Glucose-stimulated Preproinsulin Gene Expression and Nuclear trans-Location of Pancreatic Duodenum Homeobox-1 Require Activation of Phosphatidylinositol 3-Kinase but Not p38 MAPK/SAPK2*

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Exposure of islet β-cells to elevated glucose concentrations (30 versus 3 mM) prompts enhanced preproinsulin (PPI) gene transcription and the trans-localization to the nucleoplasm of pancreatic duodenum homeobox-1 (PDX-1; Rafiq, I., Kennedy, H., and Rutter, G. A. (1998) J. Biol. Chem. 273, 23241–23247). Here, we show that in MIN6 β-cells, over-expression of p110.CAAX, a constitutively active form of phosphatidylinositol 3-kinase (PI3K) mimicked the activatory effects of glucose on PPI promoter activity, whereas Δp85, a dominant negative form of the p85 subunit lacking the p110-binding domain, and the PI3K inhibitor LY 294002, blocked these effects. Similarly, glucose-stimulated nuclear trans-localization of endogenous PDX-1 was blocked by Δp85 expression, and wortmannin or LY 294002 blocked the trans-localization from the nuclear membrane to the nucleoplasm of epitope-tagged PDX1-c-myc. By contrast, SB 203580, an inhibitor of stress-activated protein kinase-2 (SAPK2)/p38 MAP kinase, had no effect on any of the above parameters, and PPI promoter activity and PDX1-c-myc localization were unaffected by over-expression of the upstream kinase MKK6 (MAP kinase kinase-6) or wild-type p38/SAPK2, respectively. Furthermore, no change in the activity of extracted p38/SAPK2 could be detected after incubation of cells at either 3 or 30 mM glucose. These data suggest that stimulation of P13K is necessary and sufficient for the effects of glucose on PPI gene transcription, acting via a downstream signaling pathway that does not involve p38/SAPK2.

Glucose homeostasis in mammals requires the proper regulation of insulin secretion from pancreatic islet β-cells. The primary signal for this activated secretion is an elevation in blood glucose concentrations, which enhances the release of stored insulin (1), the transcription (2–5) and translation (6–9) of the preproinsulin (PPI) gene, and the stability of PPI mRNA (8). However, the mechanisms by which glucose influences the expression of the preproinsulin gene are less well understood than those that control insulin secretion acutely.

The 5′ flanking region of the PPI gene has been extensively studied and a number of important regulatory elements and trans-acting factors identified (10). Particular interest has centered around the homeodomain transcription factor, PDX-1 (pancreatic duodenal homeobox-1), previously referred to as IFP-1 (11), ᵂⁱᵗᵗ−¹⁰ (12), IDX-1 (13), and IUF-1 (14). PDX-1 binds to a region upstream of the insulin gene, termed the A3 (−216CTAATG) box (15), and this binding is increased as glucose concentrations are increased in the near physiological range (0.5–30 mM) (16, 17). Although the signaling mechanisms involved are largely unclear, recent data (18) have implicated the MAP kinase family member p38 MAP kinase (the mammalian homologue of the yeast HOG1 gene, also called reactivating kinase, stress-activated protein kinase-2, SAPK2, CSBP, and Mxi2) (19) in the response of the PPI gene to glucose. These authors reported a profound inhibition of glucose-activated PPI gene expression with the pyridinyl imidazole inhibitor of p38/SAPK2, SB 203580 (20).

We have previously shown that high glucose concentrations cause the trans-location of epitope-tagged PDX-1 from the nuclear periphery to the nucleoplasm with a concomitant increase in insulin gene transcription (21). Subsequent subcellular fractionation studies have also shown nuclear trans-location of endogenous PDX-1, although in this case from the cytoplasm to the nucleus (22). Arsenite, a metabolic stress that activates nuclear translocation from the nuclear membrane to the nucleoplasm of epitope-tagged PDX-1 can be blocked by dominant negative and dominant positive forms of the enzyme, that phosphatidylinositol 3-kinase (PI3K) activity is important in glucose regulation of the PPI gene but that p38/SAPK2 is unlikely to be involved. We also provide evidence that elevated glucose concentrations may activate a related, SB 203580-inhibitable stress-activated protein kinase but that this activity is unlikely to be involved in the regulation of the preproinsulin gene.

