

LIN-10 Is a Shared Component of the Polarized Protein Localization Pathways in Neurons and Epithelia

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Summary

We tested the model that neurons and epithelial cells use a shared mechanism for polarized protein sorting by comparing the pathways for localizing basolateral and postsynaptic proteins in *C. elegans*. GLR-1 glutamate receptors are localized to postsynaptic elements of central synapses and, when ectopically expressed, to basolateral membranes of epithelial cells. Proper localization of GLR-1 in both neurons and epithelia requires the PDZ protein LIN-10, defining LIN-10 as a shared component of the basolateral and postsynaptic localization pathways. Changing the GLR-1 carboxy-terminal sequence from a group I PDZ-binding consensus (-TAV) to a group II consensus (-FYV) restores GLR-1 synaptic localization in *lin-10* mutants. Thus, these interneurons utilize at least two separate postsynaptic localization pathways.

Introduction

Both epithelial cells and neurons are structurally polarized. Epithelial cell surfaces are divided into apical and basolateral domains, whereas neurons extend axons and dendrites. The similar organization of epithelial and neuronal cell membranes into two functional domains led to the hypothesis that the trafficking machinery mediating the polarized protein localization in neurons and epithelia would be shared (Dotti and Simons, 1990). Consistent with this hypothesis, glycoproteins that are targeted to basolateral membranes of epithelial cells are localized to dendrites when ectopically expressed in neurons (Dotti and Simons, 1990; Cameron et al., 1991; Jareb and Banker, 1998). Furthermore, the same *cis*-acting sequences in epithelial proteins are required for both basolateral and dendritic targeting (Jareb and Banker, 1998). Since basolateral epithelial proteins are recognized as substrates for dendritic localization in neurons, these results suggest that a shared sorting mechanism is employed in these two cell types.

Members of the PDZ domain protein family are found at both epithelial and neuronal cell junctions (Kim, 1997; Sheng and Wyszynski, 1997). The PDZ domain is a protein-protein interaction module that binds to specific carboxy-terminal sequences of substrate proteins (Doyle et al., 1996; Songyang et al., 1997), and PDZ proteins

are proposed to act as molecular scaffolds that cluster signaling molecules at epithelial and neuronal cell junctions. Direct proof that PDZ proteins are required to localize junctional proteins has been obtained in three cases. Mutant flies lacking Discs-large (DLG) fail to localize Shaker potassium channels and Fas II adhesion molecules to larval neuromuscular junctions (Tejedor et al., 1997; Thomas et al., 1997; Zito et al., 1997). Mutations in the *C. elegans* genes *lin-2*, *lin-7*, and *lin-10* cause the normally basolateral LET-23 EGFRs to become apically localized in epithelial cells (Simske et al., 1996; Whitfield et al., 1998). Finally, InaD has been shown to localize components of the IP3 signal transduction machinery to rhabdomeres of fly photoreceptors (Shieh and Zhu, 1996; Chevesich et al., 1997; Tsunoda et al., 1997).

Since the *C. elegans* PDZ proteins LIN-2, LIN-7, and LIN-10 define a basolateral localization pathway for epithelial proteins, we tested the hypothesis that this same pathway is utilized in neurons to localize postsynaptic proteins. The synapses that we chose to study are glutamatergic synapses between a sensory neuron ASH and its interneuron targets. GLR-1 receptors are expressed in synaptic targets of ASH and are required for ASH-mediated touch sensitivity (Hart et al., 1995; Maricq et al., 1995).

We show here that LIN-10 is required for localizing GLR-1 to the ASH-to-interneuron synapses. *lin-10* encodes an ortholog of the mammalian PDZ protein X11/Mint (Whitfield et al., 1998). X11/Mint is abundantly expressed in the brain, but its physiological function is not known (Duclos et al., 1993; Borg et al., 1996; Okamoto and Südhof, 1997). Our results identify LIN-10 as a shared component of the polarized protein-sorting pathways in epithelia and neurons and provide direct evidence that PDZ proteins are required for localization of a neurotransmitter receptor to central synapses in vivo.

Results

A GLR-1::GFP Chimera Is Localized to Postsynaptic Specializations

To visualize postsynaptic specializations, we constructed a *glr-1::gfp* fusion gene, in which the GFP coding sequences were inserted into sequences encoding the cytoplasmic tail of GLR-1. We expressed GLR-1::GFP in the interneurons (using the *glr-1* promoter) and examined the subcellular localization of these chimeric receptors. In wild-type adults, GLR-1::GFP was localized in punctate structures in the nerve ring and the ventral nerve cord (Figure 1A).

Several results suggest that these punctate structures are postsynaptic specializations. Chimeric GLR-1::GFP receptors retain glutamate receptor (GluR) function in vivo, as they restore nose touch responsiveness to *glr-1* mutants (Table 1). The puncta were approximately 200–500 nm in diameter, which corresponds to the size of synaptic elements in serial section electron micrographs (White et al., 1986). The punctate structures were seen where we would expect to find synapses (i.e., the ventral

