Regulation of GLUT1 Gene Transcription by the Serine/Threonine Kinase Akt1*

Andreas Barthel, Steven T. Okino, Jinfang Liao, Kaname Nakatani, Jinping Li, James P. Whitlock, Jr., and Richard A. Roth‡

From the Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, California 94305

We used mouse hepatoma (Hepa1c1c7) cells to study the role of the serine/threonine kinase Akt in the induction of GLUT1 gene expression. In order to selectively turn on the Akt kinase cascade, we expressed a hydroxytamoxifen-regulatable form of Akt (myristoylated Akt1 estrogen receptor chimera (MER-Akt1)) in the Hepa1c1c7 cells; we verified that hydroxytamoxifen stimulates MER-Akt1 activity to a similar extent as the activation of endogenous Akt by insulin. Our studies reveal that stimulation of MER-Akt1 by hydroxytamoxifen induces GLUT1 mRNA and protein accumulation to levels comparable to that induced by insulin; therefore, activation of the Akt cascade suffices to induce GLUT1 gene expression in this cell system. Furthermore, expression of a kinase-inactive Akt mutant partially inhibits the response of the GLUT1 gene to insulin. Additional studies reveal that the induction of GLUT1 mRNA by Akt and by insulin reflects increased mRNA synthesis and not decreased mRNA degradation. Our findings imply that the GLUT1 gene responds to insulin at the transcriptional level and that Akt mediates a step in the activation of GLUT1 gene expression in this system.

The members of a family of six membrane proteins, known as the glucose transporters (GLUT1–5 and GLUT7),1 facilitate glucose uptake into mammalian cells (reviewed in Refs. 1 and 2). Glucose uptake is critical for maintaining intracellular ATP levels, and cells have evolved several strategies for regulating this process. For example, in fat and muscle, insulin stimulates the rapid translocation of the GLUT4 protein from an intracellular site to the plasma membrane, where it functions (3). In other tissues and cells, long term insulin treatment stimulates glucose uptake by increasing GLUT1 gene expression, thereby providing more transporter proteins to the cell (4, 5). The GLUT1 gene also responds to other stimuli. For example, hypoxia induces GLUT1 gene expression (6–8); this response may help protect neurons from glucose starvation and death during brain ischemia (9). In addition, increased GLUT1 gene expression is associated with oncogenic transformation of various cell types (10–12). These observations imply that the regulation of GLUT1 gene expression is relatively complex and may involve several converging signaling pathways.

The GLUT1 gene constitutes an interesting system for analyzing the mechanism by which insulin alters specific gene expression. Insulin binds to a cell surface receptor and activates an intrinsic receptor tyrosine kinase, a process that ultimately stimulates two major signaling cascades (13). One cascade leads to the activation of MAP kinase and the phosphorylation of transcription factors (14). The second cascade involves phosphatidylinositol (PI)-3 kinase (15) and numerous potential downstream effectors, including the serine/threonine kinase Akt (also called protein kinase B (PKB)) (16). Akt activity reflects contributions from three distinct isozymes (Akt1–3), which are regulated primarily by phosphorylation (16). Expression of a constitutively active Akt1 can mimic several nontranscriptional responses to insulin, including inhibition of glycolgen synthase kinase-3, activation of the p70 ribosomal S6 kinase, stimulation of GLUT4 translocation and glucose uptake, stimulation of protein synthesis, and inhibition of apoptosis (13, 16).

It is less clear whether Akt can mediate transcriptional responses to insulin (17). Several reports provide conflicting data on this point (18–22). To further address this issue, we have asked here whether Akt can induce GLUT1 gene transcription in mouse hepatoma (Hepa1c1c7) cells. We find that activation of the Akt1 kinase suffices to induce GLUT1 transcription to an extent similar to that induced by insulin. Moreover, expression of an inactive Akt1 partially inhibits the ability of insulin to stimulate GLUT1 gene expression. These results imply that Akt is a downstream effector of insulin action in inducing GLUT1 gene transcription in this cell system.

