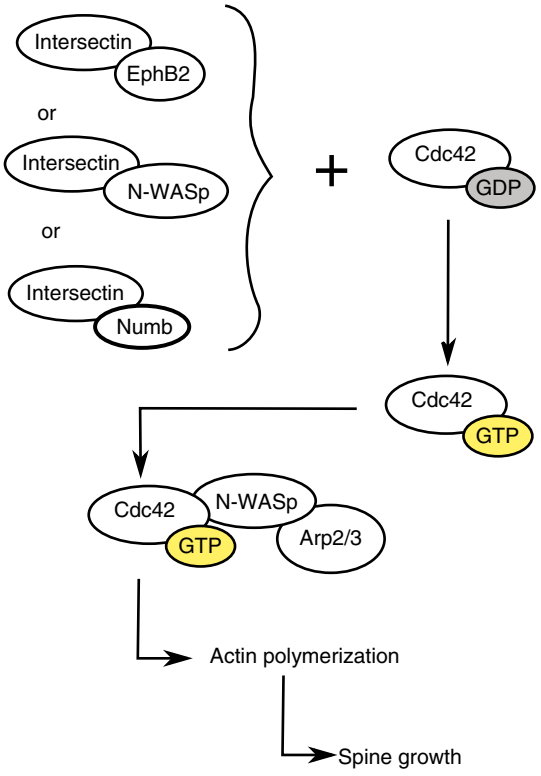

Fig. 4. The role of intersectin in dendritic spine growth. The binding of EphB2, N-WASP or Numb stimulates the ability of intersectin to act as activating guanine nucleotide exchange factor (GEF) for Cdc42. Activated, GTP-bound Cdc42 in turn recruits other factors such as Arp2/3 which stimulates actin polymerization leading to dendritic spine growth.

Numb was found to localize in puncta in the dendritic spines and to colocalize with the postsynaptic marker PSD-95, but not with the presynaptic marker synaptophysin [114]. Intersectin also colocalized with Numb. Suppression of Numb using siRNAs reduced the number of dendritic protrusions in developing neurons, but had little effect on mature neurons. It was determined that Numb enhances intersectin's GEF function for Cdc42, which in turn influences the formation of filopodia. Numb accomplishes this by competing with the SH3 DH–PH intramolecular interaction of intersectin which normally serves as a negative regulator of its GEF activity. Numb was also found to be associated with NMDAR. These findings hint at a role for intersectin and Numb in activity-dependent dendritic spine development [114].

In addition to its interaction with intersectin, Numb also interacts with EHDs. Though Numb colocalizes with AP2 and Eps15 in clathrin-coated pits and the early endosome, a significant portion is localized to intracellular vesicles that do not contain AP2. On some of these vesicles, Numb was shown to colocalize with EHD4 and Arf6 [115]. Colocalization with EHD4 was increased by the introduction of a constitutively active Arf6 mutant, which suggests that Arf6 plays a regulatory role in the association of Numb and EHD4. Further evidence of Numb's involvement in the clathrin-independent Arf6 pathway is knockdown by RNA interference, which prevented the recycling of Tac, a receptor normally recycled by the Arf6 pathway.

Notch signaling, which is regulated by Numb, also plays a role in neurite growth. Normally as neurons extend neurites the increasing number of contacts results in increased Notch receptor ligand binding. This upregulation of Notch activity results in negative feedback that arrests neurite growth [105]. Numb most likely adjusts Notch signaling via its involvement in post endocytic trafficking. From overexpression and RNA interference experiments, Numb does not affect the constitutive endocytosis and recycling of Notch but rather later sorting events which direct Notch to the lysosome for degradation. The interaction with α-adaptin and the EH domain is required for Numb influence as a Numb mutant lacking the NPF and DPF-containing region changes Notch trafficking. It still remains to be seen if Numb affects Notch receptor concentration generally or responds in a ligand-dependent manner [116].

