Akt Kinase Phosphorylation of Inositol 1,4,5-Trisphosphate Receptors*

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A consensus RXRXX(S/T) substrate motif for Akt kinase is conserved in the C-terminal tail of all three inositol 1,4,5-trisphosphate (IP₃) receptor (IP₃R) isoforms. We have shown that IP₃R can be phosphorylated by Akt kinase in vitro and in vivo. Endogenous IP₃Rs in Chinese hamster ovary T-cells were phosphorylated in response to Akt activation by insulin. LnCAP cells, a prostate cancer cell line with constitutively active Akt kinase, also showed a constitutive phosphorylation of endogenous type I IP₃Rs. In all cases, the IP₃R phosphorylation was diminished by the addition of LY294002, an inhibitor of phosphatidylinositol 3-kinase. Mutation of IP₃R serine 2681 in the Akt substrate motif to alanine (S2681A) or glutamate (S2681E) prevented IP₃R phosphorylation in COS cells transfected with constitutively active Akt kinase. Analysis of the Ca²⁺ flux properties of these IP₃R mutants expressed in COS cell microsomes or in DT40 triple knock-out (TKO) cells did not reveal any modification of channel function. However, staurosporine-induced caspase-3 activation in DT40 TKO cells stably expressing the S2681A mutant was markedly enhanced when compared with wild-type or S2681E IP₃Rs. We conclude that IP₃ receptors are in vivo substrates for Akt kinase and that phosphorylation of the IP₃R may provide one mechanism to restrain the apoptotic effects of calcium.

Inositol 1,4,5-trisphosphate receptors (IP₃R) are a family of intracellular release channels that play an essential role in evoking Ca²⁺ signals triggered by the occupation of numerous types of cell-surface receptors that are coupled to enhanced inositol-lipid turnover (1). Three different IP₃R isoforms have been identified, and most cells appear to express those coupled to enhanced inositol-lipid turnover (1). Three different IP₃R isoforms have been identified, and most cells appear to express those coupled to enhanced inositol-lipid turnover (1). Three different IP₃R isoforms have been identified, and most cells appear to express those coupled to enhanced inositol-lipid turnover (1). Three different IP₃R isoforms have been identified, and most cells appear to express those coupled to enhanced inositol-lipid turnover (1). Three different IP₃R isoforms have been identified, and most cells appear to express those coupled to enhanced inositol-lipid turnover (1).

EXPERIMENTAL PROCEDURES

Reagents—Insulin, Triton X-100 and okadaic acid were obtained from Sigma. Pfu polymerase was from Stratagene (La Jolla, CA). TransIT-LT-1 cationic lipid transfection reagent was obtained from Pan Vera Corp. (Madison, WI). Protogel-stabilized acrylamide solution was from National Diagnostic (Atlanta, GA). Horseradish peroxidase conjugated goat anti-rabbit antibody was purchased from Amersham Biosciences. Enhanced chemiluminescent substrate was obtained from Pierce (Rockford, IL). Ca²⁺ was from PerkinElmer Life Sciences. Mouse anti-chicken IgM (clone-M4) was from Southern Biotech (Birmingham, AL).

Cells and Culture Conditions—The Chinese hamster ovary T-cell line (CHO-T), which stably expresses the human insulin receptor, was a kind gift from Dr. Bryan Wolf (Childrens Hospital University of Pennsylvania, Philadelphia, PA). This cell line was cultured in F-12 (Ham’s) medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin (complete F-12), and incubated at 37 °C in a 5% CO₂, 95% air humidified incubator. The prostate cancer cell line LnCap was obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and was passaged in RPMI 1640 medium with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. The DT40 cell line containing targeted deletions of IP₃ isoforms was a kind gift of Dr. T. Kurosaki (Kansai Medical University, Moriguchi, Japan) and was cultured as described previously (13). COS-7 cells were purchased from the ATCC and were cultured as described previously (20).

DNA Constructs and Transfection—The neuronal SII(+) SII(−) type I IP₃R construct, encoding a Kozac sequence and subcloned into pCDNA 3.1, has been described previously (20). Mutants S2681A and S2681E were made using the QuikChange site-directed mutagenesis kit (Stratagene). The forward primers were designed following the manufacturer’s recommendations as follows: 5′-AGAGCCTATGCCCCGCTGTC-3′ for the S2681A mutant and ATGAGAGCCTATGGAACTG-
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GTCAGCAGT-3’ for the S2681E mutant. (codon change is shown in bold). The reverse primer was the complementary sequence of the forward primer. PCR cycling conditions were performed according to the manufacturer’s instructions, and the products were confirmed by automated sequencing (Applied Biosystems Model 377; Nucleic Acid Facility, Thomas Jefferson University, Philadelphia, PA).

