Members of the Rho subfamily of Ras-related GTP-binding proteins play important roles in the organization of the actin cytoskeleton and in the regulation of cell growth. We have shown previously that the dbl oncogene product, which represents a prototype for a family of growth regulatory proteins, activates Rho subfamily GTP-binding proteins by catalyzing the dissociation of GDP from their nucleotide binding site. In the present study, we demonstrate that the acidic phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP$_2$), provides an alternative mechanism for the activation of Cdc42Hs. Among a variety of lipids tested, only PIP$_2$ was able to stimulate GDP release from Cdc42Hs in a dose-dependent manner, with a half-maximum effect at $\sim$50 $\mu$M. Unlike the Dbl oncoprotein, which requires the presence of (free) guanine nucleotide in the medium to replace the GDP bound to Cdc42Hs, PIP$_2$ stimulates GDP release from Cdc42Hs in the absence of free guanine nucleotide. PIP$_2$, when incorporated into phosphatidylycholine carrier vesicles, binds tightly to the guanine nucleotide-depleted form of Cdc42Hs and weakly to the GDP-bound form of the GTP-binding protein but does not bind to GTP-bound Cdc42Hs, similar to what was observed for the Dbl oncoprotein. However, mutational analysis of Cdc42Hs indicates that the site that is essential for the functional interaction between PIP$_2$ and Cdc42Hs is distinct from the Dbl-binding site and is located at the positively charged carboxyl-terminal end of the GTP-binding protein. The GDP-releasing activity of PIP$_2$ is highly effective toward Cdc42Hs and Rho (and is similar to the reported effects of PIP$_2$ on Arf (Terui, T., Kahn, R. A., and Randazzo, P. A., (1994) J. Biol. Chem. 269, 28130–28135), is less effective with Rac, and is not observed with Ras, Rap1α, or Ran. The ability of PIP$_2$ to activate Cdc42Hs (or Rho) and Arf provides a possible point of convergence for the biological pathways regulated by these different GTP-binding proteins and may be related to the synergism observed between Arf and Rho subtype proteins in the stimulation of phospholipase D activity (Singer, W. D., Brown, H. A., Bokoch, G. M., and Sternweis, P. C. (1995) J. Biol. Chem. 270, 14944–14950).

The Rho subfamily of Ras-related GTP-binding proteins, which includes RhoA, Rac1, and Cdc42Hs, has been shown to regulate a diversity of cellular functions ranging from actin-mediated cytoskeletal rearrangements (Hall, 1994) to the stimulation of nuclear mitogen-activated protein kinases (Cosso et al., 1995; Minden et al., 1995; Bagrodia et al., 1995; Zhang et al., 1995), transcription (Hill et al., 1995), and DNA synthesis ( Olson et al., 1995). It is now well established that Rac1 is essential for growth factor-stimulated membrane ruffling and lamellipodia formation (Ridley et al., 1992) and acts downstream from Ras in the stimulation of cell growth (Qui et al., 1995), whereas RhoA controls the formation of stress fibers and focal adhesion complexes (Ridley and Hall, 1992). Cdc42 has been shown to be essential for bud-site assembly in Saccharomyces cerevisiae (Johnson and Pringle, 1990), for unidirectional and bidirectional cell growth in Schizosaccharomyces pombe (Miller and Johnson, 1994), and for filopodia formation in mammalian cells (Nobes and Hall, 1995; Kozma et al., 1995). The macromolecular targets for the Rho subfamily GTP-binding proteins are just now beginning to be identified. Both GDP-bound Rac1 and Cdc42Hs bind to the $\mu$, 85,000 regulatory subunit (p85) of the phosphatidylinositol 3-kinase (Zheng et al., 1994a; Tolias et al., 1995), to the p70 S6 kinase (Chou and Blenis, 1996), and to members of the p21-activated serine/threonine kinase (PAK) family (Manser et al., 1994; Martin et al., 1995; Bagrodia et al., 1995). The GDP-bound form of RhoA also has been reported to stimulate phosphatidylinositol 3-kinase activity in platelets (Zhang et al., 1993) as well as phosphatidylinositol 4-phosphate 5-kinase activity (Chong et al., 1994). In addition, it recently has been shown that RhoA and Cdc42Hs can act synergistically with Arf to stimulate phospholipase D activity (Singer et al., 1995).

