A number of guanine nucleotide exchange factors have been identified that activate Rho family GTPases, by promoting the binding of GTP to these proteins. We have recently demonstrated that lysophosphatidic acid and several other agonists stimulate phosphorylation of the Rac1-specific exchange factor Tiam1 in Swiss 3T3 fibroblasts, and that protein kinase C is involved in Tiam1 phosphorylation (Fleming, I. N., Elliott, C. M., Collard, J. G., and Exton, J. H. (1997) J. Biol. Chem. 272, 33105–33110). We now show, through manipulation of intracellular [Ca\(^{2+}\)] and the use of protein kinase inhibitors, that both protein kinase Ca and Ca\(^{2+}/\)calmodulin-dependent protein kinase II are involved in the phosphorylation of Tiam1 in vivo. Furthermore, we show that Ca\(^{2+}/\)calmodulin-dependent protein kinase II phosphorylates Tiam1 in vitro, producing an electrophoretic retardation on SDS-polyacrylamide gel electrophoresis. Significantly, phosphorylation of Tiam1 by Ca\(^{2+}/\)calmodulin-dependent protein kinase II, but not by protein kinase C, enhanced its nucleotide exchange activity toward Rac1, by approximately 2-fold. Furthermore, Tiam1 was preferentially dephosphorylated by protein phosphatase 1 in vitro, and treatment with this phosphatase abolished the Ca\(^{2+}/\)calmodulin-dependent protein kinase II activation of Tiam1. These data demonstrate that protein kinase Ca and Ca\(^{2+}/\)calmodulin-dependent protein kinase II phosphorylate Tiam1 in vivo, and that the latter kinase plays a key role in regulating the activity of this exchange factor in vitro.

The Rho family of small GTPases plays an important role in the regulation of several key cellular functions. Rho is involved in the formation of actin stress fibers and focal adhesions (1–3), Rac is required in actin polymerization associated with membrane ruffling and lamellipodia formation in fibroblasts (3, 4), and Cdc42 is important in the formation of filopodia in fibroblasts (3). Moreover, Rho family GTPases are involved in cell cycle progression (5), stimulate gene transcription through activation of the serum response factor (6), activate the Jun kinase and p38 mitogen-activated protein kinase signaling cascades (7–10), enhance Ras-triggered transformation of NIH3T3 cells (16), and that the N-terminal pleckstrin homology domain and an adjacent protein interaction domain are required for membrane localization of the exchange factor (16, 18). Phospholipids may play an important role in determining the cellular localization of Tiam1, since both PIP\(_2\) and PIP\(_3\) bind to its N-terminal pleckstrin homology domain (19), and phosphoinositide 3-kinase activity is required for activation of Rac1 by Tiam1 (20). Reversible protein phosphorylation may also be involved in the regulation of Rho family exchange factors. It has been shown that Dbl (21) and Ost (22) both exist as phosphoproteins in cells. Significantly, tyrosine phosphorylation of the oncogenes Vav (23) and Vav2 (24) by Lck results in increased GDP/GTP nucleotide exchange on Rac1 and RhoA-like GTPases, respectively, and PIP\(_3\) may enhance both phosphorylation and activation of Vav (25). In addition, we have recently demonstrated that lysophosphatidic acid (LPA), platelet-derived growth factor, and several other agonists stimulate phosphorylation of Tiam1 in Swiss 3T3 fibroblasts, via activation of protein kinase C (PKC) (26, 27), indicating that Rho exchange factors can also be phosphorylated on serine/threonine residues by a regulated mechanism.