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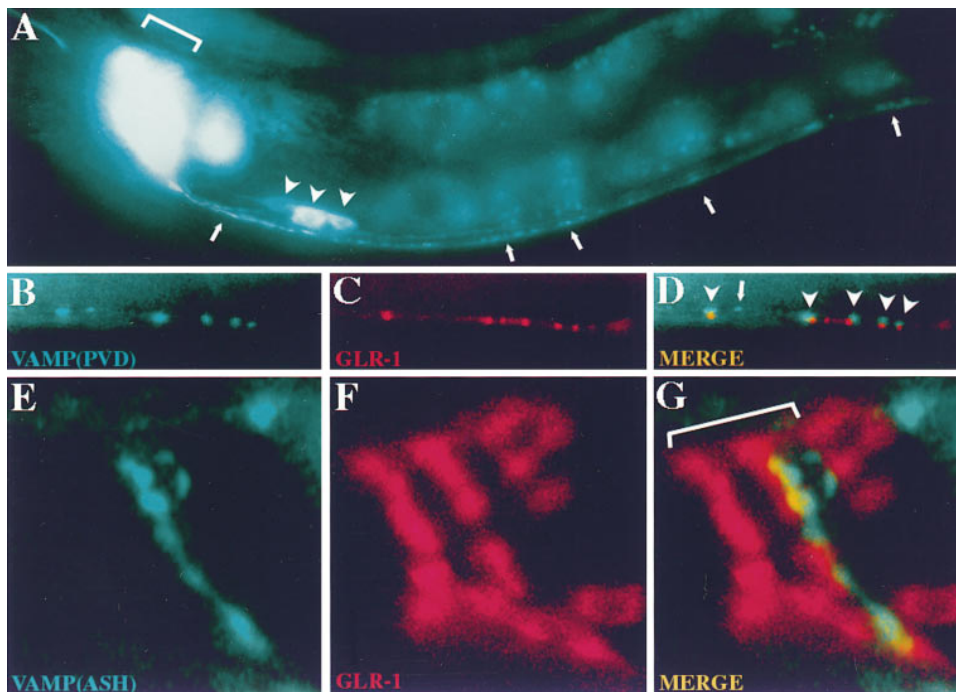


Figure 1. GLR-1 Is Localized to Postsynaptic Elements in Neurons

(A) GLR-1::GFP is expressed in the interneuron cell bodies (bracketed, arrowheads point to RIG and AVG) and is localized in a punctate pattern in the nerve ring (overexposed, bracketed) and the ventral cord (arrows). (B) VAMP::CFP expressed in PVD (utilizing the *mec-3* promoter) and (C) GLR-1::YFP expressed in the interneurons localize to adjacent puncta (arrowheads) in the ventral cord ([D], merged, yellow indicates overlap). The arrow points to a VAMP::CFP whose partner is out of the plane of focus. (E) VAMP::CFP expressed in ASH (utilizing the *asm-10* promoter) and (F) GLR-1::YFP expressed in the interneurons colocalize in the nerve ring ([G], merged, ring in brackets). All animals are shown anterior left, dorsal up.

nerve cord and nerve ring). We found 320 ± 8 ($n = 10$ animals) GLR-1::GFP puncta in young adult ventral nerve cords, and there are approximately 500 predicted synaptic inputs to the GLR-1-expressing cells (White et al., 1986). Since these interneurons are likely to express multiple neurotransmitter receptors (Hart et al., 1995; Maricq et al., 1995), it is not surprising that GLR-1 was not found at all predicted postsynaptic elements in these neurons.

Finally, if they correspond to postsynaptic elements, we would expect that GLR-1::GFP puncta would be closely apposed to presynaptic neurotransmitter release sites, which contain the synaptic vesicle-associated protein VAMP. We specifically labeled pre- and postsynaptic elements at GLR-1-containing synapses with variant GFP proteins—cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). We predicted that GLR-1::GFP would be found apposed to presynaptic specializations of the mechanosensory neurons PVD and ASH because GLR-1 is required for sensory responses mediated by these neurons (Hart et al., 1995; Maricq et al., 1995). To test this prediction, we expressed VAMP::CFP in ASH and PVD, and we expressed GLR-1::YFP in their synaptic targets, the normal site of GLR-1 expression. VAMP::CFP was localized to ventral cord puncta of the PVD sensory neuron when expressed by the *mec-3* promoter (Figures 1B–1D), whereas VAMP::CFP was localized to nerve ring puncta of the ASH sensory neuron when expressed by the *asm-10* promoter (Figures 1E–

1G). In both cases, GLR-1::YFP puncta were found closely apposed to VAMP::CFP puncta. These results demonstrate that GLR-1::GFP puncta correspond to postsynaptic elements at sensory neuron-to-interneuron synapses.

Synaptic Vesicles Are Segregated Away from GLR-1 in Interneuron Neurites

Most worm neurons have relatively simple morphologies, typically containing one or two unbranched neurites, and most worm neurites have both pre- and postsynaptic specializations and hence are neither strictly dendritic nor axonal (White et al., 1986). In this respect, worm neurons are similar to vertebrate neurons that form dendrodendritic and dendroaxonal synapses, e.g., olfactory mitral cells (Isaacson and Strowbridge, 1998). We coexpressed VAMP::CFP and GLR-1::YFP in the interneurons and found that the two chimeric proteins were localized to discrete punctate structures in the ventral nerve cord (Figures 2A–2C), consistent with the hypothesis that pre- and postsynaptic markers are segregated away from each other in neurites. We also examined these markers in animals that lack UNC-104 kinesin. As previously reported (Hall and Hedgecock, 1991; Nonet et al., 1993), VAMP::GFP was restricted to the cell bodies of GLR-1-expressing interneurons in *unc-104* mutants (Figure 2D). By contrast, GLR-1::GFP localization in *unc-104* mutants was identical to that of wild-type worms, suggesting that UNC-104 kinesin is not

Table 1. Analysis of Sensory Behaviors

Nose Touch			
Genotype	Transgene	Expression Pattern	% Responding (No. Animals)
Wild type			81 ± 06 (11)
Wild type	<i>glr-1::gfp-TAV</i>	interneurons	70 ± 06 (05)
<i>glr-1(n2461)</i>			15 ± 03 (15)
<i>glr-1(n2461)</i>	<i>glr-1::gfp-TAV</i>	interneurons	67 ± 12 (10)
<i>glr-1(n2461)</i>	<i>glr-1::gfp-FYV</i>	interneurons	54 ± 04 (40)
<i>lin-10(n1390)</i>			5 ± 02 (15)
<i>lin-10(n1853)</i>			5 ± 05 (19)
<i>lin-10(n1508)</i>			12 ± 03 (33)
<i>lin-10(n1508)</i>	<i>osm-10::lin-10</i>	ASH	9 ± 10 (60)
<i>lin-10(n1508)</i>	<i>glr-1::lin-10</i>	interneurons	69 ± 05 (34)
<i>lin-10(n1508)</i>	<i>glr-1::lin-10::GFP</i>	interneurons	43 ± 04 (45)
<i>lin-10(n1508)</i>	<i>glr-1::gfp-FYV</i>	interneurons	12 ± 02 (44)
Osmotic Avoidance			
Genotype			% Escape (No. Trials)
Wild type			2 ± 01 (4)
<i>eat-4(n2474)</i>			54 ± 06 (5)
<i>glr-1(n2461)</i>			5 ± 02 (3)
<i>lin-10(n1508)</i>			9 ± 02 (4)
<i>lin-10(n1390)</i>			3 ± 02 (3)
Volatile Avoidance			
Genotype			Seconds (No. Animals)
Wild type			6.3 ± 0.8 (20)
<i>eat-4(n2474)</i>			47.7 ± 4.2 (20)
<i>lin-10(n1508)</i>			5.2 ± 0.6 (20)
<i>lin-10(n1390)</i>			4.6 ± 0.5 (20)

