Phosphorylation of the Ras-GRF1 Exchange Factor at Ser916/898 Reveals Activation of Ras Signaling in the Cerebral Cortex*

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The Ras-GRF1 exchange factor, which is regulated by increases in intracellular calcium and the release of GTP subunits from heterotrimeric G proteins, plays a critical role in the activation of neuronal Ras. Activation of G protein-coupled receptors stimulates an increase in the phosphorylation of Ras-GRF1 at certain serine residues. The first of these sites to be identified, Ser916 in the mouse sequence, is required for full activation of the Ras exchange factor activity of Ras-GRF1 by muscarinic receptors. We demonstrate here that Ras-GRF1 is highly expressed in rat brain compared with the Sos exchange factor and that there is an increase in incorporation of [32P] into Ser916 of brain Ras-GRF1 following activation of protein kinase A. Phosphorylation of Ras-GRF1 at Ser916/898 is required for maximal induction of Ras-dependent neurite outgrowth in PC12 cells. A novel antibody (termed 2152) that selectively recognizes Ras-GRF1 when it is phosphorylated at Ser916/898 confirmed the regulated phosphorylation of Ras-GRF1 by Western blotting in both model systems of transfected COS-7 and PC12 cells and also of the endogenous protein in rat forebrain slices. Indirect confocal immunofluorescence of transfected PC12 cells using antibody 2152 demonstrated reactivity only under conditions in which Ras-GRF1 was phosphorylated at Ser916/898. Confocal immunofluorescence of cortical slices of rat brain revealed widespread and selective phosphorylation of Ras-GRF1 at Ser908. In the prefrontal cortex, there was striking phosphorylation of Ras-GRF1 in the dendritic tree, supporting a role for Ras activation and signal transduction in neurotransmission in this area.

The Ras GTases are timed molecular switches that cycle between GDP- and GTP-bound forms to control pathways of cellular growth and differentiation (1). In addition to the well-established roles for Ras in the proliferation of normal and malignant cells (2), there is increasing evidence that Ras also regulates critical functions in terminally differentiated cells such as neurons (3). The GTPase cycle is controlled by guanine nucleotide exchange factors (GEFs)3 and GTPase-activating proteins (GAPs) (4), with the balance between the effective GEF and GAP activities determining the activation state of Ras because it is the GTP-bound form that activates downstream effector pathways. The principal control in many instances may be the activation process through the activity or subcellular localization of the GEF, as there is increasing evidence that these GEFs act as kinases. Indeed, oncogenic mutations in Ras that block the GAP-catalyzed deactivation of Ras from the GTP- to GDP-bound states (7), emphasize the importance of this switch mechanism.

The Ras-GRF1 exchange factor (8), which is also termed CDC25Mm (9, 10), is highly expressed in neurons of the central nervous system (11–13). Ras-GRF1 is expressed predominantly at synapses (14), a neuronal subcellular distribution it shares with the Ras-GAP termed SynGap (15). This colocalization suggests that the proteins may reciprocally regulate Ras at synapses. Interestingly, Ras (16) and the Ras effector mitogen-activated protein kinase (17, 18) have been implicated in the control of synaptic plasticity, which is the proposed cellular corollary of memory storage, whereas mice that lack Ras-GRF1 have defects in memory (19, 20). Thus, Ras-GRF1 may participate in the regulation of synaptic plasticity in the central nervous system.

Ras-GRF1 couples heterotrimeric G proteins (21–24) and calcium signals (8, 25) to the activation of Ras. The activation of Ras-GRF1 by G protein-coupled receptors is closely associated with an increase in its phosphorylation at certain serine residues (24), with the first of these to be identified being Ser916 (in the mouse sequence, equivalent to Ser898 in the rat sequence) (26). Phosphorylation of Ser916/898, which is an in vivo and in vitro substrate for protein kinase A (PKA), is necessary for full activation of the Ras-GEF activity of Ras-GRF1 (26). The current study confirms that Ser916/898 is a physiologically relevant site of regulated phosphorylation in the endogenous Ras-GRF1 exchange factor that is expressed in rat forebrain. Localization of this regulatory phosphorylation event to the apical dendrites of prefrontal pyramidal cells suggests that Ras signaling is activated at these loci.

