THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 2, Issue of January 8, pp. 1011–1016, 1999

Printed in U.S.A.

Glucose Stimulates Translocation of the Homeodomain Transcription Factor PDX1 from the Cytoplasm to the Nucleus in Pancreatic β-Cells*

(Received for publication, June 11, 1998, and in revised form, October 7, 1998)

Wendy M. Macfarlane‡, Caroline M. McKinnon‡, Zoe A. Felton-Edkins‡, Helen Cragg‡, Roger F. L. James§, and Kevin Docherty‡¶

From the ‡Department of Molecular and Cell Biology, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD and the §Department of Surgery, University of Leicester, Leicester Royal Infirmary, Leicester LE2 7LX, United Kingdom

One of the mechanisms whereby glucose stimulates insulin gene transcription in pancreatic β-cells involves activation of the homeodomain transcription factor PDX1 (pancreatic/duodenal homeobox-1) via a stress-activated pathway involving stress-activated protein kinase 2 (SAPK2, also termed RK/p38, CSBP, and Mxi2). In the present study we show, by Western blotting and electrophoretic mobility shift assay, that in human islets of Langerhans incubated in low glucose (3 mM) PDX1 exists as an inactive 31-kDa protein localized exclusively in the cytoplasm. Transfer of the islets to high (16 mM) glucose results in rapid (within 10 min) conversion of PDX1 to an active 46-kDa form that was present predominantly in the nucleus. Activation of PDX1 appears to involve phosphorylation, as shown by incorporation of $^{32}P_i$ into the 46-kDa form of the protein. These effects of glucose could be mimicked by chemical stress (sodium arsenite), or by overexpression of SAPK2 in the β-cell line MIN6. Overexpression of SAPK2 also stimulated PDX1-dependent transcription of a –50 to –250 region of the human insulin gene promoter linked to a firefly luciferase reporter gene. The effects of glucose were inhibited by the SAPK2 inhibitor SB 203580, and by wortmannin and LY 294002, which inhibit phosphatidylinositol 3-kinase, although the effects of stress (arsenite) were inhibited only by SB 203580. These results demonstrate that glucose regulates the insulin gene promoter through activation and nuclear translocation of PDX1 via the SAPK2 pathway.

The homeodomain transcription factor PDX1 (pancreatic/duodenal homeobox-1), which has previously been called IPF1 (1), IDX1 (2), STF1 (3), or IUF1 (4), plays an important role in lineage determination in the developing endocrine pancreas (5, 6). PDX1 binds to four sites (A1, A2, A3, and A5) with the consensus sequence C(C/T)TAATG in the human insulin promoter (7). Although it can transactivate the insulin promoter (8–10), its exact role in basal insulin transcription is unclear since insulin gene transcription can occur in the absence of PDX1 (11, 12). However, there is strong evidence supporting a role for PDX1 in the mechanisms whereby glucose stimulates insulin gene transcription in response to changes in glucose concentrations (13–15). Furthermore, glucose metabolism has been shown to stimulate PDX1 DNA binding activity and insulin promoter activity in β-cells via a stress-activated signaling pathway involving stress-activated protein kinase 2 (SAPK2, also termed RK/p38, CSBP, and Mxi2) (16).

SAPK2 is a member of an expanding family of mitogen-activated protein kinase-related kinases that are activated in response to adverse stimuli such as heat, osmotic shock, ultraviolet light, DNA-damaging reagents and by proinflammatory cytokines that are produced under conditions of stress (17). In pancreatic β-cells, glucose metabolism stimulates the SAPK2 pathway by a mechanism that involves phosphatidylinositol 3-kinase (PI 3-kinase),[1] while stress (*e.g.* arsenite treatment) stimulates the SAPK2 pathway in β and other cell types by a mechanism independent of PI 3-kinase (16).

Recombinant PDX1, synthesized in *Escherichia coli*, has a molecular mass of 31 kDa, similar to that predicted from its amino acid sequence. It is inactive, insofar as it does not bind to its recognition sequence in the insulin promoter as measured by electrophoretic mobility shift assay. However, treatment of recombinant PDX1 with SAPK2 in the presence of Mg-ATP and a β-cell extract activates its DNA binding, concomitant with a shift in molecular mass from 31 kDa to 46 kDa (16). The molecular modifications responsible for this shift in molecular mass are as yet unclear. In the present study we show that PDX1 exists as the inactive 31-kDa form in the cytoplasm of human islets incubated in low (3 mM) glucose. Treatment with 16 mM glucose stimulates conversion to the active 46-kDa form, which is localized predominantly in the nucleus. These events are inhibited by SB 203580 (5 μM) (an inhibitor of SAPK2), wortmannin, and LY 294002 (inhibitors of PI 3-kinase), and mimicked by sodium arsenite and overexpression of SAPK2.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents—Radiochemicals were purchased from Amersham International (Slough, Berks, United Kingdom (UK)), sodium arsenite from Fisons (Loughborough, UK), and wortmannin from Sigma (Poole, UK). SB 203580 was a generous gift from Dr. J. Lee and Dr. P. Young (SmithKline Beecham, King of Prussia, PA). Anti-PDX1 antibody was a kind gift from Dr. C. V. Wright (Vanderbilt University, Nashville, TN). Anti-SAPK2 antibody was purchased from New England Biolabs (Cambridge, UK). Anti-upstream stimulatory factor (USF) antibody was kindly provided by Dr. M. Sawadogo (University of Texas, Houston, TX).

Oligonucleotides—Oligodeoxynucleotides were purchased from Alta Bioscience (University of Birmingham, Birmingham, UK). Single-stranded complementary oligonucleotides were annealed as described

* This work was supported by grants from the Wellcome Trust and the British Diabetic Association and by a studentship (to C. M. M.) from the British Diabetic Association. 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. Fax: 44-1224-273144.

