The Pancreatic Duodenal Homeobox-1 Protein (Pdx-1) Interacts with Histone Deacetylases Hdac-1 and Hdac-2 on Low Levels of Glucose

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We have previously demonstrated that high concentrations of glucose stimulate insulin gene expression by causing hyperacetylation of histone H4 at the insulin gene promoter. Furthermore, we have shown that the glucose-mediated hyperacetylation of histone H4 depends on the recruitment of the histone acetyltransferase p300 by the beta cell-specific transcription factor Pdx-1. In this study, we demonstrate that the histone deacetylases Hdac-1 and Hdac-2 are rapidly recruited to the insulin promoter in the mouse insulinoma cell line MIN6 when cells are switched from high to low glucose media. Moreover, we demonstrate that the beta cell-specific homeodomain protein Pdx-1 interacts with histone deacetylases Hdac-1 and Hdac-2 at low levels of glucose. In vitro studies indicate that the interaction between Pdx-1 and Hdac-1 or Hdac-2 is direct and requires the C terminus of Pdx-1. Treatment of MIN6 cells with okadaic acid, which inhibits the activity of protein phosphatases, abolishes the interaction of Pdx-1 with Hdac-1 and Hdac-2 on low levels of glucose, suggesting the requirement of a dephosphorylation event for this interaction to occur. These data indicate that insulin gene expression is decreased on low levels of glucose by recruitment of Hdac-1 and Hdac-2 to the insulin promoter by the transcription factor Pdx-1.

The production and secretion of insulin from pancreatic beta cells is regulated by changes in blood glucose levels, and defects in this regulatory process result in metabolic disorders such as diabetes. Insulin gene expression is up-regulated by increases in glucose concentration and involves several transcription factors including Pdx-1, MafA, and NeuroD (1–5). The homeodomain protein Pdx-1 is mainly expressed in the pancreatic beta cells and is required for the development and differentiation of the pancreas as well as for glucose stimulation of insulin gene transcription (6–8). Expression of Pdx-1 in the NES2Y cell line or in islets of the type 2 diabetes model Psammomys obesus, which are defective for expression of Pdx-1, restores their ability to stimulate insulin gene expression in response to increases in glucose levels (9, 10). Mutations in Pdx-1 have also been associated with the MODY4 locus (11, 12), which is a subtype of type 2 diabetes.

Modification of the N-terminal tails of histones by acetylation has been implicated in regulation of gene expression (13). In general, hyperacetylation of histone tails is associated with increased gene expression, while hypoacetylation is associated with repression of transcription or silencing (14). However, inhibition of histone deacetylase activity by treatment with trichostatin A leads to both activation and silencing of a subset of genes (15). Several histone acetyltransferases as well as histone deacetylases have been cloned to date and implicated in regulation of gene expression (16, 17). A number of transcription factors, including NFkB and the muscle-specific transcription factor MEF2 have been shown to interact with both the histone acetyltransferases and deacetylases (18–21). However, the exact mechanisms that determine the interaction of a specific transcription factor with various histone-modifying enzymes are for the most part unknown.

Histone deacetylases are divided into three different classes based on their homology, and Hdac-1 and Hdac-2 belong to the class I histone deacetylases together with Hdac-3 and Hdac-8 (17, 22). The class I histone deacetylases are 400–500 amino acid long and localized primarily to the nucleus. Histone deacetylases exist as large protein complexes, and Hdac-1/Hdac-2 have been shown to associate with co-repressor complexes Sin3A and Mi-2/NuRD (17, 22, 23). The presence of histone deacetylases in distinctive complexes with different transcription factors appears to contribute to their diverse biological activities. Multiple studies indicate that the histone deacetylase Hdac-1 and Hdac-2 contain several different post-translational modifications, including phosphorylation and sumoylation (24, 25). However, the exact role of these post-translational modifications in regulating the function and activity of these enzymes, and the tissue specificity of these modifications is unknown.

