**MafA is a dedicated activator of the insulin gene in vivo**

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**Abstract**

As successful generation of insulin-producing cells could be used for diabetes treatment, a concerted effort is being made to understand the molecular programs underlying islet β-cell formation and function. The closely related MafA and MafB transcription factors are both key mammalian β-cell regulators. MafA and MafB are co-expressed in insulin⁺/β-cells during embryogenesis, while in the adult pancreas only MafA is produced in β-cells and MafB in glucagon⁺α-cells. MafB⁻⁻/⁻ animals are also deficient in insulin⁺ and glucagon⁺ cell production during embryogenesis. However, only MafA over-expression selectively induced endogenous Insulin mRNA production in cell line-based assays, while MafB specifically promoted Glucagon expression. Here, we analyzed whether these factors were sufficient to induce insulin⁺ and/or glucagon⁺ cell formation within embryonic endoderm using the chick in ovo electroporation assay. Ectopic expression of MafA, but not MafB, promoted Insulin production; however, neither MafA nor MafB were capable of inducing Glucagon. Co-electroporation of MafA with the Ngn3 transcription factor resulted in the development of more organized cell clusters containing both insulin- and glucagon-producing cells. Analysis of chimeric proteins of MafA and MafB demonstrated that chick Insulin activation depended on sequences within the MafA C-terminal DNA-binding domain. MafA was also bound to Insulin and Glucagon transcriptional control sequences in mouse embryonic pancreas and β-cell lines. Collectively, these results demonstrate a unique ability for MafA to independently activate Insulin transcription.


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**Introduction**

Encouraging results obtained in treating type 1 diabetes by islet transplantation have stimulated efforts to develop an abundant source of functional insulin-producing cells (Shapiro et al. 2000). Islet-enriched transcription factors have been shown to play fundamental roles in pancreas development and function, especially those linked to endocrine cell-specific expression of the hormones glucagon and insulin (reviewed by Sander & German 1997, Servitja & Ferrer 2004). Expression of these hormones is crucial for maintaining glucose homeostasis, as glucagon released from α-cells stimulates the mobilization of glucose through gluconeogenesis and glycogenolysis to prevent hypoglycemia, while insulin released from β-cells promotes glucose storage. Physiological glucose levels are maintained through the counter-regulatory actions of these hormones in peripheral tissues, with defects in α- and β-cell activity resulting in the inability of diabetics to maintain normoglycemia (Del Prato & Marchetti 2004).

A key role in both islet cell development and function has been established for many transcriptional regulators of the Insulin and Glucagon genes. Transcription of these hormone genes is controlled by a number of factors, some shared, that bind to 5’ cis-regulatory elements in their enhancer region (e.g. Insulin: Pax6, Pdx1, NeuroD1 (also referred to as β2), MafA (reviewed in Melloul et al. (2002), Artner & Stein (2008)); Glucagon: Pax6 (Sander et al. 1997, Ritz-Laser et al. 1999), NeuroD1 (Naya et al. 1995), MafB (Artner et al. 2006, Nishimura et al. 2006), Brn4 (Hussain et al. 2002). Islet α- and β-cell development is impaired in Pdx6 mutant mice (Sander et al. 1997, St-Onge et al. 1997, Ashery-Padan et al. 2004), while the loss of NeuroD1 results in a severe, but general reduction in islet endocrine cells (Naya et al. 1997). Pdx1 acts upstream of Pax6 and NeuroD1, as knockout animals are pancreatic due to defects in both endocrine and exocrine cell differentiation (Jonsson et al. 1994, Ahlgren et al. 1996, Offield et al. 1996). Pdx1, NeuroD1, and MafA are also critical in mediating Insulin’s response to glucose, the most important effector of β-cell function. In addition, dysfunctional heterozygous mutations in PDX1 (Stoffers et al. 1997), NEUROD1 (Malecki et al. 1999), and PAX6 (Dumonteil et al. 1998) cause diabetes in humans, presumably due to reduced expression of target genes required for proper islet cell function.