[1] The abbreviations used are: PI 3-kinase, phosphatidylinositol 3-kinase; USF, upstream stimulatory factor; EMSA, electrophoretic mobility shift assay; PAGE, polyacrylamide gel electrophoresis; IL, interleukin; NF, nuclear factor.

previously (18), and labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Oligonucleotide B, corresponding to the A3 site of the human insulin gene promoter, is a complementary double-stranded 30-mer 5′-CCCCTGGTTAAGACTCTAATGACCCGCTGG-3′.

Isolation and Treatment of Human Islets of Langerhans—Human islets of Langerhans were isolated from the pancreas of human organ donors, and all procedures were carried out with the approval of the appropriate Ethical Committee. The pancreatic duct was cannulated *in situ* and digestion achieved by intraductal infusion of collagenase. The islets were then separated from contaminating acinar tissue by centrifugation on discontinuous density gradients of bovine serum albumin in a semi-automated system (19). The purified islets were placed in RPMI 1640 medium (Life Technologies, Inc.) containing 10% (v/v) fetal calf serum and supplemented with 400 IU/ml sodium penicillin G and 200 μg/ml streptomycin sulfate, and cultured at 37 °C in a humidified atmosphere of O_2:CO_2 (95:5) for several days prior to use. Selected islets were separated into batches of 120–150 in Hanks' buffered saline (0.12 M NaCl, 5.4 mM KCl, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.4 mM $MgSO_4$, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM $NaHCO_3$, 5% (w/v) bovine serum albumin) except where specified.

Preparation of Cell Extracts—Nuclear and cytoplasmic extracts were prepared using a modification of the method of Schreiber *et al.* (20). Cells were centrifuged for 10 s in a microcentrifuge, and resuspended in 400 μl of 10 mM Hepes, pH 7.9, containing 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 0.5 mM phenylmethanesulfonyl fluoride, 10 mM NaF, 10 mM sodium molybdate, 10 mM β-glycerophosphate, 10 mM sodium vanadate, and 10 mM *p*-nitrophenyl phosphate. Cells were allowed to swell on ice for 15 min before adding 25 μl of 10% (v/v) Nonidet P-40. The islets were then incubated for an additional 30 min on ice, vortexed for 15 s, and centrifuged for 30 s in a microcentrifuge. The cytoplasmic fraction (supernatant) was removed and frozen in small aliquots, and the pellet, which was enriched in nuclei, was resuspended in 50 μl of 20 mM Hepes, pH 7.9, containing 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, 0.1 mM *p*-aminobenzoic acid, 10 μg/ml aprotinin, 5% (v/v) glycerol, 10 mM NaF, 10 mM sodium molybdate, 10 mM β-glycerophosphate, 10 mM sodium vanadate, and 10 mM p-nitrophenyl phosphate. Nuclear extracts were then centrifuged for 2 min at 4 °C in a microcentrifuge. The supernatant was collected, aliquoted into small volumes, and stored at −70 °C. For whole cell extracts, Nonidet P-40 lysis was replaced with incubation with 1% (v/v) Triton X-100, vortexing for 1 min, and centrifugation for 2 min.

Electrophoretic Mobility Shift Assays (EMSAs)—EMSAs were performed as described previously (16). Cell extracts (0.5 μg of protein) were incubated with radiolabeled probe for 20 min at room temperature in buffer containing 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 5 mM dithiothreitol, 1 mM EDTA, and 5% (v/v) glycerol.

Cell Culture—MIN6 cells were grown in Dulbecco's modified Eagle's medium containing 5 mM glucose, supplemented with 15% heat-inactivated myoclone fetal calf serum (Sigma) and 2 mM L-glutamine, in a humidified atmosphere containing 95% air, 5% CO_2.

Plasmids—The control construct pGL-LUC is based on the plasmid pGL2 (Promega), with the thymidine kinase promoter from the herpes simplex virus cloned 5′ to the firefly luciferase gene. The construct pGL-LUC200 varies from this in that it contains 200 base pairs of the human insulin gene promotor. A −60 to −260 base pair *Hinc*II-*Pvu*II fragment from the human insulin gene promoter was blunt-ended and cloned into the *Sma*I site of the control construct. DNA was prepared using the Qiagen Endotoxin-Free Maxiprep method, and quantitated spectrophotometrically. PDX1 expression vector pCR3-PDX1 was as described previously (11). The pSG5-SAPK2 (21) expression vector was a generous gift from Dr. S. Keyse, University of Dundee.

Transfections—Cells were grown to about 80% confluence in six-well plates, and were transfected by mixing 4 μg of DNA and 54 μl of a 1 nM lipid suspension containing a 2:1 mixture of dioleoyl-L-α-phosphatidylethanolamine (Sigma) and dimethyl-dioctadecylammonium bromide (Fluka) in 1 ml of serum free Opti-MEM (Life Technologies, Inc.). The lipid-DNA complexes were allowed to form for 20 min at room temperature before being added to the washed cells. Following 5 h of incubation, 1 ml of complete medium containing 30% heat-inactivated myoclone fetal calf serum was added to the cells. After 12 h, the medium-DNA complexes were replaced by complete medium and the cells left for another 24 h before treating. Treatment of all cells started with a 5-h preincubation in Dulbecco's modified Eagle's medium containing 0.5 mM glucose. Cells were then removed from the wells and a cell pellet recovered by centrifugation at 7000 rpm for 30 s. The cell pellet was resuspended in 70 μl of 100 mM KH_2PO_4, pH 7.8, 1 mM dithiothreitol solution and lysed by freeze/thawing three times. Cell debris was removed by centrifugation at 13,000 rpm for 1 min.

Luciferase Assay—30 μl of cell extract was added to 350 μl of buffer A, pH 7.8 (15 mM $MgSO_4$, 30 mM glycylglycine, 2 mM Na_2ATP) containing 0.45 mM coenzyme A and 2.56 mM Triton X-100. To this, 150 μl of buffer G (30 mM glycylglycine) containing 0.5 mM luciferin (Sigma) was injected and the luminescence read at 560 nM using a Berthold Luma LB9501. Protein content of the cell extract was measured by taking 6 μl of extract and adding144 μl of water and 150 μl of Coomassie protein assay reagent (Pierce). Protein content was read at a wavelength of 620 nm using a Titer Tech Multiscan MCC340. A standard curve of known bovine serum albumin protein concentrations was used to calculate the protein concentrations of the samples.

