Cytosolic tail sequences and subunit interactions are critical for synaptic localization of glutamate receptors

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Summary

AMPA-type glutamate receptors mediate excitatory synaptic transmission in the nervous system. The receptor subunit composition and subcellular localization play an important role in regulating synaptic strength. GLR-1 and GLR-2 are the Caenorhabditis elegans subunits most closely related to the mammalian AMPA-type receptors. These subunits are expressed in overlapping sets of interneurons, and contain type-I PDZ binding motifs in their carboxy-terminal cytosolic tail sequences. We report that GLR-1 and GLR-2 may form a heteromeric complex, the localization of which depends on either GLR-1 or GLR-2 tail sequences. Subunit interactions alone can mediate synaptic localization as endogenous GLR-1, or GLR-2 subunits are expressed in overlapping sets of interneurons, related to the mammalian AMPA-type receptors. These AMPARs contain type-I PDZ binding motifs in their carboxy-terminal cytosolic tail sequences. We report that GLR-1 and GLR-2 are the Caenorhabditis elegans subunits most closely related to the mammalian AMPA-type receptors. These subunits are expressed in overlapping sets of interneurons, and contain type-I PDZ binding motifs in their carboxy-terminal cytosolic tail sequences. We report that GLR-1 and GLR-2 may form a heteromeric complex, the localization of which depends on either GLR-1 or GLR-2 tail sequences. Subunit interactions alone can mediate synaptic localization as endogenous GLR-1, or GLR-2 subunits can rescue the localization defects of subunits lacking tail sequences. Moreover, GLR-2 cytosolic tail sequences are sufficient to confer synaptic localization on a heterologous reporter containing a single-transmembrane domain. The localization of this GLR-2 reporter requires both a PDZ-binding motif in the GLR-2 tail sequence, and sequences outside of this motif. The PDZ protein LIN-10 regulates the localization of the reporter through the sequences outside of the PDZ-binding motif. Our results suggest that multiple synaptic localization signals reside in the cytosolic tail sequence of the receptor subunits, and that channel assembly can rescue the synaptic localization defects of individual mutant subunits as long as there are also wild-type subunits in the receptor complex.

Key words: Glutamate receptor, Trafficking, C. elegans, PDZ, LIN-10, Synapse

Introduction

Ionotropic glutamate receptors are localized to postsynaptic sites, where they function as primary mediators of excitatory synaptic transmission in the central nervous system. Glutamate receptors fall into three classes according to their pharmacological properties and sequence similarity: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type receptors, kainate receptors and N-methyl-D-aspartate (NMDA) receptors (Dingledine et al., 1999; Hollmann and Heinemann, 1994). AMPA receptor (AMPA) subunits (up to four in mammals, referred to as GluR1-R4) are multi-transmembrane-spanning proteins that assemble into tetrameric channels of differing subunit composition (Dingledine et al., 1999; Hollmann and Heinemann, 1994; Mano and Teichberg, 1998; Wenthold et al., 1992). The specific subunit composition of a given AMPAR channel plays a crucial role in determining the functional properties of that channel, including its channel opening probability, ion selectivity and cytosolic binding partners (Dingledine et al., 1999; Hollmann and Heinemann, 1994; Malinow, 2003; Sheng, 2001; Shi et al., 2001). To better understand how AMPARs function in the nervous system, it is essential to determine how individual subunits are assembled into specific complexes of channels, and how these complexes are regulated.

The postsynaptic localization of AMPARs is an important facet of their regulation, and our current understanding of how AMPARs are mobilized within cells has focused on the proteins that interact with the carboxy-terminal sequences that are exposed to the cytosol (Dong et al., 1997; Rongo et al., 1998; Sans et al., 2001; Song et al., 1998; Srivastava et al., 1998; Xia et al., 1999). The tail sequences of AMPAR subunits often contain a PDZ-binding motif, which can interact with PDZ-domain-containing proteins. Other protein sequences in the tail region are thought to be important for receptor regulation, including an NSF-interacting motif, kinase phosphorylation sites and ubiquitination sites (Burbea et al., 2002; Ehlers, 2000; Esteban et al., 2003; Lee et al., 2000; Lee et al., 2003; Osten and Ziff, 1999; Roche et al., 1996).

Regulation of receptor localization is complicated by the fact that AMPARs can assemble into tetramers comprising different subunit combinations. Recent work indicates that different compositions of receptor subunits may also play a crucial role for receptor trafficking in the neuron (Beattie et al., 2000; Greger et al., 2002; Lin et al., 2000; Shi et al., 2001). In mammals, there are four major types of AMPAR subunits: GluR1, GluR2, GluR3 and GluR4. Heteromeric channels of GluR1/GluR2 are delivered to synapses in an inducible manner in response to activity, whereas heteromeric channels of GluR2/GluR3 cycle and exchange into the synaptic membrane in a constitutive manner (Hayashi et al., 2000; Passafaro et al., 2001; Shi et al., 2001; Shi et al., 1999). The delivery of GluR2/GluR3 receptors depends on the carboxy-terminal tail sequences of these proteins, which can bind to the PDZ-domain proteins GRIP/ABP and PICK1 (Dong et al., 1997; Srivastava et al., 1998; Wyszynski et al., 1999; Xia et al., 1999). In C. elegans, PDZ, LIN-10, Synapse
tail sequences, GLR-1 and GLR-2 can be localized through a genetic approach. We find that in the absence of their cytosolic sequences, GLR-1 and GLR-2 AMPA-type subunits in nematodes using a localization. To investigate these questions, we examined the localization, or what role subunit interactions might play in about the exact sequences of GLR-1 that contribute to its functional as they are capable of fully rescuing a mechanosensory defect (Rongo and Kaplan, 1999; Leonard et al., 2002). The GLR-1::GFP chimeric receptors appear to be and subsequent endocytosis of the GLR-1 subunit (Burbea et al., 1996). The abundance of GLR-1::GFP is regulated by the ubiquitination motif in their cytosolic tail. Mutations in GLR-1 were mediated by the GluR1 carboxy-terminal tail sequence, which although it is weaker than that of glr-1 null mutants (Mellem et al., 2002). GLR-1 and GLR-2 are expressed in the interneurons that govern locomotion in the animal, and are localized postsynaptically in the nerve ring (a region of proximal neurites that circumscribe the pharynx) and along the ventral nerve cord (a fascicle of distal neurites that runs anterior to posterior along the ventral midline of the body) (Brockie et al., 2001; Mellem et al., 2002; Rongo et al., 1998). These interneurons could contain a mixture of GLR-1 and GLR-2 homomeric channels, GLR-1/GLR-2 heteromeric channels, or some combination of both.

