N-terminal Sequences Contained in the Src Homology 2 and 3 Domains of p120 GTPase-activating Protein Are Required for Full Catalytic Activity Toward Ras

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The p120 GTPase-activating protein (GAP) is a negative regulator of Ras, which has a central role in signal transduction pathways that control cell proliferation. p120 GAP accelerates the conversion of activated Ras-GTP to its inactive form, Ras-GDP, thereby inhibiting mitogenic signaling. To examine potential contributions of p120 N-terminal sequences to regulation of its C-terminal catalytic domain, we constructed deletion mutants lacking defined regions, including the variable hydrophobic region as well as the Src homology 2 (SH2) and 3 (SH3) domains. These mutant proteins were expressed in infected Sf9 insect cells from recombinant baculoviruses and assayed in vitro for their ability to stimulate the intrinsic GTPase activity of purified Ras. While deletion of the variable hydrophobic region had no effect on p120 GAP activity, deletion of the entire SH2/SH3/SH2 region severely impaired catalytic activity toward Ras. Deletion of individual SH2 and SH3 domains within this region partially inhibited p120 GAP activity. Moreover, p120 N-terminal sequences enhanced the Ras GTPase-stimulating activity of the neurofibromin GAP-related domain. These results demonstrate that sequences in the SH2/SH3/SH2 region of p120 GAP are required for full catalytic activity toward Ras. Together with earlier findings that the p120 GAP SH2 domains mediate interactions with several GAP-associated proteins, our results suggest multiple roles for the N-terminal sequences in regulating p120 GAP catalytic activity and mitogenic signaling pathways. In addition, our results raise the possibility that SH2 domain point mutations in p120 GAP detected in some basal cell carcinomas reduce catalytic activity toward Ras and thereby contribute to oncogenesis.

p120 GAP mediates its interaction with tyrosine-phosphorylated p90, which is a GAP for the Rho/Rac family of proteins (23, 24). Although p120 GAP SH2 and SH3 domains have been shown to mediate several different protein-protein interactions (25–27), there is also evidence suggesting that they may be involved in regulating GAP activity toward Ras. Previous studies showed that deletion of the entire N-terminal half of p120 GAP, including the SH2/SH3/SH2 region, leads to a significant loss in GAP activity, thus implicating N-terminal sequences as necessary for maximal p120 GAP catalytic activity (28). On the other hand, different studies have suggested a possible negative regulatory role for the p120 GAP N-terminal sequences (29). The specific domains in the p120 GAP N-terminal region that are involved in regulating catalytic activity, however, have not been defined.

To examine contributions of p120 GAP N-terminal sequences...
to GTPase-stimulating activity, we constructed deletion mutants lacking defined regions, including the variable N-terminal hydrophobic region and the SH2/SH3 domains. Analysis of these mutants showed that deletion of the entire SH2/SH3/SH2 region severely impaired p120 GAP catalytic activity toward Ras, while deletion of the individual SH2 and SH3 domains partially impaired GAP activity. Consistent with a positive role for the SH2/SH3/SH2 region, p120 N-terminal sequences enhanced the Ras GTPase-stimulating activity of the GAP-related domain (GRD) of neurofibromin. Conversely, a reciprocal domain swap with the neurofibromin N-terminal sequences did not restore activity of the p120 GAP catalytic domain. Our results suggest that sequences within the SH2/SH3/SH2 region of p120 GAP are necessary for maximal activity toward Ras and have important implications for the potential contribution of mutations in this region to oncogenesis.

**MATERIALS AND METHODS**

**Baculovirus Recombinants**—Construction of the following recombinant baculovirus vectors has been described previously (30): bGAP encoding full-length bovine p120 GAP and ΔSH encoding p120 GAP with a deletion of amino acids 166–164 of human neurofibromin, while the NF1-GAP recombinant contains amino acids 1–1167 of neurofibromin fused to amino acids 671–1149 of p120 GAP. The GRD construct contains amino acids 1125–1536 of human neurofibromin encompassing the catalytic domain of the protein. baculovirus recombinants were constructed: ΔNterm, ΔNSH2, ΔSH3, and ΔCSH2 deletion mutants, initial velocities of p120 GAP mutants were fitted to the Michaelis-Menten equation using Sigmaplot Curvefit.

