Basis for Signaling Specificity Difference between Sos and Ras-GRF Guanine Nucleotide Exchange Factors*

Received for publication, August 2, 2001, and in revised form, September 10, 2001
Published, JBC Papers in Press, September 17, 2001, DOI 10.1074/jbc.M107407200

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Sos and Ras-GRF are two families of guanine nucleotide exchange factors that activate Ras proteins in cells. Sos proteins are ubiquitously expressed and are activated in response to cell-surface tyrosine kinase stimulation. In contrast, Ras-GRF proteins are expressed primarily in central nervous system neurons and are activated by calcium/calmodulin binding and by phosphorylation. Although both Sos1 and Ras-GRF1 activate the Ras proteins Ha-Ras, N-Ras, and Ki-Ras, only Ras-GRF1 also activates the functionally distinct R-Ras GTPase. In this study, we determined which amino acid sequences in these exchange factors and their target GTPases are responsible for this signaling specificity difference. Analysis of chimeras and individual amino acid exchanges between Sos1 and Ras-GRF1 revealed that the critical amino acids reside within an 11-amino acid segment of their catalytic domains between the second and third structurally conserved regions (amino acids aa 828–838 in Sos1 and 1057–1067 in Ras-GRF1) of Ras guanine nucleotide exchange factors. In Sos1, this segment is in helix B, which is known to interact with the switch 2 region of Ha-Ras. Interestingly, a similar analysis of Ha-Ras and R-Ras chimeras did not identify the switch 2 region of Ha-Ras as encoding specificity. Instead, we found a more distal protein segment, helix 3 (aa 91–103 in Ha-Ras and 117–129 in R-Ras), which interacts instead primarily with helix K (aa 1002–1016) of Sos1. These findings suggest that specificity derives from the fact that R-Ras-specific amino acids in the region analogous to Ha-Ras helix 3 prevent a functional interaction with Sos1 indirectly, possibly by preventing an appropriate association of its switch 2 region with helix B of Sos1. Although previous studies have shown that helix B of Sos1 and helix 3 of Ha-Ras are involved in promoting nucleotide exchange on Ras proteins, this study highlights the importance of these regions in establishing signaling specificity.

Members of the Ras superfamily of GTPases function as molecular switches, cycling between the active GTP-bound and inactive GDP-bound states. They become active in cells upon interaction with guanine nucleotide exchange factors (GEFs). These GEFs promote the release of GDP from inactive GTPases, allowing their replacement with activating GTP. In the active state, the GTPases bind to and alter specific sets of “effector” molecules until the GTPases hydrolyze GTP back to GDP. This deactivating process is enhanced by GTPase-activating proteins (1).

The Ras superfamily consists of the Ras, Rho, Rab, Arf, and Ran families. The Ras family includes the Ras proteins (Ha-Ras, Ki-Ras, and N-Ras) and the TC21, R-Ras, M-Ras, Rap,Ral, Rho, Rin, Rad, Kir/Gem, Rit, and K-B-Ras proteins. These proteins are grouped because of their similar amino acid sequence, although their functions can differ quite significantly. This is because they can be activated by different upstream signals and affect different sets of downstream target proteins. Ras proteins have been the most extensively studied members of this subfamily. By activating at least three downstream signaling pathways mediated by Raf kinases, Ral-GEFs, and phosphatidylinositol 3-kinases, Ras proteins have been implicated in a wide variety of cellular processes, including enhancement of cell proliferation, differentiation, and multiple functions associated with fully differentiated cells (2). R-Ras is quite similar to Ras proteins (55% amino acid sequence identity) and can be activated by some GEFs that activate Ras, such as Ras-GRF1, and inactivated by Ras GTPase-activating proteins such as p120Ras-GAP (3). R-Ras even contains the same minimal effector domain that is responsible for binding downstream effector proteins; yet for reasons that are not understood, R-Ras displays biological properties that are distinct from those of Ras proteins. For example, unlike Ras, which activates all three downstream target proteins it binds to in cells, R-Ras activates only phosphatidylinositol 3-kinase (4). R-Ras is also a much weaker protein than Ras (5, 6). Moreover, R-Ras promotes inside-out integrin activation, whereas Ras proteins do not (7–10).