The beta cell-specific transcription factor Pdx-1 has been previously shown to interact with the histone acetyltransferases p300 and CBP, and this interaction has been demonstrated to be important for insulin gene expression (26–28). We have recently demonstrated that induction of insulin gene expression by high concentrations of glucose involves the hyperacetylation of histone H4 at the insulin gene promoter (29). At low levels of glucose, insulin gene expression is down-regulated, which correlates with deacetylation of histone H4 at the insulin promoter. In this report, we demonstrate that the beta cell-specific transcription factor Pdx-1 interacts with histone...
Glucose-regulated Interaction of Pdx-1 with HDACs

deacetylases Hdac-1 and Hdac-2 at low levels of glucose. Furthermore, Pdx-1 and Hdac-2 are associated with the insulin promoter only when glucose levels are low. Therefore, it is likely that Pdx-1 recruits Hdac-1 and Hdac-2 to the insulin gene promoter to cause deacetylation of histone H4 and down-regulation of insulin gene expression when glucose levels are low.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Mouse insulinoma-6 (MIN6) cells of passage 22 through 30 were cultured in Dulbecco's modified Eagle media (DMEM) containing 5 mM glucose, 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM glutamine, and 100 μg/ml β-mercaptoethanol (30). For the glucose regulation experiments, MIN6 cells were grown overnight in DMEM with 5 mM glucose and then washed three times with 1× PBS and transferred to 3 or 30 mM glucose containing medium without serum for 2 h unless otherwise indicated.

**Chromatin Immunoprecipitation (ChIP) Assay**—Chromatin isolation was performed as previously published (29, 31). The antibodies used for immunoprecipitation in ChIP assays were Hdac-1 (sc-7872), Hdac-2 (sc-7899), and Pdx-1 (E910) antibodies (gift from Dr. E. Seto, University of South Florida (34)). For the HDACs, the antibodies used were HDAC-1 (sc-7872), HDAC-2 (sc-7899) and c-Myc (E910) antibodies (Santa Cruz Biotechnology). All PCR reactions were performed on a Perkin-Elmer 9600 (Stratagene) in a 20-μl reaction volume as described previously (29). The linear range for each primer pair was determined empirically, using different cycle numbers and by real-time PCR using the instrument Mx4000 (Stratagene). The primers used are GAAGGCTACCTCTTGGG and GGGGGTACTGAGGCC for the mouse insulin I promoter (from −10 to −281). PCR reactions were performed at least three independent times for each sample. The PCR products obtained with the immunoprecipitated DNA were normalized to the products obtained with the total input DNA. The bands were visualized using a ChemiDoc System Imager (Bio-Rad). The PCR products obtained had the expected molecular size, and their identity was confirmed by sequencing.

**Real-time PCR**—The association of acetylated histone H4, Hdac-1, and Hdac-2 with the mouse insulin 1 promoter in cells incubated with 3 or 30 mM glucose was determined using the ChIP assay and quantified using real-time PCR with Brilliant SYBR Green QPCR Master Mix according to the manufacturer's instructions (Stratagene). The primers used to amplify the insulin promoter are described above and were used at a final concentration of 150 nM. Real-time PCR reactions were performed on a Stratagene Mx4000 according to the manufacturer's instructions. The threshold cycle (Ct) value for each PCR reaction was determined as the cycle at which the fluorescence was 10 times higher than the mean of background levels. Real-time PCR data were analyzed as described previously (32, 33). Briefly, to quantify the association of the various proteins with the insulin 1 promoter, a ΔCt value was calculated for each sample by subtracting the Ct value for the input (total DNA) from the Ct value of the immunoprecipitated sample as template. The obtained ΔCt value for the samples immunoprecipitated with specific antibodies was subtracted from the ΔCt value of samples immunoprecipitated with rabbit IgG as control to give ΔΔCt. Fold differences were calculated using the formula 2−ΔΔCt, since one cycle difference is equivalent to a 2-fold difference in amplification. Each sample was quantified in duplicate from three independent immunoprecipitations. A melt curve analysis was performed for each sample after PCR amplification to ensure that the melting curve characteristics were consistent with the presence of a single PCR product.