In this study we demonstrate that Tiam1 is phosphorylated exchange factors for Rho family GTPases have been identified (14). These exchange factors promote binding of GTP by facilitating the release of GDP from Rho proteins. Nucleotide exchange factors which act on Rho proteins contain two key conserved domains: a Dbl homology domain, which is believed to be responsible for catalyzing GDP/GTP exchange; and a pleckstrin homology domain, which seems to be important for cellular localization through interaction with lipids and/or proteins (14). Relatively little is known concerning the specificity of these exchange factors in vivo, although it has been demonstrated that Tiam1 acts as a Rac1-specific exchange factor in NIH3T3 fibroblasts, stimulating membrane ruffling and Jun kinase (15, 16), Lbc acts as a Rho-specific exchange factor, inducing stress fiber formation in Swiss 3T3 cells and foci in NIH3T3 cells (17), and Dbl stimulates Jun kinase in HeLa cells (8).

The mechanism(s) of activation of Rho family nucleotide exchange factors is not yet evident. It has been demonstrated that membrane localization of Tiam1 is required for Rac-dependent membrane ruffling and Jun kinase activation in NIH3T3 cells (16), and that the N-terminal pleckstrin homology domain and an adjacent protein interaction domain are required for membrane localization of the exchange factor (16, 18). Phospholipids may play an important role in determining the cellular localization of Tiam1, since both PIP\(_2\) and PIP\(_3\) bind to its N-terminal pleckstrin homology domain (19), and phosphoinositide 3-kinase activity is required for activation of Rac1 by Tiam1 (20). Reversible protein phosphorylation may also be involved in the regulation of Rho family exchange factors. It has been shown that Dbl (21) and Ost (22) both exist as phosphoproteins in cells. Significantly, tyrosine phosphorylation of the oncogenes Vav (23) and Vav2 (24) by Lck results in increased GDP/GTP nucleotide exchange on Rac1 and RhoA-like GTPases, respectively, and PIP\(_3\) may enhance both phosphorylation and activation of Vav (25). In addition, we have recently demonstrated that lysophosphatidic acid (LPA), platelet-derived growth factor, and several other agonists stimulate phosphorylation of Tiam1 in Swiss 3T3 fibroblasts, via activation of protein kinase C (PKC) (26, 27), indicating that Rho exchange factors can also be phosphorylated on serine/threonine residues by a regulated mechanism.
Regulation of Tiam1 by Protein Phosphorylation

by several PKC isoforms in vitro, but is selectively phosphorylated by a classical PKC isoform, PKCα, when Swiss 3T3 cells are treated with LPA. In addition, we present strong evidence that Ca[2+] 2/calcium-dependent protein kinase II (CamKII) also phosphorylates Tiam1 in Swiss 3T3 fibroblasts in response to LPA treatment and that this phosphorylation produces electrophoretic retardation on SDS-polyacrylamide gel electrophoresis. Finally, we show that phosphorylation of Tiam1 by Ca[2+] 2/calcium-dependent protein kinase II, but not protein kinase Cα, enhances its nucleotide exchange rate toward Rac1, and that this can be abrogated by treatment with protein phosphatase 1.

EXPERIMENTAL PROCEDURES

Materials—Swiss 3T3 fibroblasts were obtained from the American Type Culture Collection. Fetal bovine serum, Dulbecco’s modified Eagle’s medium (DMEM), penicillin, and streptomycin were from Life Technologies, Inc. LPA (1-octeyl) was obtained from Avanti Polar lipids. Phorbol 12-myristate 13-acetate (PMA), sodium orthovanadate, leupeptin, antipain, phenylmethylsulfonyl fluoride, sodium fluoride, sodium pyrophosphate, Tween 20, Triton X-100, and fatty acid-free bovine serum albumin were obtained from Sigma. Ro-31-8220, KN93, 12-myristate 13-acetate (PMA), sodium orthovanadate, leupeptin, 15 mM MgCl2, and 100 μM [γ-32P]ATP (specific activity 5 × 106 dpm/nmol) and phosphorylation analysis by autoradiography. Purified GST-Tiam1 (1 μl) was incubated for 20 min at 30°C in the presence or absence of the indicated amounts of purified CamKII. GST-Tiam1 was carried out in 50 mM Tris buffer, pH 7.4, containing 0.1 mg/ml BSA, 1.25 mM CaCl2, 25 μg/ml calmodulin, 15 mM MgCl2 and 100 μM ATP. Assays were carried out either using non-radio-labeled ATP and phosphorylation analysis by Western blotting or with [γ-32P]ATP (specific activity 5 × 106 dpm/nmol) and phosphorylation analysis by autoradiography.

