The APS adapter protein is recruited to the autophosphorylated kinase domain of the insulin receptor and initiates the phosphatidylinositol 3-kinase (PI3K)-independent pathway of insulin-stimulated glucose transport by recruiting CAP and c-Cbl. In this study, we have identified APS as a novel substrate for protein kinase B/Akt using an antibody that exhibits insulin-dependent immunoreactivity with a phosphospecific antibody raised against the protein kinase B substrate consensus sequence RXRXP(pS/pT) and a phosphospecific antibody that recognizes serine 21/9 of glycerol synthase 3cα/β. This phosphorylation of APS is observed in both 3T3-L1 adipocytes and transfected cells. The insulin-stimulated serine phosphorylation of APS was inhibited by a PI3-kinase inhibitor, LY290004, a specific protein kinase B (PKB) inhibitor, deguelin, and knockdown of Akt. Serine 588 of APS is contained in a protein kinase B consensus sequence for phosphorylation conserved in APS across multiple species but not found in other members of this family, including SH2-B and Lnk. Mutation of serine 588 to alanine abolished the insulin-stimulated serine phosphorylation of APS and prevented the localization of APS to membrane ruffles. A glutathione S-transferase fusion protein containing amino acids 534–621 of APS was phosphorylated by purified PKB in vitro, and mutation of serine 588 abolished the PKB-mediated phosphorylation of APS in vitro. Taken together, this study identifies APS as a novel physiological substrate for PKB and the first serine phosphorylation site on APS. These data therefore reveal the molecular cross-talk between the insulin-activated PI3-kinase-dependent and -independent pathways previously thought to be distinct and divergent.

The phosphatidylinositol 3 (PI3)2 kinase pathway is activated by a number of ligands in a variety of cellular contexts and plays a key role in a number of different cellular processes (1). In the context of insulin-stimulated glucose transport, PI3-kinase is known to be critical but not sufficient to facilitate this pathway, suggesting that other PI3-kinase independent inputs are required for the full activation of insulin-stimulated glucose transport (2). There are thought to be two pathways required for insulin-stimulated glucose transport: an IRS-PI3-kinase pathway and a PI3-kinase-independent pathway involving APS, CAP, and c-Cbl (2). These two pathways diverge at the level of the insulin receptor. The PI3-kinase-independent pathway is initiated by the binding of APS to the phosphorylated activation loop of the insulin receptor (3, 4). APS is constitutively bound to CAP and undergoes tyrosine phosphorylation on Tyr-618, allowing it to bind to the variant SH2 domain of c-Cbl (3–5). This is followed by the tyrosine phosphorylation of c-Cbl on tyrosines 700 and 774, resulting in the phosphorylated c-Cbl binding to the SH2 domain of Crk (4, 6). Following the insulin-stimulated phosphorylation of c-Cbl, the CAP/Cbl complex migrates to the caveolin-rich lipid rafts, a movement facilitated by the interaction of the CAP SOHO domain with flotillin, a protein in lipid rafts (7). This allows the Crk/C3G complex to be recruited to this microdomain, where C3G activates the small G protein TC10 (8–11). The activation of TC10 occurs independently of PI3-kinase and is crucial for insulin-stimulated Glut4 translocation (2).

An important downstream effector of the IRS-PI3-kinase pathway is the protein kinase B family (PKB, also known as Akt) (1). PKB exists in several isoforms (α, β, γ); the importance of the PKBβ (Akt2) isoform in insulin-stimulated glucose transport has been demonstrated in a number of studies using knockdown and knock-out studies (12). Insulin promotes glucose transport into target tissues, causing the exocytosis of the glucose transporter GLUT4 from intracellular vesicles to the plasma membrane (12, 13). Akt/PKB is now strongly implicated as a key kinase in the insulin-promoted storage of nutrients in muscle and adipose tissue (12, 13). However, PKB has been implicated in diverse processes ranging from carbohydrate metabolism and cell growth to anti-apoptosis and angiogenesis (1). The activation of PKB and the subsequent stimulation of glucose transport appear to be unique to insulin, even though a number of other ligands and receptors activate PKB (12, 13). In this study we have identified the APS adapter protein as a novel substrate for PKB, thus indicating the co-dependence of the PI3-kinase-independent pathway on PI3-kinase activation.

