The Islet β Cell-enriched MafA Activator Is a Key Regulator of Insulin Gene Transcription*

Received for publication, August 18, 2004, and in revised form, December 22, 2004
Published, JBC Papers in Press, January 20, 2005, DOI 10.1074/jbc.M409475200

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The islet-enriched MafA, PDX-1, and BETA2 activators contribute to both β cell-specific and glucose-responsive insulin gene transcription. To investigate how these factors impart activation, their combined impact upon insulin enhancer-driven expression was first examined in non-β cell line transfection assays. Individual expression of PDX-1 and BETA2 led to little or no activation, whereas MafA alone did so modestly. MafA together with PDX-1 or BETA2 produced synergistic activation, with even higher insulin promoter activity found when all three proteins were present. Stimulation was attenuated upon compromising either MafA transactivation or DNA-binding activity. MafA interacted with endogenous PDX-1 and BETA2 in communoprecipitation and in vitro GST pull-down assays, suggesting that regulation involved direct binding. Dominant-negative acting and small interfering RNAs of MafA also profoundly reduced insulin promoter activity in β cell lines. In addition, MafA was induced in parallel with insulin mRNA expression in glucose-stimulated rat islets. Insulin mRNA levels were also elevated in rat islets by adeno- viral-mediated expression of MafA. Collectively, these results suggest that MafA plays a key role in coordinating and controlling the level of insulin gene transcription in islet β cells.

Insulin is selectively expressed in the pancreatic β cells of the islet of Langerhans. Restricted expression is due to a unique combination of factors that stimulate through conserved enhancer region sequences located approximately between nucleotides ~340 and ~90 relative to the transcription start site (1–4). Detailed analysis has revealed that activation is primarily controlled by PAX6, PDX-1, and BETA2 binding to the C2 (~317 to ~311 bp), A3 (~201 to ~196 bp), C1 (~126 to ~101 bp), and E1 (~100 to ~91 bp) elements, respectively (5, 6). These distinct factors are enriched in islet cells, with BETA2 (7, 8) and PAX6 (9) present in all islet cell types, PDX-1 in β and a subset of δ cells (10), and only the recently isolated MafA protein exclusively in β cells (11–13). Although MafA appears to be the major regulator of C1-mediated activation (11–13), the closely related MafB protein is found in a fraction of islet β cells in vivo and is capable of activating insulin transcription in vitro (13). PDX-1, MafA, and BETA2 also control glucose-regulated transcription of the insulin gene, the principal metabolic regulator of β cell function (8, 14–17).

Gene ablation experiments performed in mice on pax6, pdx-1, and BETA2 have established a critical function for each in pancreatic development. Thus, PDX-1 is essential for the growth of the endocrine and exocrine compartments, with pancreatic development arresting at the early post-bud stage in homozygous pdx-1 mutant mice, resulting in pancreatic agenesis (18, 19). In contrast, the loss of PAX6 and BETA2 affects only islet cell development, with the number of α cells dramatically reduced in pax6 gene knock-out mice (9), and a severe, but general loss in total islet cell number in the absence of BETA2 (7, 8). Human heterozygous carriers of dysfunctional mutations in PAX6 (20), BETA2 (21), and PDX-1 (22) also contribute to the development of type 2 diabetes, presumably due to direct effects on the transcription of genes associated with β cell identity (i.e. β-glucokinase, islet amyloid polypeptide, glucose transporter type 2, as well as insulin (23–28)). Unfortunately, the role MafA plays in β cell development has not been determined. However, a significant part is strongly suggested by the exclusive presence of MafA in developing islet insulin producing cells and adult islet β cells (13, 29), its importance in pdx-1 (30) and insulin gene transcription (11–13, 29), and the general association of proteins in the large Maf family with developmental processes (i.e. MafB (31), c-Maf (32, 33), and NRL (34)).

