

Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation*

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Insulin biosynthesis and secretion are critical for pancreatic β -cell function, but both are impaired under diabetic conditions. We have found that hyperglycemia induces the expression of the basic helix-loop-helix transcription factor c-Myc in islets in several different diabetic models. To examine the possible implication of c-Myc in β -cell dysfunction, c-Myc was overexpressed in isolated rat islets using adenovirus. Adenovirus-mediated c-Myc overexpression suppressed both insulin gene transcription and glucose-stimulated insulin secretion. Insulin protein content, determined by immunostaining, was markedly decreased in c-Myc-overexpressing cells. In gel-shift assays c-Myc bound to the E-box in the insulin gene promoter region. Furthermore, in β TC1, MIN6, and HIT-T15 cells and primary rat islets, wild type insulin gene promoter activity was dramatically decreased by c-Myc overexpression, whereas the activity of an E-box mutated insulin promoter was not affected. In HeLa and HepG2 cells c-Myc exerted a suppressive effect on the insulin promoter activity only in the presence of NeuroD/BETA2 but not PDX-1. **Both c-Myc and NeuroD can bind the E-box element in the insulin promoter, but unlike NeuroD, the c-Myc trans-activation domain lacked the ability to activate insulin gene expression.** Additionally p300, a co-activator of NeuroD, did not function as a co-activator of c-Myc. In conclusion, increased expression of c-Myc in β -cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. This mechanism may explain some of the β -cell dysfunction found in diabetes.

The development of type 2 diabetes is usually associated with pancreatic β -cell dysfunction occurring together with insulin resistance. Usually β -cells can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to glucose intolerance. Once hyperglycemia becomes apparent, β -cell function deteriorates (1). The adverse

effects of chronic hyperglycemia on β -cells, called glucose toxicity, have been demonstrated by various *in vivo* (2–5) and *in vitro* studies (6, 7). After chronic exposure to hyperglycemia, insulin gene transcription and glucose-stimulated insulin secretion are suppressed.

c-Myc is a basic helix-loop-helix (bHLH)¹ transcription factor and has an important influence on cell cycle progression, cell differentiation, and the process of apoptosis (8–12). Under chronic hyperglycemic conditions *in vivo*, the expression of many β -cell-associated genes is decreased (3–5), but, in contrast, the expression of some suppressed genes is markedly up-regulated. In normal adult islets, the c-Myc expression level is very low, but c-Myc is induced in diabetic rats following partial pancreatectomy and in rats made hyperglycemic with glucose clamps (5, 13). The changes in *c-myc* gene expression are correlated with graded levels of hyperglycemia. Thus, we hypothesize that induction of c-Myc expression is involved in the β -cell dysfunction of diabetes.

Insulin enhancer elements, E-box and A-box, play an important role in regulating cell-specific expression of the insulin gene (14–19). NeuroD, also known as BETA2, binds to the E-box (20), and the pancreatic and duodenal homeobox factor-1 (PDX-1), also known as IDX-1/STF-1/IPF1, binds to the A-box (21–23). These two transcription factors are very important for insulin gene transcription. NeuroD, a member of the bHLH transcription factor family, is expressed in pancreatic and intestinal endocrine cells and neural tissue. PDX-1, a member of the homeodomain-containing transcription factor family, is expressed in the pancreas and duodenum and plays a major role in pancreatic development (24–27), in β -cell differentiation (28, 29), and in maintaining normal β -cell function probably by regulating multiple important β -cell genes (30–33). Clinically, mutations in NeuroD (34) and in PDX-1 cause maturity-onset diabetes of the young (35, 36). Mice homozygous for the null mutation in NeuroD have a striking reduction in the number of β -cells, develop severe diabetes, and die perinatally (37). The PDX-1-knockout mice are apancreatic and also develop fatal perinatal hyperglycemia (24), while heterozygous PDX-1-knockout mice have impaired glucose tolerance (38).

In this study, we examined the effects of c-Myc on insulin gene transcription and found that increased expression of c-Myc in β -cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation.

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¹ The abbreviations used are: bHLH, basic helix-loop-helix; PDX-1, pancreatic and duodenal homeobox factor-1; GFP, green fluorescent protein; PFU, plaque-forming unit; TAD, transactivation domain; CMV, cytomegalovirus; RT, reverse transcription; CAT, chloramphenicol acetyltransferase.

MATERIALS AND METHODS

Isolation and Culture of Rat Pancreatic Islets—Islets were isolated from pancreata of 200–250-g male Sprague-Dawley rats (Taconic Farms, Germantown, NY) with collagenase digestion. Briefly, the common bile duct was cannulated and injected with 6 ml of cold M199 medium containing 1.5 mg/ml collagenase (Roche Molecular Biochemicals). The islets were separated on Histopaque 1077 (Sigma) density gradient. The washed islets were picked individually under a dissecting microscope and cultured in RPMI medium (11 mM glucose, supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin sulfate) in a humidified atmosphere of 5% CO₂ at 37 °C. All animal procedures were approved by the Animal Care Committee of the Joslin Diabetes Center.

Preparation of Recombinant Adenovirus Expressing c-Myc—A recombinant adenovirus expressing c-Myc was prepared using the AdEasy system (kindly provided by Dr. Bert Vogelstein, Johns Hopkins Oncology Center) (39). In brief, the c-Myc encoding region (kindly provided by Dr. Richard D. Palmiter, University of Washington) was cloned into the *XbaI-EcoRV* site of a shuttle vector pAdTrack-CMV. To produce homologous recombination, 1.0 µg of linearized c-Myc-containing plasmid and 0.1 µg of the adenoviral backbone plasmid, pAdEasy-1, were introduced into electrocompetent *Escherichia coli* BJ5183 cells with electroporation (2,500 V, 200 ohms). Then the resultant plasmid was re-transformed into *E. coli* XL-Gold Ultracompetent Cells (Stratagene, La Jolla, CA). The plasmid was linearized with *PacI* and then transfected into the adenovirus packaging cell line 293 using LipofectAMINE (Invitrogen) which was maintained in Dulbecco's modified Eagle's medium. Ten days after transfection, cell lysate was obtained from the 293 cells. The cell lysate was added to 293 cells again, and when most of the cells were killed by the adenovirus infection and detached, cell lysate was obtained again (This process was repeated three times.). Control adenovirus expressing green fluorescent protein (Ad-GFP) was prepared in the same manner. To determine viral titers, confluent 293 cells were infected with a 1:10,000 dilution of the final lysate containing Ad-Myc (with GFP). After an 18-h incubation, the effective titer was determined by the following formula: $10^7 \times$ the average number of GFP-positive cells per field ($\times 100$ magnification), which was considered equivalent to plaque-forming units (PFU)/ml. This number was considered to be proportional to the number of infective particles in the original lysates. Isolated rat islets (~500 islets) were infected with Ad-Myc (with GFP) or Ad-GFP (without c-Myc), using a 1-h exposure to 50 µl of the adenovirus (1×10^8 PFU/ml). One hour after infection the islets were cultured for 3 days in 3 ml of RPMI medium in 6-cm bacteriologic Petri dishes.

Semiquantitative Radioactive Multiplex PCR—Total RNA was extracted from islets using Trizol (Invitrogen). After quantification by spectrophotometry, 500 ng of RNA was heated at 85 °C for 3 min and then reverse-transcribed (RT) into cDNA with 160 µM dNTP, 50 ng of random hexamers, 10 mM dithiothreitol, and 200 units of Superscript II RNase H⁻ reverse transcriptase (Invitrogen). The reactions took place for 10 min at 25 °C, 60 min at 42 °C, and 10 min at 95 °C. Polymerization reactions were performed in a Perkin-Elmer 9700 Thermocycler in a 50-µl reaction volume containing 3 µl of cDNA (20-ng RNA equivalents), 160 µM cold dNTPs, 2.5 µCi of [α -³²P]dCTP (3000 Ci/mmol), 10 pmol of appropriate oligonucleotide primers, 1.5 mM MgCl₂, and 5 units of AmpliTaq Gold DNA polymerase (PerkinElmer Life Sciences). The oligonucleotide primers and cycle number used for multiplex PCR were as follows. Insulin: (forward) TCT TCT ACA CAC CCA TGT CCC, (reverse) GGT GCA GCA CTG ATC CAC, 15 cycles; GLUT2: (forward) TGG GTT CCT TCC AGT TCG, (reverse) AGG CGT CTG GTG TCG TAT G, 20 cycles; glucokinase: (forward) TGA CAG AGC CAG GAT GGA G, (reverse) TCT TCA CGC TCC ACT GCC, 25 cycles; Kir6.2: (forward) CAT GGA GAA CGG TGT GGG, (reverse) CAG ATA GGA GGT GCG GGC, 25 cycles; SUR: (forward) ATC ACG GAA GGA GGG GAG, (reverse) TTC CGG CTT GTC GAA CTC, 28 cycles; LDH: (forward) ACA GTT GTT GGG GTT GGT G, (reverse) CCG GCT CTC TCC CTC TTG, 25 cycles; c-Myc: (forward) AGT TGG ACA GTG GCA GGG, (reverse) ACA GGA TGT AGG CGG TGG, 28 cycles; PDX-1: (forward) CGG ACA TCT CCC CAT ACG, (reverse) AAA GGG AGA TGA ACG CGG, 20 cycles; NeuroD: (forward) GCA AAG GTT TGT CCC AGC, (reverse) ACG TGG AAG ACG TGG GAG, 20 cycles. The thermal cycle profile employed a 10-min denaturing step at 94 °C followed by the number of cycles and an extension step of 10 min at 72 °C. In each step, the gene products of interest were amplified with an internal control gene (rRNA, cyclophilin, α -tubulin, or TATA-binding protein (TBP)) to correct for experimental variations between samples. The oligonucleo-

