Per-Arnt-Sim Kinase Regulates Pancreatic Duodenal Homeobox-1 Protein Stability via Phosphorylation of Glycogen Synthase Kinase 3β in Pancreatic β-Cells*

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**Background:** The enzyme PASK regulates the expression of PDX-1 and insulin in pancreatic β-cells via unknown mechanisms.

**Results:** PASK enhances PDX-1 protein stability via phosphorylation of GSK3β on Ser9.

**Conclusion:** PASK regulates insulin gene expression at least in part through inactivation of GSK3β and stabilization of PDX-1 protein.

**Significance:** We identified GSK3β as a novel target of PASK in the regulation of pancreatic β-cell function.

In pancreatic β-cells, glucose induces the binding of the transcription factor pancreatic duodenal homeobox-1 (PDX-1) to the insulin gene promoter to activate insulin gene transcription. At low glucose levels, glycogen synthase kinase 3β (GSK3β) is known to phosphorylate PDX-1 on C-terminal serine residues, which triggers PDX-1 proteasomal degradation. We previously showed that the serine/threonine Per-Arnt-Sim domain-containing kinase (PASK) regulates insulin gene transcription via PDX-1. However, the mechanisms underlying this regulation are unknown. In this study, we aimed to identify the role of PASK in the regulation of PDX-1 phosphorylation, protein expression, and stability in insulin-secreting cells and isolated rodent islets of Langerhans. We observed that glucose induces a decrease in overall PDX-1 serine phosphorylation and that overexpression of WT PASK mimics this effect. In vitro, PASK directly phosphorylates GSK3β on its inactivating phosphorylation site Ser9. Overexpression of a kinase-dead (KD) dominant negative version of PASK blocks glucose-induced Ser9 phosphorylation of GSK3β. Accordingly, GSK3β Ser9 phosphorylation is reduced in islets from pask-null mice. Overexpression of WT PASK or KD GSK3β protects PDX-1 from degradation and results in increased PDX-1 protein abundance. Conversely, overexpression of KD PASK blocks glucose-induction of PDX-1 protein. We conclude that PASK phosphorylates and inactivates GSK3β, thereby preventing PDX-1 serine phosphorylation and alleviating GSK3β-mediated PDX-1 protein degradation in pancreatic β-cells.

The production and secretion of insulin by the pancreatic β-cell are exquisitely regulated processes ensuring the maintenance of circulating glucose levels within a narrow physiological range despite large variations in energy intake and expenditure throughout the day. Dysregulation of this process leads to diabetes, a disease affecting >370 million people worldwide. The major regulator of β-cell function is glucose, which coordinately stimulates transcription of the insulin gene; splicing, stability, and translation of the insulin mRNA; processing of proinsulin into mature insulin; and insulin secretion (1). Transcription of the insulin gene is controlled by a highly complex network of transcription factors, among which pancreatic duodenal homebox-1 (PDX-1)6 plays a critical role. PDX-1 is essential for pancreas development and β-cell function in mice (2) and humans (3). Glucose stimulates several aspects of PDX-1 expression and function, including nuclear translocation (4, 5), binding to the A boxes on the insulin promoter region (6), transactivation potential (7), and protein stability (8). However, the precise mechanisms by which glucose regulates PDX-1 are largely unknown and still debated. In particular, the functional importance of glucose-stimulated PDX-1 phosphorylation is unclear. Although some studies reported a stimulatory effect of PDX-1 phosphorylation (9–12), others have come to opposite conclusions (8, 13–16). These discrepancies are partly due to the fact that specific phosphorylated residues are likely to have different functional effects. Indeed, phosphorylation of N-terminal serine residues such as Ser62 and Ser68 was reported to correlate with PDX-1 activation (12), whereas phosphorylation of C-terminal Ser268 and Ser272 by glycogen synthase kinase-3β (GSK3β) triggers PDX-1 protein degradation (8).