MafA and MafB were only recently linked to Insulin and Glucagon expression and represent the principal members of the large Maf transcription factor family expressed in the...
pancreas (Olbrt et al. 2002, Matsuoka et al. 2003, Artner et al. 2006, Nishimura et al. 2006). MafA is initially detected in insulin-producing cells at embryonic day (E)13.5 in mice, coinciding with the onset of mature β-cell differentiation (Matsuoka et al. 2004). By contrast, MafB is detected in both early (i.e., <E13.5) and late (>E13.5) phase insulin + and glucagon + cells during development (Artner et al. 2006). MafB expression becomes restricted to islet α-cells soon after birth (Artner et al. 2006, Nishimura et al. 2006), while MafA (Matsuoka et al. 2004) is always only in β-cells. The expression of MafA and MafB primarily in hormone-producing cells, and not in endocrine precursor cells, is very unusual when compared with other pancreas-enriched transcription factors. These findings indicated that MafA and MafB were both critical to transcription factors. However, only MafB−/− animals are deficient in insulin + and glucagon + cell production during embryogenesis (Artner et al. 2007, Nishimura et al. 2008), although MafA−/− mice are diabetic due to defects in adult glucose sensing and insulin secretion capacity (Zhang et al. 2005).

Ectopic expression of islet-enriched transcriptional regulators in chick embryonic endoderm has been employed to assess their independent ability to induce endocrine cell differentiation. Previous experiments demonstrated that only Ngn3 and Mty1 were capable of inducing the formation of glucagon- and somatostatin-producing cells, while many other regulators of hormone gene expression and/or pancreas cell development had no effect (Grapin-Botton et al. 2001, Gu et al. 2004). Here, we examined whether newly characterized MafA and MafB were capable of activating hormone gene expression in this in ovo assay. MafA induced the formation of insulin-producing cells in embryonic gut endoderm and co-expression of MafA with Ngn3 resulted in the formation of islet-like clusters of insulin + and glucagon + cells. The effect of MafA on insulin production did not appear to be mediated by other islet-enriched transcription factors, including Pdx1, Nkx2.2, Pax6, or Isl1. By contrast, MafB expression did not result in the production of hormone + cells. Analysis of MafA/MafB chimeric proteins indicated a special significance for the C-terminal DNA-binding domain of MafA in endogenous chick Insulin activation, although Insulin and Glucagon control sequences bound both MafA and MafB in developing α- and β-cells. Collectively, these results demonstrate a fundamental difference between closely related MafA and MafB in Insulin gene transcription, presumably reflecting their ability to recruit factors essential in cooperative activation.

Materials and Methods

ChIP assay

Staged embryos were obtained from Balb/c mice. The day of vaginal plug discovery was designated E0.5. The animal studies were approved by the Vanderbilt University Animal Care and Use Committee in accordance with current federal regulations. E18.5 pancreata, BTC3 cells, and αTC6 cells were formaldehyde cross-linked, and the sonicated protein–DNA complexes isolated under conditions described previously (Matsuoka et al. 2003, Phuc Le et al. 2005). Sonicated chromatin was incubated 12–14 h at 4 °C with rabbit α-MafA (Bethyl Laboratories, Montgomery, TX, USA) or α-MafB (Bethyl Laboratories) and the complexes isolated with A/G-agarose (Santa Cruz Biotechnology, Santa Cruz, CA, USA). PCR was performed on one-tenth of the purified immunoprecipitated DNA using Ready-to-Go PCR beads (Amersham Pharmacia Biotech) and 15 pmol of the Insulin (−379)GGAAGTCGAGAACACGCTGAGG and −40CCTCGAGACTTTGGTGTGTTGTTG, Glucagon (−353)CCAATCAAGGGATAAGACCCTTC and +7AAAGCAGCTGCCCTTCTGCACCAG, and phosphoenolpyruvate carboxykinase (PEPCK) (−43GAGTGACACCTCACGGTTGCGG and −96GCCAGGGCTTTGAGCATAGGCC) transcriptional control region primers. The amplified products were resolved on a 1%-agarose gel in Tris-acetate–EDTA buffer containing ethidium bromide.

