Sprouty2 Inhibits the Ras/MAP Kinase Pathway by Inhibiting the Activation of Raf*

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Several genetic studies in *Drosophila* have shown that the dSprouty (dSpry) protein inhibits the Ras/mitogen-activated protein (MAP) kinase pathway induced by various activated receptor tyrosine kinase receptors, most notably those of the epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR). Currently, the mode of action of dSpry is unknown, and the point of inhibition remains controversial. There are at least four mammalian Spry isoforms that have been shown to co-express preferentially with FGFRs as compared with EGFRs. In this study, we investigated the effects of the various mammalian Spry isoforms on the Ras/MAP kinase pathway in cells over-expressing constitutively active FGFR1. hSpry2 was significantly more potent than mSpry1 or mSpry4 in inhibiting the Ras/MAP kinase pathway. Additional experiments indicated that full-length hSpry2 was required for its full potency. hSpry2 had no inhibitory effect on either the JNK or the p38 pathway and displayed no inhibition of FRS2 phosphorylation, Akt activation, and Ras activation. Constitutively active mutants of Ras, Raf, and Mek were employed to locate the prospective point of inhibition of hSpry2 downstream of activated Ras. Results from this study indicated that hSpry2 exerted its inhibitory effect at the level of Raf, which was verified in a Raf activation assay in an FGF signaling context.

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**FIG. 1.** Effect of Sprys and the N and C-terminal domains of hSpry2 on the MAP kinase pathways activated by full-length FGFR1. Phosphorylation of ERK2 by FGFR1 is inhibited by hSpry2 (A). 293T cells were transfected with wild type FLAG-ERK2, FGFR1, full-length HA-Sprys, and empty vector constructs. Anti-FLAG immunoprecipitates (IP) were resolved by Western blotting analysis to detect phospho-ERK2 and ERK2 using anti-phospho-ERK1/2 (phospho-p42/44) and anti-ERK2, respectively. Other associated proteins in the cell lysates were detected using anti-FGFR1 and anti-HA. IB, immunoblots. hSpry2 inhibits FGFR1-stimulated ERK2 kinase activity (B). 293T cells were transfected with wild type FLAG-ERK2, FGFR1, full-length HA-Sprys (S1, Sprouty1; S2, Sprouty2; S4, Sprouty4), and empty vector constructs (lane C). FLAG-ERK was immunoprecipitated, and kinase activity was determined using GST-Elk as described under “Materials and Methods.” The ERK kinase activity was determined by densitometry quantitation of the 32P GST-Elk band after autoradiography. The bar graph shown represents the mean value with standard error. Neither the N- nor the C-terminal halves of hSpry2 inhibit the phosphorylation of FGFR1-stimulated ERK2 (C). 293T cells were transfected with wild type FLAG-ERK2, FGFR1, full-length cytosolic domain of FGFR1, and empty vector constructs, and the experiment was performed as in panel A. hSpry2 is an intracellular inhibitor of the Ras/MAP kinase pathway (D). 293T cells were transfected with wild type FLAG-ERK2, full-length or cytosolic domain of FGFR1, HA-Spry2, and empty vector constructs, and the experiment was performed...
activation signals down the Ras/MAP kinase pathway (17).

Apart from the genetic evidence, relatively little is known about the biochemical mode of action of the various Spry isoforms. One study demonstrated that a considerable part of the conserved C-terminal portion was involved in translocating the protein from cytosol to membrane upon RTK stimulation of cells (18). Assuming this highly conserved sequence is involved in directing the cellular localization of the protein, it is reasonable to hypothesize that the C-terminal half of the protein is involved in binding proteins that are strategically placed to execute physiological functions such as inhibition of the Ras/MAP kinase pathway.

