Distinct LIN-10 Domains Are Required for Its Neuronal Function, Its Epithelial Function, and Its Synaptic Localization[□]

Doreen R. Glodowski,* Tricia Wright,* Keri Martinowich,*
Howard Chia-Hao Chang, Douglas Beach, and Christopher Rongo

The Waksman Institute, Department of Genetics, Rutgers University, Piscataway, NJ 08854

Submitted October 8, 2004; Revised December 10, 2004; Accepted December 18, 2004
Monitoring Editor: Guido Guidotti

α -Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors (AMPA) mediate excitatory neurotransmission at neuronal synapses, and their regulated localization plays a role in synaptic plasticity. In *Caenorhabditis elegans*, the PDZ and PTB domain-containing protein LIN-10 is required both for the synaptic localization of the AMPAR subunit GLR-1 and for vulval fate induction in epithelia. Here, we examine the role that different LIN-10 domains play in GLR-1 localization. We find that an amino-terminal region of LIN-10 directs LIN-10 protein localization to the Golgi and to synaptic clusters. In addition, mutations in the carboxyl-terminal PDZ domains prevent LIN-10 from regulating GLR-1 localization in neurons but do not prevent LIN-10 from functioning in the vulval epithelia. A mutation in the amino terminus prevents the protein from functioning in the vulval epithelia but does not prevent it from functioning to regulate GLR-1 localization in neurons. Finally, we show that human Mint2 can substitute for LIN-10 to facilitate GLR-1 localization in neurons and that the Mint2 amino terminus is critical for this function. Together, our data suggest that LIN-10 uses distinct modular domains for its functions in neurons and epithelial cells and that during evolution its vertebrate ortholog Mint2 has retained the ability to direct AMPAR localization in neurons.

INTRODUCTION

The polarized trafficking and subcellular localization of specific synaptic proteins within neurons direct the flow of information within the nervous system. The localization of one synaptic component in particular, the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type ionotropic glutamate receptor (AMPA), to the postsynaptic density is a highly regulated process. AMPARs are gated by the neurotransmitter glutamate and mediate the bulk of excitatory transmission in the central nervous system. The mechanisms underlying synaptic plasticity stem, in part, from both the modulation of activity of these channels and the physical movement of these channels into and out of the synapse (Malinow *et al.*, 2000).

One of the primary ways by which neurons regulate glutamate receptor activity is by regulating the amount of these receptors that reach the synaptic membrane surface (Bolton *et al.*, 2000; Gomes *et al.*, 2003). AMPAR subunits (up to four in mammals, referred to as GluR1–R4; two in *Caenorhabditis elegans*, referred to as GLR-1 and GLR-2) are multitransmembrane spanning proteins that assemble into tetrameric channels of differing subunit composition (Hollmann and Heinemann, 1994; Dingledine *et al.*, 1999).

Our current understanding of how AMPARs are mobilized within cells has focused on the proteins that interact with the cytosolically exposed carboxyl-terminal sequences of AMPARs. Different combinations of subunits present distinct arrays of carboxyl-terminal sequences, resulting in a specific pattern of localization for a given receptor subunit combination. For example, heteromeric channels of GluR1/GluR2 are brought to synapses in response to activity, whereas heteromeric channels of GluR2/GluR3 exchange into the synaptic membrane constitutively (Shi *et al.*, 1999; Hayashi *et al.*, 2000; Passafaro *et al.*, 2001; Shi *et al.*, 2001).

To better understand how AMPARs are trafficked out of the cell body and localized to synapses, we observed the subcellular localization of channels containing the AMPAR subunit GLR-1 in *C. elegans*. Chimeric GLR-1 receptors containing green fluorescent protein (GLR-1::GFP) have been used to visualize glutamate receptors in living nematodes (Rongo *et al.*, 1998). GLR-1::GFP is localized to clusters at neuronal synapses in a manner that is dependent on Ca^{2+} signaling and the PDZ domain-containing protein LIN-10, although the mechanism of this localization is unclear (Rongo *et al.*, 1998; Rongo and Kaplan, 1999). In addition, the synaptic abundance of GLR-1::GFP is regulated by the ubiquitination and subsequent endocytosis of the GLR-1 subunit (Burbea *et al.*, 2002).

Although LIN-10 was found to be required for GLR-1 localization in neurons, mutations in *lin-10* were originally identified based on their failure to induce vulval differentiation from a set of equipotential epithelial cells, suggesting that LIN-10 functions both in epithelial cells and neurons (Kim and Horvitz, 1990; Rongo *et al.*, 1998). In *lin-10* mutants, the LET-23 epidermal growth factor receptor (EGFR),

This article was published online ahead of print in *MBC in Press* (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E04-10-0885>) on January 12, 2005.

[□] The online version of this article contains supplemental material at *MBC Online* (<http://www.molbiolcell.org>).

* These authors contributed equally to this work.

Address correspondence to: Christopher Rongo (rongo@waksman.rutgers.edu).

a key component in vulval induction, is not correctly localized to the basolateral surface (Whitfield *et al.*, 1999). LIN-10 is thought to facilitate LET-23 localization and vulval induction by forming a tripartite complex with two additional PDZ-domain-containing proteins, LIN-2 and LIN-7 (Hoskins *et al.*, 1996; Simske *et al.*, 1996; Kaech *et al.*, 1998). The LIN-7 PDZ domain directly binds to the carboxyl-terminal PDZ recognition motif in LET-23 *in vivo* because compensatory mutations in the LIN-7 PDZ domain and LET-23 PDZ recognition site can function together to facilitate LET-23 localization (Kaech *et al.*, 1998). LIN-2 in turn can bind to LIN-7, although this is not mediated by PDZ interactions (Kaech *et al.*, 1998). Indeed, the role of the LIN-2 PDZ domain is unknown; however, the PDZ domain of its mammalian ortholog CASK can bind to neuexin and syndecan, and it has been suggested that syndecan, a heparin sulfate proteoglycan, might act to present growth factor ligands to receptor tyrosine kinases like LET-23 (Hata *et al.*, 1996; Hsueh *et al.*, 1998). Thus, LIN-2 also might facilitate vulval induction by colocalizing a syndecan with LET-23 (Hsueh *et al.*, 1998). Finally, LIN-10 has been shown to bind to LIN-2 via an amino-terminal domain known as the CASK/LIN-2 interaction domain (hereafter referred to as CID) (Okamoto and Sudhof, 1997; Borg *et al.*, 1998; Kaech *et al.*, 1998; Rongo, 2001).

