Regulation of the Cool/Pix Proteins

KEY BINDING PARTNERS OF THE Cdc42/Rac TARGETS, THE p21-ACTIVATED KINASES*

Received for publication, August 10, 2001, and in revised form, December 10, 2001
Published, JBC Papers in Press, December 10, 2001, DOI 10.1074/jbc.M107704200

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The Cool (cloned-out of library)/Pix (for PAK-interactive exchange factor) proteins directly bind to members of the PAK family of serine/threonine kinases and regulate their activity. Three members of the Cool/Pix family have shown distinct regulatory activities: (i) p50





The small GTP-binding proteins Cdc42 and Rac serve as molecular switches in diverse biological response pathways, including the stimulation of cell cycle progression and gene transcription, alterations in the actin cytoskeleton, and the regulation of cell adhesion (1–7). A number of candidate downstream targets for Cdc42 and Rac have been identified, including the p21-activated serine/threonine kinases (PAKs),1 Refs. 7–10, the activated Cdc42-associated tyrosine phosphosubstrates (ACKs, Refs. 11 and 12), the p70 S6 kinase (13), IQGAPs (14–16), and the activated Cdc42-associated tyrosine phosphosubstrates (ACKs, Refs. 11 and 12), the p70 S6 kinase (13), IQGAPs (14–16), and the p50





AND THE p21-ACTIVATED KINASES

The JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 277, No. 7, Issue of February 15, pp. 5644–5650, 2002
Printed in U.S.A.
regions from adopting a conformation that inhibited access to the DH/PH domains and/or to the kinase active site of PAK. We in fact identified a family of specific binding partners for p85Cool-1 and Cool-2, named Cats (Cool-associated tyrosine phosphosubstrates) (31), which are highly similar if not identical to proteins isolated through their abilities to bind to G protein-coupled receptor kinases (called Gits) and paxillin (called PKLs) (33, 34). However, we show here that the Cats do not influence the ability of the Cool proteins to directly regulate PAK activity, although they may play a role in the recruitment of Cool proteins to endosomal membranes. Instead, we have found that an 18-amino acid region, designated T1, serves as a novel and essential element for the regulation of PAK activation, accounting for the lack of stimulatory effects on PAK activity exhibited by p50Cool-1 and p85Cool-1.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Plasmid pJ3HmPAK-3 was made as described previously (7). The plasmids pGEX-p5Cool-1-T1-HRD-PRD, pGEX-p85Cool-1-PR, pGEX-p85Cool-1ER, pGEX-p85Cool-1ER2, pGEX-p85Cool-1ER3, pGEX-p85Cool-1ER4, pGEX-p85Cool-1ER4.5, pGEX-p85Cool-1ER5, pGEX-p85Cool-1ER5K537A, pGEX-p85Cool-1ER5K537V, and pGEX-p85Cool-1ER5S358A, and pGEX-p85Cool-1ER5S358A were constructed by amplifying the appropriate bases of the open reading frame (V358A) from plasmid pET15b-p85

**Immunoprecipitation and Western Blot Analysis**—Immunoprecipitation and Western blot analyses were performed as described previously (31). The percent [3H]GDP remaining on the immunoprecipitation mixture and were performed at 22°C for 15 min. The reactions were stopped by adding 2x SDS-PAGE sample buffer containing 20 mM Tris-HCl, pH 7.4, and 15% glycerol. The phosphorylated proteins were resolved by SDS-PAGE (12% gel) and visualized by PhosphorImager analysis (Amersham Biosciences) prior to immunoblotting.