EXPERIMENTAL PROCEDURES

Materials—Minimum Eagle's medium and G418 were from Life Technologies, Inc., and other cell culture media were from UCSF Cell Culture Facility (San Francisco, CA). Total RNA was isolated using the RNasy kit from Qiagen (Chatsworth, CA). The primerase chain reaction kit was from Stratagene (La Jolla, CA). The primers for the GLUT1 and β-actin probes were from Operon Technologies Inc. (Alameda, CA), and the pGEM-T vector system was from Promega (Madison, WI). [γ-32P]ATP (3000 Ci/mmol) was from NEN Life Science Products, and the random priming polymerase chain reaction kit was from Stratagene (La Jolla, CA). The Akt-substrate peptide was synthesized in the Beckman PAN facility (Stanford, CA). Anti-GLUT1 polyclonal antiserum was from East Acres (Southbridge, MA), acrylamide was from National Diagnostics (Atlanta, GA), nitrocellulose and nylon membranes were from Schleicher & Schuell, insulin and anti-HA monoclonal antibody (12CA5) were from Roche Molecular Biochemicals, and anti-Akt1 antibodies (directed against the C terminus) were from Upstate Biochemicals (Lake Placid, NY). Anti-Akt1 and Akt3 antibodies directed against their respective pleckstrin homology domains were produced as described (23). Anti-Akt2 antibodies were a gift of Dr. Birnbaum (University of Pennsylvania) (24). Sephadex 10 microspin columns were from

* This work was supported in part by National Institutes of Health Grants DK 34926 (to R. A. R.) and ES08655 (to J. P. W.), an American Diabetes Association Mentor-Based Postdoctoral Fellowship (to K. N.), and a Feodor-Lynen Fellowship of the Alexander von Humboldt-Stiftung (to A. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Molecular Pharmacology, Stanford Medical Center, Stanford, CA 94305. Tel.: 650-725-2952; E-mail: rr@roth@stanford.edu.

1 The abbreviations used are: GLUT, glucose transporter; MER-Akt, myristoylated Akt estrogen receptor chimera; HA, hemagglutinin; PI, phosphatidylinositol.

This paper is available on line at http://www.jbc.org
Akt Activates GLUT1 Gene Expression in Hepa Cells

Amersham Pharmacia Biotech, and Express-Hyb solution was from Amersham Pharmacia Biotech, and Express-Hyb solution was from Amersham Pharmacia Biotech. Protein A-Sepharose was from Rapigest (Cambridge, MA). Protein determination was performed using the BCA kit (Pierce). 4-Hydroxytamoxifen and all other chemicals were from Sigma (St. Louis, MO).

Plasmid Constructs—The retroviral plasmids coding for HA-tagged MER-Akt1 and the inactive HA-tagged Akt1-S473A/T308A mutant were as described (20, 25), except that both constructs were expressed using the pWZL-neo retrovector vector (a gift from Dr. Garry P. Nolan, Stanford, CA).

Cell Culture—Hepa1c1c7 cells stably transfected with MER-Akt1-pWZL-neo and Akt1-S473A/T308A-pWZL-neo empty vector alone (pWZL-neo) were grown in 6-well Nunclon dishes (Nalge Nunc International, Roskilde, Denmark) in α-minimum Eagle's medium containing 10% (v/v) fetal calf serum, 100 μg/ml streptomycin, and 100 units/ml penicillin. After reaching confluency, the cells were put in hypoxic conditions by placing them in a humidified automatic CO2 incubator (Furna Scientific, model 3159) maintained at 37 °C and 1% O2, 5% CO2, and 95% N2.

Retroviral Infection—Hepa1c1c7 cells were infected with MER-Akt1-pWZL-neo, Akt1-S473A/T308A-pWZL-neo, or empty vector alone (pWZL-neo) as described previously (25); selection was performed in 200 μg/ml G418.

Isolation of Membrane Proteins and Western Blots of GLUT1—Cells were scraped in 2 ml of buffer (50 mM Heps, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1 mM dithiothreitol, 1 mM benzamidine, 1 mM phenylmethanesulfonyl fluoride), homogenized by 10 push/pull cycles using a 23 gauge needle and a 3-ml syringe, and the membranes were spun down at 4 °C for 1 h at 100,000 × g in a tabletop microcentrifuge (Beckman). The membrane pellet was washed once in the same volume of buffer, resolubilized in buffer containing 0.1% (v/v) Triton X-100, and analyzed by SDS-PAGE. The proteins were transferred to nitrocellulose membranes (2 h, 300 mA tank blotting) and the transfer was verified by Ponceau red staining before blocking the membrane with 3% bovine serum albumin in Tris-buffered saline-Tween (0.1%). The membranes were then incubated for 2 h in the first antibody (usually 1:5000 in 3% bovine serum albumin/Tris-buffered saline-Tween), washed briefly and incubated for another 1 h in secondary antibody (1:5000 in 3% fat-free milk/Tris-buffered saline-Tween). After extensive washing (Tris-buffered saline-Tween) the signal was visualized by chemiluminescence using a system containing luminol and coumaric acid.