The catalytic domains of GEFs for Ras subfamily GTPases such as Ras, R-Ras, and Ral are all similar in amino acid sequence to the first Ras-GEF identified, yeast Cdc25. Thus, they are referred to as Cdc25 homology domains (11). Most studies on Cdc25-like GEFs have investigated the Ras-activating Sos and Ras-GRF families. Sos1 and Sos2 are highly similar GEFs that contain Grb2-binding domains, allowing them to be activated by cell-surface tyrosine kinases (2, 12). Ras-GRF1 (also referred to as Cdc25Mm) (13, 14) and Ras-GRF2 (15) are also highly similar GEFs that contain calmodulin-binding domains, allowing them to be activated by elevated calcium in cells (16, 17). Ras-GRF1 can also be activated by phosphorylation (18–20). Both families of GEFs also contain DH domains that allow them to activate Rac GTPases (21–23).

Studies on Sos1 and Ras-GRF1 have revealed important insights into the mechanism of GEF action. Kinetic studies on Ras-GRF1 have shown that GEF-stimulated nucleotide exchange involves the transient creation of a ternary Ras-nucleotide-GEF complex, followed by the formation of a stable binary Ras-GEF complex. Nucleotide (usually GTP be-
cause of its excess over GDP in cells) then binds to this binary complex, promoting the release of the GEF (24). Structure/ function studies based on mutagenesis have identified three regions of Ras that interact with GEFs: the switch 1 region (aa 25–40), the switch 2 region (aa 57–65), and α-helix 3 (aa 92–104) plus loop 7 (aa 105–109) (25–36). Recent crystallographic studies on a complex between Sos1 and Ha-Ras have revealed the overall structural design of Sos1, contact points with Ha-Ras, and important insights into the potential mechanism of GEF action (37). The Sos1 structure is predominantly α-helical, with helices H and I interacting with switch 1 of Ha-Ras to open the nucleotide-binding site, allowing for exchange. Helices B, D, and G interact predominantly with switch 2 of Ha-Ras, and helix K with helix 3 of Ras apparently to stabilize GEF/Ras binding (37, 38).

Although each Cdc25-like GEF was originally thought to activate members of only one Ras subfamily, it is now appreciated that some GEFs can activate more than one. For example, although Sos1 stimulates classical Ras proteins (Ha-Ras, N-Ras, and Ki-Ras) and the closely related M-Ras (3, 39), Ras-GRF1 can activate Ras proteins, M-Ras, and the functionally distinct R-Ras (3, 40). C3G can also stimulate functionally distinct GTPases such as Rap, R-Ras, and TC21 (3, 40). To analyze the basis for signaling specificity differences in vivo, we used Ha-Ras and R-Ras mutated in their C termini (CAAX to SAAX, where A is aliphatic amino acid) to prevent their post-translational prenylation. In contrast to Ras-GRF2, Sos1 still failed to activate R-Ras under these conditions (Fig. 1B).

Specificity—Previous studies assaying GEF activity in vitro showed that the signaling specificity difference between Sos1 and Ras-GRF1 is encoded in the catalytic domains of the two proteins, which span ~500 amino acids near their C termini (40). We confirmed these findings using the in vivo assay described above. Transfection of these fragments of Sos1 (aa 564–1049) and Ras-GRF1 (aa 798–1244) into 293 cells showed that the catalytic domain of Ras-GRF1 activated Ha-Ras and R-Ras, whereas the catalytic domain of Sos1 activated only Ha-Ras (Fig. 1C).

