Suppression of Transcription Factor PDX-1/IPF1/STF-1/IDX-1 Causes No Decrease in Insulin mRNA in MIN6 Cells

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Abstract

The insulin gene transcription factor PDX-1/IPF1/STF-1/ IDX-1 plays a key role in directing β cell–specific gene expressions. Recently, impairment of PDX-1 expression or activity has been observed in β cell–derived HIT cells cultured under high glucose concentrations, and this has been suggested as a possible cause of the decrease in insulin gene transcription. To investigate the pathophysiological significance of PDX-1 as a determinant of the rate of insulin gene transcription, we suppressed its expression in β cell–derived MIN6 cells using an antisense oligodeoxynucleotide (ODN) and searched for possible changes in the β cell–specific gene expression. Treatment of MIN6 cells with an 18-mer phosphorothioate ODN complementary to a sequence starting at the translation initiation codon of PDX-1 caused a potent, concentration-dependent reduction in PDX-1 expression; addition of 2 μM antisense ODN could reduce PDX-1 expression to 14±4% of the control. There was also a decrease in its DNA binding to the insulin gene A element. Despite such suppression of PDX-1, Northern blot analysis revealed no decrease in the amount of insulin mRNA in the MIN6 cells. Similarly, no changes were detected in the transcription of the glucokinase or islet amyloid polypeptide gene, for which PDX-1 was shown to function as a transcription factor. Thus, our findings dispute the physiological significance of PDX-1 in determining the rate of insulin gene transcription. This means that other components constituting the transcription-controlling machinery need to be evaluated in order to understand the molecular basis of impaired insulin biosynthesis such as that observed due to glucose toxicity. (*J. Clin. Invest.* 1997. 100:1840–1846.) Key words: gene expression regulation • transcription factors • homeodomain protein • antisense oligodeoxynucleotide

Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is a multifactorial disease characterized by insulin resistance and impaired insulin secretion (1). Characterization of NIDDM model animals has revealed extensive degranulation of pancreatic β cells that can be observed simultaneously with the onset of overt diabetes, thus suggesting the significance of the impairment of insulin synthesis as a cause of disturbed β cell function (2, 3). To understand the molecular background for such a decrease in insulin synthesis, we need to identify the molecular components that determine the rate of insulin gene transcription and how their function is disturbed in NIDDM cases.

Among various factors that potentially inhibit insulin biosynthesis, glucose toxicity has been the main focus of recent research. Prolonged exposure of pancreatic β cells to high glucose levels exerts toxic effects on the cells (glucose toxicity), which can be characterized by impaired insulin biosynthesis and extensive degranulation (4). Recently, the pancreatic β cell–derived HIT cell has been used as a model to show that exposure of the cells to chronic high glucose concentration (5–7) or to D-ribose (8), which induces a glycation reaction, causes reduction of insulin gene promoter activity and, subsequently, decreases in insulin mRNA and insulin content. These studies also revealed a reduction in the DNA-binding activity of the β cell–specific transactivating factor PDX-1/IPF1/STF1/IDX-1 (9–11). Thus, the possibility arose that the impaired PDX-1 function per se is pathophysiologically responsible for the decrease in insulin gene promoter activities. A similar reduction in PDX-1 expression was observed in pancreatic β cells in vivo; in 90% pancreatectomized diabetic rats, chronic hyperglycemia was associated with decreased mRNA levels of both insulin and PDX-1 (12).

PDX-1 is a homeodomain-containing transcription factor which binds to the A element motif of the insulin genes and contributes to directing the genes' β cell–specific expression (9). Among the various transcription factors of the insulin gene, including those yet to be cloned, such as RIPE3b1 (13), the physiological significance is most evident for PDX-1 (14, 15). The transcription factor appears before insulin during the ontogeny of the mouse pancreas, and its expression eventually becomes restricted to the β cells in the adult (15). Besides its role in insulin gene expression, PDX-1 has been suggested to be involved in glucokinase and islet amyloid polypeptide (IAPP) gene expressions (16–18).

As a step toward elucidating the machinery that determines the rate of insulin gene transcription and understanding the molecular basis of the impaired insulin biosynthesis observed in NIDDM patients, we investigated the role of PDX-1 in determining the transcriptional rate of insulin and other β cell–specific genes. To suppress PDX-1 expression in β cell–

1. *Abbreviations used in this paper:* hInsA3, human insulin gene A3 element; IAPP, islet amyloid polypeptide; NIDDM, non–insulin-dependent diabetes mellitus; ODN, oligodeoxynucleotide.

derived MIN6 (19) and βTC1 (20) cells, we used an antisense oligodeoxynucleotide (ODN) strategy and then evaluated the effects on gene expression. We report here that, unlike suppression of another insulin gene transcription factor, E2A/Pan (21), which constitutes E element–binding IEF1 (13), suppression of PDX-1 expression causes no changes in insulin gene expression.