**Preparation of Cell Extracts and Co-immunoprecipitation Assay**—Whole cell extracts from MIN6 cells incubated with 3 or 30 mM glucose were prepared as described previously using 1% Nonidet P-40 for lysis of the cells (28). The extracts used in the co-immunoprecipitation assays were prepared in lysis buffer (10 mM Tris-Cl, pH 8.0, 140 mM NaCl, 0.5% Triton X-100, 1% sodium deoxycholate, 2 mM glutamine, and 100 μg/ml β-mercaptoethanol). After removal of cellular debris by centrifugation, the obtained supernatant was diluted with 4 volumes of dilution buffer (50 mM Tris-Cl, pH 7.5, 10% glycerol, 5 mM MgCl2, 0.2 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfon- fyl fluoride, and protease inhibitors). Immunoprecipitation with Pdx-1, Hdac-1 (sc-7872), Hdac-2 (sc-7899), and c-Myc (E910) antibodies was carried out overnight at 4 °C, followed by incubation with protein A-Sepharose 4 Fast Flow (Amersham Biosciences) for 1–2 h. The pellets were washed six times in 1 ml of Wash buffer (50 mM Tris-Cl, pH 7.5, 10% glycerol, 100 mM NaCl, 0.1% Nonidet P-40, 1 mM EDTA) and resuspended in 2× SDS sample buffer. Rabbit or mouse IgG (Sigma) was used as a negative control for nonspecific binding. Proteins were visualized using the ECL chemiluminescent detection system (Amer sham Biosciences).

**Expression and Purification of Histone Deacetylase Proteins**—The Hdac-1-GST and Hdac-2-GST constructs were kindly provided by Edward Seto, University of South Florida (34). For expression and purification of recombinant proteins, Escherichia coli BL21 (DE3) R/I/C cells expressing the various proteins were cultured in 1 liter of TB medium at 37 °C to a density of about 0.6 at 600 nm, and expression of the recombinant proteins was induced with 1 mM isopr opyl-1-thio-β-galactopyranoside for 5 h. After harvesting the cells by centrifugation, the cell pellet was resuspended in 20 ml of lysis buffer (50 mM Tris, pH 8.0, 300 mM NaCl, 5 mM imidazole, 5 mM β-mercaptoethanol, 1 mM phenylmethylsulfon fluoride, and 1 mM phenylmethylsulfon fluoride for lysis) through a French press. The cell lysate was centrifuged at 50,000×g for 2 h, and the obtained supernatant was applied on Ni-NTA agarose column (Qiagen) to purify His6-Pdx-1 and washed with 15 bed volumes of the lysis buffer containing 20 mM imidazole. The His6-Pdx-1 protein was eluted from the resin with a gradient, starting at 20 mM imidazole and ending at 500 mM imidazole in lysis buffer. The Hdac-1-GST and Hdac-2-GST were expressed using the same procedure. The lysis buffer used was 50 mM Tris-Cl, pH 7.5, 500 mM NaCl, and 2 mM β-mercaptoethanol. GST fusion proteins were purified using a glutathione-Sepharose column and by eluting with a gradient starting at 1 M and ending at 25 mM glutathione. The eluate was dialyzed against 1× PBS containing 2 mM β-mercaptoethanol. The protein concentration and purity was determined by separating the eluted proteins on SDS-PAGE together with a bovine serum albumin standard (Promega) and by staining with Coomassie Blue (Sigma).

**In Vitro Interaction Assays**—For pull-down experiments, 100 ng of recombinant GST proteins were incubated with glutathione-agarose beads (Amersham Biosciences) for 1 h. After washing the beads four times with binding buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 2 mM EDTA, 10 mM MgCl2), 100 ng of the indicated His6-tagged proteins were added and incubated with rotation at 4 °C for 1 h. The beads were then washed four times with binding buffer, and the proteins were subsequently eluted from the beads by boiling them for 10 min in 1× SDS loading buffer. The reverse pull-down experiments were performed using the same procedure but by incubating 100 ng of recombinant His6-Pdx-1 with Ni-NTA agarose. For these experiments the binding buffer also included 20 mM imidazole.

**Production of Recombinant Adenoviruses**—The full-length Pdx-1 or Pdx-1Δ1–79 adenovirus, which lacks the first 79 N-terminal amino acids containing the activation domain, was constructed using the Ad-Easy adenoviral system as described previously (35, 36). To generate the Pdx-1 or Pdx-1Δ1–79 recombinant virus, the full-length Pdx-1 cDNA or Pdx-1 lacking the first 237 bp was amplified by PCR and subcloned into the pACCMM-vmy vector in-frame with a triple Myc tagging sequence by using HindIII and MluI restriction sites to relieve Pdx-1 or Pdx-1Δ1–79 fused to Myc, and subcloned into the HindIII site of the shuttle vector pAdTrack-CMV (36). The obtained plasmids were co-transformed into E. coli BJ5183 cells with pAdEasy-1 plasmid containing the adenoviral genome. After recombination in bacteria, the obtained plasmids were transferred into E. coli DH5α cells and the prepared plasmid DNA was linearized and used to transfect HEK 293 cells using Lipofectamine (Invitrogen Life Technologies, Inc.) according to the manufacturer’s instructions. The HEK 293 cell lysate was used for additional viral amplification by several rounds of infection of HEK 293 cells. MIN6 cells were infected with a multiplicity of infection of 80–100 using a 2-h exposure to the adenovirus (35).