**Is Cat Binding Necessary for PAK Activity?**—We have been studying three members of the Cool/Pix family in some detail; p50Cool-1, p85Cool-1, and Cool-2 (Fig. 1). Given our working hypothesis that the binding of the Cat proteins to Cool-2 and/or p85Cool-1 was responsible for their distinct functional effects relative to the inhibitory activity of p50Cool-1, we set out to delineate a limit-binding domain on the Cool proteins for the Cats and to test whether this binding interaction was in fact essential for the ability of Cool proteins to promote (or permit) rather than inhibit PAK activation. We began by considering three distinguishable regions within the carboxyl-terminal domain of p85Cool-1 as potential Cat-binding sites: a histidine-rich domain (designated as 55HR, residues 419–456), a proline-rich domain (PRD, residues 461–486), and a glutamic acid-rich domain (ERD, residues 511–634) (Fig. 2A). To define which domain is capable of binding Cat, we prepared GST fusion proteins that contained each of these domains; specifically, GST-T1-HRD-PRD consisted of residues 394–487 of p85Cool-1, GST-PR contained residues 460–550, and GST-ER consisted of residues 511–640 (Fig. 2A). The immobilized GST-T1-HRD-PRD, -ER, or -ER constructs were then incubated with cell lysates expressing hemagglutinin (HA)-tagged Cat-2. We found that both GST-PR and GST-ER were able to bind Cat (Fig. 2B).

There is an overlapping region shared by the GST-PR and...
GST fusion proteins, representing residues 511–550 in p85Cool-1 (Fig. 2A). To better define the Cat-binding site, we made an additional four truncations within the ER domain, designated ER2, ER3, ER4 and ER5 (Fig. 2A). As shown in Fig. 2B, the GST-ER2 and GST-ER3 fusion constructs had the same binding affinity for Cat as GST-ER, whereas the GST-ER4 construct was only capable of weak binding and GST-ER5 showed no detectable binding activity. These data suggest that the Cat-binding site lies within residues 527–542 on p85Cool-1.

To further delineate the Cat-binding site, we prepared another truncation, ER4.5 (Fig. 2A), and found that like ER5, it was incapable of binding Cat (Fig. 2B). Taken together, these findings indicate that Cat binds between residues 527–539 on p85Cool-1 (Fig. 2C). The amino acid sequence within this region is AALEEDAQILKVI. It should be noted that a highly conserved sequence is found within residues 685–698 of Cool-2 (Fig. 2C).

To identify amino acid residues on p85Cool-1 that are essential for binding Cat, we made four mutations within the GST-ER3 background (the individual point mutations L536A, K537A, V538A, and the triple mutation L536A/K537A/V538A). We expressed and purified these different GST-ER3 mutants and performed Cat-binding assays. We found that the L536A/ER3 and [K537A/ER3 mutants were still fully capable of binding Cat (Fig. 3, lanes 2 and 3). However, both the [V538A/ER3 mutant and the [L536A/K537A/V538A/ER3 triple mutant showed little or no ability to bind Cat (Fig. 3, lanes 4 and 5). The same results were obtained when assaying p85Cool-1 interactions with Cat in cells via the co-immunoprecipitation of these proteins (data not shown). Thus, Val-538 appears to be an essential residue for the binding of p85Cool-1 to Cat.

We then examined whether the binding of Cat was in fact essential for the stimulatory effects mediated by Cool on PAK activity. We performed these experiments with Cool-2 because it is capable of strongly activating PAK, whereas p85Cool-1 typically shows little detectable stimulation (29). Because we have found that the Cool-2 triple mutation L694A/K695A/V696A does not completely eliminate its binding to Cat, to avoid any ambiguity a Cat-binding defective Cool-2 protein was generated by deleting the carboxyl-terminal end of the protein that includes the Cat-binding site (designated Cool-2/p641). Wild-type Cool-2 and the Cool-2/p641 mutant were co-transfected with HA-tagged Cat-2 into COS7 cells and binding assays were performed. The upper panel in Fig. 4A compares the relative levels of expression of the Cool-2 constructs. Under conditions where wild-type Cool-2 gave a strong binding signal (Fig. 4A, lower panel, lane 2), the Cool-2/p641 deletion mutant was unable to bind Cat (lane 3).