Methods

Materials. Radioisotopes were purchased from Amersham Japan (Tokyo, Japan). Tissue culture media were purchased from Nacalai Tesque, Inc. (Kyoto, Japan), and FBS was from ICN Biomedicals, Inc. (Costa Mesa, CA). The anti–PDX-1 (IPF1) antiserum was established recently by us (16) after immunizing a rabbit with the synthetic peptide SPQPSSIAPLRPQE representing amino acid residues 269–282 of the PDX-1 peptide (GenBank accession number X74342) (9). The anti-NeuroD polyclonal antibody (sc-1084) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Basic laboratory procedures were performed according to standard protocols (22) unless otherwise stated.

ODNs. An 18-mer phosphorothioate antisense ODN complementary to a sequence starting at the ATG initiation codon of mouse PDX-1 (5′-CTGCTCCTCACTGTTCAT-3′) and control sense and reverse phosphorothioate ODNs (5′-ATGAACAGTGAGGAG-CAG-3′ and 5′-TACTTGTCACTCCTCGTC-3′, respectively) were synthesized using an automated DNA synthesizer (model 391; Applied Biosystems, Foster City, CA).

Cell culture and ODN treatment of cells. β Cell–derived cell line MIN6 cells (19) were grown in DME supplemented with 15% heat-inactivated FBS, penicillin, and streptomycin. β Cell–derived cell line βTC1 (20) and HIT-T15 cells (1777; American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin, and streptomycin. For ODN treatment, MIN6 and βTC1 cells (∼ 80% confluent in 60-mm dishes) were washed twice with 6 ml DME (for MIN6) or RPMI 1640 (for βTC1) containing no serum. Appropriately diluted aliquots of ODN in 288 μl DME or RPMI 1640 (no serum) were preincubated at room temperature for 15 min with 288 μl DME or RPMI 1640 (no serum) containing 26 μl of LipofectAMINE® reagent (Life Technologies, Inc., Gaithersburg, MD). This mixture was added to the cells together with an additional 2.3 ml of DME or RPMI 1640 (no serum). The final concentrations of ODN were as indicated. Cells were incubated for 5 h at 37°C in the presence of 5% CO_2. Next, the serum-free medium containing LipofectAMINE was removed, and the incubation was continued with fresh (serum-containing) medium which also included appropriate concentrations of ODN. The medium was replaced every 48 h with fresh medium of the same content.

Western blot analysis. Nuclear extracts were prepared following the procedure described by Sadowski and Gilman (23). 3 μg of nuclear extract was separated by SDS-PAGE (10% resolving gel) using a Minigel® apparatus (Bio-Rad Laboratories, Hercules, CA), and transferred to polyvinylidene fluoride membranes (Immobilon-P; Nihon Millipore, Tokyo, Japan) using transfer buffer containing 20% methanol, 25 mM Tris base, and 192 mM glycine and a Mini-trans-electrophoretic transfer cell (Bio-Rad Laboratories) (100 V, 1 h). After blocking the membranes for 1–2 h at room temperature in PBS containing 3% BSA, the membranes were incubated at room temperature for 60 min in TBS buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.5) containing 1:5,000 dilution of anti–PDX-1 (IPF1) antiserum (16) or anti-NeuroD polyclonal antibody, and washed three times (10 min each time) in TBS with 0.2% Tween-20 (TBS-T). The membranes were then incubated for 60 min at room temperature in TBS containing 1:5,000 dilution of goat anti–rabbit IgG antibody (for PDX-1 detection) or porcine anti–goat IgG antibody (for NeuroD detection) coupled to horseradish peroxidase, followed by two 30-min washings with TBS-T. Immunoreactive bands were visualized by incubation

with luminol (ECL® Western Blotting Kit; Amersham Japan) and exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY). Quantification was achieved by densitometric scanning.

Gel mobility–shift analysis. 3 μg of nuclear extract was preincubated with 2 μg of poly(dI-dC) at 4°C in a 20-μl reaction mixture containing 20 mM Hepes, pH 7.9, 75 mM KCl, 2.5 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, and 3% Ficoll-Hypaque (vol/vol%). 15 min later, the binding reaction was initiated by adding 100 pg (10^4 cpm) of 5′-end ^{32}P-labeled ODN probes and, when required, nonradioactive competitor double-stranded ODNs, followed by incubation at room temperature for 30 min. In some of the binding assays, 2 μl of preimmune serum or anti–PDX-1 antiserum was added to the binding reactions 30 min before addition of the DNA probes. The samples were fractionated on 5% PAGE precooled to 4°C in 0.5× TBE (45 mM Tris base, 45 mM boric acid, 1 mM EDTA). Quantification was achieved by densitometric scanning after exposure to Kodak XAR-5 film.