We then localized the specificity region more precisely by assaying a set of chimeras between portions of the catalytic domains of Sos1 and Ras-GRF1. Recent structural analysis has revealed that the catalytic domain of Sos1 consists of two distinct α-helical domains (see Fig. 2A) (37). The N-terminal domain (aa 568–741) containing α-helices 1–6 does not interact directly with Ras, but appears to contribute to the structural integrity of the catalytic segment of Sos1. The C-terminal domain (aa 752–1044) is made up of helices A–K and contains all of the regions that interact with Ras. Although the structure of Ras-GRF1 has not been solved, it is assumed to have a similar overall structure (37). Based on this information, we generated a series of chimeras that encoded various amounts of the N-terminal segment of the catalytic domain of Sos1 and various amounts of the C-terminal segment of the catalytic domain of Ras-GRF1 (Fig. 2A). These genes were then transfected into 293 cells along with either Ha-Ras or R-Ras to test their GEF specificity as described for Fig. 1. Chimera S-A-G, which encoded all of the N-terminal domain containing helices 1–6 from Sos1 plus half of helix A (aa 546–790 in Sos1) and the C-terminal domain of Ras-GRF1 containing the second half of helix A plus helices B–K (aa 1021–1244 in Ras-GRF1) promoted nucleotide exchange in both Ha-Ras and R-Ras (Fig. 2B), indicating that the ability to activate R-Ras derives from the C-terminal segment of Ras-GRF1.

Chimera S-D-G, which encoded the N-terminal domain plus helices A–C from Sos1 (aa 546–869) and the remaining helices of the C-terminal domain from Ras-GRF1 (aa 1099–1244), retained the ability to activate Ha-Ras, but lost the ability to activate R-Ras (Fig. 2B). A similar result was obtained with chimera S-H-G, which contained the N-terminal domain plus helices A–G from Sos1 (aa 546–923) and the remaining helices from Ras-GRF1 (aa 1155–1244). These results, together with those obtained with chimera S-A-G, imply that signaling specificity resides within the segment encompassing helices A–C of the C-terminal domain.

To further define the key segment, additional chimeras were generated and assayed (Fig. 3, A and B). Chimera S-A-B-G, which contained helices 1–6 plus helix A from Sos1 (aa 546–
818) and helices B–K from Ras-GRF1 (aa 1048–1244) activated both Ha-Ras and R-Ras (Fig. 3, A and B). Thus, helix A is not involved in specificity. If we included helix B (chimera S-835-G) or helix B plus additional segments from Sos1 (chimeras S842-G, S-852-G, and S-861-G), not only was R-Ras activity lost, but Ha-Ras activity was lost as well (Fig. 3B). This was despite the fact that the mutants were expressed in cells at levels comparable to those of the functional proteins (Fig. 3C).

**Fig. 1.** Ras-GRF1 activates Ha-Ras and R-Ras, whereas Sos1 activates only Ha-Ras. 293 cells were transfected with a GTPase (GST-Ha-Ras or GST-R-Ras) and either empty vector or a vector containing a GEF (Sos1 or Ras-GRF1). 48 h later, the cells were labeled with $^{32}$PO$_4$ for 4 h, and then either GST-Ha-Ras or GST-R-Ras was isolated from cell extracts with glutathione-agarose beads. Labeled nucleotides bound to the isolated GTPases were separated by thin-layer chromatography, and the ratio of GTP to GTP$^\gamma$GDP bound to the GTPase was assessed by a PhosphorImager. A, wild-type Ha-Ras, R-Ras, Sos1, and Ras-GRF1 were used. B, wild-type Sos1 and Ras-GRF1 were used, but Ha-Ras and R-Ras mutants that could not be post-translationally processed (Ha-Ras-S$^{\delta\delta\delta}$AX and R-Ras-S$^{\delta\delta\delta}$AX) were used. C, the catalytic domains of Sos1 (aa 564–1049; SOS-C) and Ras-GRF1 (aa 798–1244; GRF1-C) and Ha-Ras and R-Ras mutants that could not be post-translationally processed were used. The levels of expression of the catalytic domains of Sos1 and Ras-GRF1 are shown. Data represent the means ± S.D. of two experiments, each performed in duplicate.
When we attempted to retain just helix B of Ras-GRF1 in the context of the rest of Sos1, the chimeric protein was not expressed (data not shown). Although this last set of chimeras lost more than just activity against R-Ras, they suggested that helix B was critical for R-Ras signaling activity. These results and those from the chimeras described for Fig. 2 suggest that the specificity region is localized within a 52-amino acid segment between amino acids 818 and 869 in Sos1 (aa 1047 and 1098 in Ras-GRF1) containing helices B and C. This hypothesis was confirmed by chimera S-AB-D-S, which was all Sos1 except for these 52 amino acids of Ras-GRF1 inserted in place of analogous Sos1 amino acids. It behaved like Ras-GRF1 and activated both Ha-Ras and R-Ras (Fig. 3, A and B).

