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BREAKTHROUGHS AND VIEWS

The Roles of PDZ-Containing Proteins in PLC-β-Mediated Signaling

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Mammalian phospholipase C-β isozymes are activated by a heterotrimeric GTP-binding protein linked to various cell surface receptors. Recent reports suggest that PDZ domain proteins play a significant role of PDZ-containing proteins in the regulation of mammalian PLC-β isozymes. PDZ-containing proteins mediate the clustering of receptors and signaling molecules and thereby regulate agonist-induced signal transduction in polarized cells such as neuronal and epithelial cells. NORPA, a *Drosophila* PLC-β, is known to be a component of a signaling complex that includes TRP and rhodopsin through interaction with INAD, a PDZ-containing protein. Mammalian PLC-β1 and -β2 isoforms interact with a PDZ-containing protein NHERF which is coupled to Trp4, a Ca^{2+} channel. In addition, PLC-β3 specifically interacts with E3KARP, another protein closely related to NHERF, through its C-terminal PDZ-binding motif. E3KARP up-regulates the PLC-β3 activation coupled to muscarinic receptor. In this review, the role of signaling complexes mediated by PDZ-containing proteins in the regulation of PLC-β isoforms will be discussed. © 2001 Academic Press

Key Words: **G-protein-coupled receptor; INAD; NHERF; E3KARP; PDZ domain; phospholipase C-β (PLC-β); Trp.**

Phospholipase C (PLC) plays a key role in signal transduction by catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield inositol-1,4,5-trisphosphate and diacylglycerol. These products respectively cause intracellular Ca^{2+} release from endoplasmic reticulum and protein kinase C activation (1). To date, 11 mammalian PLC isoforms have been identified. Based on the degree of homology on the amino acid sequences, they can be divided into four classes; PLC-β1-4, PLC-γ1-2, PLC-δ1-4 and PLC-ε (1–3). All PLC isoforms contain two regions of high sequence homology, designated as X and Y, which constitute the PLC catalytic domain (2). The PLC-β isoforms have a C-terminal extension of ~400 amino acid residues downstream of their Y domain (Fig. 1), which may be responsible for their membrane association and the regulation of their enzymatic activities (2). The regulatory mechanism of PLC-β isoforms has been extensively studied during the past decade. PLC-β is regulated by G-protein-coupled receptors which are activated by a vast number of extracellular agonists, including acetylcholine, α-adrenergic agonists, angiotensin II, bombesin, and bradykinin (4, 5). PLC-β activation is mediated by four members (α_q, α_{11}, α_{14}, and α_{16}) of the $G\alpha_q$ subfamily of heterotrimeric GTP-binding proteins. In addition, the $G_{\beta\gamma}$ subunit dissociated from $G\alpha_{i/o}$ subfamily activates PLC-β isoforms in a pertussis toxin-sensitive manner (6, 7). Each PLC-β isoform has a different sensitivity to $G\alpha_q$ and $G_{\beta\gamma}$ subunit. The $G_{\beta\gamma}$ subunit activates PLC-β in a ranking order *in vitro:* PLC-β3 > PLC-β2 > PLC-β1 (8). On the other hand, the GTPγS-stimulated $G\alpha_q$ or $G\alpha_{11}$ subunits stimulate PLC-β isoforms in the order of potency: PLC-β1 ≥ PLC-β3 > PLC-β2 (9, 10). The C-terminal region of PLC-β isoforms is required for the $G\alpha_q$-dependent activation (11).