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** The abbreviations used are: GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; PKA, protein kinase A; GST, glutathione S-transferase; H1, hemagglutinin-1; NGF, nerve growth factor; IBMX, isobutylmethylxanthine; Tricine, N-[2-hydroxy-1, 1-bis(hydroxymethyl)ethyl]glycine.
MATERIALS AND METHODS

Plasmids and Transfection—Constructs in the pKH3 mammalian expression vector (27) that encode murine Ras-GRF1, Ras-GRF1ΔN, Ras-GRF1ΔI327, and the S916A mutant have previously been described (23, 26). Ras-GRF1Δ976 was prepared by PCR using a 5′-primer with a BamHI restriction site from the template pKH3-Ras-GRF1Δ1 and verified by sequencing. Mammalian expression vectors for rat Ras-GRF1 (8), Sos1 (28), Mcy-tagged H-Ras (29), and muscarinic receptor subtypes 1 and 2 (30) were generously provided by Prof. L. A. Feig, M. Czeck, J. Roth, and M. R. Brann, respectively. COS-7 cells were transfected by calcium phosphate coprecipitation (27), and PC12 cells by electroporation (31).

Expression of recombinant wild-type Ras-GRF1-(900–983) and Ras-GRF1Δ900–983 with the S916A mutation as fusion proteins with glutathione S-transferase (GST) has been previously described (26). GST-Ras-GRF1-(632–1262) (equivalent to GST-GRF1ΔN) was expressed from the vector pGEX-GRF1-(632–1262), which was constructed by subcloning the BamHI/EcoRI insert from pKH3-GRF1ΔN (23) into pGEX-2T.

Metabolic Labeling and Digestion of Ras-GRF1—Forebrain slices cut from postnatal day 15 rat brains were labeled in six-well tissue culture plates in 1 ml/well phosphate-free Dulbecco’s modified Eagle’s medium (Invitrogen) with 2 mCi of [32P]orthophosphate (ICN, Costa Mesa, CA) for 2 h in a 37 °C incubator that was supplied with 95% O2 and 5% CO2. The slices were lysed, and Ras-GRF1 was immunoprecipitated using antibody sc-224 (Santa Cruz Biotechnology, Santa Cruz, CA) as described (23). Immunoprecipitated labeled Ras-GRF1 was processed for Western blotting and digestion with cyanogen bromide as described (26).

Development of Polyclonal Antibody 2152 against Ras-GRF1 Ser916/898—Antibodies were developed in rabbits immunized with a 13-residue phosphopeptide that represents the region flanking Ser916 of mouse Ras-GRF1. This sequence is highly conserved (with only three conservative substitutions) in rat Ras-GRF1 (8). The specificity of the antibodies was tested, and affinity-purified antibody 2152 was characterized as a selective agent for recognition of both mouse and rat Ras-GRF1 only when phosphorylated at the relevant serine (see below). Western blots were developed by enhanced chemiluminescent detection (32).

Confocal Immunofluorescence—Dual labeling indirect confocal immunofluorescence of transfected PC12 cells was performed as previously described (33). For neurite outgrowth experiments, the primary antibodies used were anti-Myc monoclonal antibody 9e10 (1:1000 dilution; Sigma) for detection of cells expressing Myc-H-Ras and anti-hemagglutinin-1 (HA1) polyclonal antibody Y-11 (1:150 dilution; Santa Cruz Biotechnology) for detection of cells expressing HA1-Ras-GRF1. The secondary antibodies used were Cy3-coupled anti-mouse antibody (1:300 dilution; Jackson Laboratories, Bar Harbor, ME) and Oregon Green-coupled anti-rabbit antibody (1:300 dilution; Molecular Probes, Inc., Eugene, OR). Pictures were taken with a ×40 water immersion lens on a Zeiss LSM510 microscope. Neurite outgrowth was quantified as previously described (34). To determine phosphorylation of Ras-GRF1, the procedure was similar, except that the primary antibodies used were anti-HA1 monoclonal antibody 12CA5 (1:500 dilution) for detection of HA1-Ras-GRF1 and polyclonal antibody 2152 (1:300 dilution) for detection of Ras-GRF1 phosphorylated at Ser916/898, and pictures were taken with a ×63 oil immersion lens.