EXPERIMENTAL PROCEDURES

Materials

Monoclonal anti-c-myc antibody 9E:10 was the kind gift of Dr. Gerard Evan (Imperial Cancer Research Fund, UK). Inhibitor kinase; Erk, extracellular-regulated protein kinase; N-C, nucleus; cytosol ratio; ATP-2, activating transcription factor-2; CMV, cytomegalo-virus; SUMO-1, small ubiquitin-related modifier-1.
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In this study, we investigated the role of PI3K and p38/SAPK2 in the regulation of preproinsulin gene expression. We used MIN6 cells, a pancreatic β-cell line, to examine the effects of these signaling pathways on insulin gene expression.

Methods

Construction of Plasmids—PDX-1-c-myc and pINS (260–600)Luc were prepared as described previously (21). Plasmid pcDNA3.MKK6* contained cDNA expressing full-length MKK6, in which Ser180 and Thr183 were mutated to Glu (24) under the control of the cytomegalovirus (CMV) immediate-early gene promoter. cDNAs encoding intact full-length p38/SAPK2 and a nonphosphorylatable form of the enzyme in which Thr180 and Tyr182 in the regulatory TGY motif were mutated to Ala and Phe, respectively, were cloned into pEX3V (25) to generate plasmids pEX3-CSBP2 WT and pEX3V-CSBP2-TAYF, respectively. A chimera between the trans-activation domain of PDX-1 (residues 2–144) (26) and the yeast Gal4 DNA binding domain was generated by polymerase chain reaction amplification (21) using primers 5′-TTTTGGATCCGT-TCA-GTA-ACA-ATG-AGC-AGT-AC (SacI site underlined) and 5′-TTTTGATCCGGG-TTG-GCTC-GGT-GTA-AGC (SacI site underlined), and the product was subcloned into the vector pSG424 (27). All plasmids were purified on a CsCl gradient (28).

Cell Culture and Transfection—MIN6 cells (29) (passages 15–25) were continuously cultured in Dulbecco’s modified Eagle’s medium containing 25 mM glucose, as described previously (21), and transferred into medium containing 3 mM glucose 24 h prior to the experiments. Jurkat cells were cultured in RPMI 1640 medium supplemented with 5% (v/v) fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. Pressure microinjection was performed as described previously (21). All reagents were obtained from Promega, Madi-}

RESULTS

Insulin Promoter Activity Is Blocked by Inhibition of PI3K and Expression of a Dominant Negative Form of the Enzyme but Is Increased by a Constitutively Active PI3K—To determine whether PI3K activation was necessary for the enhancement of PPI promoter activity in high glucose, we used a single-cell promoter reporter assay. Photon-counting digital imaging of single living MIN6 cells (31, 32) was used to compare the activity of firefly luciferase, expressed under PPI promoter control, to that of the distinct R. reniformis luciferase. The latter photoprotein was expressed under CMV promoter control and allowed correction for marked differences in the activity of the basal transcriptional/translational machinery between individual cells and cell cultures. Incubation with 30 μM versus 3 mM glucose increased insulin promoter activity by 1.6–2.2-fold (Figs. 1 and 3, A and B). The effect of glucose in this assay is specific for the PPI and other characterized glucose-sensitive promoters (e.g. the liver pyruvate kinase promoter) (31) but is not apparent with the c-fos (34), collagenase (31), or CMV3 promoters or with a truncated liver pyruvate kinase promoter lacking the glucose response element (52).

Overexpression of a mutated form of the regulatory subunit of PI3K, lacking the inter-SH2 domain (amino acids 478–513) (35), completely blocked the activation of the insulin promoter at elevated glucose concentrations (Fig. 1, A and B). Similarly, incubation of cells with the pharmacological PI3K inhibitor LY 294002 completely blocked the effects of glucose (Fig. 1C), consistent with previous results (5, 18). Indeed, in the presence of LY 294002, incubation at 30 mM glucose provoked a significant change in insulin promoter activity compared with cells cultured at 3 mM glucose (Fig. 1C).