8. Perspectives

All cells utilize a well-regulated endosomal trafficking system to direct the movement of membrane bound components. This system is responsible for controlling receptor concentrations on the cell surface, which affects how sensitive the cell is to its environment. In the brain, this system is employed in synaptogenesis, the maintenance of neural circuits and polarity, and adaptation to changing stimuli, in addition to functions essential to cell survival such as the uptake of transferrin and LDL. At various decision making points within the trafficking system EH-proteins are found. In the last decade, a large network of proteins that interact with these EH domains has been discovered, and many experiments have been performed which demonstrate the essential role of the EH domain in a variety of processes. Yet, though there are many hypotheses, a strong and thorough picture of this system and its regulators is still in the making.

So far we know that Eps15 is essential to CME of several receptors. CME oftentimes occurs repeated at the same location, as is the case in the internalization of AMPAR at the PSD. However, there are different signals which provoke internalization of AMPAR in an activity-dependent or -independent manner, and the details of these signaling pathways are not clear. Though dominant negative Eps15 mutants can block CME of AMPAR, little more is known of Eps15's role. During LTD, RalBP1, a known binding partner of several EH-proteins, is enlisted to facilitate endocytosis after phosphatase activation by NMDAR. More research is needed to undercover the missing steps in these processes.

Recycling of AMPAR is now known to play an important role in maintaining synaptic strength and as source of membrane, scaffolding

protein, and AMPAR for LTP. EHD1 is important for recycling in neurons as in other cells, though there are conflicting reports of its localization. For AMPAR exocytosis during LTP, the molecular motor MyoVb, Rab11, Rab11-FIP2, calcium influx, and NMDAR activation are all essential elements. Whether there is an exocytic microdomain to correspond to the endocytic zone is a subject of current inquiry. EHDs can bind and tubulate membrane and possibly serve as a source of membrane fission as well as bind various other regulators. What specifically the EHD proteins do is unclear. Despite structural similarity and in some cases mutual dependence for function, they can have differing localizations and effects. Their ability to transiently oligomerize, ability to bind membrane, and association with various Rabs suggest a role linking the various endosomal compartments.

The EH-protein, intersectin, has been shown to operate not only in endocytosis, but also in the morphogenesis of dendritic spines by connecting EphB receptors to the polymerization of actin via a process of amplified GEF activity. Morphogenesis is also directed in part by Numb by working with EHD4 in endosomal sorting events. The role of the Numb–EHD4 interaction in this process still needs to be investigated. Morphogenesis, synaptic plasticity, and trafficking in the postsynaptic dendritic spine are all processes that are becoming better understood, but there is still much exciting territory to explore.