Currently, genetic evidence derived entirely from studies in Drosophila indicates that the physiological role of dSpry is as an inhibitor of the Ras/MAP kinase pathway. However, the three studies that employed genetic approaches in Drosophila are not in agreement as to the mode and site of the inhibition induced by dSpry (1–3). Mammalian Spry isoforms may have physiological activities that differ from those of dSpry and from each other. In this study, we asked several questions. In biochemical terms, do the various mammalian Spry isoforms inhibit the Ras/MAP kinase pathway? What features of the protein are required for the inhibition? Where does this inhibition take place?

MATERIALS AND METHODS

Antibodies—Antibodies against phospho-ERK, phospho-JNK, phospho-p38 phospho-Akt, p38, JNK, and Akt were from New England Biolabs, anti-Gab1 was from Upstate Biotechnology (Lake Placid NY), and HA antibody was from Roche Molecular Biochemicals. Monoclonal antibodies against phosphotyrosine (PY20)-horseradish peroxidase-conjugated, anti-panERK and anti-ERK2 were from Transduction Laboratories (Lexington, KY). Polyclonal antibody against FRS2 (A872) was described previously (19). Secondary anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase, FLAG antibody, and main antibodies against phosphotyrosine (PY20)-horseradish peroxidase-conjugated secondary antibody. Immunoreactive protein bands were visualized by enhanced chemiluminescence reagent (Amersham Biosciences, Inc).

Kinase Assay and Protein Activity—Kinase activity of ERK2 was measured with an in vitro GEF-ERK2 assay using GST-Erk1 as substrate as described previously (23). Ras activity was assessed using the Ras activation assay kit from Upstate Biotechnology. Ras GTP from various treated lysates was “pulled down” using the GST fusion protein corresponding to human Ras binding domain of Raf-1 bound to agarose. The presence of Ras-GTP was detected by Western blotting using anti-Ras antibody (Upstate Biotechnology). Raf-1 kinase assays were performed using the Raf-1 immunoprecipitation kinase assay kit from Upstate Biotechnology with some modifications. Raf kinase activity was measured by a coupled assay using GST-MEK, GST-ERK, and GST-Elk or myelin basic protein as sequential substrates. The assay product was assessed using P81 phosphocellulose paper and scintillation counting. Alternatively, the radiolabeled product was analyzed by 7% SDS-PAGE and autoradiography. The assay measures the phosphotransferase activity of an in vitro kinase cascade reaction initiated by an immunocomplex containing Raf-1 (Upstate Biotechnology protocol, Ref. 24).

RESULTS

Based on the observation in mammalian embryo development that the expression of Spry isoforms coincide with the expression of FGF rather than EGF (25), we chose FGF-induced Ras/MAP kinase as our model system. Additionally we employed a cell culture system that best mimicked an inhibition of the pathway from the various in vivo systems reported: we reconstituted relatively long term stimulation of the FGF signaling pathway by overexpressing a wild type FGFR1 (fgf) and observing the effects of overexpressed Spry isoforms at various assay points downstream with emphasis on the Ras/MAP kinase pathway.

Full-length hSpry2 Is an Intracellular Inhibitor of the ERK Pathway but Not the JNK or p38 Pathways—FGFR1 was co-transfected into 293T cells with HA-tagged Spry isoforms and FLAG-tagged ERK2. Forty h after transfection, cells were lysed, and the lysates were precipitated with anti-FLAG. The immunoprecipitates and whole cell lysates were subjected to Western analysis using phospho-ERK1/2 (p42/44), ERK2, FGFR1, or HA antibodies to detect for phosphorylated ERK2 or the level of transfected proteins. The results in Fig. 1A show that hSpry2 significantly inhibits the phosphorylation of ERK2, whereas neither mSpry1 nor mSpry4 has any significant inhibitory effect.