Islet-enriched activator binding promotes the assembly of the insulin transcription complex. This process is mediated by interactions between activators themselves and by contact with the RNA polymerase II transcriptional apparatus, or indirectly through bridging coactivators. Thus, PDX-1 and BETA2 binding to the p300 coactivator or its paralogue p300/cAMP response element binding protein-binding protein (CBP)1 provides a docking and recruitment interface with the general transcriptional machinery (27, 35, 36). In this study, MafA was found to functionally interact with PDX-1 and BETA2 to promote synergistic activation of insulin enhancer-driven reporter

1 The abbreviations used are: CBP, p300/cAMP response element binding protein-binding protein; LUC, luciferase; CMV, cytomegalovirus; aa, amino acid(s); GST, glutathione S-transferase; AdV, adenovirus; siRNA, small interference RNA; E, embryonic day.

Vol. 280, No. 12, Issue of March 25, pp. 11887–11894, 2005
Printed in U.S.A.
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activity in non-β cells. MafA was also shown to play a direct and principal role in insulin gene activation in β cell lines, although p300/CBP was not involved in this response. In addition, insulin mRNA levels were found to increase in conjunction with MafA in rat islets. Our results suggest that MafA-mediated signaling is important for high level expression of the insulin gene in β cells.

MATERIALS AND METHODS

DNA Constructs—The ~238 insulin-firefly luciferase (LUC) expression plasmids contain wild-type rat insulin II gene sequences from bp ~238 to +2 and PDX-1 (A3 and A1 (37)), BET2A (E1 (38)), or MafA (C1 (29)) binding mutations. The construction of the cytomegaloivirus (CMV) enhancer-driven MafA (15), PDX-1 (39), and BET2A (36) expression vectors was described previously. MafA135–359 was made by PCR from wild type mouse MafA and subcloned into the polylinker of the CMV-driven expression vector, pEDNA3.1/Zeo (+) (Invitrogen). The arginine to alanine mutant at amino acid 265 in MafA was constructed in the CMV:MafA vector using the QuikChange® site-directed mutagenesis kit (top: 5′-CCGACTGAAACAGAAGGCGGACGCTCAAG-3′; the mutated nucleotides are underlined (Stratagene). GST:MafA chimeras of full-length, amino acids (aa) 1–233, and aa 233–359 of mouse MafA were generated by in-frame fusion with the glutathione S-transferase (GST) coding sequences in the pGEM-KG bacterial expression vector (Amersham Biosciences). The Gal4:MafA chimeras containing sequences from aa 1–359 (i.e. full-length) or aa 1–233 were constructed by subcloning PCR-amplified mouse MafA fragments into pG424 (40) to create in-frame Gal4 DNA binding domain fusion protein constructs. The proteins of the GAL4:BET2A constructs containing the hamster full-length (1-355) or aa 156–355 of BET2A were described previously (36). The CMV-driven wild type and p300 d10 mutant (deletion of aa 1680–1811) have also been described (41). The adenovirus type 5 E1A expression plasmid encodes for the wild type 243-aa protein. The adenovirus-5 expressing MafA under the control of the CMV enhancer/promoter (Adv-CMV) was generated using the mammalian expression vector, pcDNA3.1/Zeo(+) (Invitrogen). The arginine to alanine mutation at amino acid 265 in MafA was constructed in the CMV:MafA 1–359 expression vector, pcDNA3.1/Zeo(+) (Invitrogen). The proteins were immunoprecipitated with protein A-Sepharose beads (Sigma), washed three times with radioimmuno precipitation assay buffer, subjected to SDS-polyacrylamide gel electrophoresis, and then electrotransferred onto Immobilon polyvinylidene difluoride membrane (Millipore, Bedford, MA). The blot was incubated for 1 h at 4 °C in blocking buffer (10 mg Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20, and 5% nonfat dry milk) and then at 4 °C overnight with either PDX-1, BET2A (Santa Cruz Biotechnology Inc.), MafA (Bethyl Laboratories, TX), and TATA-binding protein (Santa Cruz Biotechnology Inc.) antibody. The PDX-1 polyclonal antiserum was developed to N-terminal region (aa 1–75) epitopes (39). In the MafA siRNA experiment in 293 cells, lysates from CMV:MafA (100 ng)- and MafA siRNA-pSUPER (1 µg)-transfected cells were blotted and analyzed using anti-MafA antisera. Antibody detection was performed using enhanced chemiluminescence ( Pierce Biotechnology) after incubation with a horseradish peroxidase-conjugated secondary antibody. The transcriptional activity of MafA was measured using the luciferase reporter assay. The CMV:MafA 1–233 and CMV:MafA 233–359 fusion proteins were prepared as described previously (Amersham Biosciences). Translation reactions were performed using the TNT in vitro translation kit (Promega, Madison, WI) with CMV-PDX-1, CMV:BET2A, and l-[35S]methionine. Labeled proteins were incubated with GST:MafA coupled to glutathione-Sepharose beads (Amersham Biosciences) for 1 h at 4 °C in blocking buffer (10 mg Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20, and 5% nonfat dry milk). GST-mid ست P-40, 2 mM EDTA, 10 mM MgCl2, 20 µM ZnCl2). The beads were washed three times with binding buffer, and the bound protein complexes were eluted with 1× gel loading buffer (50 mM Tris-HCl (pH 6.8), 100 mM dithiothreitol, 2% SDS, 0.1% bromphenol blue, 10% glycerol), resolved by SDS-10% polyacrylamide gel electrophoresis, and visualized by fluorography.