Northern blot analysis. Northern blots followed standard procedures using 25 μg of total RNA isolated from MIN6 cells or 10 μg of total RNA isolated from βTC1 or HIT-T15 cells. A mouse glucokinase cDNA (nucleotides 152–725) (24) and a mouse IAPP cDNA (nucleotides −4–245) (25) were prepared by reverse transcription–PCR using MIN6 total RNA as a template and subcloned individually into the pBluescript II plasmid. The mouse PDX-1 cDNA had been generated by us previously (18). The plasmid pMIn2C containing a mouse insulin II cDNA was a generous gift from Drs. J.M. Chirgwin
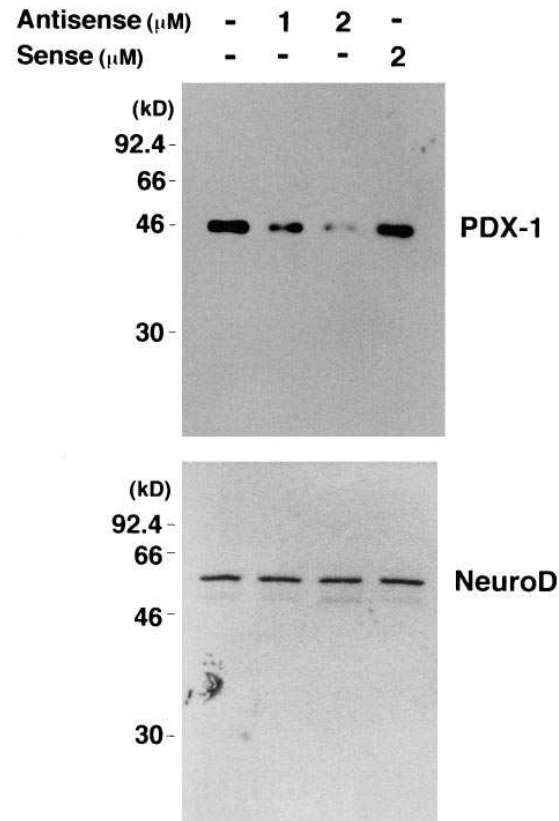

Figure 1. Effect of antisense ODN on suppression of PDX-1 expression in MIN6 cells. Western blot analysis using anti–PDX-1 antiserum (*top*) and anti-NeuroD antibody (*bottom*). MIN6 cells were exposed to 1 or 2 μM antisense or 2 μM sense ODN for 48 h. LipofectAMINE was used for the initial transfection of ODNs, and thereafter, ODNs alone were added. 3 μg each of the same nuclear extracts prepared from MIN6 cells was used for evaluation of PDX-1 and NeuroD expression.
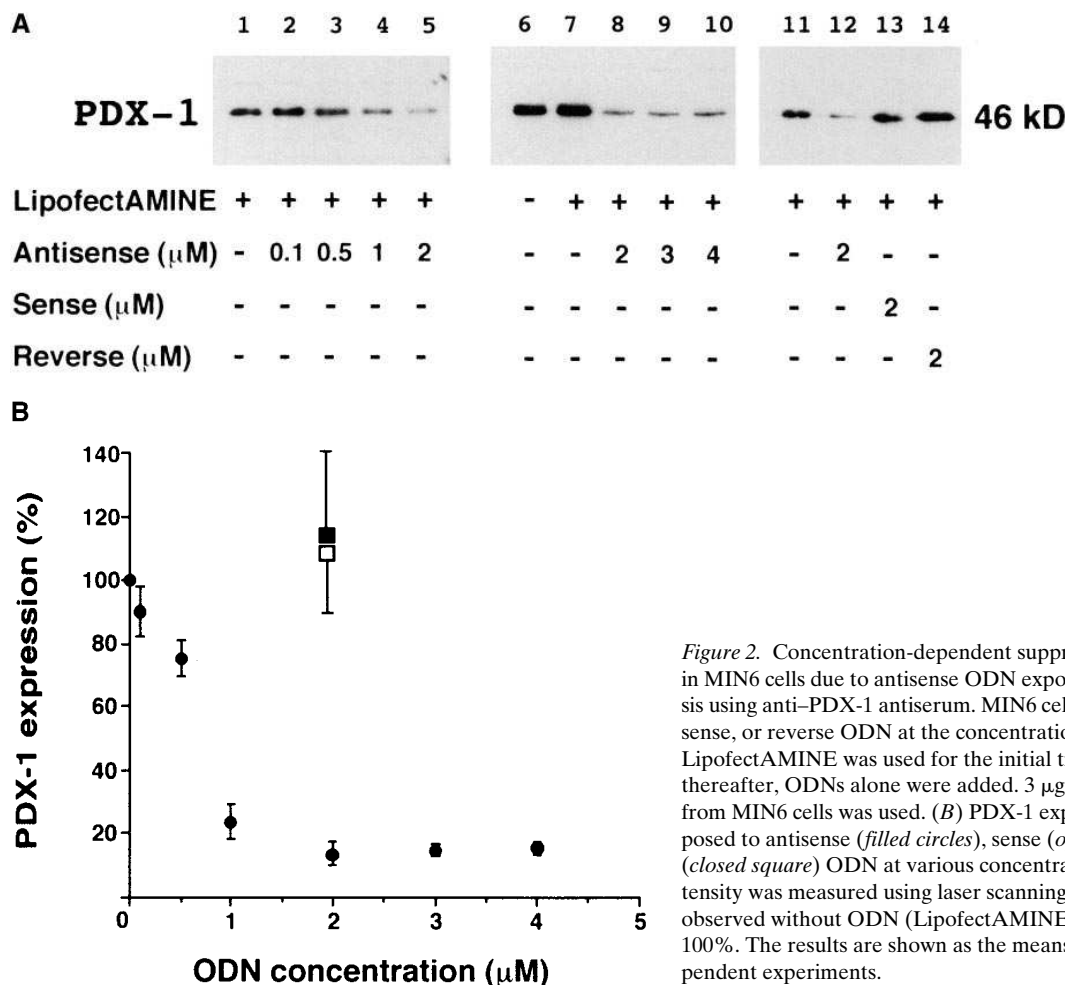
A