Forebrain slices of postnatal day 15 rat brains were fixed in 4% paraformaldehyde in phosphate-buffered saline and incubated in 50% sucrose/phosphate-buffered saline overnight at 4 °C. The slices were then permeabilized for 10 min with methanol that had been precooled to −20 °C, rinsed with phosphate-buffered saline, and blocked with phosphate-buffered saline containing 2% bovine serum albumin, 5% goat serum, and 0.25% Triton X-100 for 4 h at room temperature. Indirect confocal immunofluorescence was carried out using the following primary polyclonal antibodies: antibody 2152 (anti-Ras-GRF1 Ser916/898; 1:200 dilution); antibody sc-224 (anti-Ras-GRF1; 1:200 dilution); or, as a negative control, antibody sc-863 (anti-Ras-GRF1 antibody that is competent for Western blotting, but does not recognize the protein in immunoprecipitation or immunofluorescence protocols; 1:200 dilution). The secondary antibody used was Oregon Green-coupled anti-rabbit immunoglobulin (1:150 dilution). Pictures were taken with an Olympus Fluoview laser scanning confocal microscope using a ×20 objective.

In Vitro Phosphorylation of Ras-GRF1 Constructs—GST-Ras-GRF1-(900–983) (wild-type and mutant S916A) and GST-Ras-GRF1-(632–1262) were reacted with PKA (Sigma) as described (26).

RESULTS

Ras-GRF1 Is Highly Expressed in Rat Brain—It is now clear that there are multiple GEFs that can serve to activate Ras proteins, including those in the Sos, Ras-GRF1/CDC25Mm, Rasguanyl nucleotide-releasing protein, and other families (5). Much work has been performed on the ubiquitous Sos exchange factors that activate Ras in response to stimulation of tyrosine kinase-mediated signals (28, 35, 36). In contrast, Ras-GRF1 is expressed predominantly in the neurons of the central nervous system (11–13) and activates Ras in response to G protein-coupled and calcium signals (8, 21–24). To examine the relative expression levels of Sos and Ras-GRF1 in rat brain, a quantitative Western blot protocol was developed. Ras-GRF1 and Sos1 were expressed in COS-7 cells with identical triple-HA1 epitope tags at their N termini. The lysates from these transfections were standardized by Western blotting with anti-HA1 monoclonal antibody 12CA5. The standardized HA1-Ras-GRF1 and HA1-Sos1 lysates were then used to establish the relative sensitivities of two polyclonal antibodies directed against the Ras-GRF1 and Sos proteins (Fig. 1). The polyclonal
antibodies detected significantly more Ras-GRF1 than Sos in lysates of rat brain forebrain, even though the sensitivity of the anti-Sos antibody is greater. Correcting for the sensitivity of the antibodies, we found that there was 11-fold more Ras-GRF1 than Sos in rat forebrain slices.

Ras-GRF1 Is Phosphorylated at Ser916 in Forskolin-treated Slices of Rat Brain—Ras-GRF1 is regulated by a complex of mechanisms that include regulated phosphorylation of serine residues (24). Phosphorylation of Ser916, which is an in vivo substrate for PKA, has been shown to be necessary in model systems for full activation of the Ras-GEF activity of Ras-GRF1 by muscarinic receptors (26). To test whether phosphorylation of this residue also occurs in endogenous Ras-GRF1, we metabolically labeled rat forebrain slices with [32P]orthophosphate, stimulated the slices with forskolin to activate PKA, and immunoprecipitated Ras-GRF1. Ras-GRF1 was then cleaved at methionine residues using cyanogen bromide, and the fragments were separated by peptide PAGE (Fig. 2). Phosphorylation of mouse Ras-GRF1 at Ser916 produces a 32P-labeled fragment with an apparent mobility of ~6.5 kDa (26). The positions of the methionines that flank this phosphorylation site are conserved between mouse and rat. Transfection of COS-7 cells with rat Ras-GRF1 demonstrated that the equivalent phosphorylation (at Ser898) was revealed in a similarly sized cyanogen bromide cleavage product. Stimulation of forebrain slices of rat brain with forskolin induced the appearance of 32Pi in the equivalent fragment of endogenous Ras-GRF1, confirming that Ser898 is a site of regulated phosphorylation in the brain.