References

[1] H. Furukawa, S.K. Singh, R. Mancusso, E. Gouaux, Subunit arrangement and function in NMDA receptors, Nature 438 (2005) 185–192.
[2] S. Shi, Y. Hayashi, J.A. Esteban, R. Malinow, Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons, Cell 105 (2001) 331–343.
[3] V.N. Kharazia, R.J. Weinberg, Tangential synaptic distribution of NMDA and AMPA receptors in rat neocortex, Neurosci. Lett. 238 (1997) 41–44.
[4] S. Raghavachari, J.E. Lisman, Properties of quantal transmission at CA1 synapses, J. Neurophysiol. 92 (2004) 2456–2467.
[5] T.A. Blanpied, J.M. Kerr, M.D. Ehlers, Structural plasticity with preserved topology in the postsynaptic protein network, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12587–12592.
[6] T.A. Blanpied, D.B. Scott, M.D. Ehlers, Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines, Neuron 36 (2002) 435–449.
[7] D. Wisco, E.D. Anderson, M.C. Chang, C. Norden, T. Boiko, H. Folsch, B. Winckler, Uncovering multiple axonal targeting pathways in hippocampal neurons, J. Cell Biol. 162 (2003) 1317–1328.
[8] A.W. van Weert, K.W. Dunn, H.J. Gueze, F.R. Maxfield, W. Stoorvogel, Transport from late endosomes to lysosomes, but not sorting of integral membrane proteins in endosomes, depends on the vacuolar proton pump, J. Cell Biol. 130 (1995) 821–834.
[9] M. Babst, D.J. Katzmann, W.B. Snyder, B. Wendland, S.D. Emr, Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body, Dev. Cell 3 (2002) 283–289.
[10] F. Fazioli, L. Minichiello, B. Matoskova, W.T. Wong, P.P. Di Fiore, eps15, a novel tyrosine kinase substrate, exhibits transforming activity, Mol. Cell. Biol. 13 (1993) 5814–5828.
[11] W.T. Wong, C. Schumacher, A.E. Salcini, A. Romano, P. Castagnino, P.G. Pelicci, P. Di Fiore, A protein-binding domain, EH, identified in the receptor tyrosine kinase substrate Eps15 and conserved in evolution, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 9530–9534.
[12] A.E. Salcini, S. Confalonieri, M. Doria, E. Santolini, E. Tassi, O. Minenkova, G. Cesareni, P.G. Pelicci, P.P. Di Fiore, Binding specificity and in vivo targets of the EH domain, a novel protein–protein interaction module, Genes Dev. 11 (1997) 2239–2249.
[13] A.S. Sengar, W. Wang, J. Bishay, S. Cohen, S.E. Egan, The EH and SH3 domain Ese proteins regulate endocytosis by linking to dynamin and Eps15, EMBO J. 18 (1999) 1159–1171.
[14] F. Tebar, S. Confalonieri, R.E. Carter, P.P. Di Fiore, A. Sorkin, Eps15 is constitutively oligomerized due to homophilic interaction of its coiled-coil region, J. Biol. Chem. 272 (1997) 15413–15418.
[15] T. de Beer, R.E. Carter, K.E. Lobel-Rice, A. Sorkin, M. Overduin, Structure and asn-pro-Phe binding pocket of the eps15 homology domain, Science 281 (1998) 1357–1360.
[16] A. Lewit-Bentley, S. Rety, EF-hand calcium-binding proteins, Curr. Opin. Struct. Biol. 10 (2000) 637–643.
[17] S. Paoluzi, L. Castagnoli, I. Lauro, A.E. Salcini, L. Coda, S. Fre, S. Confalonieri, P.G. Pelicci, P. Paolo Di Fiore, G. Cesareni, Recognition specificity of individual EH domains of mammals and yeast, EMBO J. 17 (1998) 6541–6550.
[18] A. Benmerah, J. Gagnon, B. Begue, B. Megarbane, A. Dautry-Varsat, N. Cerf-Bensussan, The tyrosine kinase substrate eps15 is constitutively associated with the plasma membrane adaptor AP-2, J. Cell Biol. 131 (1995) 1831–1838.
[19] R. Carbone, S. Fre, G. Iannolo, F. Belleudi, P. Mancini, P.G. Pelicci, M.R. Torrisi, P.P. Di Fiore, eps15 and eps15R are essential components of the endocytic pathway, Cancer Res. 57 (1997) 5498–5504.
[20] R.G. Anderson, M.S. Brown, J.L. Goldstein, Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts, Cell 10 (1977) 351–364.
[21] I. Gaidarov, F. Santini, R.A. Warren, J.H. Keen, Spatial control of coated-pit dynamics in living cells, Nat. Cell Biol. 1 (1999) 1–7.
[22] E. Ungewickell, D. Branton, Assembly units of clathrin coats, Nature 289 (1981) 420–422.
[23] J.A. Ybe, B. Greene, S.H. Liu, U. Pley, P. Parham, F.M. Brodsky, Clathrin self-assembly is regulated by three light-chain residues controlling the formation of critical salt bridges, EMBO J. 17 (1998) 1297–1303.
[24] I. Gaidarov, Q. Chen, J.R. Falck, K.K. Reddy, J.H. Keen, A functional phosphatidylinositol 3,4,5-trisphosphate/phosphoinositide binding domain in the clathrin adaptor AP-2 alpha subunit. Implications for the endocytic pathway, J. Biol. Chem. 271 (1996) 20922–20929.
[25] W. Boll, H. Ohno, Z. Songyang, I. Rapoport, L.C. Cantley, J.S. Bonifacino, T. Kirchhausen, Sequence requirements for the recognition of tyrosine-based endocytic signals by clathrin AP-2 complexes, EMBO J. 15 (1996) 5789–5795.
