Previously we have shown that protein kinase C (PKC)-mediated reorganization of the actin cytoskeleton in smooth muscle cells is transmitted by the non-receptor tyrosine kinase, Src. Several authors have described how 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulation of cells results in an increase of Src activity, but the mechanism of the PKC-mediated Src activation is unknown. Using PKC isozymes purified from Spodoptera frugiperda insect cells, we show here that PKC is not able to activate Src directly. Our data reveal that the PKC-dependent Src activation occurs via the activation of the protein tyrosine phosphatase (PTP) PTPα. PTPα becomes activated in vivo after TPA stimulation. Further, we show that PKCδ phosphorylates and activates only PTPα in vitro but not any other of the TPA-responsive PKC isozymes that are expressed in A7r5 rat aortic smooth muscle cells. To further substantiate our data, we show that cells lacking PKCδ have a markedly reduced PTPα and Src activity after 12-O-tetradecanoylphorbol-13-acetate stimulation. These data support a model in which the main mechanism of 12-O-tetradecanoyl phorbol-13-acetate-induced Src activation is the direct phosphorylation and activation of PTPα by PKCδ, which in turn dephosphorylates and activates Src.

Protein kinase C (PKC) is a family of phospholipid-dependent serine/threonine kinases comprising 10 isozymes differing in their molecular domain organization of up to 4 variable and 3 constant regions and in their functions. These PKC isozymes are subdivided into three classes: (i) the “conventional” cPKCs, PKC-α, -β (βI and βII), and -γ, which can be activated by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂), diacyl glycerol (DAG), or phospholipid binding to the C1 domain and Ca²⁺ through binding to a Ca²⁺-binding site in their second constant region, C2; (ii) the “novel” nPKCs, PKC-δ, -ε, -η, -θ, which lack the C2 region and thus are Ca²⁺-independent but still DAG-, PS-, and phorbol ester-responsive; and (iii) the “atypical” aPKCs, PKC-λ/δ and -ζ, which also lack the C2 region and, in addition, are devoid of a functional DAG-binding site. Hence, the atypical PKCs are only responsive to PS but not to DAG or phorbol ester (reviewed in Refs. 1–3).

PKCs reside in the cytosol in an inactive conformation and translocate to the membrane (or other subcellular sites) upon activation, where they mediate various cellular functions through phosphorylation of target substrates. Like other kinases, PKCs have been found to be involved in intracellular signal transduction pathways that regulate cell growth, differentiation, and apoptosis, and have been implicated in the rearrangement of the cytoskeleton and migration (4).

Src-family tyrosine kinases also comprise a major group of cellular signal transducers. These tyrosine kinases can be activated by various extracellular signals and thus can modulate a variety of cellular functions, including proliferation, survival, adhesion, and migration (5). When phosphorylated at Tyr-527 Src is inactive. Activation is accomplished by dephosphorylation of this tyrosine residue, and the resulting conformational change facilitates an autophosphorylation at Tyr-416. After autophosphorylation Src is in an active state.

As described earlier, TPA activates Src in vivo (6–9). Previously, we showed in A7r5 rat aortic smooth muscle cells that PKC-induced reorganization of the actin cytoskeleton involves the activation of Src tyrosine kinase activity (10). It is therefore very likely that PKC acts upstream of Src and mediates its activation, because PKC is known to be the main receptor of TPA in cells. However, neither a model for PKC-mediated Src activation nor even an explanation for the TPA stimulation of Src activity has been described so far.

