Molecular Determinants for PICK1 Synaptic Aggregation and mGluR7a Receptor Coclustering

ROLE OF THE PDZ, COILED-COIL, AND ACIDIC DOMAINS*

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PSD-95/Disc-large/ZO-1 (PDZ) domain-containing proteins play a central role in synaptic organization by their involvement in neurotransmitter receptor clustering and signaling complex assembly. The protein interacting with protein kinase C (PICK1), a synaptic PDZ domain protein that also contains a coiled-coil and acidic domain, binds to several synaptic components including the metabotropic glutamate receptor mGluR7a. Coexpression of PICK1 and mGluR7a in heterologous cells induces coclustering of these two proteins. To examine the role of the different structural motifs of PICK1 in synaptic aggregation of PICK1 and mGluR7a coclustering, several PICK1 mutants were generated to analyze their distribution in transfected hippocampal cultured neurons and to test their ability to induce coclusters with mGluR7a when coexpressed in fibroblast cells. The PDZ and coiled-coil domains are both required, whereas the acidic region plays an inhibitory role in these processes. Our data suggest that synaptic aggregation and receptor coclustering depend on PICK1 binding to a target membrane receptor, e.g. mGluR7a, by a PDZ-mediated interaction and on PICK1 oligomerization through the coiled-coil domain. This study defined three structural signals within PICK1 regulating its synaptic localization and receptor coclustering activity, which could represent molecular substructures involved in synaptic development and plasticity.

A characteristic and important feature of central nervous system synapses is the clustering of receptors and signaling molecules at both postsynaptic and presynaptic sites, which underlies an efficient synaptic transmission in the brain. For instance, at glutamate excitatory synapses of the hippocampus, the ionotropic glutamate N-methyl-D-aspartate and a-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors are both concentrated at postsynaptic sites (1), whereas the G protein-coupled metabotropic glutamate receptor mGluR7a is specifically targeted to the presynaptic active zone of nerve terminals (2). Recent studies showed the importance of PDZ domain-containing proteins in mediating the specific synaptic aggregation of neurotransmitter receptors and in regulating receptor signaling at synapses (3–5). Among them, protein interacting with protein kinase C (PICK1) is a PDZ domain protein originally cloned on the basis of its interaction with protein kinase C (PKC) by a yeast two-hybrid screen (6). PICK1 has subsequently been shown to interact with the Eph receptor tyrosine kinases and ephrin-B ligands (7), GluR2/3/4c subunits of the AMPA receptor (8, 9), mGluR7a receptor (10, 11), and dopamine and norepinephrine transporters (12).

Functionally, PICK1 can induce coclustering of the AMPA and mGluR7a receptors in heterologous cells, suggesting a role of PICK1 in the synaptic clustering of these receptors (8–11). Indeed, disruption of the interaction between PICK1 and mGluR7a by deletion of the PICK1 binding domain in mGluR7a abolished the presynaptic clustering of recombinant mGluR7a in hippocampal neurons while retaining its axonal targeting (10). However, PICK1 might not be primarily involved in the synaptic clustering or stabilization of AMPA receptors (13) but rather was hypothesized to be involved in AMPA receptor internalization (14, 15). Blocking interaction between PICK1 and GluR2 by the infusion of competitive peptides in cultured cerebellar Purkinje cells attenuated long term depression induction (14), a process recently shown to depend on internalization events (16–18). However, blocking PICK1-GluR2 interaction had no effect on long term depression in hippocampal neurons (19). PICK1 interaction with the dopamine transporter has been implicated in the proper targeting of the latter in midbrain dopaminergic neurons (12). Thus, PICK1 could have different functions depending on the cerebral region and the specific complement and nature of PICK1 binding partners. In accordance with its biological effects, immunocytochemical data demonstrated that PICK1 is concentrated at excitatory synapses in hippocampal cultured neurons (7, 8, 10). Moreover, the presence of PICK1 immunoreactivity in synaptic vesicle fractions as well as in postsynaptic density fractions (7, 8) strongly suggests that PICK1 is expressed in both presynaptic and postsynaptic domains. PICK1 distribution has been shown to be dynamically regulated upon PKC activation, which triggered mobilization of PICK1 to excitatory synaptic sites (15).