tide primers were as follows. Cyclophilin: (forward) AAC CCC ACC GTG TTC TTC, (reverse) TGC CTT CTT TCA CCT TCC C; α -tubulin: (forward) CTC GCA TCC ACT TCC CTC, (reverse) ATG CCC TCA CCC ACG TAC; TBP: (forward) ACC CTT CAC CAA TGA CTC CTA TG, (reverse) ATG ATG ACT GCA GCA AAT CGC. The primers for rRNA was purchased from AMBION (Austin, TX). The amplimers were separated on a 6% polyacrylamide gel in 1 \times TBE buffer (45 mM Tris-HCl, 45 mM boric acid, 1 mM EDTA). The gel was dried, and the amount of [α -³²P]dCTP incorporated into each amplimer was measured with a PhosphorImager and quantified with the ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

To ensure the validity of the measurement of mRNA levels by semi-quantitative-radioactive multiplex PCR, control experiments were performed using normal rat islet cDNA to show that the amount of each amplimer obtained in a multiplex PCR was independent of the presence of other primers, excluding the possibility of strong interferences between primers as we reported previously (5). In brief, the number of cycles was adjusted to be in the exponential phase of the amplification of each product, and we verified that the amount of each PCR product in a multiplex reaction increases linearly with the amount of starting cDNA (5–40-ng RNA equivalents), ensuring that changes in the ratio of PCR product to control gene product truly reflect a change in mRNA abundance of that gene relative to the control gene.

Western Blotting—Whole cell extracts were obtained from islets infected with Ad-Myc or Ad-GFP or uninfected islets. After treatment with lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40), supernatants were collected. Ten micrograms of cell extracts were fractionated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membranes (Immun-Blot™ PVDF Membrane, Bio-Rad) using transfer buffer containing 20% methanol, 25 mM Tris base, and 192 mM glycine (300 mA, 2 h). After blocking the membranes at room temperature for 1 h in 50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20 with 5% nonfat dry milk, the membranes were incubated at 4 °C overnight in TBS buffer (50 mM Tris-HCl, 150 mM NaCl) containing a 1:1000 dilution of rabbit anti-c-Myc antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and washed three times in TBS buffer with 0.1% Tween 20 (TBS-T). The membranes were then incubated for 1 h at room temperature in TBS containing a 1:1000 dilution of anti-rabbit IgG antibody (Bio-Rad) coupled to horseradish peroxidase, followed by three 10-min washings with TBS-T. Immunoreactive bands were made visible by incubation with LumiGLO (Cell Signaling, Beverly, MA) and exposed to x-ray film (Eastman Kodak Co.).

Evaluation of Glucose-stimulated Insulin Secretion—Glucose-stimulated insulin secretion was determined by static incubation. Isolated rat islets (50 islets) were preincubated for 30 min in 2 ml of HEPES-balanced Krebs-Ringer bicarbonate buffer and then incubated for 60 min in the same buffer supplemented with 0.5% bovine serum albumin and either 2.8 mM or 16.7 mM glucose. The insulin secreted into the medium was determined with a radioimmunoassay kit (Linco Research, St. Charles, MO) using rat insulin as the standard.

Immunostaining—Islets were fixed for 60 min in 10% buffered formalin, enrobed in 2% agar to keep the pellet together through processing and embedding, immersed in the same fixative for another 90 min, washed, and stored in 0.1 M phosphate buffer until routine embedding in paraffin. Sections of these pellets were incubated at 4 °C overnight with guinea pig anti-insulin antibody (Linco Research) diluted 1:100 in PBS containing 1% bovine serum albumin and then incubated for 1 h at room temperature with Texas Red-conjugated donkey anti-guinea pig IgG (1:100) (Jackson Immunochemicals).

Gel-mobility Shift Assay—Nuclear extracts were obtained from uninfected islets and islets (~500 islets) infected with Ad-Myc and Ad-GFP. Cells were treated with 1 ml of hypotonic buffer (20 mM HEPES, pH 7.9, 20 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM Na₄P₂O₇, 1 mM Na₂VO₄, 1 mM dithiothreitol); then 50 µl of high salt buffer (420 mM NaCl and 20% glycerol in hypotonic buffer) was added to the pellet, followed by 1 h of incubation at 4 °C. The supernatants were used as nuclear extracts. Two micrograms of nuclear extract were incubated with 2 µg of poly(dI-dC) at room temperature in 20 µl of reaction buffer. The reaction buffers for binding to A-box and E-box were Buffer 1 (10 mM HEPES, pH 7.8, 0.1 mM EDTA, 75 mM KCl, 2.5 mM MgCl₂, 1 mM dithiothreitol, 3% Ficoll) and Buffer 2 (25 mM HEPES, pH 7.8, 0.2 mM EDTA, 150 mM KCl, 5 mM dithiothreitol, 9% glycerol), respectively. The binding reaction was initiated by adding ³²P-labeled double-stranded oligonucleotide probes. Double-stranded oligonucleotides reproducing the rat insulin gene PDX-1 and NeuroD binding region and surrounding sequences (ACG TCC TCT TAA GAC TCT AAT TAC CCT ACG T and ACG TTC TGG CCA TCT GCT GAT CCT ACG T) were used as binding probes. In some of the binding assays, anti-PDX-1 antiserum (30) or

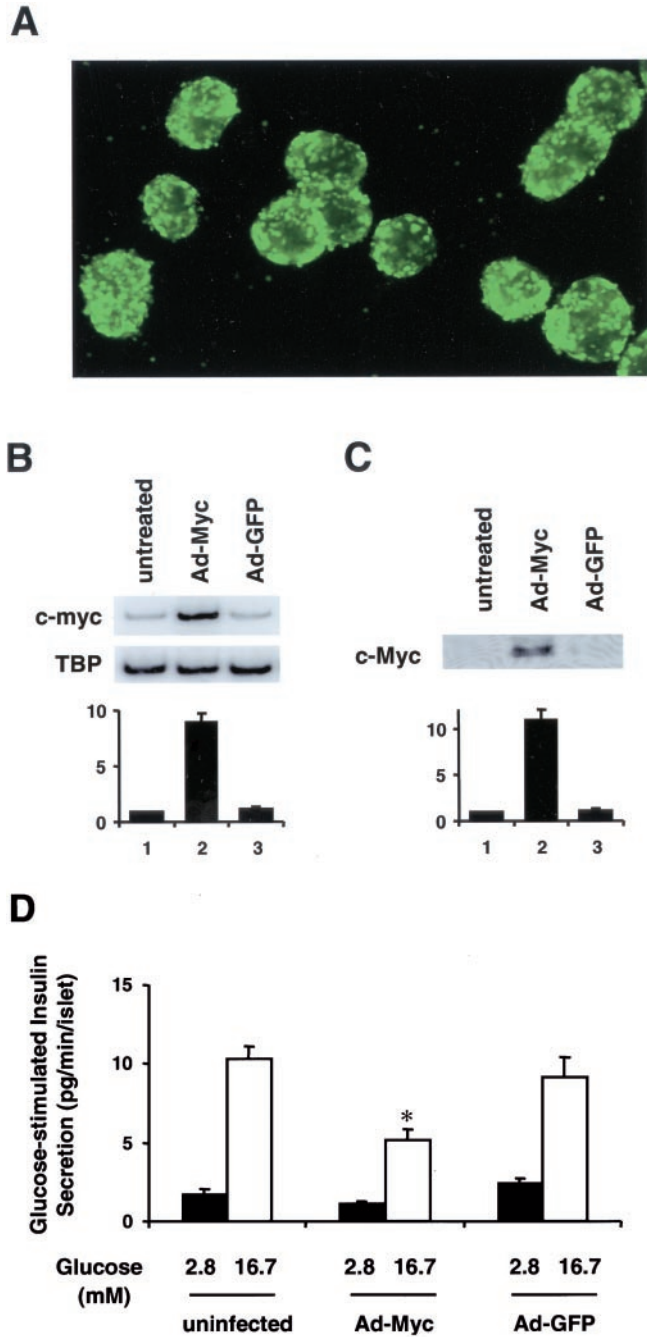


FIG. 1. Overexpression of c-Myc using adenovirus in rat pancreatic islets and its effect on glucose-stimulated insulin secretion. A, overexpression of c-Myc using adenovirus in pancreatic islets. Isolated rat islets (~500 islets) were infected with recombinant adenovirus, Ad-Myc or Ad-GFP (1×10^8 PFU/ml). The panel shows representative islets 3 days after infection with Ad-Myc. B, 3 days after infection with Ad-Myc or Ad-GFP, total RNA was isolated, and RT-PCR was done. Relative amounts of *c-myc* mRNA are expressed as mean \pm S.E. in bar graphs with those of uninfected islets being arbitrarily set at 1 ($n = 3$). C, 3 days after infection, total protein was obtained and Western blotting was done with an antibody for c-Myc. Relative protein amounts of c-Myc are expressed as mean \pm S.E. in bar graphs with those of uninfected islets being arbitrarily set at 1 ($n = 3$). D, effect of c-Myc overexpression on glucose-stimulated insulin secretion. After infection with Ad-Myc or Ad-GFP and culture in RPMI medium for 3 days, static glucose-stimulated insulin secretion was examined over 60 min with batches of 50 islets, comparing 2.8 mM and 16.7 mM glucose. Data are expressed as mean \pm S.E. in bar graphs ($n = 3$), *, $p < 0.01$.