These 52 amino acids in Sos1 and comparable ones in Ras-GRF1 are shown in Fig. 4. In an attempt to determine which amino acids within this region are most important for exchange activity against R-Ras, Sos1 amino acids were used to replace Ras-GRF1 residues at sites where divergence was most significant. The Ras-GRF1 mutants were then screened for loss of R-Ras reactivity, but retention of Ha-Ras reactivity. Eight different substitutions were made with amino acids from Sos1 distal to helix B where direct contacts between Sos1 and Ha-Ras are not made (Sos1 numbering R841E/N842T, E844N, S846E/A847E, A849V, W855I, A857E, D860Q, and C864E). None of these mutations significantly influenced the ability of Ras-GRF1 to activate R-Ras or Ha-Ras (data not shown), and neither did mutations in the N terminus of helix B. These mutations included Y820N, in which the asparagine in Sos1 is not directly involved with Ras binding. They also included the double mutant T824M/T825I, in which both methionine and isoleucine in Sos1 directly contact Ha-Ras amino acids. The properties of this last mutant suggest that unlike the comparable Sos1 amino acids Met824 and Ile825, Thr1053 and Thr1054 of Ras-GRF1 do not play a critical role in Ha-Ras binding because they are quite distinct from the Sos1 amino acids and yet changing them to Sos1 amino acids did not disrupt GEF activity.

In contrast, many changes made in the C-terminal end of helix B (F828T/N829T, N833L/L834W/I835F, and A836E/S837K/E838C) had drastic effects on Ras-GRF1 activity such that the mutants no longer activated either R-Ras or Ha-Ras (data not shown). All three of these mutations included amino acids that participate in the binding between Sos1 and Ha-Ras (Fig. 4) (37). Thus, key amino acids involved in R-Ras activation by Ras-GRF1 reside at the end of helix B; however, these sequences also appear to be involved in Ha-Ras activation. The fact that these Sos1 sequences could not substitute for Ras-GRF1 sequences adds additional evidence supporting the notion that these GEFs interact differently with target GTPases. Overall, the data indicate that the key residues endowing Ras-GRF1 with the ability to activate R-Ras and to prevent Sos1 from activating R-Ras reside in amino acids 1057–1067 of Ras-GRF1 and amino acids 828–838 of Sos1.

Mapping the Regions of Ras Proteins Involved in Signaling Specificity—Characterization of chimeras between Ha-Ras and R-Ras was used to identify key residues of the GTPases that

