Negative Regulation of Rho Family GTPases Cdc42 and Rac2 by Homodimer Formation*

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The Rho family GTPases are tightly regulated between the active GTP-bound state and the inactive GDP-bound state in a variety of signal transduction processes. Here the Rho family members Cdc42, Rac2, and RhoA were found to form reversible homodimers in both the GTP- and the GDP-bound states. The homophilic interaction of Cdc42 and Rac2, but not RhoA, in the GTP-bound state, caused a significant stimulation of the intrinsic GTPase activity, i.e. the activated form of Cdc42 and Rac2 acts as GTPase-activating proteins toward Cdc42-GTP or Rac2-GTP. The dimerization of the GTPases appeared to be mediated by the carboxyl-terminal polybasic domain, and the specific GTPase-activating effects of Cdc42 and Rac2 were also attributed to the structural determinant(s) in the same region of the molecules. Moreover, similar to the case of Cdc42 and Cdc42GAP interaction, Cdc42-GDP interacted with tetrafluoroaluminate and Cdc42-GTPγS (guanosine 5′-3'-O- (thio)triphosphate) to form a transition state complex of the GTPase-activating reaction in which the carboxyl-terminal determinant(s) of the GTPγS-bound Cdc42 plays a critical role. These results provide a rationale for the fast rate of intrinsic GTP hydrolysis by Cdc42 and Rac and suggest that dimerization may play a role in the negative regulation of specific Rho family GTPases mediated by the carboxyl-terminal polybasic domain.

Members of the Rho family GTPases of the Ras superfamily including Rho, Rac, and Cdc42 are involved in intracellular signaling events that cause reorganization of actin-cytoskeleton (1), focal adhesion formation (2), transcription activation (3, 4), cell cycle progression (5), endocytosis (6, 7), and exocytosis (8, 9). When activated by upstream signals such as growth factors and phospholipids, these proteins are converted to the active GTP-bound form from the inactive GDP-bound form (10) to interact with specific effector targets that mediate their biological functions (11, 12). The signals transduced by the small GTPases are transient; the GTP-bound Rho family proteins are capable of cleaving the phosphodiester bond linking β-phosphate of GTP to its γ-phosphate via the intrinsic GTP-hydrolysis machinery of the GTPases, a process that can be further accelerated by the GTP-activating proteins (GAPs)1. The effective cycling of these GTPases between the GTP-bound and the GDP-bound states is critical to their biological functions; mutations in the regulators of the GTPases or in the GTPases themselves effecting their GTP-binding/GTPase activity may result in transformation (10, 14, 15), morphogenetic changes (16–18), or developmental disorder (19, 20).

The mechanism of negative regulation of Rho family GTPases has been a subject of extensive investigation. The Rho GAPs, which share a homologous RhoGAP domain spanning ~180 amino acids (13), preferentially interact with the GTP-bound form of Rho proteins, resulting in a significant stimulation of the intrinsic GTPase activity. Recent mutagenesis (21) and kinetic studies (22–24), and the x-ray crystallography study of Rho and Rho GAP complex (25, 26) indicate that the effect of GAPs is exerted directly at the stage of GTP cleavage by Rho proteins. The GDP-bound Rho was found to form a stable complex with aluminum tetrafluoride and GAP and thereby to stabilize a transition state of the GAP reaction (26), further suggesting that Rho GAP contributes directly to Rho GTP-hydrolysis by supplying essential determinants to the catalytic core of the GTPase. Although Rho GAP shares no sequence homology to RasGAP or Go of heterotrimeric G-proteins, this feature of formation of a transition state of G-protein-GAP complex is apparently conserved across the panel of Ras superfamily members (27, 28) and is similar to the case of Go (29–31), raising the possibility of a divergent structural evolution that utilizes multiple opportunities to negatively regulate the GTPase activity of respective GTP-binding proteins.

