Roles and Regulation of Transcription Factor MafA in Islet β-cells

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Abstract. Insulin is a critical hormone in the regulation of blood glucose levels. It is produced exclusively by pancreatic islet β-cells. β-cell-enriched transcription factors, such as Pdx1 and Beta2, have dual roles in the activation of the insulin gene promoter establishing β-cell-specific insulin expression, and in the regulation of β-cell differentiation. It was shown that MafA, a β-cell-specific member of the Maf family of transcription factors, binds to the conserved C1/RIPE3b element of the insulin promoter. The Maf family proteins regulate tissue-specific gene expression and cell differentiation in a wide variety of tissues. MafA acts synergistically with Pdx1 and Beta2 to activate the insulin gene promoter, and mice with a targeted deletion of mafa develop age-dependent diabetes. MafA also regulates genes involved in β-cell function such as Glucose transporter 2, Glucagon-like peptide 1 receptor, and Prohormone convertase 1/3. The abundance of MafA in β-cells is regulated at both the transcriptional and post-translational levels by glucose and oxidative stress. This review summarizes recent progress in determining the functions and roles of MafA in the regulation of insulin gene transcription in β-cells.

Key words: Insulin, Transcriptional regulation, Glucose response, Glucotoxicity, Diabetes

(Endocrine Journal 54: 659–666, 2007)

Transcriptional regulators of insulin gene expression

Insulin is a polypeptide hormone that is critical in the regulation of blood glucose levels. It is produced exclusively by β-cells in the islets of Langerhans in the pancreas. A region of approximately 350 base pairs (bp) of the insulin promoter are sufficient to direct β-cell specific expression of insulin, and within this region, functionally important cis-regulatory elements and trans-acting factors have been identified (Fig. 1) (for review, see [1]).

Pdx1 is a homeodomain-containing transcription factor, and binds several elements in the insulin promoter: A1, GG2, and A3. Beta2 is a basic helix-loop-helix (bHLH) transcription factor, and binds to the E1 element of the insulin promoter as a heterodimer with the ubiquitous bHLH factor E47. Both Pdx1 and Beta2 are expressed in islet endocrine cells, and gene ablation experiments in mice have demonstrated that these proteins play critical roles in insulin gene expression, as well as in islet development and function. In humans, Pdx1 and Beta2 are responsible for the maturity-onset diabetes of the young (MODY) type 4 and 6, respectively, and mutations in these proteins have also been identified in some cases of type 2 diabetes mellitus. Genes responsible for other types of MODY, such as HNF1α (MODY3), HNF1β (MODY5), and HNF4α (MODY1), have also been shown to regulate insulin gene expression either directly by binding to the promoter, or indirectly by regulating other insulin transcription factors.

The C1 (in human) or RIPE3b (in rat) element of the insulin promoter also plays a critical role in β-cell-specific insulin gene expression, as well as in glucose-regulated insulin expression [2]. A β-cell-restricted...
C1-binding factor was identified that appears in response to glucose in pancreatic β-cell nuclear extracts [3]. Recently, MafA, a member of the Maf family of transcription factors, was identified as the C1-binding factor of the earlier study [4–7]. This review will focus in the functions and roles of MafA.

**Characteristics of Maf transcription factors**

The Maf family of transcription factors is a subgroup of the basic leucine zipper (bZip) family of transcription factors. Maf proteins are homologous to the viral Maf (v-Maf) oncoprotein, the original member of the Maf family, which was identified as the transforming element in the genome of the AS42 chicken musculoaponeurotic fibrosarcoma retrovirus in 1989 [8]. To date, genes encoding Maf family proteins have been isolated from vertebrate (mammals, birds, frogs, and fish) and from invertebrate (Drosophila) species (summarized in [9]). Maf family members are unique among bZip factors in that they contain a highly conserved extended homology region (EHR), or ancillary DNA binding region, in addition to a typical basic region, and both regions are involved in target DNA sequence recognition (Fig. 2).

