Protein Kinase A Blocks Raf-1 Activity by Stimulating 14-3-3 Binding and Blocking Raf-1 Interaction with Ras*

Nicolas Dumaz and Richard Marais‡
From the Cancer Research UK Centre for Cell and Molecular Biology, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, United Kingdom

Received for publication, April 29, 2003, and in revised form, May 30, 2003
Published, JBC Papers in Press, June 11, 2003, DOI 10.1074/jbc.C300182200

Cyclic AMP (cAMP) blocks Raf-1 activation by stimulating its phosphorylation on serine 43 (Ser43), serine 233 (Ser233), and serine 259 (Ser259). We show here that phosphorylation of all three sites blocks Raf-1 binding to Ras.GTP in vivo and that cAMP stimulates binding of 14-3-3 proteins to Ser233 and Ser259. We also show that Raf-1 and protein kinase A (PKA) form a complex in vivo that is disrupted by cAMP and that ablation of PKA by use of small interfering RNA blocks phosphorylation by cAMP. The ability of PKA to block Raf-1 activation is ablated by the PKA inhibitor H89. These studies suggest that Raf-1 and cAMP form a signaling complex in cells. Upon activation of PKA, Raf-1 is phosphorylated and 14-3-3 binds, blocking Raf-1 recruitment to the plasma membrane and preventing its activation.

In mammalian cells, growth factors, mitogens and hormones stimulate the activity of the extracellular signal-regulated protein kinases ERK1 and ERK2. The ERKs control cell growth, differentiation, and survival through the phosphorylation of multiple substrates (for review, see Ref. 1). ERKs are activated by the mitogen-activated protein kinase kinases, MEK1 and MEK2, which are activated by the MEK kinases, Raf-1, A-Raf, and B-Raf. Raf-1 activation is a complex process (for review, see Ref. 2), the first step of which is direct binding to active Ras (Ras.GTP), resulting in Raf-1 recruitment to the plasma membrane. Phosphorylation of serine 338 and tyrosine 341 in the N-region and threonine 491 and serine 495 in the activation segment is essential for Raf-1 activation (3–6). All of these phosphorylation events occur at the plasma membrane, so Ras-mediated recruitment of Raf-1 to the plasma membrane is essential for its activation.

14-3-3 proteins also play a key role in Raf-1 regulation. These small (~30 kDa) acidic dimers bind to and regulate the activity of many proteins by binding to short phosphorylated peptide motifs (7). Two 14-3-3 motifs are present in Raf-1, centered on Ser259 and serine 621 (Ser621) (2). Binding to Ser259 appears to be essential for Raf-1 activation, whereas binding to Ser259 appears to suppress activity. It was recently shown that binding of 14-3-3 to Ser259 antagonizes Raf-1 recruitment to the plasma membrane and prevents its activation by the Ras-related proteins TC21 and R-Ras (8, 9).

Cyclic AMP (cAMP) is another negative regulator of Raf-1. When cellular cAMP levels increase, Raf-1 becomes phosphorylated on three sites (Ser43, Ser233, and Ser259). These sites work independently to suppress Raf-1 activity so all three must be mutated to prevent Raf-1 inactivation by cAMP in cells (8, 10, 11). How these sites work is unclear. PKA phosphorylation of Ser43 prevents the isolated N terminus of Raf-1 from binding to Ras.GTP in vitro (12), but mutation of Ser43 to alanine does not overcome the effects of cAMP in vivo (8, 10, 11). Although 14-3-3 is known to bind to phosphorylated Ser259, its role in cAMP mediated suppression of Raf-1 activity is not known and the mechanism of action of Ser233 is unknown. Finally, it has not been established that PKA mediates these cAMP effects in cells, because cAMP activates several effectors (13), and in some cells, Ser259 can be phosphorylated by protein kinase B (14, 15).