anti-NeuroD antibody (Santa Cruz) was added to the reaction mixture 1 h before addition of the DNA probes. After the binding reactions, samples were analyzed by separation on 6% polyacrylamide gel in $1 \times$

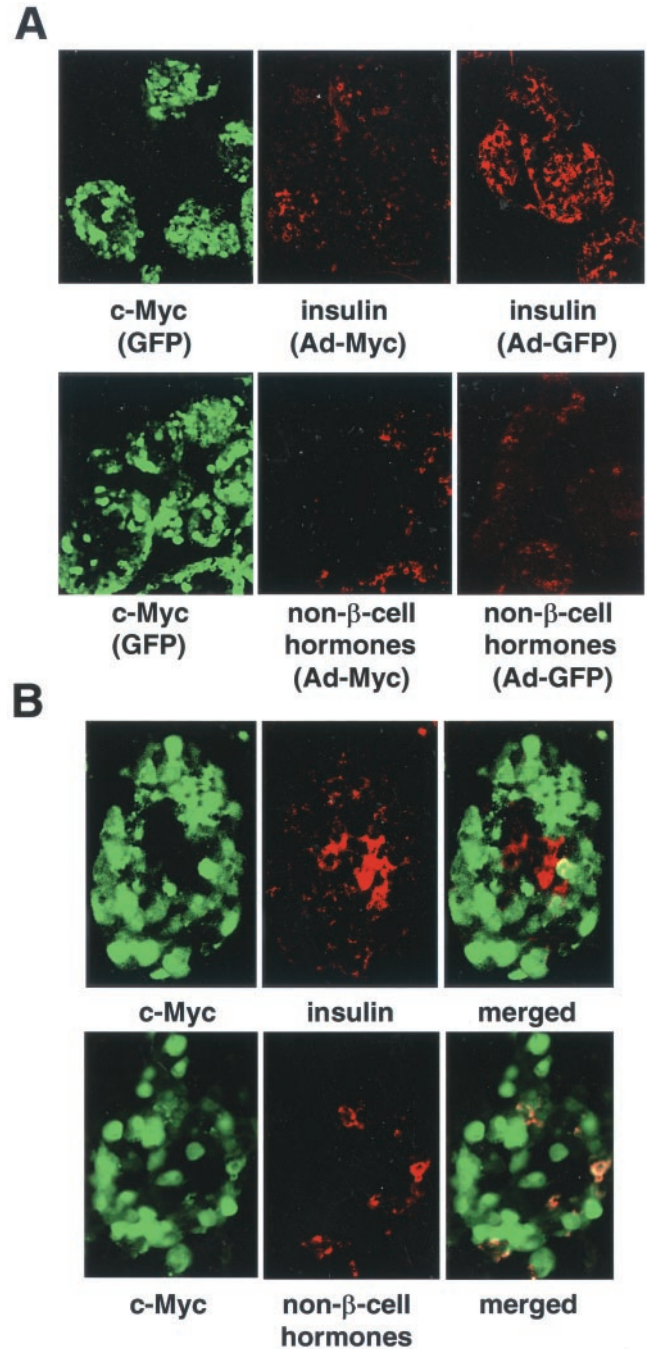


FIG. 2. Immunostaining of c-Myc-overexpressing islets. A, immunostaining for insulin and non- β -cell hormones was performed on islets 3 days after infection with Ad-Myc or Ad-GFP. The left panels show the GFP staining (green) indicating the adenoviral infected cells, and the middle and right panels show the islet hormone staining (red). As can be seen in the lower panels, the mantles of non- β -cells have clumped, and less common than often seen in native pancreas, many islets had little or no mantle. B, at higher magnification, in c-Myc-overexpressing cells (green), the insulin expression (red) is markedly reduced (upper panel). In contrast, non- β -cell hormones (red) are clearly detected even in c-Myc-overexpressing cells (green) (lower panel). The panels are presented separately as the green channel, the red channel and the merged image.

TBE buffer (45 mM Tris-HCl, 45 mM boric acid, 1 mM EDTA) followed by autoradiography.

Cell Culture and Gene Transfection— β TC1, HIT-T15, HepG2, and HeLa cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin sulfate in a humidified atmosphere of 5% CO_2 at 37 $^\circ\text{C}$. MIN6 cells were grown in the same conditions except for using Dulbecco's modified Eagle's me-