The molecular determinants within PICK1 important for synaptic clustering and receptor coclustering are unknown. Although the three-dimensional structure of PICK1 is not known, its primary structure contains motifs that predict the existence of different structural domains including a PDZ domain, a coiled-coil domain, and an acidic region constituted of a stretch of glutamic and aspartic acid residues. PDZ and coiled-coil domains are typically involved in protein-protein interaction and/or protein dimerization (20–22). Indeed, the

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‡ The abbreviations used are: AMPA, a-amino-3-hydroxy-5-methylisoxazole-4-propionate; PDZ, PSD-95/Disc-large/ZO-1; PICK1, protein interacting with protein kinase C; PKC, protein kinase C; WT, wild-type; DIV, day(s) in vitro; GAD, glutamic acid decarboxylase; TRP, transient receptor potential.
putative carboxylate binding loop in the PICK1 PDZ domain has been shown to bind the C terminus of PKC, GluR2/3, and mGluR7a (8–11, 23). To examine the role of the different structural domains of PICK1 in the mechanisms underlying PICK1 synaptic localization and mGluR7a coclustering, several Myc epitope-tagged mutant PICK1 constructs were generated to analyze their distribution in transfected hippocampal cultured neurons. In addition, their ability to induce coclusters with mGluR7a was tested when coexpressed in fibroblast cells. We show that the three domains correspond to functional signals for PICK1 synaptic localization and mGluR7a coclustering; the PDZ and coiled-coil domains are both required, whereas the acidic region plays an inhibitory role in these processes. Our data suggest that synaptic aggregation and receptor coclustering depend on PICK1 binding to a target membrane receptor, e.g., mGluR7a, by a PDZ-mediated interaction and on PICK1 oligomerization through the coiled-coil domain.

**EXPERIMENTAL PROCEDURES**

cDNA Constructs—All the PICK1 cDNA constructs used for mammalian cell expression described in this study contained a Myc-epitope tag of 12 amino acids, originally inserted into the pRK5 vector from expression plasmid produced N-terminally tagged proteins. PICK1-WT and PICK1 mutated in its PDZ domain (mutation of Lys20 and Asp28 to alanines: PICK1-KD/AA) in pCP86 (8) were subcloned into the SacI/NotI sites of the Myc-pRK5 vector. PICK1 deleted of its coiled-coil domain (PICK1-ΔCC) and of its acidic region (PICK1-ΔDE) were obtained by a two-step polymerase chain reaction strategy using appropriate primers, and the resulting polymerase chain reaction products were inserted into the SacI/Eco47III sites of Eco47III/NotI sites of Myc-pRK5-PICK1-WT for PICK1-CC and PICK1-ΔDE construction, respectively. These mutants were then subcloned into pCP97 and pCP86 (24) for yeast expression. Several other constructs were generated for the yeast two-hybrid assay. Fragments corresponding to each domain of PICK1 were synthesized by polymerase chain reaction amplification to generate the PDZ (encoding amino acids 20–110), PDZ-KD/AA (encoding amino acids 20–110 with Lys20 and Asp28 substituted to alanines), coiled-coil (CC, encoding amino acids 139–166), and acidic (DE, encoding amino acids 380–390) fragments. All of these fragments were inserted into the SacI/NotI sites of pCP97. Construction of GW1-mGluR7a and pCP97-CT7 (encoding the C-terminal tail of mGluR7a) was described previously (10).

**Neuron Culture and Transfection**—Briefly, embryonic day-18 rat hippocampi were dissociated and dissociated by trypsin and trituration. Before plating, neurons were transfected by a lipiod-mediated gene transfer method using the Effectene kit (Qiagen). Approximately 4 × 10^5 cells were incubated with 1 μg of DNA for 2 h at 37°C in the presence of 8 μl of Enhancer and 15 μl of Effectene transfection reagent, plated onto poly-L-lysine-coated coverslips in fresh medium at a density of 14,000 cells/cm². Cells were maintained in serum-free N2.1 medium suspended above a glial feeder layer cultured growing in a medium lacking leucine, tryptophan, and histidine. In addition, some diffuse labeling was often detected, most prominently in large dendrites close to the cell body. Double labeling with the synaptic marker synapsin antibody was used at 1:10,000 (Sigma); the mouse anti-NR1 (clone 9E10) and anti-GAD were used at 1:40 and 1:2 dilutions, respectively (Developmental Studies Hybridoma Bank); the rabbit anti-PICK1 (1:400) and rabbit anti-GluR7 (1:250) antibodies were generous gifts from Dr. R. Huganir (Johns Hopkins University, Baltimore, MD) and Dr. R. Molchinney (Oxford, United Kingdom). The cells were then incubated with appropriate Texas Red- or fluorescein isothiocyanate-conjugated secondary antibodies (Jackson Laboratories), and the coverslips were mounted on glass slides for microscopy analysis. Each coverslip was systematically scanned on a Zeiss Axioskop with an ×25 lens, and images of each transfected cell were acquired with a × 63 1.4 numerical aperture objective using a Photometrics SenSys cooled charge-coupled device camera and Metamorph software. The colocalization of recombinant Myc-PICK1-WT with synapsin, GluR7, and NR1 as well as PICK1-KD/AA, PICK1-ΔCC, and PICK1-ΔDE was quantified using Metamorph software as described previously (26). The fluorescence intensity and size of the enogenous and recombinant Myc-PICK1-ΔDE clusters were quantified on 60 and 150 clusters, respectively, using Metamorph software. For each Myc-PICK1 construct, between 30 and 60 transfected neurons were analyzed from 5 independent experiments. The images for presentation were prepared for printing with Adobe Photoshop.

