A Crucial Role of MafA as a Novel Therapeutic Target for Diabetes*

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MafA, a recently isolated pancreatic β-cell-specific transcription factor, is a potent activator of insulin gene transcription. In this study, we show that MafA overexpression, together with PDX-1 (pancreatic and duodenal homeobox factor-1) and NeuroD, markedly increases insulin gene expression in the liver. Consequently, substantial amounts of insulin protein were induced by such combination. Furthermore, in streptozotocin-induced diabetic mice, MafA overexpression in the liver, together with PDX-1 and NeuroD, dramatically ameliorated glucose tolerance, while combination of PDX-1 and NeuroD was much less effective. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes.

It is known that the insulin gene is specifically expressed in pancreatic β-cells and that insulin plays a crucial role in maintaining blood glucose levels. It was previously shown that an unidentified β-cell-specific nuclear factor binds to a conserved cis-regulatory element called RIPE3b in the insulin gene promoter region and is likely to function as an important transactivator for the insulin gene (1, 2). Recently, this important transactivator for the insulin gene was identified as MafA, a basic leucine zipper transcription factor (3–6). MafA controls β-cell-specific expression of the insulin gene through a cis-regulatory element called RIPE3b and functions as a potent transactivator for the insulin gene (3–8). During pancreas development, MafA expression is first detected at the beginning of the principal phase of insulin-producing cell production (4), while other important transcription factors such as the pancreatic and duodenal homeobox factor-1 (PDX-1) (8–11) and NeuroD (12, 13) are expressed from the early stage of pancreas development. In addition, while both PDX-1 and NeuroD are expressed in various types of cells in islets, MafA is the only β-cell-specific transactivator for the insulin gene. Thus, the potency of MafA as an insulin gene activator, together with its unique expression in β-cells, raises the likelihood that MafA is a principal factor of β-cell formation and function.

Insulin plays a crucial role in maintaining blood glucose levels, but in the diabetic state, chronic hyperglycemia decreases insulin gene expression and secretion. MafA DNA binding activity is also reduced under diabetic conditions in parallel with the decrease of insulin gene expression (2, 14–16). Although normoglycemia can be efficiently restored by pancreas and islet transplantation, such treatment requires life-long immunosuppressive therapy and is limited by tissue supply (17, 18). Therefore, in exploring new therapeutic methods to replace the reduced insulin in diabetes and to maintain normal glucose tolerance, it is very important to search for ways to enhance insulin gene transcription and to induce insulin-producing cells.

In this study, we show that MafA expression in the liver, together with PDX-1 and NeuroD, markedly induces insulin gene transcription and dramatically ameliorates glucose tolerance in diabetic model animals. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes and imply that such combination should be useful for replacing the reduced β-cell function found in diabetes.

**MATERIALS AND METHODS**

**Gene Transfection and Luciferase Assays—**HepG2 cells were grown in Earle’s minimal essential medium 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. The insulin 2 promoter-reporter plasmid (1.0 μg) containing 238 bp 5′-flanking sequences of the rat insulin 2 promoter region (1, 19) and 0.5 μg of the pSV-β-galactosidase control vector (Promega) were co-transfected with 1.0 μg of the MafA, PDX-1, and/or NeuroD expression plasmids (or the empty vectors) using the Lipofectamine reagent (Life Technologies). Forty-eight hours after transfection, cells were harvested for luciferase and β-galactosidase assays. Preparations of cellular extracts were assayed using a luciferase assay system (Promega). For the luciferase assay, light emission was measured with a Monolight 3010 Luminometer (Pharmingen), and β-galactosidase assays were performed with the β-galactosidase enzyme assay system (Promega). The luciferase results were normalized with respect to transfection efficiency assessed from the results of the β-galactosidase assays.

