Role of the CDC25 Homology Domain of Phospholipase Cε in Amplification of Rap1-dependent Signaling*

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Phospholipase Cε (PLCε) is a novel class of phosphoinositide-specific PLC characterized by possession of CDC25 homology and Ras/Rap1-associating domains. We and others have shown that human PLCε is translocated from the cytoplasm to the plasma membrane and activated by direct association with Ras at its Ras/Rap1-associating domain. In addition, translocation to the perinuclear region was induced upon association with Rap1-GTP. However, the function of the CDC25 homology domain remains to be clarified. Here we show that the CDC25 homology domain of PLCε functions as a guanine nucleotide exchange factor for Rap1 but not for any other Ras family GTPases examined including Rap2 and Ha-Ras. Consistent with this, coexpression of full-length PLCε or its N-terminal fragment carrying the CDC25 homology domain causes an increase of the intracellular level of Rap1-GTP. Concurrently, stimulation of the downstream kinases B-Raf and extracellular signal-regulated kinase is observed, whereas the intracellular level of Ras-GTP and Raf-1 kinase activity are unaffected. In wild-type Rap1-overexpressing cells, epidermal growth factor induces translocation of PLCε to the perinuclear compartments such as the Golgi apparatus, which is sustained for at least 20 min. In contrast, PLCε lacking the CDC25 domain translocates to the perinuclear compartments only transiently. Further, the formation of Rap1-GTP upon epidermal growth factor stimulation exhibits a prolonged time course in cells expressing full-length PLCε compared with those expressing PLCε lacking the CDC25 homology domain. These results suggest a pivotal role of the CDC25 homology domain in amplifying Rap1-dependent signal transduction, including the activation of PLCε itself, at specific subcellular locations such as the Golgi apparatus.

Ras family small GTPases direct a wide variety of intracellular signaling pathways (1–3). In mammalian cells, ∼20 proteins, such as Ras, Rap, R-Ras, Ral, Rit, Rin, and Rheb, belong to the Ras family. The best characterized members of the family are Ha-Ras, Ki-Ras, and N-Ras, which have been implicated in the regulation of cell proliferation and differentiation downstream of diverse cell surface receptors. Localization at the plasma membrane following post-translational modifications is crucial for the function of Ras proteins. On the other hand, Rap1, which was originally isolated as a Ras-related GTPase, can suppress Ki-Ras-induced transformation and thus is also termed Krev-1 (4). Inhibition of Ras-mediated pathways by Rap1 is attributable to tight binding of Rap1 to the second Ras/Rap1-binding sites of Ras effectors, such as Raf-1 and yeast adenyl cyclase, without stimulating their activities (5–7). However, it is likely that Rap1 exerts functions other than the inhibition of Ras pathways because Rap1 localizes mainly in the perinuclear compartments including the Golgi apparatus and cytoplasmic vesicles, where Ras proteins do not exist (8–11). Indeed, Rap1 is involved, for example, in the activation of integrin and subsequent cell aggregation independently of Ras pathways (12–15).

Ras family GTPases cycle between GDP-bound inactive and GTP-bound active states, serving as a molecular switch of signal transduction (1). In response to extracellular stimuli, transition from the GDP-bound state to the GTP-bound state is facilitated by GEFs (4) specific to an individual member of the Ras family, leading to the accumulation of the GTP-bound active form. Once activated, Ras family GTPases become associated with specific effectors, thereby stimulating downstream signaling pathways. Thereafter, protein-bound GTP is hydrolyzed to GDP and inorganic phosphate, causing dissociation from the effector. Thus, for the understanding of the biological function of the Ras family, it is important to identify effectors for each GTPase and to clarify the molecular mechanism underlying their activation upon binding to GTPases.