We next directly compared the effects of these Cool-2 constructs on PAK activity. The upper panels in Fig. 4B compare the relative levels of expression of the Cool proteins being examined whereas the middle panels compare the relative amounts of PAK for the different assays. As earlier reported (31), we found that the expression of wild-type Cool-2 was capable of strongly stimulating the ability of PAK to phosphorylate myelin basic protein, whereas p85Cool-1 showed little stimulatory activity (Fig. 4B, lower left panel, compare lanes 2 and 4).
mediated PAK activation. HA-tagged PAK was co-transfected with Myc-tagged p85
Cool-1, designated SH
Cool-2, we prepared a carboxyl-terminal truncation mutant of
the construct designated SH
Cool-2, which contains just the SH3,
DH, and PH domains (Fig. 5A). This mutant was transiently
transfected into COS7 cells and its effects on PAK activity were
examined. In Fig. 5B, as well as others (i.e. Figs. 5C and 7), the
upper panel compares the relative levels of expression of the
different constructs being examined, whereas the middle panel
compares the relative amounts of PAK-3 assayed. As expected,
the Cool-2 protein gave rise to a strong stimulation of PAK
activity, as assayed by the phosphorylation of an exogenous
substrate (myelin basic protein) (Fig. 5B, lower panel, compare
lanes 2 and 3). Wild type p85
Cool-1, Cool-2, or Cool-2(Δ461) into COS7 cells. PAK was
immuno precipitated with an anti-HA antibody, and its kinase activity was assayed as described under “Experimental Procedures.” The amounts of PAK (see middle panel) and Cool proteins (upper panel) were detected by immunoblot with anti-HA and anti-Myc antibody, respectively. Kinase activity was detected using myelin basic protein as a substrate (lower panel).

Identification of a New Regulatory Domain on the Cool/Pix
Proteins—Given the results described in the preceding section,
we searched for other explanations for the distinct functional
effects of the Cool/Pix proteins. We decided to consider possible
differences between the Cool-1 and Cool-2 proteins, given that
only Cool-2 is capable of directly activating PAK. Immediately
downstream from the PH domain, Cool-2 contains a serine-rich
region (designated as 90SR) whereas p85
Cool-1 contains a his-
tidine-rich region (85 HR) (see Fig. 1). Both proteins then
contain similar proline-rich (PRD) and carboxyl-terminal regions
(ERD). Note that p50
Cool-1 lacks the histidine-rich region,
as well as the proline- and glutamic acid-rich regions found in
p85
Cool-1, but contains a distinct carboxyl-terminal region (designated 50C). To examine the potential regulatory influence exerted by the carboxyl-terminal regions of p85
Cool-1 versus
Cool-2, we prepared a carboxyl-terminal truncation mutant of
Cool-1, designated SH-PH-Cool-1, which contains just the SH3,
DH, and PH domains (Fig. 5A). This mutant was transiently
transfected into COS7 cells and its effects on PAK activity were
examined. In Fig. 5B, as well as others (i.e. Figs. 5C and 7), the
upper panel compares the relative levels of expression of the
different constructs being examined, whereas the middle panel
and 3). The Cool-2(Δ461) truncation mutant, however, activated
PKA to the same extent as the wild type Cool-2 protein (Fig. 4B, lower right panel, compare lanes 1 and 3), under experimental conditions where no binding of Cat was observed. These data clearly indicate that the binding of Cat is not required for the ability of Cool-2 to stimulate PAK activity.

If the binding of the Cat proteins is not a determinant for the stimulatory capability of Cool-2, then what role do Cool-Cat interactions play in Cool function? It has been reported that PAK-Cool-Cat complexes may be involved in vesicle trafficking because Cat contains an Arf-GAP activity and localizes to endosomal membranes (35). Recently, we have found that although wild type Cool-2 co-localizes with endosomal markers (e.g. EEA1), Cool-2 mutants that are unable to bind Cat appeared to be more diffusely localized throughout the cytosol (data not shown). This leads us to suspect that Cool/Cat binding interactions are necessary for the proper translocation of Cool proteins to vesicle membranes.