Western Blotting—For Western blot analysis, 1-μg samples of cell extract were fractionated by SDS-PAGE, blotted on to ECL-nitrocellulose membrane (Amersham), and incubated for 60 min in a buffer containing 10 mM Tris-HCl, 0.05% (v/v) Tween 20, 0.5 M NaCl, and a 1:5000 dilution of anti-PDX1 antibody. The antigen-antibody complex was then detected by incubating the membrane for another 60 min in buffer containing a 1:5000 dilution of horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (ECL, Amersham).

$^{32}P_i$ Labeling and PDX1 Immunoprecipitation—MIN6 cells were washed three times in Krebs Ringer (118 mM NaCl, 4.75 mM KCl, 1.2 mM $MgCl_2$, 0.26 mM $CaCl_2$, 25 mM $NaHCO_3$) prior to addition of 0.5 mCi of $^{32}P_i/10^6$ cells in Krebs Ringer. Cells were incubated at 37 °C for 1 h, prior to addition of glucose to 0.5 or 16 mM final concentration. After 30 min cells were washed three times in Krebs Ringer and harvested, with nuclear and cytoplasmic extracts prepared as before. For PDX1 immunoprecipitation: 400-μl samples in 1× NDET (1% Nonidet P-40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris-HCl, pH 7.4) were precleared by addition of 5 μl of preimmune rabbit serum and 10 μl of protein A (in 1× NDET), with rotation at 4 °C for 2 h. Preclearing was completed by centrifugation at 13,000 rpm for 2 min at 4 °C, with the resulting pellet being discarded. 3 μl of anti-PDX1 antibody and 10 μl of protein A-Sepharose were added to 400 μl of supernatant, and PDX1 immunoprecipitated by rotation at 4 °C for 16 h. A 2-min centrifugation yielded a pellet, which was then resuspended in 0.5 ml of NDET + 0.3% SDS, with rotation at 4 °C for 2 h. Samples were then layered over 0.5 ml of NDET + 0.3% SDS/30% sucrose, and centrifuged for 5 min at 4 °C. The resulting pellet was resuspended in 0.5 ml of NDET and rotated at 4 °C for another 2 h. Finally, the samples were centrifuged for 4 min at 4 °C, with the final pellet resuspended in 30 μl of SDS-PAGE sample buffer (4% SDS, 0.02% bromphenol blue, 0.5 M sucrose, 10 mM Tris-HCl, pH 6.8, 10% β-mercaptoethanol). Samples were heated to 80 °C for 5 min prior to loading.

RESULTS

Initial experiments were undertaken to determine the effect of glucose on the molecular mass and intracellular location of PDX1. Human islets of Langerhans were incubated for 5 h in 3 mM glucose, transferred to 16 mM glucose, and PDX1 analyzed at various time intervals by Western blotting of whole cell extracts. In 3 mM glucose PDX1 was present as a 31-kDa protein (Fig. 1*A*). Within 10 min in 16 mM glucose, the 31-kDa protein was converted to the 46-kDa form. Conversion was complete within 15 min. To investigate the effect of glucose on the intracellular location of the two forms of PDX1, human islets were incubated in 3 or 20 mM glucose and cytoplasmic and nuclear fractions prepared. In 3 mM glucose, the 31-kDa form was present in the cytoplasm; however, in 16 mM glucose, the 46-kDa form was present in the cytoplasm and the nucleus (Fig. 1*B*). Treatment with acid phosphatase converted the 46-kDa form to the 31-kDa form. In the presence of excess inorganic pyrophosphate, acid phosphatase had no effect on the 46-kDa form, confirming that conversion to the 31-kDa form was associated with dephosphorylation of the 46-kDa form. The 31-kDa form was inactive as measured by EMSA (Fig. 1*C*, *lanes 1–4*), whereas the 46-kDa form present in the cytoplasm and the nucleus was active (Fig. 1*C*, *lanes 4–8*). Identification of the retarded band in Fig. 1*C* as PDX1 was confirmed by oligonucleotide and antibody competition experiments (data not shown).

The role of phosphorylation in the activation of PDX1 was investigated further by labeling of MIN6 cells with inorganic