The members of the Maf family of proteins are subdivided into two groups, large Maf and small Maf proteins, based on their structures and functions. MafA belongs to the large Maf protein group, which also includes MafB, c-Maf, and Nrl in mammals (Fig. 2). L-Maf is the chicken counterpart of MafA. The large Maf proteins have a conserved amino-terminal domain involved in their transactivator function. This domain...
is rich in Asp (D), Glu (E), Ser (S), Thr (T) and Pro (P) residues, and recent findings suggest that phosphorylation within this domain regulates the biological activity of Maf proteins [10, 11] (see below). The region between the D/E/S/T/P-rich domain and the bZip domain is relatively divergent among the family members and is referred to as the hinge region. With the exception of Nrl, this region contains stretches of poly glycine or poly histidine, which can form a flexible hinge in the protein.

The large Maf proteins (hereafter referred simply as Maf proteins) form homodimers through their leucine zipper domains and bind to consensus DNA sequences termed Maf-recognition elements (MAREs) (Fig. 2) [12, 13]. The T-MARE element (TGCTGACTCA) contains a phorbol 12-O-tetradecanoate-13-acetate (TPA)-responsive element (TRE: TGACTCA) and the C-MARE element (TGCTGACGTCAGCA) contains a cyclic AMP-responsive element (CRE: TGACGTCA), to which the AP-1 and the CREB/ATF families of bZip proteins bind, respectively. In contrast to typical bZip proteins, such as AP-1 (Fos and Jun) and ATF/CREB family members, Maf proteins bind to relatively long nucleotide sequences (13–14 bp in length). While some base mismatches in the consensus recognition sequence are allowed in Maf homodimer binding to the palindromic MAREs, GC residues flanking the central TRE or CRE seem to be critical for binding [12, 13]. Recently, Yoshida et al. showed that Maf homodimers also bind efficiently to a MARE half-site preceded by an AT-rich sequence (AT-rich plus half MARE) (Fig. 2), indicating that AT-rich sequences flanking MAREs are also important for binding [14]. Thus, Maf proteins are unique among bZip proteins in that they can bind to several types of target sequences, including palindromic MAREs and AT-rich plus half MAREs. The DNA-sequences of regulatory elements of well-characterized Maf target genes are related to these sequences [14, 15].

**Maf transcription factors as regulators of tissue-specific gene expression**

Although v-Maf was identified as a retroviral transforming protein, it is now widely accepted that Maf family proteins are regulators of a wide variety of cell- and tissue-specific gene expression, and also play a role in cell differentiation during development. For example, L-Maf (the chicken counterpart of mammalian MaFA) was identified as a transcription factor which binds to the lens-specific enhancer of the chicken αA-crystallin gene [16]. L-Maf also activates other crystallin genes, including chicken βB1- and δ1-crystallin, by binding to MARE sequences located in the promoter or enhancer regions of these genes [16]. Gain- or loss-of-function experiments in the developing chick embryo revealed that L-Maf is a key regulator of lens development [16].

c-Maf, the cellular counterpart of v-Maf, was rediscovered as a T helper 2 (Th2) lineage-specific gene product that regulates Th2-specific interleukin-4 expression [17]. Nrl, which was isolated as a gene product specifically expressed in photoreceptor cells in the retina [18], is a regulator of photoreceptor-specific rhodopsin gene expression [19, 20]. Knockout mice have revealed that Nrl is required for the development of rod photoreceptor cells [21], and missense mutations in human *nrl* are associated with autosomal dominant retinitis pigmentosa [22].

mafB was identified as the gene responsible for the phenotype of the mouse mutant kreisler, in which organization of the 5th and 6th rhombomeres (r5/r6), the segmental compartments in the developing hindbrain, is abnormal. MafB is expressed in r5/r6 during embryogenesis [23] and regulates the expression of *hoxa-3* and *hoxb-3* in r5/r6 and r5, respectively, by binding to r5/r6- and r5-specific enhancer elements [24, 25].

