RhoB Is Stabilized by Transforming Growth Factor β and Antagonizes Transcriptional Activation*

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Transforming growth factor β (TGF-β) is the prototype for an evolutionarily conserved superfamily of secreted factors implicated in diverse biological phenomena. The pleiotropic responses to TGF-β are initiated by a heteromeric receptor complex that binds and phosphorylates downstream effectors. Among these, the Smads have been extensively studied. However, less attention has been directed toward alternative downstream effectors and their participation in TGF-β signal transduction. We show that TGF-β promotes accumulation of the labile monomeric GTPase RhoB by antagonizing its normal proteolytic destruction, presumably via the 26 S proteasome. RhoB accumulates in its isoprenylated form. Transient overexpression of wild type RhoB but not its dominant negative mutant RhoB-N19 antagonizes TGF-β-mediated transcriptional activation. These results suggest a novel mechanism of regulation by TGF-β and implicate RhoB as a negative regulator of TGF-β signal transduction.

Transforming growth factor-β (TGF-β) is the prototype for a multifunctional superfamily of secreted factors. TGF-β has been implicated in diverse phenomena including growth control, production of extracellular matrix, cell adhesion and motility, and modulation of cell phenotype (1). As such, TGF-β has broad influence over normal growth and developmental programs and over the pathologic sequelae that arise from their dysfunction. TGF-β communicates with the cytoplasm through a heteromeric complex composed of type I (TβRI) and type II (TβRII) transmembrane serine/threonine kinase receptors. Upon ligand binding, TβRII transphosphorylates TβRI in a highly conserved GS domain, thereby activating TβRI kinase activity toward downstream effectors. Ligand binding specificity is conferred by TβRII, whereas signal specificity is conferred by TβRI (2).

Several studies have focused on the nature of the signal transduction machinery downstream of the TGF-β receptor complex. Genetic approaches in Drosophila melanogaster and Caenorhabditis elegans, along with supporting biochemical evidence in Xenopus laevis and mammalian systems have implicated the Smad family of proteins in signaling by TGF-β superfamily members. Evidence suggests that the Smads, through ligand-dependent phosphorylation and hetero-oligomerization, play crucial roles in growth and transcriptional regulation by TGF-β (3). Other putative downstream effectors in TGF-β signaling include FKBP12 (4), farnesyltransferase-α (5), and TRIP-1 (6), each identified through their specific interactions with TGF-β receptors. However, despite extensive investigation, a clearly defined picture of the post-receptor signaling machinery for TGF-β remains elusive.

Rho proteins, monomeric GTPases of the Ras superfamily (7), serve as molecular switches that cycle between GTP-bound (active) and GDP-bound (inactive) states. Rho proteins undergo a complex series of post-translational modifications initiated by isoprenylation of a C-terminal CAAX motif (8). Generally, Rho proteins are geranylgeranylated, although RhoB can be modified by either a geranylgeranyl or a farnesyl moiety (9). In most cases, Rho protein function requires isoprenylation (8, 10). Rho proteins regulate specific actin cytoskeletal structures, including stress fibers (RhoA/C), lamellipodia (Rac1), and filopodia (Cdc42), and make manifest the effects of growth factors and oncogenes on cell morphology (11). Additionally, Rho family members have been implicated in cell growth control as regulators of the G1/S transition and in transformation, both alone and in cooperation with oncogenic Ras (12–14). Recent evidence also suggests that Rho proteins, as regulators of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling module, are components of the intracellular relay for transcriptional activation signals that originate from the TGF-β receptor complex (15).