To determine whether activation of PI3K was alone sufficient to activate PPI promoter activity, we next over-expressed a plasma membrane-targeted form of the catalytic subunit, p110.CAAX, a constitutively active form of the catalytic subunit of PI3K (36). Co-microinjection of cells with cDNA encoding p110.CAAX and culture at 3 mM glucose increased PPI promoter activity –2-fold compared with cells co-microinjected with plasmid pcDNA3 alone (Fig. 1B). In cells stimulated for 7 h with 30 mM glucose, PPI promoter activity was increased approximately 1.6-fold by co-injection of p110.CAAX (Fig. 1B). The ratio of firefly/R. reniformis luciferases in cells expressing p110.CAAX was not significantly different after incubations at 30 and 3 mM glucose (Fig. 1B).

Cytoplasmic Nuclear Transport of PDX-1 Is Blocked by Inhibition of PI3K and Expression of a Dominant Negative Form of the Enzyme—We next examined the effects of glucose and changes in PI3K activity on the subcellular distribution of endogenous and over-expressed

References

1. G. da Silva Xavier, unpublished observation.
2. G. A. Rutter, unpublished observation.
PDX-1. Endogenous PDX-1 was probed using a polyclonal antibody to the C-terminal domain (21) and quantitative laser scanning confocal microscopy of single optical sections (Fig. 2A). Although displaying significant nucleoplasmic localization in cells incubated at 3 mM glucose (ratio of nuclear (N):cytosolic (C) PDX-1, N:C = 2.48 ± 0.12, n = 28 cells), incubation at 30 mM glucose further increased this ratio to 4.62 ± 0.21, n = 27 (p < 0.001 compared with 3 mM glucose). Co-expression of PI3K Δp85 caused a significant reduction of the nuclear/cytoplasmic ratio of endogenous PDX-1 immunoreactivity in cells cultured at 30 mM (N:C = 3.25 ± 0.33, n = 12 cells, p < 0.001) but no reduction in cells maintained at 3 mM glucose (N:C = 3.27 ± 0.29, n = 29 cells; Fig. 2A, c and d).

We have previously shown that an over-expressed PDX-1-c-myc chimera resides largely on or near the nuclear membrane in cells maintained at 3 mM glucose but is trans-located to the nucleoplasm at elevated glucose concentrations (21). Over-expressed wild-type PDX-1 (Fig. 2B, a and b), like the PDX-1-c-myc chimera (Fig. 2B, c and d), was localized largely at the nuclear membrane at 3 mM glucose, and each construct trans-located to the nucleoplasm in the presence of 30 mM glucose (Fig. 2B, a–d) (21), demonstrating that the presence of the c-myc tag had no effect on the localization of the expressed PDX-1. To quantify the effects of glucose and inhibitors, cells were counted as having either predominantly nucleoplasmic localization or localization confined to the nuclear periphery (21). Glucose-dependent trans-location of PDX-1-c-myc was dramatically reduced following treatment of cells with 100 nM wortmannin (Fig. 2B, e) or 50 μM LY 294002 (Fig. 2B, f). Indeed, in the presence of these inhibitors, nucleoplasmic localization of PDX-1-c-myc at 30 mM was reduced to levels below that apparent in control cells, maintained at 3 mM glucose (Fig. 2C).

Inhibition of the stress-activated protein kinase p38/SAPK2 with SB 203580 did not alter the effect of high glucose on PDX-1-c-myc localization (Fig. 2C). Similarly, a robust effect of glucose on the localization of endogenous PDX-1 was still apparent in the presence of this inhibitor (Fig. 2A, e and f; N:C = 7.7 ± 0.48, n = 23, and 20.2 ± 1.8, n = 25, for cells incubated 2 h at 3 and 30 mM glucose, p < 0.001 for the effect of glucose).

