Clathrin adaptor AP-1 complex excludes multiple postsynaptic receptors from axons in *C. elegans*

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Neurons are highly polarized cells with morphologically and molecularly distinct axonal and dendritic compartments. It is not well understood how postsynaptic receptors are selectively enriched in dendrites in vivo. We investigated the molecular mechanisms of dendritically polarized localization of a glutamate receptor, an acetylcholine receptor, and a ROR-type receptor tyrosine kinase in the interneuron RIA in *C. elegans*. We found that the clathrin adaptor AP-1 complex μ1 subunit UNC-101 functions cell autonomously to maintain the correct localization of these receptors in a dynamin-dependent manner. In *unc-101* mutants, instead of being dendritically enriched, all 3 receptors are evenly distributed in the axonal and dendritic compartments. Surprisingly, UNC-101 predominantly localizes to the axonal compartment, suggesting a possible transcytosis model for the dendritic targeting of neurotransmitter receptors.

polarity | trafficking | glutamate receptor | dendrite

Neurons are polarized cells that receive, process, and transmit information. These different functions are performed by morphologically and functionally distinct subcellular compartments—dendrites and axons—that contain proteins specialized for signal input or output. In principle, many different cellular mechanisms could contribute to molecular polarization in neurons (1, 2). Proteins destined for the axon or the dendrite could be selectively sorted into different transport vesicles at the transGolgi network (TGN) and selectively delivered to their proper subcellular compartment. Alternatively, steady-state differences in axonal and dendritic protein distribution could be achieved by selective stabilization of proteins by scaffolding proteins at certain areas of the plasma membrane, as well as by selective sorting and redistribution following endocytosis.

What is currently known about sorting and trafficking of axonal and dendritic proteins to their proper compartments? Three axonally localized proteins, NgCAM, Nav1.2, and VAMP, are delivered to both axons and dendrites in neurons and achieve polarization following selective endocytosis from the dendritic plasma membrane (3–6). In contrast, dendritically localized transferrin receptor appears to be directly transported to the dendrite (3). For a number of dendritically targeted proteins, structure-function analysis has revealed C-terminal tyrosine-based or dileucine-based cytoplasmic motifs that mediate dendritic targeting (7, 8).

Interesting parallels have been made between dendritic sorting in neurons and basolateral sorting in epithelia (9). A number of basolaterally sorted proteins localize to the somatodendritic region in cultured hippocampal neurons and rely on the same sorting sequences in both cell types (10). One of the key players involved in basolateral sorting in polarized epithelial cells is μ1B, the medium subunit of clathrin adaptor AP-1 complex (11). AP complexes are cytosolic tetramers that mediate sorting in secretory and endocytic pathways by promoting budding of clathrin-coated vesicles (12, 13). μ subunits are particularly important for sorting as they recognize sorting signals within cytoplasmic regions of transmembrane protein cargo (12). In epithelial cells, AP-1 medium subunit μ1B acts in recycling endosomes to mediate both targeting from TGN via the transendosomal route and postendocytic recycling to the basolateral membrane (14, 15).

AP complexes are important for sorting in neurons as well. A study by Dwyer *et al.* (16) found that AP-1 medium subunit μ1/*unc-101* is required for the localization of odorant receptors to the olfactory cilia in *Caenorhabditis elegans*, because in the absence of UNC-101, these proteins are distributed uniformly on the plasma membrane. A similar phenotype was observed for the polycystin TRPP2/PKD-2, which normally localizes to the cilia of male sensory neurons and is delocalized in *unc-101* mutants (17). A recent study showed that a disruption of another adaptor complex, AP-4, leads to the accumulation of AMPA-type glutamate receptors in axonal autophagosomes in mice (18).

Here we show that, in addition to sorting proteins to cilia, μ1/UNC-101 plays a more general role in the sorting of postsynaptic receptors to dendrites in *C. elegans*. These postsynaptic proteins include AMPA-type glutamate receptor GLR-1, α7-type nicotinic acetylcholine receptor ACR-16, and the receptor tyrosine kinase CAM-1/ROR. We show that the cytosolic domains of these postsynaptic receptors are both necessary and sufficient for their dendritic localization in a manner dependent on UNC-101. Furthermore, we find that UNC-101 localizes presynaptically, where it may be involved in the retrieval of postsynaptic proteins following endocytosis.

Results

AP-1 Subunit μ1/UNC-101 Is Required for Polarized Localization of Glutamate Receptor GLR-1 in RIA Neurons. RIA interneurons are a pair of bilaterally symmetric neurons located in the head of the worm. Each RIA neuron has a single process that extends anteriorly and then ventrally, subsequently reentering the large bundle of neuronal processes known as the nerve ring (19). The EM reconstruction revealed that the RIA process is polarized (19): the proximal region of the RIA neurite is exclusively postsynaptic (Fig. 1*A*, green), whereas the distal region is mainly presynaptic (Fig. 1*A*, red). RIA neurons express a number of postsynaptic receptors (20, 21) and are involved in several *C. elegans* behaviors, including thermotaxis (22).

We visualized the presynaptic specializations in RIA neurons in vivo by expressing synaptic vesicle-associated protein, RAB-3, fused to mCherry (23), under a RIA-specific *glr-3* promoter (20). As predicted by the EM reconstruction, this construct localized to the distal, presynaptic segment of the RIA process (Fig. 1*B*). The postsynaptic region of RIA was visualized using GLR-1, an AMPA-type glutamate receptor subunit (24, 25). *glr-1* is expressed in RIA, because electrophysiological studies have found that the majority of glutamate-induced current in RIA is abolished in *glr-1* mutants (V. Maricq, personal communication). The functional GLR-1::GFP construct (26) localized predominantly to the proximal, postsyn-

