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MafA and MafB activity in pancreatic β cells

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Summary

Analyses in mouse models have revealed crucial roles for MafA and MafB in islet β cells, with MafB required during development and MafA in adults. These two closely related transcription factors regulate many genes essential for glucose sensing and insulin secretion in a cooperative and sequential manner. Significantly, the switch from MafB to MafA expression also appears to be vital for functional maturation of the β cells produced by human embryonic stem (hES) cell differentiation. This review will summarize the discovery, distribution, and function of MafA and MafB in rodent pancreatic β cells, and describe some key questions regarding their importance to β cells.

β cell generation as a source of replacement therapy for diabetes mellitus

Pancreatic islet β cells are the only cell type in the body to secrete the insulin hormone in response to glucose. High levels of glucose stimulate insulin secretion from β cells. This hormone first acts on liver to increase glycogen synthesis and inhibit gluconeogenesis. Subsequently, insulin promotes glucose uptake and storage in skeletal muscle and adipose tissue. In addition to increased blood glucose concentrations, insulin is also secreted in response to amino acids, free fatty acids, potassium and neurotransmitters. The mass and functional capacity of β cells, in combination with peripheral insulin sensitivity, are determinants of glucose homeostasis.

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia, caused by absolute insulin deficiency due to autoimmune β cell destruction (i.e. type I diabetes) or a relative deficiency because the body cannot effectively use what is produced (type II diabetes). Since the discovery of insulin in the early 1920s, and its subsequent mass production, insulin therapy via administration of exogenous insulin has greatly improved the life expectancy of diabetics. However, patients eventually experience complications, including weight gain, kidney failure, retinopathy, nervous system damage and potentially life-threatening hypoglycemia, presumably due to the failure of exogenous insulin administration to regulate glucose levels as effectively as that provided by endogenous β cells. In 1999, a group of investigators in Canada transplanted human islets into seven type I diabetic patients, rendering them exogenous insulin-independent, with a normal or near normal glycohemoglobin levels[1]. Although most of these patients relapsed

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after three years and required external insulin treatment, this protocol provided encouragement and renewed interest for developing methods for generating sources of therapeutic β cells, as well as better protocols and reagents to prevent and minimize immunorejection.

Notably, the Canadian protocol utilized islets from two or more cadavers for each recipient, and revealed the very serious shortage of transplantable material. As a consequence, an enormous amount of effort has been focused on seeking alternative strategies to produce human islet β cells. Three major avenues have been actively pursued: 1) promoting replication of existing adult β cells; 2) directly differentiating stem cells to β cells; and 3) transdifferentiating non-β cells to a β cell fate[2].

Under normal conditions, rodent β cells replicate at a low rate in adulthood[2-4], but proliferation can be induced by glucose[5], growth factors[6], partial pancreatectomy[7, 8], and insulin resistance[8]. However, similar conditions were unable to trigger substantial β cell replication in adult humans, including partial pancreatectomy and insulin resistance[9, 10]. The discrepancy between the capacity of human and rodent islet cells for replication has recently been attributed to inter-species differences in the distribution of G1/S phase cell cycle regulators[11-15]. Notably, *in vitro* overexpression of the G1/S regulators, Cdk6 and CyclinD3, in human islets induced robust β cell proliferation (15% upon Cdk6/CyclinD3 co-transduction versus 0.3% in control)[15].

Progress has also been made in the making of insulin$^+$ cells through *in vitro* differentiation of pluripotential stem cells as well as transdifferentiation of non-β cells in mouse models. The Baetge group used this information to establish a now well-recognized *in vitro* protocol to efficiently differentiate hES cells to definitive endoderm and subsequently to hormone-producing endocrine cells. Importantly, the insulin content of these cells was similar to mature human islet β cells and insulin was secreted upon administration of KCl and cAMP, agents that promote insulin release in the absence of stimulating high amounts of glucose. However, secretion was poorly responsive to stimulating glucose concentrations, the most important physiological effector[16]. Recently these investigators were able to produce β-like cells that release insulin in response to glucose by transplanting cells produced from an early *in vitro* differentiation stage under the epididymal fat pad of immunodeficient mice[17]. A notable feature of these glucose responsive cells was their ability to now produce MafA, a transcription factor necessary for adult β cell function in the rodents[17]. This resembles the natural developmental expression profile of β cell maturation in mice[18-20], with the transition of MafB to MafA in insulin$^+$ cells appearing to be critical for activity.

Interestingly, MafA, together with Pdx1 and Ngn3, were also the islet-enriched transcription factors capable of reprogramming adult mouse pancreatic acinar cells to β-like cells[21]. These insulin$^+$ cells expressed many factors associated with β cell identity (e.g. Glut2/Slc2a2, Glucokinase, Nkx6.1, and Prohormone convertase 1/3), were ultrastructurally similar to islet β cells, secreted active insulin, and reduced high blood glucose levels upon destruction of endogenous islet β cells by streptozotocin administration, a commonly used strategy to mimic conditions found in type I diabetes[21].