In addition to G proteins, PDZ-containing proteins have been shown to be involved in the regulation of PLC-β isoforms (12, 13). The PDZ domain was originally identified as a conserved element present in three structurally related proteins; PSD-95/SAP90, DLG, and ZO-1. It consists of ~90 amino acids and binds to the short carboxy-terminal peptide sequences, X(S/T)X(V/L)-COOH, of target proteins (14, 15). A protein often contains more than one PDZ domains. Each PDZ

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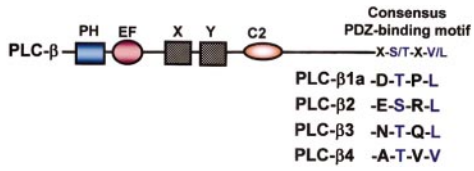

FIG. 1. Linear representation of the consensus PDZ-binding motifs and the various domains identified in PLC-β isozymes. The C-terminal four amino acids of PLC-β isozymes homologous to the potential PDZ domain-binding motifs are described. Catalytic domains X and Y as well as PH, EF-hands, C2 domains are indicated.

domain interacts with different target proteins and thereby promotes the scaffolding of signaling molecules.

ROLE OF PLC-β INTERACTION WITH INAD IN *Drosophila* PHOTOTRANSDUCTION

Insight into the physiological relevance of PDZ domain-mediated protein complex in PLC-β signaling was first provided by *Drosophila* phototransduction which involves NORPA (no-receptor-potential A), rhodopsin, protein kinase C, and *Drosophila* G protein. NORPA, a *Drosophila* PLC-β isoform, is a central component of phototransduction and forms a protein complex with rhodopsin and *Drosophila* G protein by interacting with a PDZ domain-containing protein INAD (16). The protein complex also includes two light sensitive cation-influx channel proteins (TRP and TRPL), protein kinase C (PKC), and an unconventional myosin NINAC (Fig. 2A) (16–18). INAD is localized to the