**CellCulture**—Spodoptera frugiperda (Sf9) insect cells (American Type Culture Collection) were cultured as described previously (30, 31). For protein production, Sf9 cells were singly infected with recombinant baculovirus stocks using a multiplicity of infection of 10 for each virus.

**ProteinExpression**—48 h postinfection, infected Sf9 cells were washed 3 times in cold phosphate-buffered saline and resuspended in sonication buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM MgCl₂, 10% glycerol, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM antipain, and 0.1 μM aprotinin). Cells were then lysed by sonication and clarified by centrifugation, and the soluble protein-containing supernatant was used in subsequent catalytic assays. Normalization of GAP protein levels was achieved by quantitative Western blotting analysis (23). Proteins were resolved on a 7.5% SDS gel, transferred to nitrocellulose, and probed with monoclonal anti-GAP, polyclonal anti-GAP, or polyclonal anti-GRD antibodies. Primary antibodies were detected by ECL (Amer sham Corp.) using horseradish peroxidase-conjugated antimouse or anti-rabbit antibodies, or by [125I]-protein A (ICN) and subsequent quantification by PhosphorImager (Molecular Dynamics).

**GAP Catalytic Assay**—Purified bacterially-expressed human c-Ha-Ras (13) was loaded with [γ-32P]GTP (30 Ci/mmol, DuPont NEN) in the presence of 1 mM EDTA, 1 mM diethiothreitol, 0.25 mg/ml bovine serum albumin, and 22 mM Tris-HCl, pH 7.5, at 37°C for 10 min as described previously (32, 33). GTP-loaded Ras was then incubated with insect cell lysates containing baculoviral-expressed GAPs in the presence of 5 mM MgCl₂, 13 mM Tris-HCl, pH 7.5, 0.1 mg/ml bovine serum albumin, 1 mM diethiothreitol, and 1 mM GTP at 37°C for the indicated times. Reactions were quenched on ice in 1 ml of ice-cold washing buffer (50 mM NaCl, 5 mM MgCl₂, and 25 mM Tris-HCl, pH 7.5), and filtered through nitrocellulose filters (0.45 μm, Schleicher and Schuell). Initial loading at zero time points was determined by adding GTP-loaded Ras to prequenched reactions. GAP activity was measured by monitoring the decrease in [γ-32P]GTP-Ras bound to the filter by liquid scintillation spectrometry (32, 33). For assays using full-length p120 GAP and the individual ΔNSH2, ΔSH3, and ΔCSH2 deletion mutants, initial velocities of p120 GAP activities were determined over a range of concentrations of p21 Ras-GTP. Levels of p120 GAP used in these experiments were previously determined to be in the linear range of the assay. Initial velocities of GTPase reactions in the presence of full-length p120 or deletion

\(^2\) A. Mitchell, J. Cole, D. Gutmann, and F. Collins, unpublished results.

**RESULTS**

**Deletion of p120 GAP SH2/SH3/SH2 Region Impairs its Catalytic Activity Toward Ras**—To examine potential contributions of the p120 N terminus to GAP activity, we created several p120 deletion mutants missing various regions and domains in the N-terminal portion of the protein. Mutants were constructed that lack the N-terminal variable hydrophobic region, the entire SH2/SH3/SH2 region, or the individual SH2 and SH3 domains (Fig. 1). Full-length p120 GAP and deletion mutants were expressed in Sf9 insect cells infected with recombinant baculoviruses. Infected cells were disrupted by sonication, and expression levels of soluble recombinant proteins were normalized to full-length p120 GAP by quantitative Western blot analysis for use in subsequent catalytic assays.

**GAP catalytic activity was measured by an in vitro filter-binding assay using purified, bacterially-expressed p21 c-Ha-Ras loaded with [γ-32P]GTP as substrate. Background GAP-like activity in the assay was found to be negligible when the activity of nonrecombinant baculovirus-infected Sf9 whole cell lysates was compared with the activity of Ras alone (data not shown). Using subsaturating Ras conditions, we compared full-length p120 GAP with two p120 recombinants, ΔSH and...**


ΔNterm, and the GAP activity is shown as percent of GTP hydrolysis (Fig. 2). Deletion of the SH2/SH3/SH2 region in ΔSH markedly impaired p120 GAP catalytic activity, resulting in a level of GTP hydrolysis comparable with that of nonrecombinant baculovirus-infected cell lysates alone (Fig. 2A). In contrast, deletion of the N-terminal hydrophobic region in ΔNterm had no effect on p120 GAP activity (Fig. 2B), suggesting that this region is not essential for full catalytic activity toward Ras.