To date, various proteins, such as Raf kinases (A-Raf, B-Raf, and Raf-1), Ral GEFs (RalGDS, Rlf, and Rgl), and PI3-K, have been characterized as Ras effectors on the basis of their ability to bind to Ras in a GTP-dependent manner (2). In the case of Raf-1, the RBD (amino acids 51–131) directly interacts with the effector region of Ras (amino acids 32–40). On the other hand, the domain of RalGDS responsible for the binding to Ras-GTP is designated the RA domain (16). Interestingly, x-ray crystallography revealed that the overall tertiary structure of the RA domain of RalGDS is similar to that of the Raf-1 RBD, although no extensive sequence similarity between these two domains was found (17–19). Moreover, the crystal structure of PI3-Ky

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‡ The abbreviations used are: GEF, guanine nucleotide exchange factor; RalGDS, Ral guanine nucleotide dissociation stimulator; PI3-K, phosphoinositide 3-kinase; RBD, Ras-binding domain; RA, Ras/Rap1-associating; PLC, phospholipase C; PIP, phosphatidylinositol phosphate; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; HA, hemagglutinin; DME, Dulbecco’s modified Eagle’s medium; GST, glutathione S-transferase; R1D, Rap1-interacting domain; MEK, mitogen-activated protein kinase/ERK kinase; ERK, extracellular signal-regulated kinase; KNERK, kinase-negative ERK.
was determined, demonstrating that the RBD of PI3-K has the same fold as the Raf-1 RBD and the RA domain of RaflGDS (20, 21). Collectively, the GTP-dependent interaction between Ras and its effectors is mediated by structurally conserved modules.

Mechanisms underlying the activation of effectors subsequent to Ras binding are complicated, but a primary role for Ras binding is considered to be the recruitment of effectors to the plasma membrane. For instance, Ras induces subcellular translocation of Raf-1 to the plasma membrane, where Raf-1 undergoes a variety of modifications such as serine/threonine and tyrosine phosphorylation to become fully activated (22). For full activation of Raf-1 kinase activity, however, binding of the RBD to the Ras effector region is insufficient, and an additional interaction between the cytoine-rich region of Raf-1 (amino acids 139–184) and the activator region of Ras (amino acids 26–31 and 41–53) is required (6, 23).

Recently, we and other groups identified a novel type of phosphoinositide-specific PLC, named PLCe, which possesses an RA domain that is responsible for high affinity binding to GDP-bound forms of Ras and Rap1 (24–27). Upon binding to Ras and Rap1, PLCe translocates to spatially distinct sites, the plasma membrane and the perinuclear region, respectively, where PIPs exist as substrates (25). Ras-dependent subcellular translocation and activation of PLCe were also reconstituted in vitro by the use of a liposome carrying recombinant Ras and phosphatidylinositol 4,5-bisphosphate (25). The activation of PLCe in vivo following cotransfection with constitutively activated Ras was reported as well (27). Taken together, PLCe is involved in Ras-mediated and Rap1-mediated signaling pathways, triggering the hydrolysis of PIPs at different subcellular regions depending on cellular contexts. However, it is unclear whether Ras binding exerts an allosteric effect on enzymatic activity as proposed for PI3-K activation. Also, modifications of PLCe, such as serine/threonine phosphorylation, after membrane translocation have not been reported. Thus, precise mechanisms whereby the enzymatic activity of PLCe is regulated at specific subcellular sites remain to be clarified.

In addition to the RA domain, a CDC25 homology domain was found in the N-terminal portion of PLCe. The CDC25 homology domain was originally identified in the yeast Saccharomyces cerevisiae CDC25 protein, which acts as a GEF for yeast Ras proteins. Afterward, an array of mammalian Ras GEFs, such as Sos1, So2, Ras-GRF-1, Ras-GRF-2, and Ras-GRP, were isolated, revealing that the CDC25 homology domain was conserved among all of these proteins and responsible for their GEF activity. In addition, GEFs for Rap1, such as C3G (28), Epac/cAMP-GEF (29, 30), CalDAGGEF1 (31), and RA-GEF-1/PDZ-GEF-1/RapGEF/CNrasGEF (32–35), possess the CDC25 homology domain. Hence, the CDC25 homology domain of PLCe is predicted to show GEF activity toward Ras family GTPases. The Ras exchanger motif, which is conserved among an array of Ras GEFs but is not directly implicated in catalysis (36), was not found in PLCe.