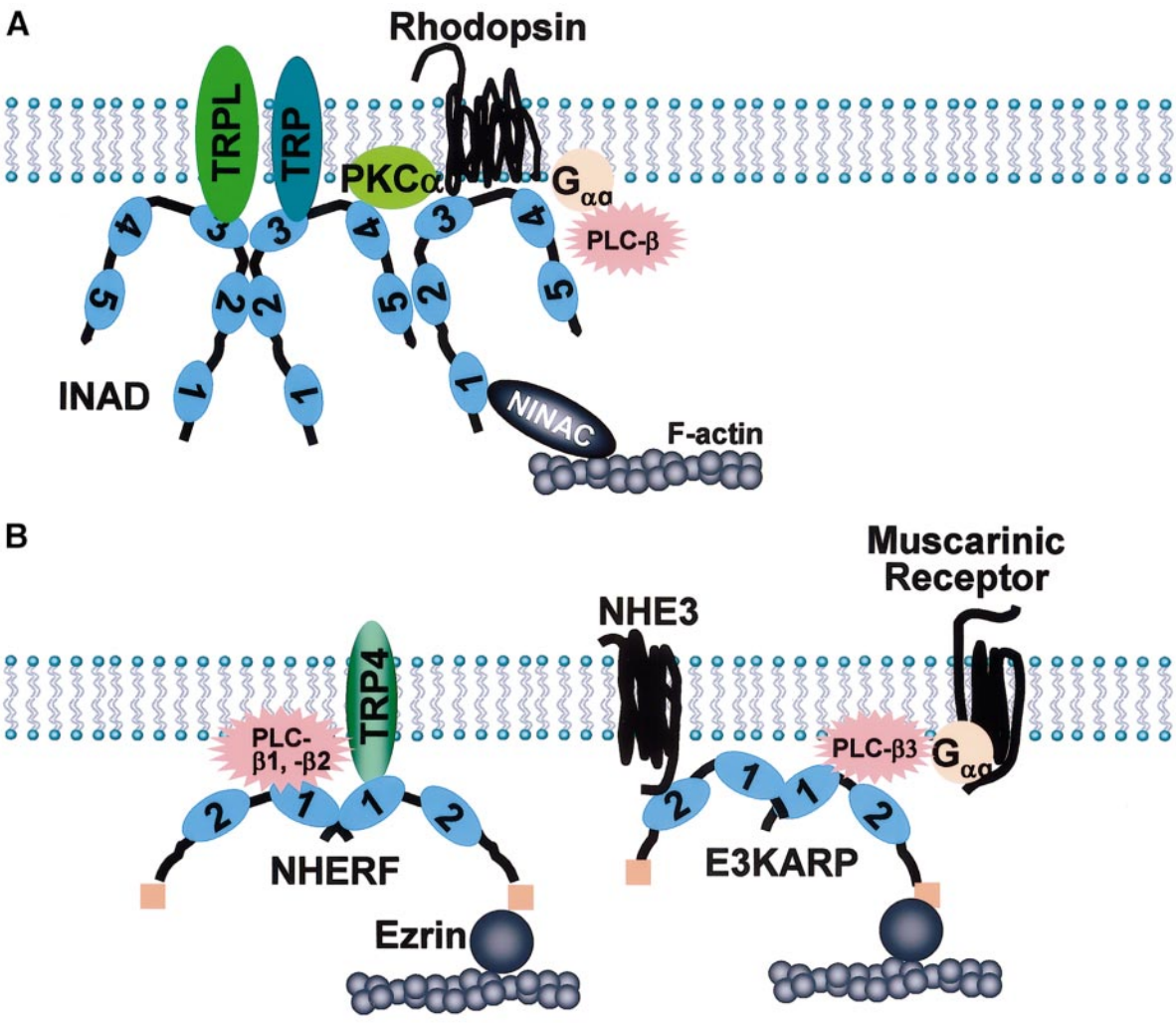

FIG. 2. Clustering of PLC-β isozymes into the protein complexes by PDZ domain proteins. (A) INAD-mediated formation of a protein complex containing NORPA (PLC-β), TRP, TRPL, protein kinase C (PKC), $G_{\alpha q}$ subunit of GTP-binding protein in *Drosophila* phototransduction. Five PDZ domains of INAD are numbered from 1 to 5. (B) Clustering of mammalian PLC-β isozymes and other signaling proteins by NHERF or E3KARP, and physical linking of the protein complex to actin cytoskeleton via ezrin. Two PDZ domains of NHERF and E3KARP are designated as 1 and 2.

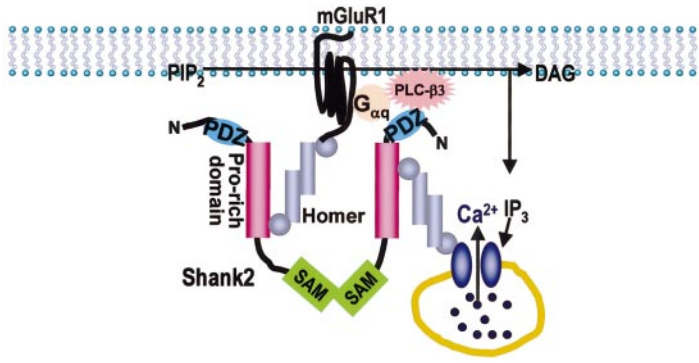

FIG. 3. Proposed model for linking of mGluR-coupled hydrolysis of phosphoinositides to intracellular calcium influx through scaffolding proteins, Shank2 and Homer. PDZ and SAM domains and proline-rich region of Shank2 are indicated.

rhabdomere, the microvillar array that forms a specialized membrane domain of photoreceptor cells in *Drosophila* eye, and contains five PDZ domains (19). The five PDZ domains differentially interact with the protein components of signaling complexes. NORPA interacts with PDZ1 as well as PDZ5 domain of INAD, whereas TRP and PKC bind with PDZ3 and PDZ2 domains, respectively. Genetic analyses indicate that INAD is required for targeting the proteins, including NORPA, PKC, and TRP (16, 18, 20) to the protein complex in the rhabdomere. Thus, INAD may act to facilitate the clustering of these proteins which are required for gating the TRP calcium channel. The association of INAD with NORPA is essential for the regulation of *Drosophila* phototransduction *in vivo* (20). According to this signal transduction pathway, an eye-specific $G_{\alpha q}$ is activated by light-stimulating rhodopsin and then the activated $G_{\alpha q}$ triggers NORPA to