**Preparation of Recombinant AdenoToviruses Expressing MafA, PDX-1, and NeuroD (Ad-MafA, Ad-PDX-1, and Ad-NeuroD)—**Recombinant adenoviruses expressing MafA, PDX-1, and NeuroD were prepared using the AdEasy system (kindly provided by Dr. Bert Vogelstein, Johns Hopkins Oncology Center) (20). In brief, the encoding region of MafA, PDX-1, and NeuroD was cloned into a shuttle vector pAdTrack-CMV. To produce homologous recombination, 1.0 μg of linearized plasmid containing MafA, PDX-1, and NeuroD and 0.1 μg of the adenoviral backbone plasmid, pAdEasy-1, were introduced into electrocompetent Escherichia coli DH5α cells by electroporation (2,500 V, 200 ohms, 25 μF). The resultant plasmids were then re-transformed into E. coli XL-Gold Ultracompetent Cells (Stratagene, La Jolla, CA). The plasmids were linearized with PciI and then transfected into the adenovirus packaging cell line 293 using Lipofectamine (Invitrogen). Ten
days after transfection, the cell lysate was collected from the 293 cells. The cell lysate was added to fresh 293 cells and when most of the cells were killed by the adenovirus infection and detached, the cell lysate was obtained again (this process was repeated three times). The control adenovirus expressing green fluorescent protein (Ad-GFP) was prepared in the same manner. The adenovirus titers were further increased to 1 x 10^10 plaque forming units (pfu/ml) using the AdenoX™ virus purification kit (Clontech). The virus titers were estimated using the AdenoX™ titer kit (Clontech).

**Induction of Hyperglycemia by Streptozotocin (STZ) and Treatment with Recombinant Adenovirus—**C57BL6 male mice (8 weeks old) (Japan SLC, Hamamatsu, Japan) were made diabetic by intraperitoneal injection of STZ (220 mg/kg) (Sigma), freshly dissolved in citrate buffer (pH 4.5). One week after STZ injection, mice were injected with 100 μl of Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP (1 x 10^10 pfu/ml) into the cervical vein. It is noted that the adenovirus injected from the cervical vein is known to be trapped only in the liver after its systematic circulation. After adenovirus injection, nonfasting blood glucose levels were measured regularly with a portable glucose meter (Precision QID, Medisense Inc., St. Charles, MA) after tail snipping. For measurement of plasma insulin levels, nonfasting blood samples were collected into heparinized capillary tubes, and plasma insulin levels were determined using an insulin-EIA test kit (Glazyme).

**Glucose Tolerance Tests—**After overnight fast, mice were injected intraperitoneally with glucose (2.0 g/kg of body weight). Blood samples were taken at various time points (0–120 min), and blood glucose levels were determined as described above.

**Northern Blot Analysis—**Ten micrograms of total RNA isolated from freeze-clamped liver tissues were electrophoresed on 1% formaldehyde-denatured agarose gel in 1 x MOPS running buffer and then transferred overnight to a Hybond-N+ membrane (Amersham Biosciences). The insulin probe was labeled with α-[32P]dCTP using the Rediprime labeling system kit (Amersham Biosciences). After overnight hybridization with a [32P]-labeled probe at 42 °C, the membranes were washed in 2 x saline/sodium phosphate/EDTA, 0.1% SDS at 42 °C. Kodak XAR film was exposed with an intensifying screen at –80 °C.

**Reverse Transcriptase-PCR Analysis—**Total RNA was extracted from frozen tissue sections using TRIzol (Invitrogen). After quantification by spectrophotometry, 2.5 μg of RNA was heated at 85 °C for 3 min and then reverse-transcribed into cDNA in a 25-μl solution containing 200 units of Superscript II RNase H

