Glucose Regulates Insulin Gene Transcription by Hyperacetylation of Histone H4*

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Induction of insulin gene expression in response to high blood glucose levels is essential for maintaining glucose homeostasis. Although several transcription factors including Beta-2, Ribe3b1, and Pdx-1 have been shown to play a role in glucose stimulation of insulin gene expression, the exact molecular mechanism(s) by which this regulation occurs is unknown. Previous data demonstrate that the transcription factors Beta-2/NeuroD1 and Pdx-1, which are involved in glucose-stimulated insulin gene expression, interact with the histone acetylase p300, suggesting a role for histone acetylation in glucose regulation of the insulin gene expression. We report that exposure of mouse insulinoma 6 cells to high concentrations of glucose results in hyperacetylation of histone H4 at the insulin gene promoter, which correlates with the increased level of insulin gene transcription. In addition, we demonstrate that hyperacetylation of histone H4 in response to high concentrations of glucose also occurs at the glucose transporter-2 gene promoter. Using histone deacetylase inhibitors, we show that increases in histone H4 acetylation cause stimulation of insulin gene transcription even in the absence of high concentrations of glucose. Furthermore, we show that fibroblasts, which lack insulin gene expression, also lack histone acetylation at the insulin gene promoter. In summary, our data support the idea that high concentrations of glucose stimulate insulin gene expression by causing hyperacetylation of histone H4 at the insulin gene promoter.

Type II diabetes is a multifactorial disease caused by a combination of defects in insulin production, insulin secretion, and insulin action. To maintain glucose homeostasis, it is imperative that insulin transcription, translation, and secretion are up-regulated in the β cells of the pancreas in response to high blood glucose levels (1). The pancreatic β cells respond to high blood glucose levels first by secreting insulin from the secretory granules followed by up-regulation of insulin gene transcription and translation as a more long term response (1). A number of other proteins have also been shown to be required for the glucose responsiveness of pancreatic β cells, including glucokinase and glucose transporter-2 (GLUT-2)\(^1\) (2, 3). The expression of GLUT-2 has been shown to be up-regulated by glucose in pancreatic β cells (4–6). The regulation of glucokinase gene expression by glucose remains unclear (7, 8); however, several of the transcription factors required for its expression are also involved in glucose regulation of gene expression (9).

Studies on the regulation of the insulin gene promoter by glucose revealed a number of enhancer elements that contribute to the glucose responsiveness of this promoter (10). This includes the E1/E2 (11), A3/A4 (12, 13), C1 (14, 15), and Za1 (16) enhancer elements. Transcription at the insulin gene promoter is regulated by various complex interactions between different transcription factors to merge signals from a variety of different pathways. The transcription factors that have been shown to be important for glucose-regulated insulin gene expression include the β helix-loop-helix protein E47/Pan1 (11) and Beta-2/NeuroD1 (17), which bind to the E elements. It also includes the β cell-specific homeodomain transcription factor Pdx-1 (18–20), which binds to the A elements, and Ribe3b1, a recently cloned glucose-regulated factor that encodes a homologue of mammalian MafA proteins that binds to the C1 element of the insulin gene promoter (14, 21–23). Although it has been shown that these transcription factors are required for glucose-stimulated insulin gene expression (9, 24–27), the exact mechanism(s) by which they stimulate insulin gene expression in response to high blood glucose levels are unknown.

Transcriptional regulation of eukaryotic genes is a very complex process that requires the cooperation of a number of transcription factors, as well as various co-activator and co-repressor proteins, which modulate histone structure (28, 29). Changes in histone modification have been shown to increase or decrease the accessibility of promoters to the transcription machinery, thereby leading to repression or activation of gene expression (28–32). A number of modifications have been shown to modulate histone structure including acetylation (33–36), phosphorylation (37), and methylation (28, 38). In the case of histone acetylation, it has been demonstrated that a cooperation between histone acetylases and deacetylases leads to activation of gene expression only in response to specific stimuli (35, 39).

