Regulation by Per-Arnt-Sim (PAS) kinase of pancreatic duodenal homeobox-1 nuclear import in pancreatic β-cells

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Abstract
The transcription factor PDX-1 (pancreatic duodenal homeobox-1) is required for normal pancreatic development and for the function of insulin-producing islet β-cells in mammals. We have shown previously that glucose regulates insulin gene expression in part through the activation and translocation of PDX-1 from the nuclear periphery to the nucleoplasm. We have also found that PASK [PAS (Per-Arnt-Sim) kinase], a member of the nutrient-regulated family of protein kinases, is activated in response to glucose challenge in β-cells and is involved in the regulation of expression of PDX-1. Purified PASK efficiently phosphorylated recombinant PDX-1 in vitro on a single site (Thr-152). To determine the impact of phosphorylation at this site, we generated wild-type and mutant (T152A, T152D and T152E) forms of PDX-1 and examined the distribution of each of these in clonal MIN6 β-cells by immunocytochemical analysis. Unexpectedly, only the T152D mutation significantly affected subcellular distribution, increasing the ratio of nuclear/cytosolic labelling at low and high glucose concentrations, suggesting that phosphorylation at Thr-152 inhibits nuclear uptake in response to glucose. Based on these results, experiments to examine the contribution of Thr-152 to the overall phosphorylation of PDX-1 in intact cells will be undertaken.

PDX-1 (pancreatic duodenal homeobox-1), also called IPF-1 (insulin promoter factor-1), is an important transcription factor expressed in β-cells in the islet of Langerhans [1,2]. Thus PDX-1 plays a pivotal part in both embryonic pancreas development and in regulating pancreatic islet β-cell-specific gene expression in adults [3].

Homzygous disruption of the Pdx-1 gene results in embryonic pancreas agenesis in mice [4,5], whereas mutations in the orthologous IPF-1 lead to the same phenotype in humans [6]. In mice, heterozygous disruption of the Pdx-1 gene led to hyperglycaemia as a result of inadequate glucose-stimulated insulin production [7]. An inactivating mutation in the Pdx-1 gene is associated with development of MODY 4 (maturity-onset diabetes of the young 4) [6], an inherited form of diabetes that results from impaired β-cell function characterized by disturbed insulin secretion. These results strongly indicate an essential role for PDX-1 in pancreas development and β-cell function.

The involvement of PDX-1 in the transcription of many genes crucial for glucose sensing and insulin gene expression indicates its irreplaceable role in mature β-cells [3]. PDX-1 binds to the promoter elements and activates transcription of many β-cell-specific genes, including those encoding the islet/liver glucose transporter gene, GLUT2 [8], as well as possibly glucokinase [9] and islet amyloid polypeptide [8].

**Figure 1** | PASK efficiently phosphorylates PDX-1 in vitro
Purified human PASK (hPASK), purified mouse PDX-1 or the combination was incubated with [γ-32P]ATP for 1 h at 25°C. Protein was then separated by SDS/PAGE and the gel was visualized by Coomassie staining (left panel) or by autoradiography (right panel). Note the hPASK autophosphorylation, and the aberrant migration of PDX-1 (calculated molecular mass, 32 kDa); PDX-1* is a C-terminal fragment of PDX-1. Molecular-mass sizes are given in kDa.
Figure 2 | Subcellular distribution of both wild-type and mutant forms of PDX-1 by immunocytochemistry

Wild-type (a–b), alanine mutant (c–d) and aspartic acid mutant (e–f) forms of PDX-1 were induced in MIN6 cells. Cells were cultured at 3 mM glucose for 16 h and then treated with 3 mM (a, c, e) or 30 mM (b, d, f) glucose for 6 h; Myc-tagged PDX-1 was detected using anti-Myc antibody (Roche) and Alexa Fluor® 568 (Molecular Probes); nuclear staining was achieved using DAPI (4′,6-diamidino-2-phenylindole). The nuclear distribution of the aspartic acid mutant form of PDX-1 appears to be a more cytosolic distribution compared with wild-type and alanine mutant forms of PDX-1.