Here we re-examine Raf-1 regulation by cAMP. Our data suggest that Raf-1 and PKA form a signaling complex in cells. When PKA is activated, it phosphorylates Raf-1, and stimulates recruitment of 14-3-3, preventing Raf-1 recruitment to the plasma membrane and subsequently blocking its activation.

MATERIALS AND METHODS

Expression Constructs—The expression vector for myc-tagged Raf-1 (pEF/mRaf-1) and Raf-1 immunoprecipitation kinase assays have been described previously (16). Alkaline-substituted mRaf-1 constructs were generated by standard PCR techniques (17) and verified by automated dideoxy sequencing procedures.

Cell Culture and Biological Techniques—COS and NIH3T3 cells were cultured and transfected with LipofectAMINE (Invitrogen), and cell extracts were prepared as described previously (16). Forskolin, isobutylmethylxanthine (IBMX), and H89 (Calbiochem) were all dissolved in dimethyl sulfoxide (Me2SO). PDGF (Sigma) was dissolved in water. Endogenous Raf-1 was immunoprecipitated with monoclonal antibody M40919.1 (Anogen, Toronto, Canada); mRaf-1 was immunoprecipitated with 9E10, and HA-tagged 14-3-3 and K-Ras with 12CA5. Complexes between Ras and Raf-1 and K-Ras were analyzed as before (9). Phospho-specific antibody to Ser259 was from New England Biolabs (94215) and antibodies to PKA Cα (sc-903), PKA Cβ (sc-904), and PKA RIα (sc-909) were from Santa Cruz.

RNA Interference—Synthetic small interfering RNA (siRNA) probes were from Dharmacon Research Inc. (Lafayette, CO). The following sequences were used: PKA Cα, AAGTGGTTTGCCACGACTGAC; PKA Cβ, AAGAGTTTCTAGCCAAAGCCA; scrambled, AACCGTCGATTTCAC-GAAACAT; and K-Ras with 12CA5. Experiments were performed in triplicate and statistical analyses were performed with the Student’s t test.

Generation of p233-specific Antibodies—Synthetic peptide Raf-1p233 (SQHRysSTPHAF; single amino acid code, where pS is a phosphorylated serine) was coupled to keyhole limpet hemocyanin (374817; Cal-

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
PKA Regulates 14-3-3 Binding to Raf-1

RESULTS

Phosphorylation on Ser43, Ser233, and Ser259 Disrupts Raf1 Binding to RasGTP—To study Raf-1 phosphorylation on Ser43, Ser233, and Ser259, endogenous Raf-1 was immunoprecipitated from NIH3T3 cells using antibody M40091.G and subjected to Western blotting using phospho-specific antibodies. M40091.G was raised in rats using standard protocols (18). For competition experiments, antibodies were preincubated with synthetic peptides (10^-7 M) and IBMX (500 |mu| M) as indicated; 10 min later the cells were treated with EGF (10 min) as indicated, and cell extracts were prepared for Ras:Raf-1 binding assays. The level of Raf-1 and Ras in 10% of the cell extracts is shown in the lower two panels and the immunoprecipitated Ras in the second panel. Raf-1 co-precipitated with Ras.GTP is shown in the first panel. Raf-1 was revealed with antibody 9E10 and Ras with the 12CA5 antibody.

Using these antibodies, we examined the kinetics of phosphorylation of Ser43, Ser233, and Ser259 on endogenous Raf-1 in NIH3T3 cells. In serum-starved cells, Ser43 and Ser259 were very weakly phosphorylated (Fig. 1B). Both were strongly phosphorylated within 60 s of forskolin/IBMX treatment and remained phosphorylated for up to 60 min (Fig. 1B). By contrast, Ser259 was phosphorylated in untreated cells, but its phosphorylation still increased 2-3-fold following forskolin/IBMX treatment (Fig. 1B). For these studies, mRaf-1 was transiently expressed in COS cells together with HA-epitope tagged K-Ras (HAK-Ras). HAK-Ras was immunoprecipitated using monoclonal antibody 12CA5, and the complexes were probed for the associated mRaf-1 by Western blotting with monoclonal antibody 9E10. In agreement with our data for the endogenous proteins (11), this binding was completely blocked when the cells were pretreated with forskolin and IBMX for 10 min (Fig. 1B). When all three sites were mutated, the ability of forskolin/IBMX to block binding to EGFR activated Ras was completely abolished (Fig. 2, lanes 18-21).

