The Islet β Cell-enriched RIPE3b1/Maf Transcription Factor Regulates pdx-1 Expression*

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Susan E. Samaras‡, Li Zhao‡, Anna Means§, Eva Henderson‡, Taka-aki Matsuoka‡, and Roland Stein‡¶

From the ‡Department of Molecular Physiology and Biophysics and the ¶Department of Surgical Oncology, Vanderbilt University Medical Center, Nashville, Tennessee 37232

Pancreatic duodenal homeobox factor-1, PDX-1, is required for pancreas development, islet cell differentiation, and the maintenance of β cell function. Selective expression in the pancreas appears to be principally regulated by Area II, one of four conserved regulatory sequence domains found within the 5′-flanking region of the pdx-1 gene. Detailed mutagenesis studies have identified potential sites of interaction for both positive- and negative-acting factors within the conserved sequence blocks of Area II. The islet β cell-enriched RIPE3b1 transcription factor, the activator of insulin C1 element-driven expression, was shown here to also stimulate Area II by binding to sequence blocks 4 and 5 (termed B4/5). Accordingly, B4/5 DNA-binding protein’s molecular mass (i.e. 46 kDa), binding specificity, and islet β cell-enriched distribution were identical to RIPE3b1. Area II-mediated activation was also unaffected upon replacing B4/5 with the insulin C1/RIPE3b1 binding site. In addition, the chromatin immunoprecipitation assay showed that the Area II region of the endogenous pdx-1 gene was precipitated by an antiserum that recognizes the large Maf protein that comprises the RIPE3b1 transcription factor. These results strongly suggest that RIPE3b1/Maf has an important role in generating and maintaining physiologically functional β cells.

Targeting of the pancreatic duodenal homeobox factor-1 (pdx-1) gene in mice has established that expression in a common progenitor cell population is essential for development of both the endocrine and exocrine compartments of the pancreas. PDX-1 acts by stimulating proliferation, branching, and differentiation of the pancreatic epithelium (1–3). In contrast, all other characterized islet endocrine (e.g. PAX6 (4, 5), Ngn3 (6), BETA2 (7), and exocrine (PTF1-p48 (8, 9)-enriched transcription factors act downstream of PDX-1 and are principally involved in islet or exocrine cell differentiation. Selective elimination of PDX-1 in mouse β cells in vivo also results in a reduction in both insulin secretion and islet β cell numbers (1). These animals become glucose intolerant and diabetic, largely because of their inability to synthesize appropriate amounts of PDX-1-regulated gene products that are involved in maintaining glucose homeostasis (1 e.g. insulin (10, 11), GLUT2 (12), and glucokinase (13)). Moreover, mutations in pdx-1 cause pancreatic agenesis (2, 3) and a form of maturity onset diabetes of the young in humans (14, 15). These data have established an essential role for PDX-1 in islet β cell development and function.

The recent success in reversing type 1 diabetes by islet transplantation has led to renewed optimism for this form of treatment (16). However, the availability of human islets is limited and will never be sufficient to treat all patients. Because islet-enriched transcription factors are essential for islet cell development, information valuable for generating transplantable cells will likely be gained by understanding how their expression is regulated. Therefore, efforts have recently focused on characterizing the transcriptional control regions in genes necessary for islet cell formation, including pdx-1 (17–23), BET2A (24), pax6 (25, 26), pax4 (27), and ngn3 (28). In specific regards to pdx-1, expression will likely be mediated by factors involved in both the differentiation and maintenance of functional β cells.

Experiments performed in transgenic animals have established that β cell-selective expression of the pdx-1 gene is regulated by sequences 5′ to the transcription start site (18, 23). Control also appears to be largely mediated by those conserved between the vertebrate pdx-1 genes (19, 20, 22). Thus, β cell-specific reporter gene expression was driven in transfection assays by areas of sequence identity shared between the chicken, mouse, and human genes (i.e. Area I, –2839/–2520 base pair (bp) (19), Area III, –1879/–1799 bp (19), Area IV, –6047/–6529 bp), or only the mouse and human genes (Area II, –2141/–1961 bp (19, 22)). In contrast, the 5′-non-conserved sequences were inactive (18, 23). A pdx-1 gene fragment spanning Areas I and II also directed transgene expression to islet β cells in vivo (termed PstBst, –2917/–1918 bp (18, 23)), although only Area II, and not Areas I (22) or III (18), functioned independently in these in vivo assays. Collectively, these data strongly suggest that Area II represents the core of the mammalian pdx-1 transcription control region.

