It has been shown that IGF-1-induced pancreatic β-cell proliferation is glucose-dependent; however, the mechanisms responsible for this glucose dependence are not known. Adenoviral mediated expression of constitutively active phosphatidylinositol 3-kinase (PI3K) in the pancreatic β-cells, INS-1, suggested that PI3K was not necessary for glucose-induced β-cell proliferation but was required for IGF-1-induced mitogenesis. Examination of the signaling components downstream of PI3K, 3-phosphoinositide-dependent kinase 1, protein kinase B (PKB), glycogen synthase kinase-3, and p70-S6-kinase (p70S6K), suggested that a major part of glucose-dependent β-cell proliferation requires activation of mammalian target of rapamycin/p70S6K, independent of phosphoinositide-dependent kinase 1/PKB activation. Adenoviral expression of the kinase-dead form of PKB in INS-1 cells decreased IGF-1-induced β-cell proliferation. However, a surprisingly similar decrease was also observed in adenoviral wild type and constitutively active PKB-infected cells. Upon analysis of extracellular signal-regulated protein kinase 1 and 2 (ERK1/ERK2), an increase in ERK1/ERK2 phosphorylation activation by glucose and IGF-1 was observed in kinase-dead PKB-infected cells, but this phosphorylation activation was inhibited in the constitutively active PKB-infected cells. Hence, there is a requirement for the activation of both ERK1/ERK2 and mammalian target of rapamycin/p70S6K signal transduction pathways for a full commitment to glucose-induced pancreatic β-cell mitogenesis. However, for IGF-1-induced activation, these pathways must be carefully balanced, because chronic activation of one (PI3K/PKB) can lead to dampening of the other (ERK1/2), reducing the mitogenic response.

The molecular defects causing obesity-linked type 2 diabetes mellitus are not well defined. However, recently it has become clear that key factors involved in causing type 2 diabetes are both impaired β-cell function (1) and a failure to increase β-cell mass to compensate for peripheral insulin resistance (2, 3). It is therefore critical that strategies be developed to replenish the loss of β-cells and/or to expand existing β-cell mass to compensate for insulin resistance. Such an approach relies on defining the mechanisms involved in regulating β-cell mitogenic signaling pathways in response to growth factors and nutrients.

Previous studies have shown that certain growth factors, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1), are important for stimulation of β-cell proliferation (4, 5). In addition, these studies showed that GH and IGF-1 are dependent on glucose at concentrations in the physiologically relevant range of 6–18 mM to increase β-cell mitogenesis. However, the basis of this glucose dependence of β-cell proliferation remains unknown.

GH signals via the janus kinase-2/signal transducer and activator of transcription-5 pathway in INS-1 β-cells. Although glucose has no independent effect on the activation of this pathway, it is nonetheless required for GH mitogenic action (4, 6). There appears to be no cross-talk of janus kinase-2 activation to insulin receptor substrate (IRS)-mediated signaling by GH. However, activation of phosphatidylinositol 3-kinase (PI3K) mediated by glucose is at least partially required for full GH-induced β-cell proliferation.

IGF-1 has been shown to activate at least two major mitogenic signaling pathways, via PI3K and mitogen-activated protein kinase (7–10). The mitogenic stimulation of receptors, such as the IGF-1 receptor, results in the phosphorylation and activation of the IRS family of proteins and the SH2-containing protein (Shc). Shc then interacts with Shc homology 2 (SH2) domain-containing proteins, such as the growth factor receptor-bound protein-2, whereas IRS proteins interact with the regulatory subunit of PI3K, p85, as well as growth factor receptor-bound protein-2. The recruitment of growth factor receptor-bound protein-2 and its association to murine sons of sevenless-1 protein (a Ras guanine nucleotide exchange factor) activates Ras, resulting in Raf-1 activation, which phosphorylates the extracellular signal-regulated protein kinase (ERK) kinase, which in turn activates ERK1/ERK2 (8). Interaction of

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1 The abbreviations used are: GH, growth hormone; IGF-1, insulin-like growth factor 1; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; SH2, Shc homology 2; ERK, extracellular signal-regulated protein kinase; PIP2, phosphatidylinositol 3,4,5-trisphosphate; PKB, protein kinase B; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK-1, 3-phosphoinositide-dependent kinase 1; GSK3, glycogen synthase kinase-3; mTOR, mammalian target of rapamycin; p70S6K, p70-S6-kinase; SGK, serum- and glucocorticoid-regulated protein kinase; SH2, SH2 of p85; m.o.i., multiplicity of infection; AdV, adenovirus; WT, wild type; CA, constitutively active; KD, kinase-dead; GFP, green fluorescent protein; Luc, luciferase.
tyrosine-phosphorylated IRS with the p85 regulatory subunit of PI3K leads to the activation of the catalytic subunit, p110, which in turn phosphorylates phosphoinositides at the 3’ position of the inositol ring, generating PIP3 (4, 6). This increase in phosphorylated phosphoinositides leads to the localization of protein kinase B (PKB) to the membrane through the interaction of PI3P and PIP2 with the pleckstrin homology domain of PKB (11). Full activation of PKB appears to be dependent on the subsequent phosphorylation of two residues, Thr-308 in the activation loop of the kinase domain and Ser-473 in the carboxyl-terminal tail (12). The protein kinase shown to phosphorylate Thr-308 is 3-phosphoinositide-dependent kinase-1 (PDK1) (13, 14), whereas the kinase responsible for phosphorylation at the Ser-473 residue has not yet been identified. PKB regulates multiple biological processes, such as cell proliferation and apoptosis, suggesting that it may phosphorylate a number of target proteins (reviewed in Ref 15). Glycogen synthase kinase-3 α and β (GSK3α/β) have been shown to be two such targets, and their phosphorylation on Ser-21 and Ser-9, respectively, negatively regulates GSK3α/β activity (16). PKB has also been implicated in catalyzing phosphorylation activation of mammalian target of rapamycin (mTOR), which then mediates the phosphorylation of p70-kDa-S6-kinase (p70S6K) (17, 18). In β-cells, glucose and IGF-1 activation of p70S6K via the IRS-mediated signal transduction pathway is dependent on PDK1 and mTOR. However, the important signaling components involved in the regulation of mitogenesis that are downstream of PDK1, such as PDK-1, PKB, mTOR, and GSK3α/β, have not been characterized in β-cells, even though it has been shown that p70S6K is activated by glucose and IGF-1 (4, 5). Therefore, in this study we set out to characterize the signaling elements downstream of PDK1 that are regulated by glucose and IGF-1 and found that PKB and p70S6K are differentially activated. These data suggested that a major part of the glucose dependence of β-cell proliferation is via a direct activation of mTOR/P70S6K, independent of PDK1/PKB activation.