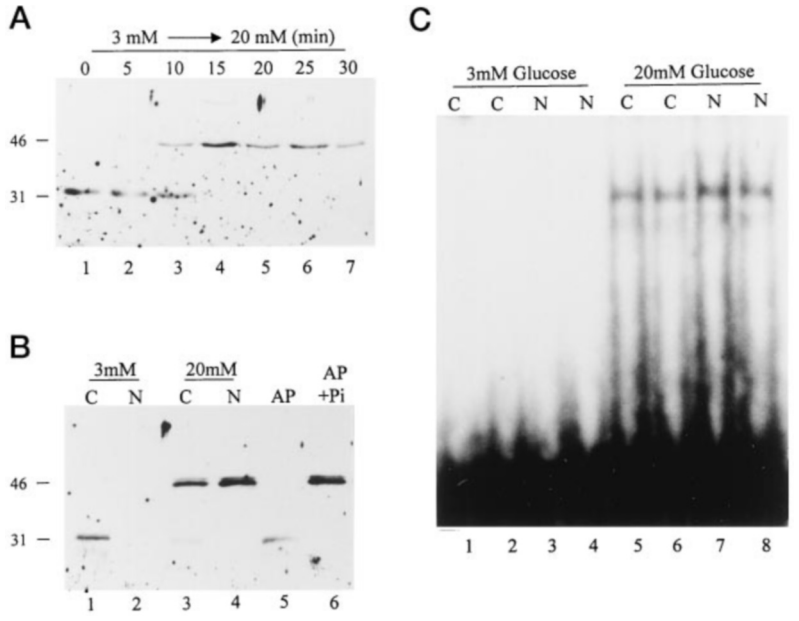

FIG. 1. **PDX1 is rapidly modified by high glucose concentrations in human islets of Langerhans.** *A*, Western blot analysis of human islets of Langerhans incubated in 3 mM glucose for 5 h, then transferred to 20 mM glucose for the time periods indicated. Whole cell extracts were prepared, and probed with a specific anti-PDX1 antibody. *B*, Western blot analysis of cytoplasmic (*C*) and nuclear (*N*) extracts prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h, or 3 mM glucose for 5 h followed by 20 mM glucose for 30 min. In *lane 5*, high glucose nuclear extract was incubated for 30 min with 10 units of potato acid phosphatase (*AP*). In *lane 6*, acid phosphatase treatment was carried out in the presence of 50 mM pyrophosphate (P_i). *C*, electrophoretic mobility shift assay of cytoplasmic (*C*) and nuclear (*N*) samples prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h, or 3 mM glucose for 5 h followed by 20 mM glucose for 30 min, using the A3 site of the human insulin gene promoter as a probe.

$^{32}P_i$ and subsequent immunoprecipitation of PDX1. At high glucose concentrations, $^{32}P_i$ was incorporated into the 46-kDa form of PDX1 (Fig. 2, *lanes 3* and *4*), but at low glucose concentrations no such incorporation was observed (*lanes 1* and *2*). Western blot analysis of the immunoprecipitated samples (Fig. 2*B*) confirmed that both forms of the protein were present, but that ^{32}P was incorporated only into the 46-kDa form of the protein, which was observed at high glucose concentrations. To evaluate the effectiveness of the biochemical fractionation of the β-cell extracts, nuclear and cytoplasmic fractions were analyzed by Western blotting using a specific anti-USF antibody. USF binds to the E2 site of the human insulin gene promoter and has a molecular size of 43 kDa (Fig. 2*C*). Blotting confirmed the presence of USF solely in the nuclear fractions, independently of glucose concentrations.

Since SAPK2 and PI 3-kinase had previously been implicated in the stimulation of PDX1 DNA binding activity, the possible involvement of these enzymes in the glucose-stimulated translocation of PDX1 to the nucleus was next investigated. Fig. 3*A* shows that glucose-stimulated conversion of PDX1 from the 31-kDa to the 46-kDa form and translocation of the 46-kDa form into the nucleus was inhibited by the SAPK2 inhibitor SB 203580, and by the PI 3-kinase inhibitors wortmannin and LY 294002 (Fig. 3A). SAPK2 is a stress-activated protein kinase, the activity of which can be stimulated by treating cells with the stress-inducing agent sodium arsenite (1 mM). The effects of glucose on PDX1 molecular mass and intracellular location were mimicked by sodium arsenite, further supporting a role for SAPK2 in these events (Fig. 3*B*). As shown previously (16) the effects of arsenite were inhibited by SB 203580 but not by wortmannin and LY 294002 (Fig. 3*B*). The IC_{50} for SB 203580 on both glucose and arsenite effects was 3 μM (data not shown).

To investigate further the involvement of SAPK2, MIN6 cells were transfected with a cDNA encoding SAPK2. Overexpression was confirmed by the appearance of a 38-kDa immunoreactive protein in extracts from transfected cells that was absent in untransfected cells (Fig. 4*A*). In untransfected MIN6 cells treated with 3 mM glucose, PDX1 was present as the 31-kDa form in the cytoplasm. Following treatment with 16 mM glucose, PDX1 was converted to the 46-kDa form, which was present in the cytoplasm and nucleus. When cells transfected with SAPK2 were incubated in 3 mM glucose, PDX1 was present as the 46-kDa form in the cytoplasm and in the nucleus (Fig. 4*B*). This result suggested that overexpression of SAPK2 resulted in conversion of PDX1 to the 46-kDa form that was localized predominantly in the nucleus. Overexpression of SAPK2 also activated PDX1 DNA binding activity in low glucose-treated MIN6 cells (Fig. 4*C*). The critical events governing modification and activation of PDX1 appear to occur in the cytoplasm, as Western blot analysis of nuclear and cytoplasmic extracts prepared from MIN6 cells overexpressing SAPK2 clearly shows that the SAPK2 protein is localized in the cytoplasm and is undetectable in the β-cell nucleus (Fig. 5*A*).

As shown previously (16), glucose stimulates the transcriptional activity of a DNA construct containing the firefly luciferase gene under the control of the thymidine kinase reporter, which is driven by a human insulin gene fragment from −60 to −260 (pGL -LUC200) (Fig. 5). Overexpression of SAPK2 in MIN6 cells had no effect on the control vector pGL-LUC, which lacked the insulin gene fragment, but gave a 5-fold stimulation of pGL-LUC200 activity, similar to that seen with 16 mM glucose (Fig. 5). The effect of overexpression of SAPK2 on the LUC200 activity was inhibited by SB 203580 (10 μM), confirming that these effects were the direct consequence of the higher SAPK2 activity in the transfected cells.

DISCUSSION

We have previously shown that glucose activates PDX1 DNA binding activity and insulin promoter activity in pancreatic β-cells via a pathway involving SAPK2 (RK/p38). We also showed that SAPK2 *in vitro* activated recombinant PDX1 DNA binding activity, concomitant with a change in the molecular mass of PDX1 from 31 kDa to 46 kDa (16). In the present study, we show that glucose stimulates the conversion of PDX1 from a 31-kDa unphosphorylated to a 46-kDa phosphorylated form in isolated human islets of Langerhans (Fig. 1) and in MIN6 cells (Fig. 4). The activation of PDX1, and translocation to the nucleus, were inhibited by SB 203580 and stimulated by arsenite and overexpression of SAPK2. This strongly supports a role for SAPK2 in these events. That glucose stimulates a rapid translocation of PDX1 to the nucleus is compatible with the results of a recent study showing that glucose stimulates translocation of a c-Myc-tagged PDX1 (IPF-1) from the nuclear periphery to the nucleoplasm (22).