Mutational analysis of 17 conserved sequence blocks within Area II revealed sites for both positive- and negative-acting regulatory factors (22). Gel shift analysis performed on the activating B8 (–2068/–2060-bp) and B14 (–2006/–1996-bp) elements demonstrated specific binding to Pax6 and Foxa2.
MATERIALS AND METHODS

Transfection Constructs—The Area II and PstBst reporter constructs were made using human (~2141/~1890-bp) and mouse (Pst/~2917 bp: Bst/~1890-bp) pdx-1 sequences (29), which were cloned directly upstream of the herpes simplex thymidine kinase (TK) promoter in a chloramphenicol acetyltransferase (CAT) expression vector, pTK(An) (34). The block transversion and insulin C1 (InsC1) substitution mutants of B4/5 were constructed in Area II:pTK and PstBst:pTK using the QuickChange mutagenesis kit (Stratagene). Each construct was determined to be correct by DNA sequencing.

Cell Transfections—Monolayer cultures of pancreatic islet β (βTC-3, HIT-T15, and Min6) and non-β (NIH3T3) cell lines were maintained as described previously (35). The LipofectAMINE reagent (Invitrogen) was used to introduce 1 μg of each of Area II:pTK or PstBst:pTK and 0.5 μg of pBSV-LUC. The activity from the Rous sarcoma virus enhancer-driven luciferase plasmid served as an internal transfection control for the pds-1:pTK constructs. Luciferase (36) and CAT (37) enzymatic assays were performed 40–48 h after transfection. Each experiment was carried out more than three times with at least two independently isolated DNA preparations.

Electrophoretic Mobility Shift Assays—Double-stranded Area II block 4 (B4, agcttTCTTTTGCAAAGCACAGCA) and block 5 (agcttAAAGCACAGCA) sequences, in which the lowercase lettering corresponds to linker sequences, were excised from plBluescriptKS2+ and Klenow-labeled with [α-32P]dATP. The InsC1 probe spans nucleotides −126 to −101 of the rat insulin II gene and was labeled as described (38). Nuclear extracts were prepared as described previously (39). Binding reactions (20 μl total volume) were conducted with 0.1–10 μg of extract protein and labeled probe (8 × 10^5 cpm) in binding buffer containing 10 mM Tris–HCl, pH 7.4, 100 mM NaCl, 2 mM diithiothreitol, 1 mM EDTA, 10% glycerol, and 1 μg of poly(dGdC) (final concentrations). The conditions for the competition analyses were the same, except that excess (see figures for amounts) of the specific competitor DNA was included in the mixture prior to the addition of probe. Anti-c-Maf antiserum (10 μg, M-153, Santa Cruz Biotechnology) was added to binding reactions 10 min prior to addition of the probe for supershift analysis. This antiserum, referred to in the text as α-c-Maf M-153, was made to a N-terminal region of c-Maf that is common to the other large Mafs and cross-reacts with each (i.e. MafA, NRL, and MafB). The samples were resolved on a 6% non-denaturing polyacrylamide gel (acrylamide:bisacrylamide ratio 29:1) and run in TGE buffer (50 mM Tris, 380 mM glycine, 2 mM EDTA, pH 8.3). The gel was dried and subjected to autoradiography.

SDS-PAGE Fractionation—βTC-3 and Min6 nuclear extracts (30 μg) were separated on a 10% SDS-polyacrylamide gel (SDS-PAGE) and then electro-transferred onto an Immobilon polyvinylidene difluoride (PVDF) membrane (Millipore). The extract lanes were cut horizontally and the mixture prior to the addition of probe. Anti-c-Maf antiserum (10 μg, M-153, Santa Cruz Biotechnology) was added to binding reactions 10 min prior to addition of the probe for supershift analysis. This antiserum, referred to in the text as α-c-Maf M-153, was made to a N-terminal region of c-Maf that is common to the other large Mafs and cross-reacts with each (i.e. MafA, NRL, and MafB). The samples were resolved on a 6% non-denaturing polyacrylamide gel (acrylamide:bisacrylamide ratio 29:1) and run in TGE buffer (50 mM Tris, 380 mM glycine, 2 mM EDTA, pH 8.3). The gel was dried and subjected to autoradiography.
into 3-mm slices. The molecular mass range of each lane fraction was determined by comparison with colored Rainbow protein markers (Amersham Biosciences). The proteins from each fraction were eluted as previously described (38) and analyzed for B4/5 and InsC1 binding activity in electrophoretic mobility shift assays.