Akt Assays—The Akt kinase assay using GSK-3 peptide (GRPRRTS-FAEG) as substrate was performed as described previously (25). In brief, cells were lysed in 400 μl of lysis buffer/well. Endogenous Akt1, Akt3, or expressed HA-tagged MER-Akt1 was immunoprecipitated from the lysates using protein A-Sepharose beads that were preabsorbed with anti-Akt1 or Akt3 PH-domain antisera or monoclonal anti-HA antibody (12CA5). Nonspecific background was measured by incubating the immunoabsorbed protein A-Sepharose beads that were preabsorbed with either normal rabbit serum or normal mouse IgG. Following the kinase reaction, the phosphorylated peptide was separated from the longer incubation time required for this latter response. For example, we observed that, at a starting concentration of 10 nM, >90% of the input insulin was degraded after 9 h of incubation with the cells (data not shown). Both responses exhibit sensitivities to insulin similar to those reported for other systems (5, 20, 27). These findings suggested that Akt activation might be linked mechanistically to GLUT1 gene expression; therefore, we performed experiments designed to test this possibility.

Expression of a Regulatable Akt1—To identify a possible relationship between Akt activity and GLUT1 gene expression, we infected Hepa1c1c7 cells with a retroviral vector encoding MER-Akt1, which is a hydroxytamoxifen-regulatable version of an epitope-tagged Akt1 (25). We verified expression of the protein by treating cells with hydroxytamoxifen, lysing them, immunoprecipitating the epitope-tagged MER-Akt1, and measuring its enzymatic activity. Our findings demonstrate that hydroxytamoxifen causes a rapid and sustained increase in Akt enzymatic activity in cells that express MER-Akt1 (Fig. 2A); however, the increase does not occur in uninfected cells (data not shown). The time course of MER-Akt1 activation by hydroxytamoxifen is only slightly slower then the time course of endogenous Akt1 activation by insulin (Fig. 2B). The maximal activity of hydroxytamoxifen-stimulated MER-Akt1 in the anti-HA antibody precipitates (about 70,000 cpm) (Fig. 2A) is about 3 times the maximal activity of insulin-stimulated endogenous Akt1 (Fig. 2B). Immunoblotting studies of total ly-
Akt Activates GLUT1 Gene Expression in Hepa Cells

Fig. 1. Insulin induces Akt1 activity and GLUT1 mRNA accumulation in a concentration-dependent fashion. A, Akt1 activity. Hepa1c1c7 cells were serum-starved overnight, incubated for 30 min with the indicated insulin concentration, and lysed; endogenous Akt1 was immunoprecipitated and assayed for enzymatic activity using GSK3-peptide as substrate. Bars indicate the means (± S.E.) of three experiments. B, GLUT1 mRNA. Hepa1c1c7 cells were treated with the indicated concentration of insulin for 18 h and lysed; total RNA was isolated and assayed for GLUT1 and actin mRNA by Northern analysis. The autoradiograms from three experiments were scanned, normalized for the amounts of actin present, and expressed as the fold induction over the nontreated controls.