Here, we show that the CDC25 homology domain of PLCe exhibits GEF activity toward Rap1 but not toward any other Ras family GTPases examined, including Rap2 and Ha-Ras. We further demonstrate that deletion of the CDC25 homology domain diminishes GEF-dependent sustained translocation of PLCe to the perinuclear region in wild-type Rap1-expressing cells. In addition, sustained increase in the Rap1-GTP level was observed in PLCe-expressing cells but not in cells expressing a PLCe mutant lacking the CDC25 homology domain. Based on these observations, a novel self-amplifying mechanism of the Rap1/PLCe pathway leading to prolonged activation is proposed.
quantitated by a bioimaging analyzer (BAS2000; Fujix). Immunoprecipitated B-Raf and Raf-1 were quantitated by immunoblotting using anti-phosphorylated ERK (number 9106; New England Biolabs Inc.) and anti-ERK (number 9102; New England Biolabs Inc.) antibodies.

Detection of ERK Phosphorylation— COS-7 cells were transfected with a combination of expression plasmids as described in the figure legends. After incubation for 24 h in DMEM supplemented with 10% fetal calf serum, the cells were starved for another 24 h in DMEM supplemented with 0.1% fetal calf serum. Thereafter, cells were harvested and dissolved in lysis buffer C (25 mM Hepes-NaOH, pH 7.3, 150 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 1 mM Na₃VO₄, 1 mM 4-2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 0.8 µM aprotinin, 15 µM E-64, 20 µM leupeptin, 50 µM bestatin, and 10 µM pepstatin A). Supernatants of centrifugation (15,000 g for 10 min at 4 °C) were used as cell extracts. Cell extracts were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting using anti-phosphorylated ERK (number 9106; New England Biolabs Inc.) and anti-ERK (number 9102; New England Biolabs Inc.) antibodies.

Fluorescence Microscopy—COS-7 cells cultured in a slide chamber were transfected with a combination of expression plasmids as described in the figure legends. After incubation for 24 h in DMEM supplemented with 10% fetal calf serum, the cells were starved for another 18 h in DMEM supplemented with 0.1% fetal calf serum. In some experiments, cells were stimulated with EGF as described in the figure legends. Following fixation with 3.7% formaldehyde and permeabilization with 0.2% Triton X-100, the cells were stained with anti-HA (12CA5; Roche Molecular Biochemicals) and tetramethylrhodamine-conjugated goat anti-mouse IgG (T2762; Molecular Probes) antibodies.

Subcellular localization of EGFP-PLCe, EGFP-PLCeΔN, HA-RAp1αWT, and HA-RAp1αWT22 was analyzed under a confocal laser microscope (MRC-1024; Bio-Rad). For staining the Golgi apparatus, serum-starved COS-7 cells were transfected with jEF-BOs-HA-Rap1αWT and an expression plasmid for either FLAG-PLCeΔN, FLAG-PLCe, or FLAG-RA-GEF-1. The GTP-bound form of Rap1α was detected by pull-down assays using GST-RalGDS-RID (upper panel). Amounts of Rap1α in aliquots of cell extracts were measured by immunoblotting using anti-HA antibody (lower panel). The representative results of three independent experiments are shown.

RESULTS

The CDC25 Homology Domain Exhibits in Vitro GEF Activity toward Rap1A—Structural features of deletion mutants employed in this study are illustrated in Fig. 1. PLCβ is a Rap1 GEF (number 9102; New England Biolabs Inc.) and anti-ERK (number 9106; New England Biolabs Inc.) antibodies.

Specificity of GEF activity in vitro. Various Ras family GTPases preloaded with [3H]GTP were incubated with excess amounts of unlabelled GTP in the presence (black bars) or absence (gray bars) of PLCβ for 20 min at 30 °C (for R-Ras at 20 °C), and [3H]GDP remaining bound to the protein was quantitated. The percentage of the zero time point is shown. The values are expressed as the means ± S.E. (n = 3).
assays (Fig. 3). PLCε did not stimulate GDP release from Ras family GTPases examined other than Rap1. Notably, Rap2 was virtually insensitive to the action of PLCε, although Rap2 exhibits high sequence similarity to Rap1.