These results highlighted the necessity of producing MafA in biologically active insulin$^+$ cells. Presently neither of these engineered β-like cells has been tested therapeutically in humans and our understanding of MafA and MafB function in islet cells *in vivo* results exclusively from rodent models. However, MafA is also enriched in human islet β cells[22], suggesting that its regulatory properties are conserved. Here, we describe the discovery, distribution, and functions of MafA and MafB in rodent pancreatic β cells, and discuss pertinent issues regarding their significance in this context. A thorough understanding of

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how these two transcription factors impact β cells will likely provide perspectives valuable to generating functional insulin$^+$ cells for therapeutic cell replacement therapy in diabetics.

MafA and MafB are principal large Maf transcription factors in pancreas

The large Maf protein family is composed of four distinct genes/proteins (see Box1), MafA, MafB, c-Maf and Nrl, which all contain N-terminal transactivation and C-terminal basic leucine-zipper DNA binding domains. The *in vitro* DNA binding properties of MafA, MafB, and c-Maf are indistinguishable[23]. c-Maf has been reported to be expressed in the pancreas[20, 24-26], while pancreatic *Nrl* expression is undetectable[27]. Signficantly, pancreas development is unaffected in *c-Maf* null mice[18].

MafA was first identified from a chicken lens cDNA expression library during a screen for proteins that bind to the lens-specific αCE2 enhancer element and activate lens *crystallin* gene transcription[28, 29], and was initially termed L-Maf (i.e. Lens-specific Maf; the chicken orthologue to mammalian MafA). However, this protein has little, if any, impact on mammalian lens fiber differentiation[30, 31]. MafA was biochemically isolated in mammalian cells from the rodent β cell-line-specific RIPE3b1 electrophoretic mobility shift binding complex that is associated with the *insulin* C1 element activation[23, 32]. Human MAFA shares 93% of homology with the mouse protein ortholog and can also activate the *insulin* promoter driven reporter expression[32]. This factor complex is crucial for glucose-responsive *insulin* transcription in β cells (discussed in detail later). Notably, MafA also appears to be the principal islet-enriched transcription factor mediating *insulin* transcription in the thymus, which is crucial for the development of self-tolerant autoantibodies[33]. Two polymorphisms in the 5-flanking promoter region of human *MAFA* have been associated with type 1 diabetes, presumably because of decreased expression in the thymus[33]. Although transgenic *Mafa* promoter-driven activity was observed in many tissues outside the pancreas in mice[34, 35], it is unclear if this represents endogenous expression.

Mammalian MafB was identified earlier than MafA as the protein product of the mutated gene in the X-ray induced *Kreisler* mutant, a hypomorphic allele caused by chromosomal inversion[36, 37]. These mice exhibit a number of abnormal behaviors, such as head tossing and running in circles, due to defects in hindbrain segmentation and otic development[37]. Later analysis of *Mafb*$^{-/-}$ mice showed that this factor controlled segmental identity in the hindbrain through activating the *Hoxb3* gene expression in rhombomere(r)5[38]. In addition, MafB plays critical roles in a variety of other cellular differentiation processes, including in kidney podocytes[39], macrophages[40-42], as well as islet α and β cells[18]. In the pancreas, MafB was originally characterized as the islet α cell-enriched[25] activator binding to the *glucagon* G1 element (located between −77 and −51 base pair (bp) relative to the transcription starting site). Recent studies have revealed that MafB is produced in both insulin$^+$ and glucagon$^+$ cells during development and necessary in α and β cell differentiation (discussed in detail later). The human *MAFB* gene was recently cloned (GenBank: AL035665.32) and is 97% identical to the mouse MafB protein, although nothing is known about its distribution or significance in the human pancreas (see Box 1).

MafA and MafB have unusual expression patterns during islet cell development

MafA and MafB are expressed in a unique tempero-spatial regulated manner in relation to other islet-enriched factors in developing and postnatal murine islet cells. MafB is expressed earlier than MafA, with the initial production detected around E10.5 in the pancreatic epithelium[20, 25]. In contrast, MafA is first produced at E13.5 and only in insulin$^+$ cells[43]. Their developmental expression pattern is also unusually late in comparison to all other

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islet-enriched transcription factors (e.g. Pdx1 (E8.5), Pax6 (E9.0), Ngn3 (E9.0), Isl1 (E9.5), Nkx2.2 (E9.5)). Moreover, these two large Maf transcription factors are mostly, if not exclusively (i.e. MafA) expressed in hormone-producing cells, a distinction from the broad distribution of other islet-enriched transcription factors in both early progenitor and committed islet cell populations. Except for a small portion of MafB$^+$Ngn3$^+$ cells, MafB is exclusively detected in glucagon$^+$ cells and insulin$^+$ cells during the primary and secondary transition[20, 25] (Box2). However, MafB disappears from β cells within two weeks of birth and becomes an adult islet α cell-specific factor, while MafA expression persists in postnatal and adult β cells[19].