![Diagram](http://www.jbc.org)
generate differential responses to Ras-GRF1 and Sos1. Sos1 interacts with multiple surfaces of Ras, including the phosphate-binding P-loop (aa 10–17), switch 1 (aa 25–40), switch 2 (aa 57–75), and helix 3/loop 7 (37). The initial chimera generated encoded the first 82 amino acids of R-Ras and the final 133 amino acids of Ha-Ras. This mutant (R-57-Ha-Ras), which contained a 26-amino acid N-terminal extension unique to R-Ras and the P-loop and switch 1 of R-Ras (aa 83 and aa 57 in Ha-Ras, respectively) joined to the switch 2 region and beyond of Ha-Ras, retained responsiveness to both Ras-GRF1 and Sos1. Thus, amino acid residues responsible for specific responses to GEFs reside in the C-terminal two-thirds of the protein. In the next chimera (R-91-Ha-Ras), the contribution of R-Ras was extended to include the switch 2 region and additional amino acids up to R-Ras residue 116 followed by Ha-Ras sequence 91 and beyond. This chimera also responded to both Ras-GRF1 and Sos1, indicating that the key residues are more C-terminal than amino acid 116 of R-Ras (aa 91 of Ha-Ras). When the R-Ras contribution was extended to amino acid 129 such that Ha-Ras contributions started at Ha-Ras amino acid 129, comparable 52 amino acids in specificity regions of Sos1 and Ras-GRF1. Amino acids 818–869 in Sos1 and amino acids 1047–1098 in Ras-GRF1 are shown along with the α-helices of Sos1 in this region. The "structurally conserved regions" (SCRs) that are highly conserved among Ras subfamily GEFs are shown. Boldface letters and arrows refer to amino acids in Ras-GRF1 substituted with amino acids from Sos1 that led to loss of activity. Open circles show amino acid substitutions in Ras-GRF1 that had no measurable effect. Amino acids that make direct contacts with Ha-Ras are indicated (*).
DISCUSSION

This study identified amino acids in both GEFs and GTPases that are responsible for the fact that Ras-GRF1 can activate Ha-Ras and the functionally distinct R-Ras, whereas Ras-GRF2 can activate only the former. We have been aided in designing experiments and interpreting results by the recent elegant studies revealing the crystal structure of Sos1 bound to Ha-Ras (37). Unfortunately, the structures of Ras-GRF1 and R-Ras have not been solved; however, it is likely that they have similar folds, and we have assumed as much for the purpose of discussion. Chimeras between Sos1 and Ras-GRF1 identified a 52-amino acid segment forming helices B and C in the catalytic domains of the GEFs as the key determinant of specificity. Merely replacing these amino acids in Sos1 with analogous Ras-GRF1 amino acids endowed Sos1 with the ability to activate R-Ras. Unfortunately, we could not do the converse experiment and show loss of R-Ras activity upon replacement of comparable Ras-GRF1 amino acids with Sos1 residues since the chimera produced lost all GEF activity. When we attempted to make chimeras with smaller regions of Sos1 replaced by analogous regions of Ras-GRF1, we again produced proteins that lost all GEF activity. However, we could exchange a variety of single and double amino acids from all regions of helix C of Sos1 into Ras-GRF1 without affecting activity against Ha-Ras or R-Ras, suggesting that helix B (not helix C) is most important.

That helix B plays a role in GEF signaling specificity is consistent with previous observations. First, structural studies have shown that amino acids within helix B of Sos1 participate in the interaction with Ha-Ras (37). Moreover, it has been observed previously that the Cdc25 family of GEF domains, which are responsible for activating Ras subfamily GTPases such as Ras, Rap, and Ral, contains four highly conserved regions (structurally conserved regions) (43). As would be expected for amino acids that generate specificity among these Cdc25 family members, helix B is not part of the structurally conserved regions and is not highly conserved among GEFs with different target GTPase specificity (see Fig. 4).