The Elevated Rap1-GTP Level and Activation of Downstream Protein Kinases Following Coexpression of the PLCε CDC25 Homology Domain—In vitro GEF activity of the CDC25 homology domain suggests that Rap1 and its downstream pathways are activated within the cell when this domain is expressed. Fig. 4 shows Rap1-GTP levels upon coexpression of indicated proteins as determined by pull-down assays using GST-Ral-GDS-RID as a probe. PLCεCDC25 as well as full-length PLCε caused elevation of Rap1-GTP levels as observed for RA-GEF-1 as a positive control (Fig. 4A). Thus, the CDC25 homology domain, in fact, acts as a GEF toward Rap1 in vivo. PLCεΔN did not induce Rap1-GTP formation, suggesting that Rap1 activation following hydrolysis of PIPs is negligible in COS-7 cells (Fig. 4B).

Raf-1 and B-Raf serine/threonine kinases have been characterized as downstream targets of Ras. Rap1 as well can bind to Raf-1 and B-Raf as Rap1 possesses the same effector region sequence compared with Ras. However, Rap1 is unable to activate Raf-1 because the interaction mediated by the cysteine-rich second binding region of Raf-1 is too strong (5, 6). On the other hand, Rap1 can activate B-Raf in PC12 and COS-7 cells (6, 40, 41). Therefore, kinase activities of B-Raf and Raf-1 were measured to assess the in vivo activity of Rap1 (Fig. 5). In parallel with elevated Rap1-GTP levels, PLCεCDC25 caused a 3-fold increase in B-Raf kinase activity when coexpressed with Rap1, which is comparable with that induced by RA-GEF-1 (Fig. 5A). Full-length PLCε stimulated B-Raf activity more potently than PLCεCDC25 and RA-GEF-1 (Fig. 5A), although the Rap1-GTP level was similar to the levels in PLCεCDC25- or RA-GEF-1-expressing cells (Fig. 4A). A part of the full-length PLCε-induced B-Raf activation may be mediated by Rap1-independent pathways stimulated by breakdown products of PIPs. In contrast, neither PLCεCDC25 nor full-length PLCε augmented Raf-1 kinase activity in Rap1-coexpressing cells (Fig. 5B). Furthermore, Raf-1 activity in Ha-Ras-expressing cells remained unchanged upon the expression of PLCεCDC25 or full-length PLCε (Fig. 5C), which is consistent with the observation that the CDC25 homology domain of PLCε did not show any GEF activity toward Ras (Fig. 3).

ERK acts downstream of Raf family kinases to exert a variety of biological functions. An antibody raised against phosphorylated ERK was employed to assess the activation (Fig. 6). PLCεCDC25 and full-length PLCε, like RA-GEF-1, induced ERK phosphorylation in Rap1-expressing cells, which is presumably mediated by endogenous B-Raf. Taken together, the CDC25 homology domain of PLCε exhibits GEF activity toward Rap1, leading to the formation of Rap1-GTP, which in turn activates the downstream B-Raf/MEK/ERK pathway within the cell.
Establishing the role of the CDC25 homology domain of PLCε as a Rap1 GEF

**Materials and Methods**

COS-7 cells were transfected with the following plasmids: pEF-BOS-HA-Rap1A V12 and pcDNA-EGFP-PLCε. Green fluorescence of EGFP is shown in panel a in the context of PLCε and PLCεΔN when expressed alone in serum-starved COS-7 cells. COS-7 cells were transfected with pcDNA-EGFP-PLCε (panel a) or pcDNA-EGFP-PLCεΔN (panel b) and analyzed under a confocal microscope. Green fluorescence of EGFP is shown in panels a and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels a and b. Green fluorescence of EGFP is shown in panels a and b. Green fluorescence of EGFP is shown in panels a and b. Green fluorescence of EGFP is shown in panels a and b.