Within this region of p85
Cool-1, there are two distinguishable stretches of sequence. One is the histidine-rich region (85HR) that is absent from both p50
Cool-2 and Cool-2 (Fig. 5A). The other is immediately downstream from the PH domain and contains 18 amino acid residues that are conserved in p50
Cool-2 but not in Cool-2 (Fig. 5A). We have designated this region as T1. To define which of these two regions may be responsible for regulating PAK activity, we further truncated 85HR, yielding the construct designated SH-T1-Cool-1, and determined the effect of this truncation mutant on PAK activity. As shown in Fig. 5C (lower panel, compare lanes 3 and 4), SH-T1-Cool-1 activated PAK activity as effectively as Cool-2 (lower panel, lane 2), whereas SH-PR-Cool-1, which contains both 85HR and the proline-rich region (Fig. 5A), was completely ineffective. It should be noted that the inability of SH-HR-Cool-1 as well as SH-PR-Cool-1 to activate PAK was not because of their inability to bind Cat (PKA. Fig. 6 shows the results of experiments in which HA-tagged PAK-3 was co-expressed with different Myc-
tagged Cool constructs, followed by precipitating PAK and Western blotting with anti-Myc antibody to detect associated
Cool proteins. We found that both SH-HR-Cool-1 and SH-PR-
in the immune complex using an anti-HA antibody. PAK-3 activity was measured as the amount of PAK-3 assayed for each condition. The expression of PAK-3 was detected using an anti-Myc antibody. PAK-3 was isolated by immunoprecipitation using an anti-PAK-3 antibody (14). The anti-Myc antibody. PAK-3 proteins were isolated by immunoprecipitation using an anti-PAK-3 antibody. The expression of PAK-3 was measured as the relative expression of the different Cool constructs (upper panel) was detected using an anti-Myc antibody. The PAK-3 proteins were isolated by immunoprecipitation using an anti-PAK-3 antibody (14).

The abilities of different truncated Cool constructs to activate PAK. A, a schematic representation of different truncated Cool-1 constructs. B, SH-PH-Cool-1 but not SH-HR-Cool-1 stimulates PAK-3 activity in vivo. COST7 cells were transfected with plasmid J3HmPAK-3 expressing HA-tagged WT PAK-3 together with plasmids expressing Myc-tagged SH-HR-Cool-1 (lane 1), SH-HR-Cool-1 (lane 2), SH-T1-Cool-1 (lane 3), Cool-2 (lane 4), and p85Cool-1 (lane 5). The lysates were incubated with anti-PAK-3 primary antibody for 1 h followed by mixing with protein A-Sepharose prebound to rabbit anti-mouse IgG for 45 min. The immunoprecipitated proteins were resolved by SDS-PAGE and electroblotted onto Immobilon P membranes (Millipore). The membranes were blotted with anti-Myc antibody (to detect the co-precipitation of Myc-tagged Cool constructs) or anti-HA antibody (for HA-PAK-3).

Cool-1 bound PAK as effectively as Cool-2 and SH-T1-Cool-1, which strongly stimulate PAK activity. Thus, taken together, these results suggested that the 85HR region has an inhibitory effect on PAK activation.

Given that the p50Cool-1 protein, which lacks the 85HR region, also is incapable of stimulating PAK activity but instead inhibits Dbl- or Cdc42-stimulated activity (29), we hypothesized that the T1 region may serve as a hinge that allows downstream regions on p85Cool-1 (85HR) and p50Cool-1 (e.g., the region designated 50C (Fig. 1)) to fold over and block access to the DH/PH domain, or the kinase domain of PAK, respectively. Based on this idea, we took a two approaches. First, we replaced the corresponding region of Cool-2 with the T1 region from Cool-1 and constructed the Cool-2 mutant designated Cool-2-T1-IN (Fig. 7A). If in fact T1 is responsible for mediating an inhibitory effect on PAK activation, then Cool-2-T1-IN should be incapable of stimulating PAK activity. Second, we replaced the entire carboxyl-terminal half of p85Cool-1, beginning with the T1 region and extending to the carboxyl terminus, with the corresponding region from Cool-2; this chimera was designated CHIM-Cool (Fig. 7A). Here the idea was that the absence of T1 should enable CHIM-Cool to activate PAK. In addition, we prepared two other Cool-2 truncation mutants, designated SH-PH-Cool-2 and SH-PR-Cool-2, that corresponded to the two Cool-1 truncation mutants SH-PH-Cool-1 and SH-PR-Cool-1, respectively (see Fig. 5A).