Quantitative immunohistochemical studies illustrated the dynamic nature of MafA and MafB production in the insulin$^+$ cell population of the mouse fetal and neonatal pancreas (Figure 1). MafB was found in almost all insulin$^+$ cells produced at E14.5 (91.7%) and E15.5 (89.3%), while only slightly reduced at the end of gestation (85%)[19]. The proportion of MafB$^+$ insulin$^+$ cells rapidly declined after birth, as very few co-expressing cells were detectable by postnatal day (P)14 (2.7%) and none by P28[19]. In contrast, a fraction of insulin$^+$ cells co-express MafA at E14.5 (45%), which increases by E18.5 (75%) to that found in adult islets (80%). *Mafa* and *Mafb* mRNA expression is regulated in a similar dynamic manner[44, 45]. The steady-state *Mafb* messenger level is roughly 6-fold higher than that of *Mafa* in E15.5-E18.5 mouse insulin$^+$ cells[44], likely explaining why there is no effect on β cell development in *Mafa* mutant mice (discussed in detail later). Significantly, *Mafa* mRNA levels increase from neonates to adults by 10-fold in rats, consistent with its important role in adult islet β cell function[45].

The unique cellular and temporal expression pattern of MafA has been attributed to roughly 10kb of DNA sequences upstream from the transcription start site. This regulatory domain contains six highly conserved segments, termed Regions (R) 1 to 6, which drive *Mafa*-like expression in β cell lines and transgenic mice[34]. In particular, mouse R3 (−8118bp to −7750 bp (relative to transcription starting site)) is highly conserved between xenopus and humans, as well as the only region of identity retained in the chicken *MAFA* gene[34]. It alone is uniquely capable of conferring β-cell-line specific reporter expression[34], while activity is lost in developing and adult β cells *in vivo* upon deletion of R3 from the R1-6 transgenic reporter[34, 35]. However, R3 was not necessary for transgenic activity of the $Mafa^{R1\text{-}6}$-driven reporter in many other non-pancreatic tissues[34, 35]. R3 activity is regulated *in vitro* by many distinct islet-enriched transcription factors, including Nkx2.2, Nkx6.1, NeuroD1, Foxa2, Pdx1, Pax6, MafB and Isl1[34, 46], all of whom are expressed earlier than MafA in the developing pancreas and are critical to β cell development and/or function[47-49]. Moreover, MafA is not present in the remaining insulin$^+$ cells of these transcription factor knockout mice, the exception being NeuroD1 wherein another closely related basic helix-loop-helix factor probably compensates. Thus, β-cell-specific *Mafa* transcription is controlled by R3 through the actions of many key islet regulators[34, 46].

Little is known about the factors influencing *Mafb* transcription, though ~8.2 kb of mouse upstream promoter sequences were capable of directing transgenic reporter expression to the pancreas[50]. The 5′-flanking region contains several potential regulatory elements, including two GC-boxes (5′-GGGCGG-3′), a palindromic sequence (5′-GTCAGCTGAC-3′), two half-Maf recognition sites (5′-GCTGAC-3′), and an E-box (5′-CAGCTG-3′; the underlined nucleotides are essential to activation). In addition, cell line based assays indicate that MafB positively regulates its own transcription[51]. However, the pancreatic transcription factors actually stimulating *Mafb* expression in developing α and β cells or silencing in postnatal β cells have not been determined.

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The importance of MafA and MafB in β cell development and function

The pancreatic transcription factors play important in pancreas development (Figure 2). Almost all these transcription factor knockout mice result in either the loss in hormone^{+} cell numbers and/or respecification to another islet cell type[47-49, 52]. For instance, Pdx1 is critical for pancreas outgrowth during development, with loss leading to pancreas agenesis in mice and humans[52-54]. This condition results in death soon after birth unless treated for the loss of insulin producing β cells, and the digestive enzymes supplied by pancreatic acinar cells. In contrast, *Ngn3* null mice are unable to produce any pancreatic islet cells during development due to the significance of this factor in the progenitor population, and die two to three days after birth from hyperglycemia[55]. Furthermore, β cells are completely lost and more grehlin^{+} ε cells are produced in mice lacking the NK homeodomain transcription factor, Nkx2.2, which is normally expressed in early pancreatic buds as well as later in Ngn3^{+} cells[56]. The pancreatic developmental phenotype of MafA and MafB null mice is distinct from essentially all other transcription factors, because MafB only impacts events required late in α and β cell maturation (Figure 1), while MafA does not affect β cell formation, despite being first detected during development in cells destined to populate the islet.