Fig. 2. Time course of Akt activation. A, activation of MER-Akt1 by hydroxytamoxifen. Cells were treated with 1 μM hydroxytamoxifen. Cells were treated with 1 μM hydroxytamoxifen for the indicated periods of time and lysed, and the expressed HA-tagged MER-Akt1 was immunoprecipitated and assayed for enzyme activity. Data points indicate the means (± S.E.) of three experiments. The inset in A shows a Western blot of total cell lysate (10 μg of protein) obtained from cells infected with either empty retroviral vector (lane 1) or vector encoding MER-Akt1 (lane 2) probed with anti-Akt1 antibody. The higher molecular mass protein is the MER-Akt1, and the smaller protein is the endogenous Akt1. B, activation of endogenous Akt by insulin. Cells were treated with 1 μM insulin for the indicated periods of time and lysed, and endogenous Akt1 was immunoprecipitated and assayed for enzyme activity. Data points indicate the means (± S.E.) of three experiments. The inset in B shows the results of an assay for the insulin-stimulated endogenous Akt3.

sates indicate that the MER-Akt1 is expressed at a level comparable to that of the endogenous Akt1 (Fig. 2A, inset). Because Akt has three distinct isoforms, we also measured the activity of endogenous Akt2 and Akt3 in Hepa1c1c7 cells after stimulation with insulin. We observed that insulin stimulates endogenous Akt3 activity (Fig. 2B, inset) to a level that is about twice the maximal amount of hydroxytamoxifen-stimulated MER-Akt1 activity (Fig. 2A), whereas these cells contain only a low level of Akt2 activity (data not shown). These results indicate that the level of hydroxytamoxifen-stimulated MER-Akt1 activity in the infected cells is about half the level of insulin-stimulated total Akt activity in the parental cells. Therefore, the findings described below using cells containing hydroxytamoxifen-stimulated MER-Akt1 do not reflect artifacts related to overexpression of Akt enzyme activity.

To demonstrate a link between Akt activity and GLUT1 gene expression, we asked whether hydroxytamoxifen induces GLUT1 mRNA accumulation in cells containing MER-Akt1. Our findings reveal that hydroxytamoxifen induces GLUT1 mRNA and GLUT1 protein to levels comparable to those that insulin induces in these cells (Fig. 3A). In control cells infected with an empty virus, hydroxytamoxifen does not induce GLUT1 mRNA or protein (Fig. 3A). We also measured the kinetics of hydroxytamoxifen-induced GLUT1 mRNA and protein accumulation; both responses reach plateaus after 24 h (Fig. 3, B and C). These kinetics are similar to those observed with insulin (data not shown). In addition, induction of GLUT1 mRNA and Akt1 activity both occur over the same concentration range of hydroxytamoxifen (Fig. 4). These findings, together with the observation that hydroxytamoxifen does not induce GLUT1 mRNA in cells that do not contain MER-Akt1, reveal that stimulation of Akt1 activity leads to an increase in GLUT1 mRNA. The simplest interpretation of these observations is that Akt1 regulates GLUT1 gene expression.

Expression of an Enzymatically Inactive Akt1—to further examine the potential link between insulin, Akt, and GLUT1 gene expression, we expressed an enzymatically inactive Akt1 mutant in Hepa1c1c7 cells. Immunoblotting studies indicate that the mutant Akt1 is expressed at levels that are comparable to those of the endogenous Akt1 protein (Fig. 5A). Induction experiments reveal that, in cells that express the mutant Akt1, the GLUT1 gene exhibits about a 50% reduction in its response to insulin (Fig. 5B). In contrast, expression of the mutant Akt1 had no effect on the response of the GLUT1 gene to hypoxia (data not shown). This selective inhibitory effect of the Akt mutant on the response of the GLUT1 gene to insulin provides additional evidence that Akt is a component of the insulin-responsive signaling pathway that regulates GLUT1 gene expression in Hepa1c1c7 cells.

Insulin and Akt Induce GLUT1 Transcription—in principle, insulin and Akt could induce GLUT1 mRNA accumulation by
increasing mRNA synthesis or by decreasing mRNA degradation. To distinguish between these possibilities, we used actinomycin D to inhibit mRNA synthesis, and we measured the half-life of GLUT1 mRNA in Hepa1c1c7 cells that stably express MER-Akt1. Our findings reveal no differences in the rates of GLUT1 mRNA decay in uninduced, insulin-induced, or hydroxytamoxifen-induced cells (Fig. 6). In all three cases, the half-life of GLUT1 mRNA is about 4 h. Therefore, these findings reveal no evidence for GLUT1 mRNA stabilization after hydroxytamoxifen or insulin treatment.