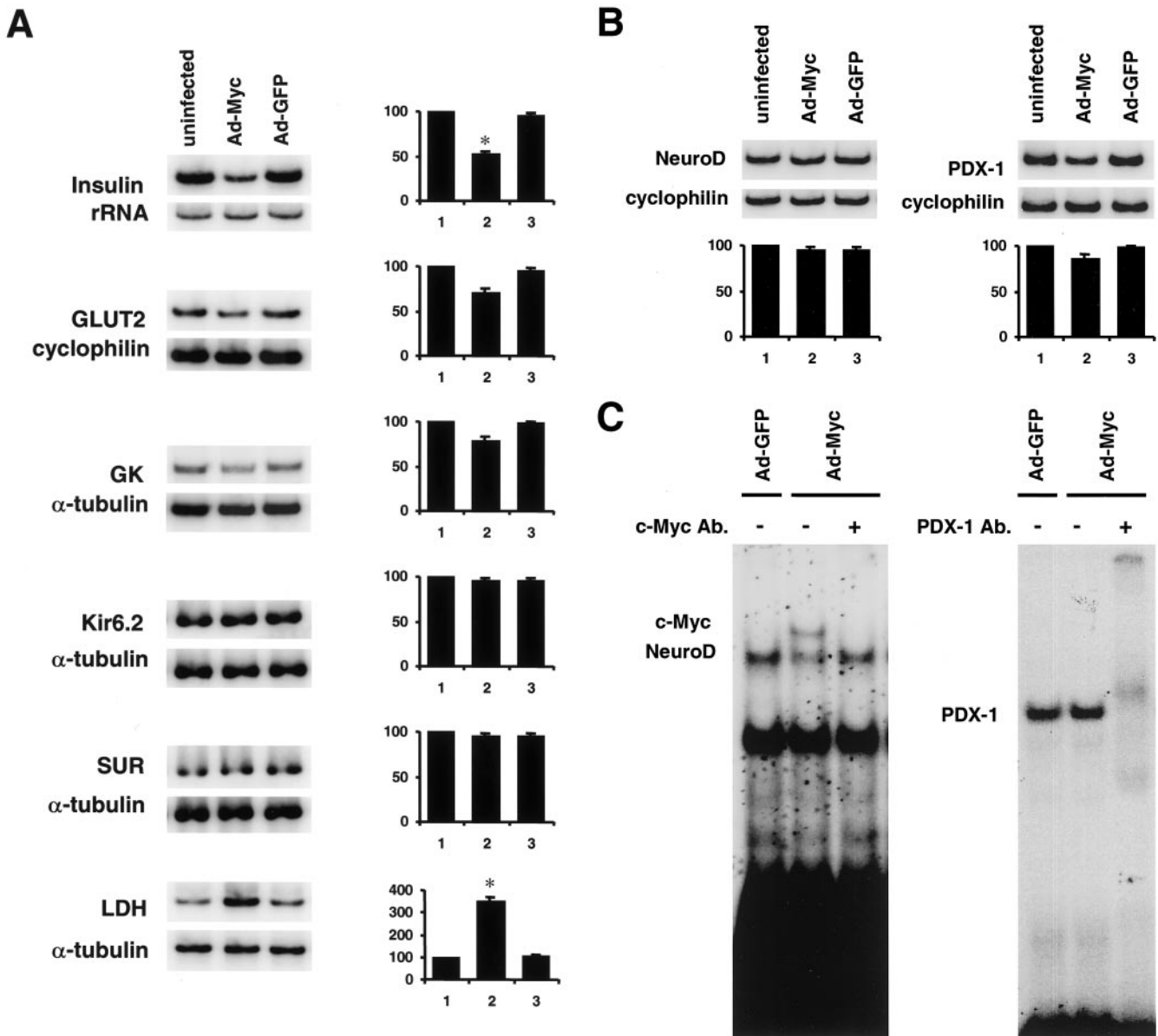


FIG. 3. Effects of c-Myc overexpression on β -cell-associated gene expression and c-Myc binding to the E-box in insulin promoter. A, effects of c-Myc overexpression on β -cell-associated gene expression. After infection with Ad-Myc or Ad-GFP and culture in RPMI medium for 3 days, total RNA was isolated from the islets and used for RT-PCR. Relative mRNA amounts of each gene (insulin, GLUT2, glucokinase, Kir6.2, SUR, and lactate dehydrogenase (*LDH*)) are expressed as mean \pm S.E. in bar graphs with those of uninfected islets being arbitrarily set at 100 ($n = 4$), *, $p < 0.01$. B, after infection with Ad-Myc or Ad-GFP and culture in RPMI medium for 3 days, total RNA was isolated from the islets, and mRNA amounts of NeuroD and PDX-1 were examined by RT-PCR. Relative mRNA amounts of each gene (NeuroD and PDX-1) are expressed as mean \pm S.E. in bar graphs with those of uninfected islets being arbitrarily set at 100 ($n = 3$). C, c-Myc binding to the E-box in insulin promoter. Three days after infection with Ad-Myc or Ad-GFP, nuclear extracts were obtained from the islets, and gel-shift assays were performed. The rat insulin gene NeuroD and PDX-1 binding regions (ACG TTC TGG CCA TCT GCT GAT CCT ACG T and ACG TCC TCT TAA GAC TCT AAT TAC CCT ACG T) were used as binding probes. In some of the binding assays, anti-c-Myc antibody or anti-PDX-1 antibody was added to the binding reactions 1 h before addition of the DNA probes. Similar results were obtained in three independent experiments.

dium (25 mM glucose) and 20% fetal calf serum. The cells were replated 24 h before transfection; then 1.0 μ g of c-Myc expression plasmid (or the empty vectors), 1.0 μ g of rat insulin II promoter-reporter (luciferase) plasmid (40) containing 238-bp 5'-flanking sequences of the rat insulin II promoter region, 1.0 μ g of the PDX-1 (41) and NeuroD (40) expression plasmids (or the empty vectors), and 0.5 μ g of pSV- β -galactosidase control vector (Promega, Madison, WI) were co-transfected into the cells with the LipofectAMINE reagent (Invitrogen) using the conditions recommended by the manufacturer. NeuroD:c-Myc-TAD (amino acids 1–143) and c-Myc:NeuroD-TAD (amino acids 189–355) fusion plasmids were made by PCR using NeuroD and c-Myc expression plasmids as templates; the resulting clones were verified by sequencing. These plasmids were transfected as above. For transfection into primary islets, 1.0 μ g of c-Myc expression plasmid (or the empty vectors), 1.0 μ g of rat insulin I promoter-reporter (CAT) plasmid (kindly provided by Dr. Michael German, University of California-San Francisco) (18) contain-

ing the 345-bp 5'-flanking sequences of the rat insulin I promoter region, and 0.5 μ g of pSV- β -galactosidase control vector were co-transfected into the islets (~ 100 islets) as described previously (42) with some modification. In brief, the plasmid DNA mixture was incubated with 10 μ l of LipofectAMINE reagent, 100 μ l of control adenovirus (Ad-GFP) (1×10^8 PFU/ml), and 100 μ l of OPTI-MEM medium for 30 min, and then the DNA/LipofectAMINE/adenovirus mixture was then added to the cells.

Luciferase and CAT Assays—Forty eight hours after the transfections, cells were harvested for luciferase and β -galactosidase assays. Preparations of cellular extracts were assayed using a luciferase assay system (Promega) and a CAT assay system (Promega). For the luciferase assay, light emission was measured with Monolight 3010 Luminometer (BD Pharmingen, San Diego, CA). β -Galactosidase assays were performed with the β -galactosidase enzyme assay system (Promega). The luciferase and CAT results were normalized with respect to trans-

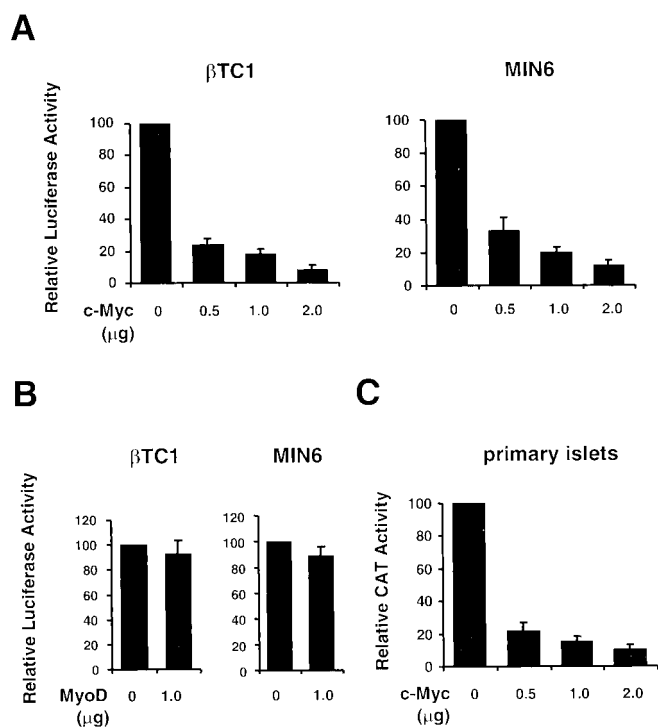


FIG. 4. Effects of c-Myc overexpression on insulin promoter activity. A, 1 μ g of rat insulin II promoter-reporter (luciferase) plasmid and 0.5 μ g of pSV- β -galactosidase control vector were co-transfected into β TC1 and MIN6 cells with 0–2.0 μ g of c-Myc expression plasmid, and then luciferase and β -galactosidase assays were performed. B, 1 μ g of rat insulin II promoter-reporter (luciferase) plasmid and 0.5 μ g of pSV- β -galactosidase control vector were co-transfected into β TC1 and MIN6 cells with 1.0 μ g of MyoD expression plasmid, and then luciferase and β -galactosidase assays were performed. C, 1 μ g of rat insulin I promoter-reporter (CAT) plasmid and 0.5 μ g of pSV- β -galactosidase control vector were co-transfected into primary islets with 0–2.0 μ g of c-Myc expression plasmid, and then CAT and β -galactosidase assays were performed. The luciferase and CAT results were normalized with respect to transfection efficiency assessed from the results of the β -galactosidase assays. Data are expressed as mean \pm S.E. in bar graphs with the basal insulin promoter activity being arbitrarily set at 100 ($n = 4$).

fection efficiency assessed from the results of the β -galactosidase assays.

Statistical Analysis—All results are presented as mean \pm S.E. in three or four independent experiments. Statistical analysis was performed using the unpaired Student's t test.

RESULTS

Adenovirus-mediated c-Myc Overexpression Impairs Insulin Biosynthesis and Secretion—To evaluate the possible implications of c-Myc induction in β -cell dysfunction, isolated rat pancreatic islets from Sprague-Dawley rats were infected with c-Myc-expressing adenovirus (Ad-Myc) or control adenovirus (Ad-GFP). Fig. 1A shows representative islets 3 days after infection with Ad-Myc. Total RNA was isolated 3 days after infection and used for RT-PCR followed by densitometric analyses. As shown in Fig. 1B, infection with Ad-Myc led to an increase in *c-myc* mRNA expression in islets as compared with uninfected cells or cells infected with Ad-GFP. Also, as shown in Fig. 1C, induction of c-Myc protein level was confirmed by Western blotting.

To examine the effects of c-Myc overexpression on glucose-stimulated insulin secretion, static incubations were performed using the islets exposed to Ad-Myc (or Ad-GFP). Three days after infection with Ad-Myc, glucose-stimulated insulin secretion was decreased, although adenovirus infection itself (Ad-GFP) did not affect insulin secretion (Fig. 1D).

To examine whether the insulin protein level is decreased by c-Myc overexpression, immunostaining for insulin was performed after infection with Ad-Myc (or Ad-GFP). Fig. 2 shows representative immunostaining 3 days after infection with the adenovirus. As shown in Fig. 2A, many islet cells were c-Myc (GFP)-positive. The percentage of non- β -cells was quite low in islets after infection with either Ad-Myc or Ad-GFP (Fig. 2A, lower panel). It should be pointed out that the mantle pattern for non- β -cells is somewhat different from the more even distribution found in sections of pancreas. It appears that some mantle cells are lost and even redistributed during the process of islet isolation with collagenase and subsequent culture. Therefore, most of the infected cells are likely to be β -cells. As shown in Fig. 2A, upper panel, insulin expression was clearly detected in islets after Ad-GFP infection (right panel) but was markedly reduced after Ad-Myc infection (middle panel). These results clearly suggest that c-Myc overexpression, but not adenovirus infection itself, suppresses the insulin expression. There was no obvious difference in non- β -cell hormone staining between Ad-Myc (Fig. 2A, lower, middle panel) and Ad-GFP group (right panel), although we cannot deny the possibility that c-Myc has some less obvious effects on non- β -cells. Furthermore, as shown in Fig. 2B, upper panel, insulin immunostaining was markedly reduced in the cells expressing c-Myc, whereas non- β -cell hormones (glucagon, somatostatin, pancreatic polypeptide) were detected even in c-Myc-overexpressing cells (Fig. 2B, lower panel). Therefore, we assume that c-Myc overexpression suppresses insulin biosynthesis, leading to lower insulin content and the secretion shown in Fig. 1D.

c-Myc Exerts a Suppressive Effect on Insulin Gene Transcription—To evaluate the possible implications of c-Myc induction for β -cell dysfunction, we examined the effects of c-Myc on β -cell-associated gene expression. As shown in Fig. 3A, 3 days after the infection, total RNA was isolated and used for RT-PCR followed by densitometric analyses. The amount of insulin mRNA was remarkably decreased in the c-Myc-overexpressing islets whereas no decrease was observed in the Ad-GFP-infected cells. GLUT2 and glucokinase mRNA levels were also moderately decreased by c-Myc overexpression, whereas the expression levels of other β -cell-specific genes, *Kir6.2* and *SUR*, did not change. Additionally, lactate dehydrogenase, which is known to be transactivated by c-Myc, was induced in islets exposed to Ad-Myc.