RhoB, whose primary structure is 88% identical to the Rho family prototype RhoA, has several distinguishing features. Most notably, RhoB is inducible by a variety of stimuli, including the growth factors epidermal growth factor and platelet-derived growth factor, genotoxic stresses from UV light and alkylating xenobiotics, and activated tyrosine kinases such as v-Src and v-Fps (16–18). Unlike other Rho family members, both RhoB mRNA and protein are labile, with half-lives of approximately 20 min and 2 h, respectively (18, 19). Additionally, RhoB is subject to post-translational palmitoylation as a secondary membrane localization signal following isoprenylation (20). This contrasts with other Rho proteins, for whom this role is presumably accomplished by an intrinsic polybasic domain immediately proximal to the CAAX motif. Because of a high degree of homology, it has been hypothesized that RhoB and RhoA have similar biological activities. Both proteins promote transactivation of the serum response element of the c-fos promoter (10, 21). Also, both RhoB and RhoA can potentiate the...
transforming activity of oncogenic Ras (13, 14, 19). However, RhoB and RhoA are localized differently in mammalian cells. Furthermore, although RhoA subcellular localization is altered in response to lysophosphatidic acid, localization of RhoB is unaffected (22). In conjunction with differences in protein stability, post-translational modifications, and inducibility by exogenous stimuli, the physiologic functions of RhoB and RhoA appear at least partially distinct.

Because of its inducibility and the previously described effects of Rho family members on TGF-β signal transduction, we explored the influence of TGF-β on RhoB and the subsequent influence of RhoB on TGF-β-mediated gene expression. We demonstrate that RhoB turnover occurs via ubiquitin-mediated destruction by the 26 S proteasome and that TGF-β exerts a stabilizing influence toward RhoB, thereby permitting its accumulation. Unlike other stimuli that induce RhoB gene transcription, TGF-β-mediated accumulation of RhoB does not correlate with changes in RhoB mRNA levels. Furthermore, transcriptional activation of the TGF-β-responsive reporter p3TP-Lux is antagonized by wild type RhoB in a dose-dependent manner but is not affected by its dominant inhibitory point mutant, RhoB-N19. These data suggest that TGF-β can regulate the abundance of some cytoplasmic effectors by controlling their destruction. Additionally, these data suggest that cells enlist multiple Rho proteins to coordinate transcriptional activation signals initiated by TGF-β.

**MATERIALS AND METHODS**

**Cell Culture, Plasmids, Antibodies, and Reagents—**Mv1Lu cells, obtained from ATCC (CCL-64), and R1B(L17) cells (28), obtained from Dr. Joan Massagué, were subcultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The pcDNA3-HA:RhoB and pcDNA3-HA:RhoB-N19 were kindly provided by Dr. George Prendergast (19). Dr. Mathias Trier provided pMT107, maintained in DMEM supplemented with 0.2% FBS. Total RNA was isolated from RhoB-treated or time matched, untreated controls using guanidinium isothiocyanate as described previously (26). Total RNA (0.5 μg) was reverse transcribed using an oligo(dT) primer. The resulting cDNA was amplified by PCR with RhoB-specific and GAPDH-specific primers simultaneously. PCR products were resolved in 2% agarose/0.5× TBE (1× TBE = 90 mM Tris, 90 mM boric acid, 2 mM EDTA) and stained with 0.8% 3:1 Agarose. PCR products were analyzed in the linear range. The RhoB-specific PCR product was isolated and subjected to automated deoxysequencing to confirm its identity.

**Determination of RhoB Half-life in Mv1Lu Cells—**Mv1Lu cells were maintained in DMEM + 10% FBS. In parallel cultures, growth medium was changed to either DMEM + 0.2% FBS or that same medium supplemented with 40 μM TGF-β1. Cultures were incubated in these conditions for 4 h. At the end of this 4-h incubation, corresponding to time 0, growth medium was changed to DMEM + 0.2% FBS and 30 μM cycloheximide, with or without coincident 40 μM TGF-β1. At the indicated times, cells were lysed in Nonidet P-40 lysis buffer and clarified by centrifugation at 15,000 × g for 15 min at 4 °C. Lysates were assayed for RhoB using either 1 μg of RhoB-specific antibody or nonspecific rabbit serum. Cell monolayers were washed and lysed in Nonidet P-40 lysis buffer as described above. Crude lysates were clarified by centrifugation and precleared with protein A-Sepharose. Supernatants were then incubated for 1 h with either 1 μg of RhoB-specific antibody or nonspecific rabbit serum. Immune complexes were collected with protein A-Sepharose and subjected to SDS-PAGE as described above. Labeled proteins were detected in fixed gels by autoradiography.