Compared with the well-studied role for PDX1 in pancreas development and β-cell function, less is known about its regulation, particularly at the post-translational level [3]. For many other transcription factors, including the steroid receptor superfamily [9] and cytokine-activated STAT (signal transducer and activator of transcription) [10], a shift from the cytosol to the nucleoplasm is a major means of activation. By using immunocytochemistry and laser-scanning confocal microscopy, previous work in our laboratory has investigated the mechanisms by which PDX-1 may be activated by glucose. PDX-1 is associated with the nuclear periphery and the nuclear membrane region in cells incubated at low glucose concentrations [11,12]. Elevated glucose concentrations stimulated the transcriptional activity of a preproinsulin promoter reporter construct monitored in a single living MIN6 β-cell and caused a concomitant translocation of PDX-1 from the nuclear periphery into the nucleoplasm. We have therefore proposed that this shift may contribute to the transactivational capacity of PDX-1 in native β-cells exposed to high extracellular glucose concentrations [11].

PAS (Per-Arnt-Sim) domains regulate the function of many intracellular signalling pathways in response to both extrinsic and intrinsic stimuli [13]. PAS domain-regulated histidine kinases are very common in prokaryotes and control a wide range of fundamental physiological processes, e.g. Rhizobia nitrogen fixation, via the oxygen-sensing protein.
FixL [14]. It has been shown that the mammalian protein kinase PASK (PAS kinase) [14] activity is stimulated in pancreatic islet β-cells by elevated glucose concentrations [15]. Importantly, by both transcriptional and post-transcriptional mechanisms, PASK activities were necessary for mediating the direct (intracellular) effect of glucose stimulation on transcription of the preproinsulin and PDX-1 genes [15].

Recombinant PDX-1 was efficiently phosphorylated by purified PASK kinase in vitro on a single site, Thr-152 (Figure 1). Prompted by the results of this in vitro experiment, wild-type and mutant (T152A, T152D and T152E) forms of PDX-1 were generated in adenoviral vectors. In order to study the regulation by PASK on the translocation of PDX-1, the T152D mutant form of PDX-1 was induced in MIN6 cells to mimic PDX-1 phosphorylation by PASK. Immunocytochemical analysis to examine the intracellular distribution of both wild-type and mutant forms of PDX-1 showed that wild-type and alanine mutant PDX-1s were more nuclear at elevated glucose concentration. In contrast, the T152D and T152E mutants displayed a more cytosolic distribution at both high and low glucose concentrations than wild-type or T152A mutant PDX-1 (Figure 2).

In the light of the above findings, adenoviruses encoding wild-type or mutant forms (as above) of Pdx-1 gene were generated for PDX-1 overexpression in a mouse pancreas-derived β-cell line, MIN6 cells, followed by 32P metabolic labelling experiments to examine the contribution of Thr-152 to the overall phosphorylation of PDX-1 in intact cells. Both wild-type and T152A mutant forms of PDX-1 were apparently phosphorylated in living β-cells. Importantly, incorporation of 32P into the Thr-152-mutated forms of PDX-1 implied that phosphorylation at other sites predominates the overall phosphorylation of PDX-1 in living β-cells.

Taken together, the present results suggest that the hypothesis that the PDX-1 phosphorylation by PASK causes translocation from the nuclear periphery to nucleoplasm would appear to be untrue. Rather, phosphorylation of PDX-1 by PASK seems to drive PDX-1 out of the nucleus.

Intriguingly, this may contribute to the effects of chronic hyperglycaemia to inhibit insulin secretion in the long term in diabetic patients. For the short-term stimulation of insulin secretion, according to the PDX-1 32P metabolic labelling phosphorylation experiment results, we assume that there must be other sites on PDX-1 that can be phosphorylated by other kinases to realize the shift of PDX-1 from the nuclear periphery to the nucleus when cells were exposed to increased glucose concentration. Results from other laboratories also suggest that phosphorylation sites Ser-61 and Ser-66 of PDX-1 by glycogen synthase kinase 3 may be involved [3].

References

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