**EXPERIMENTAL PROCEDURES**

**Materials**—All biochemicals were purchased from Sigma except where stated. Purified, activated PKBa/Akt1 was purchased from Upstate Biotechnology. The CHO.T-APS and CHO.T-APS-CAP cell lines were as previously described. 3T3-L1 adipocytes were cultured as previously described (3, 14). Anti-Myc agarose was purchased from Cell Signaling Technology. Immunoblotting was performed as previously described (14).

**Site-directed Mutagenesis**—Serine 588 of the rat APS cDNA was mutated to alanine using recombinant circle PCR with the following oligonucleotides (rat APS sequence): sense, 5′-tgcgagcagatctacggc-cacagacagt-3′, and antisense, 5′-caggtctgtggcaggtatcact-ccgccgca-3′, to introduce an alanine in place of serine 588 and a unique

---

*This work was supported by grants from the Wellcome Trust and Diabetes UK.

1 A Wellcome Senior Fellow in Clinical Science. To whom correspondence should be addressed. Tel.: 44-115-970-9488; Fax: 44-115-919-4493; E-mail: tpillay@nottingham.ac.uk.

2 The abbreviations used are: PI3, phosphatidylinositol 3-kinase; SH, Src homology; APS, adapter protein with a pleckstrin homology domain and an SH2 domain; CAP, c-Cbl-associated protein (Ponsin); CHO, Chinese hamster ovary; GST, glutathione S-transferase; PKB, protein kinase B; siRNA, small inhibitory RNA; GSK, glycogen synthase kinase; IRS, insulin receptor substrate.
BglII site. The integrity of the construct was verified by automated dideoxy sequencing. The mutant insert as a SmaI-NotI fragment was cloned into the vector prk5 containing the wild type rat APS cDNA, and both wild type and mutant cDNAs were transiently transfected into CHO.T-Hygro cells (15). The cells were serum starved for 12 h prior to experimental manipulation. Plasmid DNA was transfected into Chinese hamster ovary (CHO) cells using FuGENE as previously described (14).

**Stable Expression of S588A—Mutant APS was subcloned into the plasmid pIRES Hygro and transfected into CHO.T cells. Hygromycin-resistance clones were isolated by limiting dilution as previously described (14).**

**GST Fusion Proteins—Glutathione S-transferase (GST) fusion proteins expressing the C terminus of rat APS were expressed and purified as previously described (14).**

**Immunofluorescence Studies—CHO.T-APS cells were cultured on 19-mm coverslips and after appropriate culture conditions were washed twice with Tris-buffered saline (TBS). Immediately 4% paraformaldehyde was added for 20 min of incubation at room temperature. The paraformaldehyde was aspirated, TBS was added for 10 min, and the cells were permeabilized using 0.2% Triton X-100, phosphate-buffered saline for 10 min at room temperature. Following a brief wash with TBS, the samples were blocked with 3% bovine serum albumin, TBS (blocking buffer) at room temperature for 15 min. After blocking, goat anti-APS (C-20; Santa Cruz Biotechnology) and rabbit anti-PKB substrate (9611; Cell Signaling Technology) primary antibodies were added (1:200 in blocking buffer) for 1 h at room temperature. After the primary antibody incubation, the samples were washed three times over 5 min with TBS at room temperature, and anti-goat and anti-rabbit Alexa-Fluor secondary antibodies (1:200) were added in blocking buffer for 1 h at room temperature. After the secondary antibody incubation, the cells were washed three times over 5 min with TBS at room temperature and then mounted onto glass slides using 50% glycerol. The samples were stored at −20 °C and processed for viewing using an inverted Zeiss axiophot microscope attached to a laser illumination unit as previously described (14).