Several pieces of evidence suggest that LIN-10 is unlikely to be using LET-23 signaling as a mechanism to regulate GLR-1 localization in neurons. First, LET-23 is not expressed in the GLR-1-expressing neurons (Rongo *et al.*, 1998). Second, whereas LIN-2 and LIN-7 are essential for vulval induction, they are not required for GLR-1 localization in neurons (Rongo *et al.*, 1998). Thus, LIN-10 might interact with a different set of PDZ proteins to recognize GLR-1. Alternatively, LIN-10 might function in neurons in a different manner than the mechanism by which it functions in epithelia (Rongo *et al.*, 1998).

LIN-10 contains multiple protein-protein interaction domains. A similar domain structure is shared by two potential vertebrate orthologues of LIN-10, called Mint1 and Mint2, which are expressed exclusively in mammalian brain (reviewed in Rongo, 2001). LIN-10, Mint1, and Mint2 all have a single carboxyl-terminal PTB domain and two carboxyl-terminal PDZ domains (~85% amino acid identity for these three domains). PTB domains bind to an NPXY motif often found in phosphoproteins, whereas PDZ domains typically bind to carboxyl-terminal residues at the end of receptors and other membrane proteins (reviewed in Margolis, 1999; Garner *et al.*, 2000). LIN-10 and the Mints differ most in their amino-terminal domains (21% amino acid identity) because Mint1 and LIN-10 both contain CID, whereas Mint2 does not. Functional differences between Mint1 and Mint2 are not well understood.

To better understand the role of LIN-10 in regulating the localization of AMPARs in neurons, we used the *C. elegans* model system to characterize the domains of LIN-10 that are required to regulate GLR-1 trafficking and localization. We find that LIN-10 colocalizes with GLR-1 at the Golgi and at synaptic clusters through a specific amino-terminal region. We also find that the PTB and PDZ domains are essential for LIN-10 to regulate GLR-1 localization; however, the PDZ domains do not seem to be important for vulval induction in epithelial cells. In addition, the Mint2 vertebrate ortholog of LIN-10 can assume the functions of LIN-10 in neurons and requires its amino-terminal residues for this purpose. Together, our data suggest that LIN-10 uses distinct domains to function in neurons and epithelia.

MATERIALS AND METHODS

Genetics and Strains

Standard methods were used to handle and maintain *C. elegans* (Wood, 1988). We used the following strains: N2, *lin-10(n1508)*, *lin-10(n1390)*, *lin-10(e1439)*, *nuls25*, and *lin-15(n765ts)* (Herman and Hedgecock, 1990; Kim and Horvitz, 1990; Nonet *et al.*, 1999; Burbea *et al.*, 2002).

Transgenes and Germline Transformation

Transgenic strains were isolated by microinjecting various plasmids (typically at 50 ng/ μ l) by using either *lin-15(+)* (J. Mendel, California Institute of Technology) or *rol-6dm* (C. Mello, University of Massachusetts Medical School) as a marker. The *glr-1::rfp* transgene was generated by introducing sequences encoding monomeric red fluorescent protein (RFP) (R. Tsien, Stanford University School of Medicine) into the *HindIII* site of sequences encoding the carboxyl terminus of a *glr-1* cDNA (Campbell *et al.*, 2002). Sequences for the resulting chimeric protein were placed into the vector pV6, which contains the *glr-1* promoter. The resulting plasmid was injected into nematodes and integrated into the genome by UV/trimethylpsoralen as described previously to generate *odIs17[glr-1::rfp]* (Yandell *et al.*, 1994; Rongo *et al.*, 1998). The transgenic strain was backcrossed to a wild-type strain to remove other mutations introduced during the integration.

The following transgenes were introduced into the germline, and followed as extrachromosomal arrays. The *lin-10::gfp* transgene was generated by subcloning sequences encoding GFP in frame at the carboxyl terminus of a *lin-10* cDNA, yk114c6 (Y. Kohara, National Institute of Genetics, Japan). Sequences for the resulting chimeric protein were placed into the *glr-1* promoter vector pV6. Truncated versions of *lin-10::gfp* were generated by using a corresponding set of primers to make a polymerase chain reaction product of the partial *lin-10* cDNA, which was then used to replace sequences, in frame, encoding the full-length *lin-10* cDNA in the *lin-10::gfp* transgene. Mutated versions of *lin-10::gfp* were generated by using oligonucleotide-mediated mutagenesis to introduce sequence changes into a *lin-10* cDNA that result in amino acid substitutions. The mutated versions were then used to replace the *lin-10* cDNA in the *lin-10::gfp* transgene. The rescuing *P_{glr-1}::lin-10* transgene was generated by subcloning the full-length *lin-10* cDNA into pV6. To examine the rescuing activity of LIN-10 with various amino acid substitutions, the mutated versions of *lin-10*, generated as described above, were subcloned into pV6. The rescuing *P_{lin-31}::lin-10* transgene was generated by subcloning the full-length *lin-10* cDNA into OR138, a plasmid containing sequences encoding the *lin-31* promoter. To examine the rescuing activity of LIN-10 with various amino acid substitutions, the mutated versions of *lin-10*, generated as described above, were subcloned into OR138. All described constructs were confirmed by DNA sequencing.

To test for rescue by the Mint orthologues, the *P_{glr-1}::Mint1* and *P_{glr-1}::Mint2* transgenes were generated by subcloning the human Mint1/X11 α (AF047347) and Mint2/X11 β (AF047348) cDNAs (B. Margolis, University of Michigan Health System), respectively, into pV6. The *P_{glr-1}::M1/M2* transgene was generated by ligating sequences encoding amino acids 1–451 of Mint1 to sequences encoding amino acids 362–749 of Mint2. The *P_{glr-1}::M2/M1* transgene was generated by ligating sequences encoding amino acids 1–361 of Mint2 to sequences encoding amino acids 452–837 of Mint1. Transgenes were sequenced and characterized as described above.

Fluorescence Microscopy

GFP-, cyan fluorescent protein- (CFP), yellow fluorescent protein- (YFP), and RFP-tagged fluorescent proteins were visualized in nematodes by mounting larvae on 2% agarose pads with 10 mM levamisole at room temperature. Fluorescent images were observed using a Zeiss Axioplan II and 100 $\times$ 1.4 numerical aperture PlanApo objective and imaged with an ORCA charge-coupled device (CCD) camera (Hamamatsu, Bridgewater, NJ) by using ImagePro version 4.1 and VayTek version 6.2 software. Exposure times were chosen to fill the 12-bit dynamic range without saturation. Animals were optically sectioned (0.4 μ m), and out-of-focus light was removed with a constrained iterative deconvolution algorithm (VayTek).