Only MafB is required during mouse β cell development

MafB is required for insulin and glucagon transcription in developing α and β cells, as illustrated by the reduction in hormone^{+} cell numbers throughout embryogenesis in *Mafb*$^{-/-}$ mice[18, 57]. In particular, MafB is essential for the first wave of insulin^{+} cells produced prior to E13.5 that are not completely functional, in addition to second wave β cells. Notably, the total number of endocrine cells is unchanged in mutant mice, and many proteins associated with β cell identity continue to be made. MafB binds to and is essential for of *Insulin, Glucagon*, and *Mafa* transcription during this period. Moreover, it is required for the maintenance of key β cell gene products (like *Pdx1* and *Slc2a2* (Glucose transporter; Glut2), which are made at normal levels until ~E15.5 and then cease to be produced in the *Mafb*$^{-/-}$ pancreas by E18.5[18, 57]. Similar defects were also observed in *Kr*^{ENU} mutant mice[18, 57], a hypomorphic MafB basic DNA binding domain mutant produced by chemical mutagenesis with ethylnitrosourea (ENU)[37].

In distinction to MafB, pancreas development was unaffected in *Mafa*$^{-/-}$ mice and pancreas-specific *Mafa* (*Mafa*$^{\Delta panc}$) mutant mice[19, 31]. MafB probably acts in place of MafA in this context, since *Mafb* mRNA levels are normally 6-fold higher and elevated further (1.6-fold) in the E18.5 *Mafa*$^{\Delta panc}$ pancreas[19, 44]. Analysis of *Mafa*$^{\Delta panc}$;*Mafb*$^{-/-}$ mice demonstrated that MafA does contribute during development to β cell gene transcription (e.g. *insulin*), but this effect was only prominent in the complete absence of MafB[19]. In contrast, all the other key islet *insulin* transcriptional activators play a critical role in pancreas development. Thus, α and β cell formation is impaired in *Pax6*$^{-/-}$ mice[58-60], a severe and general reduction in islet endocrine cell numbers is found in the *NeuroD1*$^{-/-}$ mutant[61], and β cell numbers are greatly decreased upon deletion of Pdx-1 by *insulin*-driven Cre recombinase in *Pdx-1*$^{flox/flox}$ mice[62, 63]. Hence, MafA and MafB are different from most other islet-enriched transcriptional regulators in not contributing in early cell lineage specification or terminal (β and α) cell differentiation, but only in islet cell maturation and/or function.

MafA and MafB actives genes associated with glucose-stimulated insulin secretion

MafA controls glucose-responsive transcription of *insulin* and other genes in islet β cells[22, 23, 32, 64, 65]. Elevated glucose concentrations acutely regulate MafA's ability to bind and activate through the RIPE3b1/C1 DNA element[66], a key *cis*-acting element of the

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mammalian *insulin* gene[35, 67]. MafA is a very potent activator of *insulin* transcription, with this factor independently capable of stimulating endogenous *insulin* production in αTC-6 cells (a glucagon$^+$ MafB$^+$ mouse islet α cell line) and chick embryonic endoderm *in ovo*[43, 68]. In contrast, MafB independently activates *glucagon* (and not *insulin*) production in βTC-3 cells (an insulin$^+$ MafA$^+$ islet β cell line), while an N-terminal MafB: C-terminal MafA chimera can stimulate *insulin* in early chick endoderm assays[68] (see below).

Recent gene-profiling studies conducted with E18.5 wild type and $Mafb^{-/-}$ pancreata cast light on how MafB influences β cell formation[19]. Thus, gene ontology analysis revealed that MafB regulated many genes involved in aspects of mature β cell function, such as glucose sensing (e.g. *Slc2a2*), vesicle maturation (*Slc30a8*), Ca^{2+} signaling (*Camk2b*), and insulin secretion (*Nnat*). Strikingly, many of these genes were regulated in a similar way in adult $Mafa^{\Delta panc}$ islets, even though MafB was retained in a large fraction of the mutant insulin$^+$ cell population (33.4 ± 5.6% of insulin$^+$ cells expressed MafB). ($Mafa^{\Delta panc}$ mice were generated by crossing $Mafa^{fl/fl}$ mice with transgenic mice producing Cre recombinase from the $Pdx1^{5.5}$ promoter fragment early in development and in a pancreas-wide pattern[69].) Moreover, over-expression of a dominant negative form of MafA (DN-MafA) in the rat INS-1 β cell line also supported a significant role in glucose stimulated insulin secretion[22], with this mutant inhibiting by sequester wild type MafA into a dimer complex incapable of *cis*-element binding. Thus, the mRNA levels of factors involved in metabolic-secretion coupling, proinsulin processing and glucagon-like peptide 1 receptor (GLP1R) signaling were reduced by DN-MafA (i.e. *Gck, Glut2*/*Slc2a2, Pdx1, Nkx6.1, Glp1r, Pcsk1* and *Pcx*)[22].

