Trends in
Endocrinology & Metabolism

CellPress

Review

# Transcriptional mechanisms of pancreatic β-cell maturation and functional adaptation

Matthew Wortham[1] and Maike Sander[1,*]

**Pancreatic β-cells secrete insulin commensurate to circulating nutrient levels to maintain normoglycemia. The ability of β-cells to couple insulin secretion to nutrient stimuli is acquired during a postnatal maturation process. In mature β-cells the insulin secretory response adapts to changes in nutrient state. Both β-cell maturation and functional adaptation rely on the interplay between extracellular cues and cell type-specific transcriptional programs. Here we review emerging evidence that developmental and homeostatic regulation of β-cell function involves collaboration between lineage-determining and signal-dependent transcription factors (LDTFs and SDTFs, respectively). A deeper understanding of β-cell SDTFs and their cognate signals would delineate mechanisms of β-cell maturation and functional adaptation, which has direct implications for diabetes therapies and for generating mature β-cells from stem cells.**

## β-Cell function and identity are regulated by distinct processes

Insulin is the major glucose-lowering hormone whose relative or absolute deficiency underlies all forms of diabetes. Insulin is exclusively produced by pancreatic β-cells, which secrete insulin in a precisely controlled manner corresponding to the nutrient state of the organism. In type 2 diabetes (T2D), β-cells fail to respond to nutrients and cannot secrete sufficient amounts of insulin to maintain normoglycemia, while in type 1 diabetes (T1D) β-cells are destroyed by an autoimmune mechanism. Understanding how β-cell function is acquired and regulated is crucial for designing therapies to restore glucose control in diabetes. In particular, the mechanisms underlying acquisition of β-cell function have been the focus of recent interest given efforts to generate functional human β-cells from pluripotent stem cell sources. While the process of β-cell differentiation is fairly well understood [1,2], less is known about the process that equips the β-cell with the ability to regulate insulin secretion. It is clear from a large body of work that the acquisition of **β-cell identity** (see Glossary) is necessary but not sufficient for nutrient-stimulated insulin secretion, underscoring the major knowledge gap in understanding how insulin secretion first becomes coupled to nutrients in a process termed **functional maturation**. There is emerging evidence that environmental signals play an important role in β-cell functional maturation. Moreover, mature β-cells constantly monitor the nutrient environment and adjust the insulin secretory response according to changes in organismal nutrient state (**functional adaptation**). Therefore, the insulin secretory response is not a static property of mature β-cells but rather is actively fine-tuned in response to extracellular signals. Altogether, environmental signals modulate the insulin secretory response throughout lifespan (β-cell **functional plasticity**) indicating that the functional output of the β-cell at a given moment results from the interplay between extracellular signals and intrinsic properties of the β-cell.

In this review, we discuss evidence for the concept that β-cell functional plasticity is governed by environmental signals that alter gene transcription through **signal-dependent transcription factors (SDTFs)**. SDTFs are transcription factors (TFs) with dynamic activity or expression dependent upon environmental signals, exemplified by Bmal1, Creb, NFATc1, and Pparγ (Figure 1A and Table 1). We propose the effects of extracellular signals upon the insulin secretory

## Highlights

Lineage determining transcription factors (LDTFs) are required for β-cell differentiation, yet acquisition of β-cell identity is not sufficient for glucose-stimulated insulin secretion.

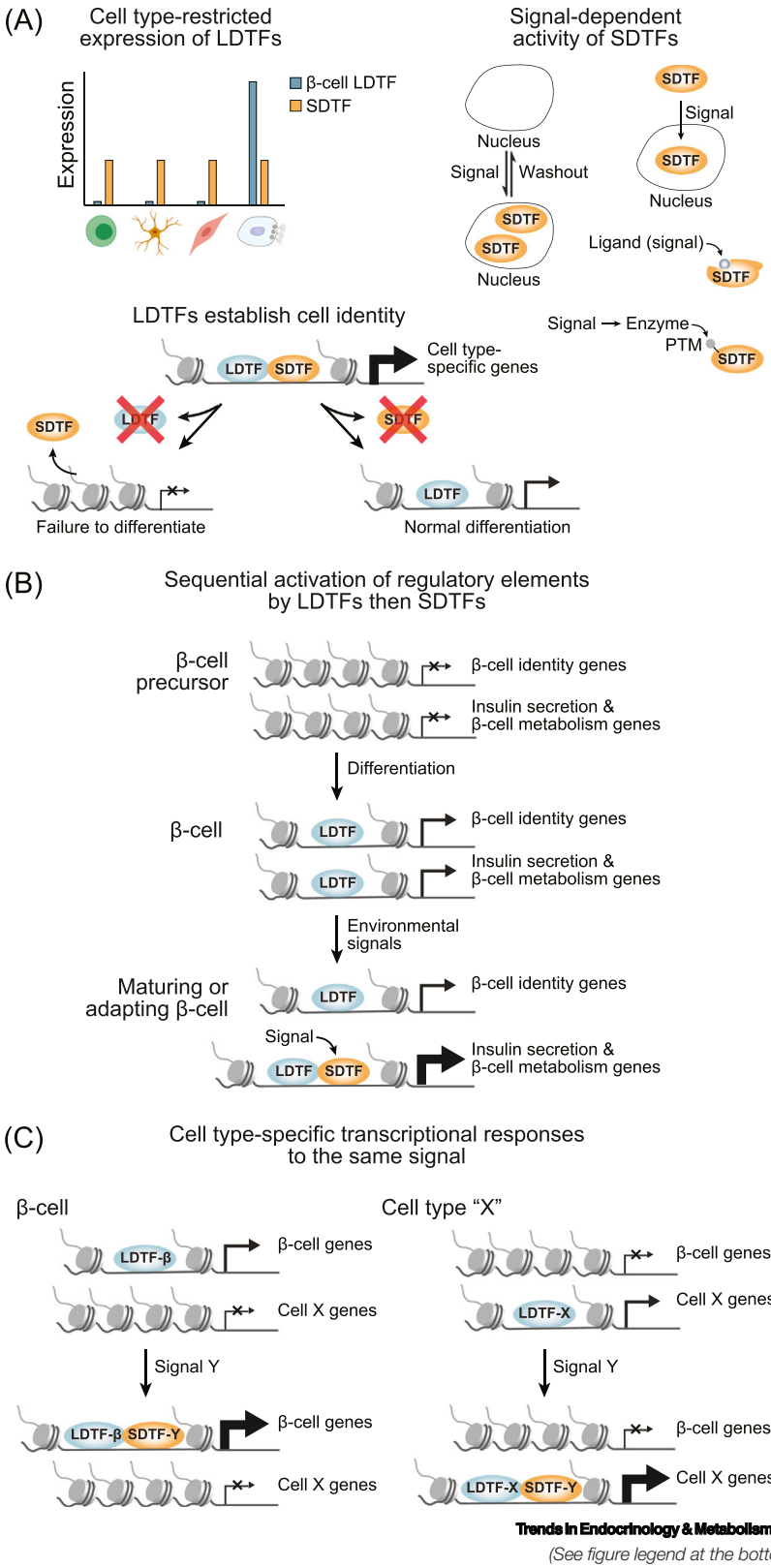
Environmental signals that regulate signal-dependent transcription factors (SDTFs) govern acquisition and adaptation of glucose-stimulated insulin secretion.