To quantify the fluorescence levels of GFP-tagged proteins, images of nematodes were captured by CCD as described above by using a constant gain and exposure time (filled the 12-bit dynamic range) for all samples. A fluorescent standard (Labsphere) showed <1% drift in signal throughout the imaging session. Images were corrected for coverslip fluorescence by subtracting a background image. Pixel intensity was measured for each animal by quantifying an area of interest that collected the fluorescent ventral nerve cord for each sample. The peak amplitude of clusters (localized signal) was calculated for each two-dimensional projection as the fractional increase over the diffuse background of ventral cord fluorescence (unlocalized baseline). Cluster outlines were automatically calculated for fluorescent signals that were two standard deviations above the unlocalized baseline by using a macro written for ImagePro. Cluster size was measured as the maximum diameter for each outlined cluster. Cluster number was calculated by counting the average number of clusters per 10 μ m of dendrite length. Images of nematodes expressing fluorescent proteins from extrachromosomal arrays were

Figure 1. LIN-10 regulates GLR-1 abundance in dendrites. SNB-1::GFP fluorescence (A and D) and GLR-1::RFP fluorescence (B and E) were observed along the ventral cord dendrites of wild-type nematodes (A–C) or *lin-10* mutants (D–F). Wild-type (100%, $n = 23$) and *lin-10* (100%, $n = 36$) animals have similar presynaptic clusters of the synaptic vesicle protein SNB-1::GFP. Whereas wild-type animals have small postsynaptic clusters of GLR-1::RFP (100%, $n = 23$) found adjacent to presynaptic SNB-1::GFP clusters, most *lin-10* mutants have large ($>2\text{-}\mu\text{m}$) clusters of GLR-1::RFP (92%, $n = 36$). Most large GLR-1 clusters can be found near SNB-1::GFP clusters. LIN-10::GFP fluorescence (G) and GLR-1::RFP fluorescence (H) were observed to colocalize along ventral cord dendrites. GLR-1::CFP fluorescence (J), mannosidase-YFP (MANS::YFP) fluorescence (K), and LIN-10::RFP fluorescence (L) were observed to colocalize at perinuclear puncta thought to be Golgi bodies (Rolls *et al.*, 2002). Merged images are shown in C, F, I, and M. Bar, 5 μm .

only captured if the array was present in all cells previously reported; animals with mosaic nervous systems were not examined.

Scoring Vulval and GLR-1 Localization Rescue

To test for rescue of GLR-1 localization defects, $P_{glr-1}::lin-10$ and its mutant variants were coinjected with *rol-6(dm)* DNA into *lin-10*; *nuls25* mutants. Multiple transgenic lines were obtained for each construct, and each line was scored as wild-type for GLR-1 localization if the animal had a predominance of 2- μm -diameter or smaller clusters, which comprise $>95\%$ of the clusters found in wild-type nematodes. Nematodes with larger clusters were scored as having the mutant phenotype. To test for rescue of vulval defects, $P_{lin-31}::lin-10$ and its mutant variants were coinjected with *myo-3::gfp* DNA (A. Fire, Stanford University School of Medicine) into *lin-10* mutants, and F1 were scored based on GFP expression in the pharynx. Each line was scored as wild-type for vulval induction if they could form a functioning vulval opening and deposit eggs 48 h into adulthood. Animals that “bagged” or failed to lay eggs were scored as having the mutant phenotype. For both assays, 20–80 animals were tested for each line, and three to five lines for each genotype.

Sequence Comparisons

LIN-10/Mint sequences from various organisms were aligned by using the CLUSTALW algorithm. *Caenorhabditis briggsae* and *Caenorhabditis remanei* orthologues were identified using the Wormbase (www.wormbase.org) and the Wash. Univ. GSC (<http://genome.wustl.edu/>) BLAST search engine. Vertebrate and nematode sequences were compared using CLUSTALW, followed by manual adjustments to the gap size by using JalView version 1.8 multiple alignment editor.

RESULTS

LIN-10 Regulates GLR-1 Abundance in Dendrites

We previously determined that GLR-1 fails to be tightly localized to synaptic clusters in *lin-10* mutants by using a GLR-1::GFP translational fusion. To examine GLR-1 localization in colocalization experiments, we generated a transgene that expresses the full-length translational fusion protein GLR-1::RFP (monomeric RFP; Campbell *et al.*, 2002). We coexpressed GLR-1::RFP with GFP-labeled synaptobrevin (SNB-1::GFP), which is localized to sites of presynaptic innervation. SNB-1::GFP, when expressed by the *glr-1* promoter, has a localization pattern that is restricted to a subpopulation of interneuron-interneuron nerve terminals (Rongo *et al.*, 1998). In wild-type nematodes, we detected GLR-1 at synaptic junctions labeled with localized

SNB-1::GFP (Figure 1, A–C). In nematodes homozygous for a *lin-10* molecular null mutation, GLR-1::RFP failed to be localized at synaptic clusters and accumulated in large structures throughout the dendrite (Figure 1, D–F). Presynaptic clusters of SNB-1::GFP were present in *lin-10* mutant dendrites (Figure 1D), demonstrating that *lin-10* is not required for synapse formation or neuronal protein trafficking per se. Rather, our results suggest that LIN-10 regulates GLR-1 localization specifically.

The Amino Terminus of LIN-10 Directs Its Localization

To begin to address how LIN-10 regulates the transport of GLR-1 receptors to synapses, we examined the localization of LIN-10 protein in neurons. We had previously generated a transgene that expresses a LIN-10::GFP fusion protein (with GFP at the carboxyl terminus) and found that this transgene rescues the *lin-10* mutant phenotype and is localized to punctate structures along the ventral cord dendrites (Rongo *et al.*, 1998; Figure 1, G–I). In a separate transgene, we replaced GFP sequences with sequences encoding monomeric RFP to generate a transgene that expresses a LIN-10::RFP fusion protein. We introduced this transgene into *lin-10* mutant nematodes to observe localization in the absence of endogenous LIN-10 protein. Both GFP- and RFP-tagged versions of LIN-10 showed similar localization patterns, allowing us to perform different combinations of colocalization experiments. We found that LIN-10::RFP was localized to perinuclear structures in the cell body and punctate structures along the ventral cord dendrites (Figure 1L; our unpublished data). To determine whether the punctate structures in the cell body correspond to Golgi-localized GLR-1 receptor, we coexpressed LIN-10::RFP, GLR-1::CFP, and mannosidase-YFP (MANS::YFP), a Golgi-resident protein, and found that they colocalized to punctate structures within the cell bodies (Figure 1, J–M) (Rongo *et al.*, 1998; Rolls *et al.*, 2002). Interestingly, LIN-10 vertebrate homologues are localized to the Golgi as well, suggesting that LIN-10 might conduct its regulatory function of receptors at one of the earliest steps in the secretory pathway (Whitfield *et al.*, 1999; Biederer *et al.*, 2002; Stricker and Huganir, 2003).