**Immunohistochemical Analyses—**Five μm sections were prepared and mounted on 4-[methacryloyloxy]ethylmethacrylate (4-ME) slides. Before incubation with antibodies, the mounted sections were rinsed with PBS three times. For detection of MafA, PDX-1, and NeuroD, the avidin-biotin complex (ABC) method was performed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). After treatment with target retrieval solution (Dako, Glostrup, Denmark) at 90 °C for 5 min, the mounted sections were incubated overnight with rabbit anti-MafA antibody, rabbit anti-PDX-1 antibody (21L), and goat anti-NeuroD antibody (N-19) (Santa Cruz Biotechnology) diluted 1:1000 in PBS containing 1% bovine serum albumin. This was followed by 1 h incubation with biotinylated anti-rabbit IgG (for MafA and PDX-1) or anti-goat IgG (for NeuroD) (Vector Laboratories) diluted 1:200. The sections were then incubated with ABC reagent for 1 h and positive reactions were visualized by incubation with the peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide. The insulin probe was labeled with [32P]dATP using the Rediprime labeling system kit (Amersham Biosciences). After overnight hybridization with a [32P]-labeled probe at 42 °C, the membranes were washed in 2 x saline/sodium phosphate/EDTA, 0.1% SDS at 42 °C. Kodak XAR film was exposed with an intensifying screen at –80 °C.

**Luciferase Assays—**Luciferase assays were performed with a Perkin-Elmer Life Sciences System. The luciferase results were normalized with respect to the β-galactosidase control vector. Data are expressed as mean ± S.E. with the basal insulin promoter activity being arbitrarily set at 1 (n = 4).

**Fig. 1** MafA expression, together with PDX-1 and NeuroD, markedly induces insulin promoter activity. A, the rat insulin promoter-reporter (luciferase) plasmid and pSV-β-galactosidase control vector were co-transfected into HepG2 cells with the MafA, PDX-1, or NeuroD expression plasmids (or the empty vector); 2 days after the transfection, luciferase and β-galactosidase assays were performed. B, the rat insulin promoter-reporter (luciferase) plasmid and pSV-β-galactosidase control vector were co-transfected with MafA expression plasmid, together with PDX-1 and/or NeuroD expression plasmid; 2 days after the transfection, luciferase and β-galactosidase assays were performed. The luciferase results were normalized with respect to the transfection efficiency assessed from the results of the β-galactosidase assays. Data are expressed as mean ± S.E. with the basal insulin promoter activity being arbitrarily set at 1 (n = 4).
For detection of insulin, the mounted sections were incubated overnight with guinea pig polyclonal anti-insulin antibody (Dako) diluted 1:1,000 in PBS containing 1% bovine serum albumin. This was also followed by 1-h incubation with biotinylated anti-rabbit IgG diluted 1:200. The sections were then incubated with ABC reagent for 1 h, and positive reactions were visualized by incubation with the peroxidase substrate solution containing 3,3′-diaminobenzidine tetrahydrochloride.

**RESULTS**

**MafA Expression in the Liver, together with PDX-1 and NeuroD, Markedly Induces Insulin Gene Expression**—To evaluate the effect of MafA, PDX-1, and NeuroD expression on insulin gene transcription in the liver, we examined insulin gene promoter activity in HepG2 cells after transfection of each expression plasmid. As shown in Fig. 1A, basal insulin promoter activity was increased by MafA alone (~80-fold increase), which was much more significant after overexpression of PDX-1 or NeuroD alone (~5-fold increase). As shown in Fig. 1B, insulin promoter activity was further increased by MafA in the presence of PDX-1 or NeuroD (~300- and ~400-fold increase, respectively). Furthermore, MafA, together with PDX-1 plus NeuroD, drastically increased insulin promoter activity (~1,200-fold increase). These results clearly show that MafA, PDX-1, and NeuroD exert strong synergistic effect on insulin promoter activity.