Understanding how these different target activities are stimulated and then integrated to yield cytoskeletal changes and nuclear activities represents a formidable challenge. One approach is to determine how Rho subfamily GTP-binding proteins are activated, since this should represent the first key step in stimulating their target activities. One mode of activation of Rho subtype GTP-binding proteins occurs through the stimulation of GDP dissociation (and consequently GTP/GDP exchange) by the family of Dbl-related proteins. The prototype for this family of guanine nucleotide exchange factors (GEFs) is the Dbl oncoprotein, which was shown to act as a GEF for Cdc42Hs and RhoA (Hart et al., 1991; Hart et al., 1994). At present, 15 members of the Dbl-related family have been identified and characterized (Cerione and Zheng, 1996), with each member containing the characteristic Dbl homology domain in tandem with a Pleckstrin homology domain. Many of these
proteins have been shown to have GEF activity including Cdc24 (Zheng et al., 1994b), Ost (Horii et al., 1994), Tian-1 (Michiels et al., 1995), and Lbc (Zheng et al., 1995). Similar to the case for the interactions between heterotrimeric GTP-binding proteins (G proteins) and agonist-stimulated heptahelical receptors (Gilman, 1987), the Dbl-related GEFs bind preferentially to and stabilize the guanine nucleotide-depleted states of Rho-like GTP-binding proteins. It is interesting that many of these Dbl-related proteins show a selective tissue distribution, whereas many of the Rho subtype proteins (e.g. Cdc42Hs, RhoA, and Rac1) appear to be ubiquitous. This suggests that additional but as yet undiscovered Dbl-related proteins exist and/or that other mechanisms may be used to stimulate the activation of Rho-subtype GTP-binding proteins. In the present study, we describe one such potential alternative mechanism where the lipid phosphatidylinositol 4,5-bisphosphate (PIP2) is able to strongly stimulate GDP dissociation from Cdc42Hs and RhoA.

EXPERIMENTAL PROCEDURES

Materials—The small GTP-binding proteins Cdc42Hs, RhoA, Rac1, and Ha-Ras were expressed and purified as glutathione S-transferase (GST) fusion proteins from Escherichia coli as described previously (Hart et al., 1994). The cDNAs encoding the K-Ras protein and Ras GRF were kind gifts from Dr. Larry Feig (Tufts Medical School) and were expressed in E. coli as GST fusion proteins. The Rap1A protein was obtained from Dr. P. Polakis (Onyx Pharmaceutical, Emoryville, CA). The cDNA encoding Ran was the generous gift of Dr. M. Rush (New York University Medical Center, New York, NY). To express Ran in E. coli as a GST fusion protein, restriction sites for NcoI and HindIII were introduced immediately adjacent to the initiation and termination codons, respectively, using the polymerase chain reaction. The polymerase chain reaction product (730 base pairs) was then ligated with NcoI/HindIII-digested pGEX-KG (Pharmacia Biotech Inc.), and the resultant GST-Ran fusion protein was purified from transformed E. coli (JM101). Automated DNA sequencing revealed no mutations in the polymerase chain reaction product. The anti-Cdc42Hs polyclonal antibody bodies were raised against the unique carboxyl-terminal sequences of Cdc42Hs as described (Shinjo et al., 1990). GST-Dbl protein was expressed in a baculovirus/Sf9 insect cell system (Hart et al., 1994), and the Cdc42Hs-GTPase-activating protein was expressed and purified from E. coli (Barford et al., 1993). The ΔC7 Cdc42Hs truncation mutant was generated by polymerase chain reaction using the plaque-forming unit DNA polymerase (Stratagene), and the resulting sequences were verified through fluorescence automated sequencing. Lipids were purchased either from Sigma or from Avanti Polar Lipids. All lipids were dissolved in chloroform, dried under nitrogen, and resuspended by sonication in 50 mM Tris-HCl (pH 8.0) immediately prior to use. The pH was adjusted as necessary with NaOH. Radioisotope-labeled guanine nucleotides were obtained from DuPont NEN.