Fig. 7B shows the results obtained with these different Cool constructs. The top panel compares the relative levels of expression of the different Cool proteins whereas the middle panel shows the relative amounts of PAK-3 for the different assays of PAK activity (bottom panel). As we expected, Cool-2-T1-IN lost the ability to stimulate PAK and behaved in a manner identical to p85Cool-1 (Fig. 7B, lower panel, lane 6). On the other hand, CHIM-Cool, like Cool-2 (lanes 4 and 9), was able to strongly stimulate PAK activity (Fig. 7B, lower panel, compare lanes 3 and 5). The truncation mutant, SH-PH-Cool-2, like SH-PH-Cool-1, stimulated PAK activity (Fig. 7B, lower panel, lane 2 and 7). However, unlike SH-PH-Cool-1, which panel shows the relative amounts of PAK-3 assayed for each condition, and the lower panel shows the assay results, as described in the legend to Fig. 4.
was not able to stimulate PAK activity, the SH-PR-Cool-2 truncation mutant lacking the T1 region was able to strongly activate PAK (Fig. 7B, lower panel, compare lanes 1 and 3). All of these data argue that the T1 region in the Cool-1 proteins is responsible for mediating the repression of PAK activation.

We went on to further examine whether the T1 region can mediate the inhibition of the guanine nucleotide exchange activity of p85\textsubscript{Cool-1}. As shown in Fig. 8, although the SH-PR-Cool-2 protein was capable of acting as a guanine nucleotide exchange factor (GEF), the chimera Cool-2-T1-IN (in which the T1 region from Cool-1 was inserted into SH-PR-Cool-2 immediately after the PH domain) did not catalyze nucleotide exchange. However, the SH-T1-Cool-1 truncation mutant did show GEF activity (Fig. 8), suggesting that the T1 region itself does not directly interact with the DH/PH domain and block its GEF activity. The GEF activities that we measured for the different Cool mutants correlated well with the effects of these mutants on PAK activation. Thus, taken together, these data argue that the T1 region in the Cool-1 proteins is responsible for mediating the repression of PAK activation.

DISCUSSION

The Cool/Pix proteins contain the tandem arrangement of DH and PH domains that are characteristic of the Dbl family of guanine nucleotide exchange factors for Rho GTP-binding proteins. Because of this, their initial discovery as PAK-binding partners led to the assumption that the Cool/Pix proteins were upstream activators of Rac and/or Cdc42, directly complexed to a Cdc42/Rac target. However, it was soon appreciated that different members of the Cool/Pix family exhibited distinct functional effects on PAK such that p50\textsubscript{Cool-1} inhibited the ability of Dbl or activated Cdc42 to stimulate PAK activity, whereas p85\textsubscript{Cool-2}/β-Pix did not interfere with Dbl- or Cdc42-mediated stimulation, and Cool-2/α-Pix strongly activated PAK (36). We originally questioned whether the distinct functional activities associated with the different Cool proteins were the outcome of a specific binding interaction between the carboxyl-terminal domains of p85\textsubscript{Cool-1}/β-Pix and Cool-2/α-Pix with a cellular protein that served as a regulatory factor. The idea was that because p50\textsubscript{Cool-1} lacked an extended carboxyl-terminal domain, it would not be capable of binding to the putative regulatory factor and thereby associated with PAK in an orientation that blocked access to the PAK active site. The binding of p85\textsubscript{Cool-2}/β-Pix or Cool-2/α-Pix to the regulatory factor would ensure the proper orientation of these Cool/Pix proteins such that they did not interfere with the kinase active site and instead permitted or even promoted PAK activation. This led to the identification of the Cat proteins, which are highly similar to the Git and PKL proteins (33, 34), and bind to p85\textsubscript{Cool-1}/β-Pix and Cool-2/α-Pix but not to p50\textsubscript{Cool-2}. However, we show here quite clearly that the binding of Cat to Cool-2 is not necessary for Cool-2-mediated activation of PAK. Preliminary results from our laboratory suggest that the role of the Cats is to ensure the proper localization of the Cool/Pix proteins, particularly to endosomes. The ability of the Cool/Pix proteins to interact with the Cat/Git/PKL proteins, which in turn bind paxillin and function as Arf-GAPs, places them at focal adhesion complexes and endosomal membranes, perhaps providing a link between cell adhesion-induced signaling and intracellular trafficking. The necessity for such a link is suggested by the findings that Arf6, long suspected to be involved in intracellular trafficking, also controls cell spreading (37, 38).