Since the purified Tiam1 preparations contained detergent and some aggregated protein, the concentration of purified Tiam1 was estimated from silver-stained gels for the stoichiometry experiments, using BSA as a standard. 0.2 ml of GST-Tiam1 was phosphorylated by PKCα (0.3 units) or CamKIIα (4 μg), for 1 h in the presence of [γ-32P]ATP (specific activity 2 × 107 dpm/nmol), as described above. The samples were separated by SDS-PAGE on 6% gels, the Tiam1 band excised from the gel and 32P incorporation assessed by scintillation counting.

Diphosphorylation of Tiam1—Purified GST-Tiam1 (10 μl) was phosphorylated with PKCα (0.3 units) or CamKIIα (4 μg), for 1 h in the presence of [γ-32P]ATP (specific activity 2 × 107 dpm/nmol), as described above. Phosphorylated GST-Tiam1 was incubated with 30 μl of glutathione-Sepharose beads for 1 h at 30°C, and the beads collected by centrifugation (3,000 × g × 5 min). The Tiam1-bound beads were washed three times with 200 μl of 50 mM Tris buffer, pH 7.0, containing 0.5 mg/ml BSA to remove the kinase, resuspended in 50 μl of the same buffer, and stored on ice until use.

Tiam1-bound beads (5 μl) were incubated with 0.3 units of purified protein phosphatase 1, 2A or 2B at 30°C for 0 and 5 min. Protein phosphatase 1-catalyzed dephosphorylation was carried out in 50 mM Tris buffer, pH 7.0, containing 0.5 mg/ml BSA and 0.2 mM MnCl2. Protein phosphatase 2B was incubated with Tiam1 in 50 mM Tris buffer, pH 7.0, containing 0.5 mg/ml BSA. Protein phosphatase 2B-catalyzed dephosphorylation was carried out in 50 mM Tris buffer, pH 7.0, containing 0.5 mg/ml BSA, 20 μg/ml calmodulin, and 1 mM CaCl2. The samples were separated by electrophoresis on 6% polyacrylamide gels and Tiam1 dephosphorylation analyzed by autoradiography.

RESULTS

Role of Protein Kinase C Isozymes in Tiam1 Phosphorylation in Vitro—In Swiss 3T3 fibroblasts, LPA stimulates threonine phosphorylation of Tiam1 through activation of PKC, and causes its electrophoretic retardation on SDS-PAGE (26). To understand further the mechanism of Tiam1 phosphorylation, we incubated purified GST-C1199-Tiam1 with several PKC isoforms to determine which isoform(s) phosphorylates the test factor. As shown in Fig. 1, all of the kinases tested phosphorylated Tiam1, indicating that PKC isoforms of the...
classical, novel, and atypical families can phosphorylate the protein in vitro. However, the different PKC isoforms phosphorylated Tiam1 to different extents. The exchange factor was preferentially phosphorylated by PKCa, -γ, and -ζ, moderately phosphorylated by PKCe, and only weakly phosphorylated by PKCβ1, -β2, and -δ. Significantly, none of the PKC isozymes tested decreased the electrophoretic mobility of Tiam1, suggesting that this was probably caused by a kinase from a different family in vivo.