The cDNA encoding the human isoform of SERCA-2b in pcDNA 3.1 was a gift from Dr. J. Lytton (University of South Florida School of Medicine). The C-terminal 191 amino acids of the type I IP₃R were expressed as a GST fusion protein after PCR amplification and cloning into the pGEX-2K vector (Amersham Biosciences). Expression and purification of the fusion protein was carried out as described previously (21).

Antibodies—The Ab against the C terminus of the type I IP₃R (CT-1 Ab) has been described previously (22). The antibody was further affinity-purified using the peptide coupled to Ultransorb beads as described by the manufacturer (Pierce). The type III IP₃R monoclonal antibody was purchased from Signal Transduction Labs (Lexington, KY). Antibodies that recognize the phospho-(Ser/Thr)-Akt substrates (Akt-S Ab) and the phospho-Akt (Ser-473)-activated form of Akt kinase (p-Akt Ab) were obtained from Cell Signaling Technology (Beverly, MA). Recombinant Akt kinase was purchased from Biomed (King of Prussia, PA.).

Cell Lysates and Immunoprecipitation—Cells were serum-starved for 24–48 h and then treated with LY29004 and/or insulin. At the end of the treatment period, the medium was aspirated and the plates were incubated for 24–48 h and then treated with LY29004 and/or insulin. At the end of the treatment period, the medium was aspirated and the plates were treated with LY29004 and/or insulin. The samples were incubated with recombinant Akt kinase protein (Clontech, Palo Alto, CA) and then immunoprecipitated overnight with IP₃R or the indicated Abs. The Abs were recovered using protein A-Sepharose beads that were washed three times in SB buffer containing 150 mM NaCl, 50 mM Tris/HCl, pH 8.5, 1 mM dithiothreitol, and 10% glycerol.

In Vivo Phosphorylation—IP₃R protein was immunoprecipitated from cerebellum microsomal membranes after solubilization in SB buffer using procedures described previously (22). The immunoprecipitated IP₃R bound to protein A-Sepharose beads was resuspended in 50 μl of phosphorylation buffer containing 50 mM Tris/HCl, pH 7.4, 10 mM MgCl₂, 1 mM dithiothreitol, 50 mM ATP, and 5 mM okadaic acid (Buffer P). The samples were incubated with recombinant Akt kinase protein (0.22 μg) and [γ-³²P]ATP (20 μCi) for 2 h at 30 °C. The beads were washed five times with buffer P and then quenched in SDS-PAGE sample buffer (50 mM Tris/HCl, pH 6.8, 1% SDS, 0.01% bromphenol blue, 140 mM 2-mercaptoethanol and 5% (v/v) glycerol). The samples were run on a 5% gel, and proteins were transferred to a nitrocellulose filter. The filter was autoradiographed before immunoblotting sequentially with Akt-S Ab and then with CT-1 Ab. GST C-terminal tail protein was used as a marker to locate the transfected cells.

RESULTS

IP₃R Phosphorylation by Akt Kinase in Vitro—The identification of Akt kinase substrates has been facilitated by the presence of a robust phosphorylation motif RXRXX(S/T) (19). A single motif of this kind is conserved within the C-terminal tail of all three IP₃R isoforms and is also conserved in IP₃R cloned from several different species, with the exception of Caenorhabditis elegans (Fig. 1A). To observe the phosphorylation directly, we incubated a GST fusion protein encoding the C-terminal tail of type I IP₃R with catalytically active Akt kinase and [³²P]ATP. The fusion protein was then isolated with glutathione-agarose and analyzed by autoradiography after SDS-PAGE (Fig. 1B). The data show that the C-terminal tail fusion protein was phosphorylated by Akt kinase. Phosphorylation could also be detected by immunoblotting with a phosphospecific Ab that detects the consensus Akt phosphorylation sequence (see Akt-S blot in Fig. 1B). Similar experiments were carried out with the full-length type I IP₃R immunoprecipitated from cerebellum lysates (Fig. 1C). In this case, a low level of [³²P] incorporation was observed even without the addition of Akt kinase, possibly reflecting the co-immunoprecipitation of endogenous kinases. The addition of Akt kinase greatly enhanced [³²P] labeling of the IP₃R. Specific phosphorylation at the Akt site was evident when the phosphorylation was detected by immunoblotting with the Akt-S Ab (Fig. 1C).