To explore the mechanism for c-Myc-mediated reduction of insulin gene expression, we examined expression levels of NeuroD and PDX-1, both of which are very important transcription factors for insulin gene expression. As shown in Fig. 3B, the expression level of NeuroD was not affected by c-Myc overexpression. The PDX-1 expression level showed a tendency to be reduced, which did not reach statistical significance. Because c-Myc is a bHLH-LZ transcription factor that binds to E-boxes, it seemed possible that c-Myc binds to the E-box in insulin promoter. To test this hypothesis, gel-mobility shift assays were performed. As shown in Fig. 3C, E-box binding complex was observed in nuclear extracts from Ad-GFP-infected islets. Nuclear extract obtained after infection with Ad-Myc showed an additional complex that was eliminated by c-Myc antibody, indicating that this band includes c-Myc protein. In contrast, the binding pattern to A-box was unaffected by c-Myc overexpression. These results suggest that c-Myc binds to the E-box in insulin promoter, and it is likely that c-Myc suppresses the insulin gene transcription by competing for binding to the E-box element.

To evaluate the effect of c-Myc overexpression on insulin gene transcription, we examined insulin gene promoter activity in several β -cell-derived cell lines (β TC1, MIN6, and HIT-T15).

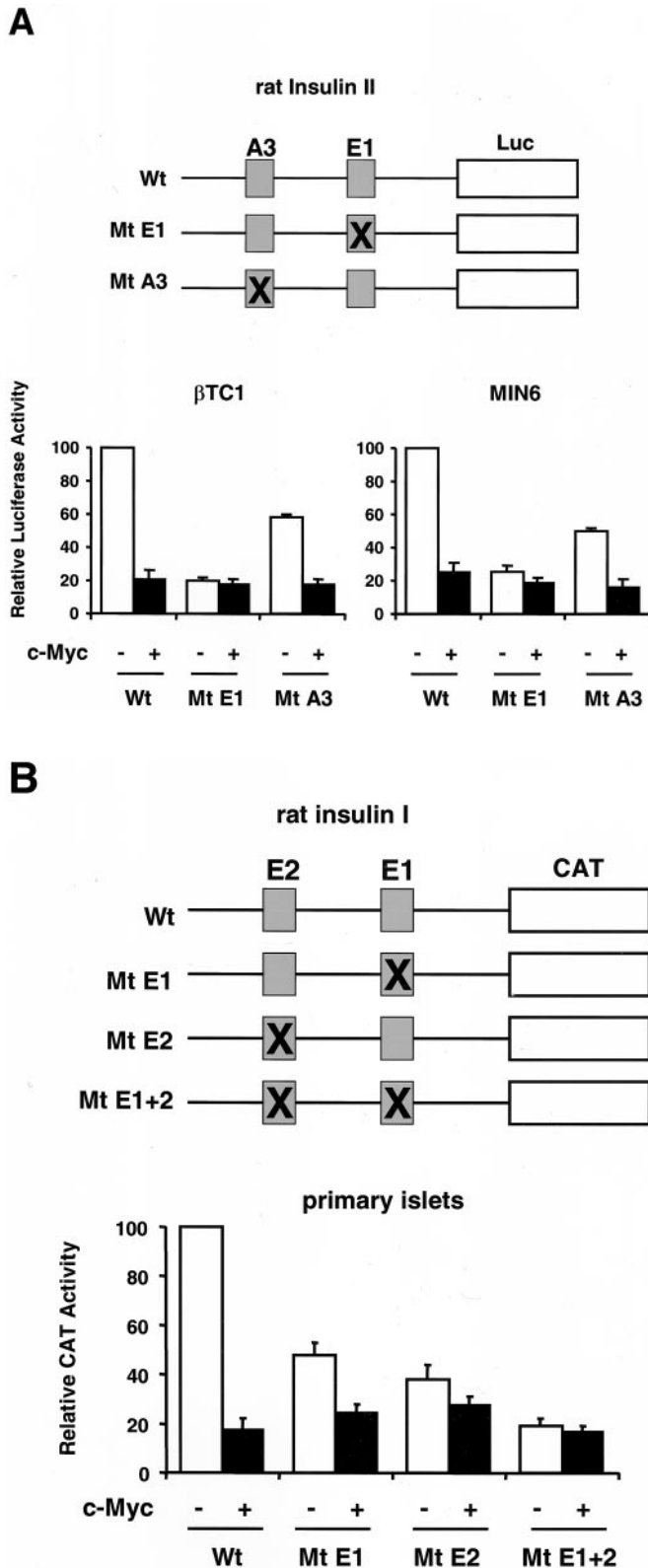


FIG. 5. Effects of E-box mutations on c-Myc-mediated suppression of the insulin promoter activity. A, wild type and E1-box or A3-box mutated (*Mt*) rat insulin II promoter-reporter (luciferase) plasmid and pSV- β -galactosidase control vector were co-transfected into β TC1 and MIN6 cells with c-Myc expression plasmid (or the empty vectors); after 48 h in culture luciferase and β -galactosidase assays were performed. It is noted that rat insulin II promoter has only one E-box. B, wild type (*Wt*) and E1-box and/or E2-box mutated rat insulin I promoter-reporter (CAT) plasmid and pSV- β -galactosidase control vector were co-transfected into primary islets with c-Myc expression plasmid (or the empty vectors); after 48 h in culture in RPMI medium,

after transient transfection of c-Myc expression plasmid. Following transfection, insulin promoter activity was decreased in a dose-dependent manner in β TC1 and MIN6 cells (Fig. 4A). Similar results were observed in HIT-T15 cells (data not shown). Because it seemed possible that other bHLH transcription factors could act in a similar manner, we examined the effect of overexpression of another bHLH transcription factor MyoD on insulin promoter activity. However, as shown in Fig. 4B, unlike c-Myc, MyoD did not exert any suppressive effect on insulin promoter activity. Furthermore, we examined the effect of c-Myc overexpression on insulin promoter activity in freshly isolated rat pancreatic islets. As shown in Fig. 4C, c-Myc-mediated reduction of insulin promoter activity was observed in primary islets.

Overexpression of c-Myc Suppresses Insulin Gene Transcription by Inhibiting NeuroD-mediated Transcriptional Activation—To examine which sequence of the insulin gene promoter region is important for c-Myc-mediated suppression of the insulin gene expression, we examined the effect of c-Myc overexpression on E1-box and A3-box mutated insulin II gene promoter-reporter plasmids. It should be noted that rat insulin II promoter has only one E-box. Although wild type insulin gene promoter activity was significantly decreased by c-Myc overexpression, the promoter activity of E1-box mutated plasmid was not affected by c-Myc overexpression. However, a reporter plasmid with mutation in the A3-box had lower basal promoter activity than wild type plasmid, and it was decreased by c-Myc overexpression (Fig. 5A). Similar experiments were performed with primary islets using E1-box and/or E2-box mutated rat insulin I gene promoter-reporter plasmids. The c-Myc expression plasmid and insulin promoter reporter fusion plasmid were co-transfected into primary islets, and CAT assays were performed. Wild type insulin promoter activity was significantly decreased by c-Myc overexpression, but this inhibitory effect was much reduced when E1- or E2-box mutated promoter activity was assessed. When both the E1- and E2-boxes were mutated, promoter activity was unaffected by c-Myc overexpression (Fig. 5B). These results suggest that c-Myc exerts suppressive effects on the insulin gene transcription mainly through the E-box. However, because E-box elements are critical for insulin promoter activity, we must acknowledge the possibility that in the absence of E-boxes insulin promoter activity is quite low, which may obscure an effect of c-Myc. Therefore, to further substantiate the results obtained in Fig. 5, we examined the effects of c-Myc on the insulin gene promoter activity in two non-insulin-producing cell lines (HeLa and HepG2) (Fig. 6). In non-insulin-producing cell lines basal insulin gene promoter activity was very low but increased after transfection of PDX-1 or NeuroD expression plasmid and increased more remarkably after simultaneous overexpression of PDX-1 and NeuroD. As shown in Fig. 6A, inhibitory effect of c-Myc on the insulin promoter activity was observed only in the presence of NeuroD, but not of PDX-1. Also, as shown in Fig. 6B, NeuroD increased the insulin promoter activity in a dose-dependent manner in the absence and presence of PDX-1, and the effect was markedly suppressed by the presence of c-Myc.