Figure 2. Localization of LIN-10 protein domains. (A) Regions of LIN-10 present in each LIN-10::GFP transgene tested are shown schematically and by amino acid number. LIN-10(1-954) is the full-length LIN-10 protein. GFP is not shown, but it is tagged at the carboxyl terminus of each construct. (B) Number of clusters (as defined as peak pixel intensities 2 SDs above the baseline) formed by the indicated GFP-tagged proteins. * $p < 0.05$ and ** $p < 0.01$ by one-way analysis of variance followed by Dunnett's multiple comparison to the GFP control. Cell body and ventral cord fluorescence from GFP alone (C and D), LIN-10(1-574)::GFP (E and F), LIN-10(1-381)::GFP (G and H), LIN-10(373-574)::GFP (I and J), LIN-10(574-766)::GFP (K and L), LIN-10(1-766)::GFP (M and N), and LIN-10(373-766)::GFP (O and P). Bar, 5 μ m. Three or more lines were observed for each transgene, with $n \geq 20$.

To determine which amino acids in the LIN-10 protein are responsible for its subcellular localization, we generated truncated variants of the LIN-10::GFP transgene by removing different sequences of the protein while placing GFP in frame at the carboxyl terminus of each partial protein. Each variant transgene was then introduced into the germline of *lin-10* mutants to observe subcellular localization in the absence of endogenous LIN-10 protein. We found that several different domains could direct the localization of GFP to distinct subcellular compartments (Figure 2). The amino-terminal half of LIN-10 (amino acids 1-574) was sufficient to be localized to perinuclear structures in the cell body (Figure 2E) and to punctate structures in the ventral cord dendrites (Figure 2F).

In an attempt to further dissect the amino-terminal domain, we found that the first 381 amino acids could direct localization to synaptic clusters and to Golgi, although less efficiently than LIN-10(1-574)::GFP (Figure 2, G and H). Fluorescent clusters of LIN-10(full length)::GFP, LIN-10(1-574)::GFP, and LIN-10(1-381)::GFP were similar in diameter and peak fluorescent intensity (our unpublished data). However, the number of LIN-10(1-381)::GFP clusters per 10 micron length of dendrite (1.2 ± 0.2) was significantly lower than for LIN-10(full length)::GFP (2.1 ± 0.3) and LIN-10(1-574)::GFP (2.2 ± 0.1), suggesting that amino acids 1-381 cannot fully recapitulate the normal localization pat-

tern (Figure 2B). The missing sequence, when expressed alone as LIN-10(373-574)::GFP, failed to direct localization to either the Golgi or synaptic clusters (Figure 2, I and J); however, we found that this sequence prevented the translocation of the fusion protein into the nucleus (Figure 2I). This was surprising because GFP alone (Figure 2C) or fused to other domains of LIN-10 similar or greater in size than LIN-10(373-574) were present both in the cytosol and the nucleus (our unpublished data), suggesting that sequences within LIN-10(373-574) can anchor the protein in the cytoplasm. Alternatively, this domain could contain a nuclear export sequence that prevents its nuclear accumulation; however, we could find no known export signal within this domain.

We also examined whether the carboxyl-terminal half of LIN-10, which contains the PTB and PDZ domains, can direct protein localization. To address this issue, we examined the localization of fusion proteins containing individual protein-protein interaction domains of LIN-10 fused to GFP. We found that none of the fusion proteins that contain carboxyl-terminal sequences were sufficient to direct localization to the Golgi or to synaptic clusters (our unpublished data). Interestingly, the PTB domain alone was sufficient to direct localization to the nucleus (Figure 2K). However, if amino-terminal sequences of LIN-10 were fused to the PTB domain, the resulting chimeric protein was retained in the

Figure 3. Amino-terminal domain of LIN-10 directs LIN-10 localization. Fluorescence from *C. elegans* neurons expressing three separate transgenes. GLR-1::CFP (A), mannosidase-YFP (B), and LIN-10(1-574)::RFP (C) colocalize to perinuclear structures in the cell body. Fluorescence from LIN-10(1-574)::GFP (E) and GLR-1::RFP (F) also colocalize to puncta in ventral cord dendrites. Merges are shown in D and G. Bar, 5 μ m.

cytoplasm. For example, LIN-10(1-766), although not localized to the Golgi, nevertheless was excluded from the nucleus (Figure 2O). We scanned the amino acid sequence of the PTB domain but failed to find any known nuclear localization signals. One explanation could be that the PTB domain, a well characterized protein-protein interaction motif, binds a protein that can translocate into the nucleus. In that case, perhaps full-length LIN-10 acts to regulate the nuclear translocation of this factor. Unexpectedly, we found that a mutation that impairs PTB domain function did not prevent nuclear accumulation of the PTB domain (our unpublished data), raising the possibility that this domain could be multifunctional.

To determine whether amino acids 1-574 of LIN-10 colocalize with GLR-1 in the Golgi, we generated a transgene, called LIN-10(1-574)::RFP, containing the amino-terminal amino acid sequences of LIN-10 fused to sequences encoding RFP. We introduced transgenes expressing LIN-10(1-574)::RFP, GLR-1::CFP, and MANS::YFP into the germline and found that their proteins colocalized to the same punctate structures within the cell body (Figure 3, A-D). To determine whether this same amino acid sequence directs localization to GLR-1-containing clusters in dendrites, we introduced the LIN-10(1-574)::GFP transgene into nematodes that express GLR-1::RFP. Wild-type endogenous LIN-10 was present to ensure that GLR-1::RFP was clustered. We found that ventral cord clusters of LIN-10(1-574)::GFP (Figure 3E) colocalized with GLR-1::RFP (Figure 3F) in dendrites. We also found that LIN-10(1-574)::RFP and GLR-1::GFP colocalized in dendrites, although the intensity of LIN-10(1-574)::RFP was less than LIN-10(1-574)::GFP (our unpublished data). Our results demonstrate that the amino terminal half of LIN-10 is sufficient to direct protein localization to Golgi and to GLR-1-containing synaptic clusters.