IP₃R Phosphorylation by Akt Kinase in Vivo—Occupation of insulin receptors leads to PI 3-kinase activation and the stimulation of Akt kinase activity (16). To examine IP₃R phosphorylation in an intact cell,
we used CHO-T cells, which overexpress human insulin receptors (23). Cell lysates were prepared in a buffer that contained a mixture of NaF, vanadate, pyrophosphate and okadaic acid to preserve the phosphorylation state of assayed proteins. Fig. 2A shows that the addition of 100 nM insulin promotes IP$_3$R phosphorylation as detected by immunoblotting with Akt-S Ab in lysates immunoprecipitated with affinity-purified IP$_3$R-1-specific Ab. In multiple experiments, the phosphorylation was evident within 5–10 min and peaked at 30–60 min. The maximal increase was 4.6 ± 0.9-fold in four separate experiments. The profile of IP$_3$R changes approximately correlated with the time course of Akt kinase activation as estimated from immunoblotting the lysates with a phosphoserine 473 of Akt kinase. Insulin-mediated IP$_3$R phosphorylation could also be demonstrated when the lysates were immunoprecipitated with Akt-S Ab and then immunoblotted with IP$_3$R-1-specific Ab (Fig. 2B). Because insulin activation of Akt kinase is secondary to PI 3-kinase stimulation, it would be anticipated that the effect of insulin on the IP$_3$R would be sensitive to inhibition by the PI 3-kinase inhibitor LY294002. Fig. 3 shows that this is indeed the case. A small amount of IP$_3$R phosphorylation can be detected in the absence of stimulation and could be due to the presence of a basal activity of Akt kinase. The data in Fig. 3 also show that the endogenous type III IP$_3$R in CHO-T cells was also phosphorylated on a Akt consensus site in response to insulin stimulation.

Loss of the tumor promoter PTEN, a negative regulator of the PI 3-kinase pathway, leads to constitutive activation of Akt kinase in several types of cancer (24). We have used LnCaP cells, a prostate carcinoma cell line devoid of functional PTEN (25), to examine the phosphorylation state of IP$_3$Rs. Fig. 3B shows that the constitutive activity of the Akt kinase in these cells is accompanied by the constitutive phosphorylation of the type I IP$_3$R as detected by Akt-S Ab. This basal phosphorylation was abolished by pretreatment with LY294002 (Fig. 3B, lane 2).

Effect of Akt Kinase Phosphorylation on IP$_3$R Channel Function—COS-7 cells have very low levels of endogenous type I IP$_3$R (20). In Fig. 4A, we have transfected the type I IP$_3$R into COS-7 cells either alone or together with a constitutively active Akt kinase (myr-Akt) (26). Lysates were immunoprecipitated with type I IP$_3$R Ab and blotted with Akt-S Ab. The only condition under which substantial phosphorylation was observed was when both type I IP$_3$R and myr-Akt were transfected together (Fig. 4A, upper panel, lane 3). Immunoblots with IP$_3$R Ab and Ser-473 phospho-Akt Ab indicated the presence of IP$_3$R and active Akt in the expected lanes.
To study the functional consequences of Akt kinase phosphorylation, we produced two mutant type I IP₃Rs in which the Akt kinase serine substrate at position 2681 was mutated to either alanine or glutamate. The latter mutation was designed to mimic the effect of Akt kinase phosphorylation. The ability of the wild-type and mutant IP₃Rs to be phosphorylated in COS-7 cells transfected with constitutively active myr-Akt is shown in Fig. 4B. Cell lysates were immunoprecipitated with type I IP₃R Ab and probed for phosphorylation at the Akt site with Akt-S Ab. As expected, there was a marked suppression of the phosphorylation signal when the Ser-2681 site was mutated to either alanine or glutamate. The small residual signal seen with the S2681E mutant may be related to the limited recognition by the Akt-S phosphopeptide Ab of the sequence context around the phosphorylation site when a glutamate residue replaces a phosphoserine residue. WT, wild-type.