Although both NeuroD and c-Myc bound to the E-box of the insulin promoter (Fig. 3C), NeuroD transactivated the insulin gene very well, but c-Myc did not (Fig. 6). In addition, p300 is known to bind to NeuroD transactivation domain (TAD) and

CAT and β -galactosidase assays were performed. Note that rat insulin I promoter has two E-boxes. The luciferase and CAT results were normalized with respect to transfection efficiency assessed from the results of the β -galactosidase assays. Data are expressed as mean \pm S.E. in bar graphs with the basal insulin promoter activity being arbitrarily set at 100 ($n = 4$).

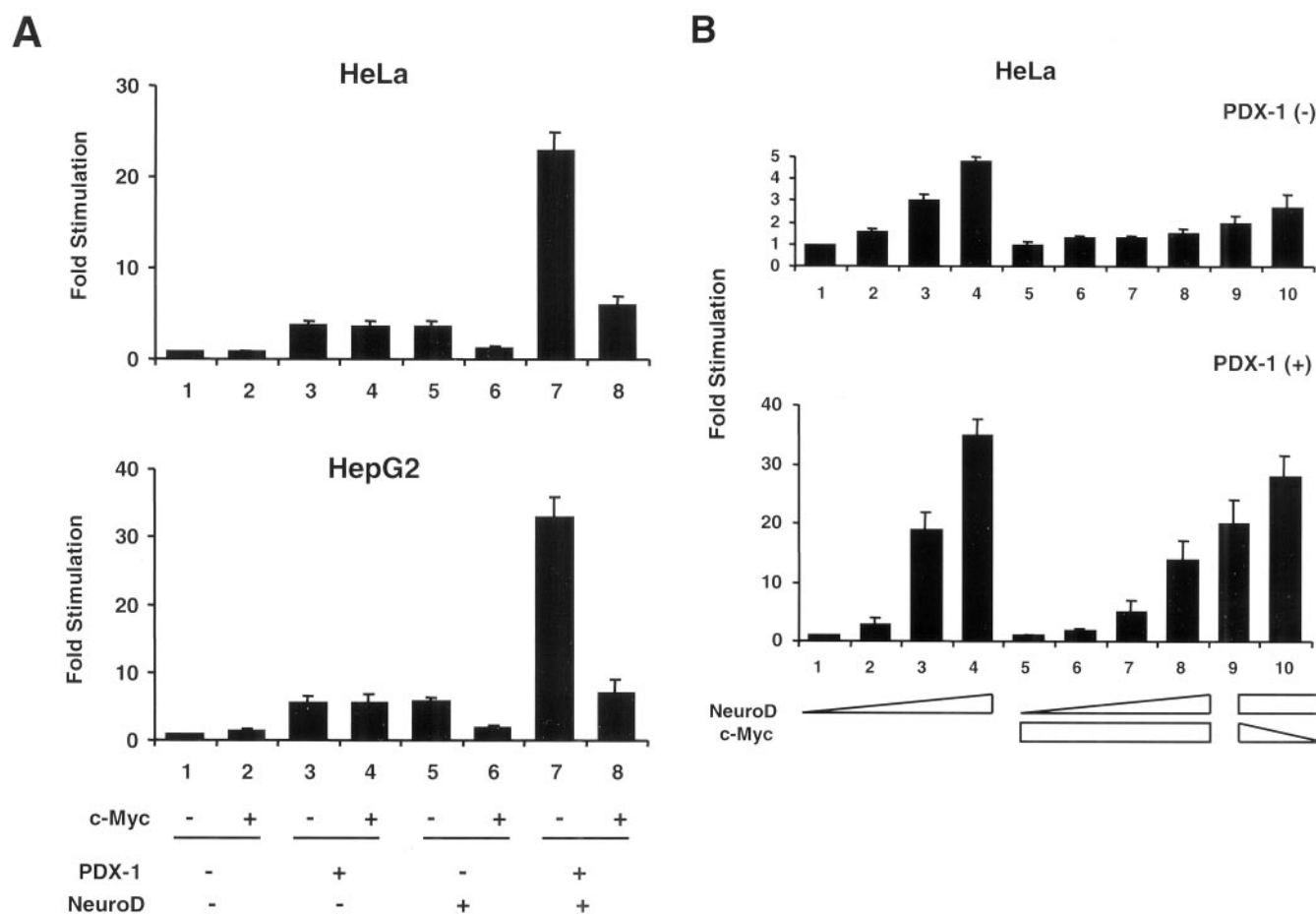


FIG. 6. Effects of c-Myc overexpression on the insulin promoter activity in HeLa and HepG2 cells. A, rat insulin II promoter-reporter plasmid and pSV- β -galactosidase control vector were co-transfected into HeLa and HepG2 cells with c-Myc expression plasmid (or the empty vectors) and PDX-1 and/or NeuroD expression plasmid (or the empty vectors); after 48 h in culture luciferase and β -galactosidase assays were performed. B, rat insulin II promoter-reporter plasmid and pSV- β -galactosidase control vector were co-transfected into HeLa cells with different amounts of c-Myc and/or NeuroD expression plasmids (or the empty vectors) (lanes 1–4, 0, 0.5, 1.0, 1.5 μ g of NeuroD; lanes 5–8, 0, 0.5, 1.0, 1.5 μ g of NeuroD plus 1.5 μ g of c-Myc, lanes 9 and 10, 1.5 μ g of NeuroD plus 1.0, 0.5 μ g of c-Myc) in the absence (upper) and presence (lower) of PDX-1 (1.0 μ g), and luciferase and β -galactosidase assays were performed. The luciferase results were normalized with respect to transfection efficiency assessed from the results of the β -galactosidase assays. Data are expressed as mean \pm S.E. in bar graphs with the basal insulin promoter activity being arbitrarily set at 1 ($n = 4$).

function as a co-activator of NeuroD (43). Thus we suspected that NeuroD-TAD is much stronger than c-Myc-TAD for the insulin gene transcription or that p300 functions as a co-activator of NeuroD but not of c-Myc. Hence, to compare the transactivational activity of c-Myc-TAD with NeuroD-TAD for the insulin gene transcription, we made fusion plasmids by replacing NeuroD-TAD with c-Myc-TAD and c-Myc-TAD with NeuroD-TAD (Fig. 7A) and examined their effects on the insulin gene promoter activity. Western blotting revealed that similar amounts of each protein were synthesized after transfection of each plasmid into HeLa cells (Fig. 7B). As shown in Fig. 7C, in insulin-producing cell lines β TC1 and MIN6, wild type NeuroD increased insulin promoter activity modestly, but NeuroD:Myc-TAD fusion protein decreased it. Also, wild type c-Myc suppressed the insulin promoter activity markedly, but the Myc:NeuroD-TAD fusion protein did not suppress as well. As shown in Fig. 7D, in HeLa cells, wild type NeuroD increased the insulin gene promoter activity, and the effect was enhanced by PDX-1 overexpression, but NeuroD:Myc-TAD did not increase it in the absence or presence of PDX-1. Also, unlike wild type c-Myc, Myc:NeuroD-TAD increased the insulin gene promoter activity. Because NeuroD and co-activator p300 are important for activating insulin gene expression, we examined the effect of p300 on NeuroD- and c-Myc-mediated transcriptional activity. As shown in Fig. 7D, p300 enhanced the effects on the

insulin gene promoter activity by wild type NeuroD and Myc:NeuroD-TAD, but the effects of wild type c-Myc and NeuroD:Myc-TAD were unchanged. These results indicate that NeuroD-TAD is much stronger than c-Myc-TAD for the insulin gene transcription and that p300 functions as a co-activator of NeuroD but probably not as a co-activator of c-Myc.

DISCUSSION

Chronic hyperglycemia leads to a decline in insulin biosynthesis and glucose-stimulated insulin secretion (1). The toxic effect of hyperglycemia on β -cells has been demonstrated with *in vivo* (2–5) and *in vitro* models (6, 7). Previously we found that hyperglycemia induces the expression of c-Myc in islets in several different diabetic models (5, 13). In this study we report that increased expression of c-Myc in β -cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. Adenovirus-mediated c-Myc overexpression suppressed insulin gene transcription and glucose-stimulated insulin secretion. Furthermore, c-Myc bound to the E-box of the insulin promoter and suppressed NeuroD-mediated transcriptional activation. Thus we postulate c-Myc mediated some of the deterioration of β -cell function found in diabetes.