**Determination of RhoB Ubiquitination and Turnover by the 26 S Proteasome—**Mv1Lu cells maintained in DMEM + 10% FBS were transfected by the DEAE-dextran technique with pMT107 and pcDNA-HA:RhoB in the combinations indicated in Fig. 4. Cultures were incubated in M/°C medium for 1 h then incubated for 4 h in M/°C medium supplemented with 200 μCi/ml [35S]methionine/cysteine with or without coincident 30 μg/ml cycloheximide. Cell monolayers were washed and lysed in Nonidet P-40 lysis buffer as described above. Crude lysates were clarified by centrifugation and precleared with protein A-Sepharose. Supernatants were then incubated for 16 h with either 1 μg of RhoB-specific antibody or nonspecific rabbit serum. Immune complexes were collected with protein A-Sepharose and subjected to SDS-PAGE as described above. Labeled proteins were detected in fixed gels by autoradiography.

**Scanning Densitometry was performed on an Alpha Innotech IS-1000 Digital Imaging System.**

**Transient Assays Using p3TP Luciferase Reporter—**Mv1Lu or R1B(L17) cells in 6-well plates were transiently transfected with pcDNA-HA:RhoB, pcDNA3-HA:RhoB-N19, and pCMV5-TjR1HA by the DEAE-dextran method as shown in the legend for Fig. 5. The constitutive reporter CMV-βgal was also included to normalize transfection efficiency. After overnight incubation in DMEM + 10% FBS, cells were incubated in DMEM + 0.2% FBS with or without 40 μg TGF-β1 for 16 h. For all assays, cells were washed with ice-cold 1× PBS. Cell lysates were prepared by scraping into Nonidet P-40 lysis buffer as described in methods. Labeled proteins were detected in fixed gels by autoradiography.
FIG. 1. TGF-β promotes accumulation of isoprenylated RhoB in Mv1Lu cells. A, Mv1Lu cells were maintained in DMEM with 10% FBS, 0.2% FBS, or 0.2% FBS supplemented with either TGF-β or lovastatin as indicated. Equivalent amounts of total cellular protein were resolved by Tricine SDS-PAGE. Proteins were electrophoretically transferred to polyvinylidene difluoride membranes and probed with antibodies against RhoB (a), RhoA (b), Rac1 (c), or Rho-GDI (d). Antigens were visualized by chemiluminescent detection using appropriate secondary antibody-horseradish peroxidase conjugates. Lovastatin treatment suggests the identity of isoprenylated RhoB (*) and its unmodified precursor (†). B, Mv1Lu cells maintained in DMEM + 0.2% FBS were switched to DMEM + 0.2% FBS and incubated for 24 h to deplete pre-existing RhoB protein. At time 0, parallel cultures were given either DMEM + 0.2% FBS or that same medium supplemented with TGF-β. At the times indicated, cell lysates were prepared for RhoB-specific immunoblot analysis as described under “Materials and Methods.” Relative distribution between unmodified and isoprenylated RhoB was estimated by scanning densitometry. No Tx, no treatment.

TGF-β Promotes Accumulation of Isoprenylated RhoB—Several stimuli have been shown to promote accumulation of RhoB mRNA (16, 17, 18). However, although more closely aligned with function, RhoB protein levels have been examined in a more limited context. We examined the abundance of RhoB, the Rho family members RhoA and Rac1, and the Rho-specific regulator Rho-GDI in Mv1Lu cells (Fig. 1A, a–d) in the presence and absence of TGF-β. Lovastatin, which abrogates isoprenoid synthesis by inhibition of hydroxymethylglutaryl-coenzyme A reductase, was used to distinguish between prenylated (†) and nonprenylated forms based upon mobility differences in Tricine SDS-PAGE. Prenylated and nonprenylated RhoB were easily distinguished, but differentially prenylated forms of RhoA and Rac1 were not readily resolved. As anticipated, no mobility differences were noted for Rho-GDI, because it is not a prenylated protein. Mv1Lu cells grown in a serum-rich medium express RhoB protein approximately equally divided between prenylated and nonprenylated forms. However, when switched to low serum medium, the abundance of RhoB protein decreases with time. A difference was noted in the rate of disappearance between prenylated and nonprenylated RhoB, with the latter disappearing more rapidly (Fig. 1A, a). When low serum medium is supplemented with 40 μM TGF-β, RhoB protein levels remain elevated, and both prenylated and nonprenylated forms are noted. In contrast to Mv1Lu cells grown in a serum-rich environment, the distribution of RhoB protein between prenylated and nonprenylated forms is skewed toward the former in the presence of TGF-β. In contrast to the dynamic nature of RhoB levels, RhoA, Rac1, and Rho-GDI levels were not significantly affected by low serum, TGF-β, or lovastatin treatment.