Effect of p38/SAPK2 Activation/Inhibition on PPI Promoter Activity—Given the failure of SB 203580 to affect PDX-1 localization, which is in contrast to recent reports (22), we next examined the effects of this inhibitor directly on PPI promoter activity (Fig. 3). p38/SAPK2 inhibition with SB 203580 was entirely without effect on the increase in PPI promoter activity observed in the presence of elevated glucose concentrations (Fig. 3A). Similarly, activation of p38/SAPK2 achieved by coinjection with a constitutively active form of the upstream kinase MKK6 (24) had no significant effect on PPI...
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factor-2 (ATF2) and the DNA-binding domain of the yeast Gal4 transcription factor (Gal4-ATF2) (27) for which the transactivational capacity was reported with a Gal4 promoter-luciferase reporter (Fig. 3, C and D) (27). Activation of Gal4-ATF2 by MKK6 was completely abolished by culture with SB 203580 (Fig. 3D). Interestingly, Gal4-ATF2 activity was increased by >2-fold in the absence of MKK6 by 30 mM glucose (Fig. 3C). This effect of high glucose was abolished by SB 203580 (Fig. 3C) and mimicked by MKK6 (Fig. 3D). No additive effect of glucose and MKK6 on Gal4-ATF2 activity was apparent.

Using a system analogous to that described above, we next examined the transactivational potential of a chimera between the N-terminal trans-activation domain of PDX-1 (residues 2–144) (26) and the Gal4 DNA binding domain (see “Experimental Procedures”). This was not significantly affected at 30 mM glucose by incubation with 10 μM SB 203580 (firefly:Renilla luciferase activities, 0.21 ± 0.053, n = 69 control cells; 0.15 ± 0.02, n = 75 cells incubated with inhibitor).

Neither Activation nor Inhibition of p38/SAPK2 Affects Glucose-stimulated Translocation of PDX-1-c-myc—The apparent absence of a role for p38/SAPK2 in PPI promoter regulation led us to examine next the effects of SB 203580 and expression of wild-type and nonphosphorylatable forms of p38/SAPK2 on the subcellular localization of PDX-1-c-myc. In common with endogenous PDX-1 (Fig. 2A, a and e) and luciferase with a rabbit polyclonal anti-PDX-1 antiserum (21) and luciferase with a rabbit polyclonal anti-luciferase antibody (51). Both primary antibodies were revealed and imaged as described under “Experimental Procedures.” Immunofluorescence at the cell surface (c and d) results from the presence of the SNAP25-luciferase construct included as a marker of transfection, which is efficiently targeted to this compartment (51). B, MIN6 cells were maintained in 3 mM glucose for 24 h before transfection with wild-type PDX-1 (a and b) or PDX-1-c-myc (c–f). After transfection, cells were cultured for a further 24 h in Dulbecco’s modified Eagle’s medium (see “Experimental Procedures”) containing 3 mM glucose. Culture was then continued for 1 h in the presence of the given inhibitors, followed by 2 h in the sustained presence of the inhibitors at 3 or 30 mM glucose as shown: a and c, 3 mM glucose; b and d, 30 mM glucose; e, 30 mM glucose plus 100 mM wortmannin (WM); and f, 30 mM glucose plus 50 μM LY 294002 (LY). Media were changed every 30 min during the stimulation period to compensate for the instability of wortmannin under these conditions. Immunocytochemistry was performed as described under “Experimental Procedures.” C, quantitation (see “Results”) of data from three separate experiments, as shown in B, in which a minimum of 100 cells was counted for each condition. SB culture with 10 μM SB 203580. Statistical significance was calculated by two-tailed t test: **, p < 0.01; *, p < 0.05 versus 3 mM glucose.