Electrophoretic Mobility Shift Assay—Batches of 350–500 islets each were washed in phosphate-buffered saline, resuspended in 400 µl of cold hypotonic buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM MgCl2, 0.5 mM dithiothreitol, 0.1 mM EDTA, 0.01 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 µg/ml aprotinin, 10 µg/ml leupeptin, 1 µg/ml pepstatin), and allowed to swell on ice for 15 min before adding 25 µl of 10% (w/v) Nonidet P-40. After vortexing vigorously, the nuclei were pelleted by centrifugation at 14,000 rpm (4 °C). The nuclei were resuspended in 50 µl of high salt buffer (20 mM HEPES (pH 7.9), 0.4 mM NaCl, 0.2 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 0.5 µg/ml aprotinin, 10 µg/ml leupeptin, and 1 µg/ml pepstatin) (17). Gel shift binding reactions (20 µl) were conducted with 5–10 µl of nuclear extract protein and radiolabeled probe at room temperature for 30 min in binding buffer (5× binding buffer, 1× EMSA buffer, 1× EDTA) (Promega, Madison, WI). Batches of 350–500 islets (13°C) were sonicated in TRIzol reagent (Invitrogen) and treated with the MessageClean kit (Promega and (μ-32P)dCTP (PerkinElmer Life Sciences). The identities of the MafA- and BET2A-containing complexes were determined by wild type element competition as well as super-shift analyses with α-MafA antisera. The binding complexes were resolved by electrophoretic separation on a 4.5% non-denaturing polyacrylamide gel and visualized by autoradiography.

Measurement of Preproinsulin mRNA Levels—Duplicate batches of 100 islets each were infected overnight with 10× plaque-forming units/ml CMV-driven MafA (AdV-CMV-MafA), the firefly luciferase (Adv-Luc (45)) control adenovirus in RPMI 1640 containing 10% fetal bovine serum and 11.1 mM glucose. Islets were then transferred to fresh RPMI 1640 containing 10% fetal bovine serum and 16.7 mM glucose, and incubated at 37 °C for 72 h. Total cellular RNA was isolated using the TRIzol reagent (Invitrogen) and treated with the MessageClean kit to remove DNA (Gene Hunter Corp., Nashville, TN). The ribonuclease protection assay (RPA) was performed with a 360-bp sequence of the rat II preproinsulin gene and a conserved 245-bp sequence of the mouse β-actin gene using the Direct Protect Lysate RPA kit (Ambion, Austin, TX) (42), with the TaqMan-based reverse transcription-PCR performed using oligonucleotides to the rat preproinsulin II and β-actin coding sequences described previously (45). This analysis was conducted with several independently transfected islet preparations.