These results not only illustrated the functional interrelationship between these closely related large Maf transcription factors in the generation of islet β cells *in vivo*, but also provided evidence indicating that MafB and MafA do not act equivalently in these processes. Interestingly, MafA levels at P2 are only 7% of adult, and these rat islet β cells have poor glucose-stimulated insulin secretion properties. However, adenoviral-mediated over-expression of MafA in P2 islets greatly stimulated this activity, while Pdx1 infection had little effect[45]. These *in vitro* data further emphasize the significance of MafA to postnatal β cell maturation and function.

The phosphorylation status of MafA regulates DNA binding activity

Both MafA and MafB are heavily phosphorylated, a post-translational modification that impacts protein stability[70-72], transactivation[70, 71, 73], and oncogenic potential[70]. For example, a priming phosphorylation at serine (S)65 in MafA or S70 in MafB is necessary for ubiquitin-mediated degradation[70, 71]. In addition, S65 phosphorylation in MafA (and likely MafB) is required for priming Glycogen Synthase Kinase 3 (GSK3) for phosphorylation at S61, threonine (T)57, T53, and S49, which enhances transactivation and transformation potential[70]. Interestingly, this post-translational modification also differentially influences the activation properties of MafA and MafB. Thus, dephosphorylation by endogenous or exogenous phosphatases only inhibits the *in vitro* DNA-binding properties of MafA, and not MafB[74].

Analysis of MafA/B chimeras revealed that phosphatase sensitivity in these DNA binding assays was imparted by MafA sequences spanning the C-terminal dimerization region (amino acids (aa) 279-359), while the homologous MafB region (aa 257-323) enabled binding even after phosphatase treatment[74]. Mutational analysis showed that phosphorylation within the MafA (or MafB) N-terminal transactivation domain, and not the C-terminal basic leucine zipper (b-Zip) region, mediated phosphorylation-dependent MafA dimer formation and DNA binding[74]. Significantly, while only wild type MafA was able to stimulate endogenous *insulin* activation, the MafBB/A chimera containing the MafA C-terminal Zip region fused to the N-terminal MafB activation domain could impart this

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property onto MafB in the chick *in ovo* electroporation assay (Figure 3)[68]. It is possible that intramolecular interactions between the phosphoamino acid-rich transactivation and b-Zip domain not only control MafA DNA-binding activity, but also create distinctive interfaces for coregulatory proteins that are uniquely important in activation.

MafA is a positive indicator of β cell health and functionality

MafA levels appear to be a sensitive barometer of the activity status of islet β cells. Changing glucose levels can positively and negatively regulate MafA activity, processes influenced by transcriptional and/or post-transcriptional mechanisms[34, 35, 67]. Acutely elevating glucose concentration stimulates the hexosamine biosynthetic pathway and directly results in increased *Mafa* and *insulin* transcription[35, 67]. However, chronically high glucose levels have a rather specific detrimental effect on MafA activity in β cell lines and diabetic *db/db* mice[75-79]. Thus, supraphysiological (glucotoxic) glucose levels inhibit MafA and Pdx1 activity and in turn reduce insulin expression. For example, *insulin* gene transcription, insulin content and glucose-induced insulin secretion are progressively and drastically decreased in HIT-T15 β cells chronically grown for months at high, supraphysiological (11.1mM) versus a lower (0.8mM) glucose concentration[80]. The decrease in Pdx1 and MafA DNA binding activity is associated with the loss of *insulin*-driven reporter activity and *insulin* mRNA levels, with the change in MafA occurring earlier and correlated more closely with the loss in expression than Pdx1[80, 81]. A similar response was observed in the βTC-6 cell line, except that MafA activity is principally affected[77]. Moreover, palmitate (a fatty acid mediator of β cell dysfunction under hyperglycemic conditions *in vivo)* decreases in MafA and Pdx-1 DNA activity in rat islets, causing reduced *insulin* expression[82]. The effect on Pdx1 activity under these conditions appears to be caused by a change in nuclear localization, while MafA mRNA processing[80], stability[75], and cellular localization[83] have been connected with inhibition.