as in panel A. The activated JNK pathway is not inhibited by Sprys (E). Cells were transfected with wild type FLAG–JNK1, FGFR1, HA-Sprys, and empty vector constructs. Anti-FLAG immunoprecipitates were resolved by Western blotting analysis to detect phospho-JNK and JNK using anti- phospho-JNK and anti-JNK, respectively. Other associated proteins in the cell lysates were detected using anti-FGFR1 and anti-HA. The activated p38 pathway is not inhibited by Sprys (F). Cells were transfected with wild type FLAG-p38, FGFR1, HA-Sprys, and empty vector constructs. Anti-FLAG immunoprecipitates were resolved by Western blotting analysis to detect phospho-p38 and p38 using anti- phospho-p38 and anti-p38, respectively. FGFR1 and Sprys were detected in the cell lysates with anti-FGFR1 and anti-HA.
Fig. 2. hSpry2 does not inhibit either the phosphorylation of FRS2α or the associated Akt path way. hSpry2 has no effect on the tyrosine phosphorylation of FRS2α (A). 293T cells were transfected with wild type FRS2α, FGFR1, FLAG-Spry2 and empty vector constructs. Aliquots of the cell lysates were resolved by SDS-PAGE and tyrosine-phosphorylated FRS2α, and other related proteins were detected by immunoblotting (IB) with respective antibodies as indicated. Spry isoforms have no effect on the phosphoinositide 3-kinase pathway activated by FGFR1 (B). 293T cells were transfected with wild type Myc-Akt, FGFR1, Gab1, FLAG-tagged Sprys, and empty vector constructs. Anti-Myc immunoprecipitates were resolved by SDS-PAGE followed by Western blotting analysis to detect phospho-Akt and Akt using anti-Akt antibodies. The results shown in Figs. 1, E and F, indicate that none of the three Spry isoforms exert any effect on the phosphorylation of JNK1 or p38. It is apparent from the data above that hSpry2 is the only isoform that inhibits a generic MAP kinase pathway, and this inhibition is confined to the ERK pathway.

hSpry2 Does Not Inhibit Either the Phosphorylation of FRS2 or the Associated Akt Pathway—ERK1/2 is somewhat distal from the receptor, and inhibition can potentially occur at various points along the pathway. To assess the site of inhibition of hSpry2, we decided to analyze key points along the canonical FGFR1 to ERK1/2 pathway.

Current evidence indicates that FRS2 is the major “distribution center” of FGFR-derived signals in mammalian cells. It has been shown to link to the Ras/MAP kinase pathway via Grb2, SHP-2, and SOS and to the Akt pathway via Grb2, Gab-1, phosphoinositide 3-kinase, and phosphoinositide-dependent kinases (17, 26). We performed experiments to investigate whether any of the Spry isoforms affected FRS2α, either via stimulation-induced tyrosine phosphorylation or by direct binding. FRS2 cDNA was co-transfected with FGFR1 and hSpry2 constructs. The cell lysates were later subjected to separation on SDS-PAGE and subsequent Western analysis employing PY20 antibody to detect tyrosine phosphorylation of FRS2α. The data in Fig. 2A show that hSpry2 has no effect on the tyrosine phosphorylation of FRS2α, which eliminates any effect of hSpry2 on the transactivation of FGFR1 as well as the ability of FGFR1 to phosphorylate FRS2α. As these experiments involve relatively long-term stimulation of the FGF pathway, it is possible that hSpry2 could either act as an in vitro competitor of the receptor or cause the induction of an exogenous inhibitor, as has been suggested elsewhere (27). The data shown preclude these possibilities as any impairment of receptor activity would present itself in the form of decreased tyrosine phosphorylation of its substrates, which is clearly not the case.

Using GST-Spry2 pull-down experiments it was also shown that FRS2α does not bind to the Spry proteins (data not shown). This further indicates that the target of hSpry2 inhibition on the FGF signaling pathway is downstream of the docker protein.

