Oxidative Stress-mediated, Post-translational Loss of MafA Protein as a Contributing Mechanism to Loss of Insulin Gene Expression in Glucotoxic Beta Cells*

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Glucose toxicity in pancreatic islet beta cells causes loss of insulin gene expression, content, and secretion due to loss of binding of transcription factors, most notably PDX-1 and RIPE-3b1 activator, to the promoter region of the insulin gene. Recently, RIPE-3b1 activator was cloned and identified as the mammalian homologue of avian Mafα/Maf-L (MafA). This enabled us to carry out more extensive studies of the role of MafA in glucotoxicity than were hitherto possible. Northern analysis of glucotoxic HIT-T15 cells revealed normal amounts of MafA mRNA, but Western analysis demonstrated a 97 ± 1% reduction in MafA protein (p < 0.0001). The proteasome is a likely site for MafA degradation as lactacystin, an irreversible proteasome inhibitor, caused an accumulation of MafA protein. Antioxidants have previously been shown to prevent the adverse effects of glucose toxicity on beta cell function both in vivo and in vitro. In the current study, chronic culturing of HIT-T15 cells with the antioxidant N-acetylcysteine (NAC) prevented loss of MafA protein (late passage = 18.9 ± 10.4% of early passage, p < 0.001; late passage with NAC = 68.7 ± 19.7% of early passage, p = not significant) and loss of DNA binding (late passage = 63.7 ± 9% of early passage, p < 0.02; late passage with NAC = 116 ± 10% of early passage, p = not significant). Additionally, transient transfection of PDX-1 or MafA cDNA into glucotoxic cells increased PDX-1 and MafA protein levels and individually increased insulin promoter activity (untreated = 34%; PDX-1 = 70%; MafA = 78% percentage of activity of early passage cells), whereas the combined transfection of MafA and PDX-1 completely restored insulin promoter activity. This recovery of promoter activity following transient transfection had no effect on endogenous insulin mRNA. However, adenoviral infection of MafA and PDX-1 significantly increased endogenous insulin mRNA levels by 93% (121 ± 9 versus 233 ± 18 density light units; n = 5, p < 0.001). We conclude that the absence of MafA protein from beta cells via chronic oxidative stress contributes importantly to the loss of endogenous insulin gene expression as glucose toxicity develops.

After the onset of type 2 diabetes, chronic hyperglycemia causes glucotoxic changes in many tissues (1, 2). Glucose toxicity in the pancreatic islet beta cell secondarily leads to further defects in beta cell function, including decreases in insulin reporter activity, gene expression, content, and secretion (3). Antioxidants have been shown to prevent these adverse changes in experimental models (4, 5). We previously observed that loss of insulin gene expression is accompanied by decreased binding to the promoter region of two important transcription factors, PDX-1 (1) (STF-1, IDX-1, IPF-1) and RIPE-3b1 activator (6–8). PDX-1 is required for pancreatic development and is a key regulator of insulin gene expression. Mutations within the RIPE-3b1/C1 element of the insulin promoter markedly reduce glucose-responsive insulin gene expression (7). We also reported that loss of RIPE-3b1 binding precedes the loss of PDX-1 binding to the insulin promoter as glucotoxicity develops (8). The chronology of these losses is likely to be important in light of a recent report that RIPE-3b1/MafA directly activates PDX-1 transcription (9).

The work in this study features the use of HIT-T15 cells, a glucose-responsive beta cell line that has proven over the past decade to reproduce the molecular changes in gene expression that are caused by glucose toxicity in vivo in animal models. Since isolated islets cannot be chronically cultured to study the adverse effects of high glucose concentrations over many months, this cell line is a valuable surrogate. In our previous work, reconstitution of late passage glucotoxic HIT-T15 cells by transient transfection with PDX-1 partially restored insulin promoter activity (8). Since RIPE-3b1 had not yet been cloned, we were unable to extend our studies with this transcription factor but hypothesized that reconstitution of the cells with both PDX-1 and RIPE-3b1 activator would lead to greater recovery of insulin promoter activity. With the recent cloning and identification of RIPE-3b1 activator as MafA (10–12), we have been able to perform new studies to examine 1) whether levels of MafA mRNA and protein are decreased in glucotoxic beta cells; 2) the mechanism of MafA protein degradation; 3) whether the antioxidant, N-acetylcysteine, can prevent glucotoxicity-induced loss of MafA protein and binding to the insulin promoter; and 4) whether overexpression of MafA and PDX-1 together can restore insulin promoter activity and mRNA levels more fully than PDX-1 alone.