PDX-1 ·························· 46 kD

	1	2	3	4	5		6	7	8	9	10		11	12	13	14
LipofectAMINE	+	+	+	+	+		-	+	+	+	+		+	+	+	+
Antisense (μM)	-	0.1	0.5	1	2		-	-	2	3	4		-	2	-	-
Sense (μM)	-	-	-	-	-		-	-	-	-	-		-	-	2	-
Reverse (μM)	-	-	-	-	-		-	-	-	-	-		-	-	-	2

B

Figure 2. Concentration-dependent suppression of PDX-1 expression in MIN6 cells due to antisense ODN exposure. (*A*) Western blot analysis using anti–PDX-1 antiserum. MIN6 cells were exposed to antisense, sense, or reverse ODN at the concentrations indicated below for 48 h. LipofectAMINE was used for the initial transfection of ODNs, and thereafter, ODNs alone were added. 3 μg of nuclear extracts prepared from MIN6 cells was used. (*B*) PDX-1 expression in MIN6 cells exposed to antisense (*filled circles*), sense (*open square*), and reverse (*closed square*) ODN at various concentrations for 48 h. The band intensity was measured using laser scanning densitometry. The amount observed without ODN (LipofectAMINE only) was set arbitrarily at 100%. The results are shown as the means±SE of at least three independent experiments.

(University of Texas Health Science Center) and M.A. Permutt (Washington University School of Medicine).

Results

Suppression of PDX-1 expression by antisense ODN. Using the antisense ODN strategy, we suppressed the expression of PDX-1 in the pancreatic β cell–derived MIN6 cells (19). A set of antisense, sense, and reverse ODNs was designed to include the 18-nucleotide coding sequences starting with the first methionine codon of the PDX-1 mRNA. To investigate whether the antisense ODN can efficiently suppress the expression of PDX-1, Western blotting analyses were performed using nuclear extracts which were isolated after incubation with the ODN for 48 h. As shown in Fig. 1, the antisense ODN could substantially suppress PDX-1 expression. The expression of another insulin gene transcription factor, NeuroD, examined as a control, was not affected (Fig. 1). We also examined the amount of PDX-1 mRNA by Northern blot analysis, but found no decrease by the antisense ODN (data not shown). This may indicate that the antisense ODN used in this study functioned via a hybrid-arrested translation rather than via RNase H–driven mRNA cleavage as suggested in the literature (21, 26).

Results of more detailed analyses of antisense PDX-1 effects are shown in Figs. 2–4. In terms of the effects of different concentrations of the antisense ODNs, representative results are shown in Fig. 2 *A*, and average data are shown as a graph in Fig. 2 *B*. The substantial suppression of PDX-1 expression was observed in MIN6 cells when kept for 48 h in the presence of a supramicromolar concentration of antisense ODN [down to 14±4% (mean±SE) under 2 μM antisense ODN]. No further decrease was observed even when concentrations > 2 μM were used (Fig. 2, *A* and *B*, lanes *8–10*). In contrast to the suppressive effect of antisense ODN, no effect was observed after addition of 2 μM sense or reverse ODN (Fig. 2 *A*, lanes *11*, *13*,
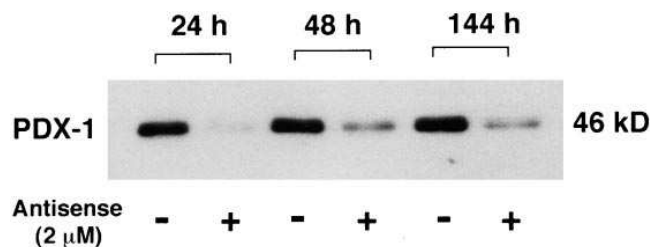

