Endothelin 1 Induces β1Pix Translocation and Cdc42 Activation via Protein Kinase A-dependent Pathway*

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p21-activated kinase (Pak)-interacting exchange factor (Pix), a Rho family guanine nucleotide exchange factor (GEF), has been shown to co-localize with Pak and form activated Cdc42- and Rac1-driven focal complexes. In this study we have presented evidence that treatment of human mesangial cells (HMC) with endothelin 1 (ET-1) and stimulation of adenylate cyclase with either forskolin or with the cAMP analog 8-Br-cAMP activated the GTP loading of Cdc42. Transient expression of constitutively active Gaα1 also stimulated Cdc42. In addition, overexpression of β1Pix enhanced ET-1-induced Cdc42 activation, whereas the expression of β1Pix SH3m(W43K), which lacks the ability to bind Pak, and β1PixDHm(L238R/L239S), which lacks GEF activity, decreased ET-1-induced Cdc42 activation. Furthermore, ET-1 stimulation induced β1Pix translocation to focal complexes. Interestingly, pretreatment of HMC with protein kinase A (PKA) inhibitors blocked both Cdc42 activation and β1Pix translocation induced by ET-1, indicating the involvement of the PKA pathway. Through site-directed mutagenesis studies of consensus PKA phosphorylation sites and in vitro PKA kinase assay, we have shown that β1Pix is phosphorylated by PKA. Using purified recombinant β1Pix(wt) and β1Pix mutants, we have identified Ser-516 and Thr-526 as the major phosphorylation sites by PKA.

The physiological and pathological responses elicited in mesangial cells ultimately require changes in the cytoskeletal organization whose architecture is modulated by the Rho family, Cdc42, Rac and RhoA. Cdc42 and Rac1 function as molecular switches (6, 7). They are converted from the GDP-bound inactive form to a GTP-bound active state by a reaction catalyzed by guanine nucleotide exchange factors (GEFs) (8). Since their identification, GEFs have become increasingly involved in mediating the effects of G protein-coupled receptor agonists. Recently, a Cdc42/Rac-GEF termed Pix (Pak-interacting exchange factor) was identified (9). Pix has a diffuse B cell lymphoma homology (DH) domain and a flanking pleckstrin homology domain, which are conserved in all of the GEFs for Rho GTPases. Pix family proteins consist of two isoforms, αPix and βPix, and recently a new splice variant of βPix designated β2Pix has been identified (10). The human Pix family bind tightly through an N-terminal SH3 domain to a conserved proline-rich Pak sequence located at the C terminus and are colocalized with Pak to form activated Cdc42- and Rac1-driven focal complexes (9). Recently, Pix has been shown to form a trimolecular complex with Pak1 and p95PKL (also known as G protein-coupled receptor kinase-interacting target, GIT1) (11). Furthermore, tyrosine-phosphorylated p95PKL can also bind paxillin (12, 13) and therefore provides the link between Pix/Pak and focal complexes through this interaction. The presence of several domains allows Pix to interact with a variety of signaling proteins and suggests that Pix might have an important role in mediating the effects of extracellular signals (2, 14–17).

The physiological and pathological responses elicited in mesangial cells ultimately require changes in the cytoskeletal organization whose architecture is modulated by the Rho family proteins. Therefore, understanding the signal transduction pathways associated with the activation of Rho family GTPase by ET and its regulation by β1Pix is of pivotal importance in delineating the effects of ET on cytoskeletal organization in mesangial cells.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—All materials for cell culturing were purchased from Invitrogen. Previously characterized SV40-transformed human mesangial cells (HMC) (18) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) in a 37 °C humidified incubator with 5% CO2. Transient transfection of cells with mammalian expression vectors

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The abbreviations used are: ET-1, endothelin 1; GEF, guanine nucleotide exchange factor; Pak, p21-activated kinase; PBD, p21 binding domain; PBS, phosphate-buffered saline; Pix, Pak-interacting exchange factor; DH, diffuse B cell lymphoma homology; PKA, protein kinase A; p95PKL, paxillin kinase linker; GST, glutathione S-transferase; PKI, PKA inhibitor; HMC, human mesangial cell; wt, wild type.
**RESULTS**