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** cAMP induces phosphorylation on Ser43, Ser233, and Ser259. A, mRaf-1 (WT) or S34A, mRaf-1 (233A) were transiently expressed in COS cells. The cells were serum-starved (24 h), left untreated (C), or treated with forskolin/IBMX (F/I; 10 min), and protein extracts were prepared. Myc-epitope-tagged proteins were captured with monoclonal antibody 9E10 for immunoblotting with phospho-specific antibody p233 (upper panels). The p233 monoclonal antibody was preincubated with competing peptides where indicated. Raf-1 levels were verified by blotting with the monoclonal antibody M40091.G (lower panel). B, NIH3T3 cells were serum-starved (24 h) and treated with forskolin/IBMX (F/I) for the indicated times when cell extracts were prepared. Endogenous Raf-1 was immunoprecipitated with monoclonal antibody M40091.G for blotting with phospho-specific antibodies to Ser43 (p43), Ser233 (p233), and Ser259 (p259). Blots were reprobed with M40091.G to reveal total Raf-1 (lower panel).

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** Phosphorylation on Ser43, Ser233, and Ser259 disrupts Raf1 binding to RasGTP. mRaf-1 (WT), S34AmRaf-1 (233A), S233AmRaf-1 (233A), or S43A,S233A,S259AmRaf-1 (43A233A259A) was transiently co-expressed with HAK-Ras (Ras) in COS cells as indicated. The cells were serum-starved (24 h) and left untreated or treated with forskolin/IBMX (F/I) as indicated; 10 min later the cells were treated with EGF (10 min) as indicated, and cell extracts were prepared for Ras:Raf-1 binding assays. The level of Raf-1 and Ras in 10% of the cell extracts is shown in the lower two panels and the immunoprecipitated Ras in the second panel. Raf-1 co-precipitated with Ras.GTP is shown in the first panel. Raf-1 was revealed with antibody 9E10 and Ras with the 12CA5 antibody.

![Diagram](https://example.com/diagram.png)
14-3-3 binds to both Ser259 and Ser259. mRaf-1 (WT), S259A-mRaf-1 (233A), or S259S259A-mRaf-1 (233A259A) were transiently co-expressed with 14-3-3 (14-3-3) in COS cells as indicated. The cells were serum-starved (24 h) and left untreated or treated with forskolin/IBMX (F/IB) for 10 min as indicated, and cell extracts were prepared for 14-3-3-Raf-1 binding assays. The level of Raf-1 and 14-3-3 in 10% of the cell extracts is shown in the lower two panels and the immunoprecipitated 14-3-3 in the second panel. Raf-1 co-precipitated with 14-3-3 is shown in the first panel. Raf-1 was revealed with antibody 9E10 and 14-3-3 with the 12CA5 antibody.

Thus, in vivo phosphorylation of each site appears to block the interaction of Raf-1 with Ras.GTP independently of the other two sites.

14-3-3 Binds to Both Ser253 and Ser259—Next we examined whether 14-3-3 binding to Raf-1 in vivo was affected by elevated cAMP. For these studies, mRaf-1 was expressed in COS cells together with HA epitope-tagged 14-3-3 (HA14-3-3). HA14-3-3 was immunoprecipitated with 12CA5 and the immunoprecipitates examined for bound mRaf-1 by Western blotting for the myc epitope. As we have demonstrated (9), mRaf-1 binds to HA14-3-3 even in resting cells (Fig. 3, lane 5). When the cells were treated with forskolin/IBMX, there was a large increase in 14-3-3 binding to Raf-1 (Fig. 3, lanes 5 and 6), which correlates with a simultaneous increase in Ser259 phosphorylation (Fig. 1B). 14-3-3 binding to Raf-1 was strongly suppressed when Ser259 was mutated to alanine, although forskolin/IBMX still elevated the amount of binding (Fig. 3, lanes 11 and 12).