**Phosphorylation of APS by PKB in Vitro—Soluble wt GST-APS (amino acids 534–621) and S588A GST-APS were purified from bacteria as previously described, eluted from the agarose beads, and resuspended in 50% glycerol prior to storage at −20 °C (16). The phosphorylation conditions are based on that described by Berwick et al. (17). For the kinase reaction, 3 μL of GST fusion protein, either wild type or S588A mutant fusion protein, were incubated with kinase buffer (20 mM Hepes, pH 7.5, 20 mM β-glycerophosphate, 1 mM EDTA, 5 mM MgCl2) in the presence of 100 μM ATP to a final volume of 20 μL. The samples were incubated at 30 °C for 40 min, and the kinase assay was terminated.
Western Blotting—The reactions were analyzed by electrophoresis using 10% NuPage Novex gels (Invitrogen), transferred onto 2-μm Invitrogen polyvinylidene difluoride filters, and Western blotted with anti-PKB substrate and phospho-GSK3 according to the manufacturer’s instructions. In all instances, immunoreactive bands were detected with the use of horseradish peroxidase-coupled anti-rabbit or anti-goat antibodies as appropriate and enhanced chemiluminescence (Supersignal; Pierce Chemical Company).

siRNA-mediated Knockdown of Akt—The Signalsilence Akt siRNA kit was purchased from Cell Signaling Technology and used to knock down Akt1 and Akt2 in CHO cells. Cells were transfected with 50 nM Akt siRNA or control siRNA 48 h prior to cell lysis. The cells were stimulated with insulin, and APS was immunoprecipitated with anti-Myc 9E10 as described above and blotted with anti-PKB substrate antibody.

RESULTS

Insulin Stimulates APS Phosphorylation on a Site Recognized by the Anti-PKB substrate Antibody in 3T3-L1 Adipocytes and CHO Cells—Initially, we determined whether there was evidence for PKB-mediated phosphorylation of APS in differentiated 3T3-L1 adipocytes (Fig. 1A), a widely used model for studies of insulin action and glucose transport. Cells were cultured under standard conditions and then serum starved and stimulated with insulin for 30 min. The lysates were incubated with immobilized anti-PKB substrate to capture PKB substrates. The immunoprecipitates were then analyzed with anti-APS antibody. The results demonstrate that APS in 3T3-L1 adipocytes is captured by the immobilized anti-PKB substrate, indicating that APS is a physiological substrate for PKB in native untransfected cells such as 3T3-L1 adipocytes (Fig. 1A). Because the interaction of the anti-PKB substrate may be indirect, we determined whether the anti-PKB substrate antibody interacted directly with APS. APS was immunoprecipitated from 3T3-L1 adipocytes stimulated with insulin, and the immunoprecipitates were probed with anti-PKB substrate antibody (Fig. 1B). APS was detected with the anti-PKB substrate antibody following insulin stimulation but not when cells were preincubated with the PI3-kinase inhibitor LY294002.

To analyze this phosphorylation further, we used transfected cells overexpressing insulin receptor and Myc epitope-tagged APS (3, 5) (Fig. 1C). In CHO.T-APS cells, insulin stimulates the phosphorylation of APS on a PKB consensus site. Cells were stimulated with insulin or fetal calf serum, and Myc epitope-tagged APS was precipitated with anti-Myc

FIGURE 2. Insulin stimulates phosphorylation of APS on a site recognized by anti-phospho-GSK3 antibody in wild type APS but not mutant S588A APS. CHO.T cells stably expressing mutant S588A APS or wild type APS were stimulated with insulin. Myc-tagged APS was immunoprecipitated and immunoblotted with anti-phospho-GSK3 as described under “Experimental Procedures.” The immunoprecipitates were also probed with anti-Myc antibody (lower panel). The experiments illustrated represent an example of three independent experiments.