Next, to evaluate the effect of ectopic expression of MafA, PDX-1, and NeuroD in the liver, we prepared MafA-, PDX-1-, and NeuroD-expressing adenoviruses (Ad-MafA, Ad-PDX-1, and Ad-NeuroD) and a control adenovirus (Ad-GFP), then injected each adenovirus into the cervical vein of 8-week-old male C57BL6 mice. To confirm that infected adenoviruses can express the target protein in the liver, we performed immunostaining for MafA, PDX-1, and NeuroD. As shown in Fig. 2A, MafA was clearly detected in the liver 3 days after infection with Ad-MafA. Similarly, PDX-1 and NeuroD were also clearly detected in the liver after infection with Ad-PDX-1 and Ad-NeuroD, respectively (data not shown). Also, to confirm that the adenovirus infected only the liver, we examined GFP expression in various tissues (brain, heart, lung, liver, spleen, pancreas, kidney, fat, and muscle). GFP was expressed only in the liver, but not in any other tissues (data not shown). After the adenovirus infection, we evaluated insulin mRNA levels by reverse transcriptase-PCR. Since it is known that rodents have two insulin genes: insulin 1 and insulin 2, we examined which insulin gene is induced by such adenoviruses. As shown in Fig. 2B, neither insulin 1 nor 2 gene expression was induced by Ad-MafA alone, but both insulin 1 and 2 were induced by Ad-MafA plus Ad-PDX-1 or Ad-NeuroD, respectively (data not shown). After the adenovirus infection, we evaluated insulin mRNA levels by reverse transcriptase-PCR. Since it is known that rodents have two insulin genes: insulin 1 and insulin 2, we examined which insulin gene is induced by such adenoviruses. As shown in Fig. 2B, neither insulin 1 nor 2 gene expression was induced by Ad-MafA alone, but both insulin 1 and 2 were induced by Ad-MafA plus Ad-PDX-1 or Ad-NeuroD, respectively (data not shown). It is noted that insulin 1 gene expression was induced only in the presence of MafA, suggesting that MafA plays a crucial role in inducing insulin gene expression. Larger amounts of insulin 1 and 2 mRNA expression were clearly observed in the liver after the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD).
The effect of the triple infection was more profound compared with that of Ad-PDX-1 plus Ad-NeuroD infection. We further evaluated insulin mRNA levels by Northern blot analysis. As shown in Fig. 2C, insulin mRNA expression was detected at all 3 days after the injection of Ad-MafA plus Ad-PDX-1 or Ad-MafA plus Ad-NeuroD. Furthermore, a much larger amount of insulin mRNA expression was induced by the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). Several insulin-positive cells (brown cells) can be clearly observed in the liver. Similar results were obtained in three independent experiments. D, male C57BL6 mice were made diabetic by intraperitoneal injection of streptozotocin (STZ) (220 mg/kg) and 1 week later were infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP. After a 6-h fast, we determined serum insulin levels in the mice. Data are expressed as mean ± S.E. (n = 3).

**Fig. 3.** MafA expression in the liver, together with PDX-1 and NeuroD, markedly induces insulin-producing cells in the liver. A, insulin content in the liver in C57BL6 mice infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP. Data are expressed as mean ± S.E. (n = 3). n.d., not detected. B, insulin content in the liver in C57BL6 mice infected with Ad-MafA, together with Ad-PDX-1 and/or Ad-NeuroD. Data are expressed as mean ± S.E. (n = 3). n.d., not detected. C, immunostaining for insulin in the liver in C57BL6 mice infected with Ad-MafA, Ad-PDX-1, and Ad-NeuroD. Several insulin-positive cells (brown cells) can be clearly observed in the liver. Similar results were obtained in three independent experiments. D, male C57BL6 mice were made diabetic by intraperitoneal injection of streptozotocin (STZ) (220 mg/kg) and 1 week later were infected with Ad-MafA, Ad-PDX-1, Ad-NeuroD, or Ad-GFP. After a 6-h fast, we determined serum insulin levels in the mice. Data are expressed as mean ± S.E. (n = 3).
NeuroD. Also, as shown in Fig. 3B, insulin content in the liver was markedly increased after the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). In addition, to reconfirm insulin protein expression in the liver, we performed immunostaining for insulin. As shown in Fig. 3C, many insulin-positive cells (brown cells) were clearly observed in the liver after the triple infection. Such effects were not observed after any double infection (Ad-MafA plus Ad-PDX-1, Ad-MafA plus Ad-NeuroD, or Ad-PDX-1 plus Ad-NeuroD) (data not shown).