The tyrosine phosphatase PTPα, a 130-kDa transmembrane PTP, is known to be activated upon TPA stimulation and subsequent phosphorylation (11, 12). Moreover, PTPα has been shown to be a physiological regulator of Src (13–15). Hence, we examined whether the TPA-induced activation of Src involves PTPα. Here we present evidence of PKC-mediated Src activation via PTPα.
PKCδ-induced Src Activation via PTPα

Cell Culture and Transfections—A7r5 rat vascular smooth muscle cells were grown in Dulbecco’s modified Eagle’s medium without phenol red containing 10% fetal calf serum and 2 mM glutamine. The medium was changed every 3 days. For transfection, A7r5 cells were seeded freshly and transfections were performed after ~24 h of culture or when cells were grown to 50–70% confluence. The expression vector for HA-PTPα and transactivator-plasmid pTet-ßTk (Invitrogen) were prepared in insect medium without serum at a ratio of 1:2 (10 μg/10-cm plate). Superfect transfections reagent (Qiagen, Hilden, Germany) was added according to the manufacturer’s recommendations. Transfected cells were examined 2 days after transfection.

Primary skin fibroblasts were isolated from newborn PKCδ−/− and PKCδ+/+ mice from the same litter as described earlier (22) and cultured in the same way as A7r5 cells. Experiments were performed between passage 3–5. Skin fibroblasts were cultured in 500 μl of media containing 50% fetal bovine serum, 3.3 g/liter lactalbumin hydrolysate, 3.3 g/liter yeast extract, 50 μg/ml gentamicin, and 2.5 μg/ml fungizone.

PKC Kinase Assays—Recombinant PKC isozymes were prepared from SF9 cells as described (21), bound to GSSH-Sepharose, and cleaved by thrombin. The activity of each isozyme was standardized in an in vitro kinase assay using myelin basic protein (MBP). One μg of MBP was incubated with different amounts (5–20 ng) of the PKC isozymes in the presence of 20 mM Tris, pH 7.5, 5 mM MgCl2, 0.1% Nonidet P-40, 0.5 mM ATP, 1.5 mM phosphatidylinerse, 10 μM ATP, 5 μc of [γ-32P]ATP (3000C/cm, Amersham Biosciences), 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride (kinase buffer) in a final volume of 50 μl for 15 min at 30 °C. The reaction was stopped by adding 10 μl of 6× SDS-sample buffer and heating to 95 °C for 5 min. Equal amounts of the sample were separated in parallel by SDS-PAGE (10%) and subjected to immunoblot analysis, using the anti-Src antibody Ab-1 to quantitate the levels of c-Src and the Src pY(416) antibody to determine the activation state of Src (16–18). To prove that equal amounts of PTPα were analyzed in each sample, an appropriate volume of 2× sample buffer was added to PTPα immunocomplexes, and the amount of PTPα was estimated by immunoblotting using the PTPα-specific antibody.

To analyze the activity of PTPα after direct phosphorylation by PKC isozymes, HA-PTPα was immunoprecipitated from serum-starved unstimulated A7r5 cells. PTPα immunocomplexes were washed once with RIPA buffer (500 mM NaCl) and twice with kinase buffer and were subjected to the kinase assay with ~20 ng of recombinant PKC isozymes in the presence of 0.5 μM TPA, 1.5 μM phosphatidylinerse, and 10 μM ATP for 30 min at 30 °C. For direct analysis of the phosphatase activity on pNPP, the phosphorylated phosphatase was washed twice with PPase assay buffer and incubated for 30 min in 200 μl of PPase assay buffer containing 10 μM pNPP. PTPα immunocomplexes were sedimented by centrifugation, an appropriate volume of 2× sample buffer was added, and the amount of PTPα was again estimated by immunoblotting using the PTPα-specific antibody. The supernatant was mixed with 200 μl of 2 μM NaOH, and the absorption at 415 nm was measured.

To measure the activity of PTPα on Src after direct PKC phosphorylation, PTPα immunocomplexes were washed twice with PPase assay buffer. The immunocomplexes were resuspended in 200 μl of PPase assay buffer containing 1 μg of purified Src, and the sample was incubated at 30 °C for 1 h with agitation. The analysis of the Src activity was performed as described above. The Src kinase assays shown in Figs. 1A and 4B were done as described earlier (10).