**Roles of MafA, Pdx1, and Beta2 in β-cells**

As described above, the C1/RIPE3b element in the insulin gene promoter plays a critical role in β-cell-specific and glucose-regulated expression of insulin [2]. A β-cell-restricted C1-binding factor that appeared in response to glucose in pancreatic β-cell nuclear extracts was originally detected by gel mobility shift analysis [3], but at the time, was not molecularly identified. In 2002 and 2003, the C1-binding factor was purified biochemically and identified as MafA [4, 6]. Using a bioinformatics approach to search for MARE-related sequences in the GenBank database, we found that the C1/RIPE3b element is similar to MARE and is conserved among species (Fig. 3A). We then isolated MafA as a β-cell restricted member of the Maf family of proteins, and showed that the C1/
RIPE3b element is a target of MafA [5, 12]. MafA is a weak transactivator of the insulin promoter when expressed alone, as are Pdx1 and Beta2. However, when these three factors are co-expressed, they synergistically and strongly activate the insulin promoter [26–29]. MafA is expressed in β-cells, but not in α-, γ- or δ-cells, in adult pancreatic islets [6, 30, 31]. In contrast, expression of Pdx1 and Beta2 is not restricted to β-cells. Therefore, β-cell-restricted expression of the insulin gene seems to be established by the synergistic action of these transcription factors. Furthermore, a direct interaction of MafA with both Pdx1 and Beta2 has been demonstrated [26]. Generally, such protein-protein interactions enhance the affinity of trans-factors for their respective cis-elements and/or co-activators, thus stabilizing DNA-protein complexes. However, the precise molecular mechanism underlying the synergistic activity of MafA, Pdx1, and Beta2 is unknown.

From a clinical point of view, it is noteworthy that simultaneous expression of MafA, Pdx1, and Beta2 in non-β-cells, such as liver cells, induced expression of the endogenous insulin gene, as well as other β-cell-specific genes, such as Glucokinase and the potassium channel subunits Kir6.2 and SUR1. These results indicate that MafA, Pdx1 and Beta2 are excellent potential targets for gene therapy approaches to the treatment of diabetes [27, 32].

Recently, it was demonstrated that mafA knockout mice develop age-dependent diabetes [26]. At birth, islets of the mafA knockout mice are morphologically normal, but with age, the mice display impaired glucose-stimulated insulin secretion (GSIS) and abnormal islet architecture. Expression of insulin, as well as the Glucose transporter 2 (GLUT2), an important component of the glucose-sensing system in β-cells, is reduced in the islets of mafA knockout mice. Similar phenotypes have been observed in pdx1 or beta2 knockout mice. Targeted disruption of pdx1 in mice results in pancreatic agenesis, which precludes further analysis, but mice with β-cell-restricted pdx1 gene ablation using cre-loxP system develop diabetes with age [33]. They also exhibit impaired glucose tolerance, abnormal islet architecture, and reduction of insulin and GLUT2 expression. Mice with targeted ablation of Beta2 exhibit diabetes due to a great reduction of β-cell number at birth, or a defect in postnatal β-cell neogenesis, depending on their genetic background [34]. Based on these observations, MafA, Pdx1, and Beta2 seem to be involved not only in insulin gene transcription, but also in proliferation and survival of β-cells in the adult pancreas.

Roles of MafA in β-cell functions are also implicated by studies of MafA overexpression in β- and non-β-cell lines [27, 35]. MafA seems to regulate not only insulin gene expression but also other genes involved in β-cell functions, such as insulin biosynthesis, insulin secretion and glucose metabolism (Fig. 3B). These genes include Prohormone convertase 1/3, potassium channel subunits Kir6.2 and SUR1, Glucagon-like peptide 1 receptor (GLP1-R), GLUT2, Glucokinase and Pyruvate carboxylase. MafA also regulates expression of other β-cell transcription factors, Pdx1, Beta2 and Nkx6.1. Thus, MafA is a key regulator of genes implicated in maintaining β-cell function.
Regulation of \textit{mafA} expression and MafA protein levels in \(\beta\)-cells

In pancreatic \(\beta\)-cells, insulin secretion and insulin gene expression are regulated by a variety of extracellular stimuli, including glucose, under both physiological and pathological conditions. Insulin gene transcription is stimulated by short-term exposure to high glucose. However, long-term or chronic exposure to high glucose, as in hyperglycemia in type 2 diabetes, leads to \(\beta\)-cell dysfunction and decreased insulin production. The molecular mechanism of \(\beta\)-cell failure (glucotoxicity) is not well understood, but oxidative stress caused by excess glycolytic reactions may play a role. Previously, C1/RIPE3b-binding activity in \(\beta\)-cells, which plays a critical role in the regulation of \(\beta\)-cell-specific and glucose-regulated expression of the insulin gene, was shown to increase in response to short-term glucose exposure, and decrease in response to chronic glucose exposure or oxidative stress [3, 36].