To test directly whether insulin and Akt activation stimulate GLUT1 gene transcription, we performed nuclear run-on experiments using Hepa1c1c7 cells that stably express MER-Akt1. Our findings indicate that insulin and hydroxytamoxifen both increase GLUT1 transcription about 3-fold (Fig. 7). These findings, together with the lack of change in mRNA degradation, imply that the induction of GLUT1 gene expression arises primarily at the level of transcription.

**DISCUSSION**

In mammalian cells, a family of GLUT proteins mediates glucose uptake, thereby profoundly influencing cellular metabolism (1–3). The GLUT1 gene, which encodes the most widely expressed glucose transporter, responds to several chemical and hormonal stimuli and makes an important contribution to the maintenance of intracellular homeostasis. Thus, an understanding of the events that control GLUT1 gene expression may provide important insights into the molecular mechanisms by which cells adapt to changes in their environment.

Here, we show that an increase in GLUT1 gene transcription substantially accounts for the accumulation of GLUT1 mRNA and protein that accompanies exposure of mouse hepatoma cells to insulin. Furthermore, we demonstrate that elevating Akt activity leads to increased GLUT1 transcription and to mRNA and protein accumulation in the absence of insulin. Finally, we show that expression of a kinase inactive Akt1 inhibits the ability of insulin to induce GLUT1 mRNA. Therefore, we infer that an increase in Akt activity is an important event in the signaling pathway through which insulin regulates GLUT1 gene expression.

Our findings are consistent with and extend prior observations that expression of a constitutively active Akt1 increases both the GLUT1 protein in 3T3-L1 adipocytes and the GLUT3 protein in L6 skeletal muscle cells (28, 29). Our observations are also consistent with a prior report implicating the p21ras protein in insulin-induced GLUT1 gene expression (30) because p21ras can activate the PI 3-kinase/Akt pathway (31). In addition, our work is consistent with studies that implicate the mammalian target of rapamycin (mTOR) in the induction of the GLUT1 mRNA by insulin (7, 32) because Akt regulates mTOR (33), and we find that rapamycin inhibits the insulin-
Akt Activates GLUT1 Gene Expression in Hepa Cells

20285

FIG. 5. Effect of a kinase inactive Akt1 on the insulin-stimulated GLUT1 mRNA accumulation in Hepa1c1c7 cells. A, expression of an inactive Akt1. Hepa1c1c7 cells were infected with either the empty retroviral vector or a retroviral vector encoding an inactive Akt1 mutant (Akt1-S473A/T308A-pWZL-neo). Amounts of the mutant Akt1 were determined by Western blotting of total cell lysates (10 μg/lane) from control cells (lane 1) and from cells infected with mutant Akt1 (lane 2). B, effect of inactive Akt1 on induction of GLUT1 mRNA by insulin. Hepa1c1c7 cells infected with either the empty retroviral vector (pWZL) or inactive Akt1 (Akt1-S473A/T308A-pWZL-neo) were serum-starved and treated for 18 h with the indicated concentrations of insulin. The cells were lysed, and total RNA was assayed for GLUT1 and actin mRNA by Northern analysis. The results were quantitated and are presented as fold inductions (± S.E.). Black bars represent data from the noninfected cells; gray bars represent the data from cells expressing inactive Akt1. Numbers in parentheses represent the number of experiments performed at the indicated insulin concentrations.

induced increase in GLUT1 mRNA in the Hepa1c1c7 cells. However, our findings are not consistent with a recent report that wortmannin, an inhibitor of the PI 3-kinase/Akt pathway, induced increase in GLUT1 mRNA in the Hepa1c1c7 cells.2 Perhaps these findings reflect differences in cell type, because the p21ras gene transcription in L6 skeletal muscle cells (32). Perhaps these findings reflect differences in cell type, because the p21ras protein also does not appear to play a role in insulin-induced GLUT1 gene transcription in L6 cells (34). It is also possible that the lack of effect of wortmannin is due to the instability of this molecule. For example, we find that LY294002, another PI 3-kinase inhibitor (35), inhibits induction of GLUT1 mRNA by insulin in Hepa1c1c7 cells.2 From a mechanistic standpoint, we envision that Akt stimulates GLUT1 transcription via phosphorylation of a particular protein(s). The target for phosphorylation might be a specific transcription factor(s) or a signaling component(s) that functions prior to the formation of a transcriptional complex at the GLUT1 promoter. The GLUT1 gene contains multiple DNA elements that enhance transcription in concert with their cognate binding proteins. Akt might influence GLUT1 gene expression by phosphorylating a protein that binds to the serum-responsive element, the cAMP-responsive element, and/or the 12-O-tetradecanoylphorbol-13-acetate-responsive element (26).