ASH sensory responses were assayed as previously described (Hart et al., 1995; Troemel et al., 1995). Errors represent SEM. *eat-4* is shown as an example of a mutant defective osmotic avoidance and volatile avoidance. For nose touch assays, each animal was tested at least ten times. For osmotic avoidance, each trial had at least 50 animals. And for volatile avoidance, each animal was tested at least three times.

required for GLR-1 transport and localization (Figure 2E). Thus, separate trafficking mechanisms are utilized to localize synaptic vesicles and GLR-1 in these neurites.

GLR-1 Is Localized to Basolateral Membranes in Epithelial Cells

Several groups have shown that basolaterally localized epithelial glycoproteins are localized to dendrites when ectopically expressed in neurons (Dotti and Simons, 1990; Cameron et al., 1991; Jareb and Banker, 1998). We

tested the converse hypothesis that the postsynaptic protein GLR-1::GFP would be localized to basolateral membranes when expressed in epithelial cells. GLR-1::GFP was ectopically expressed in the vulval precursor cells and was found to be localized to basolateral membranes (Figure 3A). Thus, GLR-1 functions as a substrate for polarized trafficking in epithelial cells.

The PDZ proteins LIN-2, LIN-7, and LIN-10 are required for localizing LET-23 EGFR to basolateral membranes of vulval precursor cells (Hoskins et al., 1996;

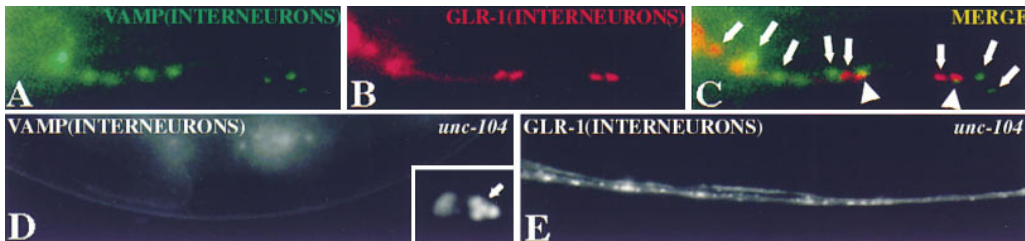


Figure 2. GLR-1 and VAMP Are Segregated Away from Each Other in Neurites

(A) VAMP::CFP and (B) GLR-1::YFP coexpressed in interneurons localize to distinct puncta (arrows) in the ventral cord ([C], merged, n = 16 animals examined). Arrowheads indicate adjacent VAMP::CFP and GLR-1::YFP elements likely to be interneuron:interneuron synapses (White et al., 1986). (D) In *unc-104(e1265)* mutants, VAMP::GFP is not found in ventral cord axons, and VAMP::GFP-containing vesicles (arrow) are trapped in the neuron cell body (inset); n = 40 animals examined. (E) GLR-1::GFP localization in *unc-104(e1265)* is similar to that of wild-type worms; n = 19 animals examined.

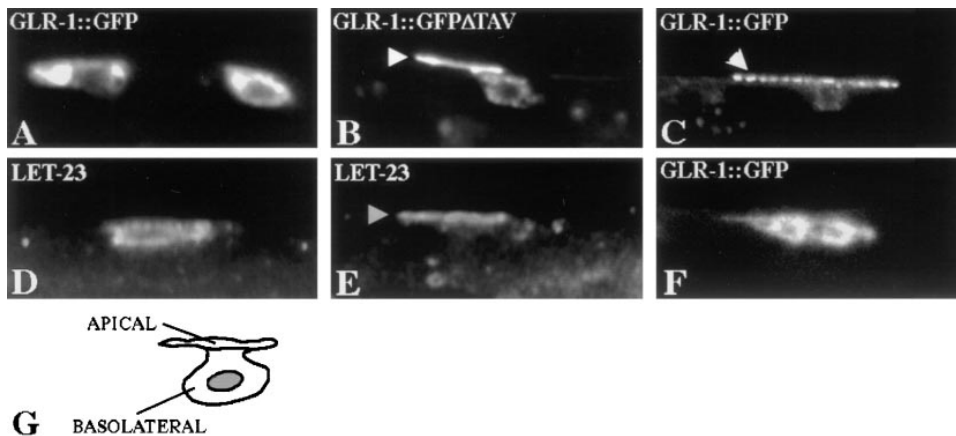


Figure 3. GLR-1 Is Localized to Basolateral Membranes in Epithelial Cells

GLR-1::GFP and GLR-1::GFP Δ TAV (lacking last 16 amino acids) expressed in the vulval precursor cells using the *lin-31* promoter. (A) In 83% of animals ($n = 88$), GLR-1::GFP shows basolateral localization, whereas (B) in 90% of animals ($n = 73$), GLR-1::GFP Δ TAV shows primarily apical staining (arrowhead). (C) In 78% of *lin-10*($n1508$) mutants ($n = 174$), GLR-1::GFP shows primarily apical localization (arrowhead). (D) 100% ($n = 22$) of wild-type worms localize LET-23 to the basolateral surface of vulval precursor cells, whereas 100% ($n = 16$) of worms that express GLR-1::GFP in their vulval precursors localize (E) LET-23 to apical (arrowhead) and (F) GLR-1::GFP to basolateral membranes. A schematic illustration of the vulva precursor cells is shown in (G).