**Measurement of Histone Deacetylase (HDAC) Activity**—Histone deacetylase activity associated with Pdx-1 or the dominant negative Pdx-1 protein was determined by incubating about 1 mg of MIN6 total cell extract with 2 μg of either mouse IgG, or c-Myc antibodies overnight. After addition of 10 μl of protein A-Sepharose, the mixture was incubated on a rotator at 4 °C for 3 h. The protein A-Sepharose beads were pelleted by centrifugation and washed four times with 1 ml of 1× PBS and resuspended in 40 μl of assay buffer. Histone deacetylase activity was quantified using the HDAC fluorescent activity assay/Drug Discovery Kit (AK-500; BIOMOL Research Laboratories). Briefly, the immunoprecipitated proteins were incubated with 100 μM acetylated substrate in 100 μl of assay buffer for 30 min at 37 °C and quantified using a fluorometer (excitation at 360 nm and emission at 450 nm) (37). This assay system allows detection of a fluorescent signal upon deacetylation of a histone substrate when treated with developer (37).
RESULTS

The Histone Deacetylases Hdac-1 and Hdac-2 Are Recruited to the Insulin Gene Promoter in the Presence of Low Concentrations of Glucose—We have previously shown that high concentrations of glucose stimulate insulin gene expression by causing the hyperacetylation of histone H4 at the insulin gene promoter (28, 29). Exposure to low concentrations of glucose triggered the deacetylation of histone H4, which correlated with decreased insulin gene expression. Treatment with trichostatin A, which inhibits histone deacetylase activity, led to increased histone H4 acetylation and insulin gene expression even at low concentrations of glucose (29). These data suggested a role for histone deacetylases in insulin gene expression at low (3 mM) glucose. To test this idea, we analyzed the recruitment of the Hdac-1 and Hdac-2 and the histone acetyltransferase p300 to the insulin gene promoter using the ChIP assay in the mouse insulinoma cell line MIN6 incubated with low (3 mM) concentrations of glucose. For this purpose, the MIN6 cells were pregrown on 30 mM glucose and shifted to 3 mM glucose, and samples were taken at 0 and 30 min. PCR products from immunoprecipitated samples and input DNA as template using primers to amplify the mouse insulin 1 promoter were separated on 8% non-denaturing PAGE gel and stained with ethidium bromide (A). B displays the real-time PCR analysis of histone H4 acetylation, p300, Hdac-1, and Hdac-2 recruitment to the insulin promoter. MIN6 cells incubated on 30 mM glucose were shifted to 3 mM glucose, and samples were taken at 0, 10, 20, 30, and 60 min and subjected to ChIP analysis. C, Western blot analysis of Hdac-1 and Hdac-2 levels in MIN6 cells incubated with 3 or 30 mM glucose for 5 h in comparison to β-actin levels.

Since Hdac-1 and Hdac-2 are recruited to the insulin promoter only in the presence of low levels of glucose, we analyzed whether the abundance of these proteins was regulated by glucose. Extracts from MIN6 cells incubated with 3 or 30 mM glucose were used for immunoblotting with Hdac-1 or Hdac-2 and β-actin antibodies. We found that the levels of Hdac-1 and Hdac-2 were similar on 3 and 30 mM glucose (Fig. 1C).