FIG. 2. Effect of glucose and changes in PI3K activity on the subcellular localization of endogenous (A) and over-expressed (B and C) PDX-1. A, untransfected cells (a, b, e, f), or cells co-transfected with Δp85 plus a SNAP25-luciferase chimera (to allow identification of transfected cells through the presence of immunoreactivity at the plasma membrane; c and d) were transfected with plasmids as indicated, then cultured for 24 h at 3 mM glucose, and preincubated (1 h) in the additional presence of 10 μM SB 203580 as indicated (e and f). The culture was then continued for 2 h at 3 (a, c, e) or 30 mM (b, d, f) glucose. Endogenous PDX-1 was probed using immunopurified polyclonal anti-PDX-1 antiserum (21) and luciferase with a rabbit polyclonal anti-luciferase antibody (51). Both primary antibodies were revealed and imaged as described under “Experimental Procedures.” Immunofluorescence at the cell surface (c and d) results from the presence of the SNAP25-luciferase construct included as a marker of transfection, which is efficiently targeted to this compartment (51). B, MIN6 cells were maintained in 3 mM glucose for 24 h before transfection with wild-type PDX-1 (a and b) or PDX-1-c-myc (c–f). After transfection, cells were cultured for a further 24 h in Dulbecco’s modified Eagle’s medium (see “Experimental Procedures”) containing 3 mM glucose. Culture was then continued for 1 h in the presence of the given inhibitors, followed by 2 h in the sustained presence of the inhibitors at 3 or 30 mM glucose as shown: a and c, 3 mM glucose; b and d, 30 mM glucose; e, 30 mM glucose plus 100 mM wortmannin (WM); and f, 30 mM glucose plus 50 μM LY 294002 (LY). Media were changed every 30 min during the stimulation period to compensate for the instability of wortmannin under these conditions. Immunocytochemistry was performed as described under “Experimental Procedures.” C, quantitation (see “Results”) of data from three separate experiments, as shown in B, in which a minimum of 100 cells was counted for each condition. SB culture with 10 μM SB 203580. Statistical significance was calculated by two-tailed t test: **, p < 0.01; *, p < 0.05 versus 3 mM glucose.

DISCUSSION

In this study, we have examined signaling pathways involved in changes in preproinsulin gene transcription and the subcellular location of endogenous and over-expressed PDX-1 in response to changes in glucose concentration.

Regulation of PPI Gene Expression by PI3K—Over-expression of PI3K led to a doubling of the insulin promoter activity at low glucose concentrations (3 mM) and a 1.6-fold increase at high glucose concentrations. A truncated form of PI3K (Δp85) lacking the inter-SH2 domain needed for interaction with the catalytic subunit of PI3K, and thus acting as a dominant negative, completely abolished this increase in insulin promoter activity. Moreover, Δp85 expression actually led to a reversal of the glucose response of the insulin promoter, that is, inhibition of promoter activity at high glucose concentrations (Fig. 2). This ability of high glucose to act in the presence of PI3K

promoter activity at either 3 or 30 mM glucose (Fig. 3B). Demonstrating the efficacy of this protein kinase to increase p38/SAPK2 activity, MKK6 co-expression dramatically increased the transactivational capacity of a simultaneously introduced construct encoding a fusion between activating transcription
blockade as an inhibitor of the PPI promoter suggests that the sugar may normally activate both activatory and inhibitory signaling pathways. Only the former pathway, which normally predominates, would appear to require PI3K activation.

**Figure 3. Effect of activation or inhibition of p38/SAPK2 on preproinsulin promoter activity.** Microinjection was performed as described in the legend to Fig. 1, with pRL-CMV and either pINS(260–60).Luc (A and B) or 0.2 mg/ml Gal4 ATF-2 (C and D). Additional plasmids were injected as follows: A, none; B, empty pcDNA3 (0.1 mg/ml−1, control (Con) or pcDNA3.MKK6, 0.15 mg/ml−1; C, empty pcDNA3; D, pcDNA3.MKK6 (0.1 mg/ml−1 each). Luciferase imaging was performed after a 6-h incubation at either 3 or 30 mM glucose. Statistical significance was calculated assuming equal variances: *, p < 0.05, and **, p < 0.01 for the effect of 30 mM glucose (panels A–C) and for the effect of MKK6 over-expression (D); +, p < 0.05, and ++, p < 0.01 for the effect of SB 203580.