Role of Ca2+/Calmodulin-dependent Protein Kinase II in Tiam1 Phosphorylation—Down-regulation of non-atypical PKC isoforms by long term PMA pretreatment, or preincubation with the protein kinase C inhibitor Ro-31-8220, reduces LPA- or platelet-derived growth factor-stimulated Tiam1 phosphorylation by approximately 75% in Swiss 3T3 cells (26, 27), suggesting that another protein kinase is also involved. Therefore, purified GST-C1199-Tiam1 was incubated with Ca2+/calmodulin-dependent protein kinase II (CamKII), a kinase with a very broad substrate specificity and widespread expression (30), to determine whether this kinase phosphorylated the exchange factor. Although some phosphorylation of Tiam1 was observed in the absence of CamKII (Fig. 2A), perhaps due to a protein kinase which co-purifies with the GST-Tiam1 (26), addition of the kinase significantly enhanced 32P phosphorylation of the exchange factor (Fig. 2A), demonstrating that this kinase can phosphorylate Tiam1. Indeed, Western blotting with antibodies confirmed that CamKII stimulated phosphorylation of Tiam1 on threonine (Fig. 2B). Significantly, in addition to phosphorylating Tiam1, CamKII induced electrophoretic retardation of the exchange factor (Fig. 2, such as is observed upon stimulation of Swiss 3T3 cells with LPA (26). Ca2+/calmodulin-dependent protein kinase II induced the Tiam1 bandshift in a concentration-dependent (Fig. 2B) and time-dependent (Fig. 2C) manner, but only in the presence of Ca2+ and calmodulin (data not shown). Intriguingly, the Tiam1 bandshift occurred in a gradual manner with time, and not as one step, suggesting that the exchange factor probably exists in several different phosphorylation states and has multiple phosphorylation sites which serve as substrates for CamKII. Indeed, when the Tiam1 protein concentration was estimated by silver staining, using BSA as a standard, stoichiometry experiments indicated that under maximal phosphorylating conditions, Tiam1 contains 10.1 ± 2.7 PKCo and 3.7 ± 0.6 CamKII phosphorylation sites.

Role of PKC and CamKII in Tiam1 Phosphorylation in Vitro—Swiss 3T3 cells were stimulated with LPA, in the presence and absence of the intracellular Ca2+ chelator BAPTA/AM, to investigate the importance of this metal ion in Tiam1 phosphorylation. The results (Fig. 3A) show that Tiam1 phosphorylation is totally abolished in the presence of the chelator, indicating that Ca2+ plays an essential role in this pathway. Together with the results obtained using protein kinase inhibitors, and PKC down-regulation (26), this suggests that LPA stimulates Tiam1 phosphorylation through activation of a classical PKC isoform and another Ca2+-dependent enzyme. Therefore, since Swiss 3T3 cells only contain PKCα, -δ, -ε, and -ζ (31), LPA must stimulate Tiam1 phosphorylation through activation of PKCo, which is the only classical Ca2+-dependent enzyme present. Significantly, BAPTA treatment also inhibited the LPA-stimulated Tiam1 bandshift (Fig. 3B), indicating that Ca2+ is required for this effect. However, the selective PKC inhibitors bisindolylmaleimide I (Fig. 3C) and Ro-31-8220 (data not shown) had no effect on the LPA-induced Tiam1 bandshift, providing further evidence that PKC does not cause this.

To confirm that CamKII is involved in LPA-induced Tiam1 phosphorylation, Swiss 3T3 cells were preincubated with the CamKII inhibitor KN93 (20 μM) for 24 h, in the presence and absence of the PKC inhibitor Ro-31-8220 (5 μM) for 1 h. As expected (26), Ro-31-8220 greatly reduced LPA-stimulated Tiam1 phosphorylation (Fig. 3D). KN93 also significantly reduced LPA-induced Tiam1 phosphorylation, and the two inhibitors together almost completely eliminated the phosphorylation (Fig. 3D). Therefore, these data strongly suggest that CamKII and PKC both contribute to the phosphorylation studied here.

To provide additional evidence that PKCo and CamKII phosphorylate Tiam1 in vivo, Swiss 3T3 cells were treated with the Ca2+ ionophore ionomycin, in the presence and absence of PMA. PMA (1 μM) alone induced limited threonine phosphorylation of Tiam1 (Fig. 3E; Ref. 26). Ionomycin (1 μM) alone stimulated Tiam1 phosphorylation to a greater extent (Fig. 3E), and enhanced the PMA-stimulated Tiam1 phosphorylation. Similar results were obtained with the ionophore A23187 (data not shown). Therefore, the observation that PMA and a Ca2+ ionophore are sufficient to stimulate Tiam1 phosphorylation is consistent with a classical PKC isoform and CamKII phosphorylating the exchange factor in vivo.