[26] A. Gallusser, T. Kirchhausen, The beta 1 and beta 2 subunits of the AP complexes are the clathrin coat assembly components, EMBO J. 12 (1993) 5237–5244.
[27] W.M. Henne, E. Boucrot, M. Meinecke, E. Evergren, Y. Vallis, R. Mittal, H.T. McMahon, FCHo proteins are nucleators of clathrin-mediated endocytosis, Science 328 (2010) 1281–1284.
[28] A. Benmerah, B. Begue, A. Dautry-Varsat, N. Cerf-Bensussan, The ear of alpha-adaptin interacts with the COOH-terminal domain of the Eps 15 protein, J. Biol. Chem. 271 (1996) 12111–12116.
[29] P. Cupers, A.P. Jadhav, T. Kirchhausen, Assembly of clathrin coats disrupts the association between Eps15 and AP-2 adaptors, J. Biol. Chem. 273 (1998) 1847–1850.
[30] F. Tebar, T. Sorkina, A. Sorkin, M. Ericsson, T. Kirchhausen, Eps15 is a component of clathrin-coated pits and vesicles and is located at the rim of coated pits, J. Biol. Chem. 271 (1996) 28727–28730.
[31] R.J. Lefkowitz, S.K. Shenoy, Transduction of receptor signals by beta-arrestins, Science 308 (2005) 512–517.
[32] S. Polo, S. Sigismund, M. Faretta, M. Guidi, M.R. Capua, G. Bossi, H. Chen, P. De Camilli, P.P. Di Fiore, A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins, Nature 416 (2002) 451–455.
[33] P.M. Okamoto, J.S. Herskovits, R.B. Vallee, Role of the basic, proline-rich region of dynamin in Src homology 3 domain binding and endocytosis, J. Biol. Chem. 272 (1997) 11629–11635.
[34] J.H. Koenig, K. Ikeda, Disappearance and reformation of synaptic vesicle membrane upon transmitter release observed under reversible blockage of membrane retrieval, J. Neurosci. 9 (1989) 3844–3860.
[35] S.L. Schmid, V.A. Frolov, Dynamin: functional design of a membrane fission catalyst, Annu. Rev. Cell Dev. Biol. 27 (2011) 79–105.
[36] A. Rothnie, A.R. Clarke, P. Kuzmic, A. Cameron, C.J. Smith, A sequential mechanism for clathrin cage disassembly by 70-kDa heat-shock cognate protein (Hsc70) and auxilin, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6927–6932.
[37] S. Mayor, R.E. Pagano, Pathways of clathrin-independent endocytosis, Nat. Rev. Mol. Cell Biol. 8 (2007) 603–612.
[38] S.B. Cantor, T. Urano, L.A. Feig, Identification and characterization of Ral-binding protein 1, a potential downstream target of Ral GTPases, Mol. Cell. Biol. 15 (1995) 4578–4584.
[39] S.H. Park, R.A. Weinberg, A putative effector of Ral has homology to Rho/Rac GTPase activating proteins, Oncogene 11 (1995) 2349–2355.
[40] V. Jullien-Flores, O. Dorseuil, F. Romero, F. Letourneur, S. Saragosti, R. Berger, A. Tavitian, G. Gacon, J.H. Camonis, Bridging Ral GTPase to Rho pathways. RLIP76, a Ral effector with CDC42/Rac GTPase-activating protein activity, J. Biol. Chem. 270 (1995) 22473–22477.
[41] M. Shipitsin, L.A. Feig, RalA but not RalB enhances polarized delivery of membrane proteins to the basolateral surface of epithelial cells, Mol. Cell. Biol. 24 (2004) 5746–5756.
[42] A. Yamaguchi, T. Urano, T. Goi, L.A. Feig, An Eps homology (EH) domain protein that binds to the Ral-GTPase target, RalBP1, J. Biol. Chem. 272 (1997) 31230–31234.
[43] M. Ikeda, O. Ishida, T. Hinoi, S. Kishida, A. Kikuchi, Identification and characterization of a novel protein interacting with Ral-binding protein 1, a putative effector protein of Ral, J. Biol. Chem. 273 (1998) 814–821.
[44] V. Jullien-Flores, Y. Mahe, G. Mirey, C. Leprince, B. Meunier-Bisceuil, A. Sorkin, J.H. Camonis, RLIP76, an effector of the GTPase Ral, interacts with the AP2 complex: involvement of the Ral pathway in receptor endocytosis, J. Cell Sci. 113 (2000) 2837–2844.
[45] P. Steiner, J.C. Sarria, L. Glauser, S. Magnin, S. Catsicas, H. Hirling, Modulation of receptor cycling by neuron-enriched endosomal protein of 21 kD, J. Cell Biol. 157 (2002) 1197–1209.
[46] C.C. Yap, D. Wisco, P. Kujala, Z.M. Lasiecka, J.T. Cannon, M.C. Chang, H. Hirling, J. Klumperman, B. Winckler, The somatodendritic endosomal regulator NEEP21 facilitates axonal targeting of L1/NgCAM, J. Cell Biol. 180 (2008) 827–842.
[47] C.M. Hales, J.P. Vaerman, J.R. Goldenring, Rab11 family interacting protein 2 associates with Myosin Vb and regulates plasma membrane recycling, J. Biol. Chem. 277 (2002) 50415–50421.
[48] C.P. Horgan, A. Oleksy, A.V. Zhdanov, P.Y. Lall, I.J. White, A.R. Khan, C.E. Futter, J.G. McCaffrey, M.W. McCaffrey, Rab11-FIP3 is critical for the structural integrity of the endosomal recycling compartment, Traffic 8 (2007) 414–430.
[49] E. Schonteich, G.M. Wilson, J. Burden, C.R. Hopkins, K. Anderson, J.R. Goldenring, R. Prekeris, The Rip11/Rab11-FIP5 and kinesin II complex regulates endocytic protein recycling, J. Cell Sci. 121 (2008) 3824–3833.