SAPK2-dependent phosphorylation of PDX1 appears to rep-

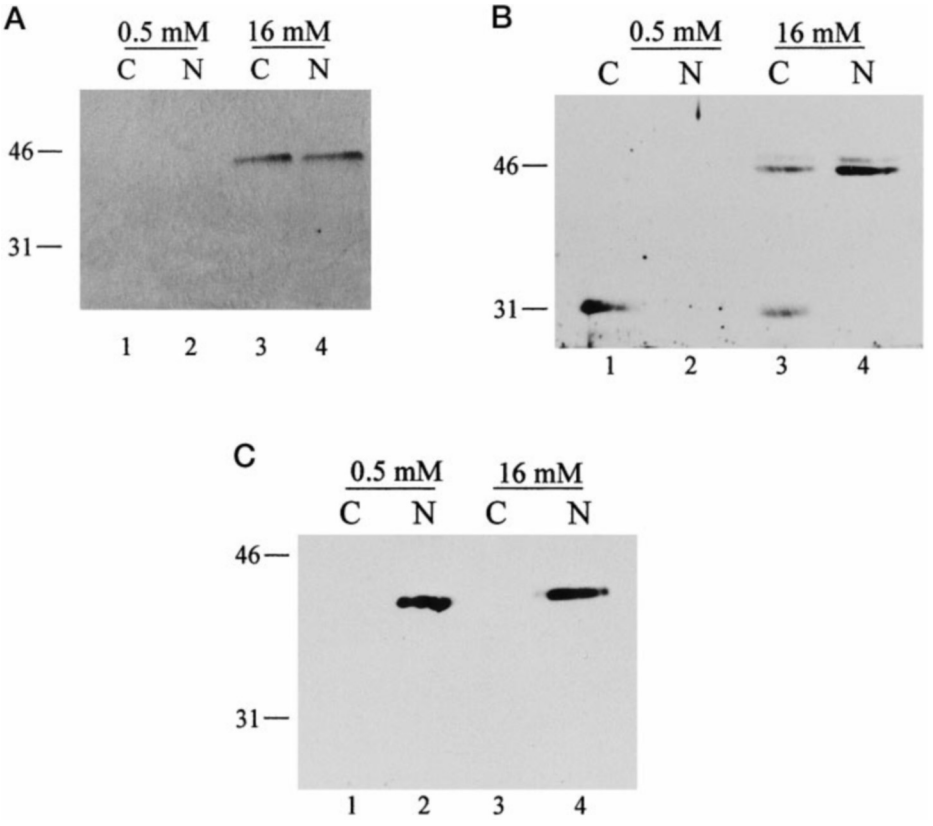

FIG. 2. **High glucose promotes incorporation of $^{32}P_i$ into the 46-kDa form of PDX1.** *A*, SDS-PAGE analysis of PDX1 immunoprecipitated from nuclear (*N*) and cytoplasmic (*C*) extracts prepared from MIN6 cells incubated for 3 h in 0.5 mM glucose, then labeled with $^{32}P_i$. Labeled cells were incubated in 0.5 mM glucose (*lanes 1* and *2*) or 16 mM glucose (*lanes 3* and *4*) for 30 min prior to the preparation of extracts. ^{32}P-Labeled PDX1 is observed in *lanes 3* and *4* at 46 kDa. *B*, Western blot analysis of immunoprecipitates described in *A*, using a specific anti-PDX1 antibody. *C*, Western blot analysis of the cytoplasmic (*C*) and nuclear (*N*) extracts from MIN6 cells incubated in 0.5 mM glucose for 5 h, or incubated in 0.5 mM glucose for 5 h followed by stimulation in 16 mM glucose for 30 min, using a specific anti-USF antibody. USF has a molecular mass of 43 kDa.