**Discussion**

In addition to the catalytic (X and Y) and regulatory (C2) domains for PLC activity, PLCε possesses CDC25 homology and RA domains, implicating Ras family GTPases in the regulation of PLCε, which is determined through the interaction of the RA domain with Ras or Rap1, is considered to be important for the function of PLCε. When coexpressed with an activated mutant of Ras, PLCε was conveyed to the plasma membrane, whereas translocation to the perinuclear region occurred when activated Rap1 was coexpressed (25). Furthermore, following EGF-dependent formation of GTP-bound forms of Ras or Rap1, PLCε was distributed to the plasma membrane and the perinuclear compartments, respectively (25). To better understand the role of the CDC25 homology domain in translocation-dependent regulation of PLCε, Rap1-mediated subcellular localization of full-length PLCε and PLCεΔN was examined (Fig. 7). Both full-length PLCε and PLCεΔN uniformly distributed in the cytoplasm without ectopically expressed Rap1 in serum-starved COS-7 cells (Fig. 7A). When coexpressed with constitutively activated Rap1 (Rap1V12), PLCεΔN, like full-length PLCε, colocalized with Rap1V12 in the perinuclear region, indicating that RA domain-dependent translocation remained unaffected after the deletion of the CDC25 homology domain (Fig. 7B). PLCε in Rap1V12-expressing cells was also costained with the Golgi apparatus (Fig. 8), indicating that PLCε translocates to the perinuclear compartments, particularly to the Golgi apparatus, upon the binding to Rap1. When serum-starved COS-7 cells expressing wild-type Rap1 were stimulated with EGF for 5 min, PLCε localized in the perinuclear region as described previously (25) (Fig. 7C). Similarly, translocation of PLCεΔN to the perinuclear region was observed upon 5 min of treatment with EGF (Fig. 7C). In marked contrast, after 20 min, EGF-induced translocation of PLCεΔN to the perinuclear region was abolished, whereas PLCε remained colocalized with Rap1 in the perinuclear region (Fig. 7D). Collectively, these results suggest that the CDC25 homology domain may play a pivotal role in the prolonged formation of Rap1-GTP in the perinuclear region upon EGF stimulation, which results in a prolonged stay of PLCε in this region. To further clarify this point, EGF-dependent increase in the level of the GTP-bound form of endogenous Rap1 was analyzed by pull-down assays in full-length PLCε- or PLCεΔN-expressing cells (Fig. 9). In the absence of ectopically expressed PLCε, EGF caused a transient increase in the Rap1-GTP level, which peaked at 5–10 min and diminished by 30 min (Fig. 9A). When full-length PLCε was expressed, the Rap1-GTP level remained elevated after 30 min of treatment. However, expression of PLCεΔN did not significantly affect the time course of EGF-induced alteration in the Rap1-GTP level (Fig. 9B). Thus, the CDC25 homology domain is involved in persistent, but not transient, activation of Rap1 following EGF treatment.

**Fig. 7.** EGF-induced translocation of PLCε. A, localization of PLCε and PLCεΔN when expressed alone in serum-starved COS-7 cells. COS-7 cells were transfected with pcDNA-EGFP-PLCε (panel a) or pcDNA-EGFP-PLCεΔN (panel b) and analyzed under a confocal microscope. Green fluorescence of EGFP is shown. B, localization of PLCε and PLCεΔN when coexpressed with Rap1A V12. COS-7 cells were transfected with the following plasmids: pEF-BOS-HA-Rap1A V12 and pcDNA-EGFP-PLCε (panels a–c); pEF-BOS-HA-Rap1A V12 and pcDNA-EGFP-PLCεΔN (panels d–f). Staining with anti-HA and tetramethylrhodamine-conjugated goat anti-mouse IgG antibodies is shown in panels a and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d. Green fluorescence of EGFP is shown in panels b and d.
ulation of PLCe. Indeed, we observed that the RA domain-mediated association of PLCe with Ras-GTP in a liposome containing phosphatidylinositol 4,5-bisphosphate as substrate enhanced the activity of PLCe (25). Translocation of PLCe to the plasma membrane was also observed when coexpressed with activated Ras (25). Therefore, it is likely that translocation of PLCe to the plasma membrane through the binding to Ras-GTP is critical for the activation. Furthermore, Kelley et al. (27) reported that PLC activity was augmented in parallel with the binding of activated Ras in COS-7 cells. On the other hand, Rap1 as well associates with the RA domain and causes translocation of PLCe to the perinuclear region (25). The activation of PLCe upon the binding to Rap1-GTP has been observed to date neither in vivo nor in vitro. However, PLCe may become activated subsequent to translocation to the perinuclear compartments in a manner similar to the Ras-dependent activation, considering that PIPs exist in the membrane of the Golgi complex and is implicated in the regulation of membrane traffic (42). Taken together, PLCe presumably acts as a downstream effector for Ras or Rap1 at distinct subcellular locations, for which the RA domain plays an essential role.