The Per-Arnt-Sim domain-containing kinase (PASK) is a nutrient-responsive serine/threonine-protein kinase highly conserved from yeast to humans (17–21). In mammals, PASK regulates glycogen synthase and is required for normal cellular activity.
energy homeostasis (22). Glucose induces PASK gene and protein expression in both isolated rat islets and insulin-secreting MIN6 cells (23, 24). At basal glucose levels, overexpression of WT PASK increased both insulin and PDX-1 expression, whereas overexpression of a kinase-dead (KD) mutant of PASK acting as a dominant negative blocked glucose-induced insulin gene expression as well as PDX-1 protein expression (23, 24), suggesting that PASK regulates the insulin gene via PDX-1. However, how PASK modulates PDX-1 activity is unknown. PASK being a serine/threonine kinase, we envisioned two possibilities: either PASK directly phosphorylates PDX-1 at a positive regulatory site, or it alleviates a negative regulation by phosphorylating another kinase such as GSK3β. This study was aimed to test these possibilities by examining the role of PASK in the phosphorylation, stability, and protein abundance of PDX-1 in pancreatic β-cells.

**EXPERIMENTAL PROCEDURES**

Reagents, Plasmids and Adenoviruses—Hyclone Dulbecco’s modified Eagles medium (DMEM) was from Thermo Fisher. RPMI 1640 medium and FBS were from Invitrogen. Cycloheximide was from Sigma. Adenoviruses encoding for luciferase, WT human (h)PASK, and KD hPASK were generated and purified as described (23, 25). Empty vector (EV), WT hPASK and KD hPASK plasmids were from Dr. Guy Rutter (Imperial College London, London, UK (26)). WT and KD GSK3β constructs were from Addgene (Cambridge, MA).

**Cell Culture, Infection, and Transfection**—MIN6 cells (passages 28–31; obtained from Dr. Guy Rutter, Imperial College London) were maintained in Hyclone DMEM containing 25 mM glucose and supplemented with 15% bovine growth serum and 0.005% β-mercaptoethanol. Cells were infected overnight with adenoviruses encoding for luciferase, WT PASK, or KD PASK at 100 multiplicities of infection/cell. HIT-T15 cells (passages 74–86; obtained from R. Paul Robertson, Pacific Northwest Diabetes Research Institute, Seattle, WA) were maintained in RPMI 1640 medium containing 11.1 mM glucose and supplemented with 10% FBS. Transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Glucose stimulation in MIN6 cells was performed as reported previously (24). Following HIT-T15 transfection, medium was changed to 1 mM glucose for 24 h and to 0.1 mM glucose 0.5% BSA for an additional 2 h, after which cells were stimulated in 0.1 mM, 1.5 mM, or 5 mM glucose.

**Animals**—All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l’Université de Montréal. Animals were housed on a 12-h light/dark cycle with free access to water and standard laboratory chow. For arterial delivery of adenoviral vectors, 250–275-g male Wistar rats (Charles River, St.-Constant, QC) were anesthetized and subjected to laparotomy. After ligation of the portal vein, the superior aorta, and the hepatic, mesenteric, and right and left renal arteries, adenoviruses encoding for luciferase, WT hPASK, or KD hPASK were injected in the celiac trunk of the inferior aorta as described (24). Islets were isolated and recovered overnight. They were precultured for 16 h at 2.8 mM glucose and then exposed to 2.8 or 16.7 mM glucose for 24 h. WT and pask-null mice were purchased from The Jackson Laboratories. At 8–12 weeks of age, pancreatic islets were isolated and recovered overnight as described previously (27).

**Western Blotting**—Protein extraction and Western blotting were performed as described previously (28). Membranes were blotted against pSer9 GSK3β (Santa Cruz Biotechnology or Cell Signaling Technologies) or total GSK3β (Santa Cruz Biotechnology), human PASK (Sigma), PDX-1 (Upstate Biotechnology), or the p85 subunit of PI3K (New England Biolabs) as indicated under “Results.” The density of protein bands was quantified using ImageJ software (National Institutes of Health).

**Cycloheximide Treatment**—100 μg/ml cycloheximide (Sigma) was added to the culture medium 24 h after transfection for 6 h. Proteins were then extracted and subjected to Western blotting as described above.

**Immunoprecipitation**—MIN6 cells were lysed in sodium orthovanadate (NaOV) buffer (100 mM NaCl, 50 mM Tris, 5 mM EDTA, 1 mM NaOV, 1% Nonidet P-40) with a complete complement of protease and phosphatase inhibitors. Total protein extracts were immunoprecipitated using protein A/G PLUS-agarose (Santa Cruz Biotechnology) and an anti-rabbit PDX-1 antibody (Upstate Biotechnology). Immunoblots were probed with an anti-goat PDX-1 antibody (R&D Systems) or an anti-mouse pSer antibody (Assaydesigns, Exeter, UK).