Statistical Analysis—The significance of the experimental findings

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RESULTS

MafA Cooperates with PDX-1 and BETA2 to Induce Insulin-driven Transcription—Preventing PDX-1, BETA2, or MafA binding drastically reduces insulin enhancer-driven expression in β cells, suggesting that these factors act in a synergistic fashion to mediate transcription. Although the exact mechanisms involved in control are unclear, experiments performed in non-β cells imply that PDX-1 and BETA2 act together to cooperatively activate expression, a process augmented by the non-DNA binding p300 coactivator (27, 35, 36). Interestingly, MafA was recently shown to be the only islet-enriched transcription factor that is first expressed in the insulin-producing progenitors that eventually populate the islet (29). In contrast, PDX-1 and BETA2 are synthesized earlier and in a broader range of islet-hormone-producing cell types (8, 10, 19, 46). MafA is also capable of independently inducing endogenous insulin gene expression in an islet α cell line (αTC-6 (29)). These data imply that MafA serves a unique role in mediating insulin gene expression.

To test if MafA functionally interacts with other islet-enriched insulin activators, PDX-1 and BETA2 expression constructs were cotransfected with MafA and insulin enhancer/promoter-driven −238 LUC reporter constructs. These experiments were conducted in HeLa cells, a non-pancreatic cell line that does not produce insulin or islet-enriched transcription factors. As expected, wild type −238 LUC activity was not influenced by either PDX-1 or BETA2 alone (35), with MafA able to independently stimulate −20-fold (Fig. 1A) (13). Transactivation was stimulated in a synergistic manner when MafA was combined with either PDX-1 (Fig. 1B) or BETA2 (Fig. 1C). Additional enhancement of insulin-driven activity was also observed in the presence of all three factors (Fig. 1D). In general, PDX-1-, BETA2-, and MafA-mediated stimulation was dependent upon activator binding, because cooperativity with MafA was essentially lost in the PDX-1 (Fig. 1B, compare −238 WT responsiveness to the A3 and A1 mutants) and BETA2 (Fig. 1C) binding site mutants, whereas activation by MafA was greatly reduced in the C1 mutant (Fig. 1D). Cooperativity was also attenuated in MafA mutants deficient in activation (i.e. MafA 135–359) or DNA-binding domain (MafA R265A) function (Fig. 1E).

MafA, PDX-1, and BETA2 had the same influence on insulin-driven expression in non-islet baby hamster kidney and NIH 3T3 cell lines (data not shown). Together, these results imply that binding of MafA to the insulin enhancer profoundly impacts upon the transcriptional potentiation properties of other key activators, like PDX-1 and BETA2.

MafB Can Act with PDX-1 and BETA2 to Stimulate Insulin Gene Expression—MafB appears to be the only other member of the large Maf family normally expressed in islet cells (13). Unlike MafA, the MafB protein is predominately expressed in adult islet α cells (Ref. 13 and data not shown). However, MafB is also detected with MafA in the insulin-producing cells.
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Fig. 2. MafB potentiates PDX-1 and BETA2-mediated transactivation of −238 WT. MafA activation of −238 WT LUC was compared with MafB in the presence of the listed effectors in HeLa cells. The normalized -fold activation ± S.E. was calculated as described in the legend to Fig. 1, *p < 0.05 versus MafA or MafB alone.

formed during the second and predominant phase of β cell formation, as well as glucagon-producing α cells during pancreas organogenesis. Transfection studies performed with MafB, PDX-1, BETA2, and −238 WT LUC in HeLa cells demonstrated that this closely related large Maf protein also acts effectively with PDX-1 and BETA2 to stimulate insulin gene expression (Fig. 2). The variation in -fold activation between MafA and MafB reflects their different N-terminal activation domain potentials (MafB > MafA (13, 47)) and not the amount of large Maf activator produced within the experiment (data not shown). These data imply that both MafA and MafB can influence insulin transcription in islet β cell progenitors. Because such cells are rare and unavailable for in vitro analysis, the studies below principally focused on determining the significance of MafA in insulin gene expression in islet β cell lines, an islet β cell model wherein only MafA is found (13).