Chimeric GLR-1 receptors tagged with the green fluorescent protein (GLR-1::GFP) can be used to visualize glutamate receptors in living animals (Rongo et al., 1998). GLR-1::GFP is localized to synaptic clusters at neuronal synapses within the C. elegans neuropil, and GLR-1::GFP synaptic localization is dependent on Ca2+ signaling and the PDZ protein LIN-10 (Rongo and Kaplan, 1999; Rongo et al., 1998). The synaptic abundance of GLR-1::GFP is regulated by the ubiquitination and subsequent endocytosis of the GLR-1 subunit (Burbea et al., 2002). The GLR-1::GFP chimeric receptors appear to be functional as they are capable of fully rescuing a glr-1 mutant for their mechanosensory defect (Rongo and Kaplan, 1999; Rongo et al., 1998).

Whereas we have begun to identify numerous genetic, trans-acting factors that regulate GLR-1 localization, we know little about the exact sequences of GLR-1 that contribute to its localization, or what role subunit interactions might play in localization. To investigate these questions, we examined the GLR-1 and GLR-2 AMPA-type subunits in nematodes using a genetic approach. We find that in the absence of their cytosolic tail sequences, GLR-1 and GLR-2 can be localized through interactions with endogenous subunits. In the absence of endogenous subunits, GLR-1 or GLR-2 are forced to form homomeric channels in vivo. In this scenario, the GLR-1 and the GLR-2 cytosolic sequences become essential for localization as homomeric channels containing cytosolic tail sequences are localized, whereas those lacking them are not. We also report that the GLR-2 tail sequence is sufficient for synaptic localization when placed into a heterologous fusion protein. We find that the PDZ-binding motif found at the end of the GLR-2 cytosolic tail sequence works together with tail sequences outside of the PDZ-binding motif to facilitate localization. Some of these sequences appear to have been conserved between vertebrate and nematode AMPAR tails.

Materials and Methods

Strains

Standard methods were used to maintain C. elegans (Wood, 1988). Transgenic strains were isolated by microinjecting various plasmids, (typically at 80 µg/ml) together with either pJM23 in lin-15(n765ts) (kindly provided by J. Mendel, Cal Tech, Pasadena, CA) or rol-6(dn) (kindly provided by C. Mello, University of Massachusetts Medical School, Worcester, MA) in wild-type animals. The lin-10 (n1508) strain was used to generate transgenic animals containing TMGFP::GLR-2 (tail) and TMGFP::GLR-2(TLF → ALE) (Whitfield et al., 1999). The glr-1 and glr-2 deletion mutant animals were generous gifts from A. Marić (Mellem et al., 2002). The nmds25 strain contains integrated glr-1::gfp (Rongo et al., 1998).

Molecular biology

The glr-1 (tailless)::gfp and glr-2 (tailless)::gfp were constructed using glr-1 and glr-2 cDNA sequences (gifts from A. Marić, University of Utah, Salt Lake City, UT and Y. Kohara, National Institute of Genetics, Mishima, Japan) from their start codon to their predicted transmembrane domain IV region. These were inserted into pPD95.75 (a gift from A. Fire, Stanford University School of Medicine, Standford, MA) in front of and in frame with GFP sequences. The resulting chimeric sequences were then placed under the control of the glr-1 and glr-2 promoter, respectively. The glr-1::gfp (pKP196), glr-1::cfp (pKP211), and glr-2::yfp (pPB60, a gift from A. Marić) transgenes contain GluR sequences through transmembrane domain IV, GFP sequences and the entire tail sequence for the respective receptor (Mellem et al., 2002; Rongo et al., 1998). The tmgfp, tmgfp::glr-1(tail), tmgfp::glr-2(tail) and tmgfp::glr-2(tlf→ sale) transgenes are encoded by plasmids pOR113, pOR38, pOR105.7, and pOR115, respectively. Sequences encoding the pes-10 signal peptide and transmembrane domain from pPD113.58 (a gift from A. Fire) were introduced in front of GFP sequences to generate pOR113, pOR38, pOR105.7 and pOR115. Sequences encoding the GLR-1 and GLR-2 cytosolic tail were placed after the GFP sequences to generate pOR38 and pOR105.7. The anh-1::cfp transgene is under the mec-3 promoter on the pKP228 plasmid (Rongo et al., 1998). Further plasmid construction details are available on request.

Sequence comparisons

GLR-1, GLR-2 and GluR tail sequences from various organisms were aligned by using the CLUSTALW algorithm. Caenorhabditis briggsae and Caenorhabditis remanei orthologs were identified using the Wormbase (www.wormbase.org) and the Wash. University GSC (http://genome.wustl.edu/) BLAST search engine. Other nematode orthologs were identified using the GSC Nematode.net BLAST search engine.
Glutamate receptor subunit interactions