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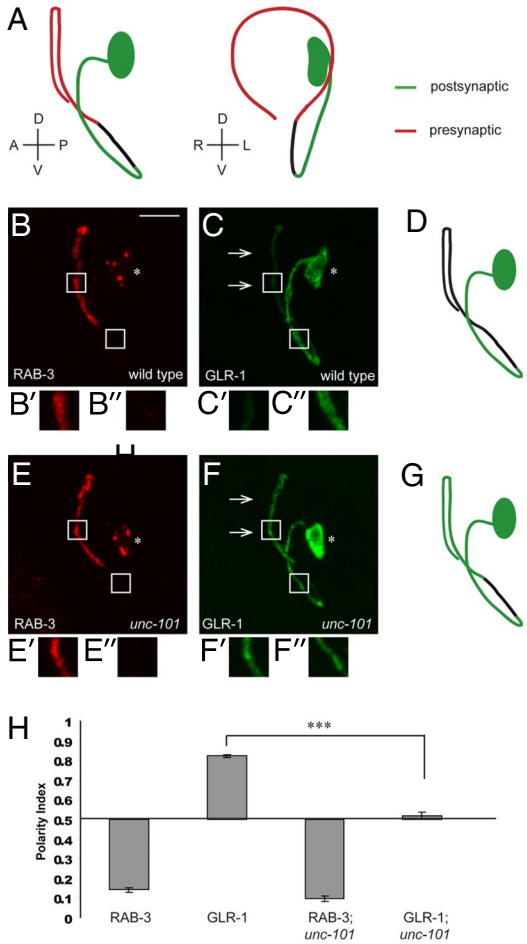

Fig. 1. AP-1 subunit μ1/*unc-101* is required for polarized localization of glutamate receptor GLR-1 in RIA neurons. (*A*) Schematic diagrams of the left RIA neuron as viewed from the left and the anterior side of the worm, respectively. RIA neurite is polarized, with a proximal region that is exclusively postsynaptic (green), followed by an asynaptic isthmus region (black), and a distal, mainly presynaptic region (red). (*B* and *C*) Representative wild-type animal expressing synaptic vesicle marker mCherry::RAB-3 (*B*) and AMPA-type glutamate receptor subunit GLR-1::GFP (*C*). For this and all subsequent images, asterisk denotes the RIA cell body; arrows point to the presynaptic region of the RIA neurite. Anterior is to the left, ventral is down. (Scale bar: 10 μm.) High-magnification views of RIA presynaptic region are shown in B′ and C′, and of postsynaptic region in B′′ and C′′. (*D*) A diagram depicting localization of GLR-1::GFP in a wild-type RIA neuron. (*E* and *F*) Representative *unc-101(m1)* animal expressing mCherry::RAB-3 (*E*) and GLR-1::GFP (*F*). GLR-1::GFP is mislocalized to the presynaptic region of the RIA neurite in *unc-101* mutants (arrows). High-magnification views of RIA presynaptic region are shown in E′ and F′, and of postsynaptic region in E′′ and F′′. (*G*) A diagram depicting localization of GLR-1::GFP in an *unc-101* RIA neuron. (*H*) Polarity index quantification for mCherry::RAB-3 and GLR-1::GFP in wild-type and *unc-101* mutants. ***$P < 0.001$; error bars, SEM.

aptic segment of RIA (Fig. 1 *C* and *D*). We next investigated the localization of RAB-3 and GLR-1 in various *C. elegans* mutants. In particular, we chose to focus on the AP-1 subunit μ1/UNC-101 because of its importance for the localization of odorant receptors to cilia in *C. elegans* (16) and the involvement of μ1B in epithelial basolateral sorting (11, 15). We found that in animals mutant for *unc-101(m1)*, a strong loss-of-function allele (27), GLR-1::GFP was uniformly distributed throughout postsynaptic and presynaptic compartments (Fig. 1 *F* and *G*), whereas mCherry::RAB-3 localization was unaffected (Fig. 1*E*). We quantified this finding by comparing average fluorescence intensities in the postsynaptic and presynaptic regions of RIA and deriving a polarity index, PI (see supporting information (SI) *Methods*). A completely axonal protein has a PI of 0, whereas an exclusively dendritically localized protein has a PI of 1. As shown in Fig. 1*H*, the PI of mCherry::RAB-3 was not affected by the *unc-101* mutation, whereas GLR-1::GFP distribution became unpolarized. Thus, μ1/UNC-101 is required for the localization of GLR-1::GFP to the postsynaptic compartment in RIA neurons. We were unable to test the requirement for the other 3 subunits of the AP-1 complex because their RNAi leads to embryonic lethality (28).

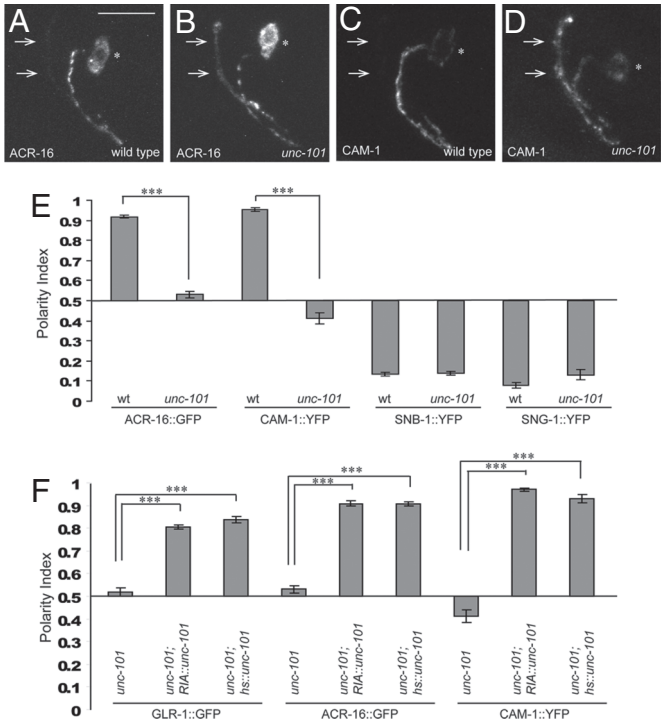

Fig. 2. μ1/*unc-101* is required for dendritic localization of 2 additional postsynaptic receptors in RIA neurons, and acts cell autonomously to maintain polarized receptor distribution. (*A* and *B*) α7-type nicotinic acetylcholine receptor subunit ACR-16::GFP localizes selectively to the RIA postsynaptic region in wild-type animals (*A*), but is mislocalized to the presynaptic region (arrows) in *unc-101(m1)* mutants (*B*). (*C* and *D*) ROR-type receptor tyrosine kinase CAM-1::YFP localizes selectively to the RIA postsynaptic region in wild-type animals (*C*) but is mislocalized to the presynaptic region (arrows) in *unc-101(m1)* mutants (*D*). (*E*) Polarity index quantification for ACR-16::GFP and CAM-1::YFP in wild-type and *unc-101(m1)* animals. In addition, polarity indices for 2 presynaptic markers, SNB-1::YFP and SNG-1::YFP are also shown. (*F*) Polarity indices quantification for GLR-1::GFP, ACR-16::GFP, and CAM-1::YFP in *unc-101(m1)* mutants expressing *unc-101* cDNA under the control of either RIA-specific *glr-3* promoter or heat-shock promoter. ***$P < 0.001$; error bars, SEM.