[50] A.M. Powelka, J. Sun, J. Li, M. Gao, L.M. Shaw, A. Sonnenberg, V.W. Hsu, Stimulation-dependent recycling of integrin beta1 regulated by ARF6 and Rab11, Traffic 5 (2004) 20–36.

[51] N. Naslavsky, J. Rahajeng, M. Sharma, M. Jovic, S. Caplan, Interactions between EHD proteins and Rab11-FIP2: a role for EHD3 in early endosomal transport, Mol. Biol. Cell 17 (2006) 163–177.

[52] L. Mintz, E. Galperin, M. Pasmanik-Chor, S. Tulzinsky, Y. Bromberg, C.A. Kozak, A. Joyner, A. Fein, M. Horowitz, EHD1—an EH-domain-containing protein with a specific expression pattern, Genomics 59 (1999) 66–76.

[53] U. Pohl, J.S. Smith, I. Tachibana, K. Ueki, H.K. Lee, S. Ramaswamy, Q. Wu, H.W. Mohrenweiser, R.B. Jenkins, D.N. Louis, EHD2, EHD3, and EHD4 encode novel members of a highly conserved family of EH domain-containing proteins, Genomics 63 (2000) 255–262.

[54] S.X. Lin, B. Grant, D. Hirsh, F.R. Maxfield, Rme-1 regulates the distribution and function of the endocytic recycling compartment in mammalian cells, Nat. Cell Biol. 3 (2001) 567–572.

[55] M. Sharma, S.S. Giridharan, J. Rahajeng, N. Naslavsky, S. Caplan, MICAL-L1 links EHD1 to tubular recycling endosomes and regulates receptor recycling, Mol. Biol. Cell 20 (2009) 5181–5194.

[56] S. Caplan, N. Naslavsky, L.M. Hartnell, R. Lodge, R.S. Polishchuk, J.G. Donaldson, J.S. Bonifacino, A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane, EMBO J. 21 (2002) 2557–2567.

[57] G.D. Henry, D.J. Corrigan, J.V. Dineen, J.D. Baleja, Charge effects in the selection of NPF motifs by the EH domain of EHD1, Biochemistry 49 (2010) 3381–3392.

[58] F. Kieken, M. Sharma, M. Jovic, S.S. Giridharan, N. Naslavsky, S. Caplan, P.L. Sorgen, Mechanism for the selective interaction of C-terminal EH-domain proteins with specific NPF-containing partners, J. Biol. Chem. 285 (2010) 8687–8694.

[59] O. Daumke, R. Lundmark, Y. Vallis, S. Martens, P.J. Butler, H.T. McMahon, Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling, Nature 449 (2007) 923–927.

[60] D.W. Lee, X. Zhao, S. Scarselletta, P.J. Schweinsberg, E. Eisenberg, B.D. Grant, L.E. Greene, ATP binding regulates oligomerization and endosome association of RME-1 family proteins, J. Biol. Chem. 280 (2005) 17213–17220.