Previous data indicate that two of the insulin gene transcription factors required for glucose-regulated expression, Beta-2/NeuroD1 and Pdx-1, interact with the histone acetylase p300 (17, 26, 40). This prompted us to investigate whether changes in histone acetylation levels play a role in regulation of insulin

\(^1\) The abbreviations used are: GLUT-2, glucose transporter-2; Ac, acetyl; TSA, trichostatin A; MIN6, mouse insulinoma 6; IP, immunoprecipitation; ChIP, chromatin IP; DMEM, Dulbecco’s modified Eagle’s medium; RT, reverse transcriptase; NHP1a, hepatocyte nuclear factor-1alpha.
Gene expression by glucose. We report that high concentrations of glucose stimulate insulin gene transcription by mediating hyperacetylation of histone H4 at the insulin gene promoter in the insulinoma cell line MIN6.

EXPERIMENTAL PROCEDURES

Cell Culture—MIN6 cells of passage 20 to 24 were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose, 10% (v/v) fetal bovine serum, 1% penicillin/streptomycin, 2 mM glutamine, and 100 μM β-mercaptoethanol (41). All experiments were carried out with MIN6 cells of passage less than 30. NIH-3T3 fibroblasts (ATCC) were maintained in DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin. For glucose regulation experiments, cells were washed three times with 1× phosphate-buffered saline and grown overnight, unless otherwise indicated, in DMEM without fetal bovine serum containing the indicated glucose concentration(s).

RNA Isolation and RT-PCR—poly(A) RNA from total RNA was isolated using the GenElute Direct mRNA Miniprep kit (Sigma) according to the manufacturer's instructions. After treatment with DNaseI (Sigma), the poly(A) RNA was reverse-transcribed using enhanced avian myeloblastosis virus reverse transcriptase (Sigma). The resulting cDNAs were used as template for PCR with oligonucleotides to amplify the insulin and β-actin genes (42). The oligonucleotide primers used are listed in Table I. The primers for the β-actin gene were designed to cross an intron so that contamination with genomic DNA can be detected, which would result in a PCR product of 330 bp versus 243 bp from the cDNA (43). PCR reactions (20-μl volume) contained 20 ng of cDNA, 300 μM dNTPs, and 2.5 pmol of oligonucletoids primers, and 1.5 units of JumpStart AccuTag LA DNA polymerase (Sigma). PCR amplification conditions were as follows: 5 min at 95 °C followed by 30 cycles of 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 30 s. The PCR products were separated on 8% non-denaturing polyacrylamide gels and stained with ethidium bromide (Sigma). The bands were visualized using the ChemiDoc System BioRad Imager (Bio-Rad) and quantified using Quantity One imaging software (Bio-Rad) as a function of both band size and band intensity (intensity/mm²).

**Chromatin Immunoprecipitation (ChIP)—**Chromatin isolation was performed as published previously (31, 44). Approximately 3 × 10⁷ MIN6 or NIH-3T3 cells were cross-linked with formaldehyde (1% final concentration). After lysis of the cells, the nuclear extracts were sonicated with glass beads (0.1 g) for five 10-s pulses at 60% power using a Tekmar Sonic Disruptor. One-third of the sample was used for immunoprecipitation with acetyl-histone H3 (K5, K14) or acetyl-histone H4 (K5, K8, K12, K16) antibodies (Upstate Biotechnology, Inc.). The samples were pre-cleared with 20 μl of blocked Pansorbin Staph A cells (Calbiochem). After 4-fold dilution of the samples in IP buffer (1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl) and incubation with 2 μg of specific antibodies or rabbit IgG (Sigma) overnight at 4 °C, the immunocomplexes were recovered by incubation with blocked Staph A cells. After washing twice in dialysis buffer (2 mM EDTA, 50 mM Tris-Cl, pH 8.0, and 0.2% Sarcosyl) and four times with IP wash buffer (1% Nonidet P-40, 100 mM Tris-HCl, pH 8.0, 500 mM LiCl, and 1% deoxycholic acid), the immunocomplexes were eluted twice from the Staph A cells (with 150 μl of 1% SDS in 50 mM NaHCO₃). The cross-links were reversed by adding 20 μl of 5 M NaCl and 1 μl of 10 mg/ml NaOH A and incubating at 65 °C for 8 h. After treating with 1.5 μl of proteinase K (10 μg/μl) the samples were extracted with phenol/chloroform and subsequently ethanol-precipitated using 20 μg of glycogen as a carrier.