RESULTS

Src Is Not Directly Activated by PKC—As shown previously (10), TPA stimulation of A7r5 cells leads to an increase of Src kinase activity in vivo. Src was immunoprecipitated from serum-starved A7r5 cells before and after TPA stimulation for 15 min, and the activity of Src was determined in immunoblot analysis using the pY(416)-specific antibody for Src. A robust increased immunoreactivity of Src from TPA-stimulated cells with the pY(416)-specific antibody was observed (Fig. 1A).

To ensure that the measurement of Src kinase activity with the Src pY(416)-specific antibody is a valid method to determine the activity of Src, we analyzed the Src activity in parallel directly in an in vitro kinase assay. Src was immunoprecipitated from A7r5 cells before and after TPA stimulation, and the kinase activity of Src was determined using acid-denatured enolase as an exogenous substrate. As shown in Fig. 1A, the kinase activity of Src measured on enolase correlates directly with the immunoreactivity of the Src pY(416)-specific antibody. A 2–3-fold increase of Src kinase activity could be observed after TPA stimulation.

Then we asked whether PKC isozymes could directly activate the kinase activity of Src, because Src has been described as a PKC substrate (23). To this end, Src was phosphorylated in vitro with the five TPA-responsive PKC isozymes that are present in wild-type A7r5 cells (α, β1, δ, ε, η). (24). Subsequently, autophosphorylation of Src at Tyr-416 was examined as a marker for Src activity utilizing a pY(416)-specific anti-
body (see above). As is evident from Fig. 1B, phosphorylation of Src with the different PKC isoforms did not result in any significant differences in the phosphorylation status of Tyr-416. As a control for equal activity of the PKC isoforms that were used in this assay, MBP was used as a substrate in an in vitro kinase assay. Incubation of MBP with the different PKC isoforms resulted in an equal incorporation of radioactively labeled phosphate, indicating a comparable activity of the five PKC isozymes (lower panel). Data shown here are representative of three experiments.

PKCɛ-induced Src Activation via PTPα

TPA Increases the Activity of PTPα against pNPP and Src—The main regulatory mechanism regulating Src activity is phosphorylation of Tyr-527. Dephosphorylation of this residue is essential for Src kinase activation. Hence, phosphatases represent plausible candidates for signal transmitters from PKC to Src. One such candidate is PTPα, which was reported to be positively regulated by PKC and which also is capable of activating Src (11, 12, 14). To test this scenario, we examined whether the activity of PTPα could be up-regulated in a TPA-dependent manner in A7r5 cells.

To determine whether TPA treatment enhances the phosphatase activity of PTPα, A7r5 cells were transfected with an HA-PTPα-encoding construct, and PTPα was immunoprecipitated with an HA-specific antibody from either TPA-stimulated (15 min) or unstimulated cells. A, the phosphatase activity was assayed using pNPP. A 2–3-fold increase of PTPase activity was detected in immunocomplexes that were derived from TPA-stimulated cells in comparison to unstimulated cells. The inserted upper panel shows a Western blot stained for PTPα, indicating that equal amounts of PTPα were assayed. Data shown are mean ± S.E. (n = 3). B, the phosphatase activity of PTPα was analyzed with Src as a physiological substrate, and the activation state of Src was determined using the pY(416)-specific antibody. Incubation of recombinant Src with PTPα immunoprecipitated from TPA-stimulated cells resulted in an increase of Src autophosphorylation at Tyr-416 in comparison to Src that was incubated with PTPα from unstimulated cells. As a control that equal amounts of PTPα and Src were analyzed in each sample, the amount of PTPα and Src detected by immunoblot analyses is shown. Data shown here are representative of three experiments.
PKCδ Phosphorylates PTPα in Vitro—Although our data prove that PKC activation in vivo results in activation of PTPα, we cannot exclude that another PKC-controlled kinase is responsible for the phosphorylation of PTPα. Hence we examined whether PKC directly phosphorylated the phosphatase in vitro. A fusion protein of the cytoplasmic domain of PTPα and GST (GST-PTPαcyt) was used as PKC substrate in an in vitro kinase assay. Only the PKC δ isoform that is present in wild-type A7r5 cells were tested (α, β, δ, ε, η) (24). The assay was performed with the same conditions used in Fig. 1B.