The above data show that Ser259 is the major cAMP-regulated 14-3-3 binding site on Raf-1 in vivo, but that another site(s) exists, so we tested whether 14-3-3 binds to Ser43 or Ser233. Mutation of Ser43 to alanine did not affect binding of 14-3-3 to Raf-1 (data not shown). However, 14-3-3 binding to Ser233A-mRaf-1 was reduced compared with wild-type mRaf-1, both in untreated and in forskolin/IBMX treated cells (Fig. 3, lanes 8 and 9). Finally, HA14-3-3 binding to mRaf-1 was completely ablated when both Ser233 and Ser259 were mutated to alanine (Fig. 3, lanes 14 and 15). These data show that there are two cAMP regulated 14-3-3 binding sites in the N terminus of Raf-1, Ser233 and Ser259. Ser259 appears to be the major 14-3-3 binding site and Ser233 a minor site.

PKA Mediates the Suppression of Raf-1 by cAMP—Finally, we tested whether the effects of cAMP were mediated by PKA. First, we examined whether Raf-1 and PKA form a complex under physiological conditions. Endogenous Raf-1 was immunoprecipitated from untreated NIH3T3 cells using antibody M40091G, and the immunocomplexes were examined for PKA by Western blotting for the PKA catalytic subunit, Ca. We found that Raf-1 immunoprecipitated from untreated cells contained PKA Ca, but the interaction between these proteins was disrupted when cells were treated with forskolin/IBMX (Fig. 4A). Furthermore, the PKA regulatory subunit, RIα, was also bound to Raf-1 in these cells, but unlike Ca, RIα binding was not disrupted when the cells were treated with forskolin/IBMX (Fig. 4B).

Next we examined the effects of PKA on Raf-1 kinase activity. Raf-1 activity was measured using an immunoprecipitation kinase cascade assay in which GST-MEK, GST-ERK, and myelin basic protein were used as sequential substrates (11). PDGF (50 ng/ml, 5 min) strongly activated Raf-1 in NIH3T3 cells, but this activation was blocked when cells were pretreated with forskolin/IBMX (Fig. 4C and see Ref. 11). However, when the cells were pretreated with forskolin/IBMX in the presence of the PKA inhibitor H89 (10 μM, 30-min pretreatment), Raf-1 activation by PDGF was no longer blocked by forskolin/IBMX pretreatment (Fig. 4C).

Since H89 is not completely specific for PKA (19), we also used RNA interference to ablate the catalytic subunits of PKA in NIH3T3 cells. There are two catalytic subunits in mice, Ca and Cb (20), so we designed siRNA probes selective for each. Treatment of NIH3T3 with these probes resulted in ablation of expression of both subunits within 72 h, whereas the scrambled control probe did not affect expression of either (Fig. 4D). The Ca and Cb probes did not affect expression of Raf-1 or the PKA regulatory subunit RII (Fig. 4D). Ablation of Ca and Cb completely blocked cAMP stimulated phosphorylation of Raf-1 on Ser43, Ser233, and Ser259 (Fig. 4D). Thus, we show for the first time that PKA is responsible for stimulating Raf-1 phosphorylation in response to cAMP in vivo.