EXPERIMENTAL PROCEDURES

Materials—The PDK-1 kinase assay kit and PDK-1 antibody were from Upstate Biotechnology Inc. (Lake Placid, NY). The phospho-GSK3α/β (Ser-21/9), total PKB, phospho-Thr380-PKB, phosphoSer473-PKB antibodies, the GSK3 kinase, and the PKB kinase assay kit were from New England Biolabs Inc. (Beverly, MA). Anti-phospho-ERK1/2 was obtained from Promega Corporation (Madison, WI), and the total ERK1/2 antisera was a gift from Dr. M. Cobb (University of Texas Southwestern Medical Center, Dallas, TX). The p70S6K antisera were generated as described (19). Monoclonal anti-c-Myc clone 9E10 was obtained from Sigma. The total GSK3 antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-rabbit and anti-sheep IgG horseradish peroxidase conjugates were from Jackson ImmunoResearch (West Grove, PA), and the anti-mouse IgG horse-radish peroxidase conjugate was from Upstate Biotechnology Inc. Wortmannin and rapamycin were from Calbiochem-Novabiochem. IGFI was purchased from Gro Pep Pty Ltd. (Adelaide, Australia). The pUSEamp expression vectors containing Myc-His-tagged mouse PKBα (wild type), myr-PKBα (constitutively active), and PKBα-K179M (kinase-dead) were from Upstate Biotechnology Inc. DNA purification kits and Superfect transfection reagents were purchased from Qiagen (Valencia, CA). Restriction enzymes were from New England Biolabs Inc. The bichromonic acid protein assay kit was purchased from Pierce. The [methyl-3H]thymidine and chemiluminescence reagent was from PerkinElmer Life Sciences. All other reagents were of analytical grade from either Sigma or Fisher.

Cell Culture—The glucose-sensitive pancreatic β-cell line INS-1 (20) was maintained in the complete medium RPMI 1640 (11.2 mM glucose) containing 5% (v/v) fetal calf serum (FCS), 10% (v/v) heat-inactivated HEPES, 2 mM glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin and incubated at 37 °C in 5% CO2 as described (20).

[3H]Thymidine Incorporation—Thymidine incorporation was measured essentially as described, with minor changes. Briefly, INS-1 cells were cultured on 6-well plates to ~60% confluence and infected with adeno virus as described below. The cells were then counted and ~1 × 106 cells were added to each well of a 96-well plate. The cells were left to attach overnight and were then made quiescent by incubation in starvation medium for 24 h. The INS-1 cells were then incubated for 24 h in starvation medium with additional glucose (10 mM IGFI-1) as indicated. During the final 4 h of this incubation 5 μCi/ml [3H]thymidine was added. The specific incorporation of [3H]thymidine into DNA was then measured by transferring the cell lysates to UniFilter-96 GF/C filter plates using the Packard cell harvester, and the radioactivity on the filters was counted with the Packard cell counter.

Stimulation and Lysis Conditions—Cells were subcultured on either 6-well or 10-cm plates to 70–80% confluence. The cells were then subjected to 24-h serum and glucose deprivation with starvation medium (RPMI 1640 medium containing 0.1% bovine serum albumin, 0.5 mM glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin) or infected with adeno virus (see “Adenovirus Infection”), when appropriate, prior to 24-h incubation with starvation medium. After the quiescent period the cells were pre-treated for 15 min with or without inhibitors as indicated, followed by incubation with fresh starvation medium with or without inhibitors with 0.5, 3, or 15 mM glucose with or without 10 nM IGFI-1 for the times indicated. The medium was removed, the cells were washed once with ice-cold phosphate-buffered saline, and the phosphate-buffered saline was replaced with ice-cold cell lysis buffer consisting of 50 mM HEPES (pH 7.5), 1% (v/v) Nonidet P-40, 2 mM EDTA, 0.5 mM sodium orthovanadate, 100 mM sodium fluoride, 1mM pyrophosphate, 4 mM EDTA, 1mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin. After sonication insoluble material was removed by centrifugation, and the samples were stored at −80 °C.

PDK-1 and PKB Kinase Assays—Cells were stimulated as described (see “Stimulation and Lysis Conditions”). The PKB immunoprecipitation and kinase assay were carried out following the procedures described in the manual, whereas some changes were made to the PDK-1 kinase assay. Active PDK-1 was immunoprecipitated as described in the manual. Briefly, ~1 μg of cell lysate was pre-cleared with 50 μl of protein G-agarose. The beads were removed, the lysate was then added to the protein G-Antibody complex, and the samples were left rotating for 2 h at 4 °C. After two washes with cell lysis buffer, followed by two washes with PDK-1 assay dilution buffer, the immunoprecipitates were resuspended in 30 μl of PDK-1 assay dilution buffer containing 10 μl (1 μg/ml) of GSK3 fusion protein and 10 μl of Mg/ATP mixture. The assay was started by the addition of 10 μl (0.5 μg/ml) of serum- and glucocorticoid-regulated protein kinase (SGK)-inactive recombinant protein. SGK is a member of the AGC subfamily of protein kinases whose kinase domain is not regulated by glucose. However, unlike other members of the AGC subfamily, SGK possess a pleckstrin homology domain enabling the assay to be carried out in the absence of PIP3. SGK is phosphorylated at the residue equivalent to Thr-308 of PKB, resulting in activation of this kinase (21). Having established conditions to ensure that the kinase activity was well within the linear portion of the reaction, samples were incubated for 7.5 min at 25 °C with continuous shaking. The reaction was terminated by the addition of 50 μl of 3X loading buffer, and samples were analyzed by immunoblotting (see “Protein Immunoblot Procedures”) with the phospho-GSK3α/β (Ser-21/9) antibody.

Construction of Adenoviruses—The P3K3 adenoviruses, inter Sro- 2 (Ad-VsI3), and the catalytic p10a subunit (Ad-Vp110) were constructed as described (22). The pUSEamp expression vectors containing Myc-His-tagged mouse PKBα (wild type), myr-PKBα (constitutively active), and PKBα-K179M (kinase-dead) were digested with the restriction enzymes HindIII and PmeI. Each of the cDNAs was inserted between the HindIII and Smal sites of pBluescript, providing the necessary restriction sites for insertion into pAdTrack-CMV between KpnI and NotI. The PKB adenoviruses were generated and purified as described (23, 24).