Figure 3. Time course of suppression of PDX-1 expression by antisense ODN. MIN6 cells were incubated with or without 2 μM antisense ODN for 24, 48, or 144 h. LipofectAMINE was used for the initial transfection, and ODNs alone were added thereafter. 3 μg of nuclear extracts prepared from MIN6 cells were used for Western blot analysis. Similar results were obtained in three independent experiments.
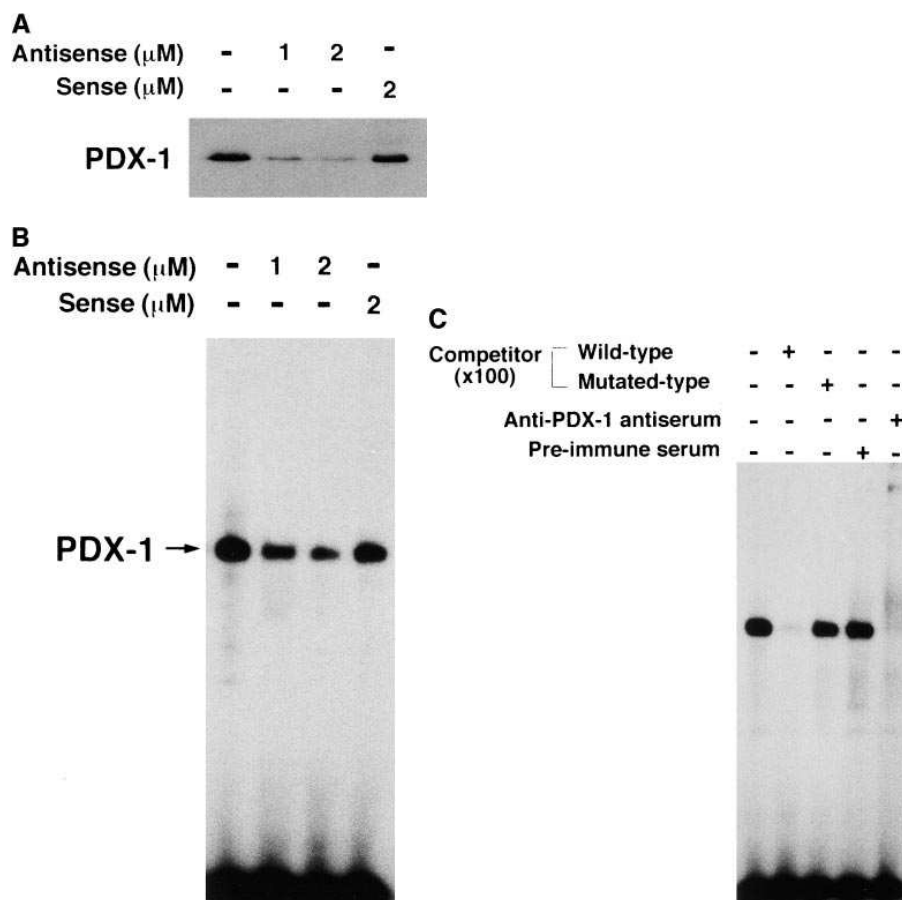
A

Antisense (μM) −   1   2   −
Sense (μM)   −   −   −   2

PDX-1

B

Antisense (μM) −   1   2   −
Sense (μM)   −   −   −   2

C

Competitor ⌈ Wild-type   −   +   −   −   −
(x100)   ⌊ Mutated-type   −   −   +   −   −
Anti-PDX-1 antiserum   −   −   −   −   +
Pre-immune serum   −   −   −   +   −

PDX-1 →

Figure 4. Inhibition of binding of PDX-1 to A element by antisense ODN. (*A*) Western blot analysis using anti–PDX-1 antiserum. MIN6 cells were exposed to 1 or 2 μM antisense or 2 μM sense ODN for 96 h. 3 μg of nuclear extracts prepared from MIN6 cells were used for Western blot analysis. The same nuclear extracts were used for gel mobility–shift analysis, shown in *B*. (*B*) Evaluation of A element–binding activity by gel mobility–shift analysis. The binding probe was a double-strand ODN reproducing the hInsA3 region and surrounding sequences (5′-CCCCTGGTTAAGACTCTAAT-GACCCGCTGG-3′). (*C*) Identification of the binding protein by gel mobility–shift analysis. Using wild-type and mutated-type (5′-CCCCTGGTTAAGACTCTccT-GACCCGCTGG-3′) competitors and preimmune and antiserum against PDX-1 as indicated, the specificity and identity of the gel–shift complex were evaluated. The nuclear extracts used were isolated from untreated MIN6 cells. *B* and *C* were parts of the same gel, and the mobilities of the complexes were identical.

and *14, and B*). Also, the gene transferring agent LipofectAMINE itself did not affect the expression level of PDX-1 (Fig. 2 *A*, lanes *6* and *7*).

The time course of the PDX-1 suppression after addition of antisense ODN to MIN6 cells was examined. As shown in Fig. 3, exposure of MIN6 cells to 2 μM antisense ODN caused a rapid decrease in PDX-1 expression within 24 h, and the suppressive effects continued until at least 144 h after the initial addition of antisense ODN. Considering the half-life of insulin mRNA, which is 6–12 h according to results obtained previously with β cell–derived RIN-5F and βTC6 cells (27, 28), this time course of suppression by 2 μM antisense ODN was suitable for evaluating the effects of PDX-1 diminution on insulin mRNA amounts. Thus, we used MIN6 cells that had been kept with antisense ODN for 96 h to evaluate the potential role of PDX-1 in determining the rate of insulin gene transcription.