**PCR Analysis of Immunoprecipitated DNA**—All PCR reactions were performed on a Robocycler Gradient 96 (Stratagene) in a 20-μl reaction volume containing 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 200 μM dNTPs, and 2 μl of primers (2.5 pmol/μl). The linear range for each primer pair was determined empirically, using different amounts of MIN6 and NIH-3T3 genomic DNA. The PCR reactions and the quantification of the obtained bands were carried out as described above. The PCR products obtained with the immunoprecipitated DNA were normalized to the products obtained with the total input DNA. The primers used for PCR are listed in Table I. A detailed PCR protocol is available upon request. All of the PCR products obtained had the expected size. The identity of the PCR products was confirmed by sequencing.

**Statistical Analysis—**Comparison of the histone acetylation or insulin mRNA levels from MIN6 cells grown on 3 or 30 mM glucose were performed using the two-tailed, unpaired Student’s t test. A p value less than 0.05 was considered statistically significant. Data are expressed as means ± S.D.

**RESULTS**

**Glucose Mediates Hyperacetylation of Histone H4 at the Insulin Gene Promoter—**To test whether high concentrations of glucose mediate changes in histone acetylation at the insulin gene promoter in the insulinoma cell line MIN6, we utilized the ChIP assay with acetyl histone H3 or acetyl histone H4 antibodies. To quantify the amount of insulin gene promoter associated with acetylated histone H3 or histone H4, the total input and the immunoprecipitated DNA were used as template for PCR with primers against the mouse insulin I gene promoter (covers the promoter region from −10 to −281). The results shown in Fig. IA indicate that expression of MIN6 cells to high concentrations of glucose (30 mM) causes an increase in histone H4 acetylation at the insulin gene promoter. However, there is no significant change in histone histone H3 levels in response to high levels of glucose.

MIN6 cells incubated with 30 mM glucose displayed an 4- to 5-fold increase in acetylated histone H4 levels compared with cells incubated with 3 mM glucose in five independent experiments (Fig. 1B). Although in this experiment the MIN6 cells were incubated overnight with low and high glucose media, we observed the increase in histone H4 acetylation even after a 2-h incubation period with 30 mM glucose (data not shown). To verify that the observed increase in histone H4 acetylation levels in response to high glucose concentrations correlated with increases in insulin gene transcription, we quantified the insulin mRNA levels in MIN6 cells grown on low or high glucose media. RT-PCR analysis performed using cDNA from low or high glucose-incubated MIN6 cells indicate a 2.5-fold increase in insulin mRNA levels in response to high glucose (30 mM) compared with the β-actin levels used as control (Fig. 1, C and D). As a control for contamination of the cDNA with genomic DNA, we employed actin primers that give an additional larger PCR product when the sample is contaminated with genomic DNA (Fig. 1, C and D). The actin histone H3 and histone H4 antibodies used in this study specifically recognize
acetylated histones in Western blots with MIN6 cell extracts (Fig. 1E).