β-Cell function is acquired in a stepwise manner whereby LDTFs initiate expression of cell type-characteristic genes, then SDTFs fine-tune gene expression to confer metabolic and functional properties to β-cells.

LDTFs guide the recruitment of SDTFs to provide cell type specificity to the transcriptional effects of environmental signals in neonatal and adult β-cells.

[1]Departments of Pediatrics and Cellular & Molecular Medicine, Pediatric Diabetes Research Center, University of California, San Diego, La Jolla, CA 92093, USA

*Correspondence:
masander@ucsd.edu (M. Sander).




## Glossary

**β-Cell identity:** the capability to express insulin and genes that participate in insulin processing, granule formation, and exocytosis.

**Disallowed genes:** genes selectively repressed in islets or β-cells compared with other tissues.

**Functional adaptation:** an increase or decrease of the insulin secretory response per β-cell.

**Functional maturation:** acquisition of glucose-responsive insulin secretion and reduction of basal insulin secretion by the β-cell.

**Functional plasticity:** a blanket term referring to both β-cell functional adaptation and functional maturation.

**Lineage-determining transcription factor (LDTF):** a constitutively active, sequence-specific transcription factor (TF) exhibiting a restricted expression pattern that is typically required for differentiation and maintenance of cell identity.

**Signal-dependent transcription factor (SDTF):** a TF broadly expressed or induced in diverse cell types that is activated by extracellular stimuli.

*(See figure legend at the bottom of the next page.)*

response are borne out through the interplay between environment-sensing SDTFs and cell type-selective **lineage-determining transcription factors (LDTFs)**. LDTFs are defined as TFs with restricted expression patterns that confer cell identity by promoting the expression of cell type-specific genes during differentiation [3] (Figure 1A). These TFs function in tandem with developmental regulation of chromatin state to establish the gene regulatory landscape in a cell, thereby creating a permissive context for later transcriptional regulation [3–6]. Once established during differentiation, gene regulatory elements can be acted upon by SDTFs, which are capable of responding to changes to the extracellular environment but do not directly impact cell identity [3] (Figure 1A,B). In essence, LDTFs provide genomic 'addresses' for recruitment of SDTFs, which fine-tune transcription of cell type-specific genes in response to environmental signals [3]. While SDTFs are activated by environmental signals in diverse cell types, their recruitment to the genome by LDTFs allows environmental signals to regulate the specialized functions of a cell [3,7] (Figure 1A,C). Upon differentiation, LDTFs endow the β-cell with its defining features including expression of insulin-processing enzymes and the insulin gene in addition to characteristic metabolic enzymes, ion channels, and the exocytotic machinery, as exemplified by the metabolic enzyme *Pcx* [1] (Table 1 and Box 1). However, despite possessing the requisite machinery for insulin secretion, newly differentiated β-cells are not yet capable of coupling the insulin secretory response to plasma glucose levels [8–10]. The time period spanning birth to early adulthood is associated with extensive changes in diet, nutrient metabolism, and the hormonal milieu, and a growing body of work has indicated that β-cells mature by sensing and responding to these changes in their environment. During functional maturation, environmental signals act upon cell type-specific gene regulatory programs acquired prenatally to regulate genes involved in insulin secretion, thereby promoting acquisition of glucose-stimulated insulin secretion (GSIS). Similarly, in mature β-cells, nutrient signals modulate the insulin secretory response through transcriptional regulation of β-cell-characteristic genes, as we have reviewed in detail elsewhere [11]. In this review, we synthesize evidence that the transcriptional program established by LDTFs is modified by environment-dependent SDTFs to promote β-cell maturation and adapt the insulin secretory response to changing nutrient environments (Figure 1). Collaborative transcriptional control by LDTFs and SDTFs was originally proposed for immune cells [3] (Figure 1). Here, we utilize the proposed model of LDTF-SDTF collaboration as a conceptual guide for the interpretation of a growing body of work regarding β-cell functional plasticity. This model can be summarized by two core properties that are largely consistent with the β-cell literature: (i) gene regulation should occur in a stepwise fashion first involving the establishment of cell type-specific regulatory elements through the activities of LDTFs followed by the adjustment of transcription via environment-dependent SDTFs (Figure 1B), and (ii) SDTFs should be capable of directly regulating cell type-specific genes due to their recruitment being guided by LDTFs, and in this way LDTF-SDTF collaboration confers cell type-specific responses to environmental signals (Figure 1C). Recurrent findings from studies of β-cell functional plasticity broadly support these properties of SDTF-LDTF collaboration in regulation of the insulin secretory response.