Distinct LIN-10 Functions Have Different Requirements for the PDZ and PTB Domains

Because the amino terminal half of LIN-10 was sufficient to direct its localization in neurons, we reasoned that the carboxyl-terminal half of LIN-10, including the PTB and PDZ domains, might be required for neuronal function. To determine whether specific domains of LIN-10 are required for its disparate functions in neurons and epithelial cells, we generated rescuing minigenes that included the full-length LIN-10 cDNA expressed by either the *glr-1* promoter, which expresses in the interneurons, or the *lin-31* promoter, which expresses in the vulval precursor epithelial cells. We called the transgenes $P_{glr-1}::LIN-10$ and $P_{lin-31}::LIN-10$, respectively. We introduced the transgenes into *lin-10* null mutants and

observed their ability to rescue GLR-1 localization or vulva induction, respectively. Introduction of the $P_{glr-1}::LIN-10$ transgene into *lin-10* mutants robustly rescued the GLR-1 localization defect (Figure 4, C and I). In addition, introduction of the $P_{lin-31}::LIN-10$ transgene into *lin-10* mutants rescued the vulval induction of these animals to levels similar to what has been observed previously (Figure 4J) (Whitfield *et al.*, 1999).

To determine which domains of LIN-10 are required for function, we introduced into the PTB and PDZ domains amino acid substitutions that have been shown to impair their function. For the PTB domain, we made a transgene, called LIN-10(F724V), in which we introduced a phenylalanine to valine substitution that impairs the ability of X11 α /Mint1 to bind to amyloid precursor protein (APP) (Borg *et al.*, 1996). For the first PDZ domain, we made a transgene, called LIN-10(KK778AA), with amino acid substitutions at lysines that are critical for function (Doyle *et al.*, 1996; Morais Cabral *et al.*, 1996). We also generated a transgene, called LIN-10(GI785PA), in which we altered the conserved GLGF domain, which adopts a critical structural orientation necessary for the PDZ domain to bind its partners (Doyle *et al.*, 1996; Morais Cabral *et al.*, 1996). For the second PDZ domain, we made a transgene, called LIN-10(RR868AA), with amino acid substitutions that prevent binding to the PDZ recognition motif (Doyle *et al.*, 1996; Morais Cabral *et al.*, 1996). Each of these variant transgenes was placed under the control of either the *glr-1* or the *lin-31* promoter and then introduced into *lin-10* null mutants to measure their ability to rescue. We also generated GFP-tagged versions of these proteins to examine the stability and localization of LIN-10 proteins harboring these mutations. We found that all of the LIN-10::GFP variants were stably produced and localized to Golgi and synaptic clusters similar to wild-type LIN-10 (Figure 4H; our unpublished data).

Interestingly, the mutation in the PTB domain impaired the ability of the transgenes to rescue both the vulval induction and the GLR-1 localization phenotypes (Figure 4, E, I, and J), suggesting that the PTB domain is critical for both functions. Surprisingly, we found that both the LIN-10(KK778AA) and LIN-10(GI785PA) transgenes, which contain mutations in the first PDZ domain, rescued the vulval induction to levels similar to wild-type LIN-10 cDNA rescue (Figure 4J; our unpublished data), but did not rescue the GLR-1 localization defects in *lin-10* mutants (Figure 4, F and I). We also found that the transgenes with mutations in the second PDZ domain rescued the vulval induction defect but did not rescue the GLR-1 localization defect (Figure 4, G, I, and J). Our results suggest that although the PTB domain is

Figure 4. Differential requirements for the PTB and PDZ domains in LIN-10 function. Fluorescence from GLR-1::GFP in the ventral cord bundle. (A) Wild-type animals. (B) *lin-10* mutants. (C) *lin-10* mutants rescued with *P_{glr-1}::LIN-10* containing wild-type cDNA. (D) *lin-10* mutants rescued with *P_{glr-1}::LIN-10(P27S)*. Mutants for *lin-10* are not rescued by *P_{glr-1}::LIN-10(F724V)* (E), which impairs the PTB domain; *P_{glr-1}::LIN-10(KK778AA)* (F), which impairs PDZ domain 1; and *P_{glr-1}::LIN-10(RR868AA)* (G), which impairs PDZ domain 2. Bar, 10 μ m. (H) Number of clusters (as defined as peak pixel intensities 2 SDs above the baseline) formed by the indicated GFP-tagged proteins. * $p < 0.05$ and ** $p < 0.01$ by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison to the GFP control. (I) Fraction of *lin-10* mutants rescued for the GLR-1 localization phenotype for the indicated transgene. (J) Fraction of *lin-10* mutants rescued for the vulval induction phenotype for the indicated transgene. * $p < 0.05$ and ** $p < 0.01$ by one-way ANOVA followed by Dunnett's multiple comparison to the no transgene control. WT indicates the wild-type LIN-10. P27S indicates the amino acid substitution found in the *n1390* allele. The other three mutations represent the corresponding transgenes in E–G. Three or more lines were observed for each transgene, with $n \geq 20$. Error bars indicate SEM.

commonly used by LIN-10, the PDZ domains are used differentially in neurons versus epithelial cells.

Mutations in *lin-10* were originally identified based on the vulval induction defect. Interestingly, 13 of the 14 isolated alleles are molecular nulls, whereas *lin-10(n1390)* is an amino acid substitution (P27S) (Whitfield *et al.*, 1999). We examined GLR-1::GFP localization in *lin-10(n1390)*. Unexpectedly, we found that GLR-1 was properly localized even though these mutants are defective for vulval induction (Figure 4, D and I), suggesting that P27, an amino-terminal residue of LIN-10, is important for vulval induction but not for GLR-1 localization. Because P27S falls within the region that we identified as essential for directing LIN-10 localization, we tested whether a *LIN-10(P27S)::GFP* transgene would produce localized LIN-10 protein. We found that *LIN-10(P27S)::GFP* is correctly localized in neurons, consistent with it being able to rescue GLR-1 localization in these cells (Figure 4H). Thus, we have identified mutations in LIN-10 protein that abolish epithelial function (P27S), neuronal function (KK778AA, GI785PA, and RR868AA), or both (F724V).

Neuronal Functions of LIN-10 Are Conserved within *Mint2*

The Mint1 and Mint2 proteins share a similar domain organization with LIN-10. We were interested in how these proteins are conserved evolutionarily, because the conservation of amino acids between distantly related species provides an indication of their sequence importance. As a first step, we examined the sequence conservation of LIN-10 orthologues between nematode species. Expressed sequence tags and genomic sequence for LIN-10 orthologues are present in *C. briggsae* and *C. remanei* and seem to be well conserved within *Caenorhabditis* (80% of the LIN-10 residues are identical (89% similar) among all three orthologues; Supplementary Figure 1). We next generated multiple tail sequence alignments for Mint1 and Mint2 from different vertebrate species (Supplementary Figure 2 and Figure 3), and used the consensus from these alignments to seed a multiple alignment with nematode LIN-10 sequences (Figure 5A). The PTB and PDZ domains are well conserved between vertebrates and nematodes, showing 61% identity