Simske et al., 1996; Whitfield et al., submitted). We found that these PDZ proteins were also required for basolateral localization of GLR-1. GLR-1::GFP expressed in *lin-2*, *lin-7*, or *lin-10* mutants was localized primarily to apical membranes of epithelial cells (Figure 3C and data not shown). Like the LET-23 EGFR, the GLR-1 carboxy-terminal tail contains a type I PDZ-binding consensus sequence (-TAV). To test whether this sequence was required for GLR-1::GFP basolateral localization, we expressed a truncated receptor lacking the final 16 residues (GLR-1::GFP Δ TAV) in the vulval precursor cells and observed that it was localized primarily to the apical surface of vulval precursor cells (Figure 3B), indicating that localization was dependent upon the carboxy-terminal tail. Our results demonstrate that GLR-1::GFP and endogenous epithelial proteins are localized to basolateral membranes by similar pathways.

If both were recognized by a common basolateral localization pathway, then ectopically expressed GLR-1::GFP might interfere with proper localization of endogenously expressed LET-23 receptors. Consistent with this model, animals expressing GLR-1::GFP in the vulval precursor cells often formed an abnormal vulva consisting of fewer cells (9.4 ± 0.5 cells, $n = 18$) than in wild-type animals (22 ± 0 cells, $n = 11$), indicating a defect in LET-23-mediated vulva induction. This vulva defect also correlated with a failure to lay eggs, resulting in a "bags of worms" phenotype in $46\% \pm 2\%$ of animals ($n = 103$), which was not observed in wild type (0%, $n = 38$). Unlike wild-type worms, which localized LET-23 to the basolateral surface of vulval precursors (Figure 3D), transgenic animals expressing GLR-1::GFP in vulva precursor cells mislocalized endogenous LET-23 receptors to the apical surface (Figure 3E), thereby mimicking the *lin-2*, *lin-7*, and *lin-10* mutant phenotypes (Hoskins et al., 1996; Simske et al., 1996; Whitfield et al., submitted). In contrast, animals that express GLR-1::GFP Δ TAV formed functional vulvas with wild-type morphology and cell numbers (21.3 ± 0.5 cells, $n = 27$). Thus, the basolateral localization pathway in epithelial cells recognizes

GLR-1 as a substrate, and GLR-1::GFP and LET-23 EGFR compete for access to this pathway.

LIN-10 Is Required for GLR-1 Localization to Central Synapses

Because GLR-1::GFP functioned as a substrate for basolateral localization, we tested whether LIN-2, LIN-7, and LIN-10 also mediate postsynaptic localization of GLR-1 in neurons. The localization of GLR-1::GFP in *lin-2* and *lin-7* null mutants was indistinguishable from that seen in wild-type animals (Figures 4A–4C). In young (L1 and L2) *lin-10* mutant larvae, the GLR-1::GFP localization pattern was normal (not shown); however, in older *lin-10* larvae and adults, GLR-1::GFP was diffusely distributed throughout the axons at the anterior end of the ventral cord but retained a punctate pattern of localization in the posterior ventral cord (Figure 4D). Although GLR-1::GFP was delocalized in older *lin-10* mutants, there appeared to be relatively abundant amounts of GLR-1::GFP in the interneuron processes. Therefore, LIN-10, but not LIN-2 or LIN-7, plays a role in localizing GLR-1 to postsynaptic specializations in vivo.

To determine if changes in GLR-1::GFP localization impaired the synaptic function of GLR-1 in vivo, we examined *lin-10* mutants for defects in behavior. We found that *lin-10* null mutants were severely defective for the nose touch response (Table 1), similar to *glr-1* mutants. These results are consistent with the idea that either the synaptic localization of GLR-1 or the concentration of GLR-1 at synaptic sites is essential for GLR-1 function in vivo. Alternatively, since diffuse GLR-1::GFP is retained in apparently abundant amounts in the neurites of *lin-10* mutants, it remains possible that the sensory defect reflects a failure to localize other proteins to the ASH-to-interneuron synapses.

LIN-10 Acts in GLR-1-Expressing Interneurons

The synaptic defects observed in *lin-10* mutants could reflect defects in either the presynaptic (i.e., ASH) or

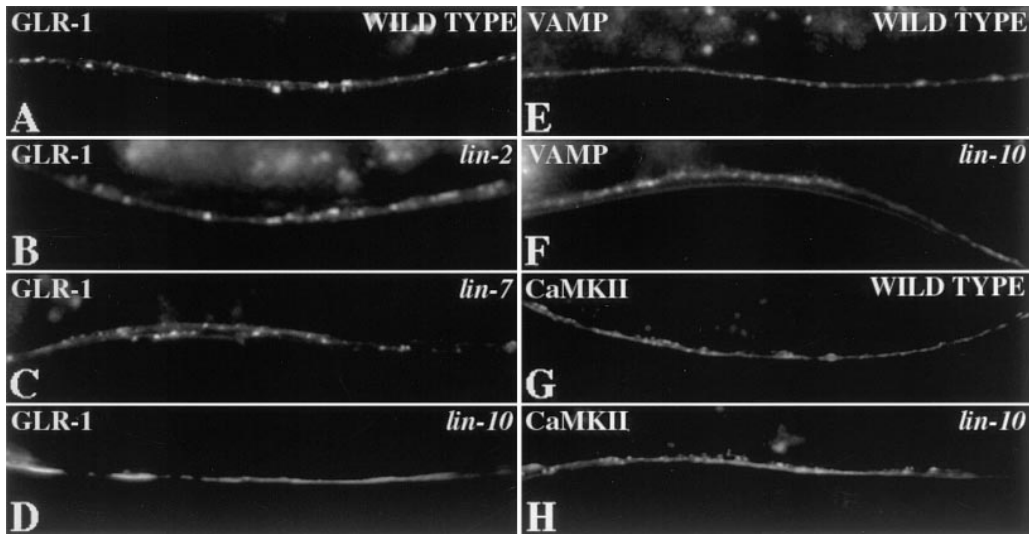


Figure 4. Postsynaptic Localization of GLR-1 Requires LIN-10

(A) GLR-1::GFP is localized in punctate structures along wild-type ventral cord axons (100%, $n = 22$). A wild-type pattern of GLR-1::GFP localization was observed in (B) *lin-2(n397)* ($n = 28$) and (C) *lin-7(n106)* ($n = 42$) mutants. (D) Diffuse GLR-1::GFP localization was observed in the anterior half of the ventral nerve cord in *lin-10(n1508)* mutants (97% were diffuse, $n = 30$). (E) VAMP::GFP expressed in the interneurons also forms punctate structures ($n = 25$), which are presumably synaptic vesicles. (F) VAMP::GFP localization is unaffected in *lin-10(n1508)* mutants ($n = 36$). CaMKII::GFP is localized in the ventral cord of (G) wild-type animals ($n = 24$), and this localization is unaffected in (H) *lin-10(n1508)* mutants ($n = 34$).