Figure 1. Mechanisms of transcriptional regulation of β-cell identity and functional plasticity. (A) Defining properties of lineage-determining transcription factors (LDTFs) and signal-dependent transcription factors (SDTFs). Although it is possible for transcription factors (TFs) to exhibit characteristics of both classes, these rules classify the vast majority of TFs studied in the context of β-cells (see Table 1). Top left, LDTFs are expressed in restricted numbers of cell types. Bottom left, LDTFs are required for differentiation of specific cell types. Right, activity or expression of SDTFs are dynamically regulated by extracellular signals. PTM, post-translational modification. (B) β-Cell-characteristic genes are activated in a stepwise manner during development and maturation first involving establishment of gene regulatory programs by LDTFs followed by fine-tuning of transcription in response to environmental signals by SDTFs. (C) LDTFs provide cell type specificity to the response to environmental signals by directing SDTFs to cell type-specific gene regulatory elements. This figure was created using BioRender (https://biorender.com/).

Table 1. Lineage-determining and signal-dependent transcription factors in the β-cell[a]

| TF | Class | Known regulators[b] | Process regulated during β-cell maturation | Process regulated in adult β-cells | Refs |
|---|---|---|---|---|---|
| Foxa2 | LDTF | | Cell identity | Regulation of GSIS | [1,2,78] |
| Glis3 | LDTF | | Cell identity | Cell survival | [1,2] |
| Hnf1α | LDTF | | GSIS | | [1,2] |
| Hnf4α | LDTF | | Regulation of GSIS | GSIS | [1,2,79] |
| Insm1 | LDTF | | Cell identity | GSIS | [1,2,35] |
| Isl1 | LDTF | | Cell identity, proliferation | *Ins1/2* transcription, GSIS | [1,2] |
| MafB | LDTF | | Cell identity | GSIS (human only) | [1,2,26] |
| NeuroD1 | LDTF | | Cell identity | GSIS | [1,2,80] |
| Nkx2.2 | LDTF | | Cell identity | Cell identity | [1,2,81] |
| Nkx6.1 | LDTF | | Cell identity, proliferation | Insulin processing | [1,2,71] |
| Pax6 | LDTF | | Cell identity | Cell identity | [1,2] |
| Pdx1 | LDTF | | Cell identity, proliferation | Cell identity | [1,2,82] |
| Rfx3 | LDTF | | Cell identity | | [1] |
| Rfx6 | LDTF | | Cell identity | GSIS | [1,2,76] |
| Atf3 | SDTF | cAMP | | *Ins1/2* transcription, GSIS | [83] |
| Atf4/5 | SDTF | ER stress | | Proliferation, survival | [84] |
| Bmal1 | SDTF | Circadian clock | GSIS | GSIS | [38,42] |
| Creb/Crtc2 | SDTF | Glp-1 via cAMP | | GSIS, survival | [27,56] |
| Egr1 | SDTF | Fatty acids | Proliferation | GSIS | [85] |
| Errγ | SDTF | Wnt4 | GSIS | GSIS | [30,43] |
| Fos/AP-1 | SDTF | cAMP, growth factors | Proliferation | GSIS | [86] |
| FoxO1 | SDTF | ROS | | Cell identity, GSIS | [11,46,48] |
| FoxO3/4 | SDTF | | | GSIS | [87] |
| MafA | SDTF | T3, cAMP, glucose | GSIS | GSIS | [24,25] |
| c-Myc | SDTF | $Ca^{2+}$, mTOR, PKCζ | Proliferation, basal insulin secretion | Proliferation | [74,88] |
| NFATc1/2 | SDTF | $Ca^{2+}$-Cn | Insulin processing, proliferation | Proliferation, GSIS | [21,89–91] |
| Nr4a1 | SDTF | ER stress, fatty acids | Proliferation | GSIS | [52,53] |
| p53 | SDTF | DNA damage | | Regulation of GSIS | [92] |
| Pparα | SDTF | Fatty acids[c] | | GSIS | [11] |
| Pparγ | SDTF | Fatty acids[c] | | GSIS | [47] |
| Pparδ | SDTF | Fatty acids[c] | | GSIS | [11,54] |
| Rev-erbα | SDTF | Circadian clock, heme[c] | | GSIS, proliferation | [93] |
| SIX2/3 | SDTF | Glucocorticoids | GSIS (human only) | | [36,37] |
| Smad2/3 | SDTF | Gdf11 | Insulin production, GSIS | Insulin production | [59,94] |
| Srebp1 | SDTF | Insulin, fatty acids | | GSIS | [11] |
| Srf | SDTF | cAMP, growth factors | Proliferation | GSIS | [60] |
| ThrA/B | SDTF | T3[c] | Proliferation, GSIS | | [25] |

## The mechanism of glucose-stimulated insulin secretion

β-Cell nutrient sensing primarily occurs through regulated glucose metabolism, which enables tight coupling between circulating glucose levels and insulin release [12] (Figure 2A,B). To achieve tight regulation of glucose metabolism, mature β-cells express metabolic enzymes and transporters whose activities are dynamic within the range of physiological substrate concentrations. Glycolysis produces ATP via substrate-level phosphorylation by pyruvate kinase [13] and also generates pyruvate, which enters the tricarboxylic acid cycle to stimulate oxidative phosphorylation (OxPhos). The increase of the ATP:ADP ratio resulting from OxPhos and pyruvate kinase activity closes $K_{ATP}$ channels on the plasma membrane, thereby inhibiting $K^+$ currents and depolarizing the cell. Voltage-gated $Ca^{2+}$ channels open in response to plasma membrane depolarization, resulting in $Ca^{2+}$ influx and activation of the insulin exocytotic machinery, thereby initiating insulin secretion (Figure 2B). The effectiveness of $Ca^{2+}$ in promoting insulin vesicle exocytosis is further modulated by signal transduction pathways and mitochondrial metabolites termed metabolic coupling factors [12,14] (Figure 2B).

In newborns, glucose metabolism in the β-cell is constitutive rather than glucose-regulated, which causes a constitutive yet partial activation of $Ca^{2+}$ influx at glucose levels spanning the physiological range [15] (Figure 2B). Incomplete restriction of glucose metabolism to the mitochondria results in low rates of OxPhos as well as insufficient production of mitochondrial metabolites that promote insulin secretion [15] (Figure 2B). In the following text, we summarize current knowledge of how β-cells rewire their metabolism to acquire the glucose-sensing mechanism characteristic of mature β-cells. We posit that glucose sensing is acquired by the exposure of β-cells to environmental signals which evoke gene expression changes via activation of SDTFs. As embryonic β-cells already express a full compendium of LDTFs, such as Foxa2, NeuroD1, Nkx2.2, Nkx6.1, and Pdx1 [1], LDTFs alone are not sufficient for the acquisition of GSIS. The processes involved in conferring β-cell identity through LDTFs have been extensively reviewed elsewhere [1]. Here, we focus on SDTFs and their role in equipping the β-cell with its characteristic functional properties.