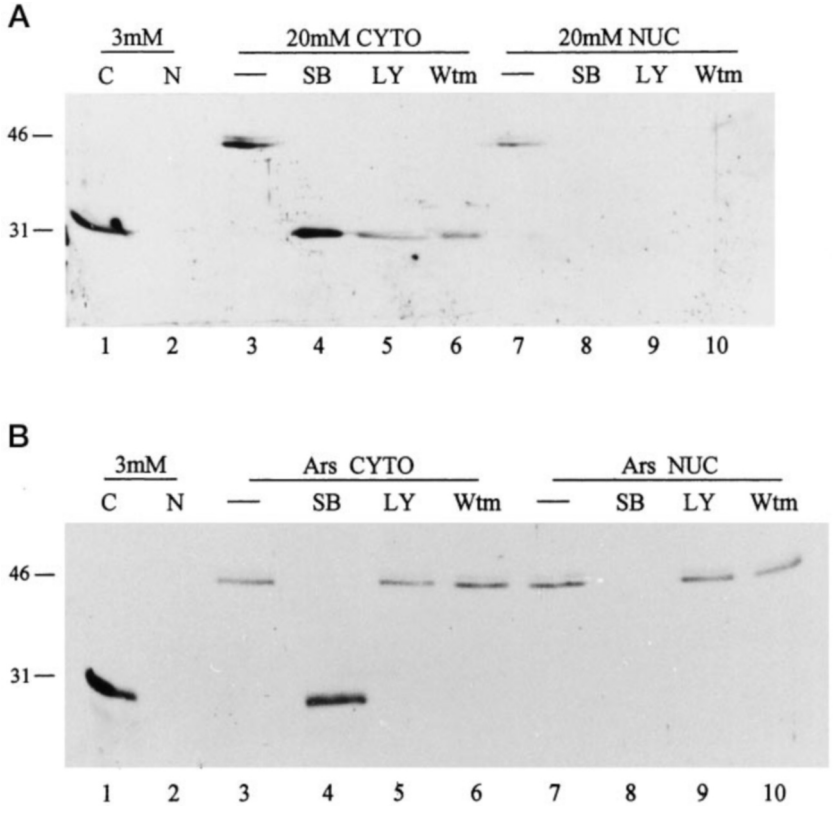

FIG. 3. **High glucose and chemical stress induce modification of PDX1.** *A*, Western blot (anti-PDX1) analysis of cytoplasmic (*C*, *lanes 3–6*), and nuclear (*N*, *lanes 7–10*) samples prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h (*lanes 1* and *2*), or 3 mM glucose for 5 h followed by 20 mM glucose for 30 min, or in 20 mM glucose for 30 min in the presence of the inhibitors indicated. SB 203580 (*SB*, 20 μM) LY 294002 (*LY*, 50 μM), and wortmannin (*Wtm*, 50 nM) were added 30 min prior to stimulation. *B*, Western blot (anti-PDX1) analysis of cytoplasmic (*C*, *lanes 3–6*), and nuclear (*N*, *lanes 7–10*) samples prepared from human islets of Langerhans incubated in 3 mM glucose for 5 h (*lanes 1* and *2*), or in 3 mM glucose for 5 h followed by 1 mM sodium arsenite for 30 min in the presence of the inhibitors indicated. SB203580 (*SB*, 20 μM) LY294002 (*LY*, 50 μM), and wortmannin (*Wtm*, 50 nM) were added 30 min prior to stimulation.

resent the critical step in glucose-induced translocation to the nucleus and activation of DNA binding activity. However, it is unclear whether phosphorylation alone accounts for the observed change in size. It is possible that phosphorylation allows or promotes a second event, which is yet to be characterized. Phosphorylation may induce a conformational change in PDX1 that affects its mobility in SDS-PAGE, but the change in size could equally result from a further post-translational modification. Many transcription factors contain nuclear localization signals within their amino acid sequences, but others, such as PDX1, do not contain recognizable nuclear localization signals and may require modification at the post-translational level.

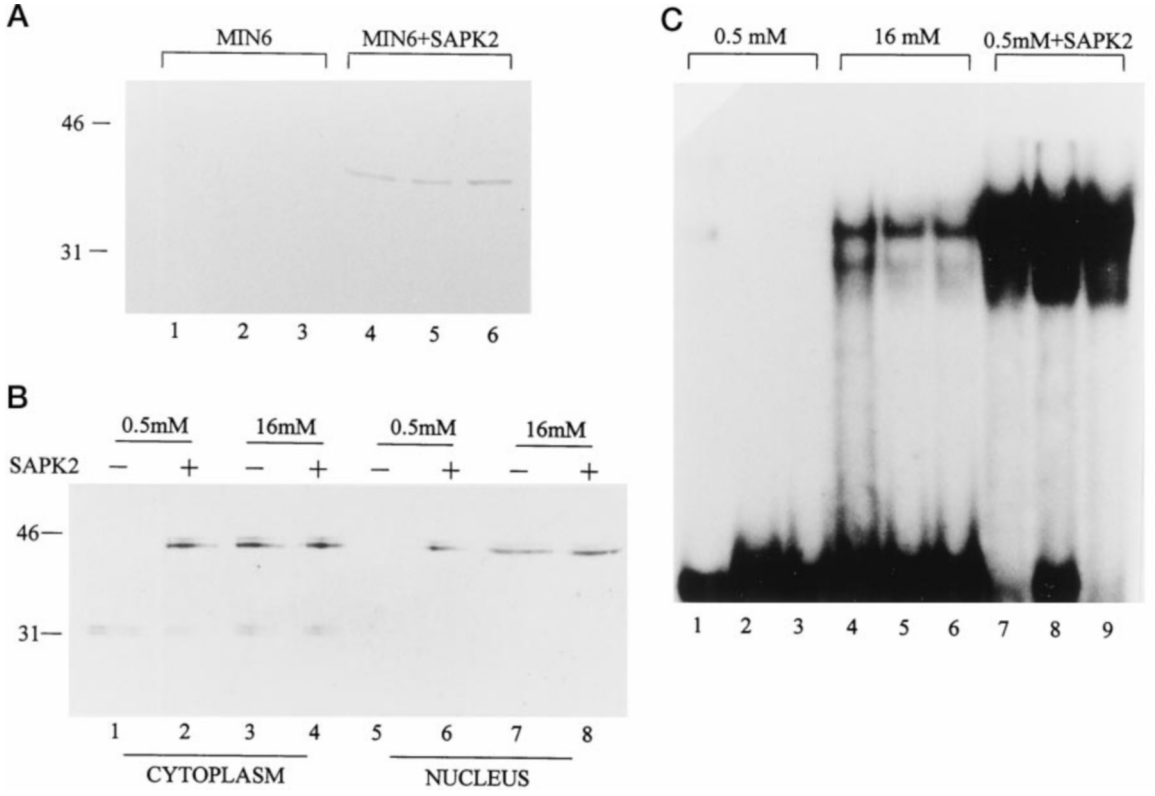

FIG. 4. **Overexpression of SAPK2 mimics the effect of high glucose on PDX1.** *A*, Western blot analysis of untransfected MIN6 cell extract (*lanes 1–3*), and extract prepared from MIN6 cells transfected with pSG-SAPK2 (*lanes 4–6*), using a specific anti-SAPK2 antibody. SAPK2 has an apparent molecular mass of 38 kDa. *B*, Western blot analysis (anti-PDX1) of cytoplasmic (*lanes 1–4*) or nuclear (*lanes 5–8*) extracts prepared from untransfected MIN6 cells (−), or MIN6 cells overexpressing SAPK2 (+). Samples were incubated in 0.5 mM glucose for 5 h, or 0.5 mM glucose for 5 h followed by stimulation for 30 min at 16 mM glucose, as indicated. *C*, electrophoretic mobility shift assay of MIN6 nuclear extracts prepared from cells incubated in 0.5 mM glucose for 5 h (*lanes 1–3*), 0.5 mM glucose for 5 h followed by 30 min at 16 mM glucose (*lanes 4–6*), or cells overexpressing SAPK2 which were incubated in 0.5 mM glucose for 5 h. EMSA analysis was performed using the A3 site of the human insulin gene promoter as probe.