DISCUSSION

A number of studies have demonstrated that agents that stimulate cAMP production in cells block Raf-1 signaling to ERK (8, 11, 12, 21, 22). However, since cAMP can activate at least three effectors (PKA, exchange factors for small G-proteins and ion exchange channels (13)), it is possible that the cAMP effects are not mediated by PKA. Here we provide unequivocal proof of the involvement of PKA. We demonstrate that when the PKA catalytic subunits are ablated using siRNA, cAMP does not stimulate phosphorylation on Ser43, Ser233, and Ser259. Although this does not prove that PKA directly phosphorylates Raf-1, it demonstrates that it is necessary. Furthermore, PKA can phosphorylate synthetic peptides representing each site in vitro, demonstrating that these are bona fide PKA sites (Ref. 11 and data not shown). We also show here that endogenous Raf-1 and PKA form a complex that is disrupted when cAMP levels in cells are elevated, and, finally, the PKA inhibitor H89 rescues Raf-1 activation in the presence of forskolin/IBMX. Although this compound inhibits several kinases (19), taken together these data demonstrate that PKA is directly involved in mediating Raf-1 phosphorylation and thereby suppressing its activation.

PKA is regulated by a diverse family of proteins called the protein kinase A anchoring proteins (AKAPs). These scaffolds bind to the PKA regulatory subunits and regulate the subcellular localization of the PKA holoenzymes. They also bind to PKA substrates, co-localizing kinase and substrate and thereby integrating diverse signaling pathways by coordinating phosphorylation of specific substrates (23). cAMP releases the catalytic subunits from this complex and it then phosphorylates local substrates. The discovery of a complex between Raf-1 and PKA is therefore particularly interesting, because it provides a molecular explanation for the integration of these two pathways. The data suggest that a specific AKAP keeps these two kinases co-localized so that when cAMP is elevated PKA can phosphorylate Ser43, Ser233, and Ser259 on Raf-1 and block signaling to MEK. Consistent with this model is the observation that whereas PKA Ca binding to Raf-1 in cells is disrupted by cAMP, RIα subunit binding was not, indicating that the putative AKAP remains bound to Raf-1 even when PKA is
PKA Regulates 14-3-3 Binding to Raf-1

We have previously demonstrated that Raf-1 only completely escapes the effects of cAMP when Ser43, Ser233, and Ser259 are all mutated to alanine (11). We show here that mutation of each also weakly restores binding of Raf-1 to Ras.GTP in vitro, although this least efficient in this respect. These data suggest that each site works independently to block binding to Ras, explaining why they work independently to block Raf-1 activation. The mechanism of action of Ser43 is unclear, but this site is just N-terminal to the Ras binding domain (RBD) of Raf-1 (amino acids 53–112 (24)) and so may work by steric hindrance or by inducing a conformational change in the RBD. Ser233 and Ser259 appear to act primarily by recruiting 14-3-3 to the N terminus of Raf-1.

Previous workers have proposed a model of Raf-1 regulation by 14-3-3. Since 14-3-3 binding to Ser259 inhibits Raf-1 activity, and because 14-3-3 is a dimer, this model proposes that 14-3-3 binds to both sites simultaneously and folds Raf-1 into an inactive conformation (2). Ras.GTP would displace 14-3-3 from CR2, simultaneously unfolding Raf-1 and recruiting it to the plasma membrane for activation. However, we show here that cAMP stimulates Ser259 phosphorylation by 2–3-fold and 14-3-3 binding by about 3-fold in vivo. Thus, in untreated cells, only about 30% of Ser259 appears to be phosphorylated and bound to 14-3-3, so the majority of Raf-1 cannot be in this 14-3-3-maintained inactive conformation. It has also been suggested that cAMP stimulates Ser421 phosphorylation and thereby blocks Raf-1 activity through an unknown mechanism (25). However, we did not find such a role. Ser421 phosphorylation was not elevated in forskolin/IBMX-treated cells, and Ser421 seems to be a rather weak 14-3-3 binding site, because binding was only visible in the S233A,S259AmRaf-1 double mutant when the Western blot was strongly overexposed (data not shown).