## Postnatal acquisition of the nutrient-sensing machinery

β-Cells first respond to glucose stimulation in the physiological range at 1–2 weeks of age in rodents [16,17] and as early as 1 year of age in humans [10] (Figure 2A). This capability requires metabolic remodeling involving acquisition of tightly regulated glucose oxidation [18] leading to reduction of basal insulin secretion and enhanced insulin secretion in stimulatory glucose (Figure 2A,B). These functional changes coincide with upregulation of metabolic genes characteristic to the mature β-cell [8,17,19]. Maturation of the β-cell metabolic program requires signals from the extracellular environment that activate SDTFs (Figure 2C and Table 1). Postnatal increases in circulating glucose are required for acquisition of GSIS in part through the $Ca^{2+}$-activated SDTF NFATc1 [16,20,21] (Figure 2C). NFATc1 binds to the promoters and regulates expression of β-cell-characteristic genes involved in glucose metabolism, such as *Gck* and *Slc2a2*, suggesting it is recruited by β-cell LDTFs that initiate expression of these genes. Additional fine-tuning of β-cell metabolism involves selective repression of a set of **disallowed genes** whose expression would enable metabolic reactions that are constitutively active at physiological nutrient levels or would shunt nutrients away from signal-generating pathways [22] (Figure 2B and Box 2). This class

Notes to Table 1:
[a]Abbreviations: cAMP, cyclic AMP; ER, endoplasmic reticulum; GSIS, glucose-stimulated insulin secretion; ROS, reactive oxygen species; T3, thyroid hormone.
[b]Regulatory relationships between LDTFs during β-cell differentiation have been reviewed in detail elsewhere [2] and are not shown here.
[c]Nuclear receptor ligand.

Box 1. Example of a gene regulated by SDTF-LDTF collaboration

Collaboration between LDTFs and SDTFs enables cells to adjust the expression level of cell type-specific genes, with LDTFs establishing a permissive chromatin state during differentiation and SDTFs fine-tuning transcriptional output. This concept is exemplified in the β-cell by the *Pyruvate carboxylase* (*Pcx*) gene, which encodes a metabolic enzyme responsible for generating mitochondrial metabolites that promote insulin secretion. In rodents, *Pcx* expression increases over the course of functional maturation and during compensation for insulin resistance [8,49]. β-Cell LDTFs bind to cell type-specific enhancers of the *Pcx* gene (shown as a genome browser snapshot of mouse *Pcx* in Figure I, data from [27,35,46,71,72]) and are collectively required for *Pcx* expression [35,71]. *Pcx* transcription is further enhanced during functional maturation by the SDTF MafA and during adaptation by several SDTFs including Pparγ and the Creb/Crtc2 complex [47,56,73]. MafA and Pparγ are not shown in the genome browser snapshot due to the absence of high-quality ChIP-seq datasets for these SDTFs in mouse islets.

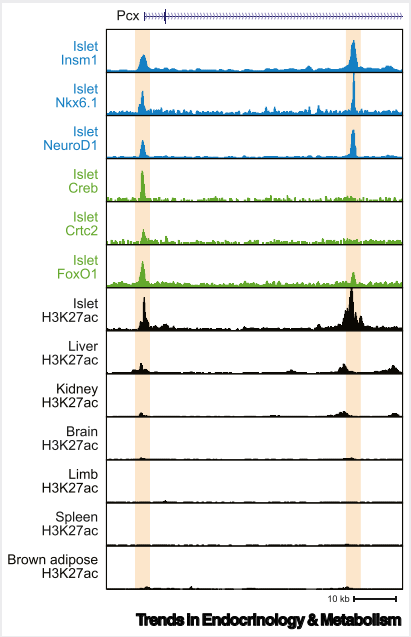



Figure I. LDTFs and SDTFs co-bind islet-specific regulatory elements of the *Pcx* gene.

of genes is exemplified by genes encoding the low $K_m$ glucose-phosphorylating enzymes Hk1-3. Gene disallowance is achieved in part by the SDTF MafA, which represses these genes [23,24]. A spike in thyroid hormone production in the first weeks of life (in rodents) increases MafA expression in the β-cell, leading to repression of disallowed genes as well as increased expression of β-cell-characteristic metabolic genes [24,25] (Figure 2C). MafA directly interacts with Pdx1 [26] and colocalizes in the genome with Pdx1 along with Foxa2 and NeuroD1 [95], suggesting these LDTFs guide recruitment of the SDTF MafA to gene regulatory elements in the β-cell. Altogether, metabolic remodeling associated with the earliest stages of β-cell functional maturation requires the concerted activities of LDTFs intrinsic to the β-cell and SDTFs responsive to changes in the postnatal environment.

The expression levels of OxPhos genes continuously increase throughout the juvenile period, suggesting these changes contribute to increasingly robust activation of $Ca^{2+}$ influx in response to glucose [29,30] (Figure 2B). Prepubescent rodent or human islets secrete less insulin than adult islets, which has been attributed to changes in expression of mitochondrial metabolic genes [29,31,32]. The transition from a milk fat-based diet to a carbohydrate-based diet during