In the absence of GAP stimulation, certain members of the Rho family, i.e. Cdc42 and Rac, have been known to contain significantly higher intrinsic GTPase activity than others (e.g. RhoA and TC10) and Rac (22–24). It was unclear how the fast rate of intrinsic GTP hydrolysis by Cdc42 and Rac come to arise and how this special property of the GTPases may contribute to their mechanism of regulation. In the present study, we found that the Rho family proteins Cdc42, Rac2, and RhoA were capable of forming reversible homodimers. The homodimer formation by Cdc42 and Rac2, but not RhoA, at the GTP-bound state, caused a marked activation of intrinsic GTPase activity. Furthermore, the specific GTPase-activating effects of Cdc42-GTP and Rac2-GTP because of the homophilic interaction were dependent upon the structural determinant(s) in the carboxyl-terminal polybasic domain of the GTPases that was required for the formation of a transition state complex of the GTPase-activating reaction. Our findings provide a rationale for the fast rate of intrinsic GTP-hydrolysis by Cdc42 and Rac and suggest that dimerization may play a role in the negative regulation of specific Rho family GTPases mediated by the carboxyl-terminal polybasic domain.

EXPERIMENTAL PROCEDURES

Materials—GDP, GTP, GTPγS, and bacterial purine nucleoside phosphorylase were from Sigma. 2-Amino-6-mercaptop-7-methylpurine ribonucleoside (MESG) and 2′(3′)-O-(N-methylanthraniloyl) (mant)-
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Results and Discussion

It has been known that the Ras proteins have slow intrinsic GTPase reaction rates (37). The apparent rate was determined to be 0.022 min\(^{-1}\) for N-Ras by an assay measuring the time course of \(\gamma\)P, release from Ras-GTP, and it did not seem to be affected by increasing concentrations of Ras (Fig. 1A). RhoA behaved similarly to Ras in this aspect, whereas the apparent rates of GTP-hydrolysis of Cdc42 and Rac2 were found to be slow.

RhoA-GTP under single turnover conditions was determined in the absence or presence of 10 \(\mu\)M respective stimulants (Cdc42GAP was at 10 nM) as indicated.

GDP were synthesized as described previously (32, 33). Recombinant human Cdc42, RhoA, Rac2, and N-Ras were expressed in Escherichia coli as His\(_{10}\)-tagged fusions using the pET expression system (22–24) and as GST fusions using the pGEX expression vector (22–24). The GAP domain of Cdc42GAP containing amino acids 205–439 was expressed as a glutathione-S-transferase (GST)-fusion using the pGEX-KG vector as described (22–24). The post-translationally modified Cdc42 was obtained in an insect expression system (34). Thrombin cleavage after affinity purification allowed removal of the N-terminal tagged sequences (22).

The carboxyl-terminal truncation mutations of Cdc42 (C-7) and RhoA (Rho-8) were generated by the polymerase chain reaction-based amplification technique using Pfu polymerase (Stratagene) (35) with primers that contained a stop codon at amino acid residues 185 (C-7) and 186 (Rho-8), respectively. The sequences of mutagenized cDNA were confirmed by automated sequencing prior to protein expression by the pET expression system.

GTPase Activity Assay—GTP-hydrolysis of the small GTPases were measured by the MERSG/phosphorylase system monitoring the free \(\gamma\)P, release as described for the cases of Cdc42, Rac1, and RhoA (22–24). Single turnover GTPase reactions were initiated by the addition of MgCl\(_2\) to a final concentration of 5 mM. Data were fitted into a single exponential to derive the apparent rate constant \(K_{app}\). For measurements of GTPase-activating reactions, 5–50 \(\mu\)l of stock solution containing the indicated amount of GAP (Cdc42GAP) or the GTP-bound G-proteins were added together with MgCl\(_2\) to the reaction mixtures. A control experiment in which the substrate GTPases were omitted was carried out in each independent experiment to provide a background. The concentration of \(\gamma\)P in the reaction solution was calculated by a factor of extinction coefficient \(\epsilon_{50\,\text{nm}} = 11,000\,\text{M}^{-1}\cdot\text{cm}^{-1}\) at pH 7.6 from the absorbance change resulting from the phosphorylase coupling reactions (32) and was used to determine the effective concentrations of the small G-proteins after one round of single turnover reaction.