μ1/*unc-101* Is Required for Localization of Multiple Postsynaptic Receptors in Several Polarized *C. elegans* Neurons. We next investigated whether UNC-101 is required for dendritic localization of other postsynaptic receptors in RIA in addition to GLR-1. ACR-16 is a nicotinic acetylcholine receptor (nAChR) subunit (29), homologous to the vertebrate nAChR subunit α7, which localizes dendritically in cultured hippocampal neurons (30). In RIA, the functional ACR-16::GFP construct (31) reproducibly localizes to the postsynaptic segment of the RIA process and is excluded from the presynaptic segment (Fig. 2 *A* and *E*). ACR-16::GFP distribution was dramatically affected in *unc-101* mutants, in which it localizes both pre- and postsynaptically (Fig. 2 *B* and *E*).

We also examined the localization of another dendritic construct, CAM-1::YFP (Fig. 2 *C* and *D*). CAM-1 is a receptor tyrosine kinase that localizes somatodendrically in a number of *C. elegans* neurons (32, 33). Its mammalian homologs, Ror1 and Ror2, also localize

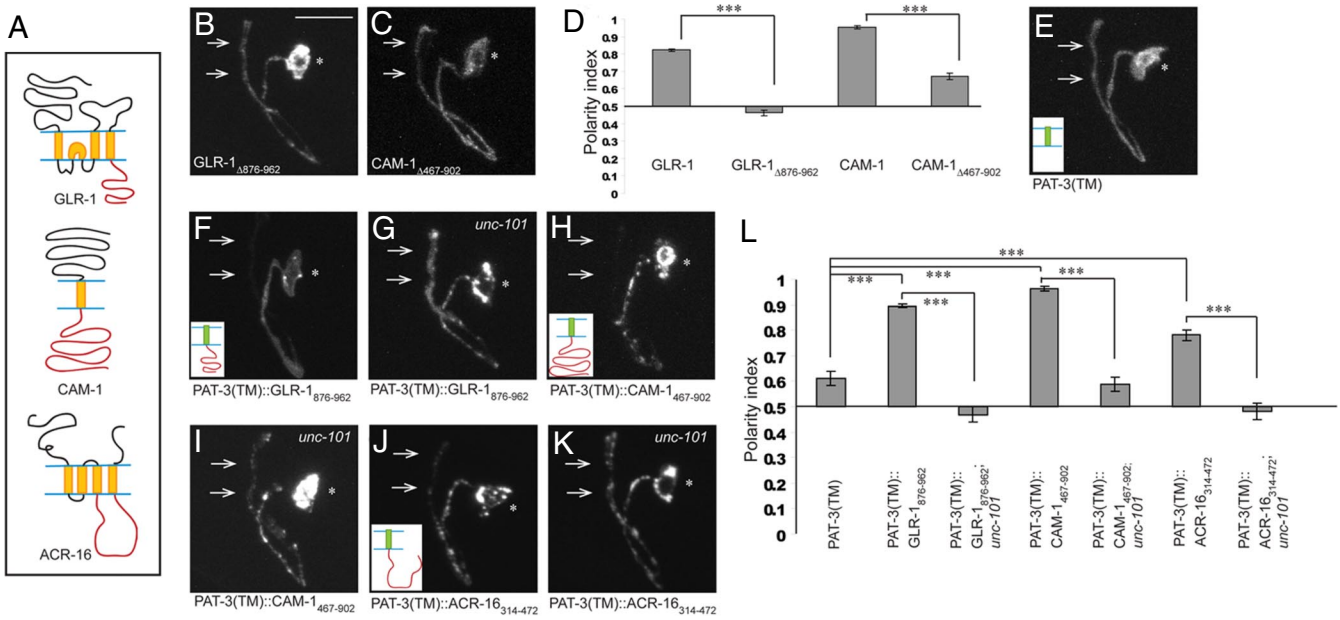

Fig. 3. Cytosolic domains of postsynaptic receptors are necessary and sufficient for dendritic localization in RIA neurons, in a manner dependent on *unc-101*. (*A*) Schematic representation of GLR-1, CAM-1, and ACR-16. Transmembrane regions are shown in yellow, key cytosolic regions in red. (*B*) Localization of the truncated GLR-1 lacking its C-terminal cytoplasmic domain, GLR-$1_{\Delta 876-962}$::YFP, in wild-type RIA neurons. (*C*) Localization of truncated CAM-1 lacking its C-terminal cytoplasmic domain, CAM-$1_{\Delta 467-902}$::YFP, in wild-type RIA neurons. (*D*) Polarity index quantification. (*E*) PAT-3(TM)::YFP is unpolarized in RIA neurons. Inset in this and subsequent images shows the schematic representation of the construct. (*F* and *G*) RIA localization of PAT-3(TM)::GLR-$1_{876-962}$::YFP in wild-type (*F*) and *unc-101(m1)* (*G*) animals. (*H* and *I*) RIA localization of PAT-3(TM)::CAM-$1_{467-902}$::YFP in wild-type (*H*) and *unc-101* (*I*) animals. (*J* and *K*) RIA localization of PAT-3(TM)::ACR-$16_{314-472}$::YFP in wild-type (*J*) and *unc-101* (*K*) animals. (*L*) Polarity index quantification for PAT-3 gain-of-function constructs. ***$P < 0.001$; error bars, SEM.

somatodendritically in cultured hippocampal neurons (34). As expected, CAM-1::YFP localized to the postsynaptic region of RIA neurons (Fig. 2 *C* and *E*) and became unpolarized in *unc-101* mutants (Fig. 2 *D* and *E*). Importantly, unlike at the worm neuromuscular junction (31), RIA ACR-16::GFP localization was not affected in *cam-1(gm122)* mutants (data not shown). Thus, ACR-16 and CAM-1 can be treated as 2 independent dendritic markers in RIA neurons.