MATERIALS AND METHODS

Cell Culture—HIT-T15 cells were maintained in RPMI 1640 media containing 10% fetal bovine serum and 11.1 mM glucose as described previously (13). Cells were categorized as early passage (p71–75) or late passage (p123–128), with each passage occurring weekly. Chronic culturing of HIT-T15 cells with N-acetyl-L-cysteine (Sigma; 0.5, 1, or 5 mM) added to the media was begun at p70.

1 The abbreviations used are: PDX-1, pancreatic/duodenal homeobox-1; RIPE-3b1, rat insulin promoter element; Mafα, mammalian homologue of avian Mafα/Maf-L; LUC, luciferase; NAC, N-acetyl-L-cysteine; DLU, density light units; GFP, green fluorescent protein; pfu, plaque-forming units; CMV, cytomegalovirus; INS, insulin.

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**Western and Northern Blot Analyses**—Nuclear extracts were prepared by the method of Schreiber et al. (14) with buffers containing 1× phosphatase inhibitor mixture set II (Calbiochem), 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 ng/μl aprotinin, and 10 ng/μl leupeptin. Protein concentrations were determined by the BCA assay (Pierce). Nuclear extracts (10 μg) were separated on 10% polyacrylamide Ready gels (Bio-Rad) and electrotransferred onto Trans-blot nitrocellulose membranes (Bio-Rad). Membranes were immunoblotted with mouse MafA antisera (1:10,000) (Bethyl Laboratories) or mouse PDX-1 antisera (1:12,000) (gift from Dr. Chris Wright, Vanderbilt University) overnight at 4 °C in 5% nonfat dry milk in phosphate-buffered saline with 0.5% Tween. The membranes were washed and probed with anti-rabbit IgG (1:1,000) (Jackson ImmunoResearch Laboratories) for 1 h at room temperature. MafA and PDX-1 proteins were detected using the ECL Western blotting detection kit (Amersham Biosciences). Membranes were stripped and reprobed with anti-TFIID (N-12) (1:200) (Santa Cruz Biotechnology) to control for protein loading. Peptide neutralization was performed to ensure MafA antibody specificity in HIT-T15 cells (data not shown). Densitometry of autoradiograms was performed using OptiQuant image analysis software (Packard). Total RNA was extracted using the RNeasy mini kit (Qiagen). MafA mRNA levels were determined by Northern blot analysis using the NorthernMax system (Ambion) with slight modification to the manufacturer’s instructions. Total RNA was electrotransferred to a nylon membrane for 2 h in 1× Tris acetate/EDTA buffer. Prehybridization at 42 °C for 1 h with ULTRAhyb-Oligo (Ambion) was followed by a 16-h hybridization with a 32P-end-labeled mouse MafA cDNA probe (CACACCACCGAGGCCTCTGGGACACGCGGCGGCGCGGTCAAGCGGCGG)611. Blots were washed according to manufacturer’s instructions. Detection of mRNA levels was performed using the Cyclone storage phosphor system (Packard), and densitometry was performed using the OptiQuant image analysis software (Packard).

**Immunoprecipitation**—Early passage HIT-T15 cells were cultured in RPMI media containing 0.8 mM glucose for 5 days. The cells were then subcultured for 72 h in RPMI media containing either 11.1 or 0.8 mM glucose. Lactacystin (Sigma), at a final concentration of 10 μM, was added during the last 12 h of the subculture period to inhibit proteasome activity. Whole cell extracts were obtained by harvesting cells in lysis buffer (140 mM NaCl, 10 mM Tris, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 10% glycerol, 1% Nonidet P-40, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 ng/μl aprotinin, 10 ng/μl leupeptin). Total MafA protein levels were measured by Western blot analysis using 30 μg of protein and immunoblotted as described above. To determine the level of MafA ubiquitination, 500 μg of cellular lysate was incubated in 500 μl of phosphate-buffered saline. Immunocomplexes were formed by the addition of 2 μg of MafA antibody with gentle rocking overnight at 4 °C. These complexes were precipitated with 25 μl of protein G agarose (Upstate Biotechnology) for 2 h at 4 °C with gentle rocking. The immunocomplexes were washed three times with phosphate-buffered saline and subjected to immunoblot analysis on 10% polyacrylamide Ready gels (Bio-Rad). Membranes were immunoblotted with anti-ubiquitin (1:1000) (Calbiochem) overnight. Membranes were washed and probed with anti-mouse IgG (1:1000) (Jackson ImmunoResearch Laboratories) for 1 h at room temperature. Detection and densitometry were performed as above.