GDP Dissociation and GTP/GDP Exchange Assays—GDP dissociation and GTP/GDP exchange assays were carried out as described previously (Hart et al., 1991). Two μg of Cdc42Hs loaded with [3H]GDP was incubated with buffer mixtures containing 100 mM NaCl, 20 mM Tris- HCl, pH 7.4, and 5 mM MgCl₂ (buffer A) with various lipids or Dbl proteins for the indicated times at room temperature. Assays monitoring the dissociation of GDP were stopped by dilution (20-μl aliquots) into 10-μl ice-cold buffer A, and the protein-bound nucleotide was trapped by filtration on nitrocellulose filters. For GTP/GDP exchange assays, 1 mM GTP was also included in the reaction buffer.

GTPyS Binding Assays—Assays monitoring the dissociation of GTPyS from Cdc42Hs were performed as described above for GDP, except that [35S]GTPyS was used, and the concentration of Cdc42Hs-GTPyS was ~0.4 μM. GTPyS binding was determined as in Hart et al. (1991). Two μg of GDP-bound Cdc42Hs were incubated in buffers containing 100 mM NaCl, 20 mM Tris- HCl, pH 7.4, 5 mM MgCl₂ (buffer A) with various lipids or Dbl proteins for the indicated times at room temperature. Assays monitoring the dissociation of GDP were stopped by dilution (20-μl aliquots) into 10-μl ice-cold buffer A, and the protein-bound nucleotide was trapped by filtration on nitrocellulose filters. For GTP/GDP exchange assays, 1 mM GTP was also included in the reaction buffer.

Liposome-Protein Complex Formation Assays—Direct binding of liposome vesicles containing PI3_P2 to Cdc42Hs was carried out by adapting the centrifugation protocol by Harlan et al. (1994). Briefly, Cdc42Hs was first loaded with GDP or GTPyS or depleted with nucleotide (Hart et al., 1994). One μg of Cdc42Hs was then added to lipid vesicles (100 μM of total volume) with 500 μM carrier PC alone or 100 μM PI3_P2, incorporated into the PC carrier vesicles through co-sonication. The mixture was incubated for 5 min before centrifugation in an ultracentrifuge Beckman airfuge (100,000 × g) for 30 min. The vesicles pelleted with this treatment were subjected to SDS-polyacrylamide gel electrophoresis and anti-Cdc42Hs Western blot analysis, and the blot was visualized by the ECL method (DuPont NEN).

RESULTS AND DISCUSSION

The mode of regulation of Rho family GTP-binding proteins has been an area of intense research investigation (Boguski and McCormick, 1993) due to the involvement of these GTP-binding proteins in the stimulation of cytoskeletal changes and transcriptional activities as well as in the regulation of cell growth. In addition to protein factors that directly stimulate the guanine nucleotide exchange activities of Rho-subtype GTP-binding proteins, for which the Dbl oncogene is a prototype (Cerione and Zheng, 1996), various phospholipids have been implicated in the regulation of the Rac GTP-binding proteins through their effects on the GDP dissociation inhibitor molecule (Chuang et al., 1993). In addition, the GTP-binding protein Arf, which undergoes GDP dissociation in response to PI2, appears to act synergistically with Rho-subtype proteins to stimulate (in a PI2-sensitive manner) phospholipase D activity (Malcolm et al., 1994; Singer et al., 1995; Moss and Vaughan, 1995). The latter finding is particularly interesting since we recently have found that Cdc42Hs is predominantly localized to Golgi membranes in mammalian cells and that its localization is influenced by different Arf mutants in a manner suggesting some type of interplay between Arf and Cdc42Hs.

Given these findings, we examined whether various phospholipids had any effect on the GTP-binding/GTPase cycle of Cdc42Hs.

Fig. 1A shows the results obtained when examining the effects of a panel of phospholipids on the rate of [3H]GDP dissociation from Cdc42Hs. Only PI2 showed a significant stimulation of GDP dissociation from Cdc42Hs. Essentially no effect was observed when phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, or phosphatidylinositol was incubated with [3H]GDP-bound Cdc42Hs. We also have not detected any effects with inositol trisphosphate under conditions where PI2 strongly stimulates [3H]GDP dissociation; however, PI2, at concentrations >500 μM, caused a slight increase in the rate of [3H]GDP dissociation (data not shown).