Phosphorylation of Ras-GRF1 at Ser916 Promotes Ras-dependent Outgrowth of Neurites in PC12 Cells—We have previously shown that phosphorylation of Ras-GRF1 at Ser916 is necessary for maximal activation of Ras-GEF activity in a biochemical assay with recombinant Ras substrate (26). Because we have shown here that this phosphorylation event occurred in the endogenous exchange factor in the brain, we investigated whether this phosphorylation plays a critical role...
in Ras activation in a neuronal context. We used the well established model of neurite extension from PC12 cells in response to Ras activation (37). The results show that expression of either the C-terminal half of Ras-GRF1 (Ras-GRF1 N) or a Myc-tagged H-Ras protein alone did not induce neurite outgrowth (Fig. 3). It has previously been demonstrated that wild-type Ras proteins (those without a constitutively activating, oncogenic mutation) are not sufficient to induce neurite outgrowth (37), and this result further suggests that the Ras-GRF1 N protein is not able to activate the endogenous Ras protein in PC12 cells to induce neurite extension. Because H-Ras is the preferred in situ substrate for Ras-GRF1 (29), we assayed for neurite extension in cotransfections of Ras-GRF1 N and wild-type H-Ras and found robust outgrowth. Deletion of the Ras-GRF1 construct to leave the C-terminal third (Ras-GRF1Δ900), although removing the Ras exchange motif sequence that is common to Ras-GEFs (5), did not reduce the H-Ras-dependent induction of neurite outgrowth. Further deletion to leave the C-terminal quarter of Ras-GRF1 (Ras-GRF1Δ976) did, however, significantly reduce neurite extension. This deletion leaves intact the CDC25 Ras-GEF domain that is necessary for biochemical activity (38) and so reveals a stimulatory function within residues 900–975 of Ras-GRF1. The same reduction in neurite extension was produced by point mutation of the Ser916 phosphorylation site to alanine (Fig. 3).

Development of an Antibody That Selectively Recognizes Ras-GRF1 Ser(P)916–The appearance of the 32P-labeled fragment of Ras-GRF1 following cyanogen bromide cleavage pro-
vides an assay for the regulated phosphorylation of Ser\textsuperscript{916/898}. This assay is time-consuming and provides poor sensitivity, however. To develop a more sensitive and powerful assay approach, antibodies that selectively recognize the Ser\textsuperscript{916/898} form of Ras-GRF1 were developed. To confirm the selectivity of antibody 2152, it was tested against recombinant Ras-GRF1 proteins that were subjected to phosphorylation by PKA (Fig. 4). The data show that antibody 2152 exhibited great selectivity for recognition of Ras-GRF1 only when it was phosphorylated, with detectable signal produced from as little as 10 ng of Ras-GRF1 Ser\textsuperscript{916}, whereas unphosphorylated Ras-GRF1 was only detected when microgram quantities were present. Note that PKA treatment of the S916A mutant protein did not produce any reactivity to antibody 2152.

Regulated Phosphorylation of Ras-GRF1 at Ser\textsuperscript{916/898}—To test whether antibody 2152 recognizes both mouse and rat Ras-GRF1 expressed in mammalian cells, it was tested against whole cell lysates of COS-7 cells that had been transfected to express Ras-GRF1 and then treated with various agonists (Fig. 5). The data show that antibody 2152 reacted with a single band that was present only in the cells transfected to express Ras-GRF1 and that this band had the same mobility as that recognized by an independent anti-Ras-GRF1 antibody (sc-863). Furthermore, it is clear that the recognition of the mouse and rat Ras-GRF1 proteins was similar and that all recognition was absolutely dependent on phosphorylation at Ser\textsuperscript{916/898}, as no reactivity occurred in cells expressing Ras-GRF1(S916A). Both forskolin and serum (the latter to a lesser extent, which is clearer when the Western blots were given prolonged exposure) stimulated an increase in the phosphorylation of Ras-GRF1 at Ser\textsuperscript{916/898}. Interestingly, treatment of the cells with thapsigargin, which increases cytosolic calcium levels (39), failed to increase phosphorylation of Ras-GRF1 at Ser\textsuperscript{916/898} above the basal level. This result supports previous observations on transfected fibroblasts that increases in calcium do not stimulate the phosphorylation of Ras-GRF1 (40), although it has been reported that Ras-GRF1 is an \textit{in vitro} substrate for calmodulin-dependent kinase II (14).