To confirm specificity of *unc-101* phenotype for postsynaptic markers, we examined the localization of 2 additional presynaptic markers (35), synaptobrevin (SNB-1::YFP) and synaptogyrin (SNG-1::YFP). The localization of these integral membrane synaptic proteins was not affected in *unc-101* mutants (Fig. 2*E* and Fig. S1 *A–D*), suggesting that UNC-101 is selectively required for postsynaptic protein localization in RIA neurons. Furthermore, to investigate if *unc-101* phenotype is selective for RIA neurons, we observed postsynaptic marker localization in 2 other polarized neurons in *C. elegans,* the motor neuron DA9 and the interneuron AVE. CAM-1::YFP localization to the dendrite and proximal axon of DA9 (32) and GLR-1::YFP localization to the postsynaptic region of AVE were both disrupted in *unc-101* mutants (Fig. S2). Together, these findings show that *unc-101* affects localization of postsynaptic receptors in a number of polarized *C. elegans* neurons.

μ1/*unc-101* Acts Cell Autonomously in RIA Neurons to Maintain Polarized Distribution of Postsynaptic Receptors. The mislocalization of postsynaptic receptors in RIA and other neurons could result from the loss of UNC-101 function in the neurons themselves or be a consequence of disruptions in their cellular environment. To distinguish between these 2 possibilities, we expressed *unc-101* cDNA under the control of the RIA-specific promoter *Pglr-3* (20) in *unc-101(m1)* mutants. This construct was able to fully rescue GLR-1::GFP, ACR-16::GFP, and CAM-1::YFP localization defects of *unc-101* mutants (Fig. 2*F* and Fig. S1 *E*, *G*, and *I*), suggesting that UNC-101 plays a cell-autonomous role in determining RIA polarity.

A priori, *unc-101* phenotype could be explained by a developmental defect in RIA polarization, in which the dendritic fate of the RIA proximal segment is not correctly specified or postsynaptic terminals are not appropriately formed. Alternatively, UNC-101 may be required to maintain ongoing trafficking of postsynaptic receptors to the dendrite, in which case we would predict that its phenotype could be rescued even after development has been completed. To distinguish between these possibilities, we expressed *unc-101* cDNA under the control of inducible heat-shock promoter (36). The transgenic animals carrying the *Phs*::*unc-101* construct were heat shocked for 2 h at the L1/L2 stage (at which point RIA polarity is already established), and the degree of rescue was assessed 24 h later. *Phs*::*unc-101* construct was able to fully rescue the GLR-1, ACR-16, and CAM-1 mislocalization defects in *unc-101* animals (Fig. 2*F* and Fig. S1 *F*, *H*, and *J*). Thus, UNC-101 is required for maintenance of postsynaptic receptor polarized distribution in RIA neurons.

Cytosolic Domains of Postsynaptic Receptors Are Necessary and Sufficient for Dendritic Localization in RIA Neurons. μ subunits of AP complexes classically mediate cargo recognition by binding tyrosine- or dileucine-based sorting motifs in the cytoplasmic portions of their targets (12). To elucidate how μ1/UNC-101 regulates postsynaptic localization of its targets, we performed structure-function experiments on GLR-1, CAM-1, and ACR-16 to identify sequences that mediate their dendritic enrichment. We hypothesized that the cytoplasmic C-terminus regions of GLR-1 and CAM-1, and the prominent cytoplasmic loop between the third and the fourth transmembrane domain of ACR-16, contain sorting information responsible for their postsynaptic enrichment (Fig. 3*A*).

To test this hypothesis, we made truncated GLR-1 and CAM-1 constructs, GLR-$1_{\Delta 876-962}$ and CAM-$1_{\Delta 467-902}$, which lack entire cytoplasmic C termini. We found that these truncated constructs were significantly less dendritically enriched than the full-length constructs (Fig. 3 *B–D*). Thus, cytoplasmic C termini of GLR-1 and CAM-1 are necessary for postsynaptic localization in RIA neurons.

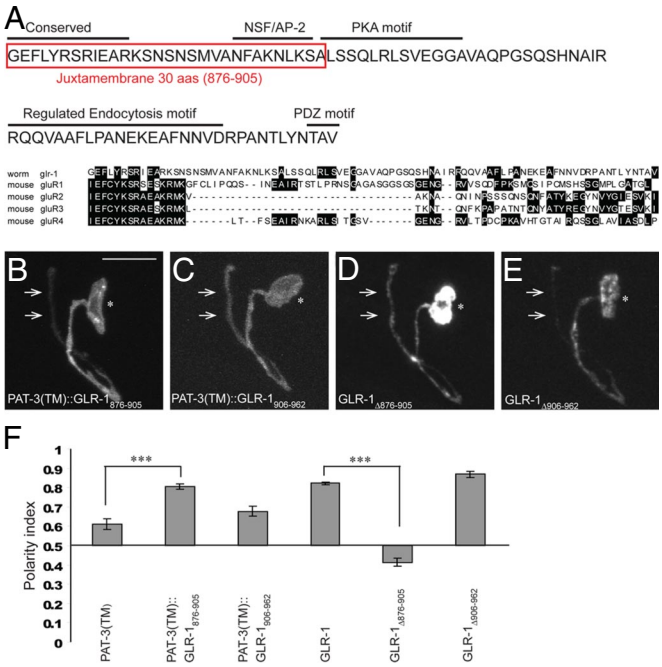

Fig. 4. Juxtamembrane 30 aa of GLR-1 are necessary and sufficient for RIA postsynaptic localization. (*A*) Amino acid sequence of GLR-1 cytoplasmic C terminus and alignment with vertebrate glutamate receptor C termini. Sequence boxed in red is necessary and sufficient for GLR-1 dendritic localization. (*B*) PAT-3 fused to juxtamembrane 30 aa of GLR-1 (PAT-3(TM)::GLR-$1_{876–905}$::YFP) is dendritically enriched in RIA neurons. (*C*) PAT-3 fused to distal 56 aa of GLR-1 (PAT-3(TM)::GLR-$1_{906–962}$::YFP) is more uniformly distributed in RIA neurons. (*D*) GLR-1 lacking juxtamembrane 30 aa (GLR-$1_{\Delta876–905}$::YFP) is unpolarized in RIA neurons. (*E*) GLR-1 lacking the distal 56 aa of its C terminus (GLR-$1_{\Delta906–962}$::YFP) localizes to the RIA dendrite. (*F*) Polarity index quantification. ***$P < 0.001$; error bars, SEM.