Common modifications promoting nuclear localization include glycosylation (23), and ubiquitination (24, 25), which modifies proteins through the addition of ubiquitin-like protein species such as SUMO1 (small ubiquitin-related modifier 1). In the case of RanGAP (Ran-GTPase activating protein) (25), phosphorylation allows the addition of SUMO1, which alters the apparent molecular mass of the protein by 20 kDa, and promotes its translocation from the cytoplasm to the nucleus. Similar events may be occurring with PDX1.

Increased binding activity of transcription factors through phosphorylation can occur by several mechanisms. Many transcription factors become phosphorylated in the cytoplasm, stimulating movement into the nucleus (26). However, this is not always the case. For example, insulin stimulates the nuclear translocation of several kinases, such as mitogen-activated protein kinase (27), which can then directly phosphorylate nuclear protein substrates, or activate other kinases (28). In the present study SAPK2 is shown to be present exclusively in the cytoplasm, suggesting that the events governing PDX1 activation occur exclusively in the cytoplasm, promoting translocation of an active PDX1 into the nucleus.

Glucose activation of PDX1 nuclear translocation occurs through a β-cell signaling pathway involving PI 3-kinase, and resulting in the activation of SAPK2. Hence, overexpression of SAPK2 can artificially promote PDX1 translocation to the nucleus even in lower glucose concentrations. In fact, overexpression of SAPK2 stimulated PDX1 binding activity to levels higher than those seen in high glucose (Fig. 4). This hyperstimulation of PDX1 by overexpression of SAPK2 occurred in 0.5 or 20 mM glucose, and appeared to reflect activation of PDX1 at maximum efficiency. In untransfected β-cells endogenous SAPK2 may represent a rate-limiting step in PDX1 activation, with glucose eliciting a controlled stimulation. Overexpression of SAPK2 to artificially high levels may therefore result in a hyperstimulation of the PDX1 activation pathway, resulting in an increased ability to phosphorylate and translocate all available PDX1 protein. This "hyperstimulation" of PDX1 binding activity did not appear to be reflected in the levels of insulin gene transcription relative to those observed in high glucose. This is not surprising, as PDX1 alone does not control transcription of the insulin gene. Several other factors (*e.g.* E1 and E2 site binding factors) are absolutely required for transcription of the insulin gene. Increased PDX1 binding activity will only exert an effect in the presence of these factors, and a maximum response would require their combined activities.

Glucose stimulates PDX1 phosphorylation and nuclear translocation through SAPK2. PDX1 is not unique in this respect; CREB, which also binds to the human insulin gene promoter (15, 16), has its binding activity controlled through alteration in its phosphorylation status. Recent studies in SK-N-MC cells have shown that CREB-dependent transcription is triggered by co-transfection with SAPK2 (29), which may act in this case through stimulation of a downstream kinase such as MAPKAPK2 (30). However, SAPK2 has been shown to directly phosphorylate several transcription factors, including ELK1, CHOP, and MEF2C (17). Stress-activated protein kinases have also been implicated in the nuclear localization of other transcription factors. In EL-4 D6/76 cells, IL-1 activation of interleukin-1 receptor-associated kinase and of stress-activated pro-

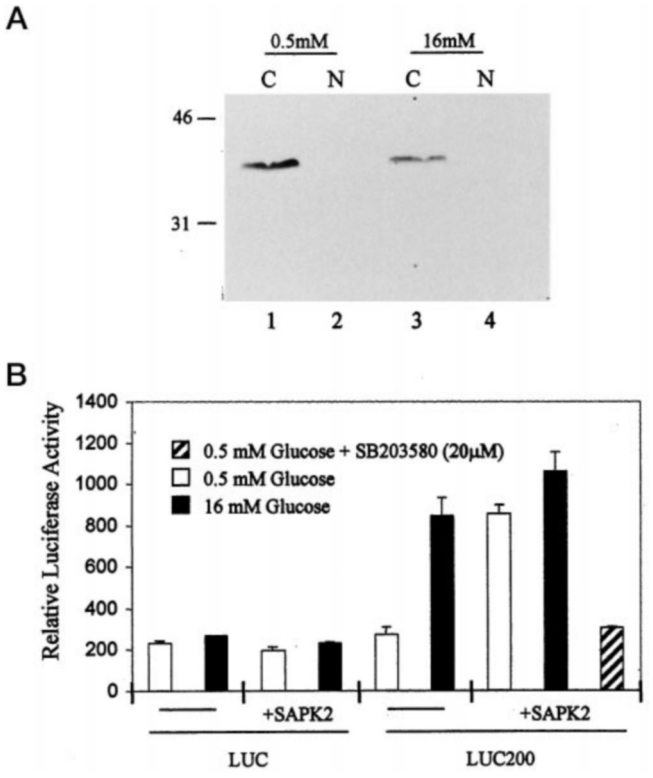

FIG. 5. **Overexpression of SAPK2 mimics the effect of high glucose concentrations on pGL-Luc200 reporter gene activity.** *A*, Western blot analysis of cytoplasmic (*C*) and nuclear (*N*) extracts prepared from MIN6 cells overexpressing SAPK2, using a specific anti-SAPK2 antibody. SAPK2 has an apparent molecular mass of 38 kDa. *B*, MIN6 cells were transfected with the control construct pGL-Luc (*LUC*, *lanes 1–4*), or with pGL-Luc200 (*LUC200*, *lanes 5–8*), and were incubated in 0.5 mM (*white*) or 16 mM glucose (*black*), or 0.5 mM glucose in the presence of 20 μM SB 203580 (*hatched*). In *lanes 3*, *4*, and *7–9*, cells were co-transfected with SAPK2 as indicated.

tein kinases, promotes translocation of transcription factors NF-κB and IL-1 NF to the nucleus, resulting in the induction of IL-2 mRNA synthesis (30). The phosphorylation induced nuclear translocation of Rel family proteins like NF-κB is induced by an extraordinary large number of agents (26), cellular stress being one example from many.