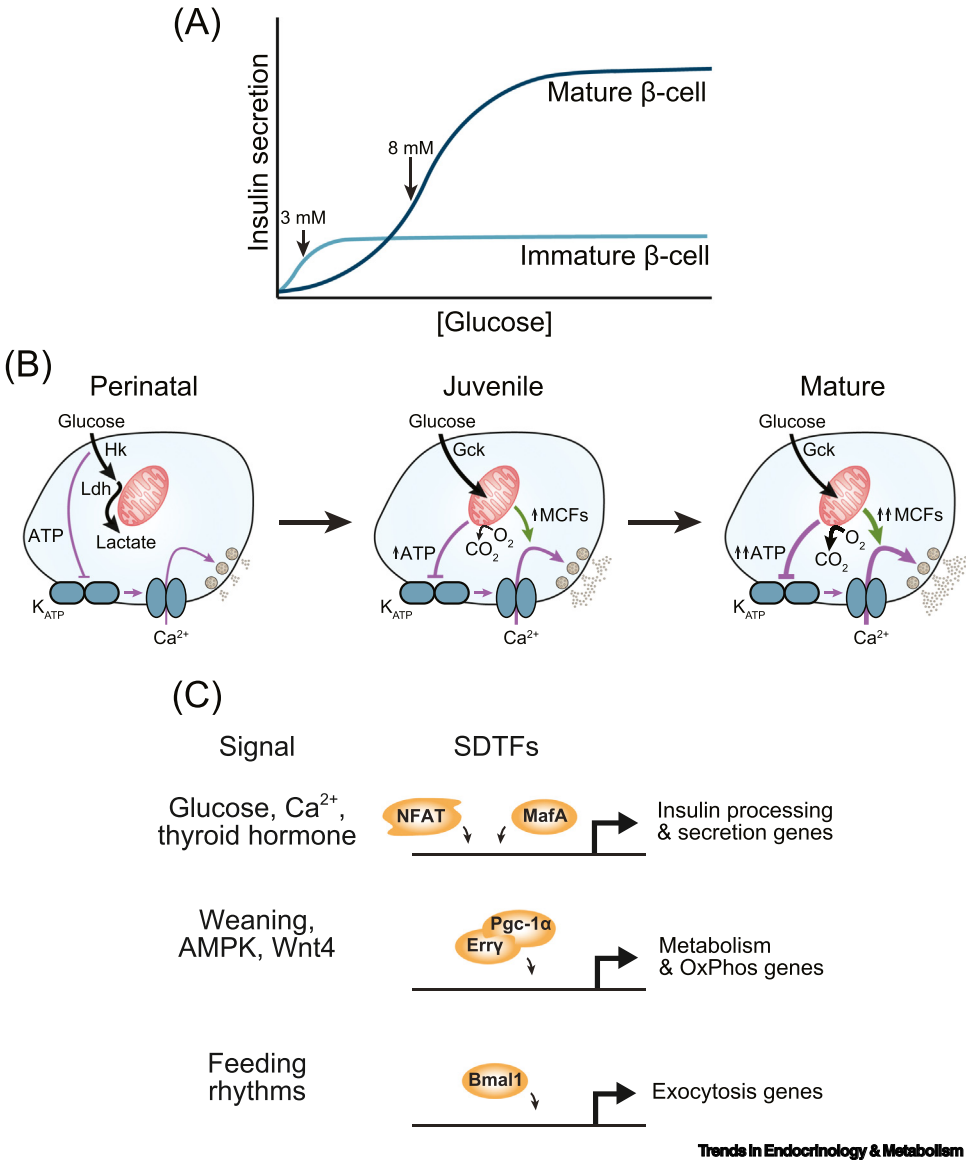



Figure 2. Transcriptional regulation of β-cell maturation. (A) The relationship between insulin secretion and glucose concentration for mature and immature β-cells, approximated from observations in [16,30]. (B) Schematic of the metabolic and functional changes of β-cells during maturation. MCFs, metabolic coupling factors. (C) Signals and their cognate signal-dependent transcription factors (SDTFs) involved in β-cell maturation. Abbreviation: OxPhos, oxidative phosphorylation.

weaning provides nutrient signals to SDTFs that promote maturation of the β-cell metabolic program. Premature weaning of mice to a chow diet accelerates β-cell functional maturation [17]. Conversely, weaning mice instead to a high-fat diet – mimicking the fat content of milk – delays the acquisition of GSIS, indicating that the change in diet composition during weaning plays a key role in β-cell maturation. Weaning to a chow diet is associated with the activation of AMPK signaling, which leads to upregulation of Pgc-1α. Pgc-1α is a coactivator of SDTFs that promotes mitochondrial function and biogenesis [33] (Figure 2C). In β-cells, the Pgc-1α-activated SDTF

Box 2. Disallowed genes

The repression of disallowed genes is necessary to establish the β-cell-characteristic metabolic program during functional maturation. As gene disallowance involves transcriptional repression rather than activation, it is unclear whether this process adheres to the properties of SDTF-LDTF collaboration. The SDTF c-Myc is known to promote expression of disallowed genes such as *Hk3* in immature β-cells, thereby contributing to the high basal insulin secretion characteristic of these cells (see Figure 2A in main text). c-Myc protein decreases in abundance as β-cells mature, leading to a reduction of basal insulin secretion in part due to downregulation of disallowed genes [74]. Postnatal reductions in circulating amino acids are likely signals leading to degradation of c-Myc protein during β-cell functional maturation [74,75]. However, because the absence of c-Myc protein is not specific to β-cells, it does not fully explain specific repression of β-cell disallowed genes. While several LDTFs including Insm1 [35], NeuroD1 [35], and Rfx6 [76] directly repress disallowed genes, these TFs are expressed much earlier than the onset of disallowed gene repression, suggesting additional mechanisms confer repressive activities to these TFs later in β-cell maturation (see Outstanding questions). Other mechanisms of disallowed gene repression that act in tandem with repressive LDTFs include microRNAs that specifically target disallowed genes [17] and epigenetic mechanisms that render the promoters of disallowed genes refractory to further regulation in mature β-cells [77]. Due to their epigenetic repression in mature β-cells, regulation of disallowed genes is restricted to the maturation process and is not thought to contribute to β-cell functional adaptation.