(78% similarity) for the PTB domain, 54% identity (75% similarity) for the first PDZ domain, and 75% identity (92% similarity) for the second PDZ domain. Sequence alignments between Mint1 and LIN-10 also indicate the presence of conserved residues in the amino terminus, particularly within CID (Figure 5A). Among the three vertebrate sequences and three nematode sequences, CID is conserved with 63% identity (67% similarity) and 82% identity (90% similarity), respectively. Between vertebrates and nematodes, CID is less well conserved (22% identity, 29% similarity). However, *C. elegans* LIN-10 is capable of binding to vertebrate CASK, suggesting that this low level of sequence

From the sequence alignments, we hypothesized that a LIN-10/Mint ancestral protein might have diverged in vertebrates into two proteins with different functions mediated by their divergent amino-terminal domains. We explored this idea by testing whether Mint1 or Mint2 could rescue the GLR-1 localization phenotype of a *lin-10* mutant. We generated $P_{glr-1}::Mint1$ and $P_{glr-1}::Mint2$ transgenes by placing their respective cDNAs under the control of the *glr-1* promoter. We introduced the transgenes into *lin-10* null mutants and observed their ability to rescue GLR-1 localization.

Figure 6. Model for LIN-10/Mint function in neurons and epithelia. (A) LIN-10 function in *C. elegans*. LIN-10 acts as a scaffold in epithelial cells by binding to LIN-2 (via the CID domain), which in turn binds to LIN-7, which can recognize the LET-23 EGFR and localize it to basolateral membranes. We found that the PTB domain and the P27 residue in the amino terminus (arrows) also are required for vulval induction. In neurons, the amino-terminal domain of LIN-10 (including CID) directs the localization of LIN-10 to the Golgi and to synapses. The PTB and PDZ domains (arrows) are likely to interact with proteins that facilitate GLR-1 delivery. (B) Mint protein function in vertebrates. We propose that the amino-terminal regions of Mint1 and Mint2 confer specificity of function on these proteins. Mint1 contains CID, which can interact with CASK and Velis, whereas we found that the amino-terminal region of Mint2 is required for AMPAR localization. MID is the Munc-18 interaction domain. The PTB and PDZ domains of both Mints interact with multiple proteins, but the functional significance of these interactions remains unclear.

Introduction of the $P_{glr-1}::Mint1$ transgene into *lin-10* mutants only mildly rescued the localization defect (Figure 5C). By contrast, the $P_{glr-1}::Mint2$ transgene rescued the GLR-1 localization defects of *lin-10* mutants to a similar level as rescue by $P_{glr-1}::LIN-10$. We also tested whether Mint1 or Mint2, expressed by the *lin-31* promoter, could rescue the vulval induction defects of *lin-10* mutants; however, we found that neither gene rescued (our unpublished data; see below for further discussion).

One explanation for why Mint2 can rescue GLR-1 localization and Mint1 cannot is that the divergent sequences in the amino-terminal half of these proteins could conduct distinct functions. Alternatively, the carboxyl-terminal half (i.e., the PTB and PDZ domains) of Mint2 might have a small number of critical sequence changes with respect to Mint1 that allow it to substitute for LIN-10. To test this hypothesis, we generated Mint protein chimeric transgenes by fusing the amino-terminal sequences of Mint1 to the carboxyl-terminal sequences of Mint2 ($P_{glr-1}::M1/M2$), and by fusing the amino-terminal sequences of Mint2 to the carboxyl-terminal sequences of Mint1 ($P_{glr-1}::M2/M1$) (Figure 5B). Whereas the $P_{glr-1}::M1/M2$ transgene did not rescue the GLR-1 localization defect, we found that the $P_{glr-1}::M2/M1$ transgene rescued to similar levels as $P_{glr-1}::Mint2$ (Figure 5C). Together, these results demonstrate that the PTB and PDZ domains of Mint1 and Mint2 are functionally equivalent with respect to GLR-1 localization but that Mint2 possesses critical sequences in its amino-terminal half that can facilitate AMPAR localization.

DISCUSSION

In this study, we used three approaches to delineate which domains of the LIN-10 protein are required for function in

neurons and in epithelial cells. First, we identified a region of the LIN-10 protein (amino acids 1–574) that is sufficient to direct localization of a heterologous protein to the Golgi and to postsynaptic densities, where full-length LIN-10 protein resides. Second, we used a *lin-10* mutant *C. elegans* strain to assay the activities of versions of LIN-10 harboring mutations in the PTB and PDZ domains. We found that the PTB and PDZ domains are required for the neuronal function of LIN-10, but only the PTB domain is required for the epithelial function of LIN-10. In addition, a single point mutation in the amino terminus of LIN-10, P27S, abolishes epithelial function but not neuronal function. Finally, we assayed Mint1 and Mint2 (two mammalian homologues of LIN-10) and chimeric versions of these proteins for their ability to rescue the GLR-1 localization defects in a *lin-10* mutant *C. elegans* strain. We found that Mint2 and a Mint2/Mint1 chimeric protein could substitute for LIN-10 function, whereas Mint1 and a Mint1/Mint2 chimeric protein could not substitute for LIN-10 function. Based upon these results, we propose that, in *C. elegans*, LIN-10 uses distinct domains to direct the localization of AMPARs in neurons and to direct the localization of EGFRs in epithelial cells (Figure 6A). Furthermore, we posit that Mint1 and Mint2 evolved to mediate distinct functions in vertebrate neurons through their divergent amino-terminal domains (Figure 6B).

It has previously been shown that, in addition to LIN-10, two other PDZ domain-containing proteins, LIN-2 and LIN-7, are required to direct the basolateral localization of EGFRs (Kaech *et al.*, 1998; Whitfield *et al.*, 1999). In vitro binding studies demonstrated that LIN-2, LIN-7, and LIN-10 form a tripartite complex wherein LIN-10 interacts with LIN-2 via the CID domain (Kaech *et al.*, 1998; Figure 6A). Currently, it is thought that this tripartite complex resides at the basolateral membrane of epithelial cells to mediate the

correct localization of EGFRs. Our work shows that the PTB domain of LIN-10 is required for vulval induction (Figure 4J), suggesting that one or more additional proteins contribute to the function of the LIN-2/LIN-7/LIN-10 complex. Perhaps the PTB domain mediates interactions with protein(s) required for the downstream signaling of EGFRs in vulval epithelial cells, because this domain is not required for localization of LIN-10 or for formation of the LIN-2/LIN-7/LIN-10 complex.