Suppression of PDX-1 binding to A element by antisense ODN. To examine whether the antisense ODN–dependent reduction of PDX-1 expression in MIN6 cells would lead to a decrease in its binding to the *cis*-acting motifs, we performed gel mobility–shift analyses using the human insulin gene A3 element (hInsA3) as a probe. The results revealed that, in accordance with the reduction of the amount of PDX-1 protein observed by Western blot analysis (Fig. 4 *A*), the intensity of the band formed with the hInsA3 probe and a nuclear protein in MIN6 cells was reduced in a dose-dependent manner by addition of antisense ODN (Fig. 4 *B*). The average band intensities in gel mobility–shift analyses for 1 and 2 μM antisense and 2 μM sense ODN lanes were 28±5, 18±5, and 80±7%, respec-

tively, of that for the control lane. The gel–shift complex was shown to include PDX-1, because the addition of anti–PDX-1 (IPF1) antiserum to the binding reaction caused diminution in band intensity (Fig. 4 *C*). These results indicated that the reduction in the protein amount of PDX-1 by antisense ODN caused a comparable reduction in its DNA-binding capacity.

Effect of PDX-1 reduction on insulin, glucokinase, and IAPP mRNA amounts. We examined whether the decrease in PDX-1 protein and DNA-binding capacity would reduce the expression of insulin gene or other potential target genes. As shown in Fig. 5, the amount of insulin mRNA did not change in cells in which PDX-1 expression was suppressed. When the band intensity for the control lane was arbitrarily set at 100%, the average intensities for 1 and 2 μM antisense and 2 μM sense ODN lanes were 82±9, 94±13, and 113±22%, respectively (*n* = 3; representative data shown in Fig. 5). Similarly, no changes were observed in the amount of glucokinase (104±6, 114±11, and 119±16%, *n* = 3; Fig. 5) or IAPP mRNA (101±7, 112±10, and 121±14%, *n* = 3; Fig. 5). Thus, these observations suggest that suppression of PDX-1 expression does not affect the transcriptional rate of these genes.

Suppression of PDX-1 and expression of insulin gene in βTC1 cells. To investigate whether similar observations would be obtained with some other immortalized β cell–derived lines, we suppressed the PDX-1 expression in βTC1 cells and investigated the expression of insulin mRNA. With the same methods and ODNs used for MIN6 cells, the expression of PDX-1 was suppressed substantially in βTC1 cells (Fig. 6; down to 24±4% with 2 μM antisense ODN, *n* = 3). Despite
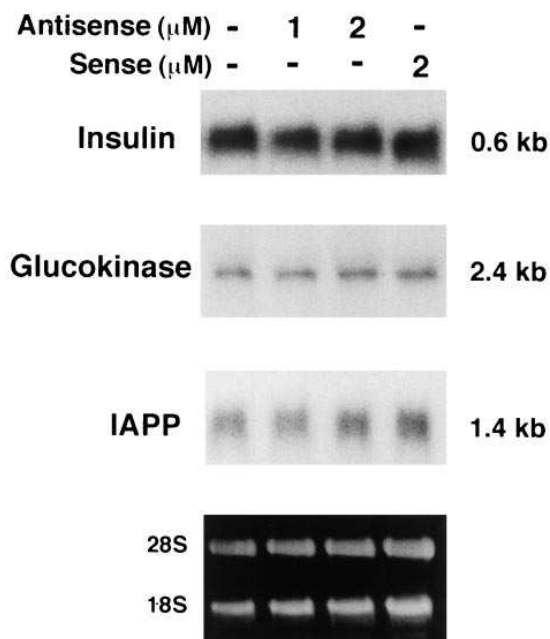
Figure 5. Absence of reduction of insulin, glucokinase, or IAPP gene expression in PDX-1–suppressed MIN6 cells. Northern blot analyses were used to evaluate a subset of β cell–specific gene expression. The binding probe was ^{32}P-labeled glucokinase, IAPP, or insulin cDNA. 25 μg of total RNA was isolated from MIN6 cells which had been exposed for 96 h to LipofectAMINE alone (lane *1*), 1 μM antisense (lane *2*), 2 μM antisense (lane *3*), and 2 μM sense ODN (lane *4*). *Bottom*, Ribosomal RNA.
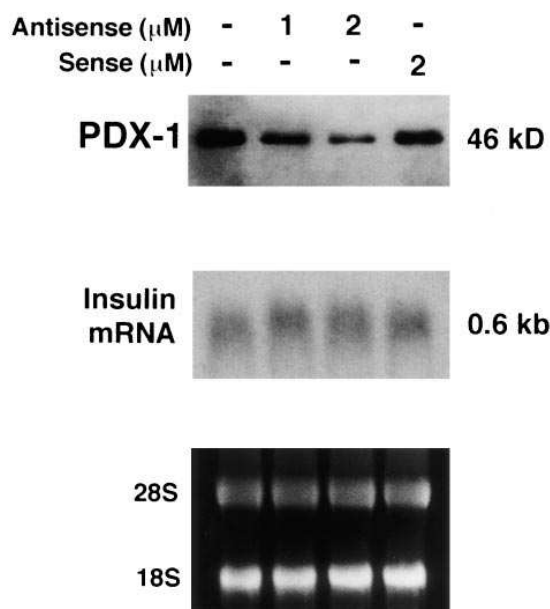