In contrast to the RA domain, the role of the CDC25 homology domain in Rap family GTPase-dependent regulation of PLCe remains obscure. Herein, we provide evidence that the CDC25 homology domain of PLCe exhibits GEF activity toward Rap1 but not Ras in vitro. These results suggest a mechanism whereby PLCe specifically amplifies Rap1 signaling by yielding Rap1-GTP, which in turn may activate PLC activity. To address this issue, we assessed the role of the CDC25 homology domain in Rap1-GTP formation in vivo and Rap1-dependent translocation to the perinuclear region by employing deletion constructs of PLCe. EGF-induced formation of Rap1-GTP exhibited a prolonged time course depending on the coexpression of PLCe, although the expression of PLCe-N exerted virtually no effect on the time course of EGF-dependent increase in the Rap1-GTP level (Fig. 9). Moreover, EGF-induced translocation of PLCe to the perinuclear region, mediated by Rap1-GTP, exhibited a prolonged time course, whereas translocation of PLCe-N was transient (Fig. 7). Considered as a whole, it is expected that Rap1-dependent PLC activation is sustained at specific subcellular sites such as the Golgi apparatus through the action of the CDC25 homology domain. It is feasible that Rap1 can serve as a regulator of multiple downstream effectors in the perinuclear compartments. Although Rap1-GTP formation in response to EGF is transient as described in Fig. 9A, a subset of Rap1 effectors including PLCe may require sustained interaction with Rap1-GTP for exerting their function. To accomplish this, a complicated regulatory mechanism involving the CDC25 homology domain as described above may be important.

A similar regulatory mechanism of Rap1 signaling is proposed for RA-GEF-1, a Rap1 GEF containing both the RA and the GEF domains (32). The RA domain of RA-GEF-1 is responsible for binding to Rap1-GTP and has a role in translocation to the perinuclear region and amplification of in vivo GEF activity of RA-GEF-1.2 Further, another Rap1 GEF, GFR/MR-GEF, possesses an RA domain that specifically interacts with M-Ras (43, 44). Although the mechanism remains obscure, the MR-GEF-dependent accumulation of Rap1-GTP within the cell was compromised upon coexpression of the activated form of M-Ras (44).

Lopez et al. (26) reported the increase in the Ras-GTP level following the expression of wild-type PLCe and its mutant deficient in PLC activity. Further, the CDC25 homology domain of PLCe induced the activation of ERK (26). However, we detected no GEF activity of PLCe toward Ras in vitro (Fig. 3), and the Ras/Raf-1 pathway was not stimulated by PLCe (Fig. 5). Additionally, no significant increase in the Ras-GTP level was observed in our assays after the expression of the CDC25 homology domain of PLCe that could activate Rap1 (data not shown). Considering our results, the activation of ERK reported by Lopez et al. (26) may possibly be mediated by the endogenous Rap1/RAF pathway, yet the reason for the discrepancy regarding Ras activation remains unclear.

The CDC25 homology domain of PLCe shows GEF activity toward Rap1, but not Rap2 in vitro (Fig. 3), whereas other Rap GEFs, including cAMP-activated Epac/cAMP-GEF (45) and RA-GEF-1 (45),3 show GEF activity toward both Rap1 and Rap2. Hence, signaling involving Rap1, but not Rap2, is enhanced through the action of the CDC25 homology domain of PLCe, and therefore PLCe may play a pivotal role exclusively downstream of Rap1, although differences in biological functions of Rap1 and Rap2 remain obscure. Intriguingly, the tuberous sclerosis-2 gene product tuberin, a GTPase-activating protein for Rap1, but not for Rap2, localizes in the Golgi apparatus (9, 46). Thus, PLCe in collaboration with tuberin may dynamically regulate the GDP/GTP state of Rap1. Future studies will reveal the physiological significance of Rap1-specific signal amplification by PLCe.