Results

Subunit interactions can mediate localization

GLR-1 and GLR-2 possess carboxy-terminal sequences similar in length and sequence to each other and to the mammalian receptors. Therefore, we wanted to examine the contribution of the cytosolic tail sequences of GLR-1 and GLR-2 to their synaptic localization by expressing subunits that lacked these cytosolic tail sequences. We reasoned that these tailless receptors would be incorporated into channels given that recent work has shown that functional heteromeric channel assembly does not require the tail sequences after transmembrane domain IV of the receptor subunit (Ayalon and Stern-Bach, 2001; Leuschner and Hoch, 1999). To test the localization of tailless receptors, we generated a transgene, glr-1(tailless)::gfp, that expresses a GLR-1::GFP protein lacking the contribution of the cytosolic tail sequences of GLR-1 and GLR-2 to their synaptic localization by expressing subunits that lacked these cytosolic tail sequences. We reasoned that these tailless receptors would be incorporated into channels given that recent work has shown that functional heteromeric channel assembly does not require the tail sequences after transmembrane domain IV of the receptor subunit (Ayalon and Stern-Bach, 2001; Leuschner and Hoch, 1999). To test the localization of tailless receptors, we generated a transgene, glr-1(tailless)::gfp, that expresses a GLR-1::GFP protein lacking...
the 87 amino acid cytosolic tail sequences (Fig. 1, top). We first introduced the glr-1(tailless)::gfp transgene into wild-type nematodes and found that GLR-1(tailless)::GFP protein was localized to ventral cord clusters (Fig. 1A), similar to full-length GLR-1::GFP (Fig. 1B). These results could suggest either that the GLR-1 tail sequences are dispensable for localization, or that GLR-1(tailless)::GFP becomes localized by its interaction with endogenous subunits. To test these two hypotheses, we introduced the glr-1(tailless)::gfp transgene into deletion mutants that lacked the endogenous subunits. In glr-1 glr-2 double-deletion mutants, GLR-1(tailless)::GFP was distributed uniformly throughout the dendrites (Fig. 1C) with few punctate clusters (Fig. 1G), indicating that the localization pattern of GLR-1(tailless)::GFP in wild-type animals is through interactions with endogenous receptor subunits. We do not believe that the increase in unlocalized receptor was due to changes in expression pattern as we detected no such changes based on cell body identifications (data not shown); rather, we believe it was due to the distribution of GFP-tagged receptor throughout the dendrite, which would otherwise be concentrated at clusters in a wild-type animal. We also generated a full-length glr-1::gfp transgene in which the tail sequences are restored, introducing it into glr-1 glr-2 double mutants (Fig. 1D). We found that GLR-1::GFP was similarly localized in both glr-1 glr-2 double mutants and in wild type. Thus, our data suggests that when GLR-1 is the only subunit available, it is capable of self-association to form homotetramers, which in turn become localized to clusters along dendrites. Moreover, the GLR-1 tail sequences are essential for the localization of GLR-1 homotetrameric channels.

We also tested for the contribution of individual endogenous GLR-1 and GLR-2 subunits by examining GLR-1(tailless)::GFP localization in single subunit deletion animals. In a glr-1 deletion mutant, GLR-1(tailless)::GFP was localized, suggesting that endogenous GLR-2 can form a heteromeric complex with GLR-1(tailless)::GFP, and facilitate GLR-1(tailless)::GFP localization in the ventral nerve cord (Fig. 1E). In a glr-2 deletion mutant, GLR-1(tailless)::GFP was also localized (Fig. 1F), suggesting that clusters of receptor along the ventral nerve cord contain homomeric GLR-1 receptor complexes comprising endogenous GLR-1 and GLR-1(tailless)::GFP. Furthermore, GLR-1(tailless)::GFP clusters in these mutants were similar in number and size to GLR-1::GFP clusters (Fig. 1G,H). Taken together, these results show that GLR-1(tailless)::GFP can become localized to ventral cord clusters if an endogenous copy of GLR-1 or GLR-2 is present, but cannot become localized when expressed in the absence of endogenous subunits. Hence, either endogenous GLR-1 or GLR-2 tail sequences can convey receptor localization through heteromeric subunit interactions.

To examine GLR-2 localization, we generated a transgene, glr-2::yfp, that expresses a GLR-2::YFP chimeric protein under the control of the glr-2 promoter (Fig. 2, top). Because the expression level of GLR-2 by its own promoter is relatively low (our unpublished work), we had difficulty detecting cluster formation along the ventral nerve cord. Therefore, we only scored GLR-2 localization to synapses proximal to the cell body and along the nerve ring, where localized GLR-2::YFP could be easily detected in a wild-type genetic background. We found that GLR-2::YFP was localized to clusters when both endogenous subunits were present (Fig. 2B) (Mellem et al., 2002). To examine the contribution of the cytosolic tail sequences of GLR-2 to its localization, we generated a transgene, glr-2(tailless)::gfp, that

![Fig. 2. GLR-2 tail sequences are required for heteromeric receptor synaptic localization and stability. (Top) The sequences of GLR-2 included in the GLR-2::YFP and GLR-2(tailless)::GFP transgenes. The arrowhead indicates the position of the fluorescent tag (between a.a. 946-947 for full-length, and after the final transmembrane domain for the tailless form) used to monitor the protein. (A,C) GLR-2(tailless)::GFP expressed in wild-type, and glr-1 and glr-2 double deletion, respectively. (B,D) Full-length GLR-2::YFP expressed in wild-type, and glr-1 and glr-2 double deletion, respectively. (A) GLR-2(tailless)::GFP can exit the neuron cell body and populate the synapses of the nerve ring (bracket) and proximal dendrites (arrows) as long as there is endogenous full-length receptor present. (B) However, GLR-2(tailless)::GFP remains in the cell body (arrowhead) when there are no endogenous subunits present. All the images were captured using either YFP or FITC filters, and were taken from the head region of the animal (anterior oriented diagonally down and to the left, dorsal up and to the left). The image in C was taken at a higher exposure to reveal the faint GFP fluorescence of the cell body; consequently, the background autofluorescence from the underlying pharyngeal tissue is also revealed. The neuron shown in all four panels is AVD. Bars, 10 µm.](image-url)
expresses a GLR-2::GFP that lacks the 59 amino acid cytosolic tail sequences. We introduced the glr-2(tailless)::gfp transgene into wild-type nematodes, and found that GLR-2(tailless)::YFP protein was localized (Fig. 2A), similar to full-length GLR-2::YFP (Fig. 2B). We also introduced the glr-2(tailless)::gfp transgene into deletion mutants that lacked both glr-1 and glr-2 subunits. In glr-1 glr-2 double mutants, GLR-2(tailless)::GFP was present at low levels, and only in the neuron cell body (Fig. 2C). By contrast, we detected the localization of full-length GLR-2::YFP receptor in a glr-1 glr-2 double deletion mutant (Fig. 2D). Taken together, these results show that GLR-2(tailless)::GFP can become localized if an endogenous copy of GLR-1 or GLR-2 is present, but cannot be transported out of the cell body when expressed in the absence of both endogenous subunits. Moreover, GLR-2(tailless)::GFP abundance is reduced in the absence of the endogenous subunits, suggesting that it might be unstable (Fig. 2C). By contrast, full-length GLR-2::YFP is localized in the absence of endogenous subunits (Fig. 2D), suggesting that the GLR-2 cytosolic tail sequences are crucial for the export and localization of GLR-2 homotetrameric channels.