To gain additional insight into the kinetics of RhoB accumulation, we examined its abundance in whole cell lysates from TGF-β-treated or matched untreated controls, preceded by 24 h of incubation in low serum medium to deplete pre-existing RhoB protein (Fig. 1B). Again, prenylated RhoB (†) was separated from nonprenylated RhoB by differential mobility in Tricine SDS-PAGE. Total RhoB protein, separated into prenylated and nonprenylated forms is depicted in Fig. 1B by scanning densitometry of identically developed immunoblots. In comparison with consistently low levels in untreated controls, TGF-β promoted a significant, time-dependent increase in the abundance of RhoB protein (approximately 8–10-fold). Furthermore, RhoB from TGF-β-treated cells accumulated predominantly in the isoprenylated form, reaching 85% prenylated RhoB in the 4-h time point. In contrast, the distribution between prenylated and nonprenylated RhoB in untreated controls fell within a range of 40–60%/60–40%, similar to the distribution found in serum-rich medium and more readily apparent with longer exposure (data not shown). We also examined the relative abundance of RhoB protein and the prenylated/nonprenylated ratio in TGF-β-treated and matched, untreated controls using the Mv1Lu-derived, TGF-β-nonresponsive cell line R1B(L17) (28). Neither RhoB protein levels nor the prenylated/nonprenylated ratio differed in R1B(L17) cells in response to TGF-β (data not shown).

RhoB mRNA Levels Are Unaffected by TGF-β—Growth factors such as epidermal growth factor and platelet-derived growth factor are known to increase the level of RhoB mRNA (16, 17). To address the possibility of changes in RhoB mRNA level, we examined the abundance of RhoB mRNA by both Northern hybridization of poly(A) RNA (Fig. 2A) and by RT-PCR (Fig. 2B). Mv1Lu cells were first incubated for 24 h in low
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The majority of protein turnover outside the lysosomal compart-
ment occurs via the 26 S proteasome by ubiquitin-dependent proteolysis (29). To gain additional insights into the mechanism of RhoB turnover in Mv1Lu cells, we examined its conjugation to ubiquitin and its subsequent elimination by the 26 S proteasome. Mv1Lu cells were transiently transfected with expression constructs encoding HA-tagged RhoB (HA-

RhoB) and octameric, hexahistidine-tagged ubiquitin (H6-ubiquitin), in the combinations shown in Fig. 4. Transfected cells were then incubated in the presence or the absence of 50 μM LLnL, an inhibitor of proteolysis by the 26 S proteasome. Ubiquitin-conjugated proteins were collected under denaturing conditions by Ni2+/NTA-agarose chromatography and subjected to SDS-PAGE and immunoblot analysis using either an α-RhoB or an α-HA antibody. These conditions permit detection of only covalent associations between ubiquitin and its targets. When 26 S proteasome activity is antagonized by LLnL, ubiquitinated forms of RhoB, confirmed by detection with both the RhoB- and HA-specific antibodies, are noted in cells transfected with both expression plasmids. No such bands are detected in mock transfected cells or in cells transfected only with a H6-ubiquitin expression construct. Electrophoretic mobilities of the ubiquitinated RhoB forms suggest that conjugation by one, two, and three ubiquitins could be easily discerned, whereas higher order conjugates were not resolved. The stabilization of RhoB in response to TGF-β in conjunction with this data suggests that TGF-β may exert negative regulatory influence toward some aspect of ubiquitin-mediated protein turnover by the 26 S proteasome.