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FIGURE 5. Calcium responses of wild-type, S2681A and S2681E IP$_3$R mutants. A, microsomes from COS cells transfected with the indicated IP$_3$R constructs and co-transfected with SERCA2b cDNA were prepared and used for a $^{45}$Ca$^{2+}$/H$_{11001}$ flux assay as described under “Experimental Procedures.” The amount of $^{45}$Ca$^{2+}$ accumulated in oxalate-loaded microsomes over a 15-min period in the presence of 10 μM IP$_3$ was measured and is expressed as a percentage of the control accumulation in the absence of IP$_3$. The experiment was done in EGTA/HEDTA Ca$^{2+}$/H$_{11001}$ buffers at free [Ca$^{2+}$] of 0.2 and 4.5 μM calibrated with a Ca$^{2+}$/H$_{11001}$-sensitive microelectrode. The data shown is the mean ± S.E. of five independent experiments. B, the IP$_3$ dose-response relationship was measured at a free [Ca$^{2+}$] of 0.2 μM. The data are the mean ± S.E. of three independent experiments. WT, wild-type. C, single cell Fura-2 imaging was carried out on DT-40 cells transfected with the indicated IP$_3$R constructs and the M3 muscarinic receptor. The procedures for sequential stimulation by increasing doses of carbachol and analysis of the data were carried out as described previously (30).
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FIGURE 6. Activation of caspase-3 in stable DT40 cell lines expressing the S2681A and S2681E mutants. A, lysates from stable cell lines expressing the S2681A and S2681E mutants were compared with the type I IP₃R levels expressed in DT40 double knock-out (DKO) cells that express only the type I IP₃R. 100 μg of lysate protein was loaded on the gels and immunoblotting was done with type I IP₃R Ab. B, the indicated cell lines were treated with 1 μM staurosporine (STS) for 3 and 6 h, and then aliquots of cells were centrifuged and lysates were assayed for caspase-3 activity as described under “Experimental Procedures.” C, COS-7 cells were transfected at ~70% confluence with wild-type, S2681A, and S2681E IP₃R constructs. At 48 h post-transfection, the cells were treated with staurosporine and assayed for caspase-3 as described for DT40 cells. The data are the mean ± S.E. of four experiments. WT, wild-type; S–A, S2681A; S–E, S2681E.

The results do not correlate with the absolute amounts of IP₃R, respectively (Fig. 4B). Thus, the differences in caspase-3 activation in COS-7 cells are unlikely to be related to differences in IP₃R expression.

DISCUSSION

Akt kinase has established roles in suppressing apoptosis, promoting growth and proliferation, and mediating many of the effects of insulin (reviewed in Refs. 16–18). The main conclusion of the present study is that IP₃R can be added to the list of potential targets of this key enzyme. We have demonstrated that this phosphorylation occurs in vitro and in vivo at a consensus Akt kinase site located in the cytosol-exposed C-terminal tail of the receptor. The generation of 3-phosphorylated phosphoinositides by PI 3-kinase is thought to initiate the activation of Akt as a result of recruitment of the enzyme to the plasma membrane. This translocation utilizes the pleckstrin homology domain in the N-terminal region of Akt. Subsequent phosphorylation of serine 473 and threonine 308 leads to complete activation of the enzyme, which can then migrate to the cytosol, nucleus, mitochondria, or Golgi apparatus to phosphorylate its various substrates. Although the exact amount of IP₃R phosphorylated in vivo has not been quantitated in the present study, we can roughly estimate this from immunoprecipitation of the phosphorylated IP₃R with Akt-S Ab. The results indicate that only a small fraction (<5%) of the total IP₃R pool is phosphorylated in COS-7 cells transfected with IP₃R and myr-Akt (data not shown). We were also unable to detect co-immunoprecipitation of endogenous or overexpressed IP₃Rs with myr-Akt (data not shown). The bulk of IP₃Rs are in the ER membrane, although smaller pools have been reported in the plasma membrane, nucleus, and the Golgi apparatus (32). There have been very few studies of Akt kinase localization in the ER (33). As far as we are aware, protein tyrosine phosphatase 1B is the sole ER substrate of Akt kinase reported in the literature (34). The finding that growth factors can increase ER PtdInsP₃ levels (35) raises the possibility that Akt kinase may be activated directly on ER membranes. Thus phosphorylation of the IP₃R could be confined to a specific subcellular pool that contains activated Akt kinase (e.g. plasma membrane, nucleus, or Golgi apparatus) or could reflect a small pool of activated Akt kinase in ER membranes. Interestingly, a constitutively active Akt kinase construct (S437D/T308D), which is active without membrane targeting (36), does not phosphorylate the IP₃R (data not shown). Further work is required to clarify the exact relationship between Akt kinase and IP₃R localization.