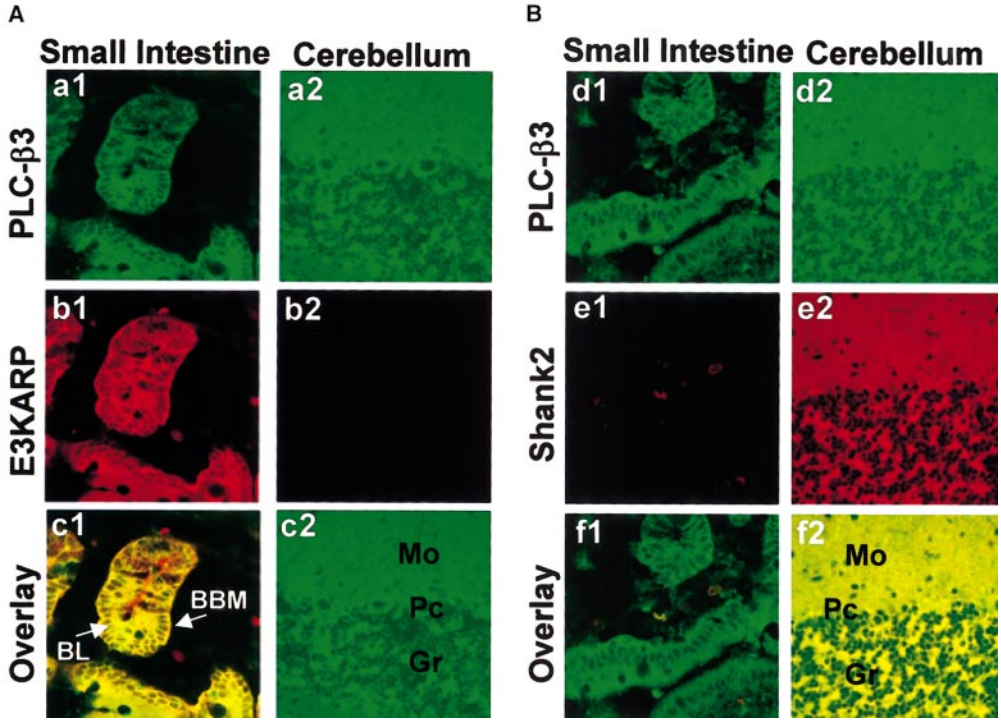

FIG. 4. Differential distribution of PLC-β3, E3KARP, and Shank2 in mouse small intestine and cerebellum. (A) Double immunofluorescence staining of PLC-β3 (green in a1 and a2) and E3KARP (red in b1 and b2) in mouse small intestine and cerebellum. The yellow color in c1 shows colocalization of PLC-β3 and E3KARP in brush border membrane (BBM) and basolateral membrane (BL) of small intestine as indicated. (B) Double immunofluorescence staining of PLC-β3 (green in d1 and d2) and Shank2 (red in e1 and e2) in mouse small intestine and cerebellum. The yellow color in f2 shows colocalization of PLC-β3 and Shank2 in Mo (molecular layer), Pc (Purkinje cell layer), and Gr (granular layer) of mouse cerebellum.

catalyze the breakdown of phosphatidylinositol 4,5-bisphosphate to generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (20). INAD-mediated targeting of NORPA into the rhabdomere may generate an activated membrane patch with a spatially restricted increase in intracellular transmitter concentration, that finally results in the coordinated gating of TRP channels sequestered to this microdomain. $G_{\alpha q}$ and NORPA are essential for visual signaling because defective mutants in either of them lead to no response to light (21, 22). Consequently, the failure to properly target these proteins to phototransduction complex impairs the light-induced activation of NORPA via G protein-mediated signaling pathway.