The finding that the Cats are not essential for Cool-2-medi-
ated activation of PAK has led us to consider alternative mechanisms that could account for the functional differences between the different members of the Cool family. We have now delineated a limit region that accounts for the distinct functional activities. This 18 amino acid region (T1) contains 4 proline residues that may form a turn that enables downstream segments of p85Cool-1 to fold back or become positioned in a way that blocks access to the active site of PAK or even prevents the guanine nucleotide exchange factor-independent activation of PAK that has been suggested to occur through the direct binding of the Cool-2/α-Pix proteins (37). The T1 region also appears to account for the inability of p85Cool-1/β-Pix to mirror the actions of Cool-2 and stimulate PAK activity, the idea being that T1 enables downstream segments of p85Cool-1 to fold back over the DH/PH domains and inhibit guanine nucleotide exchange activity. The fact that the T1 region contains a number of serine and threonine residues (8 total) also raises the possibility that it serves as a site for regulatory phosphorylation events.

Various lines of evidence have now implicated the Cool/Pix proteins as playing key roles in interfacing diverse signaling pathways. The PAK proteins, which serve as primary binding partners for Cool/Pix, have also been implicated in a variety of biological activities ranging from intracellular-mediated signal transduction to the activation of nuclear mitogen-activated protein kinases. Thus, PAK activation may serve to coordinate various cellular events and is also likely to require intricate regulation, as might be provided by the Cool/Pix proteins. Although we now have some insight into how the individual Cool proteins are able to confer distinct regulatory effects on PAK, a definitive understanding of the mechanisms underlying the negative regulatory activity of the T1 region will await structural determinations of the full-length Cool proteins. Moreover, little is known regarding to what extent each of the Cool/Pix proteins participate in the various functions of PAK. Future studies will be directed toward understanding whether multiple regulatory activities of Cool/Pix need to be exerted in the same cells or if different Cool proteins exert their specific regulatory effects in a cell-type-specific manner. It will also be interesting to see if the inhibitory effects exhibited by p50Cool-1 on PAK activity, or the lack of stimulatory effects exhibited by p85Cool-1, can be altered or reversed by cellular proteins that bind to and/or phosphorylation events that alter the orientation of the T1 region.

Acknowledgment—We thank C. Westmiller for expert secretarial assistance.