Fig. 1B shows that the PI2-stimulated dissociation of GDP from Cdc42Hs is dose-dependent, with a half-maximal effect occurring at ~50 μM PI2. At PI2 levels ≥200 μM, the rate of GDP release from Cdc42Hs was increased ~10 fold.

The stimulation of GDP dissociation by PI2 was similar to that elicited by the oncoenic Dbl protein (Hart et al., 1994). Fig. 2A shows time courses for the dissociation of [3H]GDP from Cdc42Hs in the absence of activators or in the presence of the Dbl oncogene (0.5 μM) or PI2 (100 μM). In these experiments, the dissociation of GDP was assayed in the presence of 1 mM GTP in the medium. Under these conditions, the half-time for [3H]GDP dissociation from Cdc42Hs (in the presence of phosphatidylycholine as a control) was ~25 min, whereas Dbl-catalyzed GDP dissociation occurred with a half-time of ~1.5 min, and PI2-stimulated GDP dissociation occurred with a half-time of ~2.5 min.

It is interesting that when GDP dissociation was assayed in the absence of medium GTP, dramatic differences were observed between Dbl and PI2 (Fig. 2B). Specifically, PI2 was still capable of providing a strong stimulation of the initial rate of [3H]GDP dissociation, whereas oncoenic Dbl failed to induce any detectable stimulatory effect.

These findings can be considered within the context of the

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* Erickson, J. W., Zhang, C.-J., Kahn, R. A., Evans, T., and Cerione, R. A. (1996) J. Biol. Chem. 271, in press.
generally accepted model for the interactions of guanine nucleotides and GEFs with GTP-binding proteins. It typically has been assumed that GEFs act as antagonists of guanine nucleotide binding and that conversely, nucleotide binding weakens the interactions of GEFs with GTP-binding proteins. Thus, although Dbl weakens the affinity of GDP for Cdc42Hs, the amount of GDP present (i.e. initially bound to Cdc42Hs) is sufficient to maintain occupancy of the nucleotide site, even in the presence of this GEF. However, when a high excess of GTP is included in the medium, it effectively competes with GDP for the nucleotide site, thereby resulting in Dbl-catalyzed GTP-GDP exchange. This differs from the case for PIP2, where this putative GEF is apparently more effective than Dbl in weakening the affinity of GDP for Cdc42Hs, the amount of GDP present (i.e. initially bound to Cdc42Hs) is sufficient to maintain occupancy of the nucleotide site, even in the presence of this GEF. However, when a high excess of GTP is included in the medium, it effectively competes with GDP for the nucleotide site, thereby resulting in Dbl-catalyzed GTP-GDP exchange. This differs from the case for PIP2, where this putative GEF is apparently more effective than Dbl in weakening the affinity of Cdc42Hs for guanine nucleotides. PIP2 strongly stabilizes the guanine nucleotide-depleted state of Cdc42Hs; thus, it is difficult to observe a PIP2-stimulated exchange of GDP for [35S]GTPγS. In fact, under conditions where Dbl is able to elicit a strong stimulation of [35S]GTPγS binding (when [GTPγS] = 0.5–1 μM), the addition of PIP2 shows no detectable stimulation of GTPγS binding (Fig. 2C). In addition, although PIP2 is more effective than Dbl in weakening the binding of guanine nucleotides to Cdc42Hs, it appears that guanine nucleotides also weaken the binding of PIP2 to a greater extent than the binding of Dbl. Thus, although Dbl shows a weak but measurable stimulation of [35S]GTPγS dissociation from Cdc42Hs, the addition of PIP2 (at 50–100 μM) has no effect (data not shown).