Based on our studies, we propose a different model for Raf-1 regulation by 14-3-3 in our cells. We and others recently demonstrated that 14-3-3 binding to Ser259 antagonises Raf-1 recruitment to the plasma membrane (8, 9). We propose that the majority of Raf-1 in resting cells is not phosphorylated on Ser259, and 14-3-3 is not bound to the N terminus of Raf-1, so this population can be recruited to the plasma membrane for activation. The remaining Ser259-phosphorylated population has 14-3-3 bound to its N terminus, so it cannot be recruited to the membrane or activated unless Ser259 is dephosphorylated by protein phosphatase 2A (26–28). We further propose that Raf-1 is in a complex with PKA that may be mediated by AKAPs and that cAMP releases the catalytic subunit, which then phosphorylates Raf-1 on Ser43, Ser233, and Ser259. Phosphorylated Ser43 blocks membrane recruitment by directly interfering with Raf-1 binding to Ras.GTP. Ser233 and Ser259 also block membrane recruitment, but by recruiting 14-3-3 to the Raf-1 N terminus, and 14-3-3 binds with high affinity due to the proximity of the Ser233 and Ser259 sites. Thus, these sites work in unison to suppress Raf-1 activity when cAMP is elevated.

These data fit the model recently proposed by Michael Yaffe about 14-3-3 binding (7). He observed that 14-3-3 often binds to

and PKA Cβ (PKA Ca+Cβ) as indicated. 48 h later, the cells were serum-starved (24 h) and left untreated or treated with forskolin/IBMX (F/I) for 10 min as indicated, and extracts were prepared to analyze the level of PKA Ca, PKA Cβ, and PKA RII (first three panels). Endogenous Raf-1 phosphorylation (last four panels) was analyzed as described in the legend to Fig. 1.
two sites on the same protein, one of which is a dominant site or "gatekeeper." The gatekeeper site is phosphorylated in unstimulated cells, the secondary site is not. Thus, each 14-3-3 dimer is only bound to its client through one of its two binding pockets. However, when the secondary site on the client protein becomes phosphorylated, the second 14-3-3 binding pocket engages onto that client rapidly due to the high local concentration induced by proximity. Since 14-3-3 is extraordinarily rigid, it is thought to behave as a molecular anvil deforming its bound client protein while it undergoes only minimal structural alterations (7, 29). Our data suggest that Ser259 is the gatekeeper site, while Ser233 is the secondary site, which is consistent with the fact that while Ser259 conforms to a strong 14-3-3 consensus (RSTPySTP; important residues are underlined) Ser233 conforms to a weak 14-3-3 consensus (RPySTP;235)(30). When both are phosphorylated, according to the model, 14-3-3 would bind to the N terminus of Raf-1 with high affinity and induce a conformational change that would serve to completely block membrane recruitment, possibly because it also interferes with binding to Ras.GTP. Thus, although either site can block Raf-1 activation, together they provide a much stronger block, and the main effect of cAMP is to inhibit Raf-1 membrane recruitment rather than to specifically block its kinase activity. In our model, it is still unclear what role Ser621 binding plays in Raf-1 regulation and what the relationship between Ser621 and Ser259 binding is. However, our data provide a mechanistic explanation for how phosphorylation of Ser43, Ser233, and Ser259 block Raf-1 activity and support a model in which all three sites can work independently to block Raf-1 activation by cAMP in vivo. Our results directly implicate PKA in this event for the first time.

Acknowledgment—We thank Michael Yaffe for helpful discussion.