**RESULTS**

**Recombinant Myc-PICK1 Is Selectively Clustered at Excitatory Synapses in Cultured Hippocampal Neurons**—To determine which domain of PICK1 is required for the synaptic localization and clustering properties, several Myc epitope-tagged wild-type and mutant protein constructs of PICK1 were generated and expressed in hippocampal cultured neurons by a lipid-mediated transfection. Initial experiments were conducted to determine whether the exogenously expressed recombinant wild-type Myc-PICK1 localized appropriately in neurons. Neurons were transfected either the day of plating or at 9 DIV. The distribution of the recombinant Myc-PICK1 was analyzed at 15 DIV by immunofluorescence using Myc monoclonal antibody. Myc-PICK1 expressed by transfection was distributed in clusters along dendrites and, although to a lesser extent, along axons as well (Fig. 1). In addition, some diffuse labeling was often detected, most prominently in large dendrites close to the cell body. Double labeling with the synaptic marker synapsin showed that most of the PICK1-containing clusters in both dendrites and axons were synaptic (Fig. 1). A different distribution was observed in transfected pyramidal cells and interneurons which represent the two types of cells presently in this hippocampal culture model (differentiated based on both morphological features and glutamic acid decarboxylase immunostaining), suggesting no cell type-specific targeting of PICK1. No difference in either Myc-PICK1 distribution or intensity of immunoreactivity was observed between neurons transfected the day of plating or at 9 DIV. To compare...
the PICK1 expression level between transfected and untransfected neurons, quantitative analyses were performed in the same culture after PICK1 staining to label endogenous as well as exogenous PICK1. Although the intensity of PICK1 immunoreactivity was clearly much higher in a few neurons, likely corresponding to the transfected ones, double labeling with the Myc antibody was performed to confirm that these brighter PICK1-expressing neurons corresponded to the Myc-PICK1-transfected neurons. The fluorescence intensity and size of the recombinant Myc-PICK1 clusters determined by PICK1 immunostaining were on average four and six times higher, respectively, than for the endogenous protein.

To determine whether the transfected PICK1 was selectively associated with excitatory synapses, as reported for the endogenous protein, double immunostainings were performed with markers for excitatory and inhibitory synapses (Fig. 2). Double immunolocalization of recombinant Myc-PICK1 and the GluR1 subunit of the AMPA receptor showed a good overlap of the two proteins, although some clusters of Myc-PICK1 lacked concentrations of GluR1 and vice versa. Quantitative analysis indicated that 65% of transfected Myc-PICK1 clusters colocalized with GluR1. Similarly, 54% of recombinant PICK1 clusters were associated with the N-methyl-D-aspartate receptor subunit NR1-positive synapses. By contrast, no colocalization was observed between PICK1-positive clusters and glutamic acid decarboxylase (GAD)-positive synapses, indicating that recombinant PICK1 was specifically targeted to excitatory synapses and excluded from inhibitory ones. These data indicate that even with a high level of protein expression, the recombinant Myc-PICK1 expressed by transfection in hippocampal cultured neurons was specifically concentrated at a subset of excitatory synapses. Therefore, this transfected neuron system could be exploited to elucidate the function of the different structural domains of PICK1 in synaptic clustering properties.