Taken together, the results presented in Fig. 2 would suggest...
that PIP₂ is capable of a direct interaction with Cdc42Hs and that this interaction is significantly influenced by the guanine nucleotide bound to the GTP-binding protein. Fig. 3 shows the results of such a direct binding experiment, using PC vesicles containing PIP₂. In this experiment, PC vesicles alone and PC vesicles containing PIP₂ were first incubated with Cdc42Hs, either in the guanine nucleotide-depleted state or in the GDP-bound state or the GTPγS-bound state, for 10 min at room temperature before pelleting the lipid vesicles by ultracentrifugation. Western blotting the pelleted vesicles with a specific anti-Cdc42Hs antibody revealed that Cdc42Hs, depleted of nucleotide, incubated with PC vesicles alone; lane +, 0.1 μg Cdc42Hs as a positive control.

**FIG. 3.** PIP₂ binds to the guanine nucleotide-depleted form of Cdc42Hs. One μg of nucleotide-depleted Cdc42Hs (EDTA lane), Cdc42Hs bound to GDP (GDP lane), or Cdc42Hs bound to GTPγS (GTPγS lane) was incubated with 100 μM PIP₂ incorporated in PC vesicles for 10 min before ultracentrifugation in an airfuge. The resulting pellets were subjected to an anti-Cdc42Hs Western blot. Lane −, Cdc42Hs depleted of nucleotide incubated with PC vesicles alone; lane +, 0.1 μg Cdc42Hs as a positive control.

**FIG. 4.** PIP₂ stimulates GDP dissociation from the Rho family GTP-binding proteins, Cdc42Hs and RhoA. One μg of purified Ha-Ras, Rap1a, Ran, RhoA, Rac1, or Cdc42Hs were incubated with 100 μM PIP₂ under the GDP dissociation assay conditions for 8 min.

**FIG. 5.** The carboxyl-terminal domain of Cdc42Hs is necessary for its interaction with PIP₂. One μg of a carboxyl-terminal truncated (C77) mutant of Cdc42Hs preloaded with [³H]GDP was incubated with 100 μM PIP₂ ( ), 0.5 μM GST-Dbl (○), or 1 μM GST (●), and aliquots of the reaction mixtures were added to the termination buffers at the indicated time points.

will not stimulate [³H]GDP dissociation from the K-Ras protein, under conditions where the Ras-GRF (Shou et al., 1992) has a stimulatory effect (data not shown).

The ability of PIP₂ to regulate nucleotide binding to Rho subfamily proteins, as well as Arf, and to directly associate with Cdc42Hs (see Fig. 3) suggested that the Rho subfamily GTP-binding proteins must contain a specific PIP₂-binding site. Sequence comparisons between Rho subfamily GTP-binding proteins and two PIP₂-binding proteins, Gelsolin and Villin, indicated that the carboxyl-terminal domains of Cdc42Hs, RhoA, and the Rac proteins, which contain a number of basic amino acids, shared homology with amino acid residues 140–147 of Villin and 150–169 of Gelsolin. These regions of Villin and Gelsolin have been implicated in the binding of these proteins to PIP₂ (Jannmey et al., 1992). Thus, we constructed a deletion mutant of Cdc42Hs that lacked the carboxyl-terminal seven amino acids (including two arginines that were suspected to be involved in PIP₂ binding). This truncated Cdc42Hs molecule behaves like wild-type Cdc42Hs with regard to its intrinsic GTP-binding and GTPase activities and its ability to functionally couple to the Cdc42Hs-GTPase-activating protein (data not shown). Although it undergoes a slower rate of [³H]GDP dissociation, compared to wild-type Cdc42Hs, it is still capable of interacting with Dbl and undergoing Dbl-catalyzed GDP dissociation (Fig. 5). However, the carboxyl-terminal truncated Cdc42Hs shows a markedly reduced response to PIP₂. These results then strongly argue that although Dbl and PIP₂ elicit similar effects (i.e. stimulation of GDP dissociation), they mediate these common effects from distinct binding domains on the GTP-binding protein.