Our cell biological observations suggested that GLR-1 and GLR-2 form a complex in vivo. Therefore, we tested whether GLR-1 and GLR-2 could form a complex in vitro by generating plasmids that express GLR-1 tagged with HA and GLR-2 tagged with FLAG (tags were placed in the extracellular domain of both proteins), both under the CMV promoter. We cotransfected HA::GLR-1 and FLAG::GLR-2 into COS7 cells, and solubilized the membranes of the cells with RIPA buffer after 48 hours. We immunoprecipitated protein complexes with antisera to HA, and detected both HA::GLR-1 and FLAG::GLR-2 (Fig. 3A). As a control, we found that no co-immunoprecipitation occurred when we used preimmune serum. Similarly, we immunoprecipitated with antisera to FLAG and detected HA::GLR-1.

We also generated tailless versions of our HA::GLR-1 and FLAG::GLR-2 plasmids. We cotransfected HA::GLR-1 with FLAG::GLR-2(tailless) into COS7 cells, and found that we could co-immunoprecipitate HA::GLR-1 and FLAG::GLR-2(tailless) (Fig. 3B). We also cotransfected HA::GLR-1(tailless) with FLAG::GLR-2, and found that we could coimmunoprecipitate these proteins (Fig. 3C). In both cases, no co-immunoprecipitation occurred when we used preimmune serum. Moreover, we could co-immunoprecipitate the corresponding partner regardless of whether we directly pulled down HA::GLR-1 with antisera to HA, or FLAG::GLR-2 with antisera to FLAG. Thus, GLR-1 and GLR-2 protein can form a complex, even when lacking their tails sequences.

Although GLR-1(tailless)::GFP subunits can form complexes with GLR-2, and are localized to ventral cord clusters if an endogenous copy of GLR-2 is present, it is possible that these subunits are not at the membrane surface. If this were the case, we would expect GLR-1(tailless) to have diminished function. GLR-1 mediates nose-touch sensation in interneurons by receiving glutamatergic signals from mechanosensory neurons (Hart et al., 1995; Kaplan and Horvitz, 1993). Null mutants for glr-1 are nose-touch insensitive, and exogenous GLR-1 can rescue this phenotype. We introduced either glr-1::gfp or glr-1(tailless)::gfp into glr-1 mutants, and measured their response to nose-touch. We found that both transgenes robustly rescued nose-touch sensation to near wild-type levels (Fig. 3D), suggesting that GLR-1(tailless)::GFP subunits assemble into functioning channels on sensory neuron-to-interneuron synaptic membranes.

Previous work indicates that GLR-1 and GLR-2 function together to mediate nose-touch sensation as null mutants for glr-2 are partially but not completely nose-touch insensitive (Mellem et al., 2002). Moreover, GLR-1::CFP and GLR-2::YFP subunits colocalize to clusters in the C. elegans neuron (Fig. 4E-J). To test whether GLR-2::gfp can function in nose-touch sensation, we introduced either glr-2::gfp or glr-2(tailless)::gfp into glr-2 mutants. We found that both transgenes rescued nose-touch sensation to wild-type levels (Fig. 3E), suggesting that GLR-2(tailless)::GFP subunits...
assemble into functioning channels on sensory neuron-to-interneuron synaptic membranes.

GLR-2 cytosolic tail sequences are sufficient for synaptic localization

Although our results suggested that the GLR-1 and GLR-2 cytosolic tail sequences are important for localization, we wanted to test the contribution of the subunit tail sequences separately without the influence of receptor complex formation. We engineered a transgene, tmgfp, that expresses the signal sequence and transmembrane domain from the embryonic protein PES-10 fused to GFP and under the control of the glr-1 promoter (Seydoux and Fire, 1994). We also generated versions of the tmgfp transgene that contain sequences encoding either the glr-1 or glr-2 cytosolic tail, (called tmgfp::glr-1(tail) and tmgfp::glr-2(tail), respectively; Fig. 4, top). We introduced these transgenes into wild-type nematodes and observed the localization of their fluorescent products. TMGFP protein alone failed to become localized and was distributed diffusely throughout the ventral nerve cord (Fig. 4B). Similarly, TMGFP::GLR-1(tail) was also diffusely distributed throughout the ventral nerve cord (Fig. 4C), suggesting that even though GLR-1 cytosolic tail sequences are necessary for GLR-1 homomeric channel localization, they are not sufficient to localize a heterologous membrane protein. Interestingly, we found that the TMGFP::GLR-2(tail) was localized to clusters along the ventral cord (Fig. 4D). The number of TMGFP::GLR-2(tail) clusters was about twofold lower than the number observed for full-length GLR-1::GFP (Fig. 4T). Thus, the GLR-2 cytosolic tail sequences are sufficient to direct the localization of a heterologous membrane protein, at least to a subset of clusters.

To determine whether the TMGFP::GLR-2(tail) clusters were at synapses, we examined the colocalization of TMYFP::GLR-2(tail), which contains YFP instead of GFP, and synaptic vesicle protein synaptobrevin (SNB-1) fused to CFP (SNB-1::CFP). The C. elegans ventral cord forms neuronal synapses between parallel fibers in an ‘en passant’ fashion, and synaptic vesicles can be detected in presynaptic boutons that are found adjacent to the corresponding postsynaptic element of the same synapse (White et al., 1986). In previous work, snb-1::cfp expressed under control of mec-3 promoter labeled a small number of presynaptic boutons from PVD that innervate GLR-1-expressing neurites just posterior to the vulva (Fig. 4K-M) (Nonet et al., 1998; Rongo et al., 1998). Approximately 60% of PVD presynaptic boutons innervate GLR-1-expressing neurites (White et al., 1986). As a wild-type control to measure the number of presynaptic boutons from PVD that contain an adjacent postsynaptic GLR-1 cluster, we also used a full-length glr-1::yfp transgene.
introduced full-length glr-1::yfp and snb-1::cfp into wild-type nematodes, and captured double-labeled images of GLR-1::YFP and SNB-1::CFP clusters. The images were merged and clusters were defined to be colocalized if they were found to be within 2 µm distance. We found that 51% of SNB-1::CFP-labeled boutons had an adjacent GLR-1::YFP cluster within 2 µm distance (Fig. 4M). We next generated a variant of tmgfp::glr-2(tail) that contains YFP instead of GFP. We introduced the tmyfp::glr-2(tail) and tmgfp::cfp transgenes into nematodes, and found that 28% of the SNB-1::CFP-labeled boutons were adjacent to TMYFP::GLR-2(tail) clusters (Fig. 4P,S). We believe that the probable explanation for the twofold decrease in the number of synaptically localized clusters observed between GLR-1::GFP and TMGFP::GLR-2(tail) is the twofold decrease in the total TMGFP::GLR-2(tail) clusters relative to GLR-1::GFP (Fig. 4T). Our results suggest that the GLR-2 cytosolic sequences contain information sufficient to direct localization to at least a subset of synapses.