Both the PDZ and Coiled-coil Domains Are Necessary for PICK1 Synaptic Clustering and for mGluR7a Receptor Coclustering—Analysis of the PICK1 amino acid sequence suggests the presence of three distinct structural domains: a PDZ domain (amino acids 20–110), a coiled-coil domain (amino acids 139–166), and an acidic region containing 10 acidic amino acids, either aspartic or glutamic acid decarboxylase (GAD)-positive synapses, indicating that recombinant PICK1 was specifically targeted to excitatory synapses and excluded from inhibitory ones. These data indicate that even with a high level of protein expression, the recombinant Myc-PICK1 expressed by transfection in hippocampal cultured neurons was specifically concentrated at a subset of excitatory synapses. Therefore, this transfected neuron system could be exploited to elucidate the function of the different structural domains of PICK1 in synaptic clustering properties.

The PDZ and Coiled-coil Domains Are Involved in PICK1 Synaptic Clustering and mGluR7a Coclustering—We previously reported that PICK1 and the mGluR7a receptor induced coclustering of these two proteins (10). To test whether the structural domains of PICK1, which are important for the synaptic clustering in neurons, were also functionally important for the mGluR7a coclustering activity, we analyzed the coclustering efficacy for each Myc-PICK1 mutant coexpressed with mGluR7a in fibroblast CV1 cells (Fig. 5). The number of cells exhibiting coclustering was expressed as a percentage of total cotransfected cells. Both PICK1-WT and PICK1-ΔDE induced mGluR7a receptor coclustering, with a significantly more pronounced effect for PICK1-ΔDE. By contrast, the mutation of the PDZ domain in PICK1-KD/AA and the deletion of the coiled-coil domain in PICK1-ΔCC completely abolished mGluR7a receptor coclustering. These data demonstrate the importance of the PDZ and coiled-coil domains in mGluR7a coclustering efficacy and establish the existence of common features between PICK1 synaptic clustering and mGluR7a coclustering activity.

FIG. 1. Targeting of Myc-PICK1 to synaptic sites in cultured hippocampal neurons. Neurons were transfected with Myc-PICK1 cDNA at the time of plating and double-labeled at 15 DIV for Myc and synapsin (syn). Hippocampal cultures are constituted of two types of neurons: pyramidal cells (A) and interneurons (B). The two types of cells exhibit a similar punctate Myc-PICK1 immunostaining, mainly colocalized with or opposed to the synaptic marker synapsin. Scale bar, 20 μm.
self-associate via its N-terminal half containing both the PDZ and coiled-coil domains (23). Classically, PDZ and coiled-coil domains represent structural motifs known to mediate protein-protein interaction (21, 22). To examine whether the effect of the different domains of PICK1 on the clustering properties could be related to their involvement in the homooligomeriza-

**FIG. 2.** Recombinant Myc-PICK1 is specifically clustered at excitatory synaptic sites in cultured hippocampal neurons. Neurons were transfected with Myc-PICK1 cDNA at the time of plating and immunostained at 15 DIV for the Myc epitope along with AMPA glutamate receptor subunit GluR1 (GluR1, top panels), N-methyl-D-aspartate glutamate receptor subunit NR1 (middle panels), or GAD (bottom panels). Double labeling with GluR1 or NR1 shows that Myc-PICK1 immunoreactivity colocalizes well with these receptor subunits, which are only expressed at excitatory postsynaptic sites. NR1 staining associated with dendrites from an untransfected cell within the same field can be seen. Double labeling with GAD, a marker for GABAergic inhibitory synapses, shows no overlap between Myc-PICK1 immunoreactivity and GAD-containing nerve terminals, indicating that Myc-PICK1 is excluded from inhibitory synapses. Scale bar, 10 μm.