FIGURE 3. Inhibition of PI3-kinase-dependent pathways by chemical inhibitors or RNA interference abolishes the phosphorylation of APS on a PKB consensus site. Inhibitor compounds LY294002 and deguelin were used to inhibit PI3-kinase (A) and PKB (B), respectively. Myc-tagged APS was then immunoprecipitated from the cells and blotted with anti-PKB substrate antibody. The blots were stripped and reprobed with anti-Myc antibody. The middle section in panel B shows a slightly longer exposure. C, CHO.T-APS cells were transfected with siRNA specific for Akt1 and Akt2 or control siRNA and then stimulated with insulin for 5 min. The cells were lysed, and APS was immunoprecipitated from the cells as described above and blotted with anti-PKB substrate. The experiments illustrated were performed three times and are representative.
Phosphorylation of APS by Protein Kinase B

**FIGURE 4. Mutation of serine S588 of APS to alanine abolishes the insulin-stimulated phosphorylation of APS on a PKB consensus phosphorylation site.** Serine 588 of APS contained in an RXRXXS motif was mutated to alanine by site-directed mutagenesis. Wild type and mutant APS constructs were transiently transfected into CHO.T cells that were starved and then stimulated with insulin. Myc-tagged APS was then immunoprecipitated and blotted with anti-PKBsubstrate antibody. Both LY294002 (Fig. 4A) and deguelin, a PKB inhibitor (18, 19), inhibited the insulin-stimulated phosphorylation of APS. Cells were treated with LY294002, an inhibitor of PKB, and then Myc-tagged APS was immunoprecipitated and blotted with anti-PKBsubstrate. Both LY294002 and deguelin inhibited the insulin-stimulated phosphorylation of APS to basal levels.

**FIGURE 5. PKB phosphorylates wild type APS but not mutant S588A APS in vitro.** The C terminus of APS (amino acids 534–621) was expressed as a GST fusion protein, and the purified protein was incubated with constitutively activated PKB (ΔPH) in vitro in the presence of ATP as indicated with wild type (wt) or mutant (S588A) APS. The reactions were analyzed by immunoblotting with anti-PKBsubstrate. The blots were stripped and reprobed with goat anti-APS and anti-PKB. A representative result of three different experiments is shown.

antibody and analyzed by blotting with anti-PKBsubstrate. As shown in the figure, the anti-PKBsubstrate antibody detected APS in the Myc immunoprecipitates, indicating that the results obtained in 3T3-L1 adipocytes were due to phosphorylation of endogenous APS on a PKB consensus site.

The insulin-stimulated phosphorylation of APS on a PKB consensus site was found to be dose dependent (Fig. 1D). Stimulation of cells with varying concentrations of insulin demonstrated that insulin-stimulated phosphorylation of APS was detectable at 10 ng/ml insulin. We also used phosphospecific antibodies to phosphorylated glycogen synthase kinase (GSK) 3 to determine whether the site phosphorylated in response to insulin cross-reacted (Fig. 2). The phospho-GSK3α/β (Ser-21/9) antibody recognizes GSK3α or β when these are phosphorylated by PKB at serines 21 and 9, respectively. These are contained in a PKB consensus site. Cells expressing wild type APS or mutant APS containing the S533A mutation were starved and stimulated with insulin. Myc-tagged APS was then immunoprecipitated and blotted with anti-phospho-GSK3. The results show that following insulin stimulation, the antibody detects serine phosphorylation of APS, possibly by reacting with a phosphorylated consensus sequence, but not when serine 588 is mutated to alanine.

**Effects of Inhibitors on Insulin-stimulated Serine Phosphorylation of APS**—We next wanted to confirm that the phosphorylation of APS was dependent on P13-kinase-dependent pathways and/or PKB-dependent pathways using chemical inhibitors (Fig. 3). Both LY294002 (Fig. 3A) and deguelin (Fig. 3B), a PKB inhibitor (18, 19), inhibited the insulin-stimulated phosphorylation of APS. Cells were treated with LY294002, an inhibitor of PKB, or deguelin, and then Myc-tagged APS was immunoprecipitated and blotted with anti-PKBsubstrate. Both LY294002 and deguelin inhibited the insulin-stimulated phosphorylation of APS to basal levels.

siRNA-mediated Knockdown of Akt1 and Akt2 Abolishes the Insulin-stimulated Phosphorylation of APS on a PKB Consensus Site—We used commercially available siRNA targeting Akt1 and 2 to confirm the role of Akt in mediating this phosphorylation event in intact cells (Fig. 3C). CHO cells were transfected with siRNA, and the cells were stimulated with insulin. Akt expression as detected by immunoblotting was decreased by 80%, and this abolished insulin-stimulated phosphorylation of APS and activation of GSK3.