**ET-1 Stimulates Cdc42 in Human Mesangial Cells**—We first sought to determine whether Cdc42 is activated by ET-1 in HMC. Using a pulldown assay to separate the activated GTP-bound form of Cdc42, we found that treatment of HMC with ET-1 induced the formation of the GTP-bound form of Cdc42 (Fig. 1A). ET-1 stimulates Cdc42 in a dose- and time-dependent manner, peaking at 2 min, and this activation decreases 10 min after stimulation (Fig. 1B). The dependence of Cdc42 stimulation on ET-1 concentration was determined at 5 min. Maximal stimulation was observed at a concentration of 100 nM ET-1. To confirm that ET-1 specifically stimulates Cdc42, HMC were pretreated with 10 ng/ml of *Clostridium difficile* toxin B, which was performed using Lipofectamine™ 2000 according to the manufacturer’s instructions.

**RT-PCR Analysis**—Total RNA isolated from rat mesangial cells was reverse transcribed using Superscript reverse transcriptase (Invitrogen), oligo(dT) primers (Invitrogen), and deoxynucleotide triphosphate as specified by the manufacturer. β-Fix was amplified by PCR with TITANIUM™ Taq polymerase (Clontech Laboratories, Inc.) in the presence of deoxynucleotide triphosphate, the forward primer 5'-GCTCTAGAGCTAGATTGGTCTCATCCCAAGCAGG-3' and the reverse primer 5'-GGAATCCTTAGCTGAATCCAGCAGGCAA-3' (H11032). The PCR products were subjected to electrophoresis in a 1% agarose gel. The PCR products were sequenced to confirm the identity of the PCR products. The PCR products were purified and cloned into the pCR4-TOPO vector (Invitrogen) and sequenced to confirm that ET-1 specifically stimulates Cdc42, HMC were pretreated with 10 ng/ml of *Clostridium difficile* toxin B, which was performed using Lipofectamine™ 2000 according to the manufacturer’s instructions.

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**In Vitro Kinase Assay**—The β-Fix/Pix-bound beads were resuspended in 80 μl of kinase buffer (50 mM HEPES, pH 7.5, 10 mM MgCl₂, 1 mM EGTA, 1 mM diethiothreitol, 0.015% Tween 20) and incubated with 30 units of protein kinase A catalytic subunit (Sigma), 20 μCi of [γ-32P]ATP at 30 °C for 30 min. The beads were pelleted by centrifugation and washed three times. The beads were then suspended in Laemmli sample buffer. Proteins were subjected to SDS-PAGE, Coomassie Blue staining, and autoradiography. For recombinant β-Fix and its mutants, 5 μg were used in the kinase reaction as described above.

**Measurement of cAMP**—A Biotrack cAMP enzyme immunoassay kit (Amersham Biosciences) was used to measure cyclic AMP levels in HMC according to the manufacturer’s instructions.

**PKA Assay**—SigmaCorticostrieral cAMP-dependent PKA assay (Promega) was used to measure PKA enzyme activity according to the manufacturer’s instructions. In brief, 20 μl of substrate mixture containing 100 μM kemptide peptide, 5 μM cAMP, and 5 μg of cell lysate were added in order. Then the reaction was started by adding 5 μl of the mixture containing 0.5 mM ATP and 10 μCi/μl of [γ-32P]ATP (3,000 Ci/μmol). After incubation, 10 μl of the mixture was spotted onto streptavidin-coated membrane, washed repeatedly, dried, and placed in scintillation vials for radioactive counting.

**Immunocytochemistry**—100-mm dish-cultured human glomerular mesangial cells were transfected with 10 μg of plasmid containing either wild type or mutant β-Fix. The following day, transfected cells were seeded on glass coverslips housed in a 12-well plate, where all subsequent incubations were performed. After overnight serum starvation, cells were stimulated with ET-1, washed in PBS, and fixed in 4% paraformaldehyde in PBS for 15 min. Cells were then washed, permeabilized with 0.2% Triton X-100 in PBS for 5 min, and incubated in 100 μl goat serum in PBS for 30 min. Primary antibodies against c-Myc and paxillin (Santa Cruz) were diluted 1:500 and incubated with cells for 1 h. Cells were washed several times with PBS, followed by 1-h incubation with Alexa Fluor 488 and Alexa Fluor 546 secondary antibodies (Molecular Probes) diluted 1:1,000. Cells were equilibrated and mounted in ProLong anti-fade reagent (Molecular Probes). Cells were visualized under a fluorescent microscope (E600; Nikon) and photographed using the SPOT system (Diagnostic Instruments). Cells of roughly equal and average fluorescent intensity were chosen as comparative examples.
glycosylates and inactivates Rho family proteins (19), and then stimulated with ET-1. As shown in Fig. 1C, toxin B completely inhibited ET-1-induced Cdc42 stimulation.