The activation of Akt via FRS2 and Gab1 represents a bifurcation from the Ras/MAP kinase pathway. Experiments were carried out using combinations of constructs for Myc-Akt, FGFR1, Gab1, and FLAG-tagged Spry isoforms to investigate whether hSpry2 elicited any effect on the Akt pathway. The cell lysates were subjected to precipitation with anti-Myc and assayed by Western analysis using antibodies against phospho-Akt. The results shown in Fig. 2B indicate that hSpry2 does not have any effect on the Akt pathway. Since hSpry2 did not have any effect at the level of receptor interaction with FRS2, our focus shifted to the next key point downstream; the possible effects on Ras activity.

hSpry2 Does Not Inhibit the Activity of Ras—The activation status of Ras was measured as described under “Materials and
Methods.” The main component of the assay was a recombinant protein derived from Raf that contains a domain capable of binding only to GTP-bound Ras (Raf-1 Ras binding domain). 293T cells were transfected with various combinations of constructs for FGFR1 and Spry isoforms. The results shown in Fig. 3 indicate that although transfected FGFR1 significantly enhanced the level of GTP-Ras, hSpry2 co-transfected into cells had no significant effect on these levels. Equal loading of whole cell lysates showed an inhibition of endogenous protein-ERK by hSpry2, as demonstrated in previous experiments. Similar results were obtained in three such experiments. Accumulated data therefore indicated that hSpry2 did not inhibit the Ras/MAP kinase pathway between the point of receptor activation and the activation of Ras. These data also rule out hSpry2’s sequestration of Grb2 from SOS as a likely inhibitory mechanism. It was therefore necessary to assay the effect of hSpry2 on various Spry isoforms on the status of Raf stimulation downstream of active Ras. It is apparent that there is some inhibition by mSpry1 and mSpry4, but it is very low compared with hSpry2.

hSpry2 Inhibits ERK Phosphorylation Downstream of Ras and Upstream of MEK and ERK—Experiments were carried out to locate the position of MAP kinase inhibition along the pathway from Ras to ERK. This pathway essentially involves three proteins: the G-protein Ras, which activates the protein kinase Raf; Raf which activates MeK; and MeK, which in turn activates ERK.

We took advantage of mutations in Ras, Raf, and MeK that cause an auto-activation of each of these respective proteins. Constructs were transfected into 293T cells along with FLAG-tagged ERK2 and HA-tagged Spry isoforms. In each of these experiments, the cells were lysed, and the lysates were precipitated with anti-FLAG beads and analyzed by Western blotting using phospho-ERK1/2 antibody to ascertain the activation status of ERK (MAP kinase). The data from Fig. 4A show that hSpry2 can inhibit the phosphorylation (activation) of ERK2 downstream of active Ras (V12). A distinct inhibition of phosphorylated ERK2 was also observed in the mSpry1-transfected cells; however, the degree of inhibition by hSpry2 was more profound, and we subsequently focused more on the effects of this isoform. In addition, we demonstrated that the inhibition of activated ERK2 stimulated through the Ras mutant requires the full-length hSpry2 protein since neither the C- nor N-terminal half was able to inhibit this activity (Fig. 4B). Contrary to the inhibition of activated Ras-generated signals, full-length hSpry2 did not cause any detectable inhibition of ERK2 phosphorylation when the constitutively active mutants of Raf (RafY340D) and MeK (MekS222D/S226D), respectively, were employed to activate the pathway (Fig. 4, C and D).

The above data indicate that hSpry2 is exerting its inhibitory effect at the level of activation of Raf. Raf has a complex mechanism of activation, and there currently appears to be some unidentified factors, especially kinases that may be involved. It would also appear from these data that hSpry2 should inhibit the MAP kinase pathway irrespective of what agonist stimulates it because the active Ras mutant used in this study is a generic stimulant that is independent of upstream pathways.