Within helix B, we exchanged N-terminal amino acids that differ between Sos1 and Ras-GRF1 with little effect. These included Sos1 residues that do not make direct contact with Ha-Ras, such as Leu821 and Leu822, but also those that do, such as Met824 and Ile825. Results with the latter two mutants suggest that Sos1 and Ras-GRF1 function differently because the comparable Ras-GRF1 amino acids (Thr1051 and Thr1052) are significantly distinct from their Sos1 counterparts. Moreover, replacing them with Sos1 amino acids did not alter Ras-GRF1 activity. In contrast, replacing Ras-GRF1 amino acids with Sos1 amino acids from the C-terminal end of helix B blocked Ras-GRF1 activity for R-Ras. However, these changes also blocked Ras-GRF1 activity for Ha-Ras. These substitutions involved Thr826, Thr828, Leu833, and Glu836, all of which are known to make direct contacts with Ha-Ras. These findings localized the specificity region to an 11-amino acid stretch in
helix B from Thr to Cys of Sos1 and from Phe to Glu of Ras-GRF1. The fact that Ras-GRF1 lost both R-Ras and Ha-Ras activities when Ras-GRF1 amino acids were replaced with Sos1 amino acids shows that it is difficult to disassociate basic GEF activity from target specificity. This observation adds to the argument that Ras-GRF1 and Sos1 use somewhat different mechanisms to promote guanine nucleotide exchange on Ha-Ras. A similar conclusion was reached recently in mutagenic studies of GTPases that investigated the basis for signaling specificity differences between Ras-GRF1 (Cdc25Mm) and Rap-GEF C3G (44) and Ras-GRP (45).

Helix B of Sos1 does not directly promote nucleotide release from Ha-Ras. Instead, it stabilizes Sos1/Ha-Ras interactions primarily by forming part of the interface with the switch 2 region of Ha-Ras (Fig. 7) (37, 38). These findings raised the possibility that the reason Sos1 can activate Ha-Ras (but not R-Ras) is that helix B of Sos1 can participate in binding to the switch 2 region of Ha-Ras, but not to the comparable amino acids in R-Ras. Since we found that replacement of helices B and C of Sos1 with helices B and C of Ras-GRF1 generated Sos1 activity against R-Ras, helix B of Ras-GRF1 can likely form a productive interaction with the switch 2 region regardless of whether it is part of Ha-Ras or R-Ras.

It is not obvious, however, how Sos1 can distinguish between the switch 2 regions of Ha-Ras and R-Ras because they are quite similar. In fact, previous work has shown that exchanging the one pair of differing amino acids in Ha-Ras and TC21 (which also does not respond to Sos1) has no effect on signaling specificity (3). Moreover, in the present study, chimeras of Ha-Ras and R-Ras that exchanged switch 2 regions had no effect on responsiveness to GEFs. Instead, these chimeras identified a 13-amino acid region (aa 91–103 in Ha-Ras) distal to switch 2, which constitutes helix 3 of Ha-Ras, as the key element for specificity. Merely replacing analogous amino acids in R-Ras with these 13 Ha-Ras amino acids allowed Sos1 to activate R-Ras. In addition, swapping a histidine from Ha-Ras for a glycine normally found in R-Ras at a site inside this region generated some responsiveness of R-Ras to Sos1, and swapping a glycine from R-Ras for a histidine normally found in Ha-Ras suppressed responsiveness of Ha-Ras to Sos1. These results are consistent with the fact that helix 3 has already been shown by mutagenesis studies to be involved in GEF binding (25, 31, 34).

Fig. 6. Amino acid exchange between Ha-Ras His and R-Ras Gly confirms specificity domain for GTPase responsiveness to Ras-GEFs. A, shown is a comparison of Ha-Ras and R-Ras amino acids in specificity region 91–103 of Ha-Ras and 117–129 of R-Ras with amino acids switched between the two GTPases highlighted in boldface. B and C, R-Ras His and Ha-Ras Gly, respectively, were assayed for responsiveness to the catalytic domains of either Sos1 (SOS-C) or Ras-GRF1 (GRF-C) as described in the legend to Fig. 1. The data represent the means ± S.D. of two independent experiments, each performed in duplicate.
ulate based on existing information. For example, the crystal structure of Ras and the Sos1-Ha-Ras complex reveals that helix 3 of Ha-Ras is in close proximity to switch 2 of Ha-Ras; and in particular, Val^103 of helix 3 interacts with Asp^92 of switch 2 (see Fig. 7). Since switch 2 of Ha-Ras binds to helix B of Sos1, switch 2 could be the intermediate that connects helix 3 of Ha-Ras with helix B of Sos1. A more indirect model involves helix K of Sos1 since it binds to helix 3 of Ras and is also in close proximity to helix B of Sos1 (Fig. 7). Obviously, a definitive explanation must await structural analysis of Ras-GRF1 complexes with Ha-Ras and R-Ras.