The LIN-10 protein is present in the Golgi and at the plasma membrane in both epithelial cells and neurons (Whitfield *et al.*, 1999; Figure 1). We found that an amino-terminal fragment of LIN-10, LIN-10(1–574), can colocalize with GLR-1 at the Golgi and at synapses. Presumably LIN-10(1–574) colocalizes with GLR-1 either because amino acids 1–574 are directing this localization or because amino acids 1–574 are multimerizing with the endogenous wild-type LIN-10. We favor the former possibility because in a *lin-10* null mutant background, LIN-10(1–574) forms clusters that are similar both in size and number to those in a wild-type background. Nevertheless, we cannot exclude the formal possibility that LIN-10(1–574) can cluster in a *lin-10* mutant but that these clusters are not colocalized with GLR-1, because *lin-10* mutants fail to localize GLR-1 and thus preclude any examination of colocalization with LIN-10(1–574) in these mutants.

We did not identify a region of the LIN-10 protein that localized only to the Golgi or only at the plasma membrane, but we did observe partial localization activity mediated by amino acids 1–381. Whereas full-length LIN-10 and amino acids 1–574 localized to the Golgi and to a similar number of synaptic clusters, amino acids 1–381 localized to the Golgi and to significantly fewer synaptic clusters (Figure 2). Because the sizes and fluorescent intensities of all synaptic clusters were comparable (our unpublished data), amino acids 1–381 seem to be as efficient as full-length LIN-10 at localizing to a subset of synaptic clusters. Therefore, amino acid sequences in LIN-10 may direct protein localization to specific synaptic sites. Interestingly, the CID domain is present in amino acids 1–574, but absent from amino acids 1–381. Thus, because LIN-2 and LIN-7 are not required for the neuronal function of LIN-10, the CID domain may interact with as yet unidentified neuronal proteins that play a role in directing synaptic localization of LIN-10.

Consistent with the idea that an amino-terminal region of LIN-10 directs localization, we found that mutations in the PTB and PDZ domains did not affect protein localization. By contrast, a recent report characterizing the functions of Mint1 and Mint2 in cultured mammalian neurons suggested that the PDZ domains of Mint2 are necessary, but not sufficient, for protein localization (Stricker and Haganir, 2003). One difference between this study and ours is that our experiments were performed in the absence of endogenous LIN-10 by using a *lin-10* null strain of *C. elegans*, whereas Stricker and Haganir (2003) used cultured neurons that express endogenous Mint1 and Mint2 protein.

Mint1 and Mint2 are the vertebrate homologues of LIN-10 that are expressed exclusively in brain (Okamoto and Sudhof, 1997). Like LIN-10, both Mint1 and Mint2 localize to the Golgi and to synaptic clusters (Okamoto *et al.*, 2000; Stricker and Haganir, 2003). The Mint proteins and LIN-10 share highly similar PTB and PDZ domains (Figure 5A), but they have divergent amino-terminal regions. Most notably, Mint1, but not Mint2, has a CID through which it can interact with CASK, the vertebrate homolog of LIN-2 (Borg *et al.*, 1998). Interestingly, we found that Mint2, but not Mint1, rescued AMPAR mislocalization in a *lin-10* mutant strain of

C. elegans. We also tested whether Mint1 and Mint2 could rescue the vulval defect in *lin-10* mutants, but we could not see significant rescue with either clone (our unpublished data). This result was not surprising because Mint1 has the CID but lacks P27, whereas Mint2 has P27 but lacks the CID, and both P27 and the CID are required in LIN-10 for vulval induction.

Currently, the functions of Mint1 and Mint2 remain unclear. However, based upon their subcellular localization and their ability to interact with multiple proteins (for example, Munc-18, KIF-17, presenilin-1, β APP, neurexins, glutamate receptors, and activated Arfs) it is plausible to suggest a role for these proteins in general secretory trafficking (Okamoto and Sudhof, 1997; McLoughlin *et al.*, 1999; Biederer and Sudhof, 2000; Lau *et al.*, 2000; Setou *et al.*, 2000; Hill *et al.*, 2003; Stricker and Haganir, 2003). We conclude that the divergent amino-terminal region of Mint2 is required to direct GLR-1 localization, because a M2/M1 chimeric protein could substitute for LIN-10, whereas a M1/M2 chimeric protein could not. Thus, Mint1 and Mint2 are likely to perform distinct functions in neurons, and specificity of function seems to be mediated by the amino-terminal domains of these proteins. Perhaps the Mint proteins derive specificity for particular cargo molecules from their divergent amino-terminal regions.

How does LIN-10 regulate GLR-1 translocation in neurons? Because LIN-10 colocalizes with GLR-1 at the Golgi via its amino-terminal domain, one possibility is that LIN-10 acts to regulate GLR-1 trafficking as it passes through the Golgi. Alternatively, LIN-10 might act at synapses to anchor the GLR-1 protein. Based upon *in vitro* binding studies using only the PDZ domains of Mint2 and a portion of the carboxyl-terminal tail of GluR1, it has recently been suggested that there is a direct interaction between AMPARs and Mint proteins (Stricker and Haganir, 2003). However, we previously reported an inability to detect direct binding between LIN-10 and GLR-1 by GST affinity chromatography and coimmunoprecipitation (Rongo *et al.*, 1998). Moreover, our results showing that multiple domains of LIN-10 are required for neuronal function suggest that LIN-10 is likely to work together with at least one other protein to direct localization of AMPARs. Identifying proteins that specifically interact with the domains of LIN-10 and Mint2 that are required for GLR-1 localization should provide further insight into the mechanism of LIN-10 function in neurons.

ACKNOWLEDGMENTS

We thank A. Fire, A. Fraser (Sanger Institute, Cambridge, United Kingdom), R. Herman (*Caenorhabditis* Genetics Center, University of Minnesota), J. Kaplan, Y. Kohara, B. Margolis, V. Maricq, J. Mendel, C. Mello, M. Nonet, T. Rapoport, M. Rolls, T. Stiernagle (*Caenorhabditis* Genetics Center, University of Minnesota), and R. Tsien for reagents and strains. We thank Bonnie Firestein and members of the Rongo laboratory for critically reading the manuscript. C. R. is a Pew Scholar in the Biomedical Sciences. Additional funding was provided by the National Institutes of Health grant R01-NS42023 and a grant-in-aid from the American Heart Association.

REFERENCES

- Biederer, T., Cao, X., Sudhof, T. C., and Liu, X. (2002). Regulation of APP-dependent transcription complexes by Mint/X11s: differential functions of Mint isoforms. *J. Neurosci.* 22, 7340–7351.
- Biederer, T., and Sudhof, T. C. (2000). Mints as adaptors. Direct binding to neurexins and recruitment of munc18. *J. Biol. Chem.* 275, 39803–39806.
- Bolton, M. M., Blanpied, T. A., and Ehlers, M. D. (2000). Localization and stabilization of ionotropic glutamate receptors at synapses. *Cell Mol. Life Sci.* 57, 1517–1525.