[61] B. Moren, C. Shah, M.T. Howes, N.L. Schieber, H.T. McMahon, R.G. Parton, O. Daumke, R. Lundmark, EHD2 regulates caveolar dynamics via ATP-driven targeting and oligomerization, Mol. Biol. Cell 23 (2012) 1316–1329.

[62] M. Stoeber, I. Karen Stoeck, C. Hanni, C. Karl Ernst Bleck, G. Balistreri, A. Helenius, Oligomers of the ATPase EHD2 confine caveolae to the plasma membrane through association with actin, EMBO J. 31 (2012) 2350–2364.

[63] M. Sharma, N. Naslavsky, S. Caplan, A role for EHD4 in the regulation of early endosomal transport, Traffic 9 (2008) 995–1018.

[64] H.W. Kessels, C.D. Kopec, M.E. Klein, R. Malinow, Roles of stargazin and phosphorylation in the control of AMPA receptor subcellular distribution, Nat. Neurosci. 12 (2009) 888–896.

[65] J. Lu, T.D. Helton, T.A. Blanpied, B. Racz, T.M. Newpher, R.J. Weinberg, M.D. Ehlers, Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer, Neuron 55 (2007) 874–889.

[66] N.W. Gray, L. Fourgeaud, B. Huang, J. Chen, H. Cao, B.J. Oswald, A. Hemar, M.A. McNiven, Dynamin 3 is a component of the postsynapse, where it interacts with mGluR5 and Homer, Curr. Biol. 13 (2003) 510–515.

[67] C. Racca, F.A. Stephenson, P. Streit, J.D. Roberts, P. Somogyi, NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area, J. Neurosci. 20 (2000) 2512–2522.

[68] L. Groc, M. Heine, L. Cognet, K. Brickley, F.A. Stephenson, B. Lounis, D. Choquet, Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors, Nat. Neurosci. 7 (2004) 695–696.

[69] C. Bats, L. Groc, D. Choquet, The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking, Neuron 53 (2007) 719–734.

[70] C. Tardin, L. Cognet, C. Bats, B. Lounis, D. Choquet, Direct imaging of lateral movements of AMPA receptors inside synapses, EMBO J. 22 (2003) 4656–4665.

[71] A.J. Borgdorff, D. Choquet, Regulation of AMPA receptor lateral movements, Nature 417 (2002) 649–653.

[72] M.C. Ashby, S.R. Maier, A. Nishimune, J.M. Henley, Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology, J. Neurosci. 26 (2006) 7046–7055.

[73] N.W. Gray, R.M. Weimer, I. Bureau, K. Svoboda, Rapid redistribution of synaptic PSD-95 in the neocortex in vivo, PLoS Biol. 4 (2006) e370.

[74] H.Y. Man, J.W. Lin, W.H. Ju, G. Ahmadian, L. Liu, L.E. Becker, M. Sheng, Y.T. Wang, Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization, Neuron 25 (2000) 649–662.

[75] J.W. Lin, W. Ju, K. Foster, S.H. Lee, G. Ahmadian, M. Wyszynski, Y.T. Wang, M. Sheng, Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization, Nat. Neurosci. 3 (2000) 1282–1290.

[76] S.H. Lee, L. Liu, Y.T. Wang, M. Sheng, Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD, Neuron 36 (2002) 661–674.

[77] L.A. Schwarz, B.J. Hall, G.N. Patrick, Activity-dependent ubiquitination of GluA1 mediates a distinct AMPA receptor endocytosis and sorting pathway, J. Neurosci. 30 (2010) 16718–16729.

[78] K. Han, M.H. Kim, D. Seeburg, J. Seo, C. Verpelli, S. Han, H.S. Chung, J. Ko, H.W. Lee, K. Kim, W.D. Heo, T. Meyer, H. Kim, C. Sala, S.Y. Choi, M. Sheng, E. Kim, Regulated RalBP1 binding to RalA and PSD-95 controls AMPA receptor endocytosis and LTD, PLoS Biol. 7 (2009) e1000187.