**Phosphatase Treatment**—Min6 or /H9252-H197 nuclear extract (3–5 g) was incubated for 10 min at 4°C or 30°C with and without 0.5 units of calf intestinal alkaline phosphatase (CIAP, Promega) in the presence or absence of sodium orthovanadate (Na3VO4, 10 mM) or sodium pyrophosphate (NaPPi, 10 mM) in phosphatase buffer (20 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 0.1 mM EGTA, 2 mM MgCl2, 100 μM protease inhibitor mixture (heten, Roche Diagnostics)) (10 μl total volume). The samples were analyzed for InsC1 and B4/5 binding after addition of 10 μl of gel shift binding buffer.

**Anti-phosphotyrosine Immunoprecipitation**—Immunoprecipitations using anti-Tyr(P) (4G10, Upstate Biotechnology, Lake Placid, NY) were performed as described previously (40). Briefly, SDS was added to a final concentration of 0.5% (w/v) to TC-3 nuclear extract (100 μg protein) in a buffer containing 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% glycerol, 1 mM Na3VO4, and 2 mM dithiothreitol (final concentrations) and then heated to 65°C. After diluting the SDS to 0.05%, anti-Tyr(P) or control mouse IgG was added along with protein A-Sepharose beads. The washed beads were then resuspended in 1× SDS-PAGE loading buffer and the immunoprecipitated proteins separated on a 10% SDS-polyacrylamide gel. After transfer to an Immobilon PVDF membrane, the 44–47-kDa eluted proteins were assayed for B4/5 and InsC1 binding activity.

**Immunohistochemistry**—Pancreata from 6–8-week-old mice were fixed 4–5 h in 4% paraformaldehyde at 4°C, washed, dehydrated, embedded in paraffin, and then 5-μm sections cut and mounted on glass slides. Double immunofluorescence was performed using guinea pig α-human insulin (Linco) and rabbit α-mouse c-Maf (c-Maf M-153) as primary antibodies at dilutions of 1:2000 and 1:100, respectively. Secondary antibodies were Cy3- or Cy5-labeled donkey anti-guinea pig and anti-rabbit IgG diluted to 1:500 (Jackson ImmunoResearch Laboratories). Fluorescent images were captured on a Zeiss LSM510 confocal microscope at an optical depth of 1 μm, false colors were assigned, and the images merged in Photoshop 5 (Adobe). Immunoperoxidase staining was performed with Vectastain Elite kits (Vector Labs) and with 3,3′-diaminobenzidine tetrahydrochloride substrate (Zymed Laboratories).
Inc.) according to the manufacturer’s recommendations. Rabbit anti-c-maf antibody (M-153) was diluted 1:1000.

**Chromatin Immunoprecipitation Assay**—Chromatin immunoprecipitation assays were performed with the following modifications of a described method (19, 22). The c-Maf M-153 antiserum (10 μg) was incubated with sonicated formaldehyde cross-linked TC3 chromatin. Normal rabbit IgG (10 μg, sc-2027, Santa Cruz Biotechnology) was used as a control. The protein-DNA complexes were isolated with A/G-agarose beads (Santa Cruz Biotechnology). The PCR oligonucleotides used to detect mouse control sequences were: pdx-1 Area II, 5′-GGTGGGAAATCCTTCCCTCAAG-3′ and 1927 5′-CCTTAGGGATAGCCCTCCGCTGC-3′, and phosphoenolpyruvate carboxykinase (PEPCK), 434 5′-GAGTGACACCTCACAGCTGTGG-3′ and 96 5′-GGCAGGCCCTTTGGATCATAGCC-3′. The PCR cycling parameters were 1 cycle of 95 °C/2 min and 28 cycles of 95 °C/30 s, 61 °C/30 s, 72 °C/30 s for PEPCK and 1 cycle of 95 °C/2 min and 28 cycles of 95 °C/30 s, 57.5 °C/30 s, 72 °C/30 s for Area II.