RhoB Antagonizes TGF-β-mediated Transcriptional Activation—Members of the Rho family can transactivate the TGF-β-responsive promoter p3TP-Lux in a ligand-independent manner. To explore the impact of RhoB on TGF-β signal transduction, we examined p3TP-Lux transactivation in Mv1Lu cells and their TβRII deficient, TGF-β-nonresponsive derivative, R1B(L17). Cells were transfected with p3TP-Lux reporter and either HA-RhoB or HA-RhoB-N19 expression plasmids as shown in Fig. 5. R1B(L17) cells were additionally transfected with a TβRII expression plasmid to restore TGF-β responsiveness. Luciferase activity in cell extracts was deter-

**FIG. 3. TGF-β stabilizes RhoB protein in Mv1Lu cells.** A, Mv1Lu cells were maintained in DMEM + 10% FBS. Cultures were pretreated (Pre-Tx) for 4 h in DMEM + 0.2% FBS in the presence or absence of TGF-β. To initiate the chase, new protein synthesis was abrogated in each culture by the addition of cycloheximide. Cultures were then incubated for the indicated times, and cell extracts were prepared for RhoB-specific immunoblot analysis. Total RhoB signal in the presence and absence of TGF-β for each time point was estimated by scanning densitometry and represented in the decay curves in B, C, to affirm abolition of new RhoB synthesis during the chase, 35S-labeled RhoB protein was immunoprecipitated (IP) from whole cell extracts prepared following a 4-h incubation in 100 μCi/ml [35S]methionine/cysteine either with or without coincident cycloheximide (CycHX) treatment. Both RhoB-specific antibody and normal rabbit serum (NRS) were employed.
essential regulators of TGF-β activation. Considerable evidence suggests that Smad proteins are involved in these processes, and Smad 6-ubiquitin and HA-RhoB expression plasmids as shown. Each combination was subsequently incubated in the presence or absence of the proteasome inhibitor, LLLi. Ubiquitinated proteins were collected by Ni²⁺/NTA-agarose retrieval and subjected to immunoblot analysis with antibodies directed toward either RhoB or the HA tag of the transfected protein. Ubiquitinated forms of RhoB are indicated. Ubiquitin chain length estimates are based upon the apparent molecular weights of RhoB-Ubi conjugates in SDS-PAGE.

**FIG. 4.** Ubiquitinated RhoB is degraded by the 26 S proteasome in Mv1Lu cells. Mv1Lu cells were transiently transfected with H₆-ubiquitin and HA-RhoB expression plasmids as shown. Each combination was subsequently incubated in the presence or absence of the proteasome inhibitor, LLLi. Ubiquitinated proteins were collected by Ni²⁺/NTA-agarose retrieval and subjected to immunoblot analysis with antibodies directed toward either RhoB or the HA tag of the transfected protein. Ubiquitinated forms of RhoB are indicated. Ubiquitin chain length estimates are based upon the apparent molecular weights of RhoB-Ubi conjugates in SDS-PAGE.

**FIG. 5.** RhoB antagonizes TGF-β-mediated transcriptional activation. Mv1Lu or R1B(L17) cells were transfected as indicated. TβRI expression in R1B cells restores responsiveness to TGF-β. p3TP-Lux activity was measured in lysates prepared after incubation in DMEM + 0.2% FBS with or without 40 px TGF-β. Results are reported as means ± S.E. from triplicate analysis in a representative experiment. Luciferase data are normalized to internal control β-galactosidase expression from a constitutive reporter. White bars, −TGF-β; black bars, +TGF-β.}

mined in the presence and absence of TGF-β. Equivalent expression of HA-RhoB and HA-RhoB-N19 was confirmed by α-HA immunoblot analysis in Mv1Lu cells in a parallel experiment (data not shown). In both Mv1Lu and R1B(L17) cells, HA-RhoB antagonized TGF-β-induced p3TP-Lux activity in a dose-dependent manner. HA-RhoB-N19 failed to attenuate TGF-β-mediated p3TP-Lux activity in either Mv1Lu or R1B(L17) cells. These data suggest that RhoB interacts with TGF-β signaling and is required for the inhibition of this pathway.