To test whether the same sequences are also sufficient for postsynaptic localization, we fused them in frame with the transmembrane domain of an integrin, PAT-3 (37). When tagged with YFP, PAT-3(TM) localizes in a nonpolarized fashion to both pre- and postsynaptic domains of RIA neurons (Fig. 3 *E* and *L*). However, when GLR-1 and CAM-1 C-terminus regions and ACR-16 cytoplasmic loop were fused to PAT-3(TM), these constructs displayed a striking postsynaptic localization (Fig. 3 *F*, *H*, *J*, and *L*). Thus, cytoplasmic sequences of GLR-1, CAM-1, and ACR-16 are sufficient for postsynaptic targeting in RIA neurons.

Finally, if μ1/UNC-101 is recognizing these cytoplasmic sequences and sorting them to the postsynaptic domain of RIA, we would expect that the PAT-3 gain-of-function constructs would be mislocalized in *unc-101* mutants. Indeed, when crossed into *unc-101*, PAT-3(TM)::GLR-$1_{876–962}$, PAT-3(TM)::CAM-$1_{467–902}$, and PAT-3(TM)::ACR-$16_{314–472}$ become unpolarized (Fig. 3 *G*, *I*, *K*, and *L*).

Juxtamembrane 30 aa of GLR-1 Are Necessary and Sufficient for RIA Postsynaptic Localization. Given the importance of glutamate receptors for basal neurotransmission and plasticity in the vertebrate nervous system (38), we sought to identify more specific dendritic targeting motifs within the GLR-1 C terminus. Alignment with vertebrate glutamate receptors had previously revealed a highly conserved stretch of amino acids that immediately follow the last transmembrane domain (37) (Fig. 4*A*). We hypothesized that these amino acids may be important for postsynaptic targeting of GLR-1 in RIA neurons.

To test this hypothesis, we performed further structure-function experiments and found that 30 juxtamembrane amino acids (876–905) of GLR-1 C terminus are critical for its dendritic localization in RIA neurons. PAT-3(TM)::GLR-$1_{876–905}$ construct preferentially localized to the postsynaptic domain of RIA neurons (Fig. 4 *B* and *F*), whereas PAT-3(TM)::GLR-$1_{906–962}$ localized less well (Fig. 4 *C* and *F*). Conversely, GLR-$1_{\Delta876–905}$, which lacks the juxtamembrane 30 aa, was unpolarized (Fig. 4 *D* and *F*), whereas GLR-$1_{\Delta906–962}$, which lacks the distal portion of the C terminus tail, localized well to the RIA dendrite (Fig. 4 *E* and *F*). These findings suggest that juxtamembrane 30 aa of GLR-1 are both necessary and sufficient for GLR-1 localization to the RIA postsynaptic region. Because μ subunits classically recognize tyrosine-based sorting motifs, we mutated a conserved tyrosine in the juxtamembrane region of GLR-1 into alanine (Y880A). This alteration was not sufficient to mislocalize the full-length GLR-1::GFP (data not shown), suggesting that the specific sorting motif responsible for dendritic localization of GLR-1 may be unconventional or redundant.

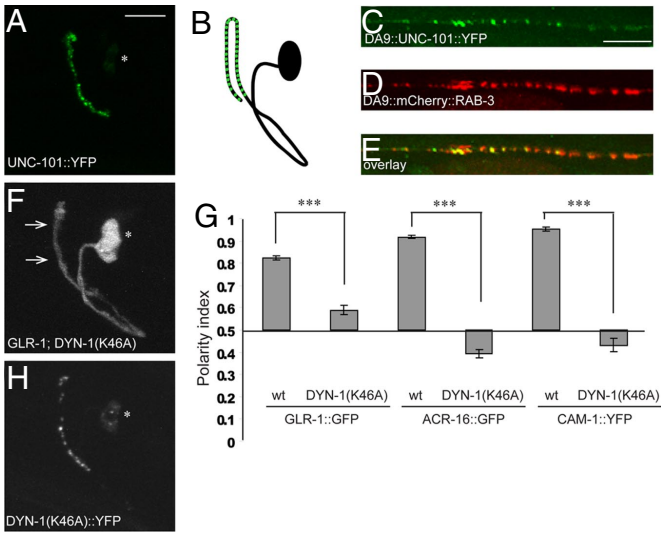

Fig. 5. μ1/UNC-101 localizes to presynaptic sites, where it may be involved in retrieval of postsynaptic receptors. (*A* and *B*) Localization of UNC-101::YFP to the presynaptic region of RIA neurons (*A*), and the accompanying schematic (*B*). (*C–E*) DA9 localization of UNC-101::YFP (*C*), mCherry::RAB-3 (*D*), and the overlay (*E*). (*F*) GLR-1::GFP is mislocalized to the presynaptic region of RIA (arrows) in the presence of dynamin DYN-1(K46A). (*G*) Polarity index quantification for GLR-1::GFP, ACR-16::GFP, and CAM-1::YFP in the absence and presence of DYN-1(K46A). ***$P < 0.001$; error bars, SEM. (*H*) DYN-1(K46A)::YFP localizes to the presynaptic region of RIA neurons.

μ1/UNC-101 Localizes to Presynaptic Sites, Where It May Be Involved in the Retrieval of Postsynaptic Receptors. To determine the subcellular site of action of UNC-101, we expressed fluorophore-tagged UNC-101 in RIA neurons. This construct was functional, as it was able to rescue GLR-1::GFP mislocalization in *unc-101* mutants (data not shown). Interestingly, we found that UNC-101::YFP localized to the presynaptic domain of the RIA process (Fig. 5 *A* and *B*). To determine if UNC-101::YFP is indeed localizing to presynaptic sites, we expressed UNC-101::YFP in the DA9 neuron, in which presynaptic sites are well segregated from each other and thus one can distinguish between axonal vs. presynaptic localization. In the DA9 neuron, UNC-101::YFP colocalized with the synaptic vesicle marker mCherry::RAB-3 (Fig. 5 *C–E*), suggesting that UNC-101 is acting at the presynaptic sites.