**RESULTS**

**B4 and B5 Affect Area II Activity**—Block mutations within conserved B2 (−2131/−2115 bp), B3 (−2110/−2102 bp), B4 (−2100/−2093 bp), and B5 (−2089/−2086 bp) reduce Area II: pTK activity in β cell lines (Fig. 1B, HIT-T15, MIN6, and βTC-3) (22). To further examine the significance of these elements in Area II activation, each was mutated within the mouse pdx-1 ‘PstBst’ region that spans Area I and Area II (Fig. 1A). In the context of the more active PstBst:pTK expression construct, the B4 and B5 mutants reduced activity to a greater extent than in Area II:pTK (Fig. 1B). Combining the B4 with B5 mutations in PstBst:pTK reduced activity further than either individual mutation (Fig. 1B). In contrast, the B2 and B3 mutants had less effect on PstBst:pTK activation (data not shown). The following experiments were designed to characterize the B4 and B5 activators in β cells.

**B4 and B5 Represent a Single cis-Element That Interacts with a β Cell-enriched Protein(s)**—To define the factors associated with B4- and B5-mediated regulation, gel shift experiments were performed with probes spanning B4, B5, and B4+B5 (B4/5), and βTC-3 or MIN6 cell nuclear extracts (Fig. 2). Identical results were obtained with MIN6 and βTC-3 cells, and they were used interchangeably in these analyses. Two common protein-DNA complexes were detected with the B4 and B4/5 probes (labeled as A and B in Fig. 2B), whereas no binding was found with B5 (data not shown). The binding affinity of B4 and B4/5 for these complexes was determined with the wild type and B4/5 double mutant site (B4/5 MT) competitors. As expected, both B4 and B4/5 reduced the levels...
of these complexes, although B4/5 was roughly 20-fold more effective (Fig. 2B). In contrast, B5 did not compete for binding (data not shown), whereas the B4/5 MT only competed away complex B, consistent with the conclusion that it is unrelated to activation (Fig. 2B). These results suggested that B4 and B5 define a single activator-binding site, which is regulated by the factor(s) found within the slower mobility complex A.

To determine the distribution of the cellular factor(s) forming complex A, binding reactions were conducted with nuclear extracts from various islet (β: Ins-1, Min6, HIT-T15; α: αTC-6) and non-islet cell types (neuronal, RC2.E10 (41, 42), NCB20 (43, 44); liver, H4IIE (45), normal rat liver; kidney, MDCK, BHK; fibroblast, NIH 3T3). Complex A was uniquely detected in the Min6 cell extracts (Fig. 2C). These results suggest that the factor(s) in activator complex A is enriched in Min6 cells.

Complex A Contains an Approximately 46-kDa Protein(s)—To estimate the size of the protein(s) in complex A, Min6 nuclear extracts were separated by SDS-PAGE and transferred to a PVDF membrane that was cut into slices to represent distinct molecular masses. The separated proteins were eluted from the membrane slices, renatured, and tested for binding to the B4/5 probe. The binding specificity of fraction 8 was identical to complex A found in unfractionated Min6 extracts (Fig. 3; data not shown). The molecular mass range of the proteins in fraction 8 was 44–47 kDa. These results indicate that complex A is composed of one or more proteins of ~46 kDa.

The 46-kDa Complex A Protein(s) Corresponds to the InsC1 Activator, RIPE3b1—Because the RIPE3b1 protein(s) that binds to and activates the InsC1 control element has the same cell-restricted distribution (38) and molecular size (see Fig. 3 and Ref. 38), we compared the binding properties of B4/5 to InsC1 (Fig. 4). Both InsC1 and B4/5 competed effectively for complex A binding when either B4/5 or InsC1 were used as probes (Fig. 4B). In addition, RIPE3b1/complex A activity was affected in the same manner by B4/5 or InsC1 mutations that either modestly (e.g. InsC1mt1, Ref. 38) or profoundly (e.g. InsC1mt3, Ref. 38) (Fig. 1B, B4/5MT) affected activity. The resulting competition patterns were consistent with each element binding the same factor(s) (Fig. 4B).

RIPE3b1 binding activity is inhibited by the actions of a tyrosine phosphatase (40). To test whether complex A formation on B4/5 is also regulated in this manner, Min6 nuclear extracts were incubated in the presence or absence of CIAP, CIAP + 10 mM Na₃VO₄, or CIAP + 10 mM NaPPi. B, βTC-3 nuclear extract was immunoprecipitated with either the anti-phosphotyrosine antibody 4G10 or normal mouse IgG. The immunoprecipitated proteins (labeled 4G10 and IgG) and whole nuclear extract were then fractionated by SDS-PAGE and transferred to PVDF membranes (Immobilon). Protein fractions 1 (63.7–62.7 kDa), 2 (41.7–53.6 kDa), and 3 (29.9–41.6 kDa) were eluted and used in B4/5 and InsC1 gel shift assays along with unfractionated βTC-3 nuclear extract.