Adenovirus Infection—The appropriate titer for each adenovirus was determined by the addition of various dilutions of each adenovirus to cells subcultured in 6-well plates (9.5 cm2) to 60% confluence (~2 × 106 cells), giving a multiplicity of infection (m.o.i.) ranging from 50 to 2000 based on 0.5–2.0 × 109 plaque forming units/ml as measured by A590. The viral stock was replaced with complete medium after 2 h, and the cells were incubated at 37 °C in 5% CO2 for ~16 h. The cells were then transfected with 2 μg of pSuper described.

Protein Immunoblot Procedures—Cell lysates were normalized for total protein after levels were determined using the bichromonic acid protein assay kit. For immunoblot analysis 25–50 μg of protein was separated by SDS-polyacrylamide gel electrophoresis. After transfer to nitrocellulose, membranes were immersed in blocking buffer containing 5% (w/v) nonfat milk, 0.1% Tween, and 0.5% (w/v) BSA. Blots were then probed overnight with the primary antibodies described.
dry milk) and incubated with gentle agitation for 1 h. This was followed by incubation overnight at 4 °C with the appropriate primary antibody diluted in primary antibody dilution buffer (10 mM Tris/HCl, pH 8.0, 150 mM NaCl, 0.1% Tween, and 5% bovine serum albumin or 10% (w/v) nonfat dry milk). After a series of washes with blocking buffer without milk, the membranes were incubated for 1 h with gentle agitation with horseradish peroxidase-conjugated secondary antibody diluted in blocking buffer. Washes were repeated as before, and the positive signals were visualized with chemiluminescence reagent and x-ray film.

RESULTS

Constitutive Activation of PI3K in INS-1 Cells Only Increases IGF-1-induced β-Cell Proliferation—It has previously been shown that activation of PI3K is required for glucose and IGF-1-induced β-cell proliferation (4, 5). As such, it was examined whether bypassing the requirement for PI3K activation by an adenoviral mediated constitutively active form of PI3K would unveil the extent of the requirement for this enzyme activity on glucose and IGF-1-induced β-cell proliferation. Previous studies have shown that adenoviral expression of the iSH2 of the p85α subunit with the catalytic p110α subunit (p110) results in constitutive activation of PI3K (22). Because of the presence of a Myc tag on each of the subunits, INS-1 cells infected with recombinant adenovirus to express iSH2 (AdV-iSH2), p110 (AdV-p110), or iSH2 + p110 (AdV-p110 + AdV-iSH2) could be confirmed by subjecting the lysates to immunoblot analysis with the anti-Myc antibody (Fig. 1A). Bands at 35 and 115 kDa correspond to the predicted molecular mass of Myc-tagged iSH2 and p110, respectively, whereas the band at 40 kDa is nonspecific (as indicated by its presence in uninfected and AdV-β-Gal-infected control cells). The β-cell mitogenesis was determined in INS-1 cells infected with the control β-Gal (AdV-β-Gal), AdV-iSH2, AdV-p110, or AdV-p110 + AdV-iSH2. The uninfected and AdV-β-Gal controls showed similar increases in [3H]thymidine incorporation to 3 or 15 mM glucose + 10 nM IGF-1 (Fig. 1B), as observed previously (4, 5). For uninfected INS-1 cells at 15 mM glucose, β-cell mitogenesis was increased 19-fold (p < 0.01), and in the additional presence of 10 nM IGF-1 was 48-fold (p < 0.005) above the 0.5 mM glucose control (Fig. 1B). In AdV-p110-infected INS-1 cells β-cell mitogenesis in response to glucose and IGF-1 was not significantly different from the uninfected control. In addition, in AdV-iSH2-infected INS-1 cells glucose-induced β-cell mitogenesis was similar to the uninfected control; however, that at 15 mM glucose + 10 nM IGF-1 was elevated to 68.2 ± 8.4-fold (n = 3) above 0.5 mM glucose control (Fig. 1B). In AdV-p110-infected INS-1 cells β-cell mitogenesis in response to glucose and IGF-1 was not significantly different from the uninfected control. In addition, in AdV-iSH2-infected INS-1 cells glucose-induced β-cell mitogenesis was similar to the uninfected control; however, that at 15 mM glucose + 10 nM IGF-1 was elevated to 68.2 ± 8.4-fold (n = 3) above 0.5 mM glucose control (Fig. 1B).

Glucose and/or IGF-1 Does Not Affect PDK-1 Kinase Activity in INS-1 Cells—PDK-1 has been suggested to lie downstream
of PI3K in signal transduction pathways (15). It was determined whether PDK-1 plays a role in the mitogenic signal transduction in INS-1 cells in response to glucose \( \pm \) IGF-1. PDK-1 activity was assessed in INS-1 cells incubated with 0.5, 3.0, or 15 mM glucose \( \pm 10 \text{ nM IGF-1} \) for 2, 10, and 40 min. PDK-1 was found to be active at 0.5 mM glucose, but neither an increase in glucose nor the presence of IGF-1 had any significant effect on further increasing PDK-1 activity (Fig. 2B). Total levels of PDK-1 in each of the samples did not vary, indicating that equivalent amounts of PDK-1 were immunoprecipitated (Fig. 2A). Thus, it would appear that PDK-1 is present in INS-1 cells and is constitutively active.

**PKB Kinase Is Stimulated by IGF-1 but Not by Glucose in INS-1 Cells—**Activation of PI3K leads to downstream activation of PKB (25). It has been proposed that PKB translocation to the plasma membrane via its pleckstrin homology domain is increased via interaction with the PIP2 and PIP3 products synthesized by increased PI3K activity at the cell plasma membrane (11). PKB membrane localization appears to be sufficient to allow PDK-1-mediated phosphorylation of PKB at Thr-308 and partial activation, although full activation requires additional phosphorylation at Ser-473 by an as yet unidentified kinase (13, 14).