Identification of Serine S588 as a Phosphorylation Site for PKB—Scanning of the APS sequence revealed that serine 588 of APS is contained in a consensus sequence that would be recognized by the anti-PKBsubstrate antibody and would be regarded as a consensus sequence for PKB. Serine 588 is contained in the sequence SRSRSX588, which conforms to the PKB consensus site RXRXXS (20). Serine 588 was mutated to alanine, and the mutant construct was transiently transfected into CHO.T cells along with the wild type construct. As seen in Fig. 4A, mutation of Ser-588 abolished the insulin-stimulated phosphorylation of APS as detected by the anti-PKBsubstrate antibody. In a separate experiment, we also analyzed the tyrosine phosphorylation of APS to demonstrate that the overall function of APS as a substrate was preserved. Mutation of serine 588 to alanine abolished the insulin-stimulated serine phosphorylation of APS but did not affect tyrosine phosphorylation of APS.

Phosphorylation of Wild Type APS but Not Mutant S588A APS by Purified PKB—We next tested whether PKB would phosphorylate APS directly in vitro to exclude the possibility that an intermediate kinase...
may be mediating this phosphorylation (Fig. 5). Amino acids 534–621 of APS were expressed as a GST fusion protein and then used as a substrate in a kinase reaction in vitro using purified constitutively activated PKB. As shown in the figure, PKB phosphorylated the wild type GST fusion protein but not the mutant GST fusion protein containing a serine to alanine substitution at 588, as detected by the anti-PKB substrate antibody.

Inhibition of PI3-Kinase-dependent Pathways Prevents the Localization of APS to Membrane Ruffles—We used immunofluorescence to determine whether inhibition of PI3-kinase would affect the localization of APS (Fig. 6). CHO.T-APS cells were serum starved and stimulated with insulin, and the cells were immunostained with anti-APS antibody and analyzed by confocal microscopy. Insulin stimulation led to the localization of APS to membrane ruffles (indicated by arrows), whereas inhibition of PI3-kinase with LY294002 resulted in the loss of APS localization to membrane ruffles (Fig. 6A).

In 3T3-L1 adipocytes the absence of membrane ruffling is well known, and it is not therefore possible to analyze the formation of ruffles. Following insulin stimulation in adipocytes, there is co-localization of APS and PKB substrate staining that is abolished in the presence of LY294002 (Fig. 6B). Mutation of serine 588 to alanine markedly reduced the localization of APS to membrane ruffles when compared with the wild type (Fig. 6C).

**DISCUSSION**

In this study we have identified a substrate for protein kinase B/Akt, the APS adapter protein. This not only defines the APS adapter protein...
Phosphorylation of APS by Protein Kinase B

as a novel in vivo substrate for PKB but also provides the first identification of a serine phosphorylation site in APS and adds to the increasing amount of information on the phosphorylation consensus recognition sequences that can be tolerated by PKB in an intact substrate.

Our evidence is based on a number of correlates. (i) The APS adapter protein is phosphorylated in intact cells, both transfected CHO cells and untransfected 3T3-L1 adipocytes, on a site recognized by the anti-PKBsubstrate antibody. (ii) A second phosphospecific antibody that recognizes a known PKB phosphorylation site in GSK3 cross-reacts with APS following insulin stimulation. (iii) Inhibition of PI3-kinase-dependent pathways in intact cells by chemical inhibitors or by using small inhibitory RNA block the recognition of APS by the antibody. (iv) Mutation of Ser-588 to alanine in full-length APS abolishes the phosphorylation in intact cells. (v) PKB phosphorylates APS directly in vitro on a site recognized by the anti-PKBsubstrate antibody. (vi) Mutation of serine 588 to alanine abolishes the phosphorylation in vitro.