In ovo electroporation

In ovo electroporation was performed as described previously (Grapin-Botton et al. 2001, Xu et al. 2006). Embryos were electroporated with cytomegalovirus (CMV) enhancer-driven pCIG vectors (Megason & McMahon 2002, Dasen et al. 2003) of mouse MafA, MafB, chimeric MafA/MafB, Ngn3, and/or Pdx1 at Hamburger & Hamilton (1951) (HH) stages 11–13. Plasmids were injected at a final concentration of 2 μg/μl, which resulted in in ovo expression of proteins within physiological levels (Dasen et al. 2003). Three pulses (50 ms, 17 V) were applied to the embryo using a BTX 830 electroporator (Genetronics, San Diego, CA, USA), which were then incubated at 38 °C for 72 h (embryonic day 5 (E5)) in a humidified chamber. Electroporated samples were checked for green fluorescence protein (GFP) expression upon tissue collection; only tissue containing GFP + cells was further analyzed.

Immunohistochemistry

Embryos were fixed in 4% paraformaldehyde for 2–4 h at 4 °C and paraffin embedded. Immunofluorescence and confocal image analyses were performed on paraffin sections as described previously (Matsuoka et al. 2003). The primary antibodies used were rabbit α-mouse MafA (1:5000), rabbit α-mouse MafA (1:1000), rabbit α-cMaf 153 (Santa Cruz Biotechnology), guinea pig α-glucagon (1:2000, Linco Research, St Charles, MO, USA), and guinea pig α-insulin (1:2000, Linco Research), with secondary biotin, Cy3−, or Cy2-conjugated donkey, α-guinea pig, and α-rabbit IgG (1:500; Jackson ImmunoResearch, West Grove, PA, USA). Peroxidase staining was performed using the DAB substrate kit (Vector Labs, Burlingame,
CA, USA). Fluorescent images were captured with a Zeiss LSM 510 confocal microscope using an optical depth of 1 μm. At least three samples were collected and analyzed for each electroporated construct.

**Real-time (RT) PCR**

Total RNA from E5 electroporated chick gut tubes was prepared using the RNeasy kit (Qiagen) and treated with DNasel using the Versagene RNA DNase kit (Gentra Systems, Minneapolis, MN, USA) to remove genomic DNA. TAQMAN RT-PCR reverse transcript reagents (Applied Biosystems, Foster City, CA, USA) were used to generate cDNAs from 0.5 μg RNA. PCRs were performed with SYBR Green Master Mix reagents (Applied Biosystems), and the results analyzed using the Applied Biosystems Prism 7000 sequence detection system and software. The following chicken cDNA primer sets were used in the amplification step: insulin top (5'-ATGTCGAGCAGCCCCCTAGTG-3'), bottom (5'-TCGTATTCCTCCTCCTGTTGAAA-3'); 18S, top (5'-AGTCCCGCTGGCTTTGTACACA-3'), bottom (5'-GATCCGAGGACCTCACTAAAC-3'). A sample point consisted of three biological replicates, with each experiment performed on three separate occasions.

**DNA constructs**

Mouse wild-type and chimeric MafA and MafB coding sequences were expressed from the pCIG vector containing a CMV enhancer, β-actin promoter, and an internal ribosome entry sequence (IRES) followed by a nuclear localized GFP (Megason & McMahon 2002). The chimeric MafA and MafB constructs were generated by PCR, using standard molecular biology techniques. The amino acid (aa) sequences contained in each were: MafA/B, A: amino acids (aa) 1–209, B: aa 187–323; MafB/A, B: aa 1–188, A: aa 206–359; MafB/A/A, B: aa 1–76, A: aa 72–359; MafA/B/A, A: aa 1–73, B: aa 77–187, A: aa 206–359. The correctness of constructs was verified by DNA sequencing. The mouse pCIG-Pdx1 and pCIG-Ngn3 expression plasmids have been described previously (Grapin-Botton et al. 2001). The glucagon-476 (Lee et al. 1992) and insulin II-238 (INS-238 Zhao et al. 2000) firefly luciferase reporters contain rat gene sequences from nucleotides –476/+10 and –238/+2 respectively.