PKB Thr-308 and Ser-473 phosphorylation and consequential regulation of PKB activity was examined in INS-1 cells incubated at 0.5, 3.0, or 15 mM glucose \( \pm 10 \text{ nM IGF-1} \) for 2, 5, 10, 20, and 40 min. PKB activity did not change significantly when cells were incubated at basal 3 mM glucose or stimulatory 15 mM glucose \( \pm 10 \text{ nM IGF-1} \) for 2, 5, 10, 20, and 40 min and then subjected to immunoblot analysis with the phospho-GSK3α/β antibody or total-GSK3α/β antibody (Fig. 3A). Neither 3 or 15 mM glucose affected IGF-1-induced PKB Thr-308 or Ser-473 phosphorylation, also correlating with a lack of influence of glucose on IGF-1-induced PKB activity. The total amount of PKB did not significantly alter with glucose/IGF-1 treatment (Fig. 3B).

GSK3α/β has been shown to be a phosphorylation substrate of PKB, where phosphorylation of GSK3α/β by PKB inhibits GSK3α/β activity (26). It was determined whether glucose and/or IGF-1 could influence endogenous GSK3α/β phosphorylation in β-cells (Fig. 4). INS-1 cells were treated at basal 3 mM glucose or stimulatory 15 mM glucose \( \pm 10 \text{ nM IGF-1} \) for 2, 5, 10, 20, and 40 min and then subjected to immunoblot analysis with the phospho-GSK3α/β antibody or total-GSK3α/β antibody (Fig. 4). Neither 3 or 15 mM glucose had any significant effect on GSK3α/β phosphorylation over the 40-min time course (Fig. 4), correlating with a lack of effect of glucose on PKB activity (Fig. 3A). In contrast, IGF-1 rapidly and markedly increased GSK3α/β phosphorylation within 2 min that was sustained throughout the 40-min time course and was independent of the glucose activation (Fig. 4). This GSK3α/β phosphorylation pattern correlated with IGF-1-induced PKB activity and phosphorylation (Fig. 3, A and B). The total amount of GSK3α/β did not alter with glucose/IGF-1 treatment (Fig. 4).

**Time-dependent Phosphorylation of p70S6K by Glucose and IGF-1—**Phosphorylation and activation of p70S6K has also been postulated to be downstream of PKB activation via activation of mTOR by PKB (17, 18). However, mTOR activation can also be mediated by nutrients such as branch chain amino acids (27–30). It was investigated whether p70S6K activation paralleled that of PKB in INS-1 cells. The activation of p70S6K protein is accompanied by phosphorylation at multiple Ser/Thr residues that retards its migration during SDS-polyacrylamide gel electrophoresis (4, 5, 31). The time-dependent activation of p70S6K was investigated in INS-1 cells incubated at 3 or 15 mM glucose \( \pm 10 \text{ nM IGF-1} \) for 2, 5, 10, 20, and 40 min. The cell lysates were then subjected to immunoblot analysis with the phospho-p70S6K antibody. The results show that the phosphorylation of p70S6K was apparent after 10 min but only in the presence of 15 mM glucose \( \pm 10 \text{ nM IGF-1} \). Phosphorylation of p70S6K was not observed until after 20 min at 3 mM glucose \( \pm 10 \text{ nM IGF-1} \) or 15 mM glucose alone (Fig. 5). There was no apparent change in p70S6K phosphorylation at 3 mM glucose over the 40-min time period (Fig. 5). The greatest level of activation was observed after 40 min of stimulation in the presence of both 15 mM glucose and 10 mM IGF-1 (Fig. 5). It should be noted that although glucose activated p70S6K, it had no apparent effect on
PKB activity (Fig. 5 versus Fig. 3). Moreover, IGF-1-induced activation of p70S6K was relatively slow compared with that of IGF-1-induced PKB activation and was enhanced by stimulatory glucose concentrations, unlike PKB activity, which was not influenced by glucose (Fig. 5 versus Fig. 3).

**Glucose-stimulated p70S6K Phosphorylation Is Partially PI3K-independent**—It was investigated whether p70S6K phosphorylation activation by glucose and IGF-1 was dependent on upstream PI3K and/or mTOR activation by the use of two specific inhibitors, wortmannin and rapamycin. Wortmannin specifically inhibits PI3K (32), and rapamycin blocks mTOR activation by forming a complex with the immunophilin.
FKBP12 to generate a potent inhibitor of mTOR (33). INS-1 cells were pre-treated with or without inhibitors and stimulated with 3 or 15 mM glucose and 10 nM IGF-1 for 40 min with or without inhibitor. To confirm that the wortmannin and rapamycin were specifically inhibiting their target, we analyzed their effects on phosphorylation of PKB (as a positive control) and ERK1/ERK2 (as a negative control) in the same INS-1 cells. In the absence of inhibitor (control) PKB was shown to be phosphorylated on Thr-308 and Ser-473 by IGF-1 but not glucose (Fig. 6A), as previously demonstrated (Fig. 3). The IGF-1-induced phosphorylation of PKB was completely inhibited by wortmannin (100 nM) but not unaffected by rapamycin (25 mM), as compared with that of the control (Fig. 6A). PKB levels were equivalent in each sample, as indicated by total PKB immunoblot analysis (Fig. 6A). ERK1/ERK2 phosphorylation activation in INS-1 cells was detected by incubation with stimulatory 15 mM glucose and was increased further with additional IGF-1 (Fig. 6B), as previously observed (4, 5, 34). Phosphorylation of ERK1/ERK2 in response to 15 mM glucose ± 10 nM IGF-1 was not significantly affected by either wortmannin or rapamycin. ERK1/ERK2 levels were equivalent in each sample, as indicated by immunoblot analysis using a total ERK1/ERK2 antibody (Fig. 6B). In control cells, the phosphorylation activation of p70S6K was stimulated in samples treated with 15 mM glucose and by 10 nM IGF-1 at both 3 and 15 mM glucose (Fig. 6C). The greatest degree of phosphorylation was observed in cells treated with 15 mM glucose + 10 nM IGF-1 (Fig. 6C). In the presence of 100 nM wortmannin, the phosphorylation of p70S6K in cells treated with 15 mM glucose or 15 mM glucose + 10 nM IGF-1 was reduced but not completely blocked, whereas phosphorylation appeared to be completely inhibited in cells stimulated with 3 mM glucose + 10 nM IGF-1 (Fig. 6C). It appeared that IGF-1 no longer potentiated 15 mM glucose-induced p70S6K phosphorylation, because p70S6K phosphorylation by 15 mM glucose + 10 nM IGF-1 in the presence of wortmannin was equivalent to that by 15 mM glucose alone (Fig. 6C). In contrast, in the presence of rapamycin, the phosphorylation activation of p70S6K by glucose and IGF-1 was completely inhibited (Fig. 6C). Effect of PKB Overexpression on Mitogenesis in INS-1 Cells—It was investigated whether increased PKB expression would influence glucose/IGF-1-induced β-cell mitogenesis. Recombinant adenoviruses were generated to express three Myc/
His-tagged constructs of PKB, consisting of a wild type (AdV-PKB-WT), a constitutively active (AdV-PKB-CA), and a kinase-dead form (AdV-PKB-KD), in INS-1 cells. The constitutively active PKB was created by fusing c-Src-derived residues, required for myristoylation, to PKB. This additional sequence directly targets PKB to the plasma membrane, leading to its constitutive activation (35). The kinase-dead form has a point mutation, K179M, removing the ATP-binding site, which results in a loss of kinase activity (36).