In addition to serving as a precursor for the second messengers IP₃ and diacylglycerol (Berridge, 1993) and for the putative messenger PIP₃ (Stephens et al., 1993), PIP₂ has been implicated as a regulator of the actin cytoskeleton, based on its ability to influence actin severing, capping, and bundling proteins in vitro (Jannmey, 1994). Thus, it is interesting that in the present studies, we find that PIP₂ binds directly to and influences the nucleotide state of GTP-binding proteins that have been implicated in cytoskeletal regulation, i.e. Cdc42Hs and RhoA. However, these findings raise a number of important issues. One has to do with the mechanism by which PIP₂ stimulates GTP dissociation and how this compares with the mechanism by which Dbl stimulates GDP dissociation and guanine nucleotide exchange. Certainly the rate-limiting step in the activation of GTP-binding proteins, which occurs as an
outcome of the exchange of GTP for bound GDP, is the dissociation of the tightly bound GDP molecule. Both Dbl and PIP$_2$ strongly catalyze this dissociation event and stabilize the nucleotide-depleted state of the GTP-binding protein. Our data, in fact, would suggest that PIP$_2$ does this even more effectively than Dbl, such that PIP$_2$ can stimulate GDP dissociation from the nucleotide binding site of Cdc42Hs in the absence of added GTP, whereas Dbl cannot. It is possible that the differences exhibited by PIP$_2$ and Dbl reflect differences in the sites on Cdc42Hs (or related proteins) that bind these agents. At the present time, we know very little about the specific sites on Cdc42Hs that are responsible for binding Dbl, although mutations in Cdc42Hs that correspond to mutations in Ras that uncouple its binding to the GEF Sos (Mosteller et al., 1994) do not uncouple Cdc42Hs from Dbl. We would speculate at this point that a key conformational change that is necessary to loosen the binding of GDP to Cdc42Hs is induced by both Dbl and PIP$_2$ from distinct (binding) sites on the Cdc42Hs molecule. Future studies will be aimed at obtaining additional information regarding the conformational change in Cdc42Hs and related Rho subfamily proteins that is necessary for this rate-limiting step for activation.

A second key issue raised by these studies concerns whether, in fact, PIP$_2$ acts as a physiological regulator of Cdc42Hs and related proteins, and if so, how? It is tempting to speculate that the actin-regulatory activities of PIP$_2$ are related to the actions of Cdc42Hs and RhoA in mediating cytoskeletal changes such as filopodia formation and/or actin stress fiber formation. This is a particularly interesting possibility given the suggestions that a cascade of Rho subfamily GTPases (i.e. RhoA, Cdc42Hs, and Rac1, and RhoA) is operating in the regulation of cytoskeletal changes in certain cells (Nobes and Hall, 1995) and that a putative target for Cdc42Hs, the phosphatidylinositol 3-kinase (Zheng et al., 1994a), which generates phosphatidylinositol compounds phosphorylated at the 3 position, may be upstream from Rac1 (Hawkins et al., 1995; Nobes et al., 1995). Thus, in addition to Dbl-related proteins, it is possible that phosphatidylinositol metabolites might serve as direct regulators of a signaling cascade that lead to changes in the actin cytoskeleton. It also is possible that phosphatidylinositol compounds work together (cooperatively) with Dbl-related proteins to activate GTP-binding proteins. We have not detected any type of cooperation between oncogenic Dbl and PIP$_2$ in the stimulation of GDP dissociation from Cdc42Hs (data not shown). However, in some cases, Dbl-related proteins have been shown to bind to Rac without stimulating GDP dissociation (Miki et al., 1993; Horii et al., 1994), and in other cases, the presence of Dbl homology domains within proteins (e.g. the Ras-GEFs, Sos and Ras-GRF) have not yet been assigned a function (Shou et al., 1992). Thus, it will be interesting to see if phosphatidylinositol compounds exert some type of regulatory or cooperative effect on the functions of these Dbl-related proteins. Finally, studies with purified phospholipase D indicate that the maximum activation of at least one isoform of the enzyme requires PIP$_2$, and the synergistic actions of the Arf GTP-binding protein (which also binds and is regulated by PIP$_2$ (Terui et al., 1995)) and either RhoA or Cdc42Hs (Singer et al., 1995). In the future, we intend to examine these possibilities further as well as determine just how PIP$_2$ levels in cells might be coordinated with the activation-deactivation cycle of Cdc42Hs and/or RhoA. For example, it is possible that signaling pathways that lead to an increase in PIP$_2$ levels will also promote the generation of guanine nucleotide-depleted Cdc42Hs and/or RhoA. Situations which then lead to a decrease in PIP$_2$ levels (e.g. stimulation of the hydrolysis of PIP$_2$ by phospholipase C enzymes) would then enable cellular GTP to bind to these GTP-binding proteins, thereby stimulating their activation. However, it also is possible that at the cellular levels of GTP (>$10 \mu$M), the exchange of GDP for GTP can occur even in the presence of high concentrations of PIP$_2$. Thus far, it has been difficult to test these levels of GTP in conventional GTP-binding assays; however, we hope in the future to be able to use fluorescence spectroscopic approaches to determine if, in fact, such a PIP$_2$-stimulated nucleotide exchange reaction is feasible.