Pdx-1 Interacts with Hdac-1 and Hdac-2 in a Glucose-dependent Manner in Vivo—Since the beta cell-specific transcription factor Pdx-1 is one of the main players in glucose regulation of insulin gene transcription, we tested whether Pdx-1 is able to interact with the histone deacetylases Hdac-1 and Hdac-2 in vivo in a glucose-dependent manner by co-immunoprecipitation experiments. Extracts from MIN6 cells incubated with 3 or 30 mM glucose were prepared and used in co-immunoprecipitation experiments with Pdx-1 or Hdac-1 antibodies. Western blot analysis of proteins from Pdx-1 immunoprecipitates with Hdac-1 or Hdac-2 antibodies indicated that these proteins co-immunoprecipitate with Pdx-1 (Fig. 2A, upper panel). The interaction of Pdx-1 with Hdac-1 and Hdac-2 was much stronger in the presence of low concentrations (3 mM) of glucose. Similar results were obtained in co-immunoprecipitation experiments with antibodies against Hdac-1 and...
Hdac-1 and Hdac-2 Interact Constitutively with a Dominant Negative Form of Pdx-1—In order to determine whether the interaction of Pdx-1 with Hdac-1 and Hdac-2 requires the activation domain of Pdx-1, MIN6 cells were infected with an adenovirus, which expresses an N-terminal-truncated version of Pdx-1 that lacks the first 79 amino acids containing the activation domain (Pdx-1A–79). This truncated protein acts as a dominant negative, because it competes with the endogenous Pdx-1 for binding to the insulin promoter but is unable to activate transcription. The truncated Pdx-1 protein was tagged with a triple human c-Myc epitope at the C terminus. To test the interaction of Pdx-1A–79 with Hdac-1 and Hdac-2, extracts from MIN6 cells incubated with 3 or 30 mM glucose were immunoprecipitated with human c-Myc, Hdac-1, or Hdac-2 antibodies. Western blot analysis of extracts immunoprecipitated with c-Myc antibodies revealed that Pdx-1A–79 was able to interact with Hdac-1 and Hdac-2 constitutively independent of the glucose concentration compared with the full-length Pdx-1 protein, which displayed interaction mainly on low glucose (Fig. 4A). Like the full-length Pdx-1, recombinant His6-Pdx-1A–79 was able to interact with both, Hdac-1 and Hdac-2 in in vitro experiments (Fig. 4B).

In support of the presented data, histone deacetylase activity associated with the full-length Pdx-1 was more than 4-fold higher in cells incubated with 3 versus 30 mM glucose (Fig. 4C). Moreover, in MIN6 cells overexpressing the dominant negative Pdx-1 protein, histone deacetylase activity associated with Pdx-1A–79 was similar on 3 versus 30 mM glucose and was higher than that observed with the full-length Pdx-1 (Fig. 4C). The histone deacetylase activity associated with full-length and truncated Pdx-1 protein was determined by immunoprecipitation with the N-terminal Pdx-1 (deleted in Pdx-1A–79) and c-Myc antibodies, respectively.

The Interaction between Pdx-1 and Hdac-1 or Hdac-2 Is Regulated by a Dephosphorylation Event—Our previous data indicate that a phosphorylation event regulates the interaction of Pdx-1 with the histone acetyltransferase p300 in response to high concentrations of glucose and that treatment with okadaic acid causes a constitutive interaction of Pdx-1 with p300 (28). Based on these data we speculated that high concentrations of glucose disrupt the interaction of Pdx-1 with Hdac-1 and Hdac-2, but causes the association of Pdx-1 with p300. To test the idea that a dephosphorylation event is required to mediate the interaction of Pdx-1 with Hdac-1/2, we incubated MIN6 cells in low glucose-containing media in the presence or absence of the 100 nM okadaic acid. After lysis of the cells, the extracts were used to immunoprecipitate Pdx-1 and the presence of Hdac-1 or Hdac-2 in Pdx-1 immunoprecipitates was detected by using antibodies against Hdac-1 or Hdac-2. The interaction between Pdx-1 and Hdac-1/2 was disrupted by treatment with okadaic acid (Fig. 5), suggesting the idea that a dephosphorylation event is required to enable the interaction of Pdx-1 with Hdac-1 and Hdac-2.

Previous data indicate that Pdx-1 changes its subcellular localization in response to different concentrations of glucose (28, 38, 39). At high levels of glucose, Pdx-1 is localized mainly to the cytoplasm and nuclear periphery and in response to low glucose it translocates to the nucleoplasm. Both, Hdac-1 and Hdac-2 appear to be localized to the nucleoplasm in the MIN6 cell line independent of the glucose concentration (data not shown).

**DISCUSSION**

Modification of N-terminal tails of histones by acetylation has been correlated with increased gene expression (13, 14). We have previously shown that glucose induces insulin gene expression by causing hyperacetylation of histone H4 at the in-
Furthermore, we found that the hyperacetylation of histone H4 at the insulin promoter by high glucose requires the recruitment of the histone acetyltransferase p300 via its interaction with the beta cell specific transcription factor Pdx-1 (28).