**DISCUSSION**

The signal transduction machinery downstream of the TGF-β receptor complex has been the focus of intense investigation. Considerable evidence suggests that Smad proteins are essential regulators of TGF-β-mediated growth inhibition and transcriptional activation (3). However, recent data also suggest a critical role for Rho proteins in TGF-β-mediated transcriptional activation (15). GTPase deficient, constitutively active mutants of Rac1, Cdc42Hs, and RhoA, and Cdc42Hs individually antagonized but collectively abolished TGF-β-mediated transcriptional activation. Furthermore, dominant negative versions of MEKK1, MKK4, SAPK/JNK, and c-Jun, components of the SAPK/JNK pathway, each abolished the positive influences of constitutively active Rho proteins on p3TP-Lux transactivation. In concert, these results suggest that TGF-β responsiveness may require the cooperation of the Rho/SAPK/JNK and Smad signal transduction cascades.

RhoB, like other members of the Ras superfamily, acts as a binary switch that cycles between GTP-bound (active) and GDP-bound (inactive) states. RhoB expression can be induced by a variety of stimuli, and evidence suggests that both RhoB mRNA and RhoB protein are labile, with half-lives of approximately 20 min and 2 h, respectively (18, 19). The inducibility and lability of RhoB, in conjunction with its post-translational modifications and its regulation by a bound guanine nucleotide, permit control of its function at several levels and as such present unique regulatory opportunities. We demonstrate that TGF-β promotes the accumulation of isoprenylated RhoB by antagonizing its destruction. Interestingly, accumulation of RhoB in its isoprenylated form may reflect a connection between TGF-β signaling and protein prenylation, suggested from the observed interaction of farnesyltransferase-α and the TGF-β type I receptor (5). The ability of TGF-β to regulate prenyltransferase activity toward RhoB is currently being explored. In contrast to other stimuli, we see no correlation between TGF-β stimulation and the abundance of RhoB mRNA. Although this cannot be eliminated as a contributing factor, our data suggest that regulated RhoB turnover is primarily responsible for the TGF-β-induced RhoB accumulation. By extension, regulated protein stability might reflect a more general mechanism by which TGF-β controls the activities of its downstream effectors to produce its diverse biological responses.

A small fraction of total cellular protein undergoes rapid turnover. Frequently these proteins are components of regulatory pathways. Their limited life span, in concert with other control mechanisms, provides restrictions on their biological influence. By extension, controlling the turnover rate of regulatory proteins, thereby influencing their steady-state levels, could dramatically affect signal transduction via the pathways in which these proteins participate. Several examples of controlled proteolysis as a means for regulating protein function have been described (29). The majority of selective protein turnover in eukaryotic cells is achieved by obligatory conjugation between lysine residues of the target protein and the C terminus of ubiquitin. Sequential ubiquitin additions yield multiubiquitin chains that direct the target protein for destruction by the 26 S proteasome (29). We show that RhoB protein is conjugated to ubiquitin in Mv1Lu cells and that ubiquitinated RhoB is subsequently eliminated by the 26 S proteasome. The participation of the 26 S proteasome and the coincident influence of TGF-β on RhoB stability suggests that TGF-β may regulate a component of the ubiquitin-dependent proteolytic machinery.