REFERENCES

1. Lowy, D. R., and Willumsen, B. M. (1993) Annu. Rev. Biochem. 62, 851–891
2. Katz, M. E., and McCormick, F. (1997) Curr. Opin. Genet. Dev. 7, 75–79
3. Bos, J. L. (1998) EMBO J. 17, 6767–6776
4. Noda, M. (1993) Biochim. Biophys. Acta 1185, 97–109
5. Hu, C.-D., Kariya, K., Kotani, G., Shirouzu, M., Yokoyama, S., and Kataoka, T. (1997) J. Biol. Chem. 272, 11702–11705
6. Okada, T., Hu, C.-D., Jin, T.-G., Kariya, K., Yamawaki-Kataoka, Y., and

2 Y. Liao, T. Satoh, X. Gao, T.-G. Jin, C.-D. Hu, and T. Kataoka, unpublished results.

3 X. Gao, et al., unpublished results.
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Kataoka, T. (1999) Mol. Cell. Biol. 19, 6057–6064
7. Shima, F., Okada, T., Kido, M., Sen, H., Tanaka, Y., Tamada, M., Hu, C.-D., Yamawaki-Kataoka, Y., Kariya, K., and Kataoka, T. (2000) Mol. Cell. Biol. 20, 26–33
8. Beranger, F., Goud, B., Tavitian, A., and de Gunzburg, J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1606–1610
9. Wieseneck, R., Mairre, J. C., Shoorinejad, F., Vass, W. C., Reed, J., Bonifacino, J. S., Resau, J. H., de Gunzburg, J., Yeung, R. S., and DeClue, J. E. (1996) Oncogene 13, 913–923
10. Matsubara, K., Kishida, S., Matsuura, Y., Kitayama, H., Noda, M., and Kikuchi, A. (1999) Oncogene 18, 1303–1312
11. York, R. D., Mulliver, D. C., Grewal, S. S., Stenberg, P. E., McCleskey, E. W., and Stork, P. J. (2000) Mol. Cell. Biol. 20, 8069–8083
12. Tsukamoto, N., Hattori, M., Yang, H., Bos, J. L., and Minato, N. (1999) J. Biol. Chem. 274, 18463–18469
13. Katagiri, K., Hattori, M., Minato, N., Irie, S., Takatsu, K., and Kinashi, T. (2000) Mol. Cell. Biol. 20, 1956–1969
14. Reedquist, K. A., Ross, E., Koop, E. A., Wolthuis, R. M., Zwartkruis, F. J., van Kooyk, Y., Salmon, M., Buckley, C. D., and Bos, J. L. (2000) J. Cell Biol. 148, 1151–1158
15. Schmidt, A., Caron, E., and Hall, A. (2001) Mol. Cell. Biol. 21, 438–448
16. Ponting, C. P., and Benjamin, D. R. (1996) Trends Biochem. Sci. 21, 422–425
17. Nasser, N., Horn, G., Herrmann, C., Scherer, A., McCormick, F., and Wittinghofer, A. (1995) Nature 375, 554–560
18. Huang, L., Weng, X., Hofer, F., Martin, G. S., and Kim, S. H. (1997) Nat. Struct. Biol. 4, 609–615
19. Huang, L., Hofer, F., Martin, G. S., and Kim, S. H. (1998) Nat. Struct. Biol. 5, 422–426
20. Walker, E. H., Perisic, O., Ried, C., Stephens, L., and Williams, R. L. (1999) EMBO J. 18, 7542–7549
21. Pacold, M. E., Suire, S., Perisic, O., Lara-Gonzalez, S., Davis, C. T., Walker, E. H., Hawkins, P. T., Stephens, L., and Williams, R. L. (2000) Cell 103, 931–943
22. Duex, G., Eisenmann, C., L., Fries, H. W., Troppmair, J., and Rapp, U. R. (1994) Trends Biochem. Sci. 19, 474–480
23. Hu, C.-D., Kariya, K., Tamada, M., Akasaka, K., Shirouzu, M., Yokoyama, S., and Kataoka, T. (1995) J. Biol. Chem. 270, 30274–30277
24. Shibatohge, M., Kariya, K., Liao, Y., Hu, C.-D., Watari, Y., Goshima, M., Shima, F., and Kataoka, T. (1998) J. Biol. Chem. 273, 6218–6222