Multiple Agonists Stimulate the Phosphorylation of Ras-GRF1 at Ser\textsuperscript{916/898} in PC12 Cells—To investigate the phosphorylation of Ras-GRF1 in a neuronal context, we coexpressed Ras-GRF1 with muscarinic receptors in PC12 cells. Western blotting of PC12 cell lysates (Fig. 6) demonstrated that activation of muscarinic receptors by carbachol, stimulation of PKA by forskolin, and activation of endogenous Trk receptors by nerve growth factor (NGF) all increased the phosphorylation of Ras-GRF1 at Ser\textsuperscript{916}. To extend these results, either HA1-Ras-GRF1 or its S916A mutant was coexpressed in PC12 cells with muscarinic receptors, and the cells were stimulated and then fixed and processed for indirect confocal immunofluorescence (Fig. 7). It is clear that antibody 2152 reactivity was again dependent on phosphorylation at Ser\textsuperscript{916}, as no reactivity was seen in untransfected cells, in transfected cells that were not stimulated, or in stimulated cells that were transfected with the Ras-GRF1(S916A) mutant. In agreement with the Western blot results, carbachol, forskolin, and NGF all induced the phosphorylation of Ras-GRF1 at Ser\textsuperscript{916}.

Regulated Phosphorylation of Ras-GRF1 at Ser\textsuperscript{916/898} in Rat Forebrain Slices—To investigate whether endogenous Ras-GRF1 is regulated by phosphorylation in a manner similar to that defined in the model systems, antibody 2152 was used to assess phosphorylation of Ras-GRF1 in rat forebrain slices. It was difficult to detect any reactivity to antibody 2152 in lysates of brain slices by Western blotting, perhaps suggesting that only a subset of Ras-GRF1 proteins (perhaps, in particular, neurons) is phosphorylated at Ser\textsuperscript{898}. When Ras-GRF1 was first immunoprecipitated from the lysate using an antibody directed against the C terminus (sc-224), the regulated phosphorylation at Ser\textsuperscript{898} became apparent by Western blotting (Fig. 8). There was little reactivity in Ras-GRF1 from untreated slices, but there were clear increases following stimulation with carbachol, forskolin, or NGF. Thus, just as in the expression systems constructed in PC12 cells, endogenous Ras-GRF1 is phosphorylated at Ser\textsuperscript{898} following activation of muscarinic or Trk receptors or PKA.

To investigate the distribution of Ras-GRF1 Ser\textsuperscript{898} in the cortex, brain slices were fixed and processed for indirect confocal immunofluorescence with antibodies 2152 (anti-Ras-GRF1 Ser\textsuperscript{916/898}) and sc-224 (anti-Ras-GRF1). Prefrontal cortex includes two classes of neurons, GABAergic interneurons and glutamate-secreting pyramidal cells. The latter class is easily recognizable by its distinctive morphology, including a prominent single apical dendrite that branches in the upper layers. In these immunolocalization studies, we observed expression of Ras-GRF1 throughout pyramidal neurons, including the cell...
The identification of activated Ras-GRF1 in the apical dendrites of prefrontal pyramidal neurons in this study is completely consistent with its postulated role as an integrator of neuronal signal transduction (41, 42).

One possibility that might have resolved the differences in the phenotypes of mice that are deficient in Ras-GRF1 would be variable compensation from the highly homologous Ras-GRF2 protein (48). Recently, however, mice deficient in Ras-GRF2 have been described to have neither an apparent phenotype nor any compensatory changes in the expression of Ras-GRF1 (49). Conversely, Ras-GRF1-deficient mice have no alteration in Ras-GRF2 expression. Furthermore, results from mice doubly deficient in both Ras-GRF1 and Ras-GRF2 were also reported. Remarkably, there is no further phenotypic effect beyond that produced by loss of Ras-GRF1 alone, suggesting that there is no overlap of function between these highly similar GEFs (49).

In this regard, it is important to note that the Ser^{898} phospho-13283rylation site, although conserved in Ras-GRF1 proteins from rodents and humans, is absent in Ras-GRF2. Thus, it is plausible that this regulatory mechanism is part of the unique function of Ras-GRF1 that cannot be replaced by Ras-GRF2.

Phosphorylation of Ras-GRF1 at Ser^{898} has previously been shown to be essential for maximal activation of Ras-GRF1 in a...
biochemical assay with recombinant Ras substrate (26). In the current study, we have shown that there is a regulatory function for residues 900–975 of Ras-GRF1 to increase Ras activation in the PC12 system and that this function is also lost by point mutation of the Ser916 phosphorylation site. Although Ras-GRF1 is phosphorylated at multiple sites in response to agonist stimulation, Ser916 is the only detectable site of phosphorylation in this region (26). These results therefore support the significance of Ras-GRF1 phosphorylation at Ser916/898 both as a marker for increased Ras activation within a neuronal context and in the physiological control of neuronal Ras activation.