We also found that ET-1 induced RhoA stimulation (Fig. 1D). However, ET-1-induced stimulation of Rac1 was not observed (data not shown).

**Effect of βPix Overexpression on ET-1-induced Cdc42 Activation**—Pix family proteins are GEFs for small GTPase proteins Cdc42/Rac (9) and have been shown to signal via these proteins. Therefore, we studied the effect of βPix and its inactive mutants on ET-1-induced Cdc42 activation in HMC. In our experiments, Cdc42 activation was measured after ET-1 treatment of HMC-overexpressing wild type rat βPix or its mutants, βPix SH3m(W43K) and βPix DHm(L238R/L239S) (9). As shown in Fig. 2A, ET-1 induced Cdc42 activation in cells expressing empty vector, and this activation was enhanced by βPix overexpression. By contrast, mutated βPix DHm(L238R/L239S), which lacks GEF activity, and SH3 domain-mutated βPix SH3m(W43K), which lacks the ability to bind to Pak, decreased ET-1-induced Cdc42 activation (Fig. 2A). The expression of βPix alone in the absence of ET-1 stimulation had no effect on Cdc42 activity (data not shown). Fig. 2B shows quantitative analysis of the results obtained by densitometry. βPix overexpression did not enhance ET-1-induced RhoA activation (data not shown), indicating that βPix specifically regulates the activation of Cdc42 by ET-1 in HMC.

**Role of PKA in Stimulation of Cdc42 by ET-1**—To determine the enzyme(s) involved in the regulation of Cdc42 activation by ET-1, we utilized different pharmacological inhibitors such as PKC inhibitor Ro31–7549, phosphatidylinositol-3-kinase inhibitor wortmannin, PKA inhibitor H-89, and PP2, a Src kinase inhibitor. We found that only H-89, a selective PKA inhibitor, blocked Cdc42 activation induced by ET-1 (Fig. 3A). The inhibitory effect of H-89 was confirmed using a myristoylated, cell-permeable peptide derivative of the naturally occurring PKA inhibitor, PKI (Fig. 3B). PKA is activated in response to cAMP generated by adenylyl cyclase. To investigate the role of PKA in Cdc42 activation, HMC were stimulated either by ET-1, cAMP analog 8-Br-cAMP, or by the adenylyl cyclase activator, forskolin. Cells transiently expressing the constitutively active α-subunit of Gs served as positive control to mimic receptor-mediated activation of adenylyl cyclase. Fig. 4A shows that ET-1 stimulated Cdc42 to a level similar to those produced by stimulating PKA, either by 8-Br-cAMP or forskolin or by expressing the constitutively active GαsQL. Treatment of the cells with H-89 inhibited Cdc42 activation induced by ET-1 and all other agonists mentioned above. These results indicate that Cdc42 activation in response to ET-1 requires PKA activity. We next wondered whether ET-1 is able to stimulate cAMP increase, which in turn activates PKA enzyme in the cells. In the presence of 3-isobutyl-1-methylxanthine, ET-1 induced a maximum of 2.5-fold cAMP accumulation over the basal level at 50 nM and higher concentrations (Fig. 4B). Next, we sought to determine whether ET-1 would stimulate PKA activity. Results show that stimulation by ET-1 (100 nM) increases PKA activity by 2-fold over non-stimulated cells (Fig. 4C). Cells expressing constitutively active GαsQL also showed an increase in PKA activity. In both cases the stimulated PKA activity was inhibited by H-89 to the basal level. Taken together, these results demonstrate that (i) activation of Gs and increase in intracellular cAMP lead to the stimulation of Cdc42, and (ii) activation of PKA plays an important role in the regulation of ET-1-mediated stimulation of Cdc42.