**Transfection assays**

Monolayer cultures of HeLa cells were maintained as described previously (Matsuoka et al. 2003). INS-238 or GLU-476 were transfected with Maf-pCIG constructs and thymidine kinase-driven renilla LUC (phRL-TK; Promega) using the lipofectamine reagent (Invitrogen). Extracts were prepared 40–48 h later and analyzed for firefly and renilla luciferase activity using the Dual-Luciferase Reporter Assay System (Promega). All of the chimeric MafA/B constructs were also found to synthesize a protein of the expected molecular weight and DNA-binding properties by western and gel shift analyses (data not shown).

**Results**

**Insulin is induced upon ectopic MafA, but not MafB, expression in developing chick endoderm**

To assess whether MafA or MafB expression could induce the production of hormone + cells in chick endoderm, mouse MafA and MafB were ectopically introduced into embryos using in ovo electroporation. The conditions used for electroporating the CMV enhancer (CMV)/β-actin promoter-driven MafA and MafB vectors favor ectopic expression in endoderm (Grapin-Botton et al. 2001, Xu et al. 2006). Embryos were electroporated at HH stage 11–13 and analyzed immunohistochemically for the presence of insulin + and glucagon + cells after 48 (i.e. when the dorsal and ventral pancreatic buds are first visible) or 72 h (when a branching epithelial architecture of the pancreas is present).

Mouse MafA and MafB expression was detected in anterior and posterior gut tubes, but not the pancreas anlage (Figs 1 and 2B). Insulin was induced in ~80% of electroporated MafA + cells, which were organized into small clusters close to the gut epithelium. However, MafA did not activate glucagon or
a number of transcriptional regulators associated with pancreas development in *in situ* hybridization or immunohistochemistry assays, including Nkx2.2, Isl1, NeuroD1, Pdx1, and Pax6 (data not shown). Furthermore, MafB did not induce glucagon, insulin, or any other analyzed islet-enriched product (Figs 1 and 2F; data not shown). These results suggest that there are unique features of MafA versus MafB, which allow *Insulin* activation, although not a more general endocrine differentiation program in this chick assay system.

**Expression of MafA and Ngn3 results in the formation of insulin- and glucagon-producing islet-like cell clusters**

Next, we examined whether co-electroporation of MafA and MafB with other regulators of β-cell differentiation could augment insulin induction and islet formation. Previous studies have shown that Ngn3 induced the differentiation of glucagon- and somatostatin-producing cells upon *in ovo* electroporation of chick gut endoderm (Grapin-Botton *et al*. 2001). Furthermore, the expression of Ngn3 promotes the activation of endogenous Pax6, Isl1, Hlxb9, NeuroD, and Nkx2.2 transcription factor expression. However, these key islet regulators were unable to activate islet hormone production upon over-expression in chick, although Pdx1 caused the formation of ectopic bud structures similar to that observed during pancreatic development (Grapin-Botton *et al*. 2001).