In this report, we demonstrate that Pdx-1 interacts also with the histone deacetylases Hdac-1 and Hdac-2 in vivo mainly at low levels of glucose. The interaction of Pdx-1 with Hdac-1 and Hdac-2 is disrupted by treatment with okadaic acid, which inhibits protein phosphatases such as PP1 and PP2A (40). In addition, we show that Hdac-1 and Hdac-2 are recruited to the insulin promoter when cells are switched from high to low levels of glucose and may be responsible for the hypoacetylation of histone H4 at the insulin promoter. Based on these and our previous data, we propose that at high concentrations of glucose, a phosphorylation event stimulates the interaction of Pdx-1 with the histone acetyltransferase p300, which becomes recruited to the insulin promoter and causes hyperacetylation of histone H4 at this promoter leading to up-regulation of insulin gene transcription (Fig. 6). At low levels of glucose, a dephosphorylation event causes Pdx-1 to dissociate from p300 and to interact with the histone deacetylases Hdac-1 and

**FIG. 3.** Pdx-1 interacts with Hdac-1 and Hdac-2 in vitro. The interaction of recombinant His$_6$-Pdx-1 with Hdac-1-GST or Hdac-2-GST was determined by incubating about 100 ng of purified protein for 1 h at 37 °C in the presence of GSH beads (A) or Ni-NTA agarose (B). After washing, the samples were separated on a 12% SDS-PAGE and blotted for Pdx-1 (A) or Hdac-1/Hdac-2 (B). Purified GST and Ni-NTA agarose were used as negative controls for nonspecific binding.

**FIG. 4.** Pdx-1 lacking the N-terminal activation domain constitutively interacts with Hdac-1 and Hdac-2. MIN6 cells were infected with wild type (WT) or the dominant negative (DN) Pdx-1 adenovirus lacking the N-terminal 79 amino acids and incubated with 3 or 30 mM glucose. After lysis of cells, wild-type Pdx-1 or dominant-negative Pdx-1 was immunoprecipitated with human c-Myc antibodies. The presence of Hdac-1 or Hdac-2 in the immunoprecipitates was detected by blotting with specific antibodies. Mouse IgG was used as a negative control for immunoprecipitation (A). The in vitro interaction of full-length Pdx-1 or dominant negative Pdx-1 fused to His$_6$ was determined by incubating about 100 ng of recombinant protein with Hdac-1-GST or Hdac-2-GST (B). The amount of histone deacetylase activity associated with wild type (WT-Pdx-1) or dominant negative Pdx-1 (Pdx-1 DN) in MIN6 cells infected with the corresponding adenoviruses and incubated with 3 or 30 mM glucose was determined by immunoprecipitation with human c-Myc antibodies (C). Histone deacetylase activity in the immunoprecipitates was determined using a fluorometric assay (37).

**FIG. 5.** Treatment of MIN6 cells with okadaic acid disrupts the interaction of Pdx-1 with Hdac-2. The interaction of Pdx-1 with Hdac-2 was determined in MIN6 cells incubated with 30 or 3 mM glucose with or without 100 nM okadaic acid (OA). After immunoprecipitation with Hdac-2 antibodies, the presence of Pdx-1 in the Hdac-2 immunoprecipitates was detected by immunoblotting with Pdx-1 antibodies. Rabbit IgG was used as a negative control. Similar data were obtained for Hdac-1 (data not shown). 3+OA, 3 mM glucose plus 100 nM okadaic acid; Hc, heavy chain.
Hdac-2. Recruitment of Hdac-1 and Hdac-2 to the insulin promoter at low levels of glucose leads to hyperacetylation of histone H4, resulting in down-regulation of insulin gene expression (Fig. 6).

Consistent with the idea that Pdx-1 regulates in insulin gene expression under low and high concentrations of glucose, Pdx-1 is associated with the insulin promoter in vivo under both conditions. However, the binding of Pdx-1 to the insulin promoter is about 2-fold increased in response to high glucose levels. It is possible that the association of Pdx-1 with the insulin promoter is enhanced by its interaction with the histone acetyltransferase p300. Alternatively, the initial acetylation of histone H4 at the insulin promoter may stabilize the binding of Pdx-1.