Hyperacetylation of Histone H4 at the GLUT-2 Promoter in Response to Glucose—To test whether glucose causes increases in histone acetylation at other β cell-specific promoters, we used the same immunoprecipitated and total DNA samples as template in PCR analysis with primers against the GLUT-2 promoter (Fig. 2). In the presence of low concentrations of glucose (3 mM), the level of acetylated histone H4 associated with the GLUT-2 promoter was minimal; however, at high concentrations of glucose (30 mM) the acetylated histone H4 levels at the GLUT-2 promoter increased drastically (Fig. 2). The level of acetylated histone H3 at the GLUT-2 promoter remained the same on low and high glucose (Fig. 2). This indicates that high levels of glucose (30 mM) cause hyperacetylation of histone H4 at both insulin and GLUT-2 gene promoters in MIN6 cells.

Histone H4 Acetylation at the Insulin Gene Promoter Is Not Increased at High Concentrations of l-Glucose—Activation of insulin gene transcription is regulated by cellular stress, as well as glucose (48). To test that the observed increase in histone H4 acetylation is not because of secondary effects such as osmotic stress caused by the high concentrations of glucose (30 mM) used in this experiment, we repeated the ChIP assay with acetyl histone H3 or histone H4 antibodies using L-glucose. Because L-glucose is not taken up by glucose transporters and thus is not metabolized, it should mimic the osmotic stress caused by high concentrations of extracellular glucose.

The analysis of acetylated histone H3 and histone H4 levels associated with the insulin gene promoter in MIN6 cells grown on low (3 mM) or high (30 mM) L-glucose indicates that there is no increase in histone H4 acetylation levels in response to high concentrations of L-glucose.
Experiments as the means of determining the promoter in response to 30 mM glucose.

It has been shown previously (1, 49) that insulin—unlike other glucose-regulated, did not change in response to increases in glucose concentration (Fig. 4, panel B). This experiment was performed with anti-acetyl histone H3 or histone H4 antibodies as indicated (panel A) and quantified as described before. The quantification of histone H4 acetylation at the insulin gene promoter from two independent experiments is shown in panel B as means ± S.D., n = 2 and is compared with the histone H4 acetylation levels in MIN6 cells incubated with 3 or 30 mM glucose.

The Decrease in Histone H4 Acetylation on Low Glucose Is Mediated by the Recruitment of Histone Deacetylases to the Insulin Gene Promoter—Histone deacetylases such as HDAC1 and HDAC2 have been shown to repress gene expression by decreasing the acetylation status of histones at specific promoters (28). Therefore, it was possible that the decrease in histone H4 acetylation on low levels of glucose (3 mM) was because of the recruitment of histone deacetylases to the insulin gene promoter. To address this question, we carried out the ChIP assay using acetyl histone H3 or histone H4 antibodies in MIN6 cells grown on media containing low or high glucose, in the presence or absence of the histone deacetylase inhibitors trichostatin A (TSA) or sodium butyrate. The inhibition of histone deacetylases in MIN6 cells resulted in equal levels of both histone H3 and H4 acetylation at the insulin gene promoter on low and high concentrations of glucose (Fig. 5A). We obtained similar results with both inhibitors; however, the acetylation levels of both histone H3 and histone H4 were consistently lower with sodium butyrate-treated samples in three independent experiments, which is likely due to additional effects that sodium butyrate has on cultured cells (50).

The level of histone H4 acetylation at the insulin gene promoter on low levels of glucose was very similar to that of high concentrations of glucose. These data suggest that the reduced level of histone H4 acetylation on low concentrations of glucose is likely because of the active recruitment of deacetylases to the insulin gene promoter.

To test whether the increase in histone H4 acetylation on low concentrations of glucose as observed with TSA treatment causes increased insulin gene transcription, we quantified the expression level of the insulin gene in MIN6 cells grown on media containing low or high concentrations of glucose treated with 0.2% acetylated histone H3 or histone H4 antibodies as indicated (panel A) and quantified as described before. Histone H4 acetylation levels increases at the GLUT-2 promoter, whose expression is not glucose-regulated, did not change in response to increases in glucose concentration (Fig. 4, third panel). This experiment again confirms that the observed hyperacetylation of histone H4 at the insulin and GLUT-2 promoters is glucose-specific.
with TSA by RT-PCR analysis. As shown in Fig. 6, the levels of insulin mRNA in MIN6 cells grown on low glucose-containing media was equal to that of high glucose-grown cells following TSA treatment. The obtained data are consistent with the idea that the decrease in histone H4 acetylation levels at the insulin gene promoter on low levels of glucose is because of the action of deacetylases and that increases in histone H4 acetylation levels correlate with increased insulin gene expression.