LINK OF PLC-β1 AND -β2 WITH MAMMALIAN TRP THROUGH NHERF

Mammalian homologues of *Drosophila* TRP have been implicated in the Ca^{2+} release from intracellular Ca^{2+} stores (23). Murine TRP4 and TRP5, as well as PLC-β1 and β2, have been known to interact with the first PDZ domain of NHERF (13). Each of these four proteins has the consensus PDZ domain-binding motif, (-X-(S/T)-X-(V/L)-COOH), at its C-terminus (Fig. 1). NHERF was originally identified as a protein factor which is required for the protein kinase A-dependent regulation of Na^+/H^+ exchanger 3 (NHE3) in epithelial cells of kidney and intestine (24). NHERF contains two PDZ domains, which interact with a variety of signaling proteins such as platelet-derived growth factor receptor (PDGFR), P2Y purinergic receptor, β2-adrenergic receptor, and a *c-yes*-associated protein YAP65 (25–28). It is thus implied that NHERF may play a role in clustering both TRP4/5 and PLC-β1/β2 as a functional analog of INAD. However, in contrast to INAD, Murine TRP4/5 and PLC-β1/β2 associate with the same PDZ domain, PDZ1, of NHERF (13). Recent report suggests that NHERF dimerizes through its PDZ domain-mediated interactions (29). Therefore, PDZ1 domain interacts with both TRP4/5 and PLC-β1/β2 isoforms, and the self-association of NHERF may be critical for linking these proteins in one protein complex. In addition, NHERF interacts with ezrin, a cytoskeletal protein, through its C-terminal 30 amino acids, called as FERM domain (30), suggesting that NHERF may link the protein complexes of PLC-β1/β2 and the calcium channels to the actin cytoskeleton by interacting with ezrin (Fig. 2B). Tang *et al.* proposed a model in which redistribution of actin cytoskeleton is involved in the regulation of the calcium channels TRP4/5 through the NHERF-mediated formation of a protein complex (13). Although the physiological role of NHERF on the regulation of PLC-β1/β2 and TRP4/5 proteins is not clear, it would be worthwhile in investigating whether NHERF would connect the phosphoinositide signaling to TRP4/5 calcium channels, thus serving as a functional analog of INAD.

PDZ-CONTAINING PROTEINS IN THE AGONIST-INDUCED ACTIVATION OF PLC-β ISOFORMS

E3KARP is highly homologous to NHERF, also containing two PDZ domains and an ezrin-binding motif. We reported that E3KARP, but not NHERF, specifically interacts with the PDZ-binding motif of PLC-β3 through its second PDZ domain (12). Overexpression of E3KARP potentiates the carbachol-induced activation of PLC-β3, whereas overexpression of a mutant form of E3KARP lacking the second PDZ domain has no effect on PLC activity. Therefore, it is likely to postulate that E3KARP modulates the carbachol-induced activation of PLC-β3 through formation of a protein complex mediated by its second PDZ domain (Fig. 2B). Results from our yeast-two-hybrid experiments revealed that PLC-β3 interacts with another PDZ domain-containing protein Shank2.[2] Shank proteins make up a new family of scaffold proteins recently identified and three members of Shank proteins (Shank1, 2, and 3) have been identified to date (31). In addition to the PDZ domain, Shank2 contains a SH3 domain, a SAM domain, and a proline-rich region (31, 32). The proline-rich region of Shank2 binds to the SH3 domain of cortactin as well as to the EVH domain of Homer, a metabotropic glutamate receptor (mGluR)-binding protein. The SAM domain is involved in the multimerization of Shank2 (31–33). It appears, therefore, that PLC-β3 associates with the PDZ domain of Shank, which further promotes complex formation with Homer, cortactin, and mGluR in neuronal cells. Moreover, the EVH domain of Homer recognizes the PPXXP motifs of mGluR and IP_3 receptor (31). In addition, Homer proteins encode a C-terminal coiled-coil domain that mediates self-multimerization between Homer proteins. Thus, mGluR can be linked to IP_3 receptor through the dimeric form of Homer. These findings suggest that the protein complex containing PLC-β3 and mGluR might be functionally coupled to the IP_3 receptor that is an effect molecule of mGluR-linked PLC activation. Therefore, scaffolding proteins including Shank and Homer may play an essential role for efficient release of intracellular calcium in response to mGluR-coupled hydrolysis of phosphoinositides (Fig. 3).