**Identification of PKA Phosphorylation Sites on βPix**—The functional activity of Cdc42 is known to be regulated by βPix, which controls its GDP/GTP-bound state. The fact that PKA inhibitors H-89 and PKI both blocked ET-1-induced Cdc42 activation prompted us to examine whether βPix was phosphorylated by PKA. Upon examination of the βPix amino acid sequence, we found two potential phosphorylation sites based on the PKA consensus sequence represented by RKXS/T. Interestingly, both potential PKA phosphorylation sites, Ser-516 and Thr-526, are highly conserved in αPix, βPix, and γPix, suggesting that these residues may be of functional significance.

To test whether Ser-516 or Thr-526 is phosphorylated by PKA, mutants of βPix were produced in which Ser-516 or Thr-526, or both, were changed to Ala by site-directed mutagenesis. In these experiments, Myc-tagged βPix(wt), βPix(S516A), βPix(T526A), and βPix(S516A/T526A) were immunoprecipitated from transiently transfected HMC using
anti-c-Myc antibody. In vitro kinase assays were performed using the purified catalytic subunit of PKA and $\beta_1$Pix immunocomplex or its mutants as substrates. As shown in Fig. 5A, PKA strongly phosphorylates wild type $\beta_1$Pix (lane 2) and $\beta_1$Pix(T526A) (lane 4), has a slight effect on $\beta_1$Pix(S516A) (lane 3), and almost no effect on $\beta_1$Pix(S516A/T526A) (lane 5). $\beta_1$Pix is not phosphorylated in the absence of PKA catalytic subunit (lane 6), indicating that PKA specifically phosphorylates $\beta_1$Pix. No phosphorylation was observed when control IgG was incubated with vehicle only. Data are representative of four independent experiments.

To further confirm that $\beta_1$Pix is a direct target for PKA phosphorylation in vitro, we performed an in vitro kinase assay using purified recombinant $\beta_1$Pix and its mutants as substrate. Autoradiography of reaction products showed that recombinant $\beta_1$Pix wt was phosphorylated by PKA (Fig. 5B, lane 1). Substitution of Ser-516, Thr-526, or both to Ala strongly reduced phosphorylation as compared with $\beta_1$Pix wt. Slight residual phosphorylation suggested that an additional residue could be targeted by PKA under these optimal conditions. Coomassie staining of gels demonstrated comparable loading of proteins (Fig. 4, lower panels). The apparent discrepancy between the ability of PKA to phosphorylate immunoprecipitated $\beta_1$Pix(T526A) (Fig. 5A, lane 4) and not recombinant $\beta_1$Pix(T526A) (Fig. 5B, lane 3) suggests the presence of other residues phosphorylated by PKA or the presence of endogenous Pak in the immunoprecipitate. Indeed, $\beta_1$Pix is a well known target for Pak phosphorylation (10). In conclusion, our results show for the first time that Ser-516 and Thr-526 are the major

FIG. 3. Cdc42 activation by ET-1 requires PKA. A, HMC were left untreated or treated for 30 min with H-89 (10 $\mu$M), Ro31-7549 (10 $\mu$M), wortmannin (100 nM), or PP2 (10 $\mu$M) before ET-1 (100 nM) was added for 5 min. Cell lysates were assayed for Cdc42 activation using the PBD assay as described under "Experimental Procedures." B, cells were treated with PKI (1 $\mu$M) before ET-1 (100 nM) was added. Lower panels indicate Cdc42 expression in total cell lysate. Results are presented as means ± S.E. of four experiments.

FIG. 4. ET-1 induces cAMP increase and PKA activation. A, HMC were treated with vehicle or H-89 for 30 min and then stimulated with ET-1 (100 nM), forskolin (20 $\mu$M), or 8-Br-cAMP (20 $\mu$M) for 5 min. Activated Cdc42 was determined by affinity precipitation with Pak-1-PBD followed by Western blotting with anti-Cdc42. B, cells were treated with different concentrations of ET-1, and cAMP increase was measured in the presence of 3-isobutyl-1-methylxanthine (300 $\mu$M) as described under "Experimental Procedures." C, cells were stimulated by ET-1 (100 nM) in the presence or absence of H-89, and PKA enzyme activity was measured as described under "Experimental Procedures." Cells expressing the constitutively active mutant of $\alpha_v$ were incubated with vehicle only. Data are representative of four independent experiments.
PKA-dependent β1Pix Translocation

**DISCUSSION**

In the present study, we have reported for the first time that ET-1 stimulates the GTPase Cdc42 by a β1Pix-mediated, PKA-dependent pathway. Moreover, we have demonstrated that ET-1-induced β1Pix translocation also requires PKA activity.