Dephosphorylation of Tiam1—We have previously established that LPA-stimulated Tiam1 phosphorylation is maximal.
at 2.5 min, begins to decrease after 10 min LPA treatment, but is still readily detectable after 60 min of LPA treatment (26). Further experiments showed that Tiam1 phosphorylation was still detectable after 3 h of LPA treatment, but that the stimulation was lost after 4 h (data not shown), presumably because of dephosphorylation. To elucidate further the mechanisms involved in controlling the level of Tiam1 phosphorylation, we investigated which phosphatases are involved in the dephosphorylation process. The results show that Tiam1 is preferentially dephosphorylated by the catalytic subunit of PP1 in vitro, when the exchange factor is phosphorylated by PKCo or CamKKII (Fig. 4). Tiam1 was also dephosphorylated by protein phosphatase 2A in vitro, but at a much slower rate (Fig. 4). Interestingly, protein phosphatase 2A slowly dephosphorylated Tiam1 when it was phosphorylated by CamKKII, but not when it was phosphorylated by PKCo.

Effects of Phosphorylation on the GDP/GTP Exchange Activity of Tiam1—Since Tiam1 acts as a Rac1-specific exchange factor in NIH3T3 fibroblasts, stimulating membrane ruffling and Jun kinase activity (15, 16), we investigated whether protein phosphorylation could affect Tiam1 GDP/GTP exchange activity toward Rac1. Purified hexahistidine-tagged Tiam1 protein was incubated with ATP in the presence or absence of purified CamKKII, and the GDP/GTP exchange rate of Tiam1 assessed by following the dissociation of [3H]GDP from Rac1. As expected (15), Tiam1 stimulated release of [3H]GDP from GST-Rac1 in a concentration-dependent (data not shown) and time-dependent manner (Fig. 5, A and B). Importantly, preincubation of Tiam1 with CamKKII stimulated the exchange ac-

**Fig. 4.** Tiam1 is preferentially dephosphorylated by protein phosphatase 1 in vitro. Purified GST-Tiam1 was phosphorylated in the presence of [γ-32P]ATP by protein kinase C (A) or Ca2+/calmodulin-dependent protein kinase II (B), then repurified using glutathione beads to remove the kinase, as described under “Experimental Procedures.” Tiam1-bound beads were incubated with 0.3 units of purified protein phosphatase 1, 2A, or 2B at 30 °C for 0 and 5 min. The samples were separated by electrophoresis on 6% polyacrylamide gels and Tiam1 dephosphorylation analyzed by autoradiography. Results are representative of three independent experiments.

DISCUSSION

The data presented here suggest that the classical PKC isozyme, PKCo, and Ca2+/calmodulin-dependent protein kinase II, both phosphorylate the Rac1-specific exchange factor, Tiam1, in response to LPA treatment of Swiss 3T3 fibroblasts. Furthermore, this phosphorylation is likely to be functionally important, since CamKKII treatment enhances the GDP/GTP exchange activity of Tiam1 (Fig. 5A). This is the first evidence that Rho family exchange factors can be activated by serine/threonine phosphorylation, and it is likely to be a general regulatory mechanism for Tiam1, since it is phosphorylated by several different agonists in Swiss 3T3 cells (26).