Lack of Insulin Gene Expression in Fibroblasts Is Associated with a Lack of Histone Acetylation at the Insulin Gene Promoter—It has been shown that acetylated histones are normally associated with promoters of actively transcribed genes (28, 29). Silent genes appear to either lack or have only minimal levels of histone acetylation at their promoter regions. To confirm our finding that increases in histone acetylation correlate with increased insulin gene transcription, we have analyzed the levels of acetylated histones associated with the insulin gene promoter in the mouse fibroblast cell line NIH-3T3, where the insulin gene is normally not expressed. For this experiment, NIH-3T3 fibroblasts grown on low or high concentrations of glucose were subjected to ChIP assay analysis using the anti-acetyl histone H3 and histone H4 antibodies. As expected, there was no detectable acetylation of either histone H3 or histone H4 at the silent insulin gene promoter in the NIH-3T3 fibroblast cell line (Fig. 7). PCR analysis of the cad promoter, using the same immunoprecipitated DNA as template, demonstrated detectable levels of histone H3 acetylation at this active promoter in NIH-3T3 cells as was seen in the MIN6 cell line.

**DISCUSSION**

Recent data indicate that the homeodomain transcription factor Pdx-1 and Beta-2/NeuroD1, required for glucose-stimulated expression of the insulin gene, interact with the histone acetylase p300 (17, 26, 40). This suggested that changes in histone acetylation might be important in the regulation of the insulin gene transcription. We report that exposure of MIN6 cells to high concentrations of glucose stimulates insulin gene expression and results in hyperacetylation of histone H4 at the insulin gene promoter.

High concentrations of L-glucose, which is not taken up by the cells, has no effect on histone acetylation excluding the possibility that hyperacetylation of histone H4 at 30 mM glucose is mediated by osmotic stress. The fact that histone H4 acetylation occurs in a glucose concentration-dependent manner also indicates that hyperacetylation of histone H4 at the insulin gene promoter in response to glucose is specific. Because the level of histone H3 acetylation is not changed in response to glucose at the insulin gene promoter, this may be responsible for maintaining basal transcription whereas hyperacetylation of histone H4 would function to up-regulate insulin gene transcription only in response to high blood glucose levels. Several transcription factors activate gene expression by mediating hyperacetylation of histone H4, including the transcription factor c-myc (51).

**Fig. 4.** Histone H4 acetylation increases in a glucose concentration-dependent manner. MIN6 cells were grown for 3 h in DMEM without glucose prior to incubation for 3 h with different concentrations of glucose as indicated. The ChIP assay was carried out using anti-acetyl histone H3 or histone H4 antibodies as described before. The PCR products obtained by using primers to amplify the insulin, GLUT-2, and cad gene promoters are shown. The data shown are representative of two independent experiments.