**hSpry2 Inhibits ERK Activation Induced by β2 Adrenergic Receptor Stimulation**—A wide range of agonists activates the Ras/MAP kinase pathway. Sprouty has been shown to inhibit signaling pathways induced by receptor tyrosine kinases. Based on our results, we were interested in investigating the effects of hSpry when a non-RTK (for instance the isoprenaline activation of β2 adrenergic receptors (β2ARs)), was used to stimulate the Ras/MAP kinase pathway. To address this, 293T cells were transfected with constructs for β2AR, HA-tagged hSpry2, and FLAG-tagged ERK2. Forty-eight h post-transfection, the cells were stimulated with isoprenaline (100 μM) for various times, the cells were lysed, and the lysates were subjected to Western analysis using anti-phospho ERK1/2 to detect the level of ERK phosphorylation. The data shown in Fig. 5 demonstrate that ERK2 phosphorylation was elevated 5 min after isoprenaline addition and that this level was sustained for the next 25 min at least. hSpry2 substantially inhibited ERK2 phosphorylation to the degree that any level of phosphorylation in the data shown was only evident at 5 min. It has been reported that isoprenaline activation of β2AR can activate EGFR by “cross-talk” and that this activation can contribute to the subsequent activation of the Ras/MAP kinase pathway (28). The point illustrated by the above experiment is that it appears hSpry2 will inhibit the Ras/MAP kinase pathway downstream of activated Ras in a manner that is not dependent on the mode of pathway activation.

The accumulated data indicate that hSpry2 is the predominant inhibitor of the Ras/MAP kinase and that its inhibitory effect is exerted at the level of Raf activation. In this case, hSpry2 should inhibit the activation of Raf.

**hSpry2 Inhibits the Activation of Raf**—The effect of the various Spry isoforms on the status of Raf stimulation downstream of active FGFR1 was analyzed. FGFR1 was transiently expressed in 293T cells, and Raf kinase activity was measured by a coupled assay using GST-MEK, GST-ERK, and GST-Elk or myelin basic protein as sequential substrates. The assay measures the phosphotransferase activity in an in vitro kinase cascade reaction initiated in the immunocomplex by active Raf-1. The results show that FGFR1 significantly stimulated the Raf kinase activity in comparison with the vector control. When the cells were co-transfected with the various Spry isoforms, it can be seen that hSpry2 profoundly reduced the level of Raf kinase activity, whereas mSpry1 and mSpry4 caused lesser inhibition (Fig. 6A). A parallel inhibition of phosphorylated ERK2 was observed with hSpry2 overexpression, using the same cell lysates and Western blotting detection (Fig. 6B). In essence, hSpry2, in comparison with mSpry1 and mSpry4, causes a more profound inhibition of the Ras/MAP kinase pathway, and this down-regulation occurs at the level of Raf kinase activation.

**DISCUSSION**

The major question asked at the onset of this study was: in biochemical terms, can we detect a point on the Ras/MAP...
kinase pathway where any of the Spry isoforms inhibit? For reasons outlined previously, we have mainly used a model protocol based around constitutive FGFR activation. The results clearly indicate that hSpry2 inhibits the Ras/MAP kinase pathway at the level of Raf activation. The other Spry isoforms tested, mSpry1 and mSpry4, displayed some inhibitory activity, but the effects are small compared with that seen with hSpry2. Other groups have reported an inhibition of the Ras/MAP kinase pathway by mSpry1 or mSpry4 in a different context (29, 30). It is possible that other activation systems may provide a different spectrum of inhibition from that shown above. However, ours is the first in-depth and comparative study addressing Spry inhibition on the FGFR signaling pathway.