**Fig. 1.** Stimulation of the GTPase activity of Cdc42 and Rac2 by the GTP\(_S\)-bound Cdc42 and Rac2, respectively. A, concentration dependence of the apparent intrinsic rates of GTP hydrolysis of Cdc42, Rac2, and RhoA compared with that of N-Ras. Recombinant G-proteins were subjected to the GTPase activity assay at various concentrations. B, Cdc42-GTP\(_S\) stimulates the GTPase activity of Cdc42-GTP. The time courses of GTP-hydrolysis by 12 \(\mu\)M Cdc42-GTP under single turnover conditions were monitored in the absence or presence of 10 \(\mu\)M Cdc42-GDP, 10 \(\mu\)M Cdc42-GTP\(_S\) or 10 nM Cdc42GAP. 12 \(\mu\)M Cdc42-GTP\(_S\) alone in the reaction buffer containing an excess amount of GTP (500 \(\mu\)M) provided a background of \(\gamma\)P release because of the basal exchange of GTP\(_S\) for GTP. C, Cdc42-GTP\(_S\) and Rac2-GTP\(_S\), but not RhoA-GTP\(_S\), possess GTPase-activating activity toward respective GTP-bound G-proteins, and isoprenylation of Cdc42 (\(^{\text{*}}\)Cdc42) has no effect on this property. \(\gamma\)P release at 2 min time point by 12 \(\mu\)M Cdc42-GTP, \(^{\text{*}}\)Cdc42-GTP (isoprenylated form), Rac2-GTP, or
increase significantly with increasing concentrations of the G-proteins bound to GTP (Fig. 1A). Titration of Cdc42-GTPγS to Cdc42-GTP under single turnover conditions resulted in a significant increase of the initial rate of the GTPase reaction, similar to the effect of the addition of Cdc42GAP, whereas addition of Cdc42-GDP had no detectable effect (Fig. 1, B and C), indicating that the activated form of Cdc42 presents a GAP activity toward Cdc42. This apparent GTPase-activating effect by Cdc42-GTPγS was not affected by isoprenylation modification since Cdc42 isolated from an insect cell expression system (*Cdc42) had a similar behavior (Fig. 1C). Similar observations were also made for Rac2 (Fig. 1C). In contrast, the GTPγS-bound RhoA lacked the ability to stimulate the GTPase activity of RhoA-GTP (Fig. 1C). Therefore, it appears that the Rho family proteins Cdc42 and Rac2, but not RhoA, possess self-stimulatory GAP activity when in the activated state. The self-stimulatory multiple turnover GTPase reactions (Fig. 1A) may provide a rationale for the previously observed fast rate of intrinsic GTP-hydrolysis by recombinant Cdc42 and Rac (22–24).

To examine the apparent homophilic interaction between molecules of the small GTPases, recombinant Cdc42 and Rac2, as well as the controls RhoA and N-Ras, were subjected to gel filtration chromatography analysis. Aside from the ~25-kDa elution peak expected for the monomeric form of the proteins, an absorption peak at ~50 kDa corresponding to the dimer position was observed in the chromatography profiles of all three Rho family GTPases, Cdc42, Rac2, and RhoA (Figs. 2A). The homodimer formation was slightly affected by replacement of bound GDP with GTPγS, which resulted in a minor shift of the overall population of the GTPases from dimers to monomers (Figs. 2A). A concentration-dependent breakdown of preformed dimers of Cdc42-GTPγS was detected from the traces of the radioactive GTPγS-bound Cdc42 eluents (Fig. 2A), indicating that the dimer formation is a reversible process. The estimated dissociation constant is at ~2 μM for (Cdc42-GTPγS)2 based upon the concentration-dependent absorption peak distributions. The property of dimer formation appeared to be specific to the Rho family members, because N-Ras at either nucleotide binding state remained exclusively monomeric (Fig. 2A). These results indicate that the Rho family GTPases Cdc42, Rac2, and RhoA can form reversible homodimers at the GTP-bound state with micromolar binding affinity. To assess whether the homophilic interaction may apply to native Rho GTPases of mammalian cells, a complex formation assay was performed using the immobilized GST-Cdc42, GST-RhoA, or GST-Ras as bait and NIH 3T3 cell lysates as the source for endogenous small GTPases. Western blot analysis of the coprecipitates of the GST fusions revealed that GST-Cdc42-GDP and GST-Cdc42-GTPγS were capable of binding to the endogenous Cdc42-GDP and Cdc42-GTPγS, respectively, but with much reduced affinity to Cdc42 of the different nucleotide binding states (Fig. 2B). Neither GST-RhoA-GDP, GST-RhoA-GTPγS, GST-Ras-GTPγS, nor GST-Ras-GTPγS interacted with Cdc42 at a detectable level (Fig. 2B) even though both the GST-RhoA and GST-Ras were able to bind to guanine nucleotides and to interact with their respective regulatory proteins (data not shown). Thus, endogenous Cdc42 of the mammalian cells is capable of forming a homophilic complex.