The PDZ-binding motif cooperates with additional sequences to mediate GLR-2 localization

The interaction of PDZ domain proteins with the PDZ-binding motif found in receptor cytosolic tail sequences is thought to play a role in receptor localization (Garner et al., 2000). PDZ-binding motifs are usually found at the last three amino acids of the carboxy-terminus of a peptide, and they can match a number of different consensus types (Bezprozvanny and Maximov, 2001). We therefore reasoned that the carboxy-terminal amino acids in GLR-2 (i.e. TLF), which match the type-I PDZ consensus, might mediate receptor localization. To test this idea, we made a transgene, tmgfp::glr-2(tlf→ale), in which we substituted alanine and glutamate for the –2 and 0 residues of the TMGFP::GLR-2(tail) protein. These substitutions have been used previously to impair type-I PDZ ligands (Zito et al., 1997). We introduced the transgene into wild-type nematodes, and we found that TMGFP::GLR-2(TLF→ALE) protein was localized to a similar number of clusters, but smaller in size when compared with TMGFP::GLR-2(tail) protein localization (Fig. 5B,E,F). Currently, only one known PDZ domain protein, LIN-10, has been shown to regulate glutamate receptor localization in C. elegans (Rongo et al., 1998). In lin-10 mutants, GLR-1 is not properly localized to clusters in the ventral cord, but instead appears to be uniformly distributed. To test whether the GLR-2 tail sequences are dependent on LIN-10 for localization, we introduced the tmgfp::glr-2(tail) transgene into lin-10(n1508) null mutants (Whitfield et al., 1999). Interestingly, the TMGFP::GLR-2(tail) protein was localized to a similar number of clusters in lin-10 mutants, but smaller in size (Fig. 5C,E,F). As the lin-10 mutant and the TLF-to-ALE substitution in the GLR-2 tail sequences both gave only a partial effect in reducing cluster size, we also tested whether LIN-10 and the PDZ recognition motif might have a compensatory effect for one another in localization. We introduced the tmgfp::glr-2(tlf→ale) transgene into lin-10(n1508) null mutants, where we observed that the synaptic localization of TMGFP::GLR-2(TLF→ALE) protein was nearly abolished (Fig. 5D,E,F). Taken together, these results suggest that the GLR-2 tail sequences are localized at the synapse by potentially two compensatory mechanisms: first, by the interaction of an unknown PDZ protein with the TLF motif, and second, by a non-PDZ-binding motif mechanism that nevertheless requires LIN-10.

Tail sequence motifs conserved between nematodes and vertebrates

Our results suggested that the cytosolic tail sequences of C. elegans glutamate receptors contain complex information that is required to direct their localization. We were interested in how these sequences are conserved evolutionarily, since the conservation of amino acids between distantly related species provides an indication of the sequence importance. First, we examined the sequence conservation of GLR-1 and GLR-2 tails between nematode species. Expressed sequence tags

![Fig. 5. GLR-2 cytosolic tail fusion protein requires the PDZ-binding motif and LIN-10 protein to be localized. We substituted the last three amino acids of the GLR-2 PDZ-binding motif (TLF→ALE) in TMGFP::GLR-2(tail) to generate TMGFP::GLR-2(TLF→ALE). (A,B) In wild-type animals, we expressed TMGFP::GLR-2(tail) (A) and TMGFP::GLR-2(TLF→ALE) (B). (C,D) In lin-10 animals, we expressed TMGFP::GLR-2(tail) (C) and TMGFP::GLR-2(TLF→ALE) (D). Both B and C show a reduced size of synaptic clusters compared with A, suggesting that both LIN-10 and the PDZ-binding motif of GLR-2 are important for synaptic localization of a GLR-2 tail chimeric protein. (D) These two pathways may compensate for each other as removal of both pathways by expressing a chimeric GLR-2 protein with a mutated PDZ-binding motif in a lin-10 background abolishes the synaptic localization pattern. Bar, 5 µm. (E) The mean number of clusters per 10 µm of ventral cord length, and (F) the mean cluster size is plotted for the given genotype. Error bars are s.e.m. for both graphs. **P<0.01 compared with TLF in wild-type by ANOVA/Dunnett’s multiple comparisons test. n=15-20 animals for each genotype.]
(ESTs) and genomic sequence for GLR-1 and GLR-2 orthologs were present in *C. briggsae* and *C. remanei*, and the tail sequences appeared to be well-conserved within *Caenorhabditis* (83 of the 87 amino acids in the GLR-1 tail were identical among all three orthologs, whereas 53 of the 59 amino acids in the GLR-2 tail were identical among all three orthologs) (Fig. 6A,B). Partial ESTs for GLR-1 orthologs, but not GLR-2, have been identified in the ongoing sequence projects for *Strongyloides stercoralis* and *Ascaris suum*. These intestinal parasitic nematodes belong to orders that are thought to have diverged from *Rhabditida*, the order containing *C. elegans*, more than 400 million years ago (Riddle, 1997). The *S. stercoralis* and *A. suum* GLR-1 tails are 48% identical (77% similar) and 44% identical (77% similar) to *C. elegans* GLR-1, respectively. Multiple alignment provided a more stringent comparison, showing that the tail sequences of nematode GLR-1 contain several stretches of amino acids that comprise a core element that is presumably required for normal GLR-1 tail sequence function (Fig. 6A,B).