Localization of μ1/UNC-101 to the presynaptic sites raised the possibility that UNC-101 acts postendocytically to retrieve postsynaptic receptors from the "inappropriate" axonal compartment. To test this hypothesis, we sought to disrupt endocytosis in RIA

neurons and see if this leads to mislocalization of postsynaptic receptors. Dynamin is a mechanoenzyme that acts as a key regulator of endocytosis by pinching off clathrin-coated vesicles from the membrane (39). To test if dynamin is required for the polarized distribution of GLR-1, we expressed in RIA neurons a dominant negative version of *C. elegans* dynamin, *dyn-1*, in which the key lysine residue in the GTP-binding site was replaced by alanine (K46A, equivalent to vertebrate K44A) (40). Dynamin^{K44A} has been broadly used to potently interfere with endocytosis in a variety of cell types (41). We observed a striking mislocalization of GLR-1, ACR-16, and CAM-1 proteins to the RIA presynaptic region in DYN-1(K46A) transgenic animals (Fig. 5 *F* and *G* and Fig. S3 *A* and *B*), consistent with the idea that endocytosis is required for proper postsynaptic protein localization. We also measured the absolute intensity of GLR-1::GFP in RIA pre- and postsynaptic segments of wild-type, *unc-101(m1)*, and DYN-1(K46A) animals, and found that the average intensity in RIA dendrite was not elevated in mutant animals compared with the wild type (Fig. S3*C*). These findings are consistent with the idea that the observed phenotypes are not a result of an overall lack of receptor turnover in the dendrite resulting in "spillover" into the axon.

We also investigated the consequences of absence of UNC-101 and presence of dominant negative dynamin in the same cell. *unc-101* animals carrying DYN-1(K46A) transgene had polarity indices that were significantly different from wild-type animals and qualitatively similar to each of the mutants alone (Fig. S3*D*). Although the interpretation of this finding is complicated by the fact that DYN-1(K46A) is a dominant-negative construct and not a null loss-of-function allele, these findings are consistent with *dyn-1* and *unc-101* acting in the same genetic pathway. Interestingly, DYN-1(K46A) tagged with YFP localized to the presynaptic domain in RIA in a pattern similar to that of UNC-101::YFP (Fig. 5*H*), suggesting that it acts mainly at the presynaptic sites to block endocytosis of postsynaptic receptors. This is consistent with previous reports that DYN-1 localizes presynaptically in *C. elegans* (42).

Our attempts at genetic loss-of-function block of endocytosis were less successful. A temperature-sensitive allele of dynamin, *dyn-1(ky51)*, did not display a defect in postsynaptic receptor localization in RIA neurons at the restrictive temperature (data not shown). However, animals carrying this hypomorphic allele regain the ability to move after initial uncoordination at the restrictive temperature, which is never observed in the *Drosophila shibire* mutants (43). This suggests that the endocytosis block in *dyn-1(ky51)* animals is not complete and may explain the discrepancy between our dominant negative and loss-of-function data.

We also sought to interfere with endocytosis by examining 2 additional mutants, AP-2 subunit μ2 *dpy-23(e840)* and AP180 *unc-11(e47)*. The vertebrate AP-2 complex has been shown to interact with the AMPA-type GluR2 receptor and mediate endocytosis of GluR2 at the postsynaptic terminals (44, 45). A mutation in the homologue of AP-2 μ2 subunit, *dpy-23(e840)*, had no obvious effect on receptor localization in RIA neurons (data not shown). However, the role of *dpy-23* in postsynaptic receptor endocytosis in *C. elegans* has not been documented, and its role in synaptic vesicle endocytosis remains to be clarified (46). Finally, clathrin adaptor AP180 *unc-11(e47)* mutants also displayed no GLR-1 polarization phenotype in RIA neurons (data not shown). This finding is surprising given the importance of *unc-11* for regulating GLR-1 abundance in the *C. elegans* ventral nerve cord (47–49), and may reflect cell-specific differences in the reliance on particular components of the endocytosis machinery.

Discussion

Information flow in neural circuits is subserved by anatomical and functional polarization of neurons into axons and dendrites. Though much progress has been made in understanding local cycling and dynamics of postsynaptic receptors (in particular, AMPA-type glutamate receptors) at the postsynaptic membrane (38), less is known about global mechanisms that specify enrichment of postsynaptic receptors in dendrites in the first place. Here we use the transparent nematode *C. elegans* to examine postsynaptic receptor sorting in vivo and with single-cell resolution. We find that μ1/UNC-101 subunit of the AP-1 adaptor complex is required for polarized localization of several postsynaptic receptors to dendrites in *C. elegans*. Furthermore, we present evidence showing that UNC-101 may be acting postendocytically at presynaptic sites to remove ectopic postsynaptic receptors from the axonal compartment, thus leading to their steady-state dendritic enrichment.

Our data, however, do not exclude a model in which UNC-101 also functions at the TGN to sort GLR-1-, CAM-1-, and ACR-16-carrying vesicles. Indeed, a previous study that examined the role of UNC-101 in targeting of olfactory receptors to cilia in sensory neurons proposed that UNC-101 acts as a dendritic sorter at the TGN (16). This conclusion was based on 2 lines of evidence: first, the authors observed a lack of phenotype in the hypomorphic *dyn-1(ky51)* mutants and in *dpy-23(e840)* mutants, which is consistent with our findings using the same alleles. It is possible that endocytosis needs to be severely compromised for a receptor localization phenotype to become apparent. Second, the authors performed live imaging experiments, convincingly showing that odorant receptors are trafficked from the cell body to the cilia in wild-type animals. This trafficking was abolished in *unc-101* mutants, suggesting that UNC-101 plays a role in the formation of "dendritic vesicles" in the cell body. It is possible that UNC-101 plays a role at both of these locales, to initially direct receptors to the right location and also to maintain their enrichment there. Because it has not been reported that *C. elegans* neurons have a diffusion barrier between axons and dendrites comparable to the axon initial segment in vertebrate neurons (50), active removal of postsynaptic receptors from the axonal segment would be necessary to combat lateral diffusion even in the presence of initial, TGN-based targeting. In epithelial cells, AP-1 μ1B subunit acts in recycling endosomes to mediate both targeting from TGN via the transendosomal route and postendocytic recycling to the basolateral membrane (14, 15). Thus, there is precedent for a μ1 subunit playing a sorting role along both secretory and endocytic pathways in the same cell type.