The discovery that helix B of Sos1 determines specificity among Ras-GEFs for activating Ha-Ras and R-Ras is striking in light of results from our recent experiments investigating the basis for signaling specificity differences between Ras-GRF1 and Sos1, the unique features involve helix B, whereas for Ha-Ras and R-Ras, they involve helix 3. R-Ras and Ras proteins have different activities in cells. For example, of the three downstream target proteins known to be activated by Ha-Ras, only phosphatidylinositol 3-kinase is activated by R-Ras (4). Also, R-Ras displays weaker oncogenic activity than Ras (5, 6); however, it can promote inside-out integrin activation, whereas Ras cannot (7). Understanding the mechanisms used by Ras-GRF1 to activate Ras and R-Ras proteins will be important to explain fully Ras-GRF1 function in neurons, where Ras and R-Ras proteins likely play unique but complementary functions.

**Acknowledgment**—We thank Dr. Jim Baleja for helpful discussions concerning the manuscript.

**REFERENCES**

1. Takai, Y., Sasaki, T., and Matosaki, T. (2001) Physiol. Rev. 81, 153–208
2. Medema, R. H., and Bos, J. L. (1993) Crit. Rev. Oncog. 4, 615–661
3. Ohba, Y., Mochizuki, N., Yamashita, S., Chan, A. M., Schrader, J. W., Hattori, S., Nagashima, K., and Matsuda, M. (2000) J. Biol. Chem. 275, 20920–20926
4. Marte, B. M., Rodriguez-Viciana, P., Wennstrom, S., Warne, P. H., and Downward, J. (1997) Curr. Biol. 7, 63–70
5. Cox, A. D., Brita, T. R., Lowe, D. G., and Der, C. J. (1994) Oncogene 9, 3281–3288
6. Self, A. J., Caron, E., Paterson, H. F., and Hall, A. (2001) J. Cell Sci. 114, 1357–1366
7. Zhang, Z., Vuori, K., Wang, H., Reed, J. C., and Ruoslahti, E. (1996) Cell 85, 61–69
8. Sethi, T., Ginsberg, M. H., Downward, J., and Hughes, P. E. (1999) Mol. Biol. Cell 10, 1799–1809
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9. Keely, P. J., Ruesy, E. V., Cox, A. D., and Parise, L. V. (1999) J. Cell Biol. 145, 1077–1088
10. Wang, B., Zou, J. X., Ek-Rylander, B., and Ruoslahti, E. (2000) J. Biol. Chem. 275, 5222–5227
11. Feig, L. A. (1994) Curr. Opin. Cell Biol. 6, 204–211
12. Bowtell, D., Fu, P., Simon, M., and Senior, P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6511–6515
13. Shou, C., Farnsworth, C. L., Neel, B. G., and Feig, L. A. (1992) Nature 358, 351–354
14. Cen, H., Papageorge, A. G., Zippel, R., Lowy, D. R., and Zhang, K. (1992) EMBO J. 11, 4007–4015
15. Fan, N. P., Fan, W.-T., Wang, Z., Zhang, L.-J., Chen, Z., and Moran, M. F. (1997) Mol. Cell. Biol. 17, 1396–1406
16. Farnsworth, C. L., Freshney, N. W., Rosen, L. B., Ghosh, A., Greenberg, M. E., and Feig, L. A. (1995) Nature 376, 524–527
17. Chen, L., Zhang, L., Greer, P., Tung, P. S., and Moran, M. F. (1993) Dev. Genet. 14, 339–346
18. Kiyono, M., Kato, J., Kataoka, T., Kaziro, Y., and Satoh, T. (2000) J. Biol. Chem. 275, 29788–29793
19. Kiyono, M., Kaziro, Y., and Satoh, T. (2000) J. Biol. Chem. 275, 5441–5446
20. Mattingly, R. R., and Macara, I. G. (1996) Nature 382, 268–272