Consistent with the view that the interaction of PDX-1-c-myc with the nuclear periphery may be important in controlling the nucleocytoplasmic partitioning of endogenous PDX-1, both parameters were regulated similarly by changes in PI3K activity (Fig. 2). In particular, the cytosol → nucleus trans-location of endogenous PDX-1 and trans-location from the nuclear periphery to the nucleoplasm of over-expressed PDX-1-c-myc both occurred via a wortmannin/LY 294002-sensitive pathway. Further, the present studies show that in the presence of either inhibitor, elevated glucose concentrations provoke a decrease in the proportion of PDX-1-c-myc located in the nucleus. This behavior may therefore provide an explanation of the inhibitory effect of high glucose on the PPI promoter during PI3K blockade (see Fig. 1 and text above).

**Figure 4. Regulation of PPI Promoter Activity and PDX-1 Trans-location by Glucose Involvement of p38/SAPK2.**—Recent studies of both PPI promoter regulation (18) and PDX-1 localization (22) have described an inhibition with the p38/SAPK2 inhibitor SB 203580 of the effects of glucose. In the present studies, neither incubation of cells with SB 203580 (Fig. 3A) nor with arsenite at 3 mM glucose (results not shown) had any effect on PPI promoter activity. Furthermore, over-expression of a constitutively active form of MKK6, a kinase immediately upstream of p38/SAPK2 (24), exerted no effect on PPI promoter activity (Fig. 3B), whereas expression of this construct strongly trans-activated Gal4 promoter activity in a SB 203580-dependent manner (Fig. 3, C and D). These observations concur with the results of other recent studies (5) that reported an absence of any effect of SB 203580 on the stimulation of PPI gene expression at 16 mM glucose. Similarly, we observed no effect of manipulating p38/SAPK2 activity on the subcellular localization of endogenous PDX-1 or PDX-1-c-myc (see above).
through we have no clear explanation for the discrepancy between these and the previously published results of MacFarlane and colleagues (16, 18, 22), it is possible that differences in experimental protocols (e.g. in studies of PPI promoter activity; microinjection versus transfection; pre-culture in 3 mM rather than 0.5 mM glucose; normalization to the activity of a nonregulated promoter) may be involved. A further possibility, which may explain the effects of SB 203580 observed in earlier studies, may be a nonspecific inhibitory effect on protein kinase B, acting downstream of PI3K.5 Nevertheless, the present study clearly indicates that elevated glucose concentrations stimulate the activity of an SB 203580-sensitive pathway, which leads to the trans-activation of the yeast Gal4-ATF2 chimera (Fig. 3C). Because elevated glucose was found to have no detectable effect on p38/SAPK2 activity measured in MIN6 cell extracts (Fig. 4C), as also observed for the glucose-responsive INS-1 β-cell line (40) and human islets,6 these data suggest that a distinct member of the SAPK family may be activated by glucose in β-cells. This activity may be more sensitive to the mild osmotic stress imposed by glucose (because of the intracellular generation of glycolytic metabolites) (41) than p38/SAPK2. This alternative SAPK2 activity may be related to a protein kinase of molecular mass 63 kDa recently identified through the use of an in-gel kinase assay (42). Whether this activity represents SAPK3/Erk6 (43) is a possibility that remains to be resolved. It now seems feasible that the activation by glucose of MAP kinase activating protein kinase-2, an immediate downstream target of p38/SAPK2 (44), observed by MacFarlane et al. (18) may have involved this or another glucose/stress-activated protein kinase.

Regulation of PI3K by Glucose—The present work indicates that the activation of PI3K is important for the regulation of PDX-1 and for stimulated preproinsulin gene transcription. Indeed, our results suggest that activation of this enzyme is both necessary and sufficient for PDX-1 trans-location and necessary for insulin promoter activation. Supporting this view, GLP-1 (glucagon-like peptide-1) was shown to increase concentrations stimulate the activity of an SB 203580-sensitive pathway, which leads to the trans-activation of the yeast Gal4-ATF2 chimera (Fig. 3C). Because elevated glucose was found to have no detectable effect on p38/SAPK2 activity measured in MIN6 cell extracts (Fig. 4C), as also observed for the glucose-responsive INS-1 β-cell line (40) and human islets, these data suggest that a distinct member of the SAPK family may be activated by glucose in β-cells. This activity may be more sensitive to the mild osmotic stress imposed by glucose (because of the intracellular generation of glycolytic intermediates and other metabolites) (41) than p38/SAPK2. This alternative SAPK2 activity may be related to a protein kinase of molecular mass 63 kDa recently identified through the use of an in-gel kinase assay (42). Whether this activity represents SAPK3/Erk6 (43) is a possibility that remains to be resolved. It now seems feasible that the activation by glucose of MAP kinase activating protein kinase-2, an immediate downstream target of p38/SAPK2 (44), observed by MacFarlane et al. (18) may have involved this or another glucose/stress-activated protein kinase.