Many G protein-coupled receptor agonists activate small GTPases that, in turn, regulate a variety of biological responses such as cell differentiation and growth (2). Our results have shown that ET-1 stimulates Cdc42 and RhoA but fails to stimulate Rac1. A critical role for Cdc42 in ET-1 signaling is further supported by recent findings (20) showing that ET-1 stimulates Cdc42, but not Rac1, in human kidney epithelial cells.

PKA is involved in ET-1-induced Cdc42 activation because PKA inhibitors blocked Cdc42 stimulation. Our results demonstrating ET-1-induced cAMP increase and activation of PKA suggest that ET-1 activates Cdc42 via cAMP production by a Gαs-dependent pathway. Indeed, we found that stimulation of adenylate cyclase by forskolin, or loading of HMC with the cAMP analog 8-Br-cAMP, induced Cdc42 activation. Further-
PKA-dependent $\beta_1$Pix Translocation

more, the expression of constitutively active Go$\alpha_1$ induced Cdc42 activation. The data presented here show cross-talk between cAMP and Cdc42. It is unknown how widespread this phenomenon is, but previous study showed that Cdc42 activation by cAMP occurs in human mast cells, Chinese hamster ovary-K1 cells, and COS-7 cells (21). Our finding that cAMP can activate Cdc42 extends the spectrum of possible pathways involved in transducing a signal from G protein-coupled receptor to Cdc42.

The activity of small GTPase proteins is regulated by GEFs that modulate their GDP/GTP-bound state. It is well established now that upon activation Cdc42 and Rac1 bind to Pak and form an active complex that regulates several signaling pathways, including cytoskeletal rearrangement (22–25). To further examine the mechanism involved in the regulation of Cdc42 stimulation, we investigated the role of the Cdc42/Rac1-GEF, $\beta_1$Pix, in ET-1-induced Cdc42 activation in HMC. Using $\beta_1$Pix and its double mutant, $\beta_1$Pix(L238R/L239S), we showed that $\beta_1$Pix acts upstream of Cdc42 and provides a key link between ET-1 receptor and Cdc42. In addition, we confirmed a direct role of PKA by establishing $\beta_1$Pix as an in vitro substrate of PKA at Ser-516 and Thr-526. The mutation of both residues inhibited Cdc42 activation and $\beta_1$Pix translocation. However, the mutation of a single residue does not have functional importance. Thr-526 is also phosphorylated by Pak (9), which may induce conformational changes in Pak. Furthermore, inhibition of PKA blocks ET-1-induced $\beta_1$Pix translocation to focal complexes (Fig. 7d). This has prompted us to speculate that phosphorylation of $\beta_1$Pix by PKA is the mechanism by which $\beta_1$Pix complex is recruited to focal complexes where it binds p53PKL (13). PKA phosphorylation of $\beta_1$Pix could also accomplish Cdc42 activation by bringing $\beta_1$Pix within the vicinity of its target Cdc42, as seen in the case of the Ras exchange factor Sos, which is brought to the membrane by binding to Grb2, thereby enabling Ras activation (26, 27). The inhibition of PKA by H-89 or the expression of $\beta_1$Pix double mutant $\beta_1$Pix(S516A/T526A) results in a very strong inhibition of $\beta_1$Pix phosphorylation by PKA. Moreover, the expression of $\beta_1$Pix phosphorylation by PKA.

![Fig. 6. ET-1-induced Cdc42 activation is mediated by PKA-dependent $\beta_1$Pix phosphorylation. HMC were transfected with HA-tagged Cdc42 alone or in combination with either $\beta_1$Pix(wt) or $\beta_1$Pix(S516A/T526A) for 24 h. Cells were stimulated with 100 nM of ET-1 (A) or 20 μM of 8-Br-cAMP (B) for 5 min and then lysed to measure GTP-bound Cdc42 using pulldown assay as described in the legend of Fig. 1. The GST-PBD bound to active Cdc42 (upper panels) was detected by immunoblotting with anti-HA tag antibody. The middle panels show the expression of $\beta_1$Pix and its mutant $\beta_1$Pix(S516A/T526A) using anti-c-Myc antibody. The lower panels show the expression of HA-Cdc42 in the total cell lysates as indicated by blotting with anti-HA tag antibody. Data are representative of four independent experiments.](http://www.jbc.org/)