PKB overexpression was analyzed by titration of adenovirus infection in INS-1 cells with subsequent PKB immunoblot analysis (Fig. 7). A GFP-expressing adenovirus (AdV-GFP) was used as a control and illustrated the endogenous levels of PKB (Fig. 7). Immunoblot analysis of INS-1 cells infected with AdV-PKB-WT, AdV-PKB-CA, or AdV-PKB-KD indicated a higher molecular weight PKB protein, in addition to the endogenous PKB (Fig. 7). This was due to the additional Myc/His tags on the adenovirally introduced PKB and was confirmed by immunoblotting with a c-Myc antibody (data not shown). The highest levels of expression (that did not adversely affect INS-1 cells) were obtained at a m.o.i. of 10 × 10^5, and this was the amount of adenovirus used for all subsequent experiments.

INS-1 cells were infected with AdV-PKB-WT, AdV-PKB-CA, AdV-PKB-KD, or a control adenovirus expressing luciferase (AdV-Luc) and were assayed for glucose/IGF-1-induced AdV-PKB-KD, or a control adenovirus expressing luciferase (AdV-Luc) and were assayed for glucose/IGF-1-induced AdV-PKB-WT, AdV-PKB-CA, AdV-PKB-KD, or AdV-GFP as a control adenovirus, made quiescent, and then incubated with 3 or 15 mM glucose or stimulatory 15 mM glucose (Fig. 8). Hence, we investigated the activation of elements in the IGF-1 signal transduction pathway in PKB-overexpressing cells (Fig. 9). INS-1 cells were infected with AdV-PKB-WT, AdV-PKB-CA, AdV-PKB-KD, or AdV-GFP as a control adenovirus, made quiescent, and then incubated with 3 or 15 mM glucose or stimulatory 15 mM glucose (Fig. 9A). The AdV-GFP-infected cells, PKB phosphorylation was only detected on Thr-308 and Ser-473 residues when stimulated with 10 nM IGF-1 at 3 mM glucose or stimulatory 15 mM glucose (Fig. 9A), as previously seen (Fig. 3). In AdV-PKB-WT-infected cells, the level of Thr-308 and Ser-473 phosphorylation, although slightly lower for the endogenous PKB compared with the AdV-GFP-infected control cells, was much greater for the adenoviral wild-type-expressed PKB (Fig. 9A). In the AdV-PKB-WT-infected cells there was increased basal phosphorylation, which was induced by IGF-1 on both Thr-308 and Ser-473 residues of PKB, independently of glucose, and this was in parallel with the endogenous PKB (Fig. 9A). In AdV-PKB-CA-infected INS-1 cells the level of phosphorylated adenovirally expressed PKB on both Thr-308 and Ser-473 residues was maximal under all conditions tested, as expected for the constitutively active variant of PKB, compared with wild type or kinase-dead PKB variants, and dampened the phosphorylation activation of endogenous PKB by IGF-1 (Fig. 9A). In AdV-PKB-KD-infected INS-1 cells, IGF-1-induced phosphorylation of Thr-308 and Ser-473 residues of endogenous PKB was similar to control AdV-GFP-infected cells. However, the levels of phosphorylation on Ser-473 of the adenovirally expressed kinase-dead PKB were markedly reduced under all conditions tested (Fig. 9A). In contrast, Thr-308 phosphorylation of the adenovirally expressed kinase-dead PKB was increased under basal conditions and stimulated by IGF-1 in a glucose-independent manner (Fig. 9A).

GSK3α/β is a downstream phosphorylation substrate of PKB, and as such its phosphorylation should parallel that of PKB. As previously seen (4, 5), our data indicated that PKB activation was involved in IGF-1-induced β-cell proliferation but not necessarily that by glucose (Figs. 3–6). As such, one might have predicted that overexpression of AdV-PKB-KD would inhibit IGF-1-induced β-cell mitogenesis at 15 mM glucose (as observed, Fig. 8), but it was surprising that overexpression of AdV-PKB-WT and especially AdV-PKB-CA also inhibited IGF-1-induced INS-1 cell proliferation at 15 mM glucose (Fig. 8). Hence, we investigated the activation of elements in the IGF-1 signal transduction pathway in PKB-overexpressing cells (Fig. 9). INS-1 cells were infected with AdV-PKB-WT, AdV-PKB-CA, AdV-PKB-KD, or AdV-GFP as a control adenovirus, made quiescent, and then incubated with 3 or 15 mM glucose or stimulatory 15 mM glucose (Fig. 9A), as previously seen (Fig. 3). In AdV-PKB-WT-infected cells, the level of Thr-308 and Ser-473 phosphorylation, although slightly lower for the endogenous PKB compared with the AdV-GFP-infected control cells, was much greater for the adenoviral wild-type-expressed PKB (Fig. 9A). In the AdV-PKB-WT-infected cells there was increased basal phosphorylation, which was induced by IGF-1 on both Thr-308 and Ser-473 residues of PKB, independently of glucose, and this was in parallel with the endogenous PKB (Fig. 9A). In AdV-PKB-CA-infected INS-1 cells the level of phosphorylated adenovirally expressed PKB on both Thr-308 and Ser-473 residues was maximal under all conditions tested, as expected for the constitutively active variant of PKB, compared with wild type or kinase-dead PKB variants, and dampened the phosphorylation activation of endogenous PKB by IGF-1 (Fig. 9A). In AdV-PKB-KD-infected INS-1 cells, IGF-1-induced phosphorylation of Thr-308 and Ser-473 residues of endogenous PKB was similar to control AdV-GFP-infected cells. However, the levels of phosphorylation on Ser-473 of the adenovirally expressed kinase-dead PKB were markedly reduced under all conditions tested (Fig. 9A). In contrast, Thr-308 phosphorylation of the adenovirally expressed kinase-dead PKB was increased under basal conditions and stimulated by IGF-1 in a glucose-independent manner (Fig. 9A).
insulin receptor substrate-1 cells and abolished in AdV-PKB-CA-infected cells. Phosphorylation was partly inhibited in AdV-PKB-WT-infected cells. p70S6K at basal 3 mM glucose was increased, but at stimulatory 15 mM glucose or in the added presence of IGF-1, p70S6K phosphorylation was not significantly different from that in AdV-GFP-infected control β-cells. In AdV-GFP-infected INS-1 cells, phosphorylation of GSK3β was comparable with that in AdV-GFP-infected cells and as such was likely to be due to endogenous PKB activity. This is consistent with previous results, which showed that the kinase-dead variant of PKB is not a dominant negative protein (15). Total levels of GSK3α/β were equivalent in all samples analyzed (Fig. 9B).