FIG. 6. Effect of insulin and hydroxytamoxifen on the half-life of GLUT1 mRNA. Hepa1c1c7 cells expressing MER-Akt1 were exposed to ethanol (as control) (A), insulin (1 μM) (B), or hydroxytamoxifen (1 μM) (C) in the presence of actinomycin D (5 μg/ml). Cells were harvested after 0, 3, or 6 h, and total RNA was assayed for GLUT1 and actin mRNA by blot hybridization. The autoradiograms shown are representative of three experiments. The amounts of GLUT1 and actin mRNA were quantified by phosphorimaging; the GLUT1 mRNA was normalized to actin and expressed as the percentage of GLUT1 mRNA at time 0. The values shown are means (± S.E.) of three experiments.

FIG. 7. Nuclear run-on experiments. Hepa1c1c7 cells expressing MER-Akt1 were exposed to ethanol (as a control), insulin (1 μM), or hydroxytamoxifen (1 μM). The cells were lysed, nuclei were isolated, and transcriptional run-on assays were performed as described under “Experimental Procedures.” The radiolabeled mRNA was hybridized with immobilized DNA probes for GLUT1 and actin. The autoradiograms from three experiments were scanned, normalized for the amounts of actin present, and expressed as the fold induction over the nontreated controls.

2 A. Barthel, S. T. Okino, J. Liao, K. Nakatani, J. Li, J. P. Whitlock, Jr., and R. A. Roth, unpublished observations.
Akt might mediate GLUT1 transcription via regulating a mammalian homolog of DAF-16. These appear to be interesting areas for future research.

Our studies demonstrate that activation of the Akt kinase cascade is sufficient to induce GLUT1 mRNA and protein accumulation via an increase in GLUT1 gene transcription. The increase in GLUT1 activity may in part account for the ability of Akt to inhibit apoptosis because glucose uptake plays a critical role in regulating intracellular ATP levels and cell viability. Akt could also mediate the increase in GLUT1 gene expression that follows hypoxia because such conditions can activate the PI 3-kinase/Akt pathway (40). However, we did not find that the PI 3-kinase inhibitor, LY294002, could block the hypoxia-induced GLUT1 mRNA accumulation. Thus, one of the roles of Akt in the maintenance of cellular homeostasis may be to integrate several types of environmental stimuli, leading to enhanced expression of a subset of genes, including GLUT1.

Acknowledgments—We thank Dr. Garry Nolan for the Phoenix retroviral packaging cell line and the retroviral vectors, Dr. Morris Birnbaum for the antibodies to Akt2, and Dr. Karlene Cimprich for a critical reading of the manuscript.