Each Domain within the SH2/SH3/SH2 Region of p120 GAP Positively Contributes to p120 GAP Catalytic Activity—To define contributions of the individual domains within the SH2/SH3/SH2 region to p120 GAP activity, additional mutants with precise deletions of the SH2 and SH3 domains (Fig. 1) were assayed for catalytic activity. We performed more extensive assays designed to saturate GAP activity with increasing concentrations of Ras-GTP in order to detect subtler effects on p120 GAP activity that might potentially result from the individual domain deletions. Full-length p120 GAP and the deletion mutants were incubated with increasing concentrations of [γ-32P]GTP-Ras ranging from 0 to 50 μM, and the initial velocity of the GTPase reaction was determined at each concentration. Initial velocities were plotted as a function of Ras concentration and fitted to the Michaelis-Menten equation. Saturation of the GAP activity was not achieved under our experimental conditions, precluding accurate determination of the catalytic constants. However, since we normalized the GAP protein levels by quantitative Western blot analysis, we can readily detect relative differences in catalytic activity of deletion mutants as compared with full-length p120 GAP.

Consistent with results obtained under sub saturating Ras concentrations, the ΔSH mutant was severely impaired in its activity toward Ras compared with full-length p120 GAP (Fig. 3A). We assayed the individual p120 domain deletion mutants and found that the ΔSH2, ΔSH3, and ΔCSH2 deletion mutants were all partially impaired to similar extents in their activity toward Ras (Fig. 3, B–D). While the activities of the individual p120 domain deletion mutants were higher than that of ΔSH, their activities were reproducibly lower than that of full-length p120. Relative levels of GAP proteins used in each assay are shown as insets to the graphs. These results indicate that the presence of each domain within the SH2/SH3/SH2 region is necessary for p120 GAP to display full catalytic activity toward Ras.

Sequences within the N Terminus of p120 GAP Stimulate Neurofibromin GAP Activity Toward Ras—We examined the effect of p120 N-terminal sequences on the catalytic activity of another Ras GAP, the NF1 gene product, neurofibromin. Recombinant proteins containing the N-terminal half of p120 GAP fused to the neurofibromin GRD (GAP-NF1) or containing the isolated neurofibromin GRD were expressed in baculovirus-infected SF9 cells (Fig. 1). Analysis of these mutants showed that the neurofibromin GRD was impaired in its GTPase-stimulating activity toward Ras but retained some activity above what was seen with the nonrecombinant vector control (Fig. 4B). When the N-terminal portion of p120 GAP was fused to the neurofibromin GRD, however, GAP activity was significantly enhanced.

To determine whether any sequence fused N-terminal to the p120 GAP catalytic domain enhances activity, a reciprocal recombinant (NF1-GAP) was analyzed that encodes the catalytic domain of p120 GAP downstream of the N-terminal portion of neurofibromin (Fig. 1). Significantly, NF1-GAP had only marginally more activity than the nonrecombinant baculovirus control for background (Fig. 4A). Unlike what was observed with the GAP-NF1 hybrid, the N-terminal sequences of neurofibromin did not have a positive effect upon p120 GAP activity. This finding is consistent with the suggestion that the enhanced activity of the p120 GAP compared with ΔSH is due to...
specific sequences within the p120 GAP N-terminal portion. Taken together with results from the SH2 and SH3 domain deletion mutants, our data demonstrate that the SH2/SH3/SH2 region of p120 GAP is required for full catalytic activity toward Ras and that the N-terminal sequences of p120 GAP stimulate neurofibromin GRD activity.