**Western and Northern Analyses for MafA**—Late passage HIT-T15 cells were subcultured for 72 h in 100-mm culture plates, 4.5 × 107 cells/plate. The cells were washed with RPMI media and infected with GFP, PDX-1, MafA, or a combination of PDX-1 and MafA adenoviruses (3 × 1012 pfu/plate) for 2 h. The wells were washed with RPMI media followed by a 24-h culture period. Nuclear extracts were prepared as described above. Electromobility shift assay was performed according to the method described by Olson et al. (6). Total RNA was extracted and analyzed by Northern blot analysis as described above. The membrane was prehybridized for 1 h followed by a 16-h hybridization at 42 °C with 32P-labeled Syrian hamster preproinsulin cDNA (15) and human β-cell cDNA (16). Two high stringency washes were performed at 55 °C for 30 min. Detection of protein binding and mRNA levels was performed using the Cyclone storage phosphor system (Packard), and densitometry was performed using the OptiQuant image analysis software (Packard).

**Statistics**—Statistical analysis was performed by unpaired Student’s t test or analysis of variance where appropriate with significance set at p < 0.05.

**RESULTS**

**Western and Northern Analyses for MafA**—MafA protein levels were measured in nuclear extracts from HIT-T15 cells (Fig. 1) using early passage (p71–75; lane 1) and late passage (p123–128; lane 2) cells chronically cultured in glucotoxic conditions (RPMI media containing 11.1 mM glucose). Late passage cells contained 4.0 ± 1.0% MafA protein of that found in early passage cells (p < 0.0001). MafA mRNA levels were not different between early and late passage cells (55.3 ± 4.0 vs. 52.5 ± 3.3).
density light units (DLU), MafA/18 S, respectively; Fig. 2). Similar results were obtained using a mouse MafA cDNA

Mechanism of Post-translational Regulation of MafA—To examine post-translational mechanisms of MafA degradation, early passage cells were cultured with or without lactacystin, an irreversible proteasome inhibitor under 0.8 and 11.1 mM glucose conditions. The accumulation of MafA protein was augmented by the addition of the proteasome inhibitor in cells cultured both in 0.8 mM glucose (3147.3 ± 380.3 versus 4812 ± 617.2 DLU; no lactacystin versus lactacystin; p < 0.03, n = 4) and in 11.1 mM glucose (4468.3 ± 659.7 versus 6712.8 ± 1281.4 DLU; p < 0.04, n = 4; Fig. 3A), suggesting that degradation occurs via the proteasome pathway. Immunoprecipitation of these cellular lysates with MafA antibody, followed by immunoblot analysis with anti-ubiquitin, indicated that MafA is ubiquitinated (Fig. 3B). Interestingly, PDX-1 was also brought down with the MafA immunoprecipitation assay, indicating that a physical interaction exists between PDX-1 and MafA. Furthermore, PDX-1 protein is also ubiquitinated (data not shown).

Preventive Effect of N-Acetylcyesteine (NAC) on the Loss of MafA Protein and MafA Binding to the Insulin Promoter in Cells Chronically Cultured in 11.1 mM Glucose—Chronic culturing of HIT-T15 cells with NAC starting at passage 70 prevented the loss of MafA protein in nuclear extracts of late passage cells (0.687 ± 0.20 DLU in 5 mM NAC-treated versus 0.189 ± 0.10 DLU in untreated cells, p < 0.02. n = 4–5; Fig. 4). The level of MafA protein in the 5 mM NAC-treated late passage cells did not differ significantly from the early passage cells. Although treatment with 5 mM NAC was necessary to have a significant effect on protein levels, a concentration of 0.5 mM NAC was sufficient to significantly improve the amount of protein binding to the C1 element of the insulin promoter (0.90 ± 0.12 versus 0.64 ± 0.05 DLU in the untreated late passage cells, p < 0.05, n = 4–6; Fig. 5). Higher concentrations of NAC (1 and 5 mM) further augmented the amount of MafA binding (1.28 ± 0.04 and 1.16 ± 0.1 DLU, respectively, p < 0.0005 versus untreated).