The spectrum of interactions seen with the various Spry isoforms are intriguing. There are no Spry proteins in Caenorhabditis elegans and only one reported in Drosophila. On current evidence, mammals have four Spry proteins, which all have smaller molecular weights than the solo Drosophila protein. All isoforms, however, appear to have evolved from the same parental protein. The C-terminal half of each protein has a highly conserved, cysteine-rich domain that is likely to be important for the location of the protein. The respective N-terminal halves are less well conserved, but there are pockets of similarity shared between the family members. For instance hSpry2 and dSpry bind directly to c-Cbl, mSpry1 binds moderately, and mSpry4 does not bind (31). It is interesting to speculate why mammals evolved four different proteins and what their various functions are. It appears that dSpry and hSpry2 have parallel effects on the branching of trachea and alveoli,
Inhibition of Raf Activation by hSpry2

Fig. 5. hSpry2 inhibits ERK activation induced by β2 adrenergic receptor stimulation. 293T cells transfected with HA-hSpry2 and β2 adrenergic receptor were serum-depleted overnight and subsequently stimulated with isoprenaline (100 μM) for various times. Cells were lysed and immunoprecipitated with FLAG antibody. The anti-FLAG immunoprecipitates (IP) were resolved by Western blotting analysis to detect phospho-ERK2 and ERK2 using anti-phospho-ERK1/2 (phospho-p42/44) and anti-ERK2, respectively. Other associated proteins in the cell lysates were detected using anti-HA. IB, immunoblots.

Fig. 6. Effect of Sprys on activated Raf-1 by FGFR1. 293T cells were transfected with empty vector (V), FGFR1 (C), or FGFR1 and FLAG-Sprys (S1, S2, and S4) constructs as indicated. Raf-1 activity was determined quantitatively by scintillation counting of the Ps1 phosphocellulose squares spotted with the assay product. Background counts were subtracted from each sample, and the resultant net counts were expressed as a percentage of the stimulated control in which no Sprouty was expressed. Aliquots of the same cell lysates were resolved by SDS-PAGE, and phospho-ERK1/2 and other related proteins were detected by immunoblotting (IB) as indicated. The data shown are representative of three independent assays. The bar graph shows the values mean with standard error. Sprouy1: S1, Sprouty2: S2, Sprouty4: S4.

respectively (1, 25). Although other mammalian Sprys have been shown to be co-expressed with various FGFs, it is not known what role they play in embryo development or signal transduction in the mature animal.

The Drosophila system, via its accessible genetics, has been invaluable for discovering novel proteins and locating them in various signaling pathways. There has been some controversy with respect to the site of action of dSpry. Originally it was thought that dSpry was a secreted protein that competed with growth factors for binding to RTKs (1). A second study indicated that dSpry was an endogenous cellular protein that was bound to the internal face of the plasma membrane via the conserved C-terminal, cysteine-rich sequence (2). Using FGFR1(cytos) to stimulate the Ras/MAP kinase pathway, we confirmed that hSpry2 exerts its inhibitory effect endogenously. Genetic analysis indicated that dSpry inhibited the Ras/MAP kinase pathway somewhere between the active RTK and the activation of Ras (2). An alternate point of action was presented when it was postulated that dSpry inhibited the Ras/MAP kinase pathway at the level of Raf activation (3). Our biochemical study, albeit in a different system, would support Raf activation as the intersection point.

Raf has a complex and variable mode of activation that depends on a number of factors with phosphorylation being a crucial event. Raf associates with various inhibitory proteins and translocates to the plasma membrane before interacting with GTP-Ras. Current concepts suggest there are immediate upstream kinases of Raf that await discovery. We currently do not know the exact mechanism whereby hSpry2 inhibits Raf activation, and because our study mostly involves transient transfection, and with the associated time lag before analysis, we cannot exclude the possibility that hSpry2 induces the expression of a specific Raf inhibitor.

It is noteworthy from our studies that hSpry2 binds c-Cbl (31) and also inhibits the Ras/MAP kinase pathway. mSpry1 and mSpry4 display mediocre effects in the majority of these studies. These data indicate that the various isoforms of Spry are almost certain to have different functions, and the full spectrum of these acts await elucidation.