Figure 6. Absence of reduction of insulin gene expression in PDX-1–suppressed βTC1 cells. βTC1 cells were exposed to 1 or 2 μM antisense or 2 μM sense ODN for 96 h. LipofectAMINE was used for the initial transfection of ODNs, and thereafter, ODNs alone were added. *Top*, Result of Northern blot analysis which used anti–PDX-1 antiserum and 3 μg of nuclear extracts. *Middle*, Result of Northern blot analysis which used 10 μg of total RNA and a ^{32}P-labeled insulin cDNA as a binding probe. Nuclear extracts and total RNA were isolated from MIN6 cells which had been exposed for 96 h to LipofectAMINE alone (lane *1*), 1 μM antisense (lane *2*), 2 μM antisense (lane *3*), and 2 μM sense ODN (lane *4*). *Bottom*, Ribosomal RNA.

this, no changes in the insulin mRNA amount were observed (control, arbitrarily set at 100%; 1 μM antisense, 109±4%; 2 μM antisense, 121±14%; 2 μM sense, 96±7%; $n = 3$).

Comparison of PDX-1 expression with that in HIT-T15 cells chronically exposed to high glucose conditions. Suppression of PDX-1 expression was coidentified with the suppression of insulin gene expression in HIT cells which had been exposed to high glucose concentration for a very long period (7, 29), and therefore was suggested to be responsible for the decrease in insulin gene expression. To allow comparison of our results with this observation in HIT-T15 cells, we examined whether the degree of decrease in PDX-1 in our study was comparable to that observed in the chronic high glucose–exposed HIT cells.

As shown in Fig. 7, the HIT-T15 cells of a late passage number (P98) showed substantial decrease in insulin mRNA content (down to 50±5% of the control [P70]; $n = 3$). The PDX-1 amount in the cells may also have decreased slightly (down to 78±12% of the control [P70]; $n = 3$); however, the degree of decrease in PDX-1 was definitely greater in our antisense ODN–treated MIN6 cells than in the HIT-T15 cells experiencing impaired insulin biosynthesis due to chronic high glucose condition. This disputes the idea that the suppression of PDX-1 is primarily responsible for the impaired insulin biosynthesis in such HIT-T15 cells.

Discussion

Loss of PDX-1 results in loss of the pancreas, showing that PDX-1 has an important physiological role in the development

of this organ (14, 15). Recently, Serup et al. and we ourselves reported that PDX-1 can contribute to the expression of a subset of β cell–specific genes when exogenously expressed in cell lines derived from pancreatic α cells (18, 30). Thus, the presence of PDX-1 is clearly important in directing the expression of β cell–specific genes. However, whereas multiple transcription factors are known to be involved in insulin or glucokinase gene expression, the physiological significance of PDX-1 as a determinant of the transcriptional rate of those genes has remained unknown.

In this study, we succeeded in substantially reducing PDX-1 expression in MIN6 cells using the antisense ODN strategy (Figs. 1–3 and 6). The reduction in protein amount also caused diminution of PDX-1 binding to the target DNA (Fig. 4). Nevertheless, we found no changes in the expression of insulin or other β cell–specific genes. Also, this seemed conserved in another β cell–derived cell line, βTC1. Although these findings dispute the physiological significance of PDX-1 in determining the rate of insulin gene transcription, they do not completely deny its involvement in driving insulin gene expression in β cells. We assume it is possible that PDX-1 may be a sufficiently abundant protein in β cell–derived cells such that diminishing its expression levels by 80–90% does not impede its overall function as a transcription factor. In this case, as long as the minimally required amount of PDX-1 is expressed in the cells, no remarkable changes in gene expression would occur in the PDX-1–suppressed β cell–derived cells. Thus, we consider it safe to conclude that the moderate suppression of PDX-1 ex-

P70 P98
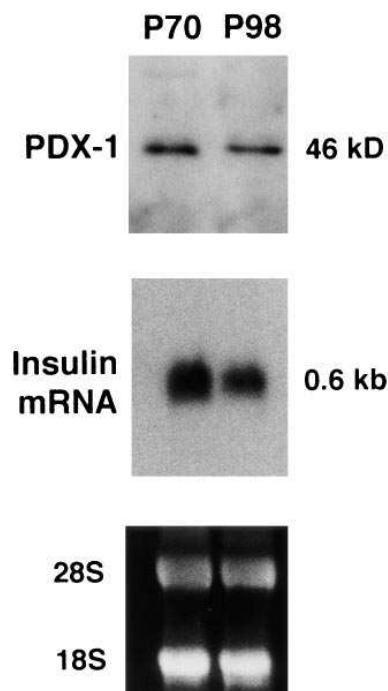
PDX-1   46 kD

Insulin
mRNA   0.6 kb

28S
18S

Figure 7. Evaluation of PDX-1 and insulin mRNA expression in HIT-T15 cells chronically exposed to high glucose. *Top*, Result of Western blot analysis which used anti–PDX-1 antiserum and 3 μg of nuclear extracts. *Middle*, Result of Northern blot analysis which used 10 μg of total RNA and a ^{32}P-labeled insulin cDNA as a binding probe. Nuclear extracts and total RNA were prepared from HIT-T15 cells of passages 70 (*P70*) and 98 (*P98*) which had been kept under high glucose condition. *Bottom*, Ribosomal RNA.

pression such as that achieved in this study is not likely to affect the gene expression pattern in the cells.