[79] J.R. Cooney, J.L. Hurlburt, D.K. Selig, K.M. Harris, J.C. Fiala, Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane, J. Neurosci. 22 (2002) 2215–2224.

[80] M. Park, J.M. Salgado, L. Ostroff, T.D. Helton, C.G. Robinson, K.M. Harris, M.D. Ehlers, Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes, Neuron 52 (2006) 817–830.

[81] M. Maletic-Savatic, R. Malinow, K. Svoboda, Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity, Science 283 (1999) 1923–1927.

[82] R.J. Wenthold, R.S. Petralia, J. Blahos II, A.S. Niedzielski, Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons, J. Neurosci. 16 (1996) 1982–1989.

[83] M.D. Ehlers, Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting, Neuron 28 (2000) 511–525.

[84] Z. Wang, J.G. Edwards, N. Riley, D.W. Provance Jr., R. Karcher, X.D. Li, I.G. Davison, M. Ikebe, J.A. Mercer, J.A. Kauer, M.D. Ehlers, Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity, Cell 135 (2008) 535–548.

[85] M. Ren, G. Xu, J. Zeng, C. De Lemos-Chiarandini, M. Adesnik, D.D. Sabatini, Hydrolysis of GTP on rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6187–6192.

[86] M. Park, E.C. Penick, J.G. Edwards, J.A. Kauer, M.D. Ehlers, Recycling endosomes supply AMPA receptors for LTP, Science 305 (2004) 1972–1975.

[87] J. Jakobsson, F. Ackermann, F. Andersson, D. Larhammar, P. Low, L. Brodin, Regulation of synaptic vesicle budding and dynamin function by an EHD ATPase, J. Neurosci. 31 (2011) 13972–13980.

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

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

[90] A. Rosenthal, M. Jouet, S. Kenwrick, Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus, Nat. Genet. 2 (1992) 107–112.

[91] V. Lemmon, K.L. Farr, C. Lagenaur, L1-mediated axon outgrowth occurs via a homophilic binding mechanism, Neuron 2 (1989) 1597–1603.

[92] H. Kamiguchi, K.E. Long, M. Pendergast, A.W. Schaefer, I. Rapoport, T. Kirchhausen, V. Lemmon, The neural cell adhesion molecule L1 interacts with the AP-2 adaptor and is endocytosed via the clathrin-mediated pathway, J. Neurosci. 18 (1998) 5311–5321.

[93] C.C. Yap, R.L. Nokes, D. Wisco, E. Anderson, H. Folsch, B. Winckler, Pathway selection to the axon depends on multiple targeting signals in NgCAM, J. Cell Sci. 121 (2008) 1514–1525.

[94] B. Sampo, S. Kaech, S. Kunz, G. Banker, Two distinct mechanisms target membrane proteins to the axonal surface, Neuron 37 (2003) 611–624.

[95] C.C. Yap, Z.M. Lasiecka, S. Caplan, B. Winckler, Alterations of EHD1/EHD4 protein levels interfere with L1/NgCAM endocytosis in neurons and disrupt axonal targeting, J. Neurosci. 30 (2010) 6646–6657.

[96] Z.M. Lasiecka, C.C. Yap, S. Caplan, B. Winckler, Neuronal early endosomes require EHD1 for L1/NgCAM trafficking, J. Neurosci. 30 (2010) 16485–16497.

[97] D.J. Keating, C. Chen, M.A. Pritchard, Alzheimer's disease and endocytic dysfunction: clues from the Down syndrome-related proteins, DSCR1 and ITSN1, Ageing Res. Rev. 5 (2006) 388–401.

[98] A. Pechstein, O. Shupliakov, V. Haucke, Intersectin 1: a versatile actor in the synaptic vesicle cycle, Biochem. Soc. Trans. 38 (2010) 181–186.

[99] M. Guipponi, H.S. Scott, H. Chen, A. Schebesta, C. Rossier, S.E. Antonarakis, Two isoforms of a human intersectin (ITSN) protein are produced by brain-specific alternative splicing in a stop codon, Genomics 53 (1998) 369–376.

[100] N.K. Hussain, M. Yamabhai, A.R. Ramjaun, A.M. Guy, D. Baranes, J.P. O'Bryan, C.J. Der, B.K. Kay, P.S. McPherson, Splice variants of intersectin are components of the endocytic machinery in neurons and nonneuronal cells, J. Biol. Chem. 274 (1999) 15671–15677.