Chronic hyperglycemia causes β cell dysfunction in type 2 diabetes in part through the generation of excessive levels of reactive oxygen species (ROS), leading to cellular damage of macromolecules including proteins, lipids, carbohydrates, and nucleic acids. Exacerbating this problem, the low levels of antioxidant enzymes in the islet render β cells more sensitive to oxidative stress (e.g. compared to liver: superoxide dismutase, 50%; glutathione peroxidase, 1%; catalase, 1%)[84, 85]. MafA is translocated to the cytoplasm[83] and p38 MAPK-mediated degradation is increased[86] by oxidative stress. In contrast, nuclear Pdx1 levels appeared to be unaffected under these circumstances in *db/db* mice[83]. Importantly, reducing ROS by transgenic β cell over-expression of glutathione peroxidase-1 in *db/db* mice not only improved blood glucose levels to near normal, but also increased islet β cell volume, insulin granulation, and MafA nuclear content[83]. It was suggested that transgenic redox protein thioredoxin expression in *db/db* β cells also reduced cell failure in part by preventing MafA inactivation[87]. Notably, loss of MafA does not cause overt β cell dysfunction *in vivo*[19, 31], suggesting that subsequent loss/inactivity of another factor(s) leads to this condition. Collectively, these results support MafA as a key regulator of glucose-stimulated insulin secretion and β cell function, perhaps representing an early indicator of cell stress in type 2 diabetes.

MafB expression is induced in maternal β cells during pregnancy

MafB is normally lost in β cells soon after birth (Figure 2). However, expression is induced in a fraction of maternal mouse insulin^{+} cells during pregnancy[44]. This contrasts with other transcription factors mediating changes in β function and/or proliferative capacity, whose expression is uniformly distributed in the islet cell population prior to pregnancy (e.g. Hnf4α[88] and Menin[89]).

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Due to fetal demands, the mother's insulin sensitivity declines by one-third during pregnancy. Both β cell function and proliferative cell mass expansion occurs in maternal islets to adapt to the bodies increased requirements for insulin. The mother develops gestational diabetes if this does not occur, as found in 3 to 7 percent of pregnancies. Measurement of β cell BrdU incorporation illustrated that maternal MafB$^+$ β cells were not undergoing cell replication[44], different to other gestational diabetes mouse models associated with transcription factor dysfunction (Menin[89], Hnf4α[88], FoxM1[90]). This suggests that MafB may have a unique role in β cell adaptation during pregnancy, possibly serving as a pre-mitotic factor to induce β cell proliferation and/or through involvement in functional enhancement during this physiological state of insulin resistance.

The potential role of MafA and MafB in islet β cell generation from non-β cells

Due to the significance of MafA in glucose-responsive transcription and adult β function, MafA has been utilized to induce β cell differentiation in both human and rodent stem cells and differentiated cell types (Table 1). This protein alone or in combination with other pancreatic transcription factors was able to induce the expression of insulin and other key β cell markers. In most cases, the generated insulin$^+$ cells improved blood glucose levels in streptozotocin-induced diabetic animals, a mouse model of type 1 diabetes. Significantly, MafA was 1 of only 3 islet factors identified in a large transcription factor screen that together effectively reprogrammed adult pancreatic acinar cells to islet β-like cells (i.e. Pdx1, Ngn3, MafA). Notably, the resultant insulin$^+$ cells not only produced many β cell identity markers but also functionally and ultrastructurally resembled endogenous β cells[21].

The ability of MafB to collaborate with other factors to generate insulin$^+$ cells has only been tested in chick gut endoderm, wherein MafB alone or together with Ngn3 was unable to induce insulin production[68]. Significantly, MafB has only been associated with functionally immature insulin$^+$ cell populations produced during the most recognized hES differentiation procedure[16, 17]. However, these cells remained dysfunctional with regard to glucose-responsive insulin secretion until MafA production was observed, a process that presently requires transplantation *in vivo*[17]. The induction of MafA is also observed during rodent pancreas development and postnatally *in vivo*, and indicates that the MafB to MafA transition may serve as a functional read out of the β-like cells produced from stem cells and/or through transdifferentiation approaches.

Hence, MafA appears to be a potent regulator essential for the generation of β-like cells. Moreover, its actions are required over MafB in specific and distinct steps of β cell maturation. Strikingly, MafA activity is inhibited by proinflammatory cytokines implicated in the pathogenesis of diabetes mellitus, like interleukin-1β and tumor necrosis factor-α. These cytokines have been detected in the pancreas of type 1 diabetic patients as well as mouse models of disease and cause MafA inactivation, β cell dysfunction and apoptosis[91, 92]. Therefore, efforts will also need to be focused on developing inhibitors for such proinflammatory cytokines to protect newly generated therapeutic cells from inactivation.