postsynaptic (GLR-1-expressing interneurons) cells. Expression of a LIN-10 cDNA in GLR-1-expressing cells was sufficient to restore nose touch response (Table 1) and normal GLR-1::GFP localization to *lin-10* mutants (Figure 5A) but did not correct the vulval defect (data not shown). By contrast, expression of LIN-10 in ASH, utilizing the *osm-10* promoter, restored neither GLR-1 localization nor the nose touch response (Figure 5B and Table 1). These results show that LIN-10 is required in the interneurons for proper localization and function of GLR-1.

We examined the subcellular distribution of LIN-10 by expressing a *lin-10::gfp* fusion gene in these interneurons. Expression of LIN-10::GFP in the interneurons re-

stored nose touch response to *lin-10* mutants, indicating that this fusion protein retains LIN-10 function (Table 1). In the ventral cord, LIN-10::CFP and GLR-1::YFP were colocalized in punctate structures, suggesting that LIN-10 is localized to glutamatergic synapses in vivo (Figures 5C–5E). In cell bodies, LIN-10::CFP and GLR-1::YFP were colocalized in perinuclear structures suggestive of ER/Golgi localization (data not shown).

LIN-10 Is Not Required for Other Aspects of Interneuron Function

What role does LIN-10 play in assembling these glutamatergic synapses? The simplest model is that LIN-10 localizes GLR-1 by directly binding to its carboxy

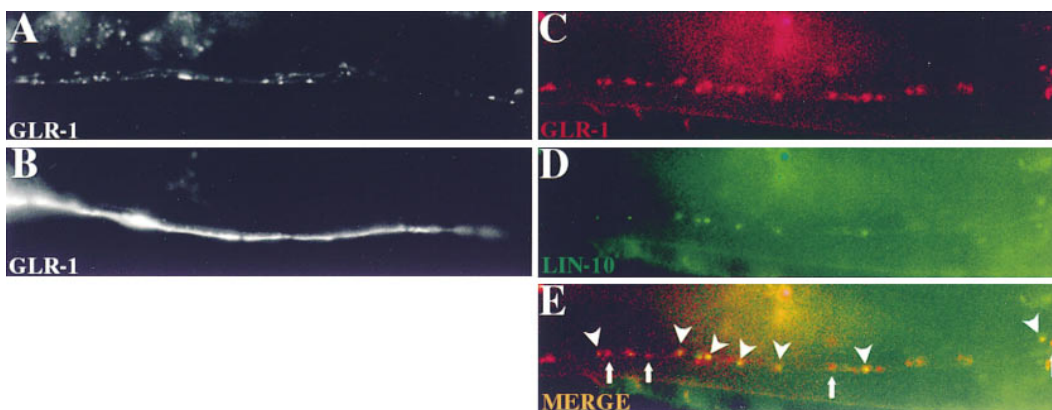


Figure 5. LIN-10 Functions in the Interneurons to Localize GLR-1

(A) Expression of a *lin-10* cDNA in the interneurons of *lin-10(n1508)* mutants restores the punctate localization of GLR-1::GFP (90% were punctate, $n = 50$), whereas (B) expression in the ASH sensory neuron does not (18% were punctate, $n = 38$). (C) GLR-1::YFP and (D) LIN-10::CFP localize to the same punctate structures (arrowheads) in ventral cord axons ([E], merged). Some GLR-1::YFP elements (arrows) do not possess a corresponding LIN-10::CFP element.

terminus. We were unable to detect binding of LIN-10 to GLR-1 in a variety of in vitro assays (data not shown), suggesting either that binding requires the presence of other factors or posttranslational modifications or that LIN-10 plays a less direct role in GLR-1 localization. We also tested whether the carboxy-terminal sequence was required for GLR-1 localization in vivo. Interestingly, GLR-1::GFP Δ TAV, which lack the last 16 amino acids including the group I consensus, are also correctly localized and retain GluR function in vivo (data not shown). The lack of dependence on the carboxy-terminal residues in this case most likely reflects the fact that GLR-1::GFP Δ TAV subunits form heteromultimeric complexes with other endogenously expressed GluRs, which provide functional localization sequences. The worm genome sequence database predicts at least ten other ionotropic GluR genes, which could encode heteromultimeric partners for GLR-1.

One possible explanation for GLR-1 delocalization is that *lin-10* interneurons are incapable of localizing all synaptic proteins. If this were the case, we would expect that other synaptic functions of the GLR-1-expressing interneurons would also be impaired in *lin-10* mutants. We tested the functional integrity of these interneurons in adult animals 36 hr after the onset of the GLR-1 delocalization defect. In addition to the nose touch response, GLR-1-expressing interneurons are required for responses to body touch and for spontaneous locomotion (Chalfie et al., 1985), both of which were normal in *lin-10* adults (data not shown). ASH-to-interneuron synapses mediate aversive responses to three distinct stimuli: nose touch, hyperosmolarity, and volatile repellents (Hart et al., 1995; Maricq et al., 1995; Troemel et al., 1995). Yet, *lin-10* adults are defective for ASH-mediated touch sensitivity but respond normally to the other ASH-mediated stimuli (Table 1). These results demonstrate that most aspects of interneuron function were normal in *lin-10* mutants, including other inputs from the ASH sensory neurons, suggesting that *lin-10* mutants do not have a general defect in localizing synaptic proteins.