Equal amounts of GST-PTPαcyt were present in the individual assays as judged by Coomassie staining. Although MBP was phosphorylated equally by all five isozymes (see Fig. 1), GST-PTPαcyt was significantly phosphorylated only by PKCδ, as shown in Fig. 3A. Hence, the cytoplasmic domain of PTPα serves as a specific substrate for PKCδ only.

To further substantiate these observations, we examined the PKC-phosphorylated PTPα utilizing two-dimensional phosphopeptide maps. Phosphorylation of PTPα with PKCδ in vitro resulted in three major spots on the map (not shown). This resembles the data presented by Tracy et al. (11), with Ser-180 being responsible for two spots and Ser-204 for one.

In Vitro Phosphorylation of PTPα by PKCδ Increases the Phosphatase Activity Significantly—To examine whether PKCδ was able to activate the PTPα phosphatase activity, HA-tagged PTPα was isolated from serum-starved cells by immunoprecipitation. The isolated protein was phosphorylated in vitro with each of the five PKC isoforms expressed in A7r5. After incubation with PKC, the phosphatase activity was determined using pNPP as a substrate for the phosphatase. As clearly evident from Fig. 3B, only previous treatment with PKCδ, but not with any other PKC isoform, increased the phosphatase activity significantly (up to 10-fold).

Next we examined whether the PTPα phosphatase activity was also increased when Src was used as a substrate. To this end, purified Src was subjected to autophosphorylation after PTPα treatment. The activity of Src was then determined in a Western blot utilizing the pY(416)-specific antibody. As is evident from Fig. 3C, PTPα that had been treated with PKCδ resulted in a significantly higher activity compared with the basal activity. In the inserted upper panel the amount of PTPα protein in each reaction, as determined by immunoblot analysis, is indicated. Data shown are mean ± S.E. (n = 3). C, Src is a substrate for PTPα. PTPα was subjected to in vitro kinase reaction with the different PKC isoforms. Subsequently, 1 μg of purified recombinant Src was incubated with the PKC-phosphorylated PTPα. Src was immunoprecipitated from the sample, and the capacity of Src to undergo autophosphorylation was determined using the pY(416)-specific antibody. As shown, only phosphorylation of PTPα with PKCδ leads to increased Src activity. Results shown are representative of three experiments.

PKCδ-induced Src Activation via PTPα

Fig. 3. PKCδ phosphorylates and activates PTPα in vitro. A, 1 μg of a GST-PTPαcyt fusion protein was subjected to an in vitro kinase assay with the five TPA-responsive isozymes under the same conditions as in Fig. 1B, fractionated by gel electrophoresis, and stained with Coomassie prior to autoradiography. Only the incubation with PKCδ resulted in a strong incorporation of radiolabeled phosphate in PTPα, whereas the other PKC isoforms did not. Data shown here are representative of three experiments. B, A7r5 cells were transfected with an HA-PTPα-construct, and PTPα was immunoprecipitated from unstimulated serum-starved cells using the HA-specific antibody. PTPα immunocomplexes were subjected to an in vitro kinase assay with the five purified recombinant PKC isoforms tested previously (see Fig. 1B) or left untreated as a control, and the phosphatase activity was assayed using pNPP as substrate. Only the incubation with PKCδ resulted in a manifest activation of the phosphatase as indicated by the strong absorbance at 415 nm, whereas the incubation with the other PKC isoforms did not result in any increased activity compared with the basal activity. In the inserted upper panel the amount of PTPα protein in each reaction, as determined by immunoblot analysis, is indicated. Data shown are mean ± S.E. (n = 3). C, Src is a substrate for PTPα. PTPα was subjected to in vitro kinase reaction with the different PKC isoforms. Subsequently, 1 μg of purified recombinant Src was incubated with the PKC-phosphorylated PTPα. Src was immunoprecipitated from the sample, and the capacity of Src to undergo autophosphorylation was determined using the pY(416)-specific antibody. As shown, only phosphorylation of PTPα with PKCδ leads to increased Src activity. Results shown are representative of three experiments.