The carboxyl-terminal polybasic domain of the Rho family proteins, spanning the last eight amino acid residues before the CAAX isoprenylation sequences (Fig. 3A), was suspected to have a role in homodimer formation because of its positively charged exposure on the surface of the molecules (38, 39). Mutants of Cdc42 with deletion of the last seven residues of the C terminus (C-7) and of RhoA with the carboxyl-terminal eight

![Fig. 2. The Rho family GTPases form reversible homodimers.](image)
residues truncated (Rho-8) were found to be present only in monomeric form (Fig. 3B). These deletion mutations did not seem to affect the tertiary folding of the proteins and maintained the full capability to interact with Rho GAP (25, 26). C-7 lost the dose dependence of the intrinsic GTPase activity of the wild-type protein (Fig. 3C), which was consistent with its inability to catalyze the GTPase reaction of itself or wild-type Cdc42 when bound to GTP\(_{\text{S}}\) (Fig. 3D). However, C-7 remained a good substrate for the GAP reaction catalyzed by wild-type Cdc42 bound to GTP\(_{\text{S}}\), suggesting that determinant(s) in the polybasic domain of Cdc42 contributes to the catalytic GAP reaction. We conclude that the carboxyl-terminal polybasic domain of the Rho family proteins mediate their homodimer formation and that structural determinants within the polybasic domain play a critical role in the self-stimulatory GAP reactions of Cdc42 and Rac2.

Interactions between Rho proteins and Rho GAP and between Ras and RasGAP have been found to undergo a transition state of the respective GAP reactions, which can be mimicked by the ternary complex of the respective G-proteins at the GDP-bound state, AIF\(_{\text{S}}\), and the respective GAPs (27, 28, 40). To determine whether the self-stimulatory GAP reaction of the Rho family homodimers, i.e. \((\text{Cdc42-GTP})_2\) and \((\text{Rac2-GTP})_2\), may be through a mechanism similar to that of GAP activations of Ras and Cdc42, a fluorescence assay originally designed to detect the formation of the ternary transition state complex of Ras-GDP, AIF\(_{\text{S}}\), and GAP for the Ras-RasGAP reaction (27) was employed to examine the case of Cdc42 homodimers. The addition of AIF\(_{\text{S}}\) or Cdc42-GTP\(_{\text{S}}\) alone did not change the emission spectrum of Cdc42 bound to the fluorescent GDP-analog mantGDP, whereas the spectrum was changed both in maximum absorption wavelength (from 448 to 430 nm) and in intensity (a 100% increase at 448 nm) when AIF\(_{\text{S}}\) and Cdc42-GTP\(_{\text{S}}\) were added together to Cdc42-mantGDP (Fig. 4A). This change in Cdc42-mantGDP fluorescence indicated a conformational change around the GTPase active site, which attributes to the formation of an analog of the GTP-bound transition state of the GTPase reaction involving Cdc42-GDP, AIF\(_{\text{S}}\), and Cdc42-GTP\(_{\text{S}}\), as were the cases for Ras-GDP, AIF\(_{\text{S}}\), and RasGAP (27) and for Cdc42-GDP, AIF\(_{\text{S}}\), and Cdc42GAP (40). Moreover, stoichiometric amounts of Cdc42-GTP\(_{\text{S}}\) to Cdc42-mantGDP were needed for this effect (Fig. 4B). The effective dissociation constant (\(K_d\)) of the transition state complex Cdc42-mantGDP-AIF\(_{\text{S}}\)-Cdc42-GTP\(_{\text{S}}\)
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One common feature of the GTPase-activating reactions is the progression from the ground state (GTP-bound state) to the transition state complex, which appears to be critical for the activation of GTPase activity. Another is the direct participation of critical amino acids from the respective GAP molecules in the GTP hydrolysis reaction to stabilize the transition state conformation of the GTPases. The findings reported in this study, that the Rho family members Cdc42 and Rac2 in the GTP-bound form forms a similar transition state complex in the homodimers and that one molecule of the dimer supplies specific structural determinant(s) to the GTPase core of the other molecule and thereby stabilizes the transition state conformation of the GTPase-activating reaction, may provide yet another example of evolutionary convergence of the mechanism of negative regulations of GTPases.