Errγ has been identified as an important regulator of mitochondrial metabolic genes whose expression increases at this stage of β-cell maturation [30,34]. In addition to its canonical target genes involved in OxPhos, Errγ regulates β-cell-specific genes involved in insulin vesicle trafficking and exocytosis [30]. How Errγ is recruited to its target sites in β-cells is still unknown. The LDTF Insm1 is a candidate, as Insm1 has been shown to bind to the promoters of exocytotic genes *Rab3a* and *Vamp2* that are also regulated by Errγ [35]. SIX2 is an SDTF shown to promote the expression of OxPhos genes in human β-cells between adolescence and adulthood [36,37], consistent with the onset of SIX2 expression in β-cells during adolescence and the upregulation of SIX2 in adulthood [31]. SIX2 also regulates β-cell-characteristic genes involved in insulin processing and exocytosis [36,37]. Analysis of genomic SIX2 binding sites in β-cells revealed enrichment of the binding motif for the LDTF MAFB, suggesting that MAFB could guide SIX2 recruitment.

The transition to solid food is also characterized by the onset of rhythmic feeding as opposed to constant nutrient intake during suckling. These changes in the pattern of nutrient intake provide entrainment signals to TFs in the core circadian clock that promote β-cell functional maturation [38] (Figure 2C). Cell-intrinsic circadian rhythms are driven by the core circadian clock, which is comprised of TFs engaged in a self-sustaining feedback loop. Analysis of binding for core clock TFs (i.e., Bmal1 and Rev-erbα) in different tissues has shown highly tissue-specific patterns of recruitment [39,40]. Motif analysis further revealed enrichment of motifs for tissue-specific LDTFs at Bmal1 and Rev-erbα binding sites [39–41]. Thus, cell type specificity of circadian gene regulation involves LDTF-mediated recruitment of core clock SDTFs resulting in tissue-specific binding distribution. The core circadian clock is not functional in β-cells of newborn mice but rather develops with the acquisition of glucose responsiveness [38]. Supporting a direct role for core circadian clock TFs in β-cell maturation, β-cell deletion of *Bmal1* prevents the acquisition of GSIS [38]. In β-cells Bmal1 has been shown to bind to the genome coincident with the LDTF Pdx1 to promote rhythmic expression of genes involved in metabolism and insulin exocytosis [42] (Figure 2C), suggesting Pdx1 guides recruitment of Bmal1 in the β-cell.

Despite recent advances, much remains unknown about how SDTFs sense and respond to changes in the environment during β-cell functional maturation. For many SDTFs, the compendium of signals that activate them in β-cells is missing or incomplete (Table 1). For example, while culture models have identified some regulators of SDTFs involved in β-cell maturation, including regulation of Errγ by Wnt4 [43] and of SIX2 by glucocorticoids [44], it remains an

open question whether this holds true at physiological concentrations of these signals *in vivo*. Identification of the physiological signals regulating SDTFs and the mechanisms whereby these signals are propagated to the nucleus will be necessary to build a complete understanding of how β-cells acquire and adapt the insulin secretory response.

## Transcriptional mechanisms of β-cell functional adaptation

In mature β-cells, changes in nutrient state are an important environmental cue for adapting the insulin secretory response to changes in organismal insulin demand, which we have reviewed in detail elsewhere [11] (Figure 3A). This adaptive response is necessary to avoid hypoglycemia in the fasted state and to prevent glucose intolerance during insulin resistance. Recurrent observations suggest that fluctuations of nutrient state in adulthood lead to quantitative changes in transcription through a pre-existing network of LDTFs that direct binding of SDTFs, thereby providing the β-cell with the ability to adjust its function in response to changing insulin demands (Table 1). Indeed, assessment of active chromatin in islets of high-fat diet-fed mice [45] or *db/db* mice [46] revealed that changes in nutrient state predominantly affect pre-existing regulatory elements rather than activate regulatory elements *de novo*. This model is consistent with epigenomic profiling of human islets indicating that enhancers unique to the β-cell are co-bound by several β-cell LDTFs and are highly enriched for binding motifs of SDTFs such as AP-1 [28] (Table 1). Mechanistic studies of the reactive oxygen species (ROS)-activated SDTF FoxO1 further support a role for SDTFs in fine-tuning transcription of LDTF-bound regulatory elements during functional adaptation. In obesity, ROS production leads to nuclear translocation of FoxO1, leading to regulation of β-cell-characteristic metabolic genes [46–48]. FoxO1 colocalizes in the genome with β-cell LDTFs such as Pdx1 and NeuroD1 [46], suggesting these LDTFs guide recruitment of FoxO1 to allow this SDTF to regulate genes involved in insulin secretion. Thus, analogous to β-cell maturation, β-cell functional adaptation involves regulation of genes involved in insulin secretion by environmental signals and cognate SDTFs [11]. The gene regulatory programs of β-cell maturation and functional adaptation have similarities but are not identical. Unique to β-cell maturation is the repression of disallowed genes (Box 2), whereas both processes involve changes in β-cell intracellular glucose metabolism [11,49–51] (Figure 3B). During obesity, moderate lipid accumulation in the β-cell activates several SDTFs that promote adaptive insulin secretion through metabolic remodeling including Nr4a1 [52,53], Pparγ [47], and Pparδ [11,54] (Figure 3C).

How an SDTF expressed in several tissues orchestrates cell type-specific transcriptional responses as a result of its recruitment by LDTFs is best illustrated by the SDTF Creb, which promotes adaptive insulin secretion in β-cells. The second messenger cAMP, which activates Creb, evokes distinct physiological responses in different cell types in part through cell type specificity of Creb target genes (Figure 3C). For example, while cAMP enhances the insulin secretory response of β-cells, in hepatocytes cAMP promotes gluconeogenesis [27,55,56]. These differences have been attributed in part to cell type specificity of genomic Creb binding sites [27]. In β-cells, the LDTF NeuroD1 recruits Creb to gene regulatory elements, thereby enabling Creb to regulate β-cell-specific genes involved in insulin secretion [27]. Knockdown of NeuroD1 reduces Creb binding at β-cell-specific sites and interferes with transcriptional activation of Creb targets unique to the β-cell without disrupting overall Creb function. Ectopic NeuroD1 expression in pancreatic exocrine cells leads to recruitment of Creb to β-cell-specific regulatory elements [27]. The example of NeuroD1 and Creb illustrates that LDTFs provide genomic 'addresses' to SDTFs to enable tissue-specific transcriptional responses to second messengers (Figure 1C). Deeper investigation of SDTF-LDTF complexes in β-cells holds promise for identifying regulatory programs capable of modulating specific aspects of β-cell function.