REFERENCES

1. Hall, A. (1998) Science 279, 509–514
2. Lonn, R., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952
3. Hynes, C. D., and Hall, A. (1995) Cell 81, 53–62
4. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
5. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
6. Consalvo, C. A., Chiarlo, M., Yu, J. C., Tani, M., Crespo, P., Xu, N., Mikita, T., and Guttinger, J. S. (1995) Cell 81, 1137–1146
7. Bagrodia, S., Taylor, S. J., Creasy, C. L., Chernoff, J., and Cerione, R. A. (1995) J. Biol. Chem. 270, 22731–22737
8. Martin, G. A., Bolog, G., McCormick, F., and Abo, A. (1995) EMBO J. 14, 1970–1978
9. Manser, E., Leung, T., Salihuddin, H., Zhao, Z. S., and Lim, L. (1994) Nature 363, 40–46
10. Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A. (1993) J. Biol. Chem. 270, 27995–27998
11. Manser, E., Leung, T., Salihuddin, H., Tan, L., and Lim, L. (1993) Nature 363, 364–367
12. Yang, W., and Cerione, R. A. (1997) J. Biol. Chem. 272, 24819–24824
13. Chou, M. M., and Blenis, J. (1996) Cell 85, 573–583
14. Hart, M., Callow, M., Souza, H., and Polakis, P. (1996) EMBO J. 15, 2997–3005
15. McCallum, S. J., Wu, W. J., and Cerione, R. A. (1996) J. Biol. Chem. 271, 21732–21737
16. Kuroda, S., Fukata, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 23363–23367
17. Symons, M., Derry, J. M. J., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Franco, U., and Abo, A. (1996) Cell 84, 723–736
18. Aspdenstrom, P., Lindberg, U., and Hall, A. (1996) Curr. Biol. 6, 70–75
19. Kolluri, R., Tokias, K. F., Carpenter, C. L., Rosen, P. S., and Kirchhausen, T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5615–5618
20. Herskomitz, I. (1995) Cell 80, 197–197
21. Brown, J. L., Stowers, L., Baer, M., Trejo, J., Coughlin, S., and Chant, J. (1996) Curr. Biol. 6, 598–605
22. Consalvo, C. A., Chiarlo, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Mikita, T., and Guttinger, J. S. (1995) Cell 81, 1137–1146
23. Adam, L., Vladiumundi, R., Kondapaka, S. B., Chernoff, J., Mendelsohn, J., and Kumar, R. (1998) J. Biol. Chem. 273, 28238–28246
24. Frost, J. A., Kinoshvatchev, A., Stigger, S., White, M. A., and Cobb, M. H. (1998) J. Biol. Chem. 273, 26191–26198
25. Sells, M., Knaus, U., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J. (1997) Curr. Biol. 7, 202–210
26. Tang, Y., Chen, Z., Ambrose, D., Liu, J., Gibbs, J. B., Chernoff, J., and Field, J. (1997) Mol. Cell. Biol. 17, 4454–4464
27. Rudel, T., and Bokoch, G. M. (1997) Science 276, 1571–1574
28. Lee, N., MacDonald, H., Reinhard, C., Halenbeck, R., Roukstis, A., Shi, T., and Williams, L. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13642–13647
29. Bagrodia, S., Taylor, S. J., Jordan, K. A., Van Aelst, L., and Cerione, R. A. (1998) J. Biol. Chem. 273, 23633–23636
30. Manser, E., Leung, T., and Lim, L. (1998) Mol. Cell. Biol. 18, 183–192
31. Bagrodia, S., Bailey, D., Lenard, Z., Hart, M., Guan, J. L., Premont, R. T., Taylor, S. J., and Cerione, R. A. (1999) J. Biol. Chem. 274, 22293–22299
32. Oh, W. K., Yoo, J. C., Jo, D., Song, Y. H., Kim, M. G., and Park, D. (1997) Biochem. Biophys. Res. Commun. 235, 784–798
33. Premont, R. T., Clain, A., Vitale, N., Freeman, J. L. R., Pitcher, J. A., Patton, W. A., Mora, J., Vaughan, M., and Leffkowitz, R. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14082–14087
34. Turner, C. E., Brown, M. C., Ferrota, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S., and Leventhal, P. S. (1998) J. Cell Biol. 145, 851–863
35. Di Cesare, A., Paris, S., Albertini, C., Dariozzi, S., Andersen, J., Mann, M., Longhi, R., and de Curtis, E. (2000) Nat. Cell Biol. 2, 521–530
36. Bagrodia, S., and Cerione, R. A. (1999) Trends Cell Biol. 9, 350–355
37. Daniel, R. H., Znke, F. T., and Bokoch, G. M. (1999) J. Biol. Chem. 274, 6047–6050
38. Song, J., Khachikian, Z., Radhakrishna, H., and Donaldson, J. G. (1998) J. Cell Sci. 15, 2257–2267