Our observation provides useful information for the understanding of β cell glucose toxicity. To date, a decrease of PDX-1 protein or its binding to the target DNA has been coidentified with a decrease in insulin biosynthesis in HIT cells kept under chronic high glucose condition (7, 29) or with D-ribose, which induces a glycation reaction (8). Therefore, PDX-1 has been suggested to be responsible for the decrease in insulin gene transcription due to glucose toxicity. Although the cell lines used were not the same, the degree of reduction in PDX-1 expression in our antisense ODN–treated cells was greater than that observed in the HIT-T15 cells of passage 98 (P98), which had reduced insulin gene expression caused by chronic high glucose condition (Figs. 1–3 and 7). We would like to note that Moran et al. seem to have found a similar phenomenon recently using HIT-T15 cells of the same age (31). Thus, our results suggest that the reduction of PDX-1 is not a primary cause for the decrease in insulin gene transcription in HIT-T15 cells.

In contrast to these observations on PDX-1–suppressed β cells, the suppression of another insulin gene transcription factor, E2A/Pan, in β cell–derived RIN cells caused dramatic attenuation of insulin gene transcription (21). E2A (E47/E12)/Pan (Pan-1/Pan-2) are ubiquitously expressed basic helix-loop-helix proteins which constitute a heterodimer, called IEF1, with the recently described tissue-specifically expressed basic helix-loop-helix protein, BETA2/NeuroD (32, 33). The heterodimer IEF1 binds to an important *cis*-motif of the insulin gene promoter E element (IEB/Nir, Far) (13), and activates transcription. Although both IEF1-binding E elements and PDX-1–binding A elements play a major role in the β cell–specific expression of the insulin gene (34, 35), their physiological roles may differ in terms of their commitment to the rate of transcription. We assume that this difference may be related to the fact that PDX-1 is a homeodomain-containing protein.

Usually, the physiological function of a homeodomain-containing transcription factor is exerted by switching its expression on and off; whether it is completely absent or present in a cell is important, for example, in the determination of cell fate (36, 37). Although speculative, this may also be the case with PDX-1; the existence of only a small amount of PDX-1 may be essential and adequate for acquiring the β cell trait in terms of the gene expression pattern. In this regard, the PDX-1 amount may be of little physiological significance as long as it is above a certain critical level.

If PDX-1 is not primarily responsible for the decrease in insulin gene transcription due to glucose toxicity, what is the responsible factor? Although E2A/Pan suppression has been shown to cause a decrease in insulin gene transcription (21) as described above, the DNA binding activity to the E element (IEF1 activity) was not reduced in HIT or βTC6 cells that had been exposed to chronic high glucose concentrations (7, 28). Therefore, it still remains obscure whether the E2A/Pan-dependent suppression of insulin gene transcription can be involved pathophysiologically in the decreased insulin synthesis in some NIDDM cases. On the other hand, the DNA-binding activity of another insulin gene transcription factor, RIPE3b1, also seemed to be suppressed by chronic exposure of HIT (38) or βTC6 cells (28) to high glucose concentrations. In the case of βTC6 cells, the binding of PDX-1 was not decreased despite the reduction of insulin gene promoter activity (28), providing support for the pathophysiological significance of reduction of RIPE3b1 activity.

In this study, we used β cell–derived cell lines rather than primary pancreatic β cells. Although both of the two cell lines used (MIN6 and βTC1) gave similar results in terms of the effects of PDX-1 suppression on insulin gene transcription, it is still possible that normal regulation of the insulin gene is altered in those cell lines. As support for this possibility, Melloul et al. showed previously that at least some portions of the insulin gene–regulating machinery may be altered in the β cell–derived cell lines (39). Therefore, similar experiments need to be performed with primary pancreatic islet cells in order to obtain conclusive evidence for the physiological role of PDX-1 in vivo.

In conclusion, our observations obtained from antisense ODN experiments indicate that a moderate decrease in PDX-1 causes no change in the expression of β cell–specific genes such as the insulin gene. Thus, the amount of PDX-1 per se does not seem to be relevant to the rate of insulin gene expression at least as long as it is above a certain level, and it is unlikely to primarily explain the decrease in insulin gene transcription caused by chronic high glucose exposure. To understand the molecular basis of β cell glucose toxicity, research needs to be aimed at other components of the insulin gene–regulating machinery.

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