It is well known that c-Myc can have a pro-apoptotic effect on cells (9–12). GFP was expressed in nuclei in islets, and thus

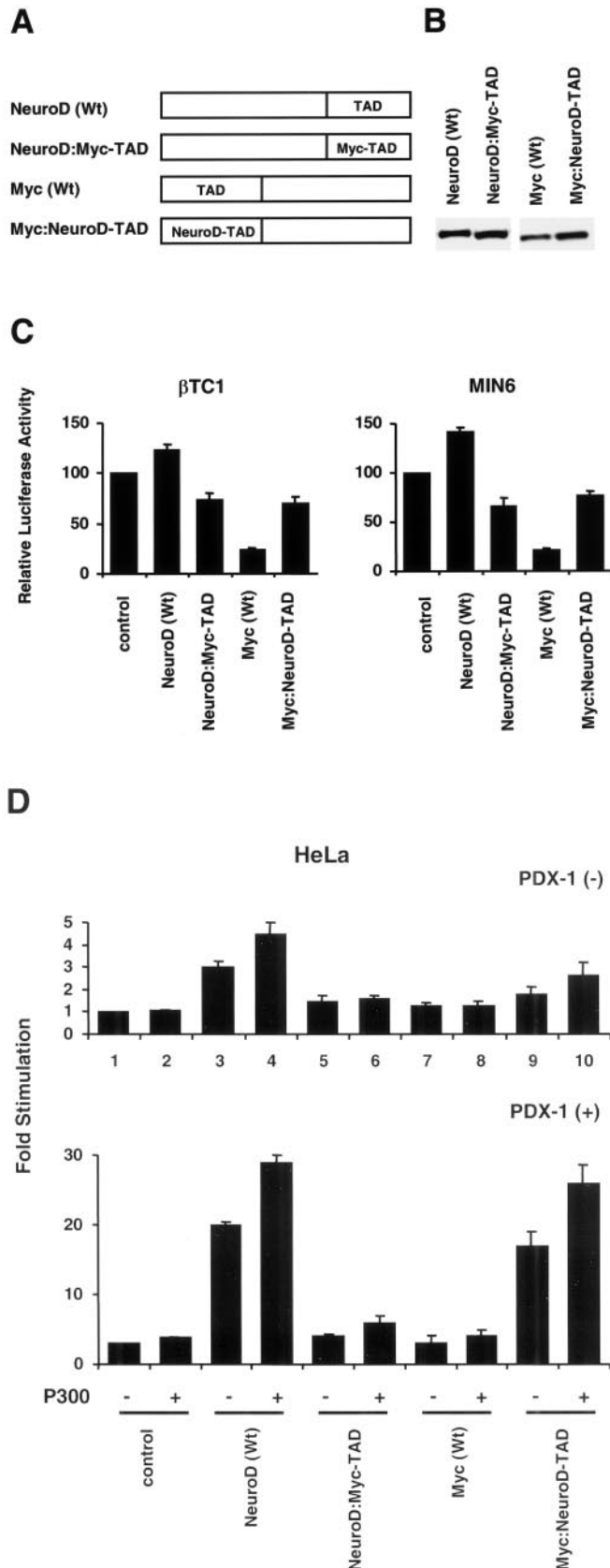


FIG. 7. Influence of p300 on NeuroD- and c-Myc-mediated effects on insulin promoter activity. A, to compare transcriptional activity of c-Myc-TAD (amino acids 1–143) with NeuroD-TAD (amino acids 189–355) on the insulin gene promoter, fusion proteins were made by replacing NeuroD-TAD with c-Myc-TAD and c-Myc-TAD with NeuroD-TAD. B, to examine synthesized protein amounts, Western blotting was done with an antibody for NeuroD (left) or for c-Myc (right) after

potential nuclear changes of apoptosis (shrinkage and fragmentation) could be assessed. In fact, apoptotic cells were detected very rarely, if at all, 3 days after infection with Ad-Myc, possibly because the exposure time was not long enough to produce substantial apoptosis. Also, as shown in Fig. 3, expression levels of several β -cell-associated genes were not affected by c-Myc overexpression. These results indicate that c-Myc-mediated suppression of the insulin gene transcription in this study is not due to induction of apoptosis or decrease of cell viability. Thus, we conclude that c-Myc exerts a suppressive effect on the insulin gene transcription in addition to its influence on cell cycle progression or the process of apoptosis (8–12); the suppressive effect of c-Myc on insulin promoter activity can occur separately from its effects upon cell viability.

c-Myc binds to the E-box in insulin promoter (Fig. 3C), but unlike NeuroD, c-Myc lacked the ability to activate insulin gene promoter (Figs. 6 and 7). Therefore, we assume that when c-Myc binds to the E-box of the insulin gene, NeuroD binding to E-box is suppressed, resulting in inhibition of insulin gene transcription. Also, it was reported that p300 binds to NeuroD-TAD and functions as a co-activator of NeuroD to transactivate the insulin gene (43). As shown in Fig. 7D, wild type NeuroD-mediated or Myc:NeuroD-TAD fusion protein-mediated transactivation of the insulin gene was enhanced by p300 overexpression, but p300 overexpression did not enhance wild type c-Myc-mediated or NeuroD:Myc-TAD fusion protein-mediated transactivation. Thus, we suggest there are several potential reasons for c-Myc-mediated suppression of the insulin gene transcription. One possibility is that transcriptional activity of c-Myc-TAD on the insulin gene is not as strong as that of NeuroD. Another is that p300, a co-activator of NeuroD, does not function as a co-activator of c-Myc. Moreover, it is known that NeuroD binds the ubiquitously expressed E2A gene product E12/E47, and this heterodimer transactivates the insulin gene (44). Although not examined in this study, such a mechanism would not be possible for c-Myc binding to the E-box of the insulin gene.

Most bHLH transcription factors bind to E-boxes and trigger transactivation of several genes, but some factors are known to inhibit transactivation. For example, bHLH transcriptional regulator Id lacks the basic amino acid residues that are required for DNA binding and inhibits the binding and activation properties of the bHLH family members by forming nonfunctional heterodimer complexes (45). Another example is proto-oncogene c-Jun, a b-Zip transcription factor that suppresses the insulin gene transcription (46, 47) by affecting the transactivation potential of the E2A gene products (48). In this study, we found that c-Myc suppressed the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. Thus we propose c-Myc as one of the factors that inhibit the effect of bHLH family proteins in addition to Id and c-Jun.

It is likely that changes in transcription factors other than

transfection of each plasmid into HeLa cells. C, wild type (Wt) NeuroD expression plasmid, NeuroD:Myc-TAD fusion plasmid, wild type c-Myc expression plasmid, or c-Myc:NeuroD-TAD fusion plasmid was cotransfected into β TC1 and MIN6 cells with rat insulin II promoter-reporter plasmid and pSV- β -galactosidase control vector; after 48 h in culture luciferase and β -galactosidase assays were performed. D, wild type NeuroD expression plasmid, NeuroD:Myc-TAD fusion plasmid, wild type c-Myc expression plasmid, or c-Myc:NeuroD-TAD fusion plasmid was cotransfected into HeLa cells with rat insulin II promoter-reporter plasmid, pSV- β -galactosidase control vector, and p300 expression plasmid (or the empty vectors) in the absence (upper) and presence of PDX-1 (lower), and luciferase and β -galactosidase assays were performed. The luciferase results were normalized with respect to transfection efficiency assessed from the results of the β -galactosidase assays. Data are expressed as mean \pm S.E. in bar graphs with the basal insulin promoter activity being arbitrarily set at 100 (C) or at 1 (D) ($n = 4$).

c-Myc, such as PDX-1, NeuroD, RIPE3b1, C/EBP β , and others, contribute to the inhibition of insulin gene expression and change in β -cell phenotype found in the diabetic state (4–7, 49, 50). It has been reported that PDX-1 expression and DNA binding activity are reduced in association with a decrease of insulin gene expression in various models of diabetes such as Zucker diabetic fatty rats (50) and rats with partial pancreatectomies (4, 5) and in β -cell-derived cell lines HIT and β TC6 after chronic exposure to high glucose concentrations (6, 7). It has also been reported that NeuroD expression is decreased in islets of rats following partial pancreatectomy (5). Because these two transcription factors are very important for insulin gene expression, it is likely that the decrease of these transcription factor expressions accounts for some of the suppression of insulin gene expression found in diabetes. Another potential contributor is the as yet uncloned transcription factor RIPE3b1, which was found to be down-regulated in HIT and β TC6 cells after exposure to high glucose concentrations (6, 7). In addition, C/EBP β expression is increased in islets from Zucker diabetic fatty rats and partial pancreatectomy rats (49). Because it has been reported that C/EBP β functions as a negative regulatory factor of insulin gene transcription (51), the increase of C/EBP β expression may also contribute to the suppression of insulin gene expression in diabetes.

Taken together, as we reported previously, c-Myc expression is enhanced in diabetic rats following partial pancreatectomy and in rats made hyperglycemic with glucose clamps, with the changes in c-myc gene expression being correlated with graded levels of hyperglycemia (5, 13). Additionally, c-myc gene transcription is directly regulated by high glucose *per se* (13, 52) and oxidative stress (53), which is provoked in islets under diabetic conditions (54) and is thought to be involved in the pathogenesis of β -cell dysfunction in diabetes (50, 55, 56). Finally, as shown in this study, increased expression of c-Myc in β -cells exerts a deleterious effect on the insulin gene transcription, probably at least in part by inhibiting NeuroD-mediated transcriptional activation. In summary, the present study indicates that c-Myc could account for some of the β -cell dysfunction of diabetes and provides a mechanism through which it inhibits insulin gene expression.