We next wanted to determine whether there are conserved motifs that are shared between nematodes and vertebrates. We generated multiple tail sequence alignments (Fig. 7) for AMPAR subunits with long tail sequences (GluR1, R2L, R4), three rat short tail AMPARs (R4S, R3, R2), and GLR-1 and GLR-2, aligned by CLUSTALW and JalView v1.8 Multiple Alignment Editor. Gray boxes indicate identities and similarities present in three or more sequences. (D) The predicted sequence of rat and *C. elegans* GluRs, emphasizing the indicated sequence motif. For the PKA motif, the underlined residue in GluR1 indicates the phosphorylated serine. For the regulated endocytosis motif, the underlined residues in GluR2 indicate the phosphorylated serine and tyrosines. (E) The predicted PDZ ligands for various rat GluRs aligned with GLR-1 and GLR-2. The type of PDZ ligand (I versus II) and its corresponding consensus is shown. ‘φ’ indicates any hydrophobic residue.
2000; Esteban et al., 2003; Lee et al., 2000; Lee et al., 2003; Roche et al., 1996) (Fig. 6D). GluR2 is phosphorylated by tyrosine kinases, leading to its regulated endocytosis, and a conserved motif surrounding the tyrosines could be identified in GluR2, R3, R4S, and GLR-1 (Ahmadian et al., 2004; Hayashi and Huganir, 2004) (Fig. 6D). A stretch of GluR2 residues known to bind NSF could be observed in R3, R4S and GLR-1 (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998) (Fig. 6D). Aside from the conserved motif found in all non-NMDA tail sequences, we could find no conserved motifs between the GLR-1 tail and the kainate receptor tails. The motifs revealed in GLR-1 were not as clearly obvious in the GLR-2 tail; however, we could identify a bipartite motif of unknown function that was conserved between GLR-2 and GluR6 (Fig. 6D). Finally, both GLR-1 and GLR-2 tails ended in a type-I PDZ ligand, similar to that found at the end of GluR1 (Fig. 6E). Taken together, our findings suggest that the GLR-1 tail (and to a lesser extent, the GLR-2 tail) contain sequence motifs found in both the vertebrate short and long tail AMPARs.

**Discussion**

We examined the GLR-1 and GLR-2 subunit tail sequences and their importance for AMPA receptor synaptic localization in either heterologous fusion proteins or in the background of heterotetrameric receptor complexes. We found that the GLR-2 tail sequence is sufficient for synaptic localization when placed into a heterologous fusion protein. In the absence of their cytosolic tail sequences, GLR-1 and GLR-2 can be localized through interactions with endogenous subunits. By contrast, both the GLR-1 and the GLR-2 cytosolic sequences are
Subunit association can facilitate localization

GLR-1 and GLR-2 are expressed in overlapping sets of interneurons that regulate locomotion, where they function to mediate nose-touch mechanosensation signaling (Brockie et al., 2001). Mutants for glr-1 are strongly defective for nose-touch sensation, whereas deletion mutants for glr-2 are only partially defective for nose-touch sensation (Hart et al., 1995; Mellem et al., 2002). Glutamate-activated currents, which are rapidly desensitizing and non-NMDA dependent, have been recorded from one of these interneurons, called AVA (Mellem et al., 2002). In glr-1 mutants, essentially all of this current is eliminated, whereas in glr-2 mutants there is a GLR-1-dependent residual current. Presumably the residual GLR-1-dependent current observed in glr-2 mutants is sufficient to mediate the partial response to nose-touch observed in these mutants. These findings suggest that GLR-1 alone can form functional glutamate-dependent channels in the absence of GLR-2 subunits, which is consistent with our findings that GLR-1 can be localized to synapses in the absence of GLR-2. Interestingly, the glutamate-dependent current is absent in glr-1 mutants, which presumably contain channels composed only of GLR-2 subunits (Mellem et al., 2002). We found that GLR-2 can be localized in the absence of GLR-1 subunits. Taken together, these findings would suggest that GLR-2 can assemble into homomeric channels and be localized to synapses, but that these channels cannot open in response to glutamate (at least in AVA) nor mediate even a partial nose-touch response.

Of the different glutamate receptor subunits found in C. elegans, only GLR-1 and GLR-2 are clear matches with AMPA-type by sequence identity (Brockie et al., 2001). GLR-3 to GLR-7 are equally similar to AMPA and kainate receptors, making their classification difficult. Thus, sequence identity at first approximation would suggest that only GLR-1 and GLR-2 can assemble into channels. Consistent with this hypothesis, mutations in glr-1 and glr-2 together can completely eliminate glutamate-gated current in the neuron AVA (Mellem et al., 2002). The subunits for GLR-4 and GLR-5 are present in AVA, yet seem to make little, if any, contribution to current. Although we currently do not know whether GLR-1 and GLR-2 form homomeric channels in wild-type animals, it seems likely that they do as GLR-1 is expressed in some neurons that lack GLR-2 (e.g. SMD, URY), and GLR-2 is expressed in some neurons that lack GLR-1 (e.g. RIA, AIA, AIB) (Brockie et al., 2001).

Tail sequences are crucial for localization

Previous work has shown that the specific subunit composition of mammalian AMPARs is important in regulating receptor synaptic localization (Hayashi et al., 2000; Passafaro et al., 2001; Shi et al., 2001; Shi et al., 1999). Localization of these heteromeric channels depends on their carboxy-terminal tail sequences (Cai et al., 2002; Dong et al., 1997; Leonard et al., 1998; Sans et al., 2001; Song et al., 1998; Srivastava et al., 1998; Wyszynski et al., 1999; Xia et al., 1999). Both GLR-1 and GLR-2 subunit tail sequences are important for synaptic localization in C. elegans neurons (Fig. 8), which suggests that C. elegans neurons might not designate specific receptor compositions for activity-dependent delivery as observed in mammalian neurons. Of course, we cannot rule out that there might be differential regulation of the receptors for synaptic localization when nematodes are presented with different environmental stimuli.

We found that the GLR-2 tail sequences are sufficient to direct the localization of a single-pass transmembrane domain. This result suggests that the cytosolic tail does not necessarily have to be in a multi-pass transmembrane protein as a requirement for its function. Moreover, it suggests that multiple cytosolic tails for a single channel are not needed for that channel’s localization, as might be predicted in a model where localization is mediated by multivalent scaffolding molecules that cluster channels together like logs in a wooden raft. Surprisingly, the GLR-1 tail sequences are not sufficient to direct the localization of a single-pass transmembrane protein. Nevertheless, our results indicate that the GLR-1 tail sequences are important for localization as homomeric GLR-1 channels that lack these sequences fail to become localized. One explanation could be that the PES-10 heterologous protein might not be the ideal context in which
to place the GLR-1 tail sequences. Alternatively, the GLR-1 tail might simply be necessary but not sufficient for localization, and thus other GLR-1 sequences could have an important role.