![Fig. 5. Complex A binding to B4/5 is sensitive to tyrosine dephosphorylation.](image-url)
A Large Maf Transcription Factor within β Cell Nuclei Binds to Area II—The RIPE3b1 transcription factor was recently isolated and shown to be a member of the large Maf transcription factor family, most likely MafA (33, 46). To determine whether the B4/5 binding complex A contains a large Maf protein, Min6 nuclear extract was preincubated with a polyclonal antiserum raised to N-terminal sequences of c-Maf shared with other members of the large Maf family. This c-Maf antiserum, termed oc-Maf M-153, cross-reacts with MafA, MafB, and NRL (46). Complex A was completely supershifted by oc-Maf M-153, whereas IgG had no effect (Fig. 4C). These results strongly suggested that complex A contains the RIPE3b1/Maf protein. Immunohistochemical analysis performed with oc-Maf M-153 on adult mouse pancreas also showed that the large Maf protein(s) of the RIPE3b1/Complex A activator was nuclear and expressed almost exclusively in insulin-producing β cells (Fig. 7).

To directly determine whether RIPE3b1/Maf binds within Area II of the endogenous pdx-1 gene, a chromatin immunoprecipitation assay was performed using formaldehyde crosslinked chromatin from βTC-3 cells. The cross-linked DNA was precipitated with the Maf antiserum and PCR-amplified with Area II and PEPCK promoter-specific primers. The Maf antibody was capable of immunoprecipitating Area II sequences, whereas the control IgG could not (Fig. 8). However, the Maf antiserum did not immunoprecipitate transcription control sequences from the PEPCK gene, which is not transcribed in β cells. These results demonstrate that RIPE3b1/Maf occupies the Area II region of the pdx-1 gene in β cells.

DISCUSSION

Area II, when compared with Area I and III, was the only pdx-1 control region capable of independently directing pancreatic β cell-selective transgene expression (18, 22). This property and the uniqueness of this domain within the human and mouse genes imply that Area II represents the core of the mammalian pdx-1 transcription unit. Mutagenesis of conserved sequence blocks within Area II revealed sites for both

\[ \text{RIPE3b1/Maf binds to the Area II region in vivo. Cross-linked chromatin from βTC-3 cells was incubated with oc-Maf M-153 antibody. The immunoprecipitated DNA was analyzed by PCR for Area II and PEPCK transcriptional regulatory sequences. As controls, PCR reactions were run on input chromatin with no DNA and with DNA obtained after precipitating with rabbit IgG.} \]

\[ \text{RIPE3b1/Maf is found within the islet β nuclei. Large Maf expression was detected in adult pancreas sections using oc-Maf M-153 antiserum. Note that large Maf protein staining was (A) localized to the nuclei of islet cells by immunoperoxidase and (B) co-localized with insulin expression by immunofluorescence. The blue-stained nuclei (A) reveal the absence of large Maf expression in surrounding acinar cells.} \]
RIPE3b1/Maf Regulates pdx-1 Transcription

positive- and negative-acting regulators, including the B8 and B14 control elements that bind the PAX6 (22) and Foxa2 (20, 23) activators, respectively. However, the rather general distribution of these two developmental regulators suggests that a more β cell-restricted factor(s) likely contributes to the expression pattern observed for Area II-driven reporters. The objective of this study was to determine whether the B2, B3, B4, and/or B5 activators had such properties. Our analysis revealed that the B4 and B5 mutants reduced expression driven by the mouse PstBst fragment spanning Areas I and II in all β cell lines tested. The B2 and B3 mutants had a lesser affect and were not analyzed further. B4 and B5 were found to comprise a single regulatory element activated by RIPE3b1/Maf, a β cell-enriched nuclear factor known for its important role in insulin gene transcription.