Furthermore, to determine whether the produced insulin is secreted into the bloodstream, we measured serum insulin levels after the adenovirus infection. We injected 220 mg/kg STZ into C57BL6 mice, and 1 week later we treated the mice with the adenoviruses. As shown in Fig. 3D, serum insulin levels were increased after infection of Ad-MafA plus Ad-PDX-1 or Ad-MafA plus Ad-NeuroD and were markedly increased after the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). Taken together, MafA expression, together with PDX-1 and NeuroD, drastically induces insulin content (Fig. 3, A–C) and secretion (Fig. 3D), in addition to induction of insulin promoter activity (Fig. 1, A and B) and mRNA expression (Fig. 2, B and C).

MafA Expression in the Liver, together with PDX-1 and NeuroD, Dramatically Ameliorates Glucose Tolerance in Diabetic Model Animals—We examined whether hepatic insulin production induced by combination of MafA, PDX-1, and NeuroD is capable of controlling blood glucose levels in STZ-induced diabetic mice. As shown in Fig. 4A, 3 days after adenovirus infection, blood glucose levels were decreased by MafA plus PDX-1, MafA plus NeuroD, or PDX-1 plus NeuroD, although no such effect was observed after infection of Ad-MafA alone. Furthermore, blood glucose levels were dramatically decreased by the triple infection (Ad-MafA, Ad-PDX-1, and Ad-NeuroD). The effects of triple infection were much more pronounced compared with those in any single or double infection. It is noted that, despite the marked effects of MafA expression, together with PDX-1 and NeuroD, the mice did not become hypoglycemia. In addition, we performed the intraperitoneal glucose tolerance test at 3 and 14 days after the adenovirus infection. As shown in Fig. 2B, there was a marked difference in glucose tolerance at any time point after glucose load between Ad-GFP-treated mice and the mice treated with Ad-MafA, Ad-PDX-1, and Ad-NeuroD.

**DISCUSSION**

In this study we examined the effects of adenovirus-mediated expression of a recently identified pancreatic β-cell-specific transcription factor MafA on insulin gene expression and glucose tolerance with and without PDX-1 and/or NeuroD and found that MafA overexpression, together with PDX-1 and NeuroD, drastically induces insulin production in the liver (Figs. 2 and 3) and ameliorates glucose tolerance in diabetic animal models (Fig. 4). The marked effects of MafA expression, together with PDX-1 and NeuroD, on insulin production and glucose tolerance indicate that MafA plays an important role in inducing insulin-producing cells and thus should be a novel therapeutic target for diabetes.
and that combination therapy should be very efficient and useful for replacing the reduced insulin biosynthesis found in diabetes and for amelioration of glucose tolerance. In addition, despite the marked effects of MafA expression, together with PDX-1 and NeuroD, the mice did not become hypoglycemia. These results imply that some glucose-mediated regulation of insulin production and/or secretion is achieved by the triple infection.

There are several possible reasons to explain why the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD) is much more effective compared with any single or double infection. One possibility is that marked induction of insulin mRNA expression by the triple infection is simply due to increase of insulin promoter activity because insulin promoter activity per se is most significantly increased by the triple transfection (Fig. 1A). Another is that the transcription partners are recruited to the insulin promoter region by MafA, together with PDX-1 and NeuroD, which would enable these transcription factors to exert strong synergistic effects and to markedly induce insulin gene expression. Both PDX-1 and NeuroD are known to associate functionally with the co-activator p300 and thereby exert strong effects on the insulin gene transcription (22–26). Thus, although not examined in this study, we assume that some transcriptional partner such as p300 contributes to the marked effects of triple overexpression on insulin gene expression. In addition, since p300 is known to interact with transcriptional adaptors such as a particular histone acetyltransferase and to be involved in chromatin remodeling complexes (27, 28), it is possible that recruited p300 to the insulin gene promoter region facilitates histone acetylation and thus contributes to the marked effects of triple overexpression on insulin gene expression.

In conclusion, MafA overexpression, together with PDX-1 and NeuroD, drastically induces insulin production in the liver and ameliorates glucose intolerance in diabetic animal models, indicating that MafA plays an important role in inducing insulin-producing cells and thus should be a novel therapeutic target for diabetes and that combination therapy should be very efficient and useful for replacing the reduced insulin biosynthesis found in diabetes and for amelioration of glucose intolerance.