The PDZ-binding motif at the carboxy-terminus of GLR-2, while not required in the TMGFP::GLR-2(tail) heterologous reporter for its localization, was needed to enhance the cluster size of the heterologous reporter. A similar observation in mammals indicated that the PDZ-binding motif of GluR2 is not required for synaptic targeting of AMPARs, but is required for complete accumulation and stabilization of the receptors (Osten et al., 2000). Interestingly, mutations in lin-10 combined with mutations in the PDZ-binding motif nearly abolished the localization of the TMGFP::GLR-2(tail) reporter. Previous results indicated that mutations in lin-10 result in two phenotypes: apparent uniform distribution of GLR-1 and increased levels of GLR-1. These dual phenotypes suggest that LIN-10 positively regulates GLR-1 localization, but negatively regulates GLR-1 levels either directly or indirectly (Rongo et al., 1998). By contrast, mutations in lin-10 result in weakened localization of TMGFP::GLR-2(tail) but do not result in increased levels of TMGFP::GLR-2(tail). Interestingly, GLR-1 tail sequences contain several critical lysines that can be ubiquitinated, resulting in the turnover of GLR-1-containing channels (Burbea et al., 2002). TMGFP::GLR-2(tail) does not have sequences similar to the GLR-1 residues that mediate GLR-1 turnover. Perhaps this explains why TMGFP::GLR-2(tail) does not accumulate in a lin-10.

Conservation of the tail sequences between C. elegans and vertebrates

Our results also indicate that multiple factors are interacting with the cytosolic tail sequences of C. elegans AMPAR. This situation is similar to that of mammalian GluR2, in which its PDZ motif and NSF binding site are thought to mediate its localization (Osten et al., 1998). No doubt additional factors that interact with AMPAR tail sequences also contribute to regulating the localization of these receptors. Several motifs have already been identified in the rat GluR1 and GluR2 carboxy-terminal tail sequences. GluR1 contains a PKC phosphorylation site and a type-I PDZ-binding ligand thought to interact with SAP97 (Ahmadian et al., 2004; Esteban et al., 2003; Lee et al., 2000; Lee et al., 2003; Roche et al., 1996). GluR2 contains PKC and tyrosine kinase phosphorylation sites, and tyrosine phosphorylation appears to regulate GluR2 endocytosis in response to insulin and growth factor signaling (Ahmadian et al., 2004; Hayashi and Huganir, 2004). GluR2 can also bind to NSF via a specific domain in the tail sequence (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998). Finally, GluR2 contains a type-II PDZ ligand thought to interact with the GRIP/ABP and PICK1 PDZ domain proteins (Dong et al., 1997; Srivastava et al., 1998; Wyszynski et al., 1999; Xia et al., 1999). We can find motifs from both of these receptors in GLR-1, suggesting that R1 and R2 tails probably derived from a metazoan ancestral AMPAR that contained motifs common to both R1 and R2. The increasing complexity required by the vertebrate nervous system, including the requirement for different compositions of receptors that can be uniquely regulated by separate activity-dependent and -independent mechanisms, probably led to the divergence of these tail sequence motifs into disparate AMPAR subunits. Presumably, such divergence arose as the ancestral forms of GluR1 lost the motifs responsible for NSF-binding and regulated endocytosis, whereas the ancestral forms of GluR2 lost the PKA motif. Both proteins kept their PDZ ligand sequences, however, which could indicate that the PDZ ligand sequence and the motifs found outside this sequence function independently, and have therefore evolved independently.

Consistent with this idea, we found that both the GLR-2 PDZ ligand, as well as sequences outside of this ligand, can function to localize GLR-2. We expect that future findings using these receptors will help determine whether the proteins that interact with these motifs have been conserved over time.

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References

Ahmadian, G., Ju, W., Liu, L., Wyszynski, M., Lee, S. H., Dunah, A. W., Taghibighoul, C., Wang, Y., Lu, J., Wong, T. P. et al. (2004). Tyrosine phosphorylation of GluR2 is required for insulin-stimulated AMPA receptor endocytosis and LTD. EMBO J. 23, 1040-1050.

Ayalon, G. and Stern-Bach, Y. (2001). Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. Neuron 31, 103-113.

Beattie, E. C., Carroll, R. C., Yu, X., Morishita, W., Yasuda, H., von Zastrow, M. and Malenka, R. C. (2000). Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat. Neurosci. 3, 1291-1300.

Bekprozvanny, I. and Maximon, A. (2001). Classification of PDZ domains. FEBS Lett. 509, 457-462.

Brockle, P. J., Madsen, D. M., Zheng, Y., Mellem, J. and Maricq, A. V. (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.

Burbea, M., Dreier, L., Dittman, J. S., Grunwald, M. E. and Kaplan, J. M. (2002). Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in C. elegans. Neuron 35, 107-120.

Cai, C., Coleman, S. K., Niemi, K. and Keinanen, K. (2002). Selective binding of synapse-associated protein 97 to Glu-R-A alpha-aminoo-5-hydroxy-3-methyl-4-isoxazole propionate receptor subunit is determined by a novel sequence motif. J. Biol. Chem. 277, 31484-31490.

Dingledine, R., Borges, K., Bowie, D. and Traynelis, S. F. (1999). The glutamate receptor ion channels. Pharmacol. Rev. 51, 7-61.

Dong, H., O’Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F. and Huganir, R. L. (1997). GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature 386, 279-284.

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

Esteban, J. A., Shi, S. H., Wilson, C., Nuriya, M., Huganir, R. L. and Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat. Neurosci. 6, 136-143.

Firestein, B. L., Brennan, J. E., Aoki, C., Sanchez-Perez, A. M., El-Husseini, A. E. and Bredt, D. S. (1999). Cypin: a cytosolic regulator of PSD-95 postsynaptic targeting. Neuron 24, 659-672.

Garner, C. C., Nash, J. and Huganir, R. L. (2000). PDZ domains in synapse assembly and signalling. Trends Cell Biol. 10, 274-280.

Gregor, I. H., Khatri, L. and Ziff, E. B. (2002). RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. Neuron 34, 759-772.

Hart, A. C., Sims, S. and Kaplan, J. M. (1995). Synaptic code for sensory
modalities revealed by C. elegans GLR-1 glutamate receptor. Nature 378, 82-84.

Hayashi, T. and Huganir, R. L. (2004). Tyrosine phosphorylation and regulation of the AMPA receptor by SRC family tyrosine kinases. J. Neurosci. 24, 6152-6160.

Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Ponce, J. C. and Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKinaseII requirement for GluR1 and PDZ domain interaction. Science 287, 2262-2267.