As PLC-β3 can interact with either E3KARP or Shank2, it is interesting to examine how PLC-β3 selects one form these two potential binding partners. A possible answer is that the tissue-specific distribution of these PDZ-containing proteins determines their availability for the interaction with PLC-β3. For example, E3KARP is expressed and colocalizes with PLC-β3

[2] Jong-Ik Hwang and Pann-Ghill Suh, unpublished data.

in the brush border membrane, but not expressed in neuronal cells. On the contrary, Shank2 is expressed in neuronal cells but not in small intestine (Fig. 4). Another question of interest is whether these PDZ-containing proteins share their role in the agonist-induced regulation of other PLC-β isozymes as NHERF associates with PLC-β1 and -β2 (13). A previous study using PLC-β1-null mice showed that PLC-β1 is involved in signal transduction in cerebral cortex and hippocampus by coupling predominantly with the muscarinic acetylcholine receptor (34). Although the effect of NHERF on the carbachol-induced PLC-β1 activation is not well defined yet, these results present a model for NHERF's participation in it similar to the positive regulation of PLC-β3 by E3KARP. Therefore, it can be suggested that the specific interaction of PLC-β isoforms with various PDZ-containing proteins may be responsible for the redundancy and diversity of agonist-induced intracellular signaling.

Several studies suggest a role of PKA in the negative regulation of PLC-β isozymes through direct phosphorylation of PLC-β isozymes (35–37). The $G_{\beta\gamma}$-induced activity of PLC-β2 is inhibited by PKA-mediated phosphorylation (35). Ali *et al.* reported that ligation of the fMLP receptor, which activates both PLC and adenylate cyclase, leads to the phosphorylation of PLC-β3 by PKA (36). The negative regulation of PLC-β through PKA-dependent phosphorylation is supported by a recent report demonstrating that PKA phosphorylates Ser1105 of PLC-β3 and inhibits its enzymatic activity stimulated by G_{α} subunit (37). Moreover, pretreatment with 8-[4-chlorophenythio]-cAMP blocks the phosphoinositide turnover induced by the activation of $G_{\alpha 1}$-coupled M1 muscarinic receptor and oxytocin receptor (37). Both β2-adrenergic receptor and PLC-β can be recruited into a protein complex by two tandem PDZ domains of NHERF or E3KARP. β2-adrenergic receptor generates cAMP from ATP by the sequential activation of G protein and adenylate cyclase, and the clustering of β2-adrenergic receptor and PLC-β may induce local elevation in the concentration of cAMP near PLC-β (Fig. 5). Although it is still unclear whether these PDZ-containing proteins participate in the PKA-dependent inhibition of PLC-β, recent reports showed that these scaffolding proteins are required for the physical linking of ezrin, PKA, and NHE3 into the protein complex (38). Therefore, these PDZ-containing proteins are likely to be involved in the PKA-dependent negative regulation of PLC-β as well as NHE3 through linking β2-adrenergic receptor, ezrin, PKA, PLC-β, and NHE3 (Fig. 5).

FORMATION OF PROTEIN COMPLEXES MEDIATED BY NHERF AND E3KARP

Through their first PDZ domains, both NHERF and E3KARP can bind to a number of receptors and ion channels; i.e., PDGFR, P2Y purinergic receptor, β2-adrenergic receptor, cystic fibrosis transmembrane regulator (CFTR), sodium bicarbonate transporters, and H^+-ATPase (25–28). Upon ligation of PDGFR by PDGF, PDGFR forms a dimer and phosphorylates various cytosolic proteins to mediate the mitogenic activities of PDGF. The binding of NHERF with PDGFR increases the receptor's dimerization and phosphorylation of PDGFR, subsequently stimulating the activation of extracellular signal-regulated kinase (ERK) (25). The first PDZ domain of NHERF can also interact with β2-adrenergic receptor in an agonist-dependent manner. This interaction between β2-adrenergic receptor and NHERF appears to modulate the endocytic sorting of β2-adrenergic receptor (39) as well as the β2-adrenergic receptor-mediated regulation of Na^+/H^+ exchange (NHE3) (28).