FIG. 3. Post-translational modification of MafA. Western blot analyses of early passage HIT-T15 cells grown in either 0.8 or 11.1 mM glucose with or without lactacystin (10 μM) were performed. A. lane 1, 0.8 mM; lane 2, 0.8 mM + lactacystin; lane 3, 11.1 mM glucose; lane 4, 11.1 mM + lactacystin probed with anti-MafA. B. MafA immunoprecipitated protein complexes from cells grown in 0.8 mM (lane 1), 0.8 mM + lactacystin (lane 2), 11.1 mM glucose (lane 3), and 11.1 mM + lactacystin (lane 4) probed with anti-ubiquitin. Representative blots from 3 experiments are shown.

Transient Transfection with PDX-1 and MafA cDNAs and Insulin Reporter Activity—Western blot analysis of HIT-T15 lysates revealed that the levels of PDX-1 and MafA proteins were increased 24 h following transfection with their respective cDNAs (Fig. 6) in both early and late passage cells. Insulin promoter activity was determined by measuring luciferase activity in HIT-T15 cells. In agreement with our previous data (8), late passage cells grown in 11.1 mM glucose had diminished promoter activity as compared with early passage cells. This decrease could be partially restored with PDX-1 (no PDX-1 = 34% of early passage; PDX-1 = 78% of early passage; Fig. 7A). Transfection with MafA cDNA also partially restored the level of promoter activity in glucotoxic cells (no MafA = 34% of early passage; MafA = 70% of early passage; Fig. 7B). Co-transfection with both MafA and PDX-1 cDNAs completely restored insulin promoter activity to the levels observed in early passage cells (Fig. 7C).

Adenoviral Overexpression of PDX-1 and MafA—Western blot analysis indicated that the levels of PDX-1 and MafA proteins in late passage cells increased following infection with their respective adenoviruses with the greatest level of expression at 24 h (Fig. 8A). Infection with the control GFP adenovirus did not affect the levels of PDX-1 or Maf A proteins as compared with uninfected cells (data not shown). The overexpressed proteins were functional as determined by their ability to bind to their respective sites on the insulin promoter (Fig. 8B). Twenty-four hours after infection, the PDX-1 adenovirus increased protein binding to the A3 site of the insulin promoter 4.3 ± 0.3-fold over the GFP-infected late passage cells (n = 3, p < 0.001). Infection with the MafA adenovirus increased protein binding to the C1 site of the insulin promoter 7.7 ± 0.8-fold over the GFP-infected cells (n = 3, p < 0.0001). Infection with the GFP control adenovirus had no effect on endogenous PDX-1 or MafA protein binding to their respective insulin promoter elements as compared with uninfected cells (data not shown).

Insulin mRNA levels were measured by Northern blot analysis 24 h after adenoviral infection (Fig. 9). Infection of early passage cells with MafA and PDX-1 adenoviruses had no effect on insulin mRNA (A). Late passage cells had significantly lower mRNA levels as compared with early passage cells (Fig. 9, B versus A, lane 1; 0.435 ± 0.05 DLU versus 4.98 ± 0.44, respectively, p < 0.001). Infection of late passage cells (Fig. 9, B and C) with the PDX-1 adenovirus increased insulin mRNA as compared with GFP-infected cells (174.2 ± 11.6 versus 120.6 ± 8.2 DLU, respectively, p < 0.009, n = 5). MafA plus PDX-1 overexpression further increased the level of insulin message to 232.8 ± 16.7 DLU (p < 0.0001 as compared with GFP infection, n = 5). MafA overexpression alone increased the level of insulin mRNA but not to a statistically significant level (152.7 ± 7.9.
DLU, \( p < 0.09, n = 5 \). Cells overexpressing PDX-1 and MafA plus PDX-1 had glucose-dependent increases in insulin mRNA and content. Cells infected with PDX-1 had insulin mRNA levels of 0.275 ± 0.010 and 0.301 ± 0.013 DLU (\( p < 0.05, n = 4 \)) when cultured in the presence of 0.8 and 11.1 mM glucose, respectively. Cells infected with MafA plus PDX-1 had insulin mRNA levels of 0.323 ± 0.028 and 0.386 ± 0.023 DLU (\( p < 0.02, n = 4 \)) when cultured in the presence of 0.8 and 11.1 mM glucose. The glucose-dependent increase in insulin mRNA caused by overexpression with MafA did not reach significance 0.262 ± 0.011 and 0.300 ± 0.044 DLU (\( p < 0.12, n = 4 \)). Insulin content was also significantly increased in cells overexpressing PDX-1 and MafA plus PDX-1 (1172.25 ± 8.95 and 970.33 ± 104.33 microunits/ml respectively, as compared with 451.67 ± 76.12 microunits/ml in GFP-infected cells; \( p < 0.01–0.001; all n = 3 \). MafA overexpression also resulted in an increase in insulin content (635.67 ± 36.25 microunits/ml), although not significantly (\( p < 0.09 \)). Static incubations of infected cells in 0.1 versus 0.8 mM glucose demonstrated increases of insulin secretion with the higher glucose concentration (data not shown), but the magnitudes of the increments were not different among the four infection conditions.