REFERENCES
1. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186
2. Avruch, J., Khokhlatchev, A., Kyriakis, J. M., Lau, Z., Tzivion, G., Vavvas, D., and Zhang, X. F. (2001) Recent Prog. Horm. Res. 56, 127–155
3. Mason, C. S., Springer, C., Cooper, R. G., Superti-Furgi, G., Marshall, C. J., and Marnas, R. (1999) EMBO J. 18, 2137–2148
4. Fabian, J. R., Daar, I. O., and Morrison, D. K. (1995) Mol. Cell. Biol. 15, 7170–7179
5. King, A. J., Sun, H., Diaz, B., Barnard, D., Miao, W., Bagrodia, S., and Marshall, M. S. (1998) Nature 396, 180–184
6. Chong, H., Lee, J., and Guan, K. L. (2001) EMBO J. 20, 3716–3727
7. Yaffe, M. B. (2002) FEBS Lett. 513, 53–57
8. Dhillon, A. S., Pollock, C., Steen, H., Shaw, P. E., Mischak, H., and Kolch, W. (2002) Mol. Cell. Biol. 22, 3237–3246
9. Light, Y., Paterson, H., and Marais, R. (2002) Mol. Cell. Biol. 22, 4984–4996
10. Sidowar, M. F., Krowkowsi, P., Lee, J. W., Collins, M. A., He, Y., and Graves, L. M. (2000) J. Biol. Chem. 275, 28688–28694
11. Duman, N., Light, Y., and Marais, R. (2002) Mol. Cell. Biol. 22, 3717–3728
12. Wu, J., Dent, P., Jelinek, T., Wolfman, A., Weber, M. J., and Sturgill, T. W. (1993) Science 260, 1065–1069
13. Beavo, J. A., and Brunton, L. L. (2002) Nat. Rev. Mol. Cell. Biol. 3, 710–718
14. Zimmermann, S., and Moelling, K. (1999) Science 286, 1741–1744
15. Komel, C., Clarke, R. A., Zimmermann, S., Nunez, L., Rossman, K., Reid, K., Moelling, K., Yanceopoulos, G. D., and Glass, D. J. (1999) Science 286, 1738–1741
16. Colledge, M., Mason, C. S., and Marais, R. (2001) Mol. Cell. Biol. 21, 2423–2434
17. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59
18. Harlow, E., and Lane, P. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
19. Davies, S. P., Reddy, H., Caivano, M., and Cohen, P. (2000) Biochem. J. 351, 95–105
20. Skalhegg, B. S., and Tasken, K. (2000) Front. Biosci. 5, D678–D693
21. Cook, S. J., and McCormick, F. (1993) Science 262, 1069–1072
22. Hafner, S., Adler, H. S., Mischak, H., Janosch, P., Heidecker, G., Wolfman, A., Pippig, S., Lohse, M., Ueffing, M., and Kolch, W. (1994) Mol. Cell. Biol. 14, 6696–6703
23. Collardey, M., and Scott, J. D. (1999) Trends Cell Biol. 9, 216–221
24. Marais, R., and Marshall, C. J. (1996) in Cell Signalling (Pawson, P. J., and Meikle, S., eds) Vol. 27, pp. 101–125, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
25. Mischak, H., Seitz, T., Janosch, P., Eulitz, M., Steen, H., Schellner, M., Philipp, A., and Kolch, W. (1996) Mol. Cell. Biol. 16, 5409–5418
26. Abraham, D., Pudar, K., Pacher, M., Kuboekoe, M., Welzel, N., Hemmings, B. A., and Marais, R. (1996) Mol. Cell. Biol. 16, 5409–5418
27. Dhillon, A. S., Meikle, S., Yazici, E., Eulitz, M., and Kolch, W. (2002) EMBO J. 21, 64–71
28. Jaumot, M., and Hancock, J. F. (2001) Oncogene 20, 3849–3858
29. Rittinger, K., Budman, J., Xu, J., Volinia, S., Cantley, L. C., Smerdon, S. J., and Yaffe, M. B. (1999) Mol. Cell. Biol. 15, 153–166
30. Yaffe, M. B., Rittinger, K., Volinia, S., Caron, P. R., Atken, A., Jeffers, H., Gamblin, S. J., Smerdon, S. J., and Cantley, L. C. (1997) Cell 91, 961–971.
Protein Kinase A Blocks Raf-1 Activity by Stimulating 14-3-3 Binding and Blocking Raf-1 Interaction with Ras
Nicolas Dumaz and Richard Marais

J. Biol. Chem. 2003, 278:29819-29823.
doi: 10.1074/jbc.C300182200 originally published online June 11, 2003

Access the most updated version of this article at doi: 10.1074/jbc.C300182200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 16 of which can be accessed free at http://www.jbc.org/content/278/32/29819.full.html#ref-list-1