Behavioral assays could be a crude measure of the synaptic connections made by these interneurons. For example, the ASH sensory behaviors might effectively assay only a subset of these connections, or these behaviors might be impaired only after the majority of connections are altered. Therefore, despite their selective behavioral defects, *lin-10* mutants might have relatively severe defects in their connectivities. To directly examine the connectivity of these interneurons, we expressed VAMP::GFP in *lin-10* mutants (Figure 4F) and found that the number and morphologies of presynaptic specializations in adult *lin-10* interneurons were indistinguishable from wild type (Figure 4E). We also visualized postsynaptic specializations in the GLR-1-expressing interneurons by expressing a calcium- and calmodulin-dependent protein kinase GFP reporter (CaMKII::GFP) and found that the number and morphologies of postsynaptic specializations in adult *lin-10* interneurons were indistinguishable from wild type (Figures 4G and 4H). These results demonstrate that other synaptic proteins are properly localized in *lin-10* mutants, indicating that LIN-10 plays a relatively specific role in the synaptic localization of GLR-1.

Parallel Pathways Localize Receptors to Synapses

Mutants lacking GLR-1 are defective for the ASH-mediated response to touch but are normally responsive to other ASH stimuli, hyperosmolarity, and volatile repellents (Hart et al., 1995; Maricq et al., 1995). Thus, in addition to GLR-1, ASH-to-interneuron synapses must contain other unidentified postsynaptic receptors mediating responsiveness to hyperosmolarity and volatile repellents. Our present results suggest that LIN-10 is required for localizing GLR-1 to these synapses but that receptors mediating the other ASH responses are localized by LIN-10-independent pathways. For example, receptors mediating the response to hyperosmolarity could be localized to the same synapses via a different PDZ protein pathway. PDZ domains that bind to carboxy-terminal sequences have been divided into two groups (Songyang et al., 1997), each of which recognizes a discrete sequence. The GLR-1 carboxy-terminal sequence -TAV matches the group I consensus (S/T, X, V). To test if other neurotransmitter receptors expressed in the same cell might be localized by a LIN-10-independent mechanism, we replaced the carboxy-terminal -TAV sequence of GLR-1 with -FYV, a consensus sequence predicted to bind to group II PDZ domains (Songyang et al., 1997). GLR-1::GFP-FYV was well expressed (Figure 6C) and restored nose touch responsiveness to *glr-1* mutants (Table 1). GLR-1::CFP-FYV and GLR-1::YFP receptors were colocalized to the same ventral cord punctate structures (Figures 6G–6I). Thus, alteration of the GLR-1 carboxy terminus did not grossly impair GLR-1 function or postsynaptic localization. Interestingly, unlike GLR-1::GFP receptors, GLR-1::GFP-FYV receptors were synaptically localized in *lin-10* mutants (Figure 6; compare panels B and D), demonstrating that a LIN-10-independent localization pathway is operative. However, GLR-1::GFP-FYV receptors failed to restore nose touch responsiveness in *lin-10* mutants (Table 1); therefore, GLR-1::GFP-FYV localization was not sufficient to restore *glr-1* signaling. The LIN-2 protein contains a group II PDZ domain and hence could potentially mediate localization of GLR-1::GFP-FYV. However, GLR-1::GFP-FYV receptors remained localized in both *lin-2* and *lin-10*; *lin-2* double mutants (Figures 6E and 6F). These results demonstrate that multiple localization pathways exist and that these pathways distinguish between different receptors based on their carboxy-terminal sequences. Moreover, these results show that in *lin-10* mutants, other trafficking pathways remain active but that GLR-1::GFP is no longer recognized as a substrate.

Discussion

Prior work led others to propose that the cellular mechanisms mediating polarized targeting of membrane proteins in neurons and epithelial cells would be shared (Dotti and Simons, 1990; Cameron et al., 1991; Jareb and Banker, 1998). We tested this hypothesis in an in vivo system by studying localization of a *C. elegans* AMPA-type GluR in neurons and in epithelial cells. We showed that GLR-1::GFP GluRs are localized to both postsynaptic elements and to basolateral membranes and that the PDZ protein LIN-10 is required for polarized

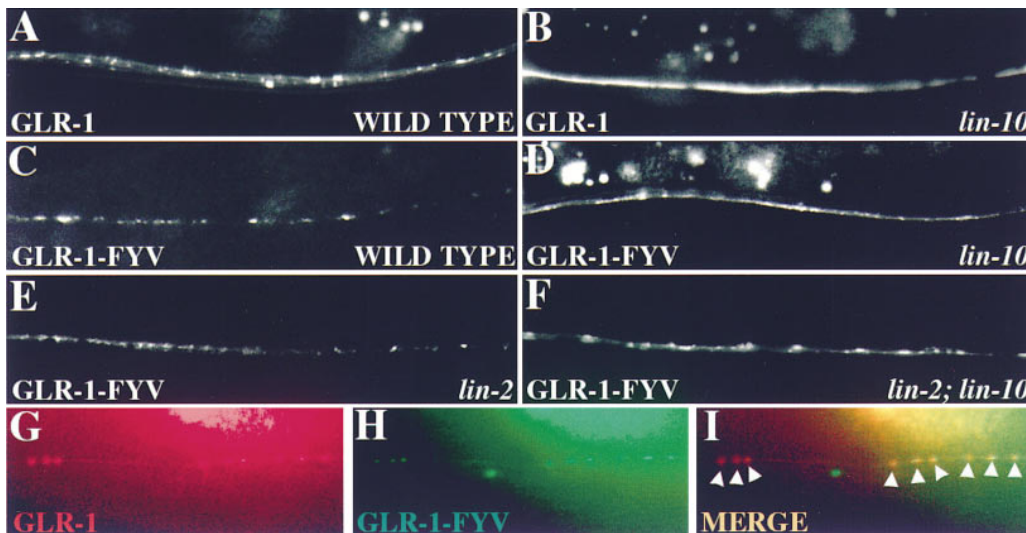


Figure 6. Two Independent Pathways Localize Postsynaptic Protein in Interneurons

(A and B) GLR-1::GFP ending in the group I sequence -TAV is localized in (A) wild-type worms ($n = 32$) but not in (B) *lin-10* mutants (20% were punctate, $n = 20$).