**DISCUSSION**

Because negative regulation of Ras activity by GAPs is one crucial mechanism the cell employs to control Ras, regulation of GAP activity is an important event for normal cell growth (8, 16, 36–38). The N-terminal portion of p120 GAP has been implicated in a variety of GAP interactions and functions in the cell. The N-terminal hydrophobic region has recently been shown to contribute to interactions with Src-family members through its proline-rich motif (39). The SH2 domains are responsible for multiple protein-protein interactions, including interactions between GAP and receptor tyrosine kinases (40, 41), nonreceptor tyrosine kinases (30, 42), as well as the GAP-associated proteins, p62 and p190 (23, 43–45). Furthermore, the intact SH2/SH3/SH2 region is capable of uncoupling a heterotrimeric G protein from muscarinic receptors (29), while the p120 GAP SH3 domain alone blocks germline vesicle breakdown in Xenopus oocytes (46) and inhibits carbachol-dependent NIH 3T3 transformation via muscarinic receptors (47, 48). In addition to affecting biological functions within different cells, the entire N-terminal half of p120 GAP has been suggested to positively regulate GAP catalytic activity in one case, and possibly inhibit it in another (28, 29). To address the question of the contribution of p120 GAP N-terminal sequences, especially its SH2/SH3/SH2 region, to p120 GAP catalytic activity toward Ras, we constructed several p120 GAP deletion mutants, expressed them in the baculovirus/Sf9 insect cell system, and assayed their ability to stimulate Ras intrinsic GTPase activity.

Consistent with earlier results involving deletion of the entire N terminus of p120 GAP (28), deletion of the SH2/SH3/SH2 domains significantly impaired p120 GAP activity toward Ras. The ΔSH mutant did not detectably stimulate GTPase activity above that of the Ras intrinsic GTPase activity as reflected by the nonrecombinant baculovirus control. Other studies indicated that the isolated p120 GAP catalytic domain displayed reduced but detectable activity (10, 12, 13, 28); this difference might be explained by different constructs or levels of protein used in the assays. By contrast, analysis of the ΔNterm mutant revealed that deletion of this region had no affect upon p120 GAP catalytic activity. These findings suggest a specific requirement for the SH2/SH3/SH2 region for maximal p120 GAP catalytic activity. We examined which domains within this SH2/SH3/SH2 region were responsible for the reduced activity of the ΔSH GAP construct. p120 GAP deletion mutants lacking the individual N-terminal SH2 or SH3 or C-terminal SH2 domains were also impaired in their ability to stimulate Ras GTPase activity, albeit to a lesser extent. No single domain deletion impaired p120 GAP activity as much as deleting the entire SH2/SH3/SH2 region did, suggesting that the complete lack of activity of the ΔSH mutant may be due to the cumulative effect of the individual domain deletions.

To explore further the stimulatory role of the p120 N-terminal sequences, we investigated whether they could affect the GAP activity of the NF1 protein, neurofibromin. When the N terminus of p120 GAP was fused with the GRD of neurofibromin (GAP-NF1), this construct exhibited much higher GAP catalytic activity than the neurofibromin GRD alone. In addition, fusion of p120 N-terminal sequences to the neurofibromin GRD stimulated activity above that of a near full-length neurofibromin recombinant containing the N-terminal neurofibromin sequences as well as the GRD. Significantly, the reciprocal domain swap did not restore p120 GAP activity, as shown by analysis of the hybrid containing the N-terminal sequences of neurofibromin and the catalytic domain of p120 GAP (NF1-GAP). This finding, taken together with the analysis of p120 GAP deletion mutants, points to sequences within the p120 GAP SH2/SH3/SH2 domains as being necessary for p120 GAP to maximally stimulate GTPase activity of Ras.

While the mechanism of how the SH2/SH3/SH2 region positively influences GAP activity remains to be determined, sequences within this region may specifically interact with the catalytic domain to stimulate activity. Alternatively, it is possible that the SH2/SH3/SH2 region contributes to the overall conformational stability of the protein, and thus large deletions in this region may cause nonspecific conformational changes that alter catalytic function. Further study will be required to distinguish between these possibilities. Nevertheless, in addition to the many other roles that these domains have, our findings demonstrate that the SH2 and SH3 domains are essential for p120 GAP to display full catalytic activity toward Ras. These results are of particular interest in light of the observation that mutations in the gene encoding p120 GAP have been detected in human basal cell carcinomas (22). Significantly, in all three cases examined, point mutations resulting in amino acid changes were detected in the C-terminal SH2 domain but not in the catalytic domain of p120 GAP. Our in