**PDX-1 Immunostaining**—MIN6 cells were seeded on poly-L-lysine-coated (Sigma) coverslips and infected overnight with adenoviruses encoding for luciferase, WT PASK or KD PASK as described above. Following glucose stimulation, cells were fixed and permeabilized with 3.7% formaldehyde and 0.2% Triton X-100. Cells were incubated with PDX-1 or hPASK antibodies followed by Alexa Fluor 488 and Alexa Fluor 546 (Invitrogen) fluorophore-conjugated secondary antibodies, respectively. Cells were mounted on coverslips with VectaShield mounting medium (Vector Laboratories) with DAPI for nuclei detection. Images were acquired using a LEICA DMI 6000B microscope and Zen Imaging software. Fluorescence was quantified using ImageJ software.

**GSK3β in Vitro Phosphorylation**—HepG2 cells expressing PASK-HA-FLAG were harvested, and PASK was purified using anti-FLAG M2 affinity gel (Sigma). PASK-HA-FLAG was eluted using a 3× FLAG peptide. 7 μl of purified PASK-HA-FLAG was incubated ± 8 μl of His-GSK3β (Abcam) in 50 mM Hepes buffer, pH 7.4, with 50 μM [γ-32P]ATP, 5 mM MgCl₂. The reaction was incubated for 30 min at 30 °C and was terminated by the addition of 4× Laemmli buffer. Samples were heated at 95 °C for 5 min and run on a 10% SDS-polyacrylamide gel at 100 V for 20 min followed by 200 V for 50 min. The gel was washed in ddH₂O for 15 min, stained with GelCode Blue (Thermo Scientific) for 1 h, and destained overnight with ddH₂O. The destained gel was visualized and subjected to autoradiography. Bands were excised, and 32P incorporation was measured by scintillation counting. For Ser9 phosphorylation, 150 ng of purified GSK3β (Sigma) was added in the final kinase reaction volume of 30 μl containing either no PASK or ~10 ng or ~100 ng of FLAG tag PASK purified from 293T cells by a FLAG affinity column. The kinase reaction was performed for 30 min at 30 °C. The reactions were stopped by adding SDS buffer. Western blotting was performed using anti-p-Ser9 GSK3β, total GSK3β (Cell Signaling Technologies), or FLAG M2 (Sigma) antibodies.
Statistics—Data are expressed as mean ± S.E. Significance was tested using Student’s paired t test, one-way analysis of variance with Dunnett’s multiple comparison test, or two-way analysis of variance with Bonferroni post hoc adjustment for multiple comparisons, as appropriate, using Instat (GraphPad Software). A value of p < 0.05 was considered significant.

RESULTS

High Glucose and Overexpression of WT PASK Decrease PDX-1 Serine Phosphorylation in MIN6 Cells—To first assess the phosphorylation state of PDX-1 in response to glucose stimulation, MIN6 cells were exposed to 2 or 11 mM glucose for 24 h. PDX-1 was then immunoprecipitated from total protein extracts and tested for serine phosphorylation by Western blotting. As shown in Fig. 1, glucose significantly decreased PDX-1 serine phosphorylation (p = 0.01; Fig. 1, A and B). In contrast, glucose had no detectable effect on threonine phosphorylation (data not shown). To test a potential role for PASK on PDX-1 serine phosphorylation, MIN6 cells were infected with adenoviruses encoding WT hPASK or luciferase as a control and exposed to 2 or 11 mM glucose for 24 h. Immunoprecipitation of total PDX-1 protein followed by Western blot analysis revealed a significant decrease in PDX-1 serine phosphorylation at low glucose in MIN6 cells overexpressing WT hPASK (p < 0.05; Fig. 1, C and D). Thus, PASK mimics the effect of high glucose on PDX-1 serine phosphorylation. Because PASK is a serine/threonine kinase, these observations suggest that it might indirectly lead to a decrease in PDX-1 serine phosphorylation by phosphorylating and inactivating another kinase which, itself, would phosphorylate PDX-1 on serine residues. A candidate for this is GSK3β, which is inactivated by phosphorylation at Ser9 in response to glucose (29, 30). When GSK3β is active (dephosphorylated), it increases PDX-1 serine phosphorylation at C-terminal residues (8) and induces its proteasomal degradation (8, 14, 15).