The reporter p3TP-Lux is frequently used as a measure of TGF-β responsiveness in mammalian cells. To explore the influence of RhoB on TGF-β signaling, we examined the impact of its expression or that of its dominant-negative mutant, RhoB-N19, on p3TP-Lux transactivation. In either Mv1Lu cells or R1B(L17) cells rendered TGF-β-responsive by TβRI cotransfection, wild type RhoB antagonized p3TP-Lux activation by TGF-β. RhoB-N19 failed to attenuate and instead perhaps potentiated p3TP-Lux activity in these cells, consistent with dominant interference with endogenous RhoB function. These
data suggest that RhoB exerts negative regulatory influence on TGF-β-induced transcriptional activation. Functionally distinct negative regulation of TGF-β signaling has also been described for FKBP12, Smad6, and Smad7 and correlated with binding to TβRI. In light of the potentiating influence of other Rho family members toward p3TP-Lux transactivation and the labile nature of RhoB, it is attractive to speculate that RhoB accumulation in response to TGF-β imposes negative but inherently self-limited restriction on the Rho-mediated aspects of TGF-β signal transduction.

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REFERENCES
1. Lyons, R. M., and Moses, H. L. (1990) Eur. J. Biochem. 187, 467–473
2. Massagué, J. (1996) Cell 85, 947–950
3. Massagué, J., Hata, A., and Liu, F. (1997) Trends Cell Biol. 7, 187–192
4. Wang, T., Li, B.-Y., Danielson, P. D., Shah, P. C., Rockwell, S., Lechleider, R. J., Martin, J., Manganaro, T., and Donahoe, P. K. (1996) Cell 86, 435–444
5. Kawabata, M., Imamura, T., Miyazona, K., Engel, M. E., and Moses, H. L. (1995) J. Biol. Chem. 270, 28628–28631
6. Chen, R.-H., Miettinen, P. J., Maruoka, E. M., Choy, L., and Derynck, R. (1995) Nature 377, 548–552
7. Van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
8. Takai, Y., Sasaki, T., Tanaka, K., and Nakashima, H. (1995) Trends Biochem. Sci. 20, 227–231
9. Lebowitz, P. F., Case, P. J., Prendergast, G. C., and Thiessen, J. A. (1997) J. Biol. Chem. 272, 15591–15594
10. Lebowitz, P. F., Du, W., and Prendergast, G. C. (1997) J. Biol. Chem. 272, 16093–16095
11. Ridley, A. J. (1995) Curr. Opin. Genet. Dev. 5, 24–30
12. Olsen, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
13. Prendergast, G. C., Khororavi-Far, R., Solski, P. A., Kurzawa, H., Lebowitz, P. F., and Der, C. J. (1995) Oncogene 10, 2298–2296
14. Qui, R. G., Chen, J., McCormick, F., and Symons, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 92, 11781–11785
15. Atifi, A., Djelloul, S., Chastre, E., Davis, R., and Guespach, C. (1997) J. Biol. Chem. 272, 1429–1432
16. Jähner, D., and Hunter, T. (1991) Mol. Cell. Biol. 11, 3682–3690
17. De Cremoux, P., Gauville, C., Clasen, V., Linares, G., Calvo, F., Tavitian, A., and Ofue, B. (1994) Int. J. Cancer 59, 408–415
18. Fritz, G., Kaina, B., and Aktories, K. (1995) J. Biol. Chem. 270, 25172–25177
19. Lebowitz, P. F., Davide, J. P., and Prendergast, G. C. (1995) Mol. Cell. Biol. 15, 6613–6622
20. Adamson, P., Marshall, C. J., Hall, A., and Tillbrook, P. A. (1992) J. Biol. Chem. 267, 20033–20038
21. Hill, C. S., and Treisman, R. (1995) Cell 80, 199–211
22. Fleming, I. N., Elliott, C. M., and Exton, J. H. (1996) J. Biol. Chem. 271, 33067–33073
23. Nakamura, T., Asano, M., Shindo-Oka, N., Nishimura, S., and Munden, Y. (1996) Biochem. Biophys. Res. Commun. 226, 688–694
24. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
25. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 7.10–7.11 and 7.26–7.29, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Chomzynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
27. Treier, M., Staeweski, L. M., and Bohmann, D. (1994) Cell 78, 787–788
28. Wizeman, J. L., Attisano, L., Carcamo, J., Zentella, A., Dadoo, J., Laibe, M., Wang, X.-F., and Massague, J. (1992) Cell 71, 1003–1014
29. Hochstrasser, M. (1997) Annu. Rev. Genet. 30, 405–439
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