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**Fig. 4.** p120 GAP N-terminal sequences enhance neurofibromin activity toward Ras. A, the catalytic activity of the NF1-GAP chimera was compared with that of full-length p120 GAP after normalizing protein levels by Western blot analysis using polyclonal anti-GAP antibody 677 as probe. B, similarly, GAP-NF1 and NF1 GRD recombinants were normalized to each other by Western blot using polyclonal anti-GRD antibody as probe. Nonrecombinant baculovirus (Baculo.) infected cell lysates were used as controls for background GTPase activity. Activity is reflected by percent hydrolysis of GTP as a function of time, with 0 and 100% activity defined by the activity of GAP-NF1 at 0 and 20 min, respectively. For all constructs, assays were performed in duplicate, and plots represent the means with standard errors shown.
intracellular location or interaction with other proteins exclude, however, that the SH2 mutations influence p120 GAP tyrants have reduced catalytic activity, which would lead to ac-
vitro findings raise the possibility that these p120 GAP mu-
region of p120 GAP.

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REFERENCES
1. Smith, M. R., DeGudicibus, S. J., and Stacey, D. W. (1986) Nature 320, 540–543
2. Zhang, K., DeClue, J., Vass, W., Papageorge, A., McCormick, F., and Lowy, D. (1990) Nature 346, 754–756
3. DeClue, J., Zhang, K., Redford, P., Vass, W., and Lowy, D. (1991) Mol. Cell. Biol. 11, 2819–2825
4. DeClue, J., Papageorge, A. G., Fletcher, J. A., Diehl, S. R., Ratner, N., Vass, W. C., and Lowy, D. R. (1992) Cell 69, 265–273
5. Huang, D., Marshall, C., and Hancock, J. (1993) Mol. Cell. Biol. 13, 2420–2431
6. Adari, H., Lowy, D. R., Williamsen, B. M., Der, C. J., and McCormick, F. (1988) Science 240, 519–521
7. Lowy, D., and Williamsen, B. (1993) Annu. Rev. Biochem. 62, 851–891
8. Trahey, M., and McCormick, F. (1987) Science 238, 542–545
9. Vogel, U. S., Dixon, R. A., Schaber, M. D., Diehl, R. E., Marshall, M. S., Sozinik, E. M., Sigal, I. S., and Gibbs, J. B. (1988) Nature 335, 90–93
10. Martin, G., Viskochil, D., Bollag, G., McCabe, P., Crosier, W., Haubruck, H., Conroy, L., Clark, R., O’Cinnel, P., Cawthon, R., Innis, M., and McCormick, F. (1990) Cell 63, 843–849
11. Moran, M., Polakis, P., McCormick, F., Pawson, T., and Ellis, C. (1991) Mol. Cell. Biol. 11, 1804–1812
12. Hetisch, L. M., and Marshall, M. (1994) Cancer Res. 54, 5439–5444
13. Marshall, M., Hill, W., N., Vogel, U., Schaber, M., Sozinik, E., Dixon, L., Sigal, I., and Gibbs, J. (1989) EMBO J. 8, 1105–1110
14. Filvaroff, E., Calautti, E., McCormick, F., and Dotto, G. P. (1992) Mol. Cell. Biol. 12, 5319–5326
15. Pawson, T., and Gish, G. D. (1992) Cell 71, 359–362
16. Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
17. Gibson, T. J., Hynes, M., Musacchio, A., Saraste, M., and Birney, E. (1994) Trends Biochem. Sci. 19, 349–353
18. Sharonin, K. M., O’Cinnel, P., Martin, G. A., Paderanga, D., Olson, K., Dinndorf, P., and McCormick, F. (1994) N. Engl. J. Med. 330, 597–601
19. Wallace, M., Marchuk, D., Andersen, L., Letcher, R., Odeh, H., Saulino, A., Fountain, J., Breteron, A., Nicholson, J., Mitchell, B., Brownstein, B., and Collins, F. (1990) Science 249, 181–186