MafA Binds Directly to PDX-1 and BETA2—Immunoprecipitation assays were performed in βTC-3 cell extracts with MafA antisera. Western blotting revealed that PDX-1 and BETA2 were coprecipitated with MafA (Fig. 3A). These complexes appear to be specific, because rabbit IgG did not precipitate either PDX-1 or BETA2. Interactions between MafA, PDX-1, and BETA2 were also detected in β cell precipitation assays conducted with PDX-1 and BETA2 antisera.

To test if MafA directly interacted with PDX-1 and BETA2, the binding properties of these factors to GST:MafA affinity columns was analyzed under conditions in which the full-length, N-terminal activation (aa 1–233), and C-terminal DNA-binding/dimerization (aa 233–359) regions of MafA served as ligands. Both PDX-1 and BETA2 bound effectively to the full-length and C-terminal region columns, whereas less binding was detected in the eluates from the GST or GST:MafA 1–233 column (Fig. 3B). Together, these results demonstrated that MafA can directly bind to BETA2 and PDX-1 in vivo.

MafA Is Important for Insulin Expression in β Cell Lines—The observation that MafA functionally and physically interacts with PDX-1 and BETA2 implies a pivotal role in promoting insulin transcription. If so, then reducing the functional levels of the activator should profoundly influence insulin gene expression in β cells. To test this proposal, MafA activity in the mouse βTC-3 and MIN6 β cell lines was suppressed by expression of either a dominant-negative-acting form of MafA (MafA135–359) or siRNAs to MafA. The influence of these MafA effectors on −238 WT LUC activity was compared with a MafA site-debilitating mutant within this insulin-driven construct (i.e. C1 mutant).

MafA135–359, which lacks the N-terminal activation domain of this basic leucine zipper containing DNA binding factor, markedly reduced −238 WT activity (Fig. 4A). Wild type MafA also slightly inhibited insulin-driven activity, presumably due to a dominant-negative effect produced by incomplete phosphorylation in this highly modified family of proteins (13, 17, 48). In addition, insulin-driven activity was decreased in MIN6 cells by siRNAs targeted to nucleotides 64–84 or 82–102 of the mouse MafA mRNA (Fig. 4B), which were the siRNAs that most effectively blocked MafA synthesis in transfection assays performed in non-islet 293 cells (Fig. 4C). In contrast, these siRNAs had little or no effect on the ubiquitously active simian virus-40 enhancer, nor did the siRNAs to MafA that only modestly reduced protein levels in 293 cells influence insulin-driven activity (i.e. 955–975 and 1054–1074 in Fig. 4, B and C). Importantly, the level of repression by either dominant-negative-acting MafA or the siRNAs was similar to the dysfunctional MafA element binding site mutant (Fig. 4), consistent with MafA serving a central role in insulin activation.

Unlike BETA2 and PDX-1, the p300 Coactivator Does Not Appear to Mediate MafA Stimulation—The non-DNA binding p300 coactivator and its parologue CBP is involved in transducing signals between PDX-1, BETA2, and the basal RNA polymerase II apparatus (27, 35, 36). In addition to this bridging function, BETA2 is uniquely modified by the intrinsic acetyltransferase activity of p300/CBP and its associated factor, p300-associated factor resulting in enhanced DNA binding and transcriptional potency (49). To examine if MafA functionally interacts with p300, HeLa cells were cotransfected with expression plasmids producing p300 and either full-length or the N-terminal activation region of MafA fused in-frame to the DNA-binding domain of the Saccharomyces cerevisiae GAL4 transcription factor. The effect of p300 on Gal4:MafA activation was compared with Gal4:BETA2 chimeras containing the coactivator binding domain (i.e. Gal4:BETA2 and Gal4:BETA2 156–355 (36)). Gal4:MafA activity was not affected by p300, whereas the positive Gal4:BETA2 control was stimulated (Fig. 5). Furthermore, MafA-mediated activation was also not impacted by p300 d110, a mutant coactivator that functions more effectively with BETA2 (36) and PDX-1 (35).