While we have previously established that LPA stimulates Tiam1 phosphorylation through activation of PKC (26), the fact that neither PKC inhibitors nor long term PMA treatment could completely abrogate this effect suggested that a second protein kinase was also involved in this pathway. This hypothesis is supported by the observation that LPA-induced electrophoretic retardation of Tiam1 is partially inhibited by staurosporine (26), but not by the PKC-specific inhibitors bisindolylmaleimide I (Fig. 3C) and Ro-31-8220, and by the fact that none of the PKC isozymes tested decreased the electrophoretic mobility of the exchange factor (Fig. 1). Several lines of evidence indicate that the second kinase involved in Tiam1 phosphorylation is Ca2+/calmodulin-dependent protein kinase
Regulation of Tiam1 by Protein Phosphorylation

II. First, the LPA-induced Tiam1 bandshift (Fig. 3B) and the PKC-independent (26) threonine phosphorylation of Tiam1 in LPA-stimulated fibroblasts (Fig. 3A) are both inhibited by BAPTA/AM, indicating that Ca\(^{2+}\) is essential for these effects. Second, the PKC-independent phosphorylation of Tiam1 in LPA-stimulated fibroblasts (26) was almost totally eliminated by the CamKII inhibitor KN93 (Fig. 3D). Furthermore, recombinant CamKII phosphorylated purified GST-Tiam1 in vitro, and incubation with this kinase also caused the distinctive Tiam1 bandshift (Fig. 2). Finally, most serine/threonine protein kinases predominantly phosphorylate serine residues, whereas CamKII can also phosphorylate threonine.

The conclusion that CamKII and PKC coordinately phosphorylate Tiam1 is supported by the fact that the exchange factor contains approximately 10.1 ± 2.7 PKC sites and 3.7 ± 0.6 CamKII sites in vitro, and PKC catalyzes 70–75% of the LPA-induced Tiam1 phosphorylation in vivo (26). Indeed, CamKII may account for the sustained nature of the Tiam1 bandshift and phosphorylation (26), since Ca\(^{2+}\) stimuli can induce CamKII to autophosphorylate to a Ca\(^{2+}\)-independent form, which retains kinase activity even after Ca\(^{2+}\) levels decline (32). The finding that Tiam1 contains 3–4 CamKII phosphorylation sites is consistent with the observation that the kinase stimulates the Tiam1 bandshift in a gradual manner, and not in one step (Fig. 3). Furthermore, Tiam1 is particularly rich in serine and threonine residues (33) and contains several potential CamKII phosphorylation consensus sequences. Although Tiam1 contains approximately 3 times more PKC sites than CamKII sites, PKC does not alter the electrophoretic mobility of the exchange factor. These results suggest that the CamKII phosphorylation sites are located close together on the Tiam1 protein, and may cause a change in the conformation of the exchange factor.

Since Ca\(^{2+}\) and PMA are intimately involved in Tiam1 phosphorylation (Fig. 3), it seems likely that it is stimulated via the PLC pathway, which generates diacylglycerol and inositol 1,4,5-trisphosphate, second messengers that activate PKC and mobilize Ca\(^{2+}\) respectively. This agrees with the facts that nanomolar concentrations of LPA activate Tiam1 phosphorylation, via a pertussis toxin-insensitive mechanism, and that Tiam1 phosphorylation is stimulated by LPA, platelet-derived growth factor, endothelin-1, bombesin, and bradykinin (26), agonists which activate PLC and PKC (34–36), but not by epidermal growth factor, which produces barely detectable phosphoinositide hydrolysis in Swiss 3T3 cells (34). Indeed, PLC-\(\gamma\) is required for platelet-derived growth factor-induced phosphorylation of Tiam1 (27), and the PLC inhibitor U-73122 abolishes Tiam1-induced cell invasion in T-lymphoma cells (37), indicating that PLC is functionally important in regulation of this exchange factor. Indeed, since CamKII is activated via the PLC pathway in many cell types (32), and CamKII activates Tiam1 (Fig. 5A), PLC probably regulates Tiam1 through stimulation of this kinase.