Inactivation of PASK Blocks Glucose-induced Ser9 GSK3β Phosphorylation in Islets—To confirm the effect of PASK on GSK3β Ser9 phosphorylation in β-cells, we overexpressed plasmids or adenoviruses encoding WT hPASK or a KD hPASK
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**FIGURE 2. PASK directly phosphorylates GSK3β on Ser9 in vitro.** A, purified WT or KD PASK was incubated with recombinant GSK3β and [γ-32P]ATP as described under “Results.” The reaction product was run on a SDS-polyacrylamide gel and stained with GelCode Blue for protein loading control (upper panel). B, quantification of GSK3β phosphorylation. Bands were excised, and 32P incorporation was measured by liquid scintillation counting. Results are expressed as mean ± S.E. (*, p ≤ 0.05). The dashed bar represents the control condition, without WT PASK (−WT), corresponding to lanes 2, 3, and 6, respectively. C, purified GSK3β was incubated with an increasing amount of PASK as described under “Experimental Procedures.” The reaction product was subjected to Western blotting using anti FLAG or p-Ser9GSK3β antibody. An anti-total GSK3β antibody was used to control for loading.

acting as a dominant negative (25) in HIT-T15 cells exposed to 0.1, 1.5, or 5 mM glucose or in isolated rat islets exposed to 2.8 or 16.7 mM glucose, respectively, for 24 h (Fig. 3, A–D). As expected, glucose significantly increased Ser9GSK3β in HIT-T15 cells (Fig. 3, A and B) and islets (Fig. 3, C and D) overexpressing the control vector. Overexpression of WT hPASK tended to increase Ser9GSK3β at 0.1 mM glucose, such that the effect of higher glucose concentrations was no longer significant (Fig. 3, A and B). Overexpression of KD hPASK completely blocked glucose-induced Ser9GSK3β phosphorylation both in HIT-T15 cells (Fig. 3, A and B) and islets (Fig. 3, C and D). In contrast, overexpression of KD hPASK did not affect ERK1/2 phosphorylation in isolated rat islets (data not shown). Consistent with these results, GSK3β Ser9 phosphorylation was significantly reduced in islets isolated from pask-null mice (Fig. 3, E and F). Taken together, our results identify GSK3β as a novel PASK substrate in pancreatic β-cells and reveal a critical role for PASK in glucose-induced Ser9GSK3β phosphorylation.

Overexpression of WT hPASK Increases PDX-1 Protein Stability via GSK3β in MIN6 Cells and Isolated Rat Islets—GSK3β regulates PDX-1 protein degradation through phosphorylation at Ser268 and Ser272 (8). To investigate whether PASK affects PDX-1 protein stability, MIN6 cells and isolated rat islets were, respectively, transfected or infected with adenoviruses encoding WT hPASK or KD hPASK and exposed to 100 μg/ml cycloheximide for 6 h, after which PDX-1 protein levels were measured by Western blotting (Fig. 4, A–D). Although cells overexpressing EV showed an ∼50% decrease in PDX-1 protein levels upon cycloheximide treatment, this decrease was completely prevented in cells overexpressing WT hPASK, but not in cells overexpressing KD hPASK (Fig. 4, A and B). Similarly, adenoviral overexpression of WT hPASK, but not that of KD hPASK, prevented the decrease in PDX-1 protein levels in islets treated with cycloheximide (Fig. 4, C and D). These results indicate that PASK regulates PDX-1 protein stability. To determine whether GSK3β is implicated in this effect, we co-expressed both KD PASK and KD GSK3β in MIN6 cells exposed to cycloheximide as described above. As shown in Fig. 4, E and F, co-expression of KD GSK3β prevented PDX-1 protein degradation in cells overexpressing KD hPASK, indicating that the regulation of PDX-1 stability by PASK requires GSK3β.

**DISCUSSION**

tl’sIn this study, we aimed to identify the mechanisms by which PASK regulates PDX-1 in the pancreatic β-cell. We found that PASK directly phosphorylates GSK3β on Ser9, prevents GSK3β-mediated PDX-1 serine phosphorylation, and increases PDX-1 protein stability and abundance (Fig. 6).