To more broadly define if MafA transactivation of insulin was influenced by p300, HeLa cells were cotransfected with −238 WT and MafA in the presence or absence of this coactivator. Activation by MafA was unaffected by p300, although the coactivator enhanced insulin enhancer-driven activity in the presence of MafA, PDX-1, and BETA2 (Fig. 6). However, stimulation by these effectors was essentially reduced to the level of MafA alone by adenovirus E1A (Fig. 6), a factor that inhibits protein-protein-mediated communication between p300/CBP and BETA2 (36) or PDX-1 (35). Collectively, the data strongly suggests that the p300/CBP coactivator does not directly influence MafA activation of the insulin gene in β cells.

Insulin Gene Expression Appears to Be Induced in Response to MafA in Islets—The most important regulator of insulin expression in islet β cells is glucose, which stimulates insulin gene transcription, protein synthesis, and secretion. Glucose-induced transcription is dependent upon MafA in β cell lines, a process mediated by increased DNA-binding factor levels (17).
To determine if the MafA in islet cells also regulated glucose-stimulated insulin mRNA expression, isolated rat islets were cultured under simulating (16.7 mM) and non-simulating (2.8 mM) glucose concentrations for 24 h. Endogenous insulin mRNA levels were induced 2.5-fold under these conditions, whereas MafA DNA binding and protein levels were increased even more profoundly (Fig. 7). In contrast, neither the levels of the insulin E1 element activator, which contains the islet-enriched BETA2 protein (16), nor the ubiquitously distributed TATA-binding protein were affected (Fig. 7, A and B).

The results in islets (Fig. 7) and H9252 cell lines (29) indicate that insulin mRNA expression increases in parallel with MafA (Fig. 5D). To examine this association in further detail, rat islets were infected with an adenovirus expressing MafA (AdV-MafA) or luciferase (AdV-LUC) and cultured for 72 h in the presence of 16.7 mM glucose. Insulin mRNA levels were increased selectively by 2-fold by AdV-MafA infection relative to H9252-actin control levels (Fig. 8). Collectively, the islet data are not only consistent with MafA being a principal mediator of insulin gene transcription in β cell in vivo but also imply that this factor may be limiting to transcription in islets.

**DISCUSSION**

Expression of the mammalian insulin gene is restricted to pancreatic β cells. Release of this hormone from the β cell promotes the storage of metabolic fuels and is tightly regulated by nutritional conditions in vivo, with disturbances in production and release contributing to hyperglycemia in various types of diabetes. The mechanisms involved in driving β cell-type-specific transcription appear to principally involve the cis-acting elements located within the proximal enhancer region. The activator factors binding to this region are thought to result in the recruitment of the general transcription apparatus to the nearby promoter, providing transcriptional synergy through interactions between activators and components of the basal transcription apparatus. In the present study, MafA was shown to act in conjunction with other known insulin enhancer regulatory factors (BETA2 and PDX-1) to promote synergistic transcription of the insulin gene in non-β cell lines. Limiting MafA activity in β cell lines was also found to compromise insulin transcription, whereas overexpression elevated steady-state insulin mRNA levels in islets. Together, these results suggest that MafA likely has a profound influence on transcription of the insulin gene in vivo.

The first molecular sign of pancreatic development is the restricted expression of PDX-1, which occurs prior to insulin transcription at embryonic day E8.5 (19, 46). PAX6 (9, 50) and BETA2 (8) are detected around E10, preceding the production of the first insulin-positive cells around E10.5. However, the few insulin-positive cells found at this time lack specific products required for mature islet β cell function and are not believed to populate the islet (51, 52). Fully differentiated β cells first appear around E13 at the start of a massive wave of β cell

**FIG. 3.** MafA binds to PDX-1 and BETA2 in vivo. A, immunoprecipitation (IP) analysis was performed with βTC-3 extracts and anti-MafA antisera or rabbit IgG (rIgG); the precipitates were Western-blotted (WB) with (left, top) anti-PDX-1 or (right, top) anti-BETA2 antibody. We also perform the IP with either anti-PDX-1 (left, bottom) or anti-BETA2 antibody (right, bottom) and WB with MafA antisera. The input illustrates the levels of PDX-1, BETA2 or MafA in 2% of the cell extract. B, radiolabeled (top) PDX-1 or BETA2 was then eluted, separated by 10% SDS-polyacrylamide gel electrophoresis, and detected by autoradiography. The input lane represents the volume fraction used in the binding assay. Bottom, the asterisk denotes the location of the GST:MafA chimera in a Coomassie Blue-stained 10% SDS-polyacrylamide gel.
differentiation termed the “secondary transition” (53). Because MafA is first detected in this insulin-producing cell population, we sought to determine if this factor cooperated with other insulin gene enhancer factors to stimulate transcription.