ERK1/ERK2 phosphorylation activation was investigated in PKB-overexpressing INS-1 cells (Fig. 9C). In AdV-GFP-infected INS-1 cells, ERK1/ERK2 phosphorylation was increased by 15 mM glucose, compared with basal 3 mM glucose and further enhanced on addition of 10 nM IGF-1 (Fig. 9C). Surprisingly, 15 mM glucose ± 10 nM IGF-1-induced ERK1/ERK2 phosphorylation was partly inhibited in AdV-PKB-WT-infected INS-1 cells and abolished in AdV-PKB-CA-infected β-cells. In contrast, ERK1/ERK2 phosphorylation activation by 15 mM glucose ≥ IGF-1 was not inhibited in AdV-PKB-KD-infected β-cells but, if anything, was enhanced (Fig. 9C). Total ERK1/ERK2 levels were equivalent in all samples examined (Fig. 9C). The inhibition of glucose ≥ IGF-1 ERK1/ERK2 phosphorylation in the presence of constitutively active PKB strongly suggests that there is cross-talk between the PI3K/PKB and ERK1/ERK2 signaling pathways.

The phosphorylation state of p70S6K in response to 40 min of incubation with glucose and IGF-1 was examined in adenoaxenial mediated PKB-overexpressing INS-1 cells (Fig. 9D). Phosphorylation of p70S6K was not detected in the AdV-GFP-infected control cells at basal 3 mM glucose, slightly increased at 3 mM glucose + 10 nM IGF-1 or 15 mM glucose alone, and further enhanced after treatment with 15 mM glucose + 10 nM IGF-1 (Fig. 9D), similar to observations in uninfected INS-1 cells (Fig. 6C). Similar patterns of p70S6K phosphorylation were observed in AdV-PKB-WT, AdV-PKB-KD-infected INS-1 cells (Fig. 9D). In AdV-PKB-CA-infected INS-1 cells, phosphorylation of p70S6K at basal 3 mM glucose was increased, but at stimulatory 15 mM glucose or in the added presence of IGF-1, p70S6K phosphorylation was not significantly different from that in AdV-GFP-infected control β-cells (Fig. 9D). These results suggested that constitutive activation of PKB did not overly affect glucose/IGF-1-induced phosphorylation activation of p70S6K.

**DISCUSSION**

Previous studies have not only shown that PI3K is important for glucose-dependent β-cell mitogenesis (4, 5) but that glucose induces the activity of PI3K (37). Hence, to gain more insight into the mechanisms involved in glucose-dependent IGF-1 β-cell mitogenesis, we studied the effects of overexpressing constitutively active PI3K by infecting INS-1 cells with AdV-p110 and AdV-iSH2. However, because AdV-p110- and AdV-iSH2-infected INS-1 cells only showed IGF-1-enhanced β-cell mitogenesis (Fig. 1B), it suggested that the glucose-dependent signaling events must lie elsewhere. This led us to focus on events downstream of PI3K.

Two such components downstream of PI3K are PDK-1 and PKB (15). The proposed model for PKB activation describes a complex process with a number of steps, one of which involves the protein PDK-1 (15). The first step is translocation of PKB from the cytosol to the plasma membrane, which is induced by increased levels of PIP3, synthesized at the plasma membrane by PI3K. The interaction of PIP3/PIP2 with the pleckstrin homology domain of PKB causes a conformational change allowing phosphorylation at Thr-308 and Ser-473, which appear to be important steps for full activation of PKB. PDK-1 has been shown to phosphorylate PKB at Thr-308; however, the kinase that phosphorylates PKB at Ser-473 has not yet been identified. We first analyzed the activity of PDK-1 in response to glucose and IGF-1. PDK-1 activity did not respond to glucose or IGF-1 in INS-1 cells (Fig. 2B). Hence, it appeared that PDK-1 exists in an active state under basal conditions in pancreatic β-cells and does not appear to be stimulated in response to glucose or IGF-1. This is consistent with reports in other cells that showed that PDK-1 is constitutively active in vivo (38, 39).

In addition, the expression of AdV-PKB-CA, which is constitutively active because of a membrane-targeting motif attached to the amino terminus, in INS-1 cells is sufficient to localize PKB to the membrane, resulting in phosphorylation of the Thr-308 and Ser-473 residues in unstimulated cells (Fig. 9A).
These results suggest that there is a significant amount of active PDK-1 present at the membrane of unstimulated cells. Therefore, although PDK-1 is an important component of the PI3K pathway, it does not appear to be a key regulatory component of the glucose or IGF-1 mitogenic response in pancreatic β-cells.

Similar studies were carried out to determine whether PKB activity was induced by glucose. However, only IGF-1 stimulated the activation of PKB, whereas glucose had no significant effect (Fig. 3). This lack of response to glucose is consistent with the data obtained from INS-1 cells infected with the constitutively active PI3K in which mitogenesis was only enhanced by IGF-1 and not glucose alone (Fig. 1B). Interestingly, the results from the time course of IGF-1-enhanced PKB phosphorylation show that phosphorylation of PKB at Ser-473 increases at a slower rate compared with PKB Thr-308 phosphorylation (Fig. 3B). Hence, the slower migrating species could be PKB phosphorylated at both Thr-308 and Ser-473. As such, these results suggested that phosphorylation of PKB at Thr-308 was first, followed by phosphorylation at Ser-473. Intriguingly, the PKB kinase-dead variant, although inactive (as confirmed by lack of GSK3α/β phosphorylation), was still phosphorylated at Thr-308 in a manner regulated by IGF-1. However, lack of phosphorylation at Ser-473 of the kinase-dead PKB suggested that Ser-473 phosphorylation of PKB might well be an autophosphorylation event. If so, the slight amount of IGF-1-induced kinase-dead PKB Ser-473 phosphorylation observed was probably due to endogenous PKB activity (Fig. 9A). These data are consistent with a recent report suggesting that Thr-308 is phosphorylated first, followed by autophosphorylation of the Ser-473 residue of PKB (40).