PASK is an evolutionary conserved, nutrient-responsive serine/threonine kinase (17, 21, 32). In Saccharomyces cerevisiae, the PASK orthologs Psk1 and Psk2 regulate the partitioning of glucose between storage as glycogen or utilization (33). In mammalian cells, PASK is responsive to the nutrient environment (23) and regulates several signaling pathways involved in the maintenance of energy homeostasis (32), including glyco-
synthase at Ser640 (22). The precise mechanisms by which PASK detects changes in intracellular nutrient concentrations are unknown, but most likely involve the binding of a nutrient-derived small molecule to the N-terminal PAS domain which induces a conformational change and unmasks the kinase domain of the protein (32). In pancreatic β-cells, PASK expres-
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FIGURE 5. PASK enhances PDX-1 protein abundance. A, MIN6 cells were infected with adenosviruses encoding luciferase, WT hPASK, or KD hPASK and exposed to 0.5 or 16 mM glucose for 6 h. PDX-1 (a, e, i, m, q, and u) and PASK (c, g, k, o, s, and w) were visualized by fluorescence microscopy (×40). Nuclei were stained using DAPI (b, f, j, n, r, and v). B, three replicate experiments were quantified. Results are expressed as mean ± S.E. (error bars). **, p < 0.01; ***, p < 0.001.

FIGURE 6. A model for PASK regulation of PDX-1 protein stability through inactivation of GSK3β. In pancreatic β-cells, glucose increases PASK expression and activity. PASK phosphorylates and inactivates GSK3β, alleviating PDX-1 serine phosphorylation and preventing its degradation.

Although these findings were not confirmed in another study (34). Importantly, however, PASK mRNA levels are reduced and are no longer responsive to glucose in islets from type 2 diabetic humans (35). Recently, we have identified two rare mutations in the pask gene associated with early onset diabetes (26). One of these induced an ~2-fold increase in PASK activity, and its expression in islets increased basal insulin secretion and gene expression, supporting an important role for PASK in human β-cell function.

In response to changing glucose levels, PDX-1 undergoes a number of post-translational modifications that modulate its stability, subcellular localization, and binding to the insulin gene promoter. These include phosphorylation (4, 8, 9, 12–14, 16, 37–42), SUMOylation (43), and O-GlcNAcylation (44, 45). SUMOylation of PDX-1 increases its nuclear localization as well as its protein stability and is correlated with an increase in insulin promoter activity (43). PDX-1 contains at least two sites of O-GlcNAcylation that increase its DNA binding activity (44, 45). PDX-1 can be phosphorylated on multiple residues, but the functional role of these specific phosphorylation sites remains poorly understood. In fact, although phosphorylation of PDX-1 in response to glucose was initially proposed to regulate its nuclear translocation, more recent studies have shown that the
effects on PDX-1 function depend on the phosphorylated residues. Phosphorylation downstream of the mitogen-activated protein kinase p38 and phosphatidylinositol 3-kinase was shown to stimulate PDX-1 nuclear localization (4, 9) whereas phosphorylation on Ser^{269} by homeodomain-interacting protein kinase 2 (HIPK2) has been shown to lead to nuclear exclusion (37). Phosphorylation of Thr^{152} by PASK has also been reported (16), although whether this occurs in vivo remains to be demonstrated. In this study, we were not able to detect a significant effect of glucose on Thr phosphorylation of immunoprecipitated PDX-1 (data not shown). Phosphorylation also regulates PDX-1 DNA binding activity (39, 41) as well as its interaction with transcriptional co-factors (42, 46). Finally, phosphorylation has also been shown to regulate PDX-1 stability (Refs. 8, 14 and see below).