![Fig. 7. PKA-dependent $\beta_1$Pix translocation to focal complexes. Upper panels, cells were transiently transfected with Myc-tagged $\beta_1$Pix(wt) for 24 h (a–f), stained for Myc epitope expression, and viewed under a fluorescence microscope as described under "Experimental Procedures." Cells were left unstimulated (a), stimulated with ET-1 (100 nM) for 15 min (b, c), or treated with 10 μM of H-89 (d) or with 1 μM PKI (f) for 30 min prior to ET-1 stimulation. e, non-stimulated cells treated with PKI. g and h, cells were transfected with $\beta_1$Pix(S516A/T526A) for 24 h later, the cells were left untreated (g) or stimulated with ET-1 for 15 min (h). b and c, representative images of stimulated cells. Lower panels, double immunofluorescence staining was carried out using monoclonal anti-c-Myc and polyclonal anti-paxillin antibodies. Cells were stimulated by ET-1 for 30 min. Representative images from more than four independent experiments are shown.](http://www.jbc.org/)
Pak2-β1Pix complex translocation through phosphorylation of β1Pix on Ser-525 and Thr-526 (28, 29). However, in these studies Pix translocation was found to occur independently of Rac1 activation. Considering that Pak acts downstream of PKA, the phosphorylation of β1Pix by PKA might provide the first step before Pak phosphorylation provides the signal for β1Pix complex to be recruited to focal complexes. β1Pix is usually associated with a multicomplex of proteins binding to paxillin and Pak, an effector of Cdc42 function associated with filopodia formation (30, 31). This complex is linked to paxillin through an ADP-ribosylation factor GAP protein, p95PKL (13). Although ADP-ribosylation factor GTPases play a critical role in vesicle transport, they have been involved in regulating the actin cytoskeleton (32) and the translocation to the plasma membrane of focal adhesion proteins such as paxillin (33). Alignments between different Pix isoforms show that Ser-516 and Thr-526 are highly conserved, indicating that these residues may have a functional importance in the formation of these signaling complexes. The physiological significance of β1Pix phosphorylation remains unclear. Increasing evidence indicates that phosphorylation is an important mechanism of regulation of GEFs of the diffuse B cell lymphoma family. Although it has been shown that the phosphorylation status of β1Pix has no effect on its GEF activity (9), recent findings show that the activation of Rac1 is dependent on β1Pix phosphorylation (29).

We can speculate that PKA regulation of Cdc42 activation through phosphorylation of β1Pix may have an important consequence for regulating mesangial cell migration. Indeed, it has been shown that increased Cdc42 activity results in increased cell motility (34). During initial stages of mesangial cell migration new protrusions are formed at the leading edge of the cell. It can be hypothesized that PKA amplifies Cdc42 activation through β1Pix phosphorylation, resulting in the initiation of microspike formation and cell protrusion. This scenario implies that activated Cdc42 is localized at the leading edge of migrating cells. In summary, our findings describe a new ET-1 receptor-initiated pathway that involves the activation of εAMP/PKA pathway and results in the activation of β1Pix translocation and Cdc42; the findings identify important venues for future work regarding the role of this pathway in mesangial cell migration.