**FIG. 3.** Mutation in the PDZ domain and deletion of the coiled-coil domain disrupt PICK1 synaptic aggregation. Neurons were transfected at the time of plating with different Myc-PICK1 cDNAs as indicated on the figure and were immunostained at 15 DIV with a Myc antibody. For each construct, a representative picture of the Myc immunostaining obtained in dendrites and axons is presented. Labeled axons were identified as thin caliber processes extended far from the transfected cell body. For each construct, no difference in the distribution of Myc immunoreactivity was noticed between dendrites and axons. Mutation of the PICK1 PDZ domain by substitution of the Lys-Asp into Ala-Ala residues (Myc-PICK1-KD/AA) disrupts the synaptic clustering of the PICK1 protein. Similarly, the mutant Myc-PICK1-ΔCC, deleted of the coiled-coil domain, exhibits a diffuse labeling throughout the processes. By contrast, deletion of the acidic region (Myc-PICK-ΔDE) does not abolish the synaptic aggregation. The distribution of this PICK1 mutant is similar to the one observed for PICK1-WT. Scale bars, 7 μm.
The three structural domains of PICK1 deduced from its primary sequence played an important role in PICK1 synaptic aggregation and mGluR7a coclustering activity. PICK1 mutated in the PDZ domain or lacking the coiled-coil domain was no longer localized at synapses and lost the ability to cluster the mGluR7a receptor. We found that mutation of the predicted carboxylate binding loop of the PDZ domain abolished the direct interaction with mGluR7a receptor.
tor, while the ability of PICK1 to self-associate was maintained. On the other hand, deletion of the coiled-coil domain suppressed the other potential site of PICK1 self-association but did not affect binding with mGluR7a. These results suggest that synaptic localization and receptor coclustering depend on both PICK1 self-association through the coiled-coil domain and binding to a target membrane receptor, e.g. the mGluR7a receptor, by a PDZ-mediated interaction. In addition, we showed that the acidic region of PICK1 plays an inhibitory role in PICK1 clustering properties.

The PDZ mutant protein PICK1-KD/AA was not able to cocluster mGluR7a in heterologous cells or to localize at synapses in hippocampal neurons. Because this PICK1 mutant form did not bind mGluR7a (10, 11), the absence of coclustering activity was expected. The impairment of synaptic clustering in neurons was more surprising. Given that mutation of the two amino acid residues Lys27-Asp30 to Ala-Ala has been shown to specifically disrupt the interaction between PICK1 and several of its presynaptic and postsynaptic target proteins such as GluR2/3 (8, 9) and mGluR7a (10, 11), the simplest explanation for the diffuse neuronal distribution of the PICK1 PDZ mutant is that the target receptor plays an active role in the synaptic recruitment and/or stabilization of PICK1. In Drosophila photoreceptors, the PDZ protein INAD acts as a scaffold protein to assemble a signaling complex involved in the phototransduction initiated by light activation of rhodopsin (29). This signaling complex contains phospholipase C, the transient receptor potential (TRP) ion channel, and eye-PKC and is discretely localized in rhabdomeres of the photoreceptor cell. The proper distribution of this complex depends on INAD, as demonstrated in inad null mutant flies (29). In turn, the rhabdomeric localization of INAD depends on the interaction with TRP as demonstrated in trp null or trp mutant flies (30, 31). Thus, INAD is required for anchoring the signaling complex in the rhabdomeres, but in turn the INAD-target-interacting protein TRP is required to localize INAD to the rhabdomeres. It has also been shown in transfected neuron cultures that the PDZ domain protein PSD-95 could be localized in axons as a result of interactions with the membrane protein shaker-type K+ channel Kv1.4 (32) and that transfected Kv1.4 also depended on the interaction with PSD-95 to be selectively localized in axons (32) and in postsynaptic sites (33). Our data suggest the existence of a similar dual dependence to properly localize the PDZ protein PICK1 and its target binding proteins. We previously showed that the mGluR7a receptor lacking the PICK1 PDZ binding site failed to localize at presynaptic sites (10). Conversely, our present data show that the PDZ mutant PICK1 lacking the interaction site with its binding partners mGluR7a and GluR2/3 was no longer concentrated at presynaptic or postsynaptic sites. This interdependent relationship could enhance PICK1-receptor complex stability at synaptic sites and could prevent the build-up of excess PICK1 molecules at synapses without bound receptor.

The deletion of the coiled-coil domain also abolished the synaptic localization of PICK1. Coiled-coil domains correspond to peptidic motifs mediating protein-protein interaction typically involved in dimerization of a wide variety of proteins (20, 21). Here we showed by a yeast two-hybrid assay that the PICK1 coiled-coil domain can mediate PICK1 self-association. Our data suggest thus that PICK1 homooligomerization through the coiled-coil domain is central to the mechanism of both synaptic aggregation and receptor coclustering in fibroblast cells. Dimerization or multimerization of other proteins interacting with membrane receptors or channels has been shown to play a critical role in their clustering functions. For example, rapsyn-induced nicotinic acetylcholine receptor aggregation in heterologous systems required rapsyn self-association through tetratricopeptide repeat domains (34). Similarly, the multimerization of PSD-95 by its N-terminal part was necessary to induce K+ channel Kv1.4 clustering in heterologous cells (35).