Mutation of either B4 or B5 reduced activation of the PstBst-driven fragment, and all of our subsequent analysis focused on characterizing their activator(s). Using B4, B5, or B4/5 in gel shift assays revealed that a specific, β cell-enriched complex bound to these sequences (Fig. 2). The results of the transfection studies performed with B4, B5, and B4/5 mutants in PstBst were also consistent with this conclusion (Fig. 1). Because the size and cellular distribution of the specific B4/5 binding complex was similar to the InsC1/RIPE3b1 complex, the binding and functional properties of InsC1 were compared with B4/5. InsC1 not only specifically and effectively competed with B4/5 in gel shift assays but also was functionally indistinguishable in transfections performed with InsC1 substitution mutants in PstBst (Fig. 6). In addition, the apparent dependence upon tyrosine phosphorylation for RIPE3b1 binding to InsC1 (40) was also found with B4/5 (Fig. 5).

The RIPE3b1 activator was recently independently isolated from βTC-3 (data not shown) and HIT T-15 cells (46) using a biochemically based InsC1 affinity matrix chromatography strategy. Maf, a member of the large Maf family of basic leucine zipper proteins, was identified by mass spectrophotometric analysis of the purified fractions. The presence of Maf in the RIPE3b1 complex was further demonstrated using an antisemur that cross-reacts with N-terminal epitopes common to all the proteins in the large Maf family (Fig. 4C). These results confirmed the primary basis for concluding that Maf is the RIPE3b1 activator (46). This is likely to be correct for β cell lines; however, we have used specific molecular and immunological reagents to show that other members of the large Maf family are expressed in the adult islet β cells, specifically c-Maf and MafB (data not shown). Because Maf, c-Maf, and MafB are equally capable of binding and activating B4/5-like sequences in vitro (Ref. 48 and data not shown), these results suggest that several members of the large Maf family may control RIPE3b1-like-mediated transcription in the β cell. This broader composition of the RIPE3b1/Maf activator is also consistent with the importance of the large Maf proteins in myeloid (49, 50) and lens (51–53) cell differentiation.

Importantly, the large Maf-recognizing antisemur demonstrated that a protein(s) in this family binds to Area II control region sequences in intact β cells (Fig. 8). In addition, this antisemur also supershifted the β cell-enriched A/RIPE3b1 complex formed with the B4/5 probe (Fig. 4C) and immunohistochemically localized Maf protein in the nuclei of mouse islet β cells (Fig. 7). We and others have also shown that the large Maf proteins can activate islet target gene expression in transfection assays (33, 46). As a consequence, we conclude that RIPE3b1/Maf binds to B4/5 and activates Area II-mediated transcription in β cells. Furthermore, because only Maf was identified during the biochemical isolation and characterization of the RIPE3b1/InsC1 binding complex, it is most likely the principal large Maf activator in β cells.

Collectively, the data presented here and elsewhere demonstrate that insulin and pdx-1 are bona fide transcriptional targets for RIPE3b1/Maf control. Inspection of the consensus large Maf binding motif (TG(C)6, GCA, Ref. 48) also revealed a greater similarity of B4/5 to InsC1 than was initially apparent (Fig. 6). Interestingly, the presence of this consensus binding site in the transcription control region of other selectively expressed genes suggests a general, but significant, role in controlling β cell-specific expression (e.g. islet-specific glucose-6-phosphatase catalytic subunit related protein (54), —183/–165; islet amyloid polypeptide (55–57), —540/–528 hp). It is likely that the β cell-enriched RIPE3b1/Maf activator acts in concert with more widely distributed factors (e.g. PAX6 and Foxa2) to mediate selective expression.

The functional cooperativity observed between the large Mafs and PAX6 in activating lens gene expression may also be of relevance to both insulin and pdx-1 in the β cell. Thus, PAX6 directly regulates c-Maf gene expression during lens development (58, 59), and together they function cooperatively to stimulate crystalline gene expression in differentiating lens fibers (58–61). It is also intriguing to consider that the loss in β cell function upon eliminating insulin receptor (62) or insulin receptor substrate-2 (63, 64) signaling may be mediated, at least in part, by directly effecting Maf activation. Thus, because signaling by all of these effectors in β cells is imparted by tyrosine phosphorylation, large Maf activation would be directly influenced in the absence of insulin receptor and/or insulin receptor substrate-2 function, resulting in reduced insulin and pdx-1 transcription as seen in islets of Ins2–/– mice (47). In support of this theory, β cell mass and function are restored upon transgenic expression of PDX-1 in Ins2–/– mice (47). Our studies are currently focused on determining the nature of the interactions between RIPE3b1/Maf and other factors that are required for assembly of the pdx-1 and insulin transcription complexes and, more broadly, the significance of RIPE3b1/Maf in pancreatic development.

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