The Rho exchange factors Vav and Vav2 are also regulated through protein phosphorylation (23, 24). However, these proteins are activated through tyrosine phosphorylation by Lek (23, 24), whereas Tiam1 is activated via threonine phosphorylation (26), by CamKII (Fig. 5A). Interestingly, while Vav is totally inactive until phosphorylated (23), both Vav2 (24) and Tiam1 (Fig. 5) have a low basal rate of exchange activity. This basal activity may partially explain why phosphorylation does not stimulate the exchange activity of Tiam1 (Fig. 5A) or Vav2 (24), as much as Vav (23). Alternatively, Tiam1 may be regulated by additional factors. However, phosphorylation by PKC does not appear to be this signal, since it does not affect Tiam1 GDP/GTP exchange activity (Fig. 5B). Protein phosphatase 1 treatment had no significant effect on control Tiam1 activity (Fig. 5C), suggesting that the basal exchange activity is not due to serine/threonine phosphorylation of the protein in Sf9 cells. This eliminates the possibility that PKC does not regulate

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**Fig. 5.** Effect of Ca\(^{2+}\)/calmodulin-dependent protein kinase II, protein kinase C, and protein phosphatase 1 on the GDP/GTP exchange activity of Tiam1. Nucleotide exchange reactions were carried out as described under "Experimental Procedures," using [\(^{3}H\)]GDP-loaded GST-Rac1 as substrate, for the indicated times. Exchange assays were carried out in the presence of autophosphorylated CamKII [\(^{\circ}\)], Tiam1 [\(^{\circ}\)], or Tiam1 plus CamKII [\(^{\circ}\)] (A), autophosphorylated PKC [\(^{\circ}\)], Tiam1 [\(^{\circ}\)], or Tiam1 plus PKC [\(^{\circ}\)] (B), or Tiam1, Tiam1 plus CamKII, Tiam1 plus PP1, or Tiam1, CamKII, and PP1 [\(^{\circ}\)]. C, shaded bars, 0 min; striped bars, 20 min. Results are the mean ± standard error of at least three independent experiments.
Tiam1 activity in vitro because of prior phosphorylation of a key PKC regulatory site. On the other hand, PKC treatment eliminated the CamKII-stimulated activation of Tiam1 (Fig. 5C), returning Tiam1 exchange activity to basal levels. Therefore, unlike the activation of p115 by Ga13 (38), Tiam1 activation is due to reversible phosphorylation rather than a direct protein-protein interaction.

The function of Tiam1 phosphorylation by PKC is not yet apparent. It remains possible that phosphorylation plays a role in the regulated membrane localization of the exchange factor (16). Alternatively, this phosphorylation may regulate the activity of Tiam1 against other potential GTPases. It is clear that CamKII and PKC phosphorylate different sites on Tiam1, since only CamKII causes the electrophoretic retardation (Fig. 2) and activation (Fig. 5A) of Tiam1. However, a possible interaction between the two kinases in the regulation of the phosphorylation and activation of Tiam1 has not been explored. It is also not yet apparent how CamKII activates Tiam1, but it seems likely that the phosphorylation causes a key change in the conformation of Tiam1. This could involve reorientation of the Dbl homology domain and pleckstrin homology domains (39), perhaps allowing the GTPase easier access to its binding site, or enhancing the GDP/GTP exchange reaction by another mechanism.

Rac1 affects many cellular processes, including gene transcription activated by the serum response factor (6), membrane ruffling and lamellipodia formation (3, 4), activation of the Jun kinase pathway (7–9), cell cycle progression (5), and phospholipase D activity (40). Moreover, LPA regulates several signaling pathways that involve Rho family GTPases including stress fiber formation (1), gene transcription through activation of the serum response factor (6), and phospholipase D (41). The work of Collard and associates indicates that Tiam1 produces the same cytoskeletal changes as induced by Rac1 and also activates the Jun kinase pathway (15, 16). However, it is not yet clear if the other pathways are regulated by Tiam1 in vivo, or if other exchange factors are involved in the effects of Rac1 on the cytoskeleton and these other signaling processes. Further work will also be required to determine the role of PKC in Tiam1 regulation, and elucidate the molecular mechanism by which CamKII activates Tiam1.

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