A second key conclusion from the present study is that phosphorylation of the IP₃R does not modify IP₃-s-mediated Ca²⁺ channel function. This conclusion was based on experiments utilizing IP₃Rs that were mutated at the 2681 position to either alanine or glutamate. Neither of the mutants showed significantly different behavior from wild-type receptors in either COS-7 cells or after transient transfection into IP₃R TKO DT40 cells. This conclusion is in agreement with the finding that the dominant negative form of Akt kinase or LY294002 does not affect bradykinin-mediated intracellular Ca²⁺ release in porcine endothelial cells (37). LY294002, which suppressed the Akt kinase activation mediated by carbachol in CHO cells or IgM in DT40 cells, also had no effect on the ability of these agonists to mobilize intracellular Ca²⁺ as measured with Fura-2 (data not shown).

What then is the functional role of IP₃R phosphorylation by Akt kinase? Studies in DT40 and Jurkat cells have established that genetic ablation of IP₃R isoforms induces resistance to apoptotic stimuli (9, 29). In bladder cancer cell lines, the small interfering RNA-induced suppression of type I IP₃R prevented apoptosis mediated by cisplatin (39). It has been generally assumed that these findings reflect a requirement for intracellular Ca²⁺ release for one or more steps in the apoptotic cascade.
A component of this cascade that shows such a Ca$^{2+}$ requirement is the activation of caspase-3. Thus caspase-9 and -3 are not activated by various stimuli in Jurkat cells deficient in type I IP$_3$R (40). Assefa et al. (31) have shown that restoration of type I IP$_3$R into DT40 TKO cells restores a normal caspase-3 activation and apoptotic response to stimulation by staurosporine or IgM. Interestingly, they also showed that the same effect could be observed with an IP$_3$R mutant that was functionally inactive as an IP$_3$-sensitive channel as a result of the deletion of the N-terminal 225 amino acids (31). Type-I IP$_3$Rs have been shown to be caspase-3 substrates with cleavage generating a 95-kDa C-terminal membrane fragment (41, 42). This “channel-only” fragment has been reported to be constitutively open and to induce leakage of Ca$^{2+}$ from the ER when transiently expressed in COS cells (43). Stable expression of the 95-kDa fragment in DT40 TKO cells was also able to support caspase-3 activation and apoptosis, whereas a mutant IP$_3$R that could not be cleaved by caspase-3 was inactive in these assays (31). These studies emphasize that the role of the type I IP$_3$R in apoptosis may be independent of its function in IP$_3$-mediated Ca$^{2+}$ release.

The results in Fig. 6 are in agreement with the findings of others that IP$_3$Rs play a role in sustaining caspase-3 activation (31, 40). We find that the non-phosphorylatable S2681A mutant is more effective in this regard than wild-type IP$_3$Rs or the S2681E mutant. These data show that one functional phosphorylation site and regulate channel function (38, 47). However, IP$_3$R sensitization by Ca$^{2+}$ from the ER to the mitochondria in several models of apoptosis (47, 48). The caspase-cleaved IP$_3$R, rather than the full-length form, seems particularly important for sustaining apoptosis in DT40 cells (31). Our findings raise the possibility that Akt phosphorylation of the IP$_3$R may suppress Ca$^{2+}$ leakage through the channel-only 95-kDa caspase-3 cleavage fragment or inhibit in some other manner the IP$_3$R-mediated transfer of Ca$^{2+}$ from the ER to the mitochondria.

Other possible IP$_3$-independent functional effects of IP$_3$R phosphorylation can be envisaged. More than 25 proteins have been found to interact with IP$_3$Rs, leading to the suggestion that the receptor may function in ER membranes to assemble and integrate signaling systems (49). Cytochrome $c$ and Bcl-2 both bind to the IP$_3$RC-terminal tail containing the Akt phosphorylation site and regulate channel function (38, 47). However, IP$_3$R interaction with cytochrome $c$ (data not shown) or Bcl-2 $\beta$ was not modified by Akt phosphorylation. Altered interactions of the phosphorylated IP$_3$R with other target proteins cannot presently be excluded.