PKCδ-induced PTPα activity in PKCδ−/− fibroblasts after TPA stimulation. The inset immunoblot shows that equal amounts of PTPα were analyzed in both samples.

To test whether the diminished activation of PTPα in PKCδ−/− fibroblasts also resulted in a diminished activation of Src after TPA stimulation, we analyzed the activity of Src before and after TPA stimulation. Src was immunoprecipitated from PKCδ−/− and −/− before and after TPA stimulation for 15 min, and the activity of Src was analyzed by Western blot using the Src pY(416)-specific antibody (Fig. 4B, upper panel) and directly in an in vitro kinase assay using acid-denatured enolase as a substrate (lower panel). As shown in Fig. 4B, TPA stimulation of wild-type fibroblasts resulted in a roughly 3-fold Src activation as compared with the non-stimulated cells. As expected from our previous results, cells from PKCδ−/− mice
Fig. 4. Cells from PKCδ knockout mice show decreased TPA-stimulated PTPα and Src activity. Dermal fibroblasts from PKCδ knockout (+/–) or matching wild-type (+/+ or −/−) mice were serum-starved overnight and then stimulated with TPA for 15 min or left untreated, and the activity of PTPα and Src was determined. A, PTPα was immunoprecipitated from these cell lysates and analyzed for activity using pNPP as a substrate. PKCδ −/− cells showed an increase of PTPα activity after TPA stimulation, whereas the activity of PTPα from −/− cells was unaffected by the TPA treatment. The inserted upper panel shows a Western blot stained for PTPα, indicating that equal amounts of PTPα were assayed. Data shown are mean ± S.E. (n = 3). B, Src was immunoprecipitated from PKC δ−/+ and PKCδ −/− cells before and after TPA stimulation for 15 min, and the activity was determined using the Src pY(416)-specific antibody (upper panel) or in an in vitro kinase assay using acid-denatured enolase (lower panel). To demonstrate that equal amounts of Src were present, the level of Src protein was estimated by immunoblotting using the Src-specific antibody. Results shown are representative of three experiments.

Shook only a marginal increase of Src activity after TPA stimulation. These data reveal that the lack of PKCδ results in an impaired ability of the cells to activate PTPα, and thereby Src, upon TPA stimulation in vivo.

Discussion

Several laboratories, including ours, have shown previously that TPA treatment of cells leads to the activation of Src. The molecular mechanism, however, has long been elusive. Because our initial observations showed that TPA increases Src activity, we first evaluated the effect of TPA on Src. Using specific PKC inhibitors we could easily demonstrate that this effect was transmitted via PKC, while phorbol ester receptors like β-chimaerins and Ras-GRPs have been excluded from being responsible for the observed effects (Ref. 10 and data not shown).

The activation of Src has been described before in detail (14). Src activation is dependent on dephosphorylation at Tyr-527.

Subsequently, Src autophosphorylates at Tyr-416 to obtain full tyrosine kinase activity (Fig. 5). These data led to the assumption that an immediate activator of Src is a phosphatase, rather than a kinase like PKC. As we show here, phosphorylation of Src with purified PKC isozymes does not result in the activation of Src. Hence, PKC must be upstream of a phosphatase and essential for the TPA-induced Src activation.

We have shown evidence that PTPα directly activates Src kinase after previous activation of PTPα only by PKCδ but not by any other PKC isozyme tested. These experiments were performed in vitro and in vivo to substantiate our conclusions.