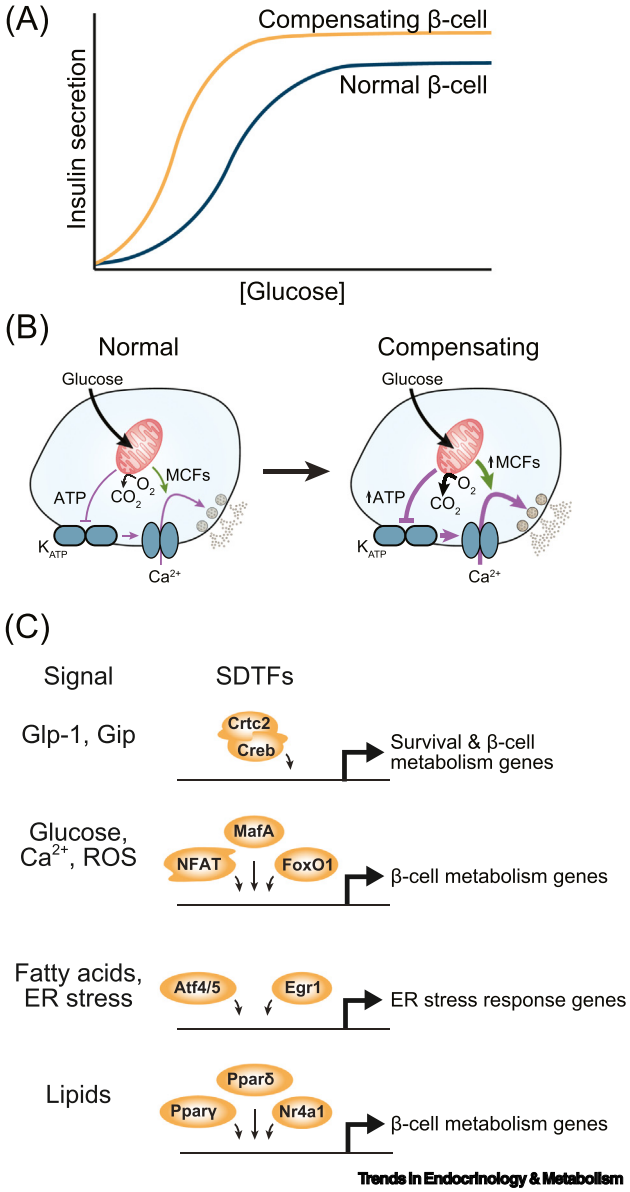



Figure 3. Transcriptional regulation in the β-cell during functional adaptation. (A) The relationship between insulin secretion and glucose concentration during compensation for insulin resistance relative to normal β-cells, based on observations in [50,51]. (B) Schematic of the metabolic and functional changes in β-cells during compensation for insulin resistance. MCFs, metabolic coupling factors. (C) Signals and their cognate signal-dependent transcription factors (SDTFs) involved in compensation for insulin resistance. ER, endoplasmic reticulum; ROS, reactive oxygen species.

## Relevance of collaborative transcriptional regulation to therapeutic strategies in diabetes

Intensive study of pancreatic development for the purpose of developing β-cell replacement therapies has led to the design of pluripotent stem cell differentiation protocols that generate insulin-producing cells expressing nearly a full complement of β-cell LDTFs [57]. Despite these advances, β-cells produced by current differentiation protocols exhibit several functional defects, including high basal insulin secretion, lower first phase insulin secretion compared with primary β-cells, and reduced or absent second phase insulin secretion [57–59]. As reviewed here, environmental signals and their cognate SDTFs play fundamental roles in acquisition and adjustment of the insulin secretory response. However, our understanding of SDTFs in the β-cell represents a major knowledge gap in β-cell biology that lags behind that of β-cell LDTFs. The catalog of SDTFs

necessary for β-cell functional plasticity is almost certainly incomplete. Unbiased analysis of genes correlating with maturation state of single β-cells suggests a role for a number of SDTFs including Atf3, Srf, and the AP-1 family TFs in β-cell functional maturation [60]. Our group recently compared motif enrichment at active chromatin sites in islets from fed and fasted mice, which revealed AP-1 and ETS families as candidate SDTFs for mediating β-cell functional adaptation [61]. The recent advent of technologies for mapping chromatin state at the single cell level [62] should aid the unbiased identification of SDTFs involved in the regulation of β-cell functional plasticity. Application of these emerging technologies to conditions associated with β-cell functional plasticity should shed light onto this process and help identify strategies for promoting maturation of β-cells derived from pluripotent stem cells or enhancing functional adaptation of endogenous β-cells.

The environment of incipient diabetes has been shown to activate SDTFs that promote β-cell dysfunction, which could be targets for diabetes prevention. Activation of gene regulatory elements in response to cytokine treatment mimicking the inflammatory environment of T1D revealed that LDTFs can direct recruitment of SDTFs mediating β-cell dysfunction such as IRF family TFs [63]. Similarly, the systemic environment of T2D can activate maladaptive transcriptional programs through SDTFs that impair insulin secretion [64,65]. Animal models of severe T1D or T2D have additionally revealed impaired expression of LDTFs and cell type-specific genes in the β-cell together with ectopic expression of non-pancreatic hormones [66–68]. The extent to which these phenomena also occur in human diabetes is under intense investigation. Nevertheless, these findings suggest that the environments of T1D and T2D disrupt β-cell function and identity in a process distinct from a simple reversal of developmental β-cell differentiation and maturation. Further exploration of how β-cell transcriptional regulation is remodeled by the stressful environments associated with T1D and T2D has potential to reveal novel pathogenic mechanisms and therapeutic targets.

## Outstanding questions

Are SDTFs appropriately activated during *in vitro* differentiation of β-cells from pluripotent stem cells? If not, what environmental signals or SDTFs are missing from current β-cell differentiation protocols?

How do TFs that normally activate gene expression repress disallowed genes during β-cell maturation?

What is the role of the epigenome in the response to environmental signals promoting β-cell functional plasticity?

What are the upstream signals that couple environmental cues to SDTF activity in β-cells?

How do SDTFs contribute to β-cell failure in T1D and T2D?