REFERENCES
1. Mueckler, M., Hresko, R. C., and Sato, M. (1997) Biochem. Soc. Trans. 25, 951–954
2. Olson, A. L., and Pessin, J. E. (1996) Annu. Rev. Nutr. 16, 235–256
3. Holman, G. D., and Cushman, S. W. (1994) Annu. Rev. Nutr. 14, 133, 2540–2544
4. Garcia de Herreros, A., and Birnbaum, M. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8252–8256
5. Flier, J. S., Mueckler, M. M., Usher, P., and Lodish, H. F. (1987) Science 235, 1492–1495
6. Bashan, N., Burdett, E., Hundal, H. S., and Klip, A. (1992) J. Biol. Chem. 267, 264, 6587–6595
7. Kozlovsky, N., Rudich, A., Potashnik, R., Ebina, Y., Murakami, T., and Bashan, N. (1997) J. Biol. Chem. 272, 33367–33372
8. Behroz, A., and Ismail-Beigi, F. (1997) J. Biol. Chem. 272, 5555–5562
9. Lee, W. H., and Bondy, C. A. (1995) Endocrinology 133, 2540–2544
10. Birnbaum, M. J., Haspel, H. C., and Rosen, O. M. (1987) Science 235, 1495–1498
11. Hiraki, Y., Garcia de Herreros, A., and Birnbaum, M. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8252–8256
12. Flier, J. S., Mueckler, M. M., Usher, P., and Lodish, H. F. (1987) Science 235, 1492–1495
13. Shepherd, P. R., Withers, D. J., and Siddle, K. (1998) Biochem. J. 333, 471–490
14. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186
15. Toker, A., and Cantley, L. C. (1997) Nature 387, 673–676
16. Coffre, P. J., Jin, J., and Woodgett, J. R. (1998) Biochem. J. 335, 1–13
17. O’Brien, R. M., and Granner, D. K. (1996) Physiol. Rev. 76, 1109–1161
18. Wang, D., and Sul, H. S. (1988) J. Biol. Chem. 263, 25420–25426
19. Cirky, S. B., Uddin, S., Danilkovich, A., Gao, S., Klippel, A., and Untermann, T. G. (1998) J. Biol. Chem. 273, 6482–6487
20. Liu, J., Barthel, A., Nakatani, K., and Roth, R. A. (1998) J. Biol. Chem. 273, 27320–27324
21. Agati, J. M., Yeagley, D., and Quinn, P. G. (1998) J. Biol. Chem. 273, 18751–18759
22. Dickens, M., Svitek, C. A., Culbert, A. A., O’Brien, R. M., and Tavare, J. M. (1998) J. Biol. Chem. 273, 20144–20149
23. Krook, A., Kawano, Y., Song, X. M., Efendic, S., Roth, R. A., Wallberg-Henriksson, H., and Zierath, R. J. (1997) Diabetes 46, 2110–2114
24. Calera, M. R., Martinez, C., Liu, H., El-Jack, A. R., Birnbaum, M. J., and Pitch, P. F. (1998) J. Biol. Chem. 273, 7201–7204
25. Kohn, A. D., Barthel, A., Kevakina, K. S., Boge, A., Wallach, B., Summers, S. A., Birnbaum, M. J., Scott, P. H., Lawrence, J. C., Jr., and Roth, R. A. (1998) J. Biol. Chem. 273, 11937–11943
26. Todaka, M., Nishiyama, T., Murakami, T., Saito, S., Ito, K., Kanai, F., Kan, M., Ishii, K., Hayashi, H., Shichiri, M., and Ebina, Y. (1994) J. Biol. Chem. 269, 29265–29270
27. Walker, K. S., Deak, M., Paterson, A., Hudson, K., Cohen, P., and Alessi, D. R. (1998) Biochem. J. 331, 299–308
28. Kohn, A. D., Summers, S. A., Birnbaum, M. J., and Roth, R. A. (1996) J. Biol. Chem. 271, 31372–31378
29. Hajduch, E., Alessi, D. R., Hemmings, B. A., and Hndal, H. S. (1998) Diabetes 47, 1006–1013
30. Hausdorff, S., Prangion, J., and Birnbaum, M. (1994) J. Biol. Chem. 269, 21391–21394
31. Liu, A. X., Testa, J. R., Hamilton, T. C., Jove, R., Nicolaia, S. Y., and Cheng, J. Q. (1998) Cancer Res. 58, 2973–2977
32. Somwar, R., Sumitani, S., Taha, C., Sweeney, G., and Klip, A. (1998) Am. J. Physiol. 275, E618–E625
33. Scott, P. H., Brunn, G. J., Kohn, A. D., Roth, R. A., and Lawrence, J. C., Jr. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7772–7777
34. Taha, C., Mitsumoto, Y., Liu, Z., Skolnik, E. Y., and Klip, A. (1995) J. Biol. Chem. 270, 24678–24681
35. Vlahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. P. (1994) J. Biol. Chem. 269, 5241–5248
36. Du, K., and Montminy, M. (1998) J. Biol. Chem. 273, 32377–32379
37. Zelzer, E., Levy, V., Kahana, C., Shilo, B. Z., Rubinsteine, M., and Cohen, B. (1998) EMBO J. 17, 5085–5094
38. Stancoisky, I., and Baltimore, D. (1997) Cell 91, 299–302
39. Paradis, S., and Ruvkun, G. (1998) Genes Dev. 12, 2488–2498
40. Mature, N. M., Chen, E. Y., Laderoute, K. R., and Giaccia, A. J. (1997) Blood 90, 3322–3331