REFERENCES
1. Hacohen, N., Kramer, S., Sutherland, D., Hiromi, Y., and Krasnow, M. A. (1998) Cell 92, 253–263.
2. Casey, T., Vinos, J., and Freeman, M. (1999) Cell 96, 655–665.
3. Reich, A., Sapir, A., and Shilo, B. (1999) Development 126, 4139–4147.
4. de Maximy, A. A., Nakatake, Y., Moncada, S., Itoh, N., Thiery, J. P., and Bellucci, S. (1999) Mech. Dev. 81, 213–216.
5. Minowada, G., Jarvis, L. A., Chi, C. L., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M. A., and Martin, G. R. (1999) Development 126, 4465–4475.
6. Teif, J. D., Lee, M., Smith, S., Leinwand, M., Zhao, J., Bringas, P., Jr., Crowe, D. L., and Warburton, D. (1999) J. Cell Biol. 145, 219–222.
7. Ip, Y. T., and Davis, R. J. (1998) Curr. Opin. Cell Biol. 10, 205–219.
8. Schaffer, H. J., and Weber, M. (1999) Mol. Cell. Biol. 19, 2435–2444.
9. Whitmarsh, A. J., and Davis, R. J. (1999) J. Mol. Med. 74, 589–607.
10. Sturgill, T. W., and Wu, J. (1991) Biochem. Biophys. Acta 1092, 350–357.
11. Ferrell, J. E., Jr. (1996) Trends Biochem. Sci. 21, 460–466.
12. Marais, R., and Marshall, C. J. (1996) Cancer Surv. 27, 101–125.
13. Segal, R. A., and Greenberg, M. E. (1996) Annu. Rev. Neurosci. 19, 463–489.
14. Bar-Sagi, D., and Feramisco, J. R. (1985) Cell 42, 841–848.
15. Nozaki, K., Go, M., Ogura, A., Liu, D. G., Amano, T., Takano, T., and Ikawa, Y. (1985) Nature 318, 73–75.
16. Schlessinger, J. (1994) Curr. Opin. Genet. Dev. 4, 25–30.
17. Khouhara, H., Hadari, Y., Sipkale-Kroizman, T., Schilling, J., Bar-Sagi, D., Lux, I., and Schlessinger, J. (1997) Cell 89, 693–702.
18. Lim, J., Wong, E. S., Ong, S. H., Yuoff, P., and Low, B. C. (2000) J. Biol. Chem. 275, 32837–32845.
19. Lim, Y. P., Low, B. C., Lim, J., Wong, E. S., and Guy, G. R. (1999) J. Biol. Chem. 274, 19025–19034.
20. Ong, S. H., Guy, G. R., Hadari, Y. R., Laks, S., Gotob, N., Schlessinger, J., and Lux, I. (2000) Mol. Cell. Biol. 20, 979–989.
21. Low, B. C., Lim, Y. P., Lim, J., Wong, E. S., and Guy, G. R. (1999) J. Biol. Chem. 274, 33133–33130.
22. Zheng, C. F., and Guan, K. L. (1993) J. Biol. Chem. 268, 23933–23939.
23. Zheng, C. F., and Guan, K. L. (1994) EMBO J. 13, 1121–1131.
24. Zhang, B. H., and Guan, K. L. (2000) EMBO J. 19, 5429–5439.
25. Chambers, D., and Mason, I. (2000) Mol. Biol. Cell 11, 361–364.
26. Ong, S. H., Hadari, Y. R., Gotob, N., Schlessinger, J., and Lux, I. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6074–6079.
27. Glende, J., Fenten, G., Seemann, M., Sturz, A., and Thierauch, K. H. (2000) Mol. Biol. Cell 9, 91–99.
28. Maudsley, S., Friesen, K. L., Zamech, A. M., Miller, W. E., Ahn, S., Daaka, Y., Lefkowitz, R. J., and Luttrell, L. M. (2000) J. Biol. Chem. 275, 9572–9580.
29. Impagniello, M. A., Weitzer, S., Gannon, G., Compagni, A., Cotton, M., and Christofori, G. (2001) J. Cell Biol. 152, 1087–1098.
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