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REFERENCES

1. Berridge, M. J. (1993) Nature 361, 315–325
2. Patel, S., Joseph, S. K., and Thomas, A. P. (1999) Cell Calcium 25, 247–264
3. Wojcikiewicz, R. J. H. (1995) J. Biol. Chem. 270, 11678–11683
4. Supattapone, S., Danoff, S. K., Theibert, A., Joseph, S. K., Steiner, J., and Snyder, S. H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8747–8750
5. Joseph, S. K., and Ryan, S. V. (1993) J. Biol. Chem. 268, 23059–23065
6. Komalavilas, P., and Lincoln, T. (1994) J. Biol. Chem. 269, 8701–8707
7. Schlossmann, J., Ammendola, A., Ashman, K., Zong, X., Huber, A., Neubauer, G., Wang, G. X., Allescher, H. D., Korth, M., Wilm, M., Hofmann, F., and Ruth, P. (2000) Nature 404, 197–201
8. Ferris, C. D., Huganir, R. L., Bredt, D. S., Cameron, A. M., and Snyder, S. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2232–2235
9. Jayaraman, T., and Marks, A. R. (1997) Mol. Cell. Biol. 17, 3005–3012
10. Cui, J., Matkovich, S. J., DeSouza, N., Li, S., Rosenblit, N., and Marks, A. R. (2004) J. Biol. Chem. 279, 16311–16316
11. Malathi, K., Koyzahana, S., Ho, M., Soghoian, D., Li, X., Silane, M., Berenstein, A., and Jayaraman, T. (2003) J. Cell. Biochem. 90, 1186–1196
12. Ferris, C. D., Cameron, A. M., Bredt, D. S., Huganir, R. L., and Snyder, S. H. (1991) Biochem. Biophys. Res. Commun. 175, 192–198
13. Wagner, L. E., Li, W. H., and Yule, D. I. (2003) J. Biol. Chem. 278, 45811–45817
14. Bolte, S., M. Alzayady, K., Xu, Q., and Wocjkiewicz, R. J. (2004) FEBS Lett. 557, 181–184
15. Rooney, T. A., Joseph, S. K., Queen, C., and Thomas, A. P. (1996) J. Biol. Chem. 271, 19817–19825
16. Whitman, E. L., Cho, H., and Birnbaum, M. I. (2002) Trends Endocrinol. Metab 12, 444–451
17. Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A., and Sugimoto, C. (2003) Oncogene 22, 8983–8998
18. Brazil, D. P., Yang, Z. Z., and Hemmings, B. A. (2004) Trends Biochem. Sci. 29, 233–242
19. Lawlor, M. A., and Alessi, D. R. (2001) J. Cell Sci. 114, 2903–2910
20. Boehning, D., and Joseph, S. K. (2000) J. Biol. Chem. 275, 21492–21499
21. Boehning, D., and Joseph, S. K. (2000) EMBO J. 19, 5450–5459
22. Joseph, S., and Samanta, S. (1993) J. Biol. Chem. 268, 6477–6486
23. Endemann, G., Yonezawa, K., and Roth, R. A. (1990) J. Biol. Chem. 265, 396–400
24. Cantley, L. C., and Neel, B. G. (1999) Nat. Rev. Mol. Cell Biol. 2, 437–465
25. Tsunoda, T., Koga, H., Yokomizo, A., Tatsugami, K., Eto, M., Inokuchi, J., Hirata, A., Michikawa, T., Inoue, T., and Mikoshiba, K. (2004) Science's STKE 2004 re1
26. Wang, G. X., Allescher, H. D., Korth, M., Wilm, M., Hofmann, F., and Ruth, P. (2000) Nature 404, 197–201
27. Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A., and Sugimoto, C. (2003) Oncogene 22, 8983–8998
28. Brazil, D. P., Yang, Z. Z., and Hemmings, B. A. (2004) Trends Biochem. Sci. 29, 233–242
29. Lawlor, M. A., and Alessi, D. R. (2001) J. Cell Sci. 114, 2903–2910
30. Boehning, D., and Joseph, S. K. (2000) J. Biol. Chem. 275, 21492–21499
31. Lawlor, M. A., and Alessi, D. R. (2001) J. Cell Sci. 114, 2903–2910
32. Vermassen, E., Parys, J. B., and Mauger, J. P. (2004) Biochim. Biophys. Acta 1661, 19817–19825
33. Endemann, G., Yonezawa, K., and Roth, R. A. (1990) J. Biol. Chem. 265, 396–400
34. Cantley, L. C., and Neel, B. G. (1999) Nat. Rev. Mol. Cell Biol. 2, 437–465
35. Tsunoda, T., Koga, H., Yokomizo, A., Tatsugami, K., Eto, M., Inokuchi, J., Hirata, A., Michikawa, T., Inoue, T., and Mikoshiba, K. (2004) Science's STKE 2004 re1
36. Wang, G. X., Allescher, H. D., Korth, M., Wilm, M., Hofmann, F., and Ruth, P. (2000) Nature 404, 197–201
37. Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A., and Sugimoto, C. (2003) Oncogene 22, 8983–8998