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REFERENCES
1. Sharma, A., and Stein, R. (1994) Mol. Cell. Biol. 14, 871–879
2. Sharma, A., Fusco-DeMane, D., Henderson, E., Efrat, S., and Stein, R. (1995) Mol. Endocrinol. 9, 1456–1466
3. Matsuzuka, T. A., Zhao, L., Artner, I., Jarrett, H. W., Friedman, D., Means, A., and Stein, R. (2003) Mol. Cell. Biol. 23, 6049–6062
4. Matsuzuka, T. A., Artner, I., Henderson, E., Means, A., Sander, M., and Stein, R. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 3260–3265
5. Ollbrt, M., Rud, J., Moss, L. G., and Sharma, A. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 6737–6742
6. Katsaka, K., Han, S. I., Shioda, S., Hirai, M., Nishizawa, M., and Handa, H. (2002) J. Biol. Chem. 277, 49903–49910
7. Kajihara, M., Sone, H., Amemiya, M., Katoh, Y., Isoogai, M., Shimano, H., Yamada, N., Takahashi, S. (2003) Biochem. Biophys. Res. Commun. 312, 831–842
8. Katsaka, K., Shioda, S., Ando, K., Sakagami, K., Handa, H., and Yasuda, K. (2004) J. Mol. Endocrinol. 32, 9–20
9. Miller, C. P., McGehee, R. E., and Habener, J. F. (1994) EMBO J. 13, 1145–1156
10. Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S., and Montminy, M. R. (1993) Mol. Endocrinol. 7, 1275–1283
11. Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. (1994) Nature 373, 606–609
12. Naya, F. J., Stellrecht, C. M. M., and Tsai, M.-J. (1995) Mol. Endocrinol. 9, 1009–1019
13. Naya, F. J., Huang, H., Qiu, Y., Mutoh, H., DeMayo, F., Leiter, A. B., and Tsai, M.-J. (1997) Genes Dev. 11, 2323–2334
14. Poitout, V., Olson, L. K., and Robertson, R. P. (1996) J. Clin. Invest. 97, 1041–1046
15. Moran, A., Zhang, H. J., Olson, L. K., Harmon, J. S., Poitout, V., and Robertson, R. P. (1997) J. Clin. Invest. 99, 524–539
16. Robertson, R. P. (2004) J. Biol. Chem. 279, 42351–42354
17. Robertson, R. P. (2004) N. Engl. J. Med. 350, 694–705
18. Weir, G. C., and Bonner-Weir, S. (1997) Diabetes 46, 1247–1256
19. Hoffman, G., C. W. G., Peshavaria, M., Henderson, E., Shieh, S.-Y., Tsai, M.-J., Teitelman, G., and Stein, R. (1997) J. Biol. Chem. 272, 2930–2933
20. He, T.-C., Zhou, S., DaCosta, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 2509–2514
21. Watada, H., Kajimura, Y., Umayadzha, Y., Matsuzuka, T., Kaneto, H., Fujitani, Y., Kamada, T., Kawamori, R., and Yamasaki, Y. (1996) Diabetes 45, 1478–1488
22. Put, V., Sharma, A., and Stein, R. (1998) Mol. Cell. Biol. 18, 2957–2964
23. Mutoh, H., Naya, F. J., Tsai, M.-J., and Leiter, A. B. (1998) Genes Dev. 12, 820–830
24. Sharma, A., Moore, M., Marrora, E., Lee, J. E., Qiu, Y., Samaras, S., and Stein, R. (1999) Mol. Cell. Biol. 19, 704–713
25. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2002) Mol. Cell. Biol. 22, 412–429
26. Stanoevich, V., Habener, J. F., and Thomas, M. K. (2004) Endocrinology 145, 2918–2928
27. Qiu, Y., Guo, M., Huang, S., and Stein, R. (2004) J. Biol. Chem. 279, 9796–9802
28. Mosley, A. L., Corbett, J. A., and Ozcan, S. (2004) Mol. Endocrinol. 18, 2279–2290