A known substrate of PKB is GSK3α/β, a protein kinase implicated in several biological and metabolic processes (26). GSK3α/β phosphorylation by PKB leads to down-regulation of its activity. However, only IGF-1 stimulated GSK3α/β phosphorylation at serine (Fig. 3B). Hence, the slower migrating species could be PKB phosphorylated at both Thr-308 and Ser-473. As such, these results suggested that phosphorylation of PKB at Thr-308 was first, followed by phosphorylation at Ser-473. Intriguingly, the PKB kinase-dead variant, although inactive (as confirmed by lack of GSK3α/β phosphorylation), was still phosphorylated at Thr-308 in a manner regulated by IGF-1. However, lack of phosphorylation at Ser-473 of the kinase-dead PKB suggested that Ser-473 phosphorylation of PKB might well be an autophosphorylation event. If so, the slight amount of IGF-1-induced kinase-dead PKB Ser-473 phosphorylation observed was probably due to endogenous PKB activity (Fig. 9A). These data are consistent with a recent report suggesting that Thr-308 is phosphorylated first, followed by autophosphorylation of the Ser-473 residue of PKB (40).
Differential Activation of PKB and p70S6K in β-Cells

Hydration, whereas glucose had no effect (Figs. 4 and 9B). This lack of response to glucose and the stimulation of GSK3α/β phosphorylation by IGF-1 correlated with the activation of PKB (Fig. 4). Hence, these results suggest that GSK3α/β is activated by IGF-1 in a glucose-independent manner. However, it is not clear what lies downstream of IGF-1-mediated GSK3α/β inhibition in β-cells and if it relates to the control of β-cell mitogenesis. Hence the potential biological role of GSK3α/β in β-cells awaits future experimental investigation. Additional proteins that are important in mitogenic signaling are mTOR and p70S6K. These enzymes are activated, in part, by a PI3K-dependent pathway and are critical for cell proliferation (18, 41–43). Although a role for mTOR in mitogenesis has been implicated, little is known about the molecular mechanisms involved in regulating the activation of this large molecular mass protein. The mitogenic activation of p70S6K is also complex due to a requirement for hierarchical phosphorylation at multiple sites (31, 44). However, there is substantial evidence that mTOR mediates the phosphorylation of p70S6K, because rapamycin, which interacts with and inhibits mTOR, blocks the activation of p70S6K in response to mitogenic stimuli (45). A number of reports exist that strongly suggest that p70S6K is downstream of PKB via the activation of mTOR (17, 18). However, the phosphorylation activation of p70S6K in pancreatic β-cells did not correlate with the activation of PKB. In this regard, the activation of p70S6K was much slower compared with PKB activation (Fig. 5 versus Fig. 3), and whereas glucose stimulated p70S6K activation (Figs. 5 and 9), glucose had no effect on upstream control of PKB activity (Fig. 3). In addition, p70S6K phosphorylation activation by glucose was not inhibited by the PI3K-specific inhibitor, wortmannin, in contrast to an apparent complete inhibition of IGF-1-induced p70S6K and PKB phosphorylation activation by wortmannin (Fig. 6). In addition, overexpression of AdV-PKB-KD did not appear to change the activation of p70S6K in response to IGF-1 and/or glucose compared with the AdV-PKB-WT (Fig. 9D), even though complete phosphorylation activation of AdV-PKB-KD did not occur (Fig. 9A). However, the mTOR-specific inhibitor, rapamycin, completely abolished p70S6K activation phosphorylation by IGF-1 and glucose (Fig. 6D). Hence, taken together, these results strongly support a model in which p70S6K activation by glucose is via mTOR, bypassing a requirement for PKB activation in β-cells. This is consistent with other reports that have shown p70S6K activation via mTOR, independent of PKB, by other nutrient sources such as branched chain amino acids (27, 28, 30). In addition, studies in yeast suggest that mTOR is involved in regulating cellular responses to nutrient availability (46). In contrast, IGF-1 activated the PI3K/PKB/mTOR signaling pathway. Therefore the mitogenic stimulation by glucose is, at least in part, PKB-independent, and mTOR may mediate the glucose-dependent aspect of the stimulated phosphorylation of p70S6K. Hence, mTOR may be one major component regulating glucose-dependent β-cell mitogenesis in addition to the glucose activation of ERK1/ERK2 (34). Although it is clear that glucose metabolism is required, the secondary signals that activate kinases to induce the activation of signaling proteins, such as mTOR and ERK1/ERK2, have yet to be identified. One possible candidate is Ca2+, because the inhibition of glucose-induced Ca2+ levels in β-cells prevents the phosphorylation of ERK1/ERK2, suggesting that ERK1/ERK2 phosphorylation is in part regulated by a Ca2+-dependent protein kinase (34). It was not too surprising that the kinase-dead form of PKB, which does not appear to act as a dominant negative protein, had little effect on β-cell mitogenesis, due to compensation by endogenous PKB (Fig. 8). However, it was unexpected that overexpression of the wild type or constitutively active forms of PKB did not increase β-cell mitogenesis. The phosphorylation activation of p70S6K by glucose and IGF-1 was not enhanced by overexpression of the wild type or constitutively active forms of PKB (Fig. 9D), reaffirming the importance of glucose activation bypassing PKB and acting directly through mTOR activation (as discussed above). Surprisingly, the constitutively active PKB inhibited the IGF-1 and/or glucose phosphorylation activation of ERK1/ERK2 (Fig. 9C). One possible mechanism by which this may occur is through inhibition of Ras1 activity, because studies have shown that this enzyme is negatively regulated by PKB phosphorylation (47, 48). Hence, these PKB inhibitory effects on the ERK1/ERK2 pathway probably contribute to the decrease in β-cell mitogenesis observed in IGF-1-treated cells expressing the constitutively active or wild type forms of PKB. Interestingly, the ERK1/ERK2 phosphorylation levels in IGF-1- and/or glucose-stimulated INS-1 cells expressing the kinase-dead form of PKB were slightly higher compared with those in control-infected cells (Fig. 9C). Therefore, although PKB activity is diminished in AdV-PKB-KD-expressing INS-1 cells, the ERK1/ERK2 signaling pathway appears to be more active. These observations of adenovirus-mediated PKB overexpression in β-cells strongly suggest that there is a requirement for both the PI3K and ERK1/ERK2 signaling pathways for glucose-dependent IGF-1-induced β-cell mitogenesis. Moreover, the data imply that there is a tightly controlled balance between PKB and ERK1/ERK2 signaling pathway elements, both kinetically and of their expression levels; otherwise, negative cross-talk between the pathways would likely dampen signal transduction. It should also be considered that other targets downstream of PKB, such as BAD, caspase-9, and forkhead transcription factors (reviewed in Ref. 49), which affect the mitogenesis and/or survival of β-cells, may contribute to control of β-cell mass and warrant further investigation. These elements downstream of PKB may be important factors that play a major role in the pathogenesis of type 2 diabetes if adversely regulated by prolonged hyperglycemia and/or hyperlipidemia, which in turn could contribute to the failure of β-cell expansion. Alternatively, if PKB downstream elements can be carefully controlled pharmacologically, this may provide a means of preserving or even enhancing β-cell mass as a potential therapy for both type 1 and 2 diabetes (2).Acknowledgement—We are grateful to Cynthia Jacobs in the preparation of this manuscript.