These studies focused on interactions with BETA2 and PDX-1, which have been shown to act together to mediate insulin activation (35, 54). Here we show that in transfected non-H9252 cells, MafA led to significantly higher insulin-driven activity when combined with BETA2 and PDX-1 (Fig. 1). The removal of MafA activity from H9252 cell lines also clearly supported the importance of this factor in insulin gene expression (Fig. 4). Thus, insulin reporter gene activity was reduced to a level approaching a dysfunctional MafA binding site mutant upon coexpression of the wild type insulin reporter with either a dominant negative-acting MafA (i.e. MafA135–359) or siRNAs targeted to nucleotides 64–84 and 82–102 siRNAs to MafA were effective at reducing MafA protein levels. The endogenous β-actin level was used to control for loading.

FIG. 4. Reducing MafA activity levels causes a dramatic loss in insulin-driven activity in β cells. −238 WT LUC (0.25 μg) was transfected into βTC-3 and/or MIN6 cells with either MafA135–359 (1 μg) (A) or MafA siRNA expression constructs (64–84, 82–102, 955–975, and 1054–1074) (1 μg) (B). The impact of these conditions on MafA-mediated activation was compared with a dysfunctional MafA binding site mutant in −238 LUC (−238 C1 mt). The normalized relative activity ± S.E. is the ratio of −238 LUC activity plus effector to the wild type reporter gene alone. *, P < 0.05 versus −238 WT LUC alone. C, Western blot analysis of 293 cells transfected with MafA (0.1 μg) and MafA siRNA (1 μg) expression constructs demonstrated that the 64–84 and 82–102 siRNAs to MafA were effective at reducing MafA protein levels. The endogenous β-actin level was used to control for loading.

under these conditions is consistent with a defining role in controlling insulin transcription unit activity.

Strikingly, MafA is the only islet-enriched transcription factor expressed exclusively in islet β cells or in such a restricted fashion during islet cell development (29). Collectively, these observations indicate that MafA is critical for the assembly and function of the insulin gene transcription complex. Although cooperative interactions with this factor likely lead to the high level of specificity in insulin gene activation and to a high level of transcriptional synergy in islet β cells, MafA is not absolutely essential for expression. This conclusion is based upon the presence of insulin positive cells during the early stages of pancreatic development, prior to MafA expression (29). Furthermore, MafA is also not found in the few insulin-positive cells present in Nkx6.1 transcription factor null mice (29), which result in a profound and specific reduction in neogenesis of secondary transition β-cells (55). These results led to the proposal that MafA acts downstream of Nkx6.1 during a late stage of β cell formation. As a consequence, islet insulin expression will likely be severely compromised in MafA knock-out
mice, although MafB may provide some level of compensation. Low level insulin gene expression is observed in insulin transcription factor knockdown mice (PAX6 (9), PDX-1 (19), and BET2A (8)), demonstrating that interactions between a subset of these activator proteins provides some level of transcriptional activation.

The insulin activators not only function to induce localization of the basal transcriptional machinery to the promoter, but also by recruiting chromatin-modifying complexes. Thus, chromatin remodeling and covalent histone modifications are important for the function of transcriptional activators. For example, the acetylation of core histones by p300/CBP and P/CAF, which are involved in MafA-mediated activation (Fig. 5). However, MafA may be involved in recruiting a distinct chromatin-modifying activity such as SWI/SNF, which uses the energy of ATP hydrolysis to alter nucleosome structure and/or facilitate nucleosome mobility (58).

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J. Biol. Chem. 2005, 280:11887-11894.
doi: 10.1074/jbc.M409475200 originally published online January 20, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M409475200

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