In addition to the coiled-coil domain, we found that the PDZ domain of PICK1 can also mediate self-association. This finding is similar to the self-association and heteromultimerization of GRIP and the related protein ABP via a subset of their PDZ domains (36) but is more surprising for the single PDZ domain protein PICK1. The PDZ domain-mediated self-association of PICK1 was not abolished by mutations that abolish receptor ligand binding. However, the requirement of the coiled-coil domain for coclustering of PICK1 with mGluR7a suggests that binding of the PDZ domain to a receptor ligand renders this domain unable to simultaneously mediate self-association. Thus, it is tempting to speculate that PDZ domain-mediated self-association might be a mechanism to regulate availability of the PDZ domain to a receptor ligand.

Contrasting with what was observed for the PDZ and coiled-coil domains, deletion of the acidic region located in the C-terminal part of PICK1 notably increased the extent of PICK1 synaptic aggregation as well as its ability to cluster mGluR7a. Thus, this acidic region played a negative regulatory role in these processes. Because acidic amino acid sequences have been found in some proteins to be involved in inter- and intramolecular interactions to modulate the function of proteins (37, 38), we tested whether the fragment containing the acidic region could bind to PICK1 in the yeast two-hybrid system. No interaction was evidenced, suggesting that the mechanism by which the acidic region affects PICK1 localization and mGluR7a coclustering did not involve intramolecular interactions. However, we can not rule out the participation of yet unknown proteins in intermolecular interactions with the PICK1 acidic domain. An attractive possibility is that the PICK1 acidic region may directly bind Ca2+. Stretches of aspartic and glutamic acid residues have been shown in several other proteins such as calreticulin (39) and casequestrin (40) to bind Ca2+. Ca2+ binding can lead to conformational changes and modulation of interactions with protein partners, as demonstrated for calmodulin (41).

Although associated with the vast majority of excitatory synapses, transfected PICK1-WT and PICK1-DE were not associated with all of them. This result implies that mechanisms other than the intrinsic structural features of PICK1 play a role in the regulation of PICK1 synapse targeting and/or stabilization. It has been shown recently that PKC activation

**Fig. 6. Interaction of PICK1 mutants with PICK1-WT and the C-terminal tail of mGluR7a in the yeast two-hybrid system.** Interactions were tested by cotransformation of yeast with full-length PICK1 fused to the GAL4 DNA activation domain and with various PICK1 constructs or fragments fused to the GAL4 binding domain. Positive clones leading to expression of the three reporter genes histidine, adenine, and β-galactosidase are indicated by +. Only the fragment corresponding to the PICK1 acidic region (DE fragment) does not interact with PICK1-WT. When the interaction of the different PICK1 mutants was tested with the C-terminal tail of mGluR7a (CT7), only the PDZ mutant PICK1-KD/AA does not interact with CT7 (−).

| PICK1-KD/AA | PICK1-DE | PICK1-WT |
|-------------|----------|----------|
| +           | +        | +        |

PDZ fragment: +

PDZ-KD/AA fragment: +

coiled coil fragment: +

DE fragment: −
in cultured neurons enhanced PICK1 synaptic localization, suggesting that phosphorylation events are involved in PICK1 neuronal subcellular distribution (15). Although there was no direct demonstration that this effect correlated with PKC-induced PICK1 phosphorylation, previous studies showed that PICK1 could be phosphorylated in vitro by PKC (6) in accordance with the presence of several consensus phosphorylation sites for PKC in PICK1. Thus phosphorylation, perhaps by modifying coiled-coil-mediated oligomerization or PDZ domain associations, could regulate PICK1 distribution and function.

Based on our data, the simplest model for PICK1 synaptic aggregation and its ability to cocluster a target membrane-associated receptor requires two specific elements: binding to the target membrane receptor by a PDZ-mediated interaction and PICK1 self-association through the coiled-coil domain. This model takes into account the fact that the PICK1-interacting receptors exist as dimeric or heteromeric forms, as shown for mGluR7a (10) and the AMPA receptor (42), respectively, thus allowing for rafting of PICK1 dimers and multimeric receptors with multiple PICK1-binding sites. In addition, the PICK1 acidic region represents an inhibitory module in synaptic organization by their involvement in neurotransmitter receptor clustering and signaling complex assembly. Changes in the molecular composition of synapses are thought to underlie some aspects of synaptic plasticity. In this study, we defined three structural signals within PICK1 regulating its synaptic localization and receptor coclustering activity. These motifs could correspond to molecular substrates involved in synaptic development and plasticity.

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