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5 C. Marshall, personal communication.

6 G. A. Rutter and M. Dickens, unpublished observation.
PI3K activity in INS-1 cells and to increase insulin mRNA and the DNA binding activity of PDX-1, an effect inhibited by LY 294002 (45).

Because PI3K is a downstream target of insulin receptor substrate-1 and -2, and insulin exerts its effects via PI3K in "classical" insulin-responsive cells (46), a role for insulin has been suggested in the activation of the preproinsulin gene (47, 48). Direct evidence for this role of insulin came from a study in which insulin secreted from the β-cell (both rat islets and HIT-T15 cells) was shown to have a feed forward effect on its biosynthesis by enhancing preproinsulin gene transcription (5). This autocrine response was further shown to involve the insulin receptor PI3K, p70S6 kinase, and calmodulin kinase pathways. The involvement of PDX-1 in this novel pathway of PPI gene activation is uncertain, although unpublished observations by MacFarlane et al. (22) have supported a role for insulin in activation of PDX-1 and the PPI gene via a pathway involving PI3K and p38/SAPK2 but not a rapamycin-sensitive pathway. We have also examined the effect of insulin on PPI promoter activity in MIN6 cells but found no discernible effect at concentrations of 100 nM (34). Similarly, no change in the transcriptional response to glucose was observed after the near complete blockade of insulin secretion (34). Thus, the role of insulin secretion in the regulation of insulin gene transcription remains controversial (48). Furthermore, we have recently noted that the trans-location to the plasma membrane of the exchange factor ARNO (ADP-ribosylation factor nucleotide opening site) (49), which is mediated by binding to a pleckstrin homology domain of phosphatidylinositol 3,4,5-trisphosphate generated by PI3K, is weakly stimulated by glucose but unaffected by insulin concentrations below 1 μM.7 These observations support the view that the activation of PI3K by glucose is likely to involve a direct intracellular signaling mechanism that is independent of insulin secretion.

Conclusion—Glucose stimulates PPI promoter activity via an intracellular signaling pathway involving PI3K and protein kinase C but not p38/SAPK2 or Erk-1/2. Part of this response involves trans-location to the nucleus of PDX-1. The molecular mechanisms involved in triggering the shift of PDX-1 remain to be elucidated. Although it has been proposed (22) that a shift in the apparent molecular mass of PDX-1 (from 31 → 46 kDa) may also be involved in PDX-1 nuclear translocation, we have observed no such change in the apparent molecular mass of either endogenous or over-expressed PDX-1 c-myc by Western analysis of MIN6 cell extracts, with PDX-1 migrating at a molecular mass close to 46 kDa in extracts of cells incubated at either 3 or 30 mM glucose (results not shown). Furthermore, although these data suggest that PDX-1 (calculated apparent molecular mass close to 31 kDa) exists as a covalently modified species in MIN6 cells, we have recently found that this is unlikely to be the result of attachment of the candidate molecule SUMO-1 (small ubiquitin-related modifier-1; apparent molecular mass ~12 kDa) (50) involved in targeting Ran-GAP to the nuclear pore complex protein, RanBP2. Thus, antibodies to SUMO-1 fail to immunoprecipitate PDX-1, and conversely, anti-PDX-1 polyclonal antibody does not immunoprecipitate SUMO-1.8 The current studies demonstrate that PI3K activity has a direct effect on the control of insulin gene transcription and the regulation of PDX-1 localization. Whether PI3K is activated directly by glucose, or indirectly by secreted insulin (48), remains to be determined.

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