20. Viskochil, D., Buchberg, A., Xu, G., Cawthon, R., Stevens, J., Woffl, R., Culver, M., Carey, J., Copeland, N., Jenkins, N., White, R., and O’Cinnel, P. (1990) Cell 62, 187–192
21. Viskochil, D., White, R., and Cawthon, R. (1993) Annu. Rev. Neurosci. 16, 183–205
22. Friedman, E., Gemen, P., Martin, G., and McCormick, F. (1993) Nature Genetics 5, 242–247
23. Bryant, S. S., Briggs, S., Smithgall, T., Martin, G., McCormick, F., Chang, J. H., Parsons, S., and Jove, R. (1995) J. Biol. Chem. 270, 17974–17972
24. Ridley, A. J., Self, A. J., Kasmi, F., Paterson, H. F., Hall, A., Marshall, C. J., and Ellis, C. (1993) EMBO J. 12, 5151–5160
25. Anderson, D., Koch, C., Grey, L., Ellis, C., Moran, M., and Pawson, T. (1990) Science 250, 979–982
26. Koch, C. A., Anderson, D., Moran, M. P., Ellis, C., and Pawson, T. (1991) Science 252, 668–674
27. Skolnik, E. Y., Margolis, B., Mohammad, M., Lowenstein, E., Fischer, R., Drees, A., Ulrich, A., and Schlessinger, J. (1991) Cell 65, 83–90
28. Gideon, P., John, J., Froch, M., Lautwein, A., Clark, R., Scheffer, J., and Withnghofer, A. (1992) Mol. Cell. Biol. 12, 2050–2056
29. Martin, G., Yatani, A., Clarke, R., Conroy, L., Polakis, P., Brown, A., and McCormick, F. (1992) Science 255, 192–194
30. Park, S., Marshall, M., Gilbs, J., and Jove, R. (1992) J. Biol. Chem. 267, 11612–11618
31. O’Reilly, D., Miller, L., and Luckow, V. (1992) Baculovirus Expression Vectors: A Laboratory Manual, W. F. Freeman and Co., New York
32. Golubic, M., Tanaka, K., Dobrowolski, S., Wood, D. T., Marshall, M., Tamanini, F., and Stacey, D. W. (1991) EMBO J. 10, 2897–2903
33. Greib, E. M., Roelski, H., Dobrowolski, S., Wolfman, A., and Stacey, D. W. (1992) Oncogene 7, 2151–2159
34. Chang, J.-H., Sutherland, W. M., and Parsons, S. J. (1995) Methods Enzymol., 254, 430–445
35. Gutmann, D., Wood, D. I., and Collins, F. S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9658–9662
36. Field, J., Broek, D., Kataoka, T., and Wigler, M. (1987) Mol. Cell. Biol. 7, 2128–2133
37. Field, J., Nikawa, J., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A., and Wigler, M. (1988) Mol. Cell. Biol. 8, 2159–2165
38. Gilbs, J. B., Schaber, M. D., Allard, W. J., Sigal, I. S., and Scolnick, E. M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5026–5030
39. Briggs, S. D., Bryant, S. S., Jove, R., Sanderson, S. D., and Smithgall, T. E. (1995) J. Biol. Chem. 270, 14718–14724
40. Krasan, D. R., Morrison, D. K., Wong, G., McCormick, F., and Williams, L. T. (1990) Cell 61, 125–133
41. Kazlauska, A., Ellis, C., Pawson, T., and Cooper, J. (1990) Science 247, 1578–1581
42. Brott, B., Decker, S., O’Brien, M., and Jove, R. (1991) Mol. Cell. Biol. 11, 5059–5067
43. Mcglade, J., Brunskrotch, B., Anderson, D., Mbanamu, G., Settiner, J., Dedhar, S., Kazakis-Astochi, M., Chen, L., and Pawson, T. (1993) EMBO J. 12, 3073–3081
44. Settiner, J., Albrit, C. F., Foster, L. C., and Weinberg, R. A. (1992) Nature 359, 153–154
45. Wong, G., Muller, O., Clark, R., Conroy, L., Moran, M., Polakis, P., and McCormick, F. (1992) Cell 69, 551–558
46. Duchesne, M., Shweigheffer, F., Parker, F., Clerc, F., Frobert, Y., Thang, M., and Touque, B. (1993) Science 259, 525–528
47. Xu, J., F., McCormick, F., and Gutfkind, J. S. (1994) Oncogene 9, 597–601
48. Mattingly, R., Sorisky, A., Brann, M., and Macara, I. (1994) Mol. Cell. Biol. 14, 7943–7952
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