Concluding remarks

Studies on MafA and MafB using mouse models have revealed crucial roles in islet β cell formation and function, with MafB required during development and MafA in adults. Gene expression analyses disclosed that the two closely related transcription factors regulate key β cell genes in a cooperative and sequential manner. Research in mouse models as well as *in*

vitro hES cell differentiation showed that the switch from $MafB^+$ to $MafA^+$ appears vital to functional maturation of β cells.

However, many unanswered questions remain to be addressed to completely understand how MafA and MafB work in β cells. For example, do MafA and MafB act equivalently, and thus, do differences in expression levels explain the *Mafb* and *Mafa* knockout phenotypes. If so, what is the threshold level required for these factors to successfully activate β cell gene transcription? If MafA and MafB have distinct capabilities, as suggested by biochemical and cell line-based studies, how are the differences implemented? One potential mechanism is recruitment of discrete binding co-regulatory partners. Ironically there are hundreds of coregulatory factors (http://www.nursa.org), yet only a hand full have been linked to β cells, presently limited to p300/CBP-associated factor in regards to MafA[70]. In addition, it is also vital to understand the driving force (e.g. signaling pathways) and executors (e.g. transcription factors and epigenetic factors) that silence MafB and drive high MafA expression in postnatal β cells, and how these elements can be employed in the development of β cells for therapeutic purposes. Last, but certainly not least, our information on human MAFA and MAFB function is presently limited to their actions as oncogenes in cancer (see Box1, multiple myelomas and angioimmunoblastic T-cell lymphomas)[93]. Thus, it is crucial to determine if the highly conserved human orthologs of MafA (i.e. 93% to rodent) and MafB (97%) have similar functional properties in the pancreas, since clearly the ultimate goal is to translate the knowledge obtained from model organisms to humans.

Abbreviation

Pdx1	pancreatic duodenal homeobox1
Ngn3	neurogenin 3
MafA	musculoaponeurotic fibrosarcoma oncogene family, A
MafB	musculoaponeurotic fibrosarcoma oncogene family, B
Pax6	paired box gene 6
Isl1	Islet1 transcription factor, LIM/homeodomain
Nkx2.2	NK2 transcription factor related, locus 2
MAPK	mitogen-activated protein kinases

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Box 1

The Maf transcription factor family

The Maf transcription factor family is a subgroup of the basic leucine-zipper (bZIP) family. The family members are named after v-Maf, the oncogenic component of the avian retrovirus AS42 originally isolated from a spontaneous musculoaponeurotic fibrosarcoma (Maf) in chicken[94]. Many *maf*-related genes have been cloned in human, mouse, rat, frog, quail, chick, zebrafish, and *Drosophila* by homology to the v-Maf bZIP domain[95]. In addition, Maf proteins show high conservation in the extended homology region (EHR) or ancillary DNA binding region, a small domain N-terminal to a basic amino acid rich region. The EHR in combination with this basic region imparts the DNA binding specificity to Maf proteins, which is 13-14 base pairs longer than the 8 base pair core binding site of other bZIP factors, like AP1 and CREB[96-98]. Maf proteins recognize several types of DNA-binding sequences, including palindromic Maf-recognition elements (MAREs, both T-MARE and C-MARE)[97, 98] and AT-rich plus half MAREs[99].

The Maf protein family is subdivided into two major groups based on their molecular size, small (149-162 amino acids) and large (236-370 amino acids). Small Maf proteins (i.e. MafF, MafG and MafK) all lack a distinct transactivation domain. Small Maf homodimers can compete with large Maf proteins for *cis*-element binding and repress gene transcription[100, 101]. On the other hand, they can also form heterodimers with other bZIP factors such as the Cap-n-collar (CNC) family proteins and transactivate genes important to stress signaling, hematopoiesis, CNS function, inflammatory response and oncogenesis[102]. In contrast, the large Maf proteins (i.e. MafA, MafB, c-Maf, and Nrl) are characterized by the presence of a transactivation domain in the N-terminal region. These proteins are all recognized as key regulators of tissue specific gene expression and cellular differentiation in, for example, brain, retina, lens, kidney and pancreas [93, 103]. In addition, the large Maf proteins can transform primary cells and serve as oncogenes in human cancers[93], specifically c-Maf in angioimmunoblastic T-cell lymphomas[104], and multiple myelomas[105, 106] as well as MafB in multiple myelomas[107].