Co-expression of Ngn3 and MafA led to the formation of islet-like structures, with insulin-producing cells located in the core and the more plentiful Ngn3-induced glucagon cells on the periphery (Fig. 2A). Ngn3 alone induces glucagon+ cell formation, but not insulin+ (Supplementary Fig. 1, which can be viewed online at [http://joe.endocrinology-journals.org/content/vol198/issue2/; Grapin-Botton *et al*. 2001]). There was no apparent change in insulin+ cell number upon further addition of Pdx1, or after Pdx1 + MafA treatment (Fig. 2A). In addition, MafB had no effect on insulin+ and glucagon+ cell number when co-electroporated with Ngn3 (Fig. 2F). Co-electroporation of MafA with MafB also did not result in the production of more insulin-producing cells than with MafA alone (Supplementary Fig. 1). A 2.4-fold induction of *Insulin* expression in chick gut endoderm was observed with MafA and Ngn3 (Fig. 2D). Co-expression of MafA with Ngn3 and/or Pdx1 produces hormone+ cell clusters. (A) Double immunofluorescence staining of E5 chick embryo sections electroporated with MafA, Ngn3, and/or Pdx1 expression plasmids, as indicated. MafA (green) was detected only in insulin+ cells (red), and not in glucagon+ cells (red). MafA was present in 95% (152 out of 159 cells) and 93% (91 out of 98 cells) of insulin+ cells in MafA + Ngn3− or MafA + Ngn3 + Pdx1-treated cells respectively. (B) Peroxidase staining for MafA and insulin (brown) in E5 chick embryo sections electroporated with MafA + Ngn3 + Pdx1. Mouse MafA was not detected in the pancreas anlage (insert); as expected, insulin was present in both. (C) Schematic of the chick digestive system. Electroporated cells were detected only in the anterior and posterior gut tubes (orange). The black line indicates the plane of section shown in (B). (D) MafA electroporation induces *Insulin* mRNA expression in E5 chick endoderm. All of the expression data were derived by real-time PCR analysis and are expressed as the normalized fold difference relative to the pCIG vector alone. *P*<0.05, as determined by ANOVA analysis. The values shown represent mean ± s.d. (E) Triple immunofluorescence staining of E5 chick embryo sections electroporated with MafA + Ngn3 + Pdx1. MafA (green), Pdx1 (blue), and Ngn3 (red) are co-expressed in electroporated cells. (F) Double immunofluorescence staining of E5 chick embryo sections electroporated with MafB and Ngn3 expression plasmids. Co-expression of MafB and Ngn3 does not induce insulin (red) expression, while glucagon+ cells (red) were detected. Scale bar = 10 μM.
mRNA in MafA+ Ngn3− and MafA+ Ngn3+ Pdx1-treated samples was observed over vector or Ngn3-electroporated cells (Fig. 2D), which is significant considering the small percentage of insulin+ cells produced by electroporation. The absence of other cooperating factors normally present in developing β-cells presumably prevented higher level Insulin expression. Notably, the insulin+ and glucagon+ cells detected were in a region of endoderm distinct from the developing pancreas (Fig. 2B and C).

The MafA DNA-binding/dimerization domain is a major determinant of insulin induction

MafA/MafB chimeric proteins were generated to identify which region(s) of MafA are critical for Insulin induction. The level of identity between MafA and MafB is the greatest within their N-terminal activation and C-terminal DNA-binding/dimerization domains (Fig. 3). MafA and MafB activity can be assayed in a very sensitive manner in HeLa cell lines, as both Insulin- and Glucagon-driven reporter constructs are activated to high levels in transient transfection assays (Matsuoka et al. 2003, Artner et al. 2006, Nishimura et al. 2006). By contrast, little to no change in reporter activity is observed in MafA- and MafB-transfected α- or β-cell lines (data not shown).

Each of the MafA/B chimeric constructs was capable of independently activating the transfected hormone-driven reporter constructs in HeLa cells (Fig. 3B), a result expected considering that MafA and MafB have similar transactivation and protein stability properties (Matsuoka et al. 2003, Nishizawa et al. 2003, Zhao et al. 2005). The subtle difference in relative activation of the N-terminal spanning MafA/B constructs likely reflects their activation domain strength, as determined in mammalian cell transfection experiments (Nishizawa et al. 2003, Zhao & Stein unpublished).

The most active insulin-inducing chimeric construct was MafB/A/A in the in ovo assay, which contains the N-terminal aa 1–77 transactivation domain of MafB fused to aa 77–351 of the hinge and DNA-binding region of MafA (Fig. 4). Like wild-type MafA, the activity of MafB/A/A was enhanced in the presence of Ngn3. By contrast, a larger N-terminal (aa 1–187) MafB region fusion, MafB/A, only induced endogenous insulin expression in the presence of Ngn3. The same property was found with MafA/B/A, wherein only the hinge-spanning region of MafA was exchanged (i.e. aa 77–187). Significantly, MafA/B, which contains the MafB DNA-binding/dimerization domain, was inactive alone or in the presence of Ngn3 (Fig. 4), like MafB (Fig. 2F). As expected none of the chimeric Maf proteins were capable of influencing glucagon cell production (Supplementary Fig. 2, which can be viewed online at http://joe.endocrinology-journals.org/content/vol198/issue2/). However, both MafA/B and MafB activated hormone-driven expression in HeLa cells (Fig. 3). Together, these results suggest that the MafA DNA-binding/leucine-zipper dimerization domain is indispensable for Insulin activation in the in ovo assay, while the activation domain of either MafA or MafB was functional.