The utility of the anti-PKBsubstrate antibody has now been demonstrated in numerous studies and has been used to identify substrates for protein kinase B/Akt (7, 17, 21, 22). The antibody recognizes the serine-phosphorylated consensus sequence RXRXXS, in the rat APS cDNA, this is contained in a single site at SRSRSNS588 with serine 588 corresponding to the phosphorylated residue. This serine residue, located in the C terminus of APS, is also conserved across a wide range of species including mammalian (human, mouse, rat) and non-mammalian sequences (chicken, blowfish, pufferfish, and frog) (Fig. 7), implying evolutionary and functional importance. Interestingly, this phosphorylation site is not conserved in other members of this protein family, such as the SH2-B isoforms and Lnk. We have previously shown that APS and SH2-B differ in their functional capacity in relation to tyrosine phosphorylation (15); this has been confirmed by detailed structural analysis (23, 24). Therefore, APS and SH2-B differ in the ability to couple to downstream signaling pathways. The lack of a similar PKB consensus site in SH2-B and Lnk would create conditions for directing these molecules to distinct subcellular compartments. This is supported by the effects of a PI3-kinase inhibitor on the localization of APS analyzed by immunofluorescence. Inhibition of PI3-kinase leads to the loss of APS from membrane ruffles. Thus, the function of this insulin-stimulated serine phosphorylation may be to direct APS to a specific subcellular compartment.

The derivation of the consensus PKB phosphorylation site, RXRXXS(T/S), has emerged from studies that have utilized synthetic peptide libraries. The short peptide sequence ARKRERAYSGFHHGA was found to be the optimal substrate for phosphorylation (20). The arginine at −5 does not appear to be critical for phosphorylation by PKB, although this decreases the \( V_{\text{max}}/K_m \) for phosphorylation by PKB. Interestingly, the mouse sequence contains a histidine at position −5. The arginine residue at −3 was found to be critical for phosphorylation of this peptide substrate in vitro. Unsurprisingly, the critical arginine at −3 is conserved between all of the species shown. Many known PKB substrates contain a serine in the −2 position, and this is observed in the sequences for APS. In addition, the online motif identification program Scansite (25) gives serine 588 a score of 0.5750, which is greater than several known substrates of PKB, including BAD and FKHL1.

It is not known whether APS contains a PKB docking site (16) or whether this is required for PKB substrates in the same way as c-Jun NH2-terminal kinase. However, it appears that only amino acids 534–621 are required for phosphorylation by PKB, at least in vitro, which may imply that the putative docking site, if present, lies within this region. This is certainly a hypothesis that can be tested in future experiments.

In summary, our study has shown that serine 588 of APS is a newly identified target for protein kinase B in intact cells and in vitro and is the first serine phosphorylation site identified on APS to date. Furthermore, our data imply that the neoclassical PI3-kinase "independent" pathway receives an input from the classical PI3-kinase pathway, indicating a degree of molecular cross-talk. Therefore, these pathways are not mutually exclusive as previously thought but may in fact converge at the level of PKB, a critical component in the pathway leading to insulin-stimulated glucose transport. Furthermore, the interaction of PKB with APS adds to the list of previously identified interactions for this activation loop-binding protein that include CAP, c-Cbl, and Asb6 (4, 5, 14). The precise function of this PKB-mediated phosphorylation event is not entirely clear but may be responsible for regulating cellular localization and will be the subject of future investigation.

Acknowledgments—We thank Tim Self for assistance with the confocal microscopy and Zamal Ahmed for useful discussions.