**DISCUSSION**

The recent identification of RIPE-3b1 as MafA (10–12) enabled us to perform the studies described in this report. We show that, as with PDX-1, MafA protein is virtually absent in glucotoxic HIT-T15 cells. However, in contrast to the decrease
in the level of PDX-1 mRNA, MafA mRNA levels remain normal in glucoxic cells, suggesting post-translational modification of the protein. In the current study, we show that MafA protein is ubiquitinated and that blocking the proteasomal pathway with lactacystin leads to an accumulation of MafA. The antioxidant, NAC, which prevents disappearance of PDX-1 in glucotoxic states (17), also prevented loss of MafA protein and its binding to the insulin promoter in these cells. Furthermore, transient transfection with either transcription factor improved defective insulin promoter activity, and transfection with both fully restored this activity. Combined adenoviral overexpression of PDX-1 and MafA partially restored endogenous insulin gene expression and insulin content in glucoxic HIT-T15 cells.

Maf proteins are a family of transcription factors that are key regulators in cellular differentiation. The oncprotein v-Maf (musculoaponeurotic fibrosarcoma) was the first identified member of the Maf family (18). These transcription factors all contain a conserved basic leucine zipper (bZIP) domain necessary for their dimerization and DNA binding properties. Three members are referred to as small Mafs: MafG, MafF, and MafK. The additional four large Maf members are c-Maf, MafB, neural retina leucine zipper, and MafA (L-Maf), which have a well conserved N-terminal transactivation domain. The MafA gene was originally isolated from avian neuroretina cells (19) and recently identified in pancreatic islets (10, 11, 20, 21). Within the islet, MafA is localized to the beta cell, whereas expression of MafB (12) and c-Maf (21) is limited to the alpha cell. Furthermore, MafA has been found to be required for the formation and development of mature islet beta cells (20). Regulation of the insulin gene by MafA (11, 12, 20, 22) occurs via binding to the RIPE-3b1/C1 element of the insulin promoter, which is similar to the binding sequence referred to as Maf-recognition element (MARE). Loss of this essential activator of insulin gene transcription greatly compromises insulin gene expression.

We have proposed that chronic oxidative stress is an important mechanism for glucoxic toxicity (17). Treatment of Zucker diabetic fatty rats (5) and db/db mice (4) with NAC protected against the progression of type 2 diabetes. Performance of molecular studies designed to examine mechanisms of glucoxic toxicity is not possible with isolated islets because they do not withstand chronic cultures over many months. Therefore, we have used HIT-T15

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**Fig. 6.** Transfection with MafA and PDX-1 cDNAs increases their respective protein levels. A representative Western blot of nuclear extracts from transfected HIT-T15 cells is shown. Lanes 1–4 from early passage cells (p71–75); lanes 5–8 from late passage cells (p123–128) grown in media containing 11.1 mM glucose. Lanes 1 and 5, transfected with CMV control; lanes 2 and 6, transfected with PDX-1; lanes 3 and 7, transfected with MafA; lanes 4 and 8, transfected with Pdx-1 and MafA. Blots were probed with anti-MafA (47 kDa) (upper panel) and anti-Pdx-1 (46 kDa) (lower panel).