Our results confirm a significant decrease in the overall serine phosphorylation of PDX-1 in response to glucose stimulation, in agreement with a previous study (8). The inverse relationship between the increase in PDX-1 protein levels and its degree of serine phosphorylation suggests that phosphorylation at specific serine residues is associated with degradation of the protein (8, 14). Indeed, Humphrey et al. (8) have shown that GSK3β phosphorylation of PDX-1 at C-terminal Ser^{268} targets the protein for proteasomal degradation and that glucose alleviates GSK3β-mediated degradation of PDX-1 via inactivation of GSK3β by the Ser/Thr protein kinase Akt. Consistent with this, PDX-1 protein expression is reduced in pancreatic β-cells overexpressing GSK3β (8, 15) and, conversely, is increased upon loss of GSK3β (36) or Akt overexpression (8). An et al. (37) have come to opposite conclusions regarding Ser^{269} (corresponding to Ser^{268} in humans, as in the Humphrey et al. study (8)). Even though glucose decreased its phosphorylation, neither PDX-1 stability nor its transactivation potential was affected, suggesting that GSK3β and/or HIPK2 target more than one site, which would explain the difference in the observed effects. In the present study we identified an additional mechanism by which glucose stabilizes PDX-1, namely via phosphorylation of GSK3β by PASK. Although we cannot unequivocally conclude from our data that direct phosphorylation of Ser^{5} GSK3β mediates PASK stabilization of PDX-1, this possibility is highly likely considering our observations that PASK directly phosphorylates GSK3β in vitro and that overexpression of PASK mimics the effects of glucose on PDX-1 serine phosphorylation. The increase of GSK3β Ser^{5} phosphorylation at basal glucose in response to WT PASK overexpression was not statistically significant, suggesting that this event might be necessary but not sufficient for the full effect of glucose on GSK3β Ser^{5} phosphorylation. A role for PASK in this process is supported by our finding in islets isolated from pask-null mice where GSK3β Ser^{5} phosphorylation is significantly reduced. This does not exclude the possibility that PASK might also phosphorylate GSK3β at additional other residues. Nevertheless, our data strongly suggest that PASK inactivates GSK3β because phosphorylation at Ser^{5} is a well known inactivating regulation (29, 30). Boucher et al. (14) showed that GSK3β can also phosphorylate PDX-1 on Ser^{61} and Ser^{66}, which leads to its degradation in conditions of oxidative stress. In contrast, Khoo et al. (12) observed that ERK1/2 phosphorylates PDX-1 in vitro on Ser^{61} and Ser^{66} residues, leading to an increase in insulin gene promoter activity. The apparent discrepancy between these studies may be explained by the fact that Boucher et al. (14) examined endogenous PDX-1 phosphorylation whereas Khoo et al. (12) used an in vitro approach. Clearly, further studies are required to better understand the mechanisms and functional impact of Ser^{61} and Ser^{66} phosphorylation of PDX-1 and to determine whether PASK and ERK1/2 act through different mechanisms to modulate PDX-1 and insulin gene expression.

Previously we have shown that overexpression of WT hPASK increases PDX-1 protein levels at low glucose and that overexpression of KD hPASK blocks the glucose effect (24). By performing a cycloheximide chase experiment, here we found that PDX-1 protein stability was enhanced in MIN6 cells overexpressing WT hPASK but not the KD form in both MIN6 cells and isolated rat islets. Consistent with previous data (24, 26), overexpression of WT hPASK did not affect PDX-1 mRNA expression (data not shown), consistent with a mainly post-translational effect. Importantly, overexpression of KD GSK3β blocked PDX-1 degradation in cells overexpressing KD PASK, thus demonstrating the requirement for GSK3β in PASK regulation of PDX-1 stability.

Although glucose-induced nuclear translocation of PDX-1 had been reported by subcellular fractionation (4) or with an exogenously expressed protein (5, 9, 16, 37, 38), fewer studies have been able to demonstrate similar subcellular redistribution of endogenous PDX-1 by immunohistochemistry (9, 38). In our experiments PDX-1 was expressed predominantly in the nucleus of MIN6 cells even at low glucose levels. Consistent with our previous findings (24), overexpression of WT PASK dramatically increased PDX-1 protein abundance at low glucose levels, whereas overexpression of KD PASK blocked glucose-induced PDX-1 protein abundance. Although we cannot exclude a potential effect of glucose or PASK on PDX-1 subcellular localization, our results suggest that regulation of protein stability and abundance is predominant in this system.

In conclusion, we have demonstrated a novel role for PASK in modulating PDX-1 protein stability, identifying GSK3β as a new PASK target in the maintenance of pancreatic β-cell function. These observations suggest that PASK could potentially be targeted therapeutically to enhance PDX-1 expression and activity and thereby improve pancreatic β-cell function in type 2 diabetes.

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