Muscarinic stimulation was previously demonstrated to increase the phosphorylation and activity of Ras-GRF1 in mouse brain explants (23); thus, stimulation of rat brain Ras-GRF1 Ser916 phosphorylation by carbachol treatment was expected. Activation of muscarinic receptor signaling in the cortex is also correlated with memory function in humans (50), with broad biochemical assay with recombinant Ras substrate (26). In the context of its function that can select the downstream targets of their substrates GTPases (67, 68). Thus, it is essential to consider the cellular and subcellular context within which the GEFs act to activate Ras signal transduction. In this regard, the selectivity and sensitivity of anti-Ras-GRF1 Ser916/898 antibody 2152 will provide a powerful approach toward the elucidation of Ras activation pathways in central neurons.

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31. McKiernan, C. J., Stabilia, P. F., and Macara, I. G. (1996) Mol. Cell. Biol. 16, 4985–4995
32. Mattingly, R. R., Felczak, A., Chen, C. C., McCabe, M. J., Jr., and Rosenspire, A. J. (2001) Toxcol. Appl. Pharmacol. 176, 162–168
33. Lounsbury, K. M., Richards, S. A., Curey, K. L., and Macara, I. G. (1996) J. Biol. Chem. 271, 32834–32841
34. Yang, H., Xiao, Z.-c., Becker, B., Hillenbrand, R., Rougon, G., and Schachner, M. (1999) J. Neurosci. Res. 55, 687–703
35. Corbalan-Garcia, S., Margarit, S. M., Galron, D., Yang, S. S., and Bar-Sagi, D. (1998) Cell 94, 881–891
36. Innocenti, M., Tenca, P., Frettel, E., Paretta, M., Tocque, A., Di Fiore, P. P., and Scita, G. (2002) J. Cell Biol. 156, 125–136
37. Bar-Sagi, D., and Feramisco, J. R. (1985) Science 228, 841–848
38. Coccetti, P., Monzani, E., Alberghina, L., Casella, L., and Martegani, E. (1998) Biochim. Biophys. Acta 1383, 292–300
39. Mattingly, R. R., and Garrison, J. C. (1998) FEBS Lett. 429, 225–230
40. Mattingly, R. R. (1998) In Vitro Mol. Toxicol. 11, 57–62
41. Finkbeiner, S., and Dalva, M. B. (1998) Bioessays 20, 691–695
42. Orban, P. C., Chapman, P. F., and Brambilla, R. (1999) Trends Neurosci. 22, 38–44
43. Tonini, R., Franceschetti, S., Parello, D., Sala, M., Mancinelli, E., Timinini, S., Brusetti, R., Sancini, G., Brambilla, R., Martegani, E., Sturani, E., and Zippel, R. (2001) Mol. Cell. Neurosci. 18, 691–701
44. Izzet, J. M., Tremp, G. L., Leonard, J. P., Multon, M. C., Ret, G., Schweighoffer, F., Tocque, B., Bluet-Pajot, M. T., Cormier, V., and Dautry, F. (1998) Nature 393, 125–126
45. Costa, R. M., Federov, N. B., Kogan, J. H., Murphy, G. G., Stern, J., Ohno, M., Kucherlapati, R., Jacks, T., and Silva, A. J. (2002) Nature 418, 14376–14381
46. Fleming, I. N., Elliott, C. M., Buchanan, F. G., Downes, C. P., and Exton, J. H. (1999) J. Biol. Chem. 274, 12753–12758
47. Ichiba, T., Hashimoto, Y., Nakayama, M., Kurashida, Y., Tanaka, S., Kurata, T., and Matsuda, M. (1999) J. Biol. Chem. 274, 14376–14381
48. Ebinu, J. O., Bottorff, D. A., Chan, E. Y., Stang, S. L., Dunn, R. J., and Stone, J. C. (1998) Science 280, 1082–1086
49. Hart, M. J., Jiang, X., Kasaas, T., Roseve, W., Singer, W. D., Gilman, A. G., Downward, J. (1998) Science 280, 1082–1086
50. Mattingly, R. R., Milstein, M. L., and Mirkin, B. L. (2001) Cell. Signal. 13, 499–505
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