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REFERENCES

- Weir, G. C., Laybutt, D. R., Kaneto, H., Bonner-Weir, S., and Sharma, A. (2001) *Diabetes* **50** (suppl.), 154–159
- Bonner-Weir, S., Trent, D. F., and Weir, G. C. (1983) *J. Clin. Invest.* **71**, 1544–1553
- Tokuyama, Y., Sturis, J., DePaoli, A. M., Takeda, J., Stoffel, M., Tang, J., Sun, X., Polonsky, K. S., and Bell, G. I. (1995) *Diabetes* **44**, 1447–1457
- Zangen, D. H., Bonner-Weir, S., Lee, C. H., Latimer, J. B., Miller, C. P., Habener, J. F., and Weir, G. C. (1997) *Diabetes* **46**, 258–264
- Jonas, J.-C., Sharma, A., Hasenkamp, W., Iikova, H., Patane, G., Laybutt, R., Bonner-Weir, S., and Weir, G. C. (1999) *J. Biol. Chem.* **274**, 14112–14121
- Sharma, A., Olson, L. K., Robertson, R. P., and Stein, R. (1995) *Mol. Endocrinol.* **9**, 1127–1134
- Poitout, V., Olson, K., and Robertson, R. P. (1996) *J. Clin. Invest.* **97**, 1041–1046
- Evan, G. I., and Littlewood, T. D. (1993) *Curr. Opin. Genet. Dev.* **3**, 44–49
- Evan, G. I., and Littlewood, T. D. (1998) *Science* **281**, 1317–1322
- Dang, C. V. (1999) *Mol. Cell. Biol.* **19**, 1–11
- Laybutt, D. R., Weir, G. C., Kaneto, H., Lebet, J., Palmiter, R. D., Sharma, A., and Bonner-Weir, S., *Diabetes*, in press
- Pelengaris, S., Rudolph, B., and Littlewood, T. (2000) *Curr. Opin. Genet. Dev.* **10**, 100–105
- Jonas, J.-C., Laybutt, D. R., Steil, G. M., Trivedi, N., Pertusa, J. G., Casteelle, M. V. D., Weir, G. C., and Henquin, J.-C. (2001) *J. Biol. Chem.* **276**, 35375–35381
- Karlsson, O., Edlund, T., Moss, J. B., Rutter, W. J., and Walker, M. D. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 8819–8823
- Crowe, D. T., and Tsai, M.-J. (1989) *Mol. Cell. Biol.* **9**, 1784–1789
- Whelan, J., Cordle, S. R., Henderson, E., Weil, P. A., and Stein, R. (1990) *Mol. Cell. Biol.* **10**, 1564–1572
- German, M. S., Moss, L. G., Wang, J., and Rutter, W. J. (1992) *Mol. Cell. Biol.* **12**, 1777–1788
- German, M. S., and Wang, J. (1994) *Mol. Cell. Biol.* **14**, 4067–4075
- Robinson, G. L. W. G., Peshavaria, M., Henderson, E., Shieh, S.-Y., Tsai, M.-J., Teitelman, G., and Stein, R. (1994) *J. Biol. Chem.* **269**, 2452–2460
- Naya, F. J., Stelrecht, C. M. M., and Tsai, M.-J. (1995) *Genes Dev.* **9**, 1009–1019
- Ohlsson, H., Karlsson, K., and Edlund, T. (1993) *EMBO J.* **12**, 4251–4259
- Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S., and Montminy, M. R. (1993) *Mol. Endocrinol.* **7**, 1275–1283
- Miller, C. P., McGehee, R. E., and Habener, J. F. (1994) *EMBO J.* **13**, 1145–1156
- Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) *Nature* **37**, 606–609
- Ahlgren, U., Jonsson, J., and Edlund, H. (1996) *Development* **122**, 1409–1416
- Offield, M. F., Jetton, T. L., Labosky, P., Ray, M., Stein, S., Magnuson, M., Hogan, B. L. M., and Wright, C. V. E. (1996) *Development* **122**, 983–985
- Kaneto, H., Miyagawa, J., Kajimoto, Y., Yamamoto, K., Watada, H., Umayahara, Y., Hanafusa, T., Matsuzawa, Y., Yamasaki, Y., Higashiyama, S., and Taniguchi, N. (1997) *J. Biol. Chem.* **272**, 29137–29143
- Guz, Y., Montminy, M. R., Stein, R., Leonard, J., Gamer, L. W., Wright, C. V. E., and Teitelman, G. (1995) *Development* **121**, 11–18
- Bonner-Weir, S., Taneja, M., Weir, G. C., Tatarkiewicz, K., Song, K.-H., Sharma, A., and O'Neil, J. J. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 7999–8004
- Watada, H., Kajimoto, Y., Umayahara, Y., Matsuoaka, T., Kaneto, H., Fujitani, Y., Kamada, Y., Kawamori, R., and Yamasaki, Y. (1996) *Diabetes* **45**, 1478–1488
- Waeber, G., Thompson, N., Nicod, P., and Bonny, C. (1996) *Mol. Endocrinol.* **10**, 1327–1334
- Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., and Edlund, H. (1998) *Genes Dev.* **12**, 1763–1768
- Wang, H., Maechler, P., Ritz-Laser, B., Hagenfeldt, K. A., Ishihara, H., Philippe, J., and Wollheim, C. B. (2001) *J. Biol. Chem.* **276**, 25279–25286
- Malecki, M. T., Jhala, U. S., Antonellis, A., Fields, L., Doris, A., Orban, T., Saad, M., Warram, J. H., Montminy, M., and Krolewski, A. S. (1999) *Nat. Genet.* **23**, 323–328
- Stoffers, D. A., Ferrer, J., Clarke, W. L., and Habener, J. F. (1997) *Nat. Genet.* **17**, 138–139
- Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L., and Habener, J. F. (1997) *Nat. Genet.* **15**, 106–110
- Naya, F. J., Huang, H., Qiu, Y., Mutoh, H., DeMayo, F., Leiter, A. B., and Tsai, M.-J. (1997) *Genes Dev.* **11**, 2323–2334
- Dutta, S., Bonner-Weir, S., Montminy, M., and Wright, C. (1998) *Nature* **392**, 560
- He, T.-C., Zhou, S., DaCosta, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 2509–2514
- Sharma, A., and Stein, R. (1994) *Mol. Cell. Biol.* **14**, 871–879
- Peshavaria, M., Henderson, E., Sharma, A., Wright, C. V., and Stein, R. (1997) *Mol. Cell. Biol.* **17**, 3987–3996
- Sander, M., Griffen, S. C., Huang, J., and German, M. S. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 11572–11577
- Sharma, A., Moore, M., Marcora, E., Lee, J. E., Qiu, Y., Samaras, S., and Stein, R. (1999) *Mol. Cell. Biol.* **19**, 704–713
- Glick, E., Leshkowitz, D., and Walker, M. D. (2000) *J. Biol. Chem.* **275**, 2199–2204
- Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L., and Weintraub, H. (1990) *Cell* **61**, 49–59
- Inagaki, N., Maekawa, T., Sudo, T., Ishii, S., Seino, Y., and Imura, H. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 1045–1049
- Henderson, E., and Stein, R. (1994) *Mol. Cell. Biol.* **14**, 655–662
- Robinson, G. L. W. G., Henderson, E., Massari, M. E., Murre, C., and Stein, R. (1995) *Mol. Cell. Biol.* **15**, 1398–1404
- Seufert, J., Weir, G. C., and Habener, J. F. (1998) *J. Clin. Invest.* **101**, 2528–2539
- Tanaka, Y., Gleason, C. E., Tran, P. O. T., Harmon, J. S., and Robertson, R. P. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 10857–10862
- Lu, M., Seufert, J., and Habener, J. F. (1997) *J. Biol. Chem.* **272**, 28349–28359
- Kaneto, H., Suzuma, K., Sharma, A., Bonner-Weir, S., King, G. L., and Weir, G. C. (2002) *J. Biol. Chem.* **277**, 3680–3685
- Li, D. W., and Spector, A. (1997) *Mol. Cell. Biochem.* **173**, 59–69
- Ihara, Y., Toyokuni, S., Uchida, K., Odaka, H., Tanaka, T., Ikeda, H., Hiai, H., Seino, Y., and Yamada, Y. (1999) *Diabetes* **48**, 927–932
- Deleted in Proof
- Kaneto, H., Kajimoto, Y., Miyagawa, J., Matsuoaka, T., Fujitani, Y., Umayahara, Y., Hanafusa, T., Matsuzawa, Y., Yamasaki, Y., and Hori, M. (1999) *Diabetes* **48**, 2398–2406
- Kaneto, H., Xu, G., Song, K.-H., Suzuma, K., Bonner-Weir, S., Sharma, A., and Weir, G. C. (2001) *J. Biol. Chem.* **276**, 31099–31104

MECHANISMS OF SIGNAL TRANSDUCTION:

Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation

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