MafA and MafB bind within the 5' promoter region of Insulin and Glucagon in mouse endocrine islet cells

Since MafA specifically activates endogenous Insulin hormone expression in chick embryos and mammalian pancreatic cell lines (Matsuoka et al. 2004, 2007), we next analyzed whether this resulted from differentially binding to Insulin and Glucagon transcriptional regulatory sequences. MafB is expressed

![Figure 3](http://joe.endocrinology-journals.org/content/vol198/issue2/)

Each of the MafA/MafB chimeras can activate Insulin- and Glucagon-driven expression in cell lines. (A) Schematic of MafA, MafB, and the chimeric proteins. The sequence identity within the conserved activation (aa 1–75) and DNA-binding/dimerization regions is shown. (B) The ability of MafA, MafB, and the chimeric proteins to activate Glucagon- and Insulin-driven reporter constructs in HeLa cells was compared. The chimeric constructs were capable of activating Insulin- and Glucagon-driven reporter constructs. Normalized luciferase activities ± s.d. are presented relative to the pCIG vector alone (n=3). *P<0.05.
essentially in all insulin$^+$ and glucagon$^+$ cells in the E18.5 mouse pancreas (Artner et al. 2006, Nishimura et al. 2006), while MafA is only in insulin$^+$ cells (Matsuoka et al. 2004). As expected, MafB was bound to the Insulin and Glucagon control regions in ChIP assay (Artner et al. 2006, 2007). Strikingly, α-Maf antibodies also precipitated these hormone control regions in E18.5 pancreata, although chromatin treated with normal rabbit IgG did not (Fig. 5A). In addition, MafA antibodies did not precipitate control sequences from the PEPCK gene, which is not expressed in islet endocrine cells. MafA was also capable of selectively binding to Insulin and Glucagon control sequences in βTC3 cells, an insulin$^+$ β-cell line that produces MafA and not MafB ((Matsuoka et al. 2003), Fig. 5B), while MafB occupied the same hormone transcriptional control region in an α-cell line, αTC6.

The ChIP data demonstrate that MafA and MafB are bound to the Insulin and Glucagon promoters during development and likely in adult islets. Binding is indicative of positive control in developing α- and β-cells, but may also suggest an involvement in negative regulation during this time and in adults. Significantly, MafA was recently shown to act as both an activator and repressor of CHOP-10/GADD153 transcription in β-cells (Lawrence et al. 2007).

**Discussion**

Previous studies have established that MafA and MafB are critical to β-cell function (Zhang et al. 2005) and α- and β-cell differentiation (Artner et al. 2007, Nishimura et al. 2008) respectively. To assess whether MafA and MafB were both sufficient to initiate the differentiation of hormone-producing cells, we performed in ovo electroporation experiments in developing chick endoderm. MafA was uniquely capable of inducing Insulin expression in this model system, whereas MafB did not activate either Insulin or Glucagon transcription nor could a variety of other key pancreatic transcription factors, including Pdx1, Isl1, Pax6, Pax4, Hb9, and MNR2 (Fig. 2; (Grapin-Botton et al. 2001)). On the other hand, such distinct functional dissimilarities were not revealed between MafA and MafB in transient co-transfection assays with Insulin-, Glucagon-, or lens Crystallin-driven reporters (Yoshida & Y asuda 2002, Matsuoka et al. 2003, Nishizawa et al. 2003, Nishimura et al. 2006), cell transformation experiments (Nishizawa et al. 2003), or in vitro gel shift assays (Matsuoka et al. 2003).