(C–I) The group I tail of GLR-1 was replaced with the group II tail -FYV to generate GLR-1::GFP-FYV. When introduced into either (C) wild-type worms ($n = 68$) or (D) *lin-10* mutants ($n = 26$), GLR-1::GFP-FYV is localized in a punctate pattern similar to (A) GLR-1::GFP. GLR-1::GFP-FYV is also localized in (E) *lin-2* ($n = 18$) and (F) *lin-10; lin-2* double mutants ($n = 37$). (G) GLR-1::YFP and (H) GLR-1::CFP-FYV colocalize in ventral cord axons (I), merged, arrowheads indicate overlap); $n = 39$ animals examined.

localization of GLR-1::GFP in both cell types. Therefore, our results confirm this hypothesis, and they identify LIN-10 as a shared component of the basolateral and postsynaptic pathways.

In addition, we showed that LIN-10 is required for the function of a set of central glutamatergic synapses in vivo. Given that the mammalian LIN-10 orthologs, the X11/Mint proteins, are abundantly expressed in the brain (Duclos et al., 1993; Borg et al., 1996; Okamoto and Südhof, 1997), we speculate that these proteins are also involved in localizing GluRs to synapses in the mammalian brain. Consistent with this possibility, we showed that mammalian X11 can restore proper postsynaptic localization of GLR-1::GFP in *lin-10* mutants (C. R. and J. M. K., unpublished observations), suggesting that the mammalian proteins retain this function. Thus, our results also have broad implications for the mechanisms underlying synaptic localization of neurotransmitter receptors in other systems.

Because we failed to detect LIN-10 binding to GLR-1 in vitro, it is possible that LIN-10 plays an indirect role in receptor targeting. LIN-10 might be required for some general aspect of interneuron development or function, and delocalization of GLR-1 is the indirect consequence of this generalized defect. Alternatively, LIN-10 is part of a protein complex mediating GLR-1 localization, even though it may not bind directly to GLR-1. Our results strongly favor the latter hypothesis. GLR-1 and LIN-10 are colocalized to synapses in vivo, consistent with a direct function of LIN-10 in postsynaptic targeting rather than a more indirect effect involving proteins or processes outside of the synapse. The ectopic expression of GLR-1 in epithelial cells is a test of how directly LIN-10 interacts with GLR-1. If this interaction were massively indirect, it is unlikely that GLR-1 would be recognized by

the LIN-2/7/10 basolateral targeting system in epithelial cells. By contrast, if the LIN-10/GLR-1 interaction is relatively direct, then one would strongly predict that GLR-1 would be recognized as a substrate for basolateral targeting, as we showed. At a minimum, other proteins required for GLR-1 localization must be present in both neurons and epithelial cells. Interneurons lacking LIN-10 correctly localized other synaptic proteins and retained many behavioral functions; hence, *lin-10* mutations do not cause a global defect in synapse formation or interneuron function. Finally, the requirement for LIN-10 in GLR-1 localization was bypassed by altering the carboxy-terminal sequence of GLR-1, suggesting the mutant interneurons fail to localize GLR-1 due to a failure to recognize the GLR-1 carboxy terminus. These results strongly support the model that LIN-10 is part of a protein complex that recognizes GLR-1 and mediates its localization to postsynaptic elements.

Separate Targeting Mechanisms for Synaptic Vesicles and GLR-1

How are pre- and postsynaptic specializations built along the lengths of a single neurite? We showed that pre- and postsynaptic proteins are segregated away from each other in neurites. Mutations in the *unc-104* kinesin gene block transport of synaptic vesicles and their associated proteins to presynaptic terminals of worm interneurons (Hall and Hedgecock, 1991; Nonet et al., 1993) but have no effect on the transport of GLR-1 to postsynaptic terminals in the same neurites. By contrast, *lin-10* mutations prevent localization of GLR-1 to postsynaptic terminals but do not alter trafficking of VAMP to presynaptic terminals of these same cells. These data suggest that separate mechanisms function within a single neurite to localize synaptic vesicles and

GLR-1 receptors to pre- and postsynaptic specializations, respectively.

Comparison of the Basolateral and Postsynaptic Pathways

Although LIN-10 is shared by the basolateral and postsynaptic localization pathways, the defects in epithelial and neuronal localization are subtly different in *lin-10* mutants. In *lin-10* mutant epithelial cells, LET-23 and GLR-1 are localized primarily to the apical rather than the basolateral surface. This result is consistent with prior work in mammalian systems showing that in the absence of basolateral localization signals, many membrane proteins contain cryptic apical localization signals that result in apical localization by default (Keller and Simons, 1997). By contrast, in *lin-10* mutant neurons, GLR-1 is diffusely distributed rather than being localized to pre- or postsynaptic compartments. This difference is consistent with recent results, suggesting that specific signals are necessary for presynaptic localization (Jareb and Banker, 1998).

Another difference between epithelial and neuron localization is that all three PDZ proteins contribute equally to basolateral localization in epithelial cells, whereas only LIN-10 is needed for postsynaptic localization of GLR-1::GFP. One explanation for the latter result is that LIN-2, LIN-7, and LIN-10 function at independent steps in LET-23 basolateral localization and that only one of these steps, the LIN-10-dependent step, is shared with neurons. Consistent with this view, LIN-7 is localized to epithelial cell junctions (Simske et al., 1996), whereas LIN-10 is perinuclear in epithelial cells (Whitfield et al., submitted). Alternatively, LIN-10 may function as part of a complex of PDZ proteins composed of different components in epithelial cells and neurons. LIN-2, LIN-7, LIN-10, and their mammalian orthologs (CASK, Velis, and Mint/X11) form a tripartite complex (Kaeck et al., 1998 [this issue of *Cell*]; Butz et al., 1998 [this issue of *Cell*]). Presumably, GLR-1::GFP is recognized by this complex when it is ectopically expressed in epithelial cells, whereas in neurons, GLR-1::GFP might be recognized by a complex containing LIN-10 with partners that substitute for LIN-2 and LIN-7.