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REFERENCES

Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A. (1995) J. Biol. Chem. 270, 27985–27988
Barford, D. T., Zheng, Y., Kuang, W.-J., Hart, M. J., Evans, T., Cerione, R. A., and Ashkenazi, A. (1993) J. Biol. Chem. 268, 26059–26062
Berridge, M. J. (1990) Nature 361, 315–325
Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
Cerione, R. A., and Zheng, Y. (1996) Curr. Opin. Cell Biol. 8, 216–222
Chong, L. D., Traynor-Kaplan, A., Bokoch, G. M., and Schwartz, M. A. (1994) Cell 79, 507–513
Chou, M. M., and Blenis, J. (1996) Cell 85, 575–583
Chuang, T.-H., Bohl, B. P., and Bokoch, G. M. (1993) J. Biol. Chem. 268, 26206–26211
Coo, O. A., Chiaribelli, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutfkind, J. S. (1995) Cell 81, 1137–1146
Gilman, A. G. (1987) Annu. Rev. Biochem. 56, 615–649
Hill, C. S., Wynne, J., and Treisman, R. (1995) J. Biol. Chem. 270, 1137–1146
Horii, Y., Beeler, J. F., Sakaguchi, K., Tachibana, M., and Miki, T. (1994) EMBO J. 13, 4776–4786
Janmey, P. A. (1994) Annu. Rev. Physiol. 56, 189–191
Janmey, P. A., Lamb, J., Allen, P. G., and Matsudaoka, P. T. (1992) J. Biol. Chem. 267, 11818–11823
Johnson, D. I., and Pringle, J. R. (1990) J. Cell Biol. 111, 143–152
Kozma, R. S., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Biol. Cell 15, 1942–1952
Miki, T., Smith, C. L., Long, J. E., Eva, A., and Fleming, T. P. (1993) J. Biol. Chem. 268, 462–465
Miller, P. J., and Johnson, D. I. (1994) Mol. Cell. Biol. 14, 1075–1083
Minden, A., Lin, A., Claret, F.-X., Abo, A., and Karin, M. (1995) Cell 81, 1147–1157
Moffat, K., and Vaughan, M. (1995) J. Biol. Chem. 270, 12527–12530
Mosteller, R. D., Han, J., and Broek, D. (1994) Mol. Cell. Biol. 14, 1104–1112
Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
Nobes, C. D., Hawkins, P., Stephens, L., and Hall, A. (1995) J. Cell Sci. 108, 233–239
Olson, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
Qi, R.-G., Chen, J., Kirn, D., McCormick, F., and Symons, M. (1995) Nature 374, 457–459
Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
Singer, W. D., Brown, H. A., Bokoch, G. M., and Sternweis, P. C. (1995) J. Biol. Chem. 270, 14944–14950
Terui, T., Kahn, R. A., and Randazoo, P. A. (1994) J. Biol. Chem. 269, 28130–28135
Zhang, J., King, W. G., Dillon, S., Hall, A., Feig, L., and Rittenhouse, S. E. (1993) J. Biol. Chem. 268, 22521–22526
Zheng, Y., Bagrodia, S., and Cerione, R. A. (1994a) J. Biol. Chem. 269, 18777–18780
Zheng, Y., Cerione, R. A., and Bender, A. (1994b) J. Biol. Chem. 269, 2369–2372
Zheng, Y., Olson, M. F., Hall, A., Cerione, R. A., and Toksz, D. (1995) J. Biol. Chem. 270, 9031–9034

3 Y. Zheng, unpublished results.