μ subunits classically mediate cargo recognition by binding to motifs in the cytosolic sequences of target proteins (12). In agreement with this, we found that cytosolic sequences of the tested postsynaptic receptors are both necessary and sufficient for dendritic targeting in RIA neurons. Interestingly, the equivalent cytosolic sequences of their vertebrate homologues (GluR1 C-terminus region and α7 nAChR M3-M4 loop) are sufficient for dendritic localization in cultured hippocampal neurons (30, 51). These findings suggest that the mechanisms of dendritic targeting may be evolutionarily conserved, which is remarkable given that *C. elegans* neurons are morphologically quite different from vertebrate hippocampal neurons. In addition to being smaller and simpler, 2 of the neurons used in this study (RIA and AVE) have a pseudounipolar morphology, with a single neurite that is functionally partitioned into an axon and a dendrite. Furthermore, vertebrate neurons contain the axon initial segment, which acts as a diffusion barrier between axon and dendrites and helps maintain neuronal polarity (50, 52); this feature is likely not found in *C. elegans* neurons. The evolutionary conservation even in such morphologically different cell types highlights the fundamental importance of this mechanism of dendritic targeting.

Further structure-function analysis of GLR-1 C terminus showed that the juxtamembrane 30 aa are necessary and sufficient for dendritic localization. This result is in agreement with the finding that the proximal 39 aa of GluR1 tail are sufficient for dendritic targeting in vertebrate neurons (51). The juxtamembrane 30 aa of GLR-1 contain a proximal, highly conserved 12 aa sequence that is present in vertebrate glutamate

receptors (37). This highly conserved sequence has been found to be critical for AMPA-stimulated GluR2 endocytosis in HEK cells (53), and for endocytosis and endocytic sorting of NMDA receptors (54). In addition, the juxtamembrane tail portions of glutamate receptors are known to interact with actin-associated protein 4.1N (GluR1) (55), fusion-associated ATPase NSF (GluR2) (56, 57), and the adaptor complex AP-2 (GluR2) (45). Thus, this region of glutamate receptor C termini appears crucial for various endocytic and exocytic processes, as well as interactions with the cytoskeleton. Interestingly, distal C-terminus PDZ motifs of AMPA-type glutamate receptors have been implicated in their synaptic clustering/retention in both vertebrates (58, 59) and in *C. elegans* (26, 37). Yet, these PDZ motifs are not required for glutamate receptor dendritic targeting (51, and this study), suggesting that molecular mechanisms specifying "global targeting" (to the dendrite) and "local targeting" (to the synapse) are at least partly distinct.

Materials and Methods

Strains and Genetics. Worms were raised on OP50 *E. coli* seeded NGM plates at 22 °C. Wild-type animals were N2 Bristol strain. The following strains were provided by the *Caenorhabditis* Genetics Center: DR1 *unc-101(m1)* I; NG2615 *cam-1(gm122)* II; CB840 *dpy-23(e840)* X; CB47 *unc-11(e47)* I; and CX51 *dyn-1(ky51)* X.

Cloning and Constructs. Plasmids and transgenic strains were generated using standard techniques (60). Transgenic strain list and detailed subcloning information are included in *SI Methods*.