**Fig. 5.** The decrease in histone H4 acetylation levels on low glucose is due to the recruitment of histone deacetylases to the insulin gene promoter. MIN6 cells were grown on media containing 3 or 30 mM glucose for 12–18 h with or without TSA or sodium butyrate (NaB) as indicated. ChIP assays were performed using anti-acetyl histone H3 or histone H4 antibodies. The PCR products obtained using primers to amplify the insulin gene promoter are shown in panel A. The quantification of the intensity of the obtained PCR products from three independent experiments is shown in panel B for the TSA and in panel C for the sodium butyrate treatment as means ± S.D., n = 3.
High concentrations of glucose also lead to hyperacetylation of histone H4 at the GLUT-2 gene promoter, indicating that glucose regulation of β cell-specific gene expression may be in general mediated by increases in histone H4 acetylation. Recent data indicate that HNF1α is required for expression of the GLUT-2 gene but not of the insulin I and II genes (52, 53). HNF1α has been shown to regulate GLUT-2 expression by direct binding to its promoter and by causing hyperacetylation at the GLUT-2 promoter via recruitment of histone acetylases (52, 54). Both acetylated histone H3 and histone H4 levels decrease in the HNF1α homozygous knockout mice (52). Interestingly, HNF1α is also required for expression of the transcription factors Pdx-1 and Beta-2/NeuroD1 (53). We have observed only changes in histone H4 acetylation levels at the GLUT-2 promoter whereas histone H3 acetylation did not significantly change in response to high concentrations of glucose. The region of the GLUT-2 promoter analyzed in this study covers the sequences from −523 to −738 with respect to the transcription initiation site, which is more upstream than the region used in the studies with HNF1α (52). This specific promoter region was chosen, because it has been shown previously (55) to be sufficient for glucose regulation of GLUT-2 gene expression and contains binding sites for the transcription factor Pdx-1.

This is the first example of nutrient regulation of gene expression by mediating changes in histone acetylation. However, the expression of several genes have been shown to be regulated through modulation of histone acetylation in response to external stimuli. For example, the stimulus for up-regulation of interferon-β expression is viral infection, which requires various factors including NF-κB, CBP, Gcn5, TBP, TAF1, TAF5, and RNA polymerase II (56). The recruitment of these factors, as well as increased transcription, correlates with increases in the acetylation levels of histones H3 and histone H4 at this promoter (57). Acetylated histones are associated with promoters of actively transcribed genes whereas silent genes appear to either lack or have only minimal histone acetylation at their promoter regions (28). Consistent with this idea, the fibroblast NIH-3T3 cell line where the insulin gene is silent lacks histone acetylation at the insulin gene promoter.

Tissue-specific expression of genes is mediated by the action of specific transcription factors that can modulate the histone structure at specific promoters by interacting with co-activators or co-repressors. For instance, the muscle-specific transcription factor MyoD has been shown to be essential for myoblast differentiation through activation of a number of muscle-specific genes (58). In undifferentiated myoblasts, MyoD has been shown to interact with class I histone deacetylases HDAC1 and HDAC2; however, it interacts with the histone acetylases PCAF and p300 to drive transcription in myotubes (59, 60). Because the region of the insulin gene promoter amplified by PCR in our studies contains binding sites for Pdx-1, Beta-2, and Ribe3b1, it is likely that one or several of these transcription factors are responsible for mediating the hyperacetylation of histone H4 in response to high concentrations of glucose. Indeed Pdx-1 and Beta-2/NeuroD1 have been shown to interact with the histone acetylase p300 (17, 26, 40). Furthermore, it has been demonstrated that introduction of a recombinant Pdx-1 adenovirus into livers of mice leads to the expression of endogenous, otherwise silent, genes for mouse insulin I and II, suggesting that Pdx-1 itself is sufficient to activate insulin gene expression in the liver (61). These data suggest the idea that high concentrations of glucose cause hyperacetylation of histone H4 at the insulin gene promoter by the recruitment of the histone acetylase p300 via Pdx-1 and/or Beta-2 that leads to up-regulation of insulin gene transcription.

This idea is also supported by the fact that another chromatin-associated protein, HMGI(Y), has been shown to bind to the A3/A4 region of the insulin promoter and to enhance the in vitro DNA binding of a complex containing Pdx-1 and E47/ Pan-1 transcription factors (25). The HMGI(Y) family of proteins has been implicated in the formation of enhanceosome, which are complexes that allow synergistic regulation of promoter regions by a number of transcriptional factors (62). Treatment of MIN6 cells with histone deacetylase inhibitors results in increased histone H4 acetylation at the insulin gene promoter independent of glucose concentration, which leads to increased insulin gene transcription, even in the absence of glucose regulation of histone H4 acetylation.