It has been proposed that glucose increases endogenous insulin mRNA levels and insulin promoter activity in HIT T15 cells via exocytosis of insulin leading to autocrine stimulation of the β-cell insulin receptor (31). The insulin stimulatory effects on mRNA levels were mediated through PI 3-kinase, p70 S6 kinase, and calmodulin kinase pathways. We have also found that insulin (1–10 ng/ml) stimulates PDX1 DNA binding activity in human islets of Langerhans and the human insulin promoter (pGL-LUC200) activity in MIN-6 cells.[2] However, in our study the effects of insulin were inhibited by the PI 3-kinase inhibitors wortmannin (50 nM) and LY 294002 (10 μM) and by the SAPK2 inhibitor SB 203580 (7 μM), but not by the p70 S6 kinase inhibitor rapamycin (50 μM). This suggests that modulation of endogenous insulin mRNA levels by insulin may involve additional pathways to those described for the activation of PDX1.

In summary, glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic β-cells via a cell signaling pathway involving SAPK2. This translocation is associated with a 5-fold increase in insulin promoter activity, and may represent a pivotal event in the nutrient regulation of insulin mRNA production.

Acknowledgment—We thank Prof. P. Cohen for helpful suggestions and provision of reagents.

[2] H. Wu, W. M. Macfarlane, and K. Docherty, unpublished results.

REFERENCES

1. Ohlsson, H., Karlsson, K., and Edlund, T. (1993) *EMBO J.* **12,** 4251–4259
2. Miller, C. P., McGehee, R. E., Jr., and Habener, J. F. (1993) *EMBO J.* **13,** 1145–1156
3. Leonard, J., Peers, B., Johnson, T., Ferreri, S. L., and Montminy, M. R. (1993) *Mol. Endocrinol.* **7,** 1275–1283
4. Boam, D. W. S., and Docherty, K. (1989) *Biochem. J.* **264,** 233–239
5. Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) *Nature* **371,** 606–609
6. Guz, Y., Montminy, M. R., Stein, R., Leonard, J., Gamer, L. W., Wright, C. V. E., and Teitelman, G. (1995) *Development* **121,** 11–18
7. Clark, A. R., Petersen, H., Read, M. L., Scott, V., Michelsen, B., and Docherty, K. (1993) *FEBS Lett.* **329,** 139–143
8. Petersen, H. V., Serup, P., Leonard, J., Michelsen, B. K., and Madsen, O. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 10465–10469
9. Peers, B. J., Leonard, J., Sharma, S., Teitelman, G., and Montminy, M. R. (1995) *Mol. Endocrinol.* **8,** 1798–1806
10. Serup, P., Jensen, J., Andersen, F. G., Jorgensen, M. C., Blume, N., Holst, J. J., and Madsen, O. D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 9015–9020
11. Macfarlane, W. M., Cragg, H., Docherty, H. M., Read, M. L., James, R. F. L., Aynsley-Green, A., and Docherty, K. (1997) *FEBS Lett.* **413,** 304–308
12. Kajimoto, Y., Watabe, H., Matsuoka, T., Kaneto, H., Fujitani, Y., Miyazaki, J.-I., and Yamasaki, Y. (1997) *J. Clin. Invest.* **100,** 1840–1846
13. Melloul, D., Ben-Neriah., Y., and Cerasi, E. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3865–3869
14. Macfarlane, W. M., Read, M. L., Gilligan, M., Bujalska, I., and Docherty, K. (1994) *Biochem. J.* **303,** 625–631
15. Marshak, S., Totary, H., Cerasi, E., and Melloul, D. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 15057–15062
16. Macfarlane, W. M., Smith, S. B., James, R. F. L., Clifton, A. D., Doza, Y. N., Cohen, P., and Docherty, K. (1997) *J. Biol. Chem.* **272,** 20936–20944
17. Cohen, P. (1997) *Trends Cell Biol.* **7,** 353–361
18. Boam, D. S., Clark, A. R., and Docherty, K., (1990) *J. Biol. Chem.* **265,** 8285–8296
19. Ricordi, C., Lacy, P. E., and Scharp, D. W. (1989) *Diabetes* **38,** 140–145
20. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) *Nucleic Acids Res.* **17,** 6419
21. Groom, L. A., Sneddon, A. A., Alessi, D. R., Dowd, S., and Keyse, S. M. (1996) *EMBO J.* **15,** 3621–3632
22. Rafiq, I., Kennedy, H. J., and Rutter, G. A. (1998) *J. Biol. Chem.* **273,** 23241–23247
23. Duverger, E., Pellerin-Mendes, C., Mayer, R., Roche, A., and Monsigny, M (1995) *J. Clin. Sci.* **108,** 1325–1332
24. Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchiar, F (1997) *Cell* **88,** 97–107
25. Matunis, M. J., Coutavas, E., and Blobel, G (1996) *J. Cell Biol.* **135,** 1457–1470
26. Thanos, D., and Maniatis, T (1995) *Cell* **80,** 529–532
27. Chen, R., Sarnecki, C., and Blenis, J. (1992) *Mol. Cell. Biol.* **12,** 915–927
28. Traverse, S., Gomez, N., Paterson, H., Marshall, C., and Cohen, P. (1992) *Biochem. J.* **288,** 351–355
29. Kim, J., and Kahn, C. R. (1997) *Biochem. J.* **323,** 621–627
30. Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., and Comb, M. J. (1996) *EMBO J.* **15,** 4629–4642
31. Leibiger, I. B., Leibiger, B., Moele, T, and Berggren, P.-O. (1998) *Mol. Cell* **1,** 933–938