The carboxyl-terminal region of Rho family proteins represents the most divergent sequences in the family. Most members contain a stretch of two to six poly-lysine and/or arginine residues immediately before the isoprenylation CAAX sequence. Not unlike the similar structure of K-Ras, this polybasic domain of S. cerevisiae Cdc42 has been suggested to have a role in membrane attachment (41) and, in the case of human Cdc42, may be involved in interaction with acidic phospholipids such as phosphatidylinositol 4,5-bisphosphate (42). Our observation of the dimer-formation and the GTPase-activating capability of wild-type and C-terminal mutant (C-7) of Cdc42 further suggest that the C-terminal region of certain Rho family proteins is essential for dimerization and self-stimulatory GAP activity and may be involved in a non-symmetric homodimer configuration: one molecule of Cdc42 contributes the C-terminal domain containing the binding and catalytic determinant(s) (such as a critical arginine residue) to function as a GAP, whereas the other molecule may act as a substrate to interact with this “GAP” through a distinct region of the molecule such as the GTP-binding core. Based upon studies of the carboxyl-terminal hypervariable region of Ras, it was expected that the C-terminal domain might not contain definable tertiary structure (37). Our findings that the carboxyl-terminal...
polybasic domain can act only as a GAP when bound to GTP in the homophilic interaction of Cdc42 and Rac2 and that synthetic polypeptides corresponding to the domain sequences are capable of competing with the homodimer formation yet contain no GAP activity indicate that the conformation of this region of Rho proteins is sensitive to the nucleotide binding state of the GTPases. Moreover, all Rho family proteins examined containing the polybasic sequences at the carboxyl termini, including the additional mammalian members Rac1, RhoC, and RhoG, have been found capable of forming homodimers or higher oligomers, whereas others lacking polybasic residues in the region, e.g. TC10 and RhoB, are in monomeric form, suggesting that the polybasic nature of the domain is critical in mediating homophilic interactions of specific Rho family GTPases. The fact that RhoA is found to form homodimers but yet is unable to stimulate the GTPase activity in the homophilic interaction further implies that additional unique structural determinant(s) is required to elicit the GTPase-activating effect.

Whether the observed homodimer formation and self-stimulatory GTPase-activating activity of Cdc42 and Rac2 bear physiological significance remains unclear. Given that these Rho family proteins are expected to assemble directly or indirectly to large molecular complexes in cells (1, 2, 11) and the potential dual roles as both activator and down-regulator of their own signals of the GTPases at the activated state, it will be a particularly challenging issue to address. Although the $K_{cat}$ values of the self-stimulatory GAP reaction of the GTP-bound homodimers may be an order of magnitude lower than that catalyzed by Rho GAP, the observed $K_m$ of the Cdc42-GTP homodimers and the effective $K_m$ of the transition state complex are comparable with that of Cdc42-RhoGAP interaction (−2 μM) and Ras-RasGAP interaction (−1.3 μM) respectively (22, 27). The relatively high abundance and ubiquitous distribution of Rho family proteins also make it an attractive possibility that the homophilic interaction may have a role in the regulation of these small GTPases. Future work to examine the structural determinant(s) of the carboxyl-terminal polybasic domain of Cdc42 and Rac2 involved in the dimer formation and catalysis and the site(s) of the GTPase hydrolysis core of the substrate required for the interaction should provide additional insight for the mechanism of dimer formation.