### Glucose Regulation of Histone H4 Acetylation

**Figure 6.** Inhibition of histone deacetylases in MIN6 cells causes constitutive expression of the insulin gene. MIN6 cells grown on low glucose (3 mM) or high glucose (30 mM) containing media were treated with or without 100 ng/ml TSA for 15 h. After isolation of total RNA, RT-PCR analysis was performed to determine the mRNA levels of insulin and β-actin as control. The obtained PCR products were resolved on a PAGE gel (panel A), and the intensity of the bands was quantified (panel B). The data from two independent experiments are expressed as means ± S.D., n = 2.

| Glucose (mM) | 3 | 30 |
|-------------|---|----|
| IP-Ac H3    |   |    |
| IP-Ac H4    |   |    |
| 0.2% Input  |   |    |

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**Figure 7.** NIH-3T3 fibroblasts lack histone acetylation at the insulin gene promoter. NIH-3T3 cells were grown on media containing 3 or 30 mM glucose for 12–18 h before being used in ChIP assays with acetyl histone H3 or H4 antibodies. The obtained PCR products were resolved on a PAGE gel (panel A), and the intensity of the bands was quantified (panel B). The data from two independent experiments are expressed as means ± S.D., n = 2.

| Glucose (mM) | 3 | 30 |
|-------------|---|----|
| IP-Ac H3    |   |    |
| IP-Ac H4    |   |    |
| 0.2% Input  |   |    |

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**Figure 8.** Effect of glucose on histone acetylation in NIH-3T3 fibroblasts. NIH-3T3 fibroblasts were grown on media containing 3 or 30 mM glucose for 12–18 h. After isolation of total RNA, RT-PCR analysis was performed to determine the mRNA levels of insulin and β-actin as control. The obtained PCR products were resolved on a PAGE gel (panel A), and the intensity of the bands was quantified (panel B). The data from two independent experiments are expressed as means ± S.D., n = 2.

| Glucose (mM) | 3 | 30 |
|-------------|---|----|
| IP-Ac H3    |   |    |
| IP-Ac H4    |   |    |
| 0.2% Input  |   |    |
high concentrations of glucose. These data indicate that the decreased levels of histone H4 acetylation observed on low glucose are because of the active recruitment of histone deacetylases to the insulin gene promoter. Class I histone deacetylases, specifically HDAC1 and HDAC2, have been implicated in repression of gene expression (63). It is possible that on low concentrations of glucose, expression of the insulin gene is kept low by the recruitment of deacetylases such as HDAC1 and HDAC2. At high concentrations of glucose the recruitment of a histone acetylase, such as p300, could cause up-regulation of insulin gene expression by hyperacetylation of histone H4. It has been demonstrated that the interplay between histone acetyltransferases and histone deacetylases is the key to the dynamics of chromatin structure and function. Indeed, NF-κB has been shown to interact with both p300 and HDAC1 in a phosphorylation-dependent manner (64). Another transcription factor, HIF-1, is also negatively regulated by HIF1α (65). However, it associates with p300/CBP to function as a transcriptional activator. The association of HIF-1 with HDAC1 or p300 is regulated by the O2 concentration (66). Experiments are under way to test the recruitment of HDACs and p300 to the insulin gene promoter in a glucose-dependent manner.

Our data indicate that up-regulation of insulin gene expression in response to high glucose levels is mediated by hyperacetylation of histone H4. Because this up-regulation of insulin gene expression at high glucose concentrations is essential in maintaining glucose homeostasis, dysregulation of histone acetylation levels at the insulin gene promoter may be an important cause of insulin insufficiency leading to diabetes. Understanding the role of histone modification in glucose-stimulated insulin gene expression may be valuable for the engineering of non-β cells such as the liver to produce and secrete insulin in a glucose-dependent manner.

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