Fig. 5. Schematic representation of the model for the PKCδ-mediated activation of Src. Upon activation, PKCδ phosphorylates PTPα on two serine residues, thereby shifting the phosphatase in an active conformation. PTPα dephosphorylates Src at Tyr-527; the resulting conformational changes facilitate an autophosphorylation at Tyr-416. After autophosphorylation Src is in an active state.
that are expressed in A7r5 rat smooth muscle cells (24) were used. In these assays, only PKCδ was able to phosphorylate a GST fusion protein containing the entire cytoplasmic domain of PTPα (amino acids 176–802). Similar results were obtained using PTPα immunoprecipitated from A7r5 cells (data not shown).

Analysis of PTPα protein phosphorylated by PKCδ using a two-dimensional phosphotryptic map showed a similar pattern of phosphopeptides that had been shown before by Tracy et al. (11). From these data we conclude that the in vivo phosphorylation sites within PTPα that have been described before are phosphorylated by PKCδ.

We next set out to determine whether the observed phosphorylation of PTPα by PKCδ resulted in increased activity of the phosphatase. As shown in Fig. 4, PKCδ induced an increase in PTPα activity as measured using pNPP dephosphorylation. It also led to an increase in Src activity, as reflected in pY(416) levels of baculovirus-synthesized Src. Thus, while other kinases may contribute to the activation of PTPα (28), our data show that phosphorylation by PKCδ was sufficient to increase its phosphatase activity.

Utilizing cells derived from PKCδ knockout mice, we were able to further substantiate our findings. As we show here, PKCδ−/− cells showed no induction of PTPα activity after TPA stimulation. In accordance with these data, we found only a marginal induction of Src activity after TPA stimulation in these cells, indicating that the PKCδ-mediated phosphorylation of PTPα is a critical moment for the activation of Src after TPA stimulation.

However, a low but yet significant induction of Src activity can consistently be observed in PKCδ−/− cells after TPA stimulation. This finding indicates that additional, yet less prominent, mechanisms of TPA-induced Src activation exist, the nature of which remains to be identified. However, the reduced basal and induced levels of Src activity might explain why these cells have a severe defect in migration and cell cycle progression.²

Because both the colocalization of PKCδ and Src (9, 29) and the colocalization of PTPα and PKCδ (30) have been described, our data suggest a model according to which these molecules (most likely together with additional associated proteins like RACK) reside in a signaling complex, as has been described for the mitogen-activated protein kinases (reviewed in Ref. 26). According to our data, we propose a model in which the main mechanism of TPA-induced Src activation is the direct phosphorylation and activation of PTPα by PKCδ, which in turn dephosphorylates and activates Src.

Acknowledgments—We thank Helle Chlebusch and Meike Hillmann (Department of Nephrology, Medical School, Hannover, Germany) for excellent technical assistance. We thank Jeroen den Hertog (Hubrecht Laboratories, Utrecht, Netherlands) for the PTPα-specific antibody, Val Brunton (Beatson Laboratories, Glasgow, UK) for the Src baculovirus, and David Shalloway (Cornell University, Ithaca, NY) for the inducible HA-tagged PTPα constructs. We also thank Fred Mushinski for critically reading the manuscript and Annely Haase (Veterinary School, Hannover, Germany) for help preparing the manuscript.