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1. Horton AC, Ehlers MD (2003) Neuronal polarity and trafficking. *Neuron* 40:277–295.
2. Winckler B, Mellman I (1999) Neuronal polarity: Controlling the sorting and diffusion of membrane components. *Neuron* 23:637–640.
3. Burack MA, Silverman MA, Banker G (2000) The role of selective transport in neuronal protein sorting. *Neuron* 26:465–472.
4. Wisco D, *et al.* (2003) Uncovering multiple axonal targeting pathways in hippocampal neurons. *J Cell Biol* 162:1317–1328.
5. Garrido JJ, *et al.* (2001) Identification of an axonal determinant in the C-terminus of the sodium channel Na(v) 1.2. *EMBO J* 20:5950–5961.
6. Sampo B, Kaech S, Kunz S, Banker G (2003) Two distinct mechanisms target membrane proteins to the axonal surface. *Neuron* 37:611–624.
7. West AE, Neve RL, Buckley KM (1997) Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. *J Neurosci* 17:6038–6047.
8. Rivera JF, Ahmad S, Quick MW, Liman ER, Arnold DB (2003) An evolutionarily conserved dileucine motif in Shal K+ channels mediates dendritic targeting. *Nat Neurosci* 6:243–250.
9. Dotti CG, Simons K (1990) Polarized sorting of viral glycoproteins to the axon and dendrites of hippocampal neurons in culture. *Cell* 62:63–72.
10. Jareb M, Banker G (1998) The polarized sorting of membrane proteins expressed in cultured hippocampal neurons using viral vectors. *Neuron* 20:855–867.
11. Folsch H, Ohno H, Bonifacino JS, Mellman I (1999) A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell* 99:189–198.
12. Nakatsu F, Ohno H (2003) Adaptor protein complexes as the key regulators of protein sorting in the post-Golgi network. *Cell Struct Funct* 28:419–429.
13. Boehm M, Bonifacino JS (2002) Genetic analyses of adaptin function from yeast to mammals. *Gene* 286:175–186.
14. Ang AL, *et al.* (2004) Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. *J Cell Biol* 167:531–543.
15. Gan Y, McGraw TE, Rodriguez-Boulan E (2002) The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane. *Nat Cell Biol* 4:605–609.
16. Dwyer ND, Adler CE, Crump JG, L'Etoile ND, Bargmann CI (2001) Polarized dendritic transport and the AP-1 mu1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron* 31:277–287.
17. Bae YK, *et al.* (2006) General and cell-type specific mechanisms target TRPP2/PKD-2 to cilia. *Development* 133:3859–3870.
18. Matsuda S, *et al.* (2008) Accumulation of AMPA receptors in autophagosomes in neuronal axons lacking adaptor protein AP-4. *Neuron* 57:730–745.
19. White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci B* 314:1–340.
20. Brockie PJ, Madsen DM, Zheng Y, Mellem J, Maricq AV (2001) Differential expression of glutamate receptor subunits in the nervous system of Caenorhabditis elegans and their regulation by the homeodomain protein UNC-42. *J Neurosci* 21:1510–1522.
21. Tsalik EL, *et al.* (2003) LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. *Dev Biol* 263:81–102.
22. Mori I, Ohshima Y (1995) Neural regulation of thermotaxis in Caenorhabditis elegans. *Nature* 376:344–348.
23. Mahoney TR, *et al.* (2006) Regulation of synaptic transmission by RAB-3 and RAB-27 in Caenorhabditis elegans. *Mol Biol Cell* 17:2617–2625.
24. Hart AC, Sims S, Kaplan JM (1995) Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. *Nature* 378:82–85.
25. Maricq AV, Peckol E, Driscoll M, Bargmann CI (1995) Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor. *Nature* 378:78–81.
26. Rongo C, Whitfield CW, Rodal A, Kim SK, Kaplan JM (1998) LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* 94:751–759.
27. Lee J, Jongeward GD, Sternberg PW (1994) unc-101, a gene required for many aspects of Caenorhabditis elegans development and behavior, encodes a clathrin-associated protein. *Genes Dev* 8:60–73.
28. Shim J, Sternberg PW, Lee J (2000) Distinct and redundant functions of mu1 medium chains of the AP-1 clathrin-associated protein complex in the nematode Caenorhabditis elegans. *Mol Biol Cell* 11:2743–2756.
29. Touroutine D, *et al.* (2005) acr-16 encodes an essential subunit of the levamisole-resistant nicotinic receptor at the Caenorhabditis elegans neuromuscular junction. *J Biol Chem* 280:27013–27021.
30. Xu J, Zhu Y, Heinemann SF (2006) Identification of sequence motifs that target neuronal nicotinic receptors to dendrites and axons. *J Neurosci* 26:9780–9793.
31. Francis MM, *et al.* (2005) The Ror receptor tyrosine kinase CAM-1 is required for ACR-16-mediated synaptic transmission at the C. elegans neuromuscular junction. *Neuron* 46:581–594.
32. Klassen MP, Shen K (2007) Wnt signaling positions neuromuscular connectivity by inhibiting synapse formation in C. elegans. *Cell* 130:704–716.
33. Sieburth D, *et al.* (2005) Systematic analysis of genes required for synapse structure and function. *Nature* 436:510–517.
34. Paganoni S, Ferreira A (2003) Expression and subcellular localization of Ror tyrosine kinase receptors are developmentally regulated in cultured hippocampal neurons. *J Neurosci Res* 73:429–440.
35. Nonet ML (1999) Visualization of synaptic specializations in live C. elegans with synaptic vesicle protein-GFP fusions. *J Neurosci Methods* 89:33–40.
36. Stringham EG, Dixon DK, Jones D, Candido EP (1992) Temporal and spatial expression patterns of the small heat shock (hsp16) genes in transgenic Caenorhabditis elegans. *Mol Biol Cell* 3:221–233.
37. Chang HC, Rongo C (2005) Cytosolic tail sequences and subunit interactions are critical for synaptic localization of glutamate receptors. *J Cell Sci* 118:1945–1956.
38. Shepherd JD, Huganir RL (2007) The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu Rev Cell Dev Biol* 23:613–643.
39. McNiven MA, Cao H, Pitts KR, Yoon Y (2000) The dynamin family of mechanoenzymes: Pinching in new places. *Trends Biochem Sci* 25:115–120.
40. van der Bliek AM, *et al.* (1993) Mutations in human dynamin block an intermediate stage in coated vesicle formation. *J Cell Biol* 122:553–563.
41. Damke H (1996) Dynamin and receptor-mediated endocytosis. *FEBS Lett* 389:48–51.
42. Labrousse AM, Shurland DL, van der Bliek AM (1998) Contribution of the GTPase domain to the subcellular localization of dynamin in the nematode Caenorhabditis elegans. *Mol Biol Cell* 9:3227–3239.
43. Clark SG, Shurland DL, Meyerowitz EM, Bargmann CI, van der Bliek AM (1997) A dynamin GTPase mutation causes a rapid and reversible temperature-inducible locomotion defect in C. elegans. *Proc Natl Acad Sci USA* 94:10438–10443.
44. Carroll RC, *et al.* (1999) Dynamin-dependent endocytosis of ionotropic glutamate receptors. *Proc Natl Acad Sci USA* 96:14112–14117.
45. Lee SH, Liu L, Wang YT, Sheng M (2002) Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. *Neuron* 36:661–674.
46. Dittman JS, Kaplan JM (2006) Factors regulating the abundance and localization of synaptobrevin in the plasma membrane. *Proc Natl Acad Sci USA* 103:11399–11404.
47. Burbea M, Dreier L, Dittman JS, Grunwald ME, Kaplan JM (2002) Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in C. elegans. *Neuron* 35:107–120.
48. Grunwald ME, Mellem JE, Strutz N, Maricq AV, Kaplan JM (2004) Clathrin-mediated endocytosis is required for compensatory regulation of GLR-1 glutamate receptors after activity blockade. *Proc Natl Acad Sci USA* 101:3190–3195.
49. Dreier L, Burbea M, Kaplan JM (2005) LIN-23-mediated degradation of beta-catenin regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of C. elegans. *Neuron* 46:51–64.
50. Winckler B, Forscher P, Mellman I (1999) A diffusion barrier maintains distribution of membrane proteins in polarized neurons. *Nature* 397:698–701.
51. Ruberti F, Dotti CG (2000) Involvement of the proximal C terminus of the AMPA receptor subunit GluR1 in dendritic sorting. *J Neurosci* 20:RC78.
52. Hedstrom KL, Ogawa Y, Rasband MN (2008) AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. *J Cell Biol* 183:635–640.
53. Lin JW, *et al.* (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. *Nat Neurosci* 3:1282–1290.
54. Scott DB, Michailidis I, Mu Y, Logothetis D, Ehlers MD (2004) Endocytosis and degradative sorting of NMDA receptors by conserved membrane-proximal signals. *J Neurosci* 24:7096–7109.
55. Shen L, Liang F, Walensky LD, Huganir RL (2000) Regulation of AMPA receptor GluR1 subunit surface expression by a 4.1N-linked actin cytoskeletal association. *J Neurosci* 20:7932–7940.
56. Nishimune A, *et al.* (1998) NSF binding to GluR2 regulates synaptic transmission. *Neuron* 21:87–97.
57. Song I, *et al.* (1998) Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors. *Neuron* 21:393–400.
58. Xia J, Zhang X, Staudinger J, Huganir RL (1999) Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. *Neuron* 22:179–187.
59. Leonard AS, Davare MA, Horne MC, Garner CC, Hell JW (1998) SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. *J Biol Chem* 273:19518–19524.
60. Mello C, Fire A (1995) DNA transformation. *Methods Cell Biol* 48:451–482.