REFERENCES

1. Swenne, I., Borg, L. A., Crane, C. J., and Schnell Landstrom, A. (1992) Diabetologia 35, 939–945
2. Rhodes, C. J. (2000) J. Mol. Endocrinol. 24, 303–311
3. Withers, D. J., Gutierrez, J. S., Tewary, H., Burks, D. J., Ren, J. M., Previs, S., Zhang, Y., Bernai, D., Pons, S., Shulman, G. I., Bonner-Weir, S., and White, M. F. (1998) Nature 391, 900–904
4. Cousin, S. P., Hugl, S. R., Myers, M. G., Jr., White, M. F., Reifel-Miller, A., and Rhodes, C. J. (1999) Biochem. J. 344, 649–658
5. Hugl, S. R., White, M. F., and Rhodes, C. J. (1998) J. Biol. Chem. 273, 17712–17719
6. Billestrup, N., Hansen, J. A., Hansen, L. H., Moldrup, A. H., Gaagaard, E. D., and Nielsen, J. H. (1998) Endocr. J. 45, suppl. 41–45
7. Myers, M. G., Jr., Grammer, T. C., Wang, L. M., Sun, X. J., Pierre, J. H., Blienis, J., and White, M. F. (1994) J. Biol. Chem. 269, 28783–28789
8. Kodawadi, T., Tobe, K., Honda-Yamamoto, R., Tanemoto, H., Kaburagi, Y., Momomura, K., Ueki, K., Takahashi, Y., Yamauchi, A., Akamatsu, Y., and Yasuzaki, Y. (1996) Endocr. J. 43, suppl. 33–41
9. Benito, M., Valverde, A. M., and Lorenzo, M. (1996) Int. J. Biochem. Cell Biol. 28, 499–510
10. Yenush, L. and White, M. F. (1997) Bioessays 19, 491–500
11. Downward, J. (1998) Science 273, 673–674
12. Alesi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. (1996) EMBO J. 15, 6541–6551
13. Alesi, D. R., James, S. R., Downes, C. P., Gaffney, P. R., Reese, C. B., and Cohen, P. (1997) Curr. Biol. 7, 261–269
14. Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F., and Hawkins, P. T. (1997) Science 277, 567–570
15. Vanhaesebroeck, B., and Alessi, D. R. (2000) Biochem. J. 346, 561–576
16. Cross, D. A., Alesi, D. R., Cohen, P., Andjelkovic, M., and Hemmings, B. A.
Differential Activation of PKB and p70^{56K} in β-Cells

Yano, H., Nakanishi, S., Kimura, K., Hanai, N., Saitoh, Y., Fukui, Y., Nonomura, Y., and Matsuda, Y. (1993) J. Biol. Chem. 268, 25846–25856

Brown, E. J., and Schreiber, S. L. (1996) Cell 86, 517–520

Kohn, A. D., Summers, S. A., Birnbaum, M. J., and Roth, R. A. (1996) J. Biol. Chem. 271, 31772–31778

Franke, T. F., Yang, S. I., Chan, T. O., Datta, K., Kaslaukas, A., Morrison, D. K., Kaplan, D. R., and Tsichlis, P. N. (1995) Cell 81, 727–736

Cousin, S. P., Hugl, S. R., Wrede, C., Kajio, H., Myers, M. G., Jr., and Rhodes, C. J. (2001) Endocrinology, 142, 229–240

Alessi, D. R., Deak, M., Casamayor, A., Caudwell, F. B., Morrice, N., Norman, D. G., Gaffney, P., Reese, C. B., MacDougall, C. N., Harbison, D., Ashworth, A., and Bownes, M. (1997) Curr. Biol. 7, 776–789

Casamayor, A., Morrice, N. A., and Alessi, D. R. (1999) Biochem. J. 342, 287–292

Toker, A., and Newton, A. C. (2000) J. Biol. Chem. 275, 8271–8274

Dumont, F. J., and Su, Q. (1996) Life Sci. 58, 373–395

Proud, C. G. (1996) Trends Biochem. Sci. 21, 181–185

Chou, M. M., and Brenis, J. (1995) Curr. Opin. Cell Biol. 7, 806–814

Pullen, N., and Thomas, G. (1997) FEBS Lett. 410, 78–82

Dufner, A., and Thomas, G. (1999) Exp. Cell Res. 253, 100–109

Thomas, G., and Hall, M. N. (1997) Curr. Opin. Cell Biol. 9, 782–787

Zimmermann, S., and Moelling, K. (1999) Science 286, 1741–1744

Guan, K. L., Figueroa, C., Brtva, T. R., Zhu, T., Taylor, J., Barber, T. D., and Vojtek, A. B. (2000) J. Biol. Chem. 275, 27354–27359

Datta, S. R., Brunet, A., and Greenberg, M. E. (1999) Genes Dev. 13, 2965–2977
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