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Box 2

Primary and secondary transitions during pancreas development

Hormone-expressing cells are produced during two sequential regulatory transitions[108], often referred to as the primary and secondary transition. They differ in cell ultrastructure and hormone/enzyme content. The "primary transition" (prior to E13.5 in mice) is related to organ determination, that is when the foregut region is destined to form the pancreas. The first glucagon$^+$ cells in mice are seen in the pancreatic epithelium at E9[109], with a few insulin$^+$ cells found one day later. During this early phase, only a small number of cells contain islet-like granules. The majority of pancreatic epithelial cells are at the "protodifferentiated" state, lacking secretory granules and producing low levels of terminal gene products (e.g. insulin and amylase)[108]. Although the physiological roles of these early cells are still unknown, it is generally believed that (at least) the insulin$^+$ cells are dysfunctional. Notably, they do not express the principal glucose transporter of β cells, Glut2/Slc2a2[109, 110].

The "secondary transition" is a relatively short period (between E13.5 and E15.5 in mice), characterized by an immense increase in pancreas-specific protein synthesis. The majority (if not all) islet β cells are formed during this transition[109, 111]. The protein concentrations of β cell-enriched products increase between 100- and 1,000-fold[112]. Similarly, exocrine cell differentiation is initiated at this time, accompanied by exponential increases in acinar enzyme gene expression, development of large amounts of rough endoplasmic reticulum, and formation of zymogen granules. It is also at this time that the structure of the adult organ begins to be recognizable. After the secondary transition, expansion of both the exocrine and endocrine pancreas occurs through proliferation of differentiated endocrine cell types. Endocrine cells migrate and aggregate to form islets, and become responsive to glucose after birth[113-115].

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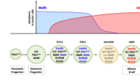

Figure 1. Roles of MafA and MafB in mouse β cells

MafA and MafB are expressed in a dynamic temporal pattern (top panel)[19, 20] and required at distinct stages in mouse β cells (bottom panels). MafB is critical to β cell terminal differentiation through regulation of key β genes (blue). Thus MafB binds and directly initiates the *insulin* and *MafA* transcription in developing β cells. In addition, it is required at E18.5 for expression of β cell factors involved in glucose-stimulated insulin secretion (e.g. *pdx1, slc2a2*, and *slc30a8*). MafA regulates most of these β cell genes (red) in adult when *MafB* in silenced.

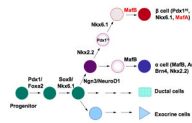

Figure 2. A cascade of transcription factors plays important roles in pancreas development

The schematic diagram is a simplified model indicating transcription factors expressed at each stage of rodent pancreas development. The large Maf transcription factors MafB and MafA are located at the bottom of the cascade. MafB is required for the terminal differentiation of α and β cells while MafA is critical for β cell function in adult.

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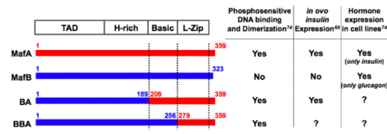

Figure 3. Summary of the differences between MafA and MafB in phosphatase-sensitive DNA binding and endogenous hormone gene activation assays

The indicated MafB/A chimeras have been tested for phosphatase-sensitive DNA binding in gel shift assays, and for their ability to activate endogenous *insulin* and *glucagon* gene expression in both the chicken *in ovo* electroporation and cell line based assays. “?”, to be determined.

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Table 1

Differentiation or transdifferentiation experiments that use MafA to generate β cells.

Model	Original cell type	Other TF* administrated	Delivery method	Conversion efficiency**	β-cell or endocrine markers induced	Other β cell characteristics	Glucose-stimulated insulin secretion/c-peptide production	Improve hyperglycemia in animals / transplanted animals	Refs
In vitro differentiatied Mouse ES cell line A2lox	stem cell line	none	doxycycline inducible transgene	n.a.	Insulin, Slc2a2, Gck,	n.a.	yes	n.a.	116
Placenta-derived multipotent stem cells	stem cell	none	lentivirus	n.a.	Insulin, Slc2a2, Nkx2.2	n.a.	yes	yes	117
Live mouse	exocrine cell	Pdx1, Ngn3	adenovirus injection	>20%	Insulin, Slc2a2, Gck, Pcsk1, Nkx6.1, NeuroD, Nkx2.2	cell size, ultrastructure	n.a.	yes	21
AR42J-B13 cells	pancreatic progenitor-like cell line	Ngn3, Nkx6.1	adenovirus	n.a.	Insulin, Slc2a2, Pdx1, Nkx2.2	n.a.	n.a.	n.a.	118
Live mouse	hepatocyte	Ngn3, NeuroD2	adenovirus injection	0.5-1%	Insulin	n.a.	n.a.	yes	119
Live rat	intestinal epithelial cell	none	oral administration of adenovirus	n.a.	Insulin, Pdx1, Pcsk1, Kir6.2	n.a.	n.a.	yes	120
Chicken embryo	gut endoderm cells	alone or with Ngn3	electroporation	n.a.	Insulin	n.a.	n.a.	n.a.	68

*TF, transcription factors.

**Conversion efficiency is determined as percentage of insulin$^+$ cells over the infected/electroporated cells.