The second PDZ domains of NHERF and E3KARP interact with NHE3, and the negative effect of these PDZ-containing proteins on NHE3 activity is mediated by the direct interaction with ezrin, which is an anchoring protein for PKA (38). Through the association with ezrin, PKA is linked to the protein complex containing NHE3, and inhibits NHE3 activity by phosphorylating NHE3. In addition, the second PDZ domain of NHERF binds the C-terminus of YAP65, a c-*yes*-associated protein. The YAP65 associates with c-*yes* and is implicated in the localization of c-*yes* in the apical membrane of airway epithelial cells through interaction with NHERF (26). These observations suggest that the subapical protein complexes, including the nonreceptor tyrosine kinase c-*yes* and NHERF, may regulate the apical signal transduction pathways leading to changes in ion transport, cytoskeletal organization, or gene expression in epithelial cells (26). PLC-β isoform could be a component of the protein complexes, and the PDZ-containing protein may link PLC-β to other signaling molecules including muscarinic receptor, metabotropic glutamate receptor, TRP4, and IP_3 receptor.

CONCLUSION AND PERSPECTIVES

We describe here that PLC-β1 and -β2 interact with the first PDZ domain of NHERF, whereas PLC-β3 binds with the second PDZ domain of E3KARP through its C-terminal PDZ-binding motif. The PDZ domains of NHERF and E3KARP are involved in membrane localization and complex formation of signaling components. Both these proteins have two distinct PDZ domains, which recruit signaling molecules. However, it is still unclear how these PDZ-containing proteins themselves stimulate the enzymatic activities of PLC-β isozymes regulated by G-protein-coupled receptors. To define the molecular mechanism of the activation of PLC-β, the molecular identities of other components of the protein complex containing PLC-β isozymes must

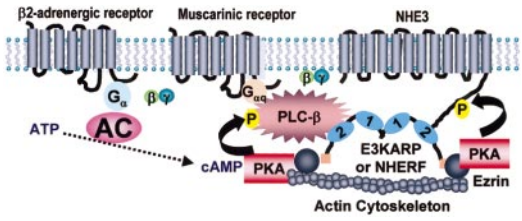

FIG. 5. Possible role of E3KARP and NHERF in PKA-dependent regulation of PLC-β isoforms and NHE3. Two PDZ domains of NHERF and E3KARP are indicated as 1 and 2. G_{α}, alpha subunit of GTP-binding proteins; β and γ, β and γ subunits of GTP-binding proteins; AC, adenylate cyclase; PKA, protein kinase A; NHE3, Na^+/H^+ exchanger 3.

be elucidated. An interesting aspect is the specificity of the interaction between PLC-β isozymes and PDZ-containing proteins. PLC-β3 interacts with distinct PDZ-containing proteins of E3KARP and Shank2, in intestinal epithelium and neuronal cells, respectively, suggesting that the interaction of PLC-β isoforms with different PDZ-containing proteins is dependent on cell type. On the other hand, PLC-β1 and -β2 isoforms interact with NHERF, indicating that each PLC-β isoform may interact with distinct PDZ-containing proteins. Thus, the specific interaction of PDZ-containing proteins with PLC-β isozymes and the differential expression of PDZ-containing proteins may diversify the PLC-β signaling induced by G-protein-coupled receptors. In addition to this research interest, it will also be interesting to study how the PDZ-containing proteins may link the PLC-β-activated signaling to other signaling components including calcium channel TRP4 and IP_3 receptor by recruiting protein components into the complex.

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