REFERENCES

1. Brazil, D. P., Yang, Z. Z., and Hemmings, B. A. (2004) Trends Biochem. Sci. 29, 233–242
2. Saltiel, A. R., and Pessin, J. E. (2002) Trends Cell Biol. 12, 65–71
3. Ahn, M. Y., Katzenakis, K. D., Bheda, F., and Pillaig, T. S. (2004) J. Biol. Chem. 279, 21526–21532
4. Liu, J., Kimura, A., Baumann, C. A., and Saltiel, A. R. (2002) Mol. Cell. Biol. 22, 3599–3609
5. Ahmed, Z., Smith, B. J., and Pallay, T. S. (2000) FEBS Lett. 475, 31–34
6. Liu, J., DeYoung, S. M., Hwang, J. B., O’Leary, E. E., and Saltiel, A. R. (2003) J. Biol. Chem. 278, 36754–36762
7. Kane, S., Sano, H., Liu, S. C., Asara, J. M., Lane, W. S., Garner, C. C., and Lienhard, G. E. (2002) J. Biol. Chem. 277, 22115–22118
8. Chang, L., Adams, R. D., and Saltiel, A. R. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 12835–12840
9. Chiang, S. H., Baumann, C. A., Kanzaki, M., Thurmond, D. C., Watson, R. T., Neudauer, C. L., Macara, I. G., Pessin, J. E., and Saltiel, A. R. (2001) Nature 410, 944–948
10. Chiang, S. H., Hou, J. C., Hwang, J. P., and Saltiel, A. R. (2002) J. Biol. Chem. 30, 30
11. Chiang, S. H., Hwang, I., Legendre, M., Zhang, M., Kimura, A., and Saltiel, A. R. (2003) EMBO J. 22, 2679–2691
12. Welsh, G. I., Hirs, L., Berwick, D. C., Dell, G., Wherlock, M., Birkin, R., Leney, S., and Tavare, J. M. (2005) Biochem. Soc. Trans. 33, 346–349
13. Whiteman, E. L., Cho, H., and Birnbaum, M. J. (2002) Trends Endocrinol. Metab. 13, 444–451
14. Wilcox, A., Katzenakis, K. D., Bheda, F., and Pillaig, T. S. (2004) J. Biol. Chem. 279, 38881–38888
15. Ahmed, Z., and Pallay, T. S. (2003) Biochem. J. 371, 405–412
16. Lawlor, M. A., and Alessi, D. R. (2001) J. Cell Sci. 114, 2903–2910
17. Berwick, D. C., Hirs, I., Heesom, K. J., Moule, S. K., and Tavare, J. M. (2002) J. Biol. Chem. 277, 33895–33900
18. Chun, K. H., Kosmider, J. W., II, Sun, S., Pezzuto, J. M., Lotan, R., Hong, W. K., and Lee, H. Y. (2003) J. Natl. Cancer Inst. 95, 291–302
19. Bortul, R., Tazzari, P. L., Billi, A. M., Tabellini, G., Mantovani, I., Cappellini, A., Grafone, T., Martinelli, G., Conte, R., and Martelli, A. M. (2005) Br. J. Haematol. 129, 677–686
20. Obata, T., Yaffe, M. B., Leparc, G. G., Piro, E. T., Maegawa, H., Kashiwagi, A., Kikkawa, R., and Cantley, L. C. (2000) J. Biol. Chem. 275, 36108–36115
21. Berwick, D. C., and Tavare, J. M. (2004) Trends Biochem. Sci. 29, 227–232
22. Jiang, Z. Y., Zhou, Q. L., Holik, J., Patel, S., Leszyk, J., Coleman, K., Chouinard, M., and Czech, M. P. (2005) J. Biol. Chem. 280, 21622–8
23. Hu, J., Liu, J., Ghirolando, R., Saltiel, A. R., and Hubbard, S. R. (2003) Mol. Cell 12, 1379–1389
24. Hu, J., and Hubbard, S. R. (2005) J. Biol. Chem. 280, 18943–18949
25. Yaffe, M. B., Leparc, G. G., Lai, J., Obata, T., Volinia, S., and Cantley, L. C. (2001) Nat. Biotechnol. 19, 348–353