- Borg, J. P., Ooi, J., Levy, E., and Margolis, B. (1996). The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. *Mol. Cell. Biol.* 16, 6229–6241.
- Borg, J. P., Straight, S. W., Kaech, S. M., de Taddeo-Borg, M., Kroon, D. E., Karnak, D., Turner, R. S., Kim, S. K., and Margolis, B. (1998). Identification of an evolutionarily conserved heterotrimeric protein complex involved in protein targeting. *J. Biol. Chem.* 273, 31633–31636.
- 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.
- Campbell, R. E., Tour, O., Palmer, A. E., Steinbach, P. A., Baird, G. S., Zacharias, D. A., and Tsien, R. Y. (2002). A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA* 99, 7877–7882.
- Dingledine, R., Borges, K., Bowie, D., and Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* 51, 7–61.
- Doyle, D. A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996). Crystal structure of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell* 85, 1067–1076.
- Garner, C. C., Nash, J., and Haganir, R. L. (2000). PDZ domains in synapse assembly and signalling. *Trends Cell Biol.* 10, 274–280.
- Gomes, A. R., Correia, S. S., Carvalho, A. L., and Duarte, C. B. (2003). Regulation of AMPA receptor activity, synaptic targeting and recycling: role in synaptic plasticity. *Neurochem. Res.* 28, 1459–1473.
- Hata, Y., Butz, S., and Sudhof, T. C. (1996). CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neuroligins. *J. Neurosci.* 16, 2488–2494.
- Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C., and Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 287, 2262–2267.
- Herman, R. K., and Hedgecock, E. M. (1990). Limitation of the size of the vulval primordium of *Caenorhabditis elegans* by *lin-15* expression in surrounding hypodermis. *Nature* 348, 169–171.
- Hill, K., Li, Y., Bennett, M., McKay, M., Zhu, X., Shern, J., Torre, E., Lah, J. J., Levey, A. I., and Kahn, R. A. (2003). Munc18 interacting proteins: ADP-ribosylation factor-dependent coat proteins that regulate the traffic of beta-Alzheimer's precursor protein. *J. Biol. Chem.* 278, 36032–36040.
- Hollmann, M., and Heinemann, S. (1994). Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17, 31–108.
- Hoskins, R., Hajnal, A. F., Harp, S. A., and Kim, S. K. (1996). The *C. elegans* vulval induction gene *lin-2* encodes a member of the MAGUK family of cell junction proteins. *Development* 122, 97–111.
- Hsueh, Y. P., Yang, F. C., Kharazia, V., Naisbitt, S., Cohen, A. R., Weinberg, R. J., and Sheng, M. (1998). Direct interaction of CASK/LIN-2 and syndecan heparan sulfate proteoglycan and their overlapping distribution in neuronal synapses. *J. Cell Biol.* 142, 139–151.
- Kaech, S. M., Whitfield, C. W., and Kim, S. K. (1998). The LIN-2/LIN-7/LIN-10 complex mediates basolateral membrane localization of the *C. elegans* EGF receptor LET-23 in vulval epithelial cells. *Cell* 94, 761–771.
- Kim, S. K., and Horvitz, H. R. (1990). The *Caenorhabditis elegans* gene *lin-10* is broadly expressed while required specifically for the determination of vulval cell fates. *Genes Dev.* 4, 357–371.
- Lau, K. F., McLoughlin, D. M., Standen, C., and Miller, C. C. (2000). X11 alpha and x11 beta interact with presenilin-1 via their PDZ domains. *Mol. Cell Neurosci.* 16, 557–565.
- Malinow, R., Mainen, Z. F., and Hayashi, Y. (2000). LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.* 10, 352–357.
- Margolis, B. (1999). The PTB domain: the name doesn't say it all. *Trends Endocrinol. Metab.* 10, 262–267.
- McLoughlin, D. M., Irving, N. G., Brownlee, J., Brion, J. P., Leroy, K., and Miller, C. C. (1999). Mint2/X11-like colocalizes with the Alzheimer's disease amyloid precursor protein and is associated with neuritic plaques in Alzheimer's disease. *Eur. J. Neurosci.* 11, 1988–1994.
- Morais Cabral, J. H., Petosa, C., Sutcliffe, M. J., Raza, S., Byron, O., Poy, F., Marfatia, S. M., Chishti, A. H., and Liddington, R. C. (1996). Crystal structure of a PDZ domain. *Nature* 382, 649–652.
- Nonet, M. L., Holgado, A. M., Brewer, F., Serpe, C. J., Norbeck, B. A., Holleran, J., Wei, L., Hartwig, E., Jorgensen, E. M., and Alfonso, A. (1999). UNC-11, a *Caenorhabditis elegans* AP180 homologue, regulates the size and protein composition of synaptic vesicles. *Mol. Biol. Cell* 10, 2343–2360.
- Okamoto, M., Matsuyama, T., and Sugita, M. (2000). Ultrastructural localization of mint1 at synapses in mouse hippocampus. *Eur. J. Neurosci.* 12, 3067–3072.
- Okamoto, M., and Sudhof, T. C. (1997). Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. *J. Biol. Chem.* 272, 31459–31464.
- Passafium, M., Piech, V., and Sheng, M. (2001). Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat. Neurosci.* 4, 917–926.
- Rolls, M. M., Hall, D. H., Victor, M., Stelzer, E. H., and Rapoport, T. A. (2002). Targeting of rough endoplasmic reticulum membrane proteins and ribosomes in invertebrate neurons. *Mol. Biol. Cell* 13, 1778–1791.
- Rongo, C. (2001). Disparate cell types use a shared complex of PD2 proteins for polarized protein localization. *Cytokine Growth Factor Rev.* 12, 349–359.
- Rongo, C., and Kaplan, J. K. (1999). CaMKII 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.
- Setou, M., Nakagawa, T., Seog, D. H., and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* 288, 1796–1802.
- 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.
- 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.
- Simske, J. S., Kaech, S. M., Harp, S. A., and Kim, S. K. (1996). LET-23 receptor localization by the cell junction protein LIN-7 during *C. elegans* vulval induction. *Cell* 85, 195–204.
- Stricker, N. L., and Haganir, R. L. (2003). The PDZ domains of mLin-10 regulate its trans-Golgi network targeting and the surface expression of AMPA receptors. *Neuropharmacology* 45, 837–848.
- Whitfield, C. W., Benard, C., Barnes, T., Hekimi, S., and Kim, S. K. (1999). Basolateral localization of the *Caenorhabditis elegans* EGF 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, NY: Cold Spring Harbor Laboratory.
- Yandell, M. D., Edgar, L. G., and Wood, W. B. (1994). Trimethylpsoralen induces small deletion mutations in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 91, 1381–1385.