REFERENCES
1. Tapon, N., and Hall, A. (1997) Curr. Opin. Cell Biol. 9, 86–94
2. Ridley, A. J. (1996) Curr. Biol. 6, 1256–1264
3. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
4. Perona, R., Montaner, S., Sanijger, L., Sanchez, P. I., Bravo, R., and Lacoal, J. C. (1997) Genes Dev. 11, 463–475
5. Olson, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1276–1277
6. C. Lamaze, C., Chuang, T. H., Terlecky, I. J., Bokoch, G. M., and Schmid, S. L. (1996) Nature 382, 177–179
7. Schmalzing, G., Richter, H. P., Hansen, A., Schwartz, W., Just, I., and Aktories, K. (1995) J. Cell Biol. 130, 1319–1332
8. O’Sullivan, A. J., Brown, A. M., Freeman, H. N. M., and Gomperts, B. D. (1996) Mol. Biol. Cell 7, 397–408
9. Norman, J. C., Fries, L. S., Ridley, A. J., and Koffler, A. (1996) Mol. Biol. Cell 7, 1429–1442
10. Cerione, R. A., and Zheng, Y. (1996) Curr. Opin. Cell Biol. 8, 216–222
11. Hall, A. (1998) Science 279, 509–513
12. Van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–3011
13. Lamarche, N., and Hall, A. (1994) Trends Genet. 10, 436–440
14. Lin, R., Bagrodia, S., Cerione, R. A., and Manor, D. (1997) Curr. Biol. 7, 794–797
15. Michiels, F., Habets, G. M., Stam, J. C., van der Kamen, R. A., and Collard, J. G. (1995) Nature 378, 338–340
16. Luo, L., Liao, Y. J., Jan, L. Y., and Yon, Y. N. (1994) Genes Dev. 8, 1787–1792
17. Eaton, S., Wepf, R., and Simons, K. (1996) Curr. Biol. 6, 1277–1289
18. Murphy, A. M., and Montell, D. J. (1996) J. Cell Biol. 133, 617–630
19. Luo, L., Hensch, T. K., Ackerman, L., Barbé, S., Jan, L. Y., and Jan, Y. N. (1996) Nature 379, 837–840
20. Pasteris, N. G., Cadle, A., Logie, L. J., Porteous, M. E. M., Schwartz, C. E., Stevenson, R. E., Glover, T. W., Wieryo, R. G., and Gorski, J. L. (1994) Cell 79, 669–678
21. Li, R., Zhang, B., and Zheng, Y. (1997) J. Biol. Chem. 272, 32830–32835
22. Zhang, B., Wang, Z-X., and Zheng, Y. (1997) J. Biol. Chem. 272, 21999–22007
23. Zhang, B., Chernoff, J., and Zheng, Y. (1998) J. Biol. Chem. 273, 8776–8782
24. Rittinger, K., Walker, P., Eccleston, J. F., Nurmahomed, K., Owen, D., Laue, E., Gamblin, S. J., and Smardon, S. J. (1997) Nature 386, 693–697
25. Rittinger, K., Walker, P. A., Eccleston, J. F., Smardon, S. J., and Gamblin, S. J. (1997) Nature 389, 758–762
26. Mittal, R., Ahmadian, M. R., Goody, R. S., and Wittinghofer, A. (1996) Science 273, 115–117
27. Ahmadian, M. R., Mittal, R., Hall, A., and Wittinghofer, A. (1997) FEBS Lett. 406, 315–318
28. Bourne, H. R. (1997) Nature 389, 673–675
29. Sprang, S. R. (1997) Science 277, 329–330
30. Noel, J. P. (1997) Nat. Struct. Biol. 4, 677–680
31. Webb, M. R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4884–4887
32. Hiratsuka, T. (1983) Biochim. Biophys. Acta 742, 496–508
33. Zheng, Y., Fischer, D. J., Santos, M. F., Tiggi, G., Pasteris, N. G., Gorski, J. L., and Xu, Y. (1996) J. Biol. Chem. 271, 33169–33172
34. Li, R., and Zheng, Y. (1997) J. Biol. Chem. 272, 4671–4679
35. Zheng, Y., Bagrodia, S., and Cerione, R. A. (1994) J. Biol. Chem. 269, 18727–18730
36. Bourne, H. R., Sanders, D. A., and McCormick, F. (1991) Nature 349, 117–127
37. Wei, Y., Zhang, Y., Derewenda, U., Liu, X., Minor, W., Nakamoto, R. K., Somlyo, A. V., Somlyo, A. P., and Derewenda, Z. S. (1997) Nat. Struct. Biol. 9, 699–702
38. Feldham, J. L., Dotsch, V., Raza, S., Manor, D., Cerione, R. A., Sutcliffe, M. J., Wagner, G., and Oswald, R. E. (1997) Biochemistry 36, 8755–8766
39. Hoffman, G. R., Nassar, N., Oswald, R. E., and Cerione, R. A. (1998) J. Biol. Chem. 273, 4392–4399
40. Davis, C. R., Richman, T. J., Delibaka, S. B., Collins, C. C., and Johnson, D. I. (1998) J. Biol. Chem. 273, 849–858
41. Zheng, Y., Glaven, J. A., Wu, W. J., and Cerione, R. A. (1996) J. Biol. Chem. 271, 23815–23819

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