[101] S. Thomas, B. Ritter, D. Verbich, C. Sanson, L. Bourbonniere, R.A. McKinney, P.S. McPherson, Intersectin regulates dendritic spine development and somatodendritic endocytosis but not synaptic vesicle recycling in hippocampal neurons, J. Biol. Chem. 284 (2009) 12410–12419.

[102] C.D. Nobes, A. Hall, Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia, Cell 81 (1995) 53–62.

[103] F. Irie, Y. Yamaguchi, EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP, Nat. Neurosci. 5 (2002) 1117–1118.

[104] M. Das, E. Scappini, N.P. Martin, K.A. Wong, S. Dunn, Y.J. Chen, S.L. Miller, J. Domin, J.P. O'Bryan, Regulation of neuron survival through an intersectin-phosphoinositide 3′-kinase C2beta-AKT pathway, Mol. Cell. Biol. 27 (2007) 7906–7917.

[105] A.S. Kim, L.T. Kakalis, N. Abdul-Manan, G.A. Liu, M.K. Rosen, Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein, Nature 404 (2000) 151–158.

[106] N.K. Hussain, S. Jenna, M. Glogauer, C.C. Quinn, S. Wasiak, M. Guipponi, S.E. Antonarakis, B.K. Kay, T.P. Stossel, N. Lamarche-Vane, P.S. McPherson, Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP, Nat. Cell Biol. 3 (2001) 927–932.

[107] R. Torres, B.L. Firestein, H. Dong, J. Staudinger, E.N. Olson, R.L. Huganir, D.S. Bredt, N.W. Gale, G.D. Yancopoulos, PDZ proteins bind, cluster, and synaptically colocalize with Eph receptors and their ephrin ligands, Neuron 21 (1998) 1453–1463.

[108] E. Santolini, C. Puri, A.E. Salcini, M.C. Gagliani, P.G. Pelicci, C. Tacchetti, P.P. Di Fiore, Numb is an endocytic protein, J. Cell Biol. 151 (2000) 1345–1352.
[109] T. Uemura, S. Shepherd, L. Ackerman, L.Y. Jan, Y.N. Jan, numb, a gene required in determination of cell fate during sensory organ formation in *Drosophila* embryos, Cell 58 (1989) 349–360.
[110] T.W. Koh, P. Verstreken, H.J. Bellen, Dap160/intersectin acts as a stabilizing scaffold required for synaptic development and vesicle endocytosis, Neuron 43 (2004) 193–205.
[111] B. Marie, S.T. Sweeney, K.E. Poskanzer, J. Roos, R.B. Kelly, G.W. Davis, Dap160/intersectin scaffolds the periactive zone to achieve high-fidelity endocytosis and normal synaptic growth, Neuron 43 (2004) 207–219.
[112] W. Zhong, J.N. Feder, M.M. Jiang, L.Y. Jan, Y.N. Jan, Asymmetric localization of a mammalian numb homolog during mouse cortical neurogenesis, Neuron 17 (1996) 43–53.
[113] E. Frise, J.A. Knoblich, S. Younger-Shepherd, L.Y. Jan, Y.N. Jan, The *Drosophila* Numb protein inhibits signaling of the Notch receptor during cell–cell interaction in sensory organ lineage, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 11925–11932.
[114] T. Nishimura, T. Yamaguchi, A. Tokunaga, A. Hara, T. Hamaguchi, K. Kato, A. Iwamatsu, H. Okano, K. Kaibuchi, Role of numb in dendritic spine development with a Cdc42 GEF intersectin and EphB2, Mol. Biol. Cell 17 (2006) 1273–1285.
[115] C.A. Smith, S.E. Dho, J. Donaldson, U. Tepass, C.J. McGlade, The cell fate determinant numb interacts with EHD/Rme-1 family proteins and has a role in endocytic recycling, Mol. Biol. Cell 15 (2004) 3698–3708.
[116] M.A. McGill, S.E. Dho, G. Weinmaster, C.J. McGlade, Numb regulates post-endocytic trafficking and degradation of Notch1, J. Biol. Chem. 284 (2009) 26427–26438.