A third notable difference is that the GLR-1 delocalized phenotype in *lin-10* mutants occurs only in older larvae and adults, whereas the LET-23 defect is apparent as soon as expression is detected (Simske et al., 1996). One explanation for the late onset of the postsynaptic localization defect is that initial targeting of GLR-1 to synapses does not require LIN-10 but that LIN-10 is needed to selectively retain GLR-1 at synapses. In this model, *lin-10* mutants would be expected to initially localize GLR-1 early in development but fail to maintain localization as development proceeded. Alternatively, GLR-1-containing synapses formed in young larvae may differ from those formed later, such that only the latter synapses require LIN-10. This might be the case if early and late synapses have different sets of colocalized receptors or signaling molecules.

Other Synaptic Proteins Localized by LIN-10

We showed that LIN-10 plays a relatively specific role in localizing GLR-1 to sensory neuron-to-interneuron

synapses. Nevertheless, two results suggest that LIN-10 is required to localize synaptic proteins other than GLR-1. First, GLR-1::GFP is properly localized in L1 and L2 *lin-10* larvae, yet these animals are not responsive to nose touch. Second, GLR-1::GFP-FYV receptors are synaptically localized in *lin-10* mutants but fail to restore nose touch responsiveness to these mutants. We hypothesize that LIN-10 is needed to localize both GLR-1 and other factors also required for the nose touch response. LIN-10 could interact with multiple synaptic proteins via its two PDZ domains and PTB domain, thereby localizing several synaptic proteins in vivo. Similar models have been proposed for the function of other multivalent PDZ domain proteins, e.g., InaD (Shieh and Zhu, 1996; Chevesich et al., 1997; Tsunoda et al., 1997).

Implications for Synapse Formation and Plasticity

The ASH-to-interneuron synapses mediate the responses to three distinct stimuli and are therefore polymodal. Previous work showed that different postsynaptic receptors are required for the different ASH sensory modalities. We propose that different neurotransmitter receptors are recruited to the ASH-to-interneuron synapses through the action of distinct PDZ proteins. Thus, the touch response is mediated by LIN-10 recruitment of GLR-1 to these synapses, whereas additional (as yet unidentified) synaptic receptors mediate the other ASH-mediated behaviors and are localized by class II PDZ proteins. Independent pathways for localizing specific postsynaptic elements would provide a mechanism to alter the mixture of neurotransmitter receptors at synapses. These postsynaptic localization pathways could be independently regulated to change synaptic structure and function during development or in response to experiences.

Experimental Procedures

Transgenes and Germline Transformation

Transgenic strains were isolated by microinjecting various plasmids (typically at either 50 or 100 ng/ μ l) using either *lin-15(+)* (J. Mendel), *rol-6dm* (C. Mello), or *ttx-3::gfp* (O. Hobert) as a marker.

CFP is GFP with the following amino acid substitutions: Y66W, N146I, M153T, V163A, and N212K (Heim and Tsien, 1996; Ormo et al., 1996). The CFP plasmid was generated by making N212K in PD115.46 (A. Fire). YFP is GFP with the following amino acid substitutions: S65G, V68L, S72A, and T203Y (R. Tsien).

GLR-1 Expression Constructs

KP#196 *glr-1::gfp* contains GFP inserted at a HindIII site (nt 8218) of the *glr-1* plasmid C06XP (V. Maricq), conserving the last 16 codons of *glr-1*. Three independent lines (*nuls23*, *nuls24*, *nuls25*) carrying an integrated version of this transgene showed identical punctate GLR-1::GFP localization. GLR-1::GFP in the text refers to *nuls25*. KP#221 *glr-1::YFP* contains YFP in the HindIII site of the *glr-1* cDNA (V. Maricq). KP#200 and KP#202 contain GLR-1::GFP and GLR-1::GFP Δ TAV, respectively, in the *lin-31* expression vector pB253. The GLR-1::GFP and GLR-1::GFP-FYV minigenes (KP#232 and KP#233, respectively) contain GFP in the HindIII site of the *glr-1* cDNA downstream of the *glr-1* promoter. The carboxy-terminal sequence -TAV was changed to -FYV by PCR.

VAMP Expression Constructs

KP#198 *glr-1::VAMP::GFP* contains VAMP::GFP (M. Nonet) downstream of the *glr-1* promoter. KP#215 *glr-1::VAMP::CFP* was generated by introducing CFP into VAMP by splice-overlap PCR and ligation into pV6. KP#228 *mec-3::VAMP::CFP* contains VAMP::CFP ligated into PD57.56. KP#230 *osm-10::VAMP::CFP* contains VAMP::

CFP downstream of the *osm-10* promoter (A. Hart and J. M. K., unpublished).

LIN-10 Expression Constructs

KP#199 *glr-1::lin-10* contains the *lin-10* cDNA (yk114c6, Y. Kohara) in pV6. KP#201 *glr-1::lin-10::GFP* was generated by ligating yk114c6 into KP#240. KP#205 *osm-10::lin-10* contains yk114c6 behind the *osm-10* promoter. KP#229 *glr-1::lin-10::CFP* was generated by ligating yk114c6 into KP#239.

CaMKII Expression Constructs

KP#234 *glr-1::CaMKII::GFP* contains a CaMKII cDNA (K11E8.1), amplified from the RB1 cDNA library (R. Barstead) ligated into KP#241.

Fluorescence Microscopy

Fixed animals were immunostained with anti-LET-23 (C. Whitfield) antibodies as described (Finney and Ruvkun, 1990). CFP and YFP constructs were visualized using custom filter sets (Chroma): CFP, D414/30X exciter, 505DCXR dichroic, and HQ505LP emitter; YFP, HQ487/15 exciter, 505DCXR dichroic, and HQ505LP emitter. Images were photographed on 35 mm film and digitized with a film scanner. For double labelings, images were pseudocolored and merged using Adobe Photoshop.

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