As indicated above, MafA was identified as the C1/RIPE3b-binding factor, providing evidence of the underlying molecular mechanisms of these responses to glucose exposure.

As illustrated in Fig. 4, both transcriptional and post-translational processes have been implicated in the regulation of MafA by acute and chronic glucose exposure of \(\beta\)-cells. Levels of \textit{mafA} mRNA and MafA protein are increased under high glucose conditions [5, 26]. Recently, Kitamura \textit{et al.} showed that glucose and oxidative stress regulate \textit{mafA} expression at the transcriptional level through FoxO1, a forkhead transcription factor involved in oxidative stress responses and metabolism [37]. Raum \textit{et al.} identified a \textit{cis}-regulatory region of \textit{mafA} approximately 8 kb upstream of the transcription start site, which regulates its \(\beta\)-cell-specific expression [38]. Pdx1, FoxA2, and Nkx2.2, which are all important transcriptional regulators of \(\beta\)-cell formation and function, regulate transcription of \textit{mafA} by binding to this \textit{cis}-regulatory region [38].

Harmon \textit{et al.} have shown that chronic exposure of a \(\beta\)-cell line to high glucose leads to a decrease in MafA protein without a corresponding change in \textit{mafA}.
mRNA level, indicating a post-translational mechanism of control of MafA protein stability by oxidative stress [39]. Lawrence et al. have reported that MafA forms a complex with the NFAT transcription factor on the insulin promoter and activates transcription in response to high glucose stimuli. Under chronic glucose exposure, MafA is replaced by C/EBPβ, which negatively regulates the insulin promoter [40].

Recently, we found that MafA is constitutively phosphorylated at multiple serine and threonine residues in the amino-terminal region (Fig. 4) [41]. Ser65 of MafA is first phosphorylated by an unidentified kinase (priming kinase), and then glycogen synthase kinase 3 (GSK3) sequentially phosphorylates Ser61, Thr57, Thr53 and Ser49. We have also shown that MafA protein is degraded by the proteasome in low glucose conditions, and multiple phosphorylation at these residues is a prerequisite for degradation. The molecular mechanism by which phosphorylated MafA undergoes rapid degradation in response to low glucose is still unknown. Recently, Vanderford et al. showed that MafA induction by glucose is mediated by hexosamine biosynthetic pathway [42]. Therefore, O-GlcNAc modification of MafA and/or proteasome subunits may be involved in the regulation of MafA protein abundance.

In obesity, or in type 2 diabetes, prolonged exposure of β-cells to fatty acids also causes dysfunction of β-cells (lipotoxicity), including defects in glucose-induced insulin secretion and insulin transcription. The molecular mechanisms underlying lipotoxicity are unknown, but recently it was shown that exposure of β-cells to palmitate decreases insulin expression through reduction of mafA mRNA expression, as well as nuclear exclusion of Pdx1 [43].

Based on these findings, MafA abundance in β-cells appears to be regulated by various extracellular stimuli at multiple levels, including transcription and protein stability. MafA activity as transcriptional activator may also be regulated by post-translational modifications such as phosphorylation.

**Conclusion and future prospects**

Investigations into the molecular mechanisms of gene regulation by MafA have recently emerged. Elucidation of MafA regulation in β-cells will enable us to develop a comprehensive understanding of the pathophysiology of β-cells and the molecular mechanisms of glucotoxicity and lipotoxicity. It will also inform the development of efficient gene therapy applications in the area of β-cell neogenesis, β-cell transdifferentiation, or β-cell generation from ES cells, for the cure of diabetes.

**References**


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