Several other transcription factors have been shown to interact with both histone acetyltransferases and deacetylases in a regulated manner. The interaction of MyoD with Hdac-1 in undifferentiated cells represses the expression of muscle-specific genes (41). On the other hand, association of MyoD with the histone acetyltransferases p300 and PCAF is important for myogenesis (42). The transcription factor CREB (cAMP response element-binding protein) interacts with the histone deacetylases Hdac-1 and Hdac-2 (43) as well as with the histone acetyltransferases CBP and p300 when it is phosphorylated (44, 45).

Our data indicate that the N-terminal activation domain of Pdx-1, which is required for its interaction with the histone acetyltransferases p300 and CBP (26, 27), is not essential for its interaction with Hdac-1 and Hdac-2 in vitro as well as in vivo. Although several proteins have been shown to interact with Pdx-1 via its N-terminal activation domain, so far only one protein called PCIF1 has been identified to interact with Pdx-1 via its C terminus (46). Interestingly, it has been demonstrated that PCIF1 inhibits the ability of Pdx-1 to activate transcription (46). However, it is not known whether PCIF1 is in a complex with Pdx-1 and Hdac-1/2. It also remains to be determined whether PCIF1 is able to interact with the histone deacetylases Hdac-1/2.

Treatment of MIN6 cells with okadaic acid, which inhibits the activity of the protein phosphatases PP1 and PP2A, disrupts the localization of Pdx-1 to nuclear periphery (28, 38, 39) and also inhibits its interaction with Hdac-1 and Hdac-2 on low levels of glucose. Therefore it is possible that the localization of Pdx-1 to nuclear periphery is important for its interaction with Hdac-1 and Hdac-2. Alternatively, the localization of Pdx-1 to nuclear periphery and its interaction with Hdac-1 and Hdac-2 at low concentrations of glucose may be independent processes, because Hdac-1 and Hdac-2 appear to be localized to the nucleoplasm independent of the glucose concentration (data not shown). Nevertheless, both the localization of Pdx-1 to nuclear periphery and its interaction with Hdac-1 and Hdac-2 at low levels of glucose requires a dephosphorylation event. It is likely that Pdx-1 becomes phosphorylated at high concentrations of glucose which enables it to interact with the histone acetyltransferase p300 to up-regulate insulin gene expression (Fig. 6). At low concentrations of glucose, dephosphorylation of Pdx-1 may cause its dissociation from p300 and promote its interaction with Hdac-1 and Hdac-2 to down-regulate insulin gene expression (Fig. 6). In agreement with this proposed mechanism, we found that Pdx-1 is a phosphoprotein, however Pdx-1 appears to be phosphorylated independent of glucose levels (data not shown). It is likely that Pdx-1 becomes phosphorylated at different residues depending on the concentration of glucose, which may determine its interaction with p300 or Hdac-1/2. However, it is also possible that exposure to low levels of glucose causes the dephosphorylation of Hdac-1 and Hdac-2, which may promote their interaction with Pdx-1. Indeed, previous data indicate that Hdac-1 and Hdac-2 are phosphorylated by protein kinase CK2, which disrupts the complex formation between Hdac-1 and Hdac-2 with the co-repressor Sin3 (24, 47, 48).

Interestingly, like Pdx-1, the transcription factor CREB interacts with both histone acetyltransferases as well as histone deacetylase Hdac-1. Phosphorylation of CREB by PKA at Ser-133 stimulates its interaction with histone acetyltransferases (44, 45), while association of Hdac-1 and CREB in unstimulated cells blocks the phosphorylation of CREB at Ser-133 (43). Furthermore, it has been shown that in unstimulated cells, Hdac-1 promotes the dephosphorylation of CREB by forming a stable complex with protein phosphatase PP1 (43). Since okadaic acid is an inhibitor of PP1, it is possible that on low levels glucose, the interaction of Hdac-1 with Pdx-1 may stimulate the dephosphorylation of Pdx-1 and thereby disrupt its interaction with the histone acetyltransferase p300. As shown for the transcription factor CREB, association of Pdx-1 with Hdac-1 and PP1 or PP2A could result in coordinated dephosphorylation of Pdx-1 and deacetylation of histone H4 at the insulin promoter at low levels of glucose. This would result in down-regulation of insulin gene expression at low concentrations of glucose.

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