**Fig. 7.** MafA and PDX-1 co-transfection completely restores insulin promoter activity. Insulin promoter activity was measured by firefly luciferase activity corrected for transfection efficiency with pRL-CMV measured by Renilla luciferase activity in early passage (p71–75) and late passage (p123–128) cells chronically cultured in 11.1 mM glucose. A, transfection with CMV control (0 ng) or PDX-1 cDNA (12.5 or 25 ng); B, transfection with CMV control (0 ng) or MafA cDNA (12.5 or 25 ng); and panel C, transfection with CMV control (0 ng) or both MafA and PDX-1 cDNAs (12.5 or 25 ng of each) (n = 7–10, *p < 0.05 late versus early).

**Fig. 8.** Infection with MafA and PDX-1 adenoviruses increases their protein levels and protein binding to their respective elements on the insulin promoter. A representative Western blot of whole cell extracts from late passage HIT-T15 cells is shown. A, upper panel, PDX-1-infected cells immunoblotted with anti-PDX-1 (46 kDa); lower panel, MafA-infected cells immunoblotted with anti-MafA (47 kDa). The concentration of each adenovirus ranged from 0 to 5 × 10^9 pfu/well. B, a representative electromobility shift assay of nuclear extracts from late passage HIT-T15 cells infected with adenovirus at 3 × 10^10 pfu/plate. Upper panel, protein binding to the A3 element of the insulin promoter. Lane 1, GFP-infected cells; lane 2, PDX-1-infected cells; lower panel, protein binding to the C1 element of the insulin promoter. Lane 1, GFP-infected cells; lane 2, MafA-infected cells.
cells cultured under high glucose concentrations for periods up to 1 year. In all respects, this beta cell line reproduces the losses in PDX-1, MafA, insulin mRNA, insulin content, and glucose-induced insulin secretion that have been found in animal models. For example, NAC prevented the loss of insulin message and promoter activity and significantly improved glucose-stimulated insulin secretion in late passage HIT-T15 cells chronically cultured with media containing 11.1 mM glucose (5). Loss of PDX-1 protein and decreased binding to the insulin promoter could be prevented by culturing cells with NAC (17). In the current study, we show that NAC also has beneficial effects on MafA by preserving protein expression and binding to the insulin promoter. This finding is consistent with the observation that MafA binding to the human insulin promoter can be abolished with oxidizing agents (23) and further supports the role of oxidative stress as a mechanism for glucose toxicity.

Complete recovery of insulin promoter activity with the combined transient overexpression of PDX-1 and MafA supports our hypothesis that both factors were required for normal activity. However, the incomplete recovery of endogenous insulin message in the adenoviral infection studies suggests that a greater duration of exposure to overexpressed proteins may be needed for full restoration of insulin mRNA and/or that other factors involved in insulin transcription may also be diminished in glucotoxic cells. Prior to recognizing the RIPE-3b1 activator as MafA, cooperativity between proteins binding to the RIPE3a and RIPE3b elements had been observed (24, 25).

Homo- and heterodimers formed between Mafs and other basic leucine zipper factors (for a review, see Ref. 26) can lead to positive or negative transcriptional regulation depending on the combination. The small Maf proteins, as homo- or heterodimers, can bind to Maf-recognition elements and act as transcriptional repressors (27). Since oxidative stress has been shown to induce the levels of MafG (28), it is possible that these small Mafs may also have an effect on insulin gene regulation. Alternatively, proteins interfering with positive regulators may facilitate the down-regulation of the insulin gene (29). Beta cells exposed to high glucose levels have increased gene expression of Id-1 and Id-3 (30) and up-regulated CCAAT/enhancer-binding protein/β levels (31), all of which can negatively affect insulin gene expression (31, 32). This inhibition is likely due to their interference with E47 homodimer or heterodimer formation (29).

In conclusion, the recent identification of RIPE-3b1 as MafA has allowed us to now demonstrate that protein levels of this insulin gene transcription factor are virtually absent in glucotoxic beta cells. In contrast to the transcription defect leading to PDX-1 protein loss, this work indicates that the defect leading to MafA protein loss is post-translational. MafA is ubiquitinated, and blocking the proteasomal pathway with lactacystin resulted in an increase in MafA protein levels. Combined transient transfection of PDX-1 and MafA completely normalizes insulin promoter activity, and combined adenoviral overexpression of PDX-1 and MafA increases endogenous insulin gene expression.
expression in glucotoxic HIT-T15 cells. That NAC prevents the loss of both MafA and PDX-1 proteins and their binding to the insulin promoter in glucotoxic HIT-T15 cells supports our hypothesis that chronic oxidative stress is a central mechanism for glucotoxicity and beta cell dysfunction.

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