The inability of MafB to stimulate hormone expression in the in ovo electroporation assay implies that cooperating factors mediating activation during mouse pancreatic development are lacking in chick endoderm. Certain key Insulin stimulatory factors must also be absent in mammalian α-cells (e.g. αTC6 cells), precluding activation even while bound to endogenous control sequences (Fig. 5). The more limited activity of MafB was expected, since its relatively broad expression pattern in other endodermal tissues, including cells in the villi of the duodenum (Artner et al. 2006), does not support a master regulatory function. By contrast, MafA is unusual in being exclusively expressed in insulin$^+$ cells within the pancreas, consistent with the observations supported here of a fundamental role in promoting Insulin transcription. We believe that both MafA and MafB are critical to mammalian β-cell maturation, with MafB acting during development and MafA principally after birth. The data from MafA (Zhang et al. 2005) and MafB (Artner et al. 2007, Nishimura et al. 2008) mutant mice...
MafA induces insulin expression

MaFA and MafB bind to the Insulin and Glucagon promoter region in vivo. Formaldehyde-cross-linked chromatin from (A) E18.5 pancreatic and (B) \( \beta \)
TC3 and \( \alpha \)TC6 cells was incubated with antibodies raised to MafA or MafB, as indicated. Immunoprecipitated DNA was analyzed by PCR with primers specific to transcriptional regulatory sequences of mouse Insulin, Glucagon, and PEPCK. As controls, PCR was performed with input DNA (1:100 dilution), DNA precipitated by normal rabbit immune serum, and DNA that was precipitated in the absence of antiserum.

MafA and MafB are expressed preferentially in insulin-producing cells in vivo. The inability of MafA (or MafB) to activate endogenous Glucagon (or Insulin) did not appear to reflect differential binding, as both large Maf factors were capable of specifically binding and stimulating Insulin- and Glucagon-driven reporters in HeLa cells. Sequences within the N-terminal transactivation domain region of MafA may also selectively contribute to in vivo activation, since MafA was more active than the MafB/A/A chimera. Although we cannot rule out that protein stability contributes to this difference, it is noteworthy that distinguishing activation domain functions have been observed in other systems (Yoshida & Yasuda 2002, Nishizawa et al. 2003).

The inability of MafA (or MafB) to activate endogenous Glucagon (or Insulin) did not appear to reflect differential binding, as both large Maf factors were capable of specifically binding and stimulating Insulin- and Glucagon-driven reporter expression in gel shift and transient transfection assays (Matsuoka et al. 2003, Artner et al. 2006, Nishimura et al. 2006). However, these in vitro experiments do not account for the native chromatin structure of endogenous genes, which may preclude physiological regulation. As a consequence, ChIP assays were performed to determine whether MafA and MafB physically associated with endogenous Insulin and Glucagon control sequences. We observed occupancy of Insulin and Glucagon flanking control region sequences in both \( \beta \)TC3 and \( \alpha \)TC6 cells by MafA and MafB (Fig. 5). MafA binding to Glucagon in E18.5 pancreata demonstrated that occupancy was also observed in developing \( \beta \)-cells in vivo. Although the physiological significance of this observation is unclear, it suggests that MafA may act as a repressor of Glucagon transcription in \( \beta \)-cells, an activity associated with...
Pdx1 (Ritz-Laser et al. 2003) and recently for MafA in the context of the CHOP-10/GADD153 gene (Lawrence et al. 2007). Repression of glucagon transcription by Pdx1 is mediated by the recruitment of Hdac-1 and Hdac-2 (Mosley & Ozcan 2004). Importantly, these results demonstrate that MafA and MafB directly bind to the Insulin and Glucagon promoter, but that activation is context dependent.

In conclusion, many investigators are working to generate functional insulin-producing β-like cells from various stem and adult progenitor sources. Although some success has been obtained in generating immature islet-like cells, these appear to be of limited functional value (Segev et al. 2004). The collective results demonstrate a fundamental role for MafA in Insulin gene expression, suggesting that its presence will be essential for generating functional β-cells for therapeutic transplantation.

Declaration of Interest

The authors declare no conflict of interest.

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