REFERENCES

1. Yoshimura, A., Iwasaki, S., Inui, K., Ideura, T., Koshikawa, S., Yanagisawa, M., and Masaki, T. (1995) Kidney Int. 48, 1290–1297
2. Arai, H., Hori, S., Aramori, I., Okubo, H., and Nakanishi, S. (1990) Nature 342, 730–732
3. Sakurai, T., Yanagisawa, M., Takuya, Y., Miyaizaki, H., Kimura, S., Goto, K., and Masaki, T. (1990) Nature 348, 732–735
4. Simonson, M. S., Wann, S., Mene, P., Dubyak, G. R., Kester, M., Nakazato, Y., Sedor, J. R., and Dunn, M. J. (1989) J. Clin. Invest. 83, 708–712
5. Gomez-Garre, D., Ruiz-Ortega, M., Ortego, M., Largo, R., Lopez-Armada, M. J., Plaza, J. J., Gonzales, E., and Egido, J. (1996) Hypertension 27, 885–892
6. Asperenstrom, P. (1999) Exp. Cell Res. 246, 20–25
7. Mackay, D. J., and Hall, A. (1998) J. Biol. Chem. 273, 20685–20688
8. Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
9. Manser, E., Foo, T. H., Koh, C. G., Zhao, Z. S., Chen, X. Q., Tan, L., Tan, I., Leung, T., and Lim, L. (1998) Mol. Cell 1, 183–192
10. Koh, C. G., Manser, E., Zhao, Z. S., Ng, C. P., and Lim, L. (2001) J. Cell Sci. 114, 4239–4251
11. Ku, G. M., Yablonski, D., Manser, E., Lim, L., and Weiss, A. (2001) EMBO J. 20, 457–465
12. Bagrodia, S., Bailey, D., Lenard, Z., Hart, M., Guan, J. L., Premont, R. T., Taylor, S. J., and Cerione, R. A. (1999) J. Biol. Chem. 274, 22393–22400
13. Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S., McDonald, A. R., Bagrodia, S., Thomas, S., and Leventhal, P. S. (1999) J. Cell Biol. 145, 851–863
14. Li, Z., Hannigan, M., Mo, Z., Liu, B., Lu, W., Wu, Y., Smrcka, A. V., Wu, G., Li, L., Liu, M., Huang, C. K., and Wu, D. (2003) Cell 114, 215–227
15. Zegers, M. M., Forget, M. A., Chernoff, J., Mostov, K. E., ter Beest, M. B., and Hansen, S. H. (2003) EMBO J. 22, 4155–4165
16. Meili, R., and Firtel, R. A. (2003) Cell 114, 153–156
17. Chahdi, A., Sorokin, A., Dunn, M. J., and Landry, Y. (2004) Biochem. Biophys. Res. Commun. 317, 384–389
18. Sraer, J. D., Delarue, F., Hagege, J., Feunteun, J., Pined, F., Nguyen, G., and Rondeau, E. (1996) Kidney Int. 49, 267–270
19. Prepens, U., Just, I., Hofmann, F., and Aktories, K. (1997) Adv. Exp. Med. Biol. 419, 349–353
20. Miyamoto, Y., Yamauchi, J., and Itch, H. (2003) J. Biol. Chem. 278, 29980–29990
21. Feoktistov, I., Goldstein, A. E., and Biagioni, I. (2000) Mol. Pharmacol. 58, 903–910
22. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J. (1997) Curr. Biol. 7, 202–210
23. Amano, M., Mukui, H., Uno, Y., Chihara, K., Matsui, T., Hamaejima, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 271, 648–650
24. Hall, A. (1994) Annu. Rev. Cell Biol. 10, 31–54
25. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
26. McCormick, F. (1983) Nature 303, 15–16
27. Aronheim, A., Engelberg, D., Li, N., al Alawi, N., Schlessinger, J., and Karin, M. (1994) Cell 78, 849–861
28. Shin, E. Y., Shin, K. S., Lee, C. S., Woo, K. N., Quan, S. H., Soong, N. K., Kim, Y. G., Cha, C. I., Kim, S. E., Park, D., Bokoch, G. M., and Kim, E. G. (2002) J. Biol. Chem. 277, 44417–44430
29. Shin, E. Y., Woo, K. N., Lee, C. S., Koo, S. H., Kim, Y. G., Kim, W. J., Bae, C. D., Chang, S. I., and Kim, E. G. (2004) J. Biol. Chem. 279, 1994–2004
30. Bagrodia, S., and Cerione, R. A. (1999) Trends Cell Biol. 9, 350–355
31. Daniels, R. H., and Bokoch, G. M. (1999) Trends Biochem. Sci. 24, 350–355
32. Song, J., Khachikian, Z., Radhakrishna, H., and Donaldson, J. G. (1998) J. Cell Sci. 111, Pt. 15, 2257–2267
33. Norman, J. C., Jones, D., Barry, S. T., Hilt, M. B., Cockcroft, S., and Critchley, D. R. (1998) J. Cell Biol. 143, 1981–1995
34. Crean, J. K., Furlong, F., Finlay, D., Mitchell, D., Conway, B., Brady, H. R., Godson, C., and Martin, F. (2004) FASEB J. 18, 1541–1543
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