## Concluding remarks

Glucose-regulated insulin secretion is acquired in a process distinct from β-cell differentiation in part through environmental signals, and insulin secretion is continually adjusted throughout the lifespan in response to changes in the nutrient environment. Here, we reviewed evidence supporting a mechanism whereby β-cell LDTFs endow the β-cell with characteristic ion channels and the machinery to process and exocytose insulin, whereas SDTFs act upon the transcriptional program established by LDTFs to mature and adapt the insulin secretory response in response to environmental signals (Figures 1B, 2C, and 3C).

While the conceptual model of LDTF-SDTF collaboration is intended to distill an abundance of observations from the literature, it is almost certainly a simplification of the complex transcriptional regulation that occurs *in vivo*, and there will likely be exceptions to the generalized rules discussed in this review (Figure 1). The operative definitions of LDTFs and SDTFs (Figure 1A) leave open the possibility for individual TFs to exhibit characteristics of both classes and therefore defy strict categorization. In some cases, environmental signals have been shown to activate gene regulatory elements *de novo* without pre-existing chromatin priming or TF binding [63], indicating that not all regulatory elements undergo sequential activation (Figure 1B). Finally, little is known about the specific mechanisms of SDTF cooperation with LDTFs in the β-cell. With the exception of Creb recruitment by NeuroD1 [27], there are few mechanistic studies of this process. Cooperative, rather than sequential, binding of SDTFs and LDTFs remains possible, and transient binding and dissociation of TFs to DNA could confound interpretations of sequential binding of different TFs as inferred through static assays such as chromatin immunoprecipitation sequencing (ChIP-seq) [69]. While gain- or loss-of-function experiments followed by ChIP and gene expression assays are the gold standard for delineating the logic of TF binding and direct gene regulation [3], such datasets have not been generated for many TFs discussed here. With increased sensitivity of assays to

map TF binding [70], it will be possible to characterize mechanisms of SDTF recruitment by LDTFs in β-cells. In writing this review, we hope to stimulate further studies of collaborative transcriptional control in the β-cell (see Outstanding questions) with the expectation that a deeper understanding of these transcriptional networks will lead to improved human stem cell-derived β-cell models and novel strategies for enhancing adaptive insulin secretion or preventing β-cell decompensation.

## Declaration of interests

The authors declare no competing interests.

## References


1. Dassaye, R. *et al.* (2016) Transcription factor regulation of pancreatic organogenesis, differentiation and maturation. *Islets* 8, 13–34
2. Arda, H.E. *et al.* (2013) Gene regulatory networks governing pancreas development. *Dev. Cell* 25, 5–13
3. Heinz, S. *et al.* (2015) The selection and function of cell type-specific enhancers. *Nat. Rev. Mol. Cell Biol.* 16, 144–154
4. Wang, A. *et al.* (2015) Epigenetic priming of enhancers predicts developmental competence of hESC-derived endodermal lineage intermediates. *Cell Stem Cell* 16, 386–399
5. Lee, K. *et al.* (2019) FOXA2 is required for enhancer priming during pancreatic differentiation. *Cell Rep.* 28, 382–393.e387
6. Alvarez-Dominguez, J.R. *et al.* (2020) Circadian entrainment triggers maturation of human in vitro islets. *Cell Stem Cell* 26, 108–122.e110
7. Mullen, A.C. *et al.* (2011) Master transcription factors determine cell-type-specific responses to TGF-beta signaling. *Cell* 147, 565–576
8. Jermendy, A. *et al.* (2011) Rat neonatal beta cells lack the specialised metabolic phenotype of mature beta cells. *Diabetologia* 54, 594–604
9. Rorsman, P. *et al.* (1989) Failure of glucose to elicit a normal secretory response in fetal pancreatic beta cells results from glucose insensitivity of the ATP-regulated K+ channels. *Proc. Natl. Acad. Sci. U. S. A.* 86, 4505–4509
10. Henquin, J.C. and Nenquin, M. (2018) Immaturity of insulin secretion by pancreatic islets isolated from one human neonate. *J. Diabetes Investig.* 9, 270–273
11. Wortham, M. and Sander, M. (2016) Mechanisms of beta-cell functional adaptation to changes in workload. *Diabetes Obes. Metab.* 18, 78–86
12. Campbell, J.E. and Newgard, C.B. (2021) Mechanisms controlling pancreatic islet cell function in insulin secretion. *Nat. Rev. Mol. Cell Biol.* 22, 142–158
13. Lewandowski, S.L. *et al.* (2020) Pyruvate kinase controls signal strength in the insulin secretory pathway. *Cell Metab.* 32, 736–750.e735
14. Kalwat, M.A. and Cobb, M.H. (2017) Mechanisms of the amplifying pathway of insulin secretion in the beta cell. *Pharmacol. Ther.* 179, 17–30
15. Boschero, A.C. *et al.* (1990) D-glucose and L-leucine metabolism in neonatal and adult cultured rat pancreatic islets. *Mol. Cell. Endocrinol.* 73, 63–71
16. Blum, B. *et al.* (2012) Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat. Biotechnol.* 30, 261–264
17. Jacovetti, C. *et al.* (2015) Postnatal beta-cell maturation is associated with islet-specific microRNA changes induced by nutrient shifts at weaning. *Nat. Commun.* 6, 8084
18. Stolovich-Rain, M. *et al.* (2015) Weaning triggers a maturation step of pancreatic beta cells. *Dev. Cell* 32, 535–545
19. Dhawan, S. *et al.* (2015) DNA methylation directs functional maturation of pancreatic beta cells. *J. Clin. Invest.* 125, 2851–2860
20. Aguayo-Mazzucato, C. *et al.* (2006) Restructuring of pancreatic islets and insulin secretion in a postnatal critical window. *PLoS One* 1, e35
21. Zhao, J. *et al.* (2019) In vivo imaging of beta-cell function reveals glucose-mediated heterogeneity of beta-cell functional development. *eLife* 8, 41530
22. Pullen, T.J. and Rutter, G.A. (2013) When less is more: the forbidden fruits of gene repression in the adult beta-cell. *Diabetes Obes. Metab.* 15