Hollmann, M. and Heinemann, S. (1994). Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31-108.

Kaplan, J. M. and Horvitz, H. R. (1993). A dual mechanosensory and chemosensory neuron in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 90, 2227-2231.

Klocker, N., Bunn, R. C., Schnell, E., Caruana, G., Bernstein, A., Nicoll, R. A. and Brodli, D. S. (2002). Synaptic glutamate receptor clustering in mice lacking the SH3 and Gk domains of SAP97. Eur. J. Neurosci. 16, 1517-1522.

Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F. and Huganir, R. L. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405, 955-959.

Lee, H. K., Takamiyai, K., Han, J. S., Man, H., Kim, C. H., Rumbaugh, G., Yu, S., Ding, I., He, C., Petralia, R. S. et al. (2003). Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112, 631-643.

Leonard, A. S., Davare, M. A., Horne, M. C., Garner, C. C. and Hell, J. W. (1998). SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. J. Biol. Chem. 273, 19518-19524.

Leuschner, W. D. and Hoch, W. (1999). Subtype-specific assembly of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunits is mediated by their n-terminal domains. J. Biol. Chem. 274, 16907-16916.

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

Malinow, R. (2003). AMPA receptor trafficking and long-term potentiation. Philos. Trans. R. Soc. Lond. Ser. B 358, 707-714.

Mamo, I. and Teichberg, V. I. (1998). A tetrameric subunit stoichiometry for a glutamate receptor-channel complex. NeuroReport 9, 327-331.

Mariéq, A. V., Peckol, E., Driscoll, M. and Bargmann, C. I. (1995). Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor. Nature 378, 78-81.

Mellem, J. E., Brockie, P. J., Zheng, Y., Madsen, D. M. and Mariéq, A. V. (2002). Decoding of polyomodal sensory stimuli by postsynaptic glutamate receptors in C. elegans. Neuron 36, 933-944.

Nishimune, A., Isaac, J. T., Molnar, E., Noel, J., Nash, S. R., Tagaya, M., Collingridge, G. L., Nakashiba, S. and Henley, J. M. (1998). NSF binding to GluR2 regulates synaptic transmission. Neuron 21, 87-97.

Nonet, M. L., Saffee, O., Zhao, H., Rand, J. B. and Wei, L. (1998). Synaptic transmission deficits in Caenorhabditis elegans synaptobrevin mutants. J. Neurosci. 18, 70-80.

Osten, P. and Ziff, E. B. (1999). AMPA receptor forms a biochemically functional complex with NSF and alpha- and beta-SNAPs. Ann. New York Acad. Sci. 868, 558-560.

Osten, P., Srivastava, S., Inman, G. J., Vilim, F. S., Khatri, L., Lee, L. M., States, B. A., Einheber, S., Milner, T. A., Hanson, P. I. et al. (1998). The AMPA receptor GluR2 C terminus can mediate a reversible, ATP-dependent interaction with NSF and alpha- and beta-SNAPs. Neuron 21, 99-110.

Passafaro, M., Pich, V. and Sheng, M. (2001). Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat. Neurosci. 4, 917-926.

Riddle, D. L., Blumenthal, T., Meyer, B. J. and Priss, J. R. (eds) (1997). C. elegans II. (Cold Spring Harbor Monograph Series). Plainview, NY: Cold Spring Harbor Laboratory Press.

Roche, K. W., O’Brien, R. J., Mammen, A. L., Bernhardt, J. and Huganir, R. L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16, 1179-1188.

Rongo, C. and Kaplan, J. K. (1999). CaMKinII regulates the density of central glutamatergic synapses in vivo. Nature 402, 195-199.

Rongo, C., Whitfield, C. W., Rodal, A., Kim, S. K. and Kaplan, J. M. (1998). LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. Cell 94, 751-759.

Rosenmund, C., Stern-Bach, Y. and Stevens, C. F. (1998). The tetrameric structure of a glutamate receptor channel. Science 280, 1596-1599.

Sams, N., Racca, C., Petralia, R. S., Wang, Y. X., McCallum, J. and Wenthold, R. J. (2001). Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. J. Neurosci. 21, 7506-7516.

Seydoux, G. and Fire, A. (1994). Soma-germ-line asymmetry in the distributions of embryonic RNAs in Caenorhabditis elegans. Development 120, 2823-2834.

Sheng, M. (2001). Molecular organization of the postsynaptic specialization. Proc. Natl. Acad. Sci. USA 98, 7058-7061.

Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold, R. J., Svoboda, K. and Malinow, R. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284, 1811-1816.

Shi, S., Hayashi, Y., Esteban, J. A. and Malinow, R. (2001). Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105, 331-343.

Song, I., Kamboj, S., Xia, J., Dong, H., Liao, D. and Huganir, R. L. (1998). Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors. Neuron 21, 393-400.

Srivastava, S., Osten, P., Vilim, F. S., Khatri, L., Inman, G., States, B., Daly, C., DeSouza, S., Abagyan, R., Valtschanoff, J. G. et al. (1998). Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein APB. Neuron 21, 581-591.

Wenthold, R. J., Yokotani, N., Doi, K. and Wada, K. (1992). Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies. Evidence for a hetero-oligomeric structure in rat brain. J. Biol. Chem. 267, 501-507.

White, G. J., Southgate, E., Thomson, J. N. and Brenner, S. (1986). The structure of the nervous system of C. elegans. Philos. Trans. R. Soc. London B 314, 1-340.

Whitfield, C. W., Benard, C., Barnes, T., Hekini, S. and Kim, S. K. (1999). Basolateral localization of the Caenorhabditis elegans epidermal growth factor receptor in epithelial cells by the PDZ protein LIN-10. Mol. Biol. Cell 10, 2087-2100.

Wood, W. B. (1988). The nematode C. elegans. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Wyszynski, M., Valtschanoff, J. G., Naisbit, S., Dunah, A. W., Kim, E., Standaert, D. G., Weinberg, R. and Sheng, M. (1999). Association of AMPA receptors with a subset of glutamate receptor-interacting protein in vivo. J. Neurosci. 19, 6528-6537.

Xia, J., Zhang, X., Staudinger, J. and Huganir, R. L. (1999). Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1. Neuron 22, 179-187.

Zito, K., Fetter, R. D., Goodman, C. S. and Isacoff, E. Y. (1997). Synaptic clustering of FascinII and Shaker: essential targeting sequences and role of Dlg. Neuron 19, 1007-1016.