25. Song, C., Hu, C.-D., Masago, M., Kariya, K., Yamawaki-Kataoka, Y., Shibatohge, M., Wu, D., Satoh, T., and Kataoka, T. (2001) J. Biol. Chem. 276, 2752–2757
26. Lopez, I., Mak, E. C., Ding, J., Hamm, H. E., and Lomasney, J. W. (2001) J. Biol. Chem. 276, 2758–2765
27. Kelley, G. G., Reks, S. E., Ondrake, J. M., and Smreka, A. V. (2001) EMBO J. 20, 743–754
28. Gotob, T., Hattori, S., Nakamura, S., Kitayama, H., Noda, M., Takai, Y., Kaibuchi, K., Matsui, H., Hatefi, O., Takahashi, H., Kurata, T., and Matsuda, M. (1995) Mol. Cell. Biol. 15, 6746–6753
29. de Rooij, J., Zwartkruis, F. J., Verheijen, M. H., Cool, R. H., Nijman, S. M., Wittinghofer, A., and Bos, J. L. (1998) Nature 396, 474–477
30. Kawasaki, H., Springett, G. M., Mochizuki, N., Toki, S., Nakaya, M., Matsuda, M., Housman, D. E., and Graybiel, A. M. (1998) Science 282, 2275–2279
31. Kawasaki, H., Springett, G. M., Toki, S., Canales, J. J., Harlan, P., Blumenstiel, J. P., Chen, E. J., Bany, I. A., Mochizuki, N., Ashbacher, A., Matsuda, M., Housman, D. E., and Graybiel, A. M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13278–13283
32. Lian, Y., Kariya, K., Hu, C.-D., Shibatohge, M., Goshima, M., Okada, T., Watari, Y., Gao, X., Jin, T.-G., Yamawaki-Kataoka, Y., and Kataoka, T. (1999) J. Biol. Chem. 274, 37815–37820
33. de Rooij, J., Boenink, N. M., van Triest, M., Cool, R. H., Wittinghofer, A., and Bos, J. L. (1999) J. Biol. Chem. 274, 38125–38130
34. Ohtsuka, T., Hata, Y., Ide, N., Yasuda, T., Inoue, E., Inoue, T., Mizoguchi, A., and Takai, Y. (1999) Biochem. Biophys. Res. Commun. 265, 38–44
35. Pham, N., Cheglakov, I., Koch, C. A., de Hoog, C. L., Morlan, M. F., and Rotin, D. (2000) Curr. Biol. 10, 555–558
36. Borja-Altamira, A. P., Margariti, S. M., Bar-Sagi, D., and Kuriyan, J. (1998) Nature 394, 337–343
37. Kiyono, M., Satoh, T., and Kaziro, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4826–4831
38. Kiyono, M., Suzuki, N., and Kataoka, T. (1999) Science 289, 683–686
39. Wang, Q. J., Bhattacharyya, D., Garfield, S., Nacro, K., Marquez, V. E., and Blumberg, P. M. (1999) J. Biol. Chem. 274, 37233–37239
40. Vossler, M. R., Yao, H., York, R. D., Pan, M. G., Rim, C. S., and Stork, P. J. (1997) Cell 89, 73–82
41. York, R. D., Yao, H., Dillon, T., Ellig, C. L., Eckert, S. P., McCleskey, E. W., and Stork, P. J. (1998) Nature 392, 622–626
42. Roth, M. G. (1999) Trends Cell Biol. 9, 174–179
43. Ichiba, T., Hoshi, Y., Eto, Y., Tajima, N., and Kurihara, Y. (1999) FEBS Lett. 457, 85–89
44. Roskoski, R., Jr., Castello, A. F., and Quilliam, L. A. (2000) J. Biol. Chem. 275, 34901–34908
45. Ohta, Y., Mochizuki, N., Matsuo, K., Yamashita, S., Nakaya, M., Hashimoto, Y., Hamaguchi, M., Kurata, T., Nagashima, K., and Matsuda, M. (2000) Mol. Cell. Biol. 20, 6074–6083
46. Wienecke, R., Konig, A., and DeClue, J. E. (1995) J. Biol. Chem. 270, 16109–16114
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