21. Nimmal, A. S., Yatsula, B. A., and Bar-Sagi, D. (1998) Science 279, 566–563
22. Fan, W.-T., Koch, C. A., de Hoog, C. L., Fan, N. P., and Moran, M. F. (1998) Curr. Biol. 8, 935–938
23. Kiyono, M., Satoh, T., and Kaziro, Y. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4826–4831
24. Lenzen, C., Cool, R. H., Prinz, H., Kuhlmann, J., and Wittinghofer, A. (1998) Biochemistry 37, 7420–7430
25. Willumsen, B. M., Papageorge, A. G., Kung, H., Bekesi, E., Robine, T., Johnson, M., Vass, W. C., and Lowy, D. R. (1996) Mol. Cell. Biol. 6, 2646–2654
26. Fasano, O., Crecot, J. B., De Vendittis, E., Zahn, R., Feger, G., Vitelli, A., and Parmeggiani, A. (1998) EMBO J. 7, 3375–3383
27. Mitsu, M. Y., Jacquet, E., Poulet, P., Kung, H., Gidion, P., Schlichting, I., Wittinghofer, A., and Parmeggiani, A. (1992) EMBO J. 11, 2391–2397
28. Fasano, O., Crecot, J. B., Di Blasi, F., Seranita, G., Mira, M., and Parmeggiani, A. (1992) EMBO J. 11, 2391–2397
29. Kiyono, M., Kaziro, Y., and Satoh, T. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3544–3548
30. Segal, M., Willumsen, B. M., and Levitzky, A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5564–5568
31. Mosteller, R. D., Han, J., and Brock, D. (1994) Mol. Cell. Biol. 14, 1104–1112
32. Quilliam, L. A., Kato, K., Rubin, K. M., Hisaka, M. M., Huff, S. Y., Campbell-Burk, S., and Der, C. J. (1994) Mol. Cell. Biol. 14, 1113–1121
33. Leonardsen, L., DeClue, J. E., Lybaek, H., Lowy, D. R., and Willumsen, B. M. (1996) Oncogene 13, 2177–2187
34. Quilliam, L. A., Hisaka, M. M., Zhong, S., Lowry, A., Mosteller, R. D., Han, J., Drugan, J. K., Brock, D., Campbell, S. L., and Der, C. J. (1996) J. Biol. Chem. 271, 11076–11082
35. Day, G. J., Mosteller, R. D., and Brock, D. (1998) Mol. Cell. Biol. 18, 7444–7454
36. Boriack-Sjodin, P. A., Margarit, S. M., Bar-Sagi, D., and Kuriyan, J. (1998) Nature 394, 337–343
37. Hall, B. E., Yang, S. S., Boriack-Sjodin, P. A., Kuriyan, J., and Bar-Sagi, D. (2001) J. Biol. Chem. 276, 27629–27637
38. Quilliam, L. A., Castron, A. F., Rogers-Graham, K. S., Martin, C. B., Der, C. J., and Bi, C. (1999) J. Biol. Chem. 274, 23850–23857
39. Gotto, M. N., Nishida, Y., Tomida, M., Hatake, O., Nakamura, S., Matsuoka, M., and Hattori, S. (1997) J. Biol. Chem. 272, 18602–18607
40. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 61–68
41. Gotto, M. N., Nishida, Y., Tomida, M., Hatake, O., Nakamura, S., Matsuoka, M., and Hattori, S. (1997) J. Biol. Chem. 272, 18602–18607
42. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 61–68
43. Gotto, M. N., Nishida, Y., Tomida, M., Hatake, O., Nakamura, S., Matsuoka, M., and Hattori, S. (1997) J. Biol. Chem. 272, 18602–18607
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J. Biol. Chem. 2001, 276:47248-47256.
doi: 10.1074/jbc.M107407200 originally published online September 17, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107407200

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