REFERENCES

1. Goodnight, J., Mischak, H., and Mushinski, J. F. (1994) Adv. Cancer Res. 64, 159–269

2. Toker, A. (1998) Front. Biosci. 3, D1134–D1147

3. Newton, A. C. (1997) Curr. Opin. Cell Biol. 9, 161–167

4. Keenan, C., and Kelleher, D. (1998) Cell Signal. 10, 225–232

5. Thomas, S. M., and Brugge, J. S. (1997) Annu. Rev. Cell Dev. Biol. 13, 513–609

6. Bruce-Staskal, P. J., and Bouton, A. H. (2001) Exp. Cell Res. 264, 296–306

7. Schlaepfer, D. D., Jones, K. C., and Hunter, T. (1998) Mol. Cell. Biol. 18, 2571–2585

8. Xian, W., Rosenberg, M. P., and DiGiovanni, J. (1997) Oncogene 14, 1435–1444

9. Shannagam, M., Krett, N. L., Peters, C. A., Maizels, E. T., Murad, F. M., Kawakatsu, H., Rosen, S. T., and Hunzicker-Dunn, M. (1998) Oncogene 16, 1649–1654

10. Brandt, D., Gimona, M., Hillmann, M., Haller, H., and Mischak, H. (2002) J. Biol. Chem. 277, 20963–20971

11. Tracy, S., van der Geer, P., and Hunter, T. (1995) J. Biol. Chem. 270, 10587–10594

12. den Hertog, J., Sap, J., Pals, C. E., Schlessinger, J., and Kruijer, W. (1995) Cell Growth Differ. 6, 303–307

13. Harder, K. W., Moller, N. P., Peacock, J. W., and Jrk, F. R. (1998) J. Biol. Chem. 273, 31890–31900

14. Zheng, X. M., Resnick, R. J., and Shalloway, D. (2000) EMBO J. 19, 984–987

15. den Hertog, J., Pals, C. E., Peppelenbosch, M. P., Tertoolen, L. G., de Laat, S. W., and Kruijer, W. (1993) EMBO J. 12, 3789–3798

16. Fincham, V. J., Brunton, V. G., and Frame, M. C. (2000) Mol. Cell. Biol. 20, 6518–6536

17. Owens, D. W., McLean, G. W., Wyke, A. W., Parasekva, C., Parkinson, E. K., Frame, M. C., and Brunton, V. G. (2000) Mol. Cell. Biol. 20, 51–64

18. Sanna, P. P., Bertone, F., Cammalleri, M., Tallent, M. K., Siggins, G. R., Bloom, F. E., and Francesconi, W. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8653–8657

19. den Hertog, J., Tracy, S., and Hunter, T. (1994) EMBO J. 13, 3020–3032

20. Goodnight, J., Mischak, H., Kolch, W., and Mushinski, J. F. (1995) J. Biol. Chem. 270, 9991–10001

21. Kazanietz, M. G., Arcos, L. B., Bahador, A., Mischak, H., Goodnight, J., Mushinski, J. F., and Blumberg, P. M. (1993) Mol. Pharmacol. 44, 298–307

22. Leitges, M., Mayr, M., Braun, U., Mayr, U., Li, C., Pfister, G., Ghaffari-Tahrizi, N., Baser, G., Hu, Y., and Xu, Q. (2001) J. Clin. Invest. 108, 1555–1562

23. Gould, K. L., Woudgett, J. R., Cooper, J. A., Buss, J. E., Shalloway, D., and Hunter, T. (1985) Cell 42, 849–857

24. Fukumoto, S., Nishizawa, Y., Hoshi, M., Koyama, K., Ohno, S., and Morii, H. (1997) J. Biol. Chem. 272, 13816–13822

25. Zheng, X. M., Wang, Y., and Pallen, C. J. (1992) Nature 359, 336–339

26. Garrington, T. P., and Johnson, G. L. (1999) Curr. Opin. Cell Biol. 11, 211–218

27. Zheng, X. M., Resnick, R. J., and Shalloway, D. (2002) J. Biol. Chem. 277, 21922–21929

28. Brautigan, D. L., and Pinnall, F. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6696–6700

29. Chang, B. Y., Chiang, M., and Cartwright, C. A. (2001) J. Biol. Chem. 276, 20346–20356

30. Stetak, A., Cserrmely, P., Ulrich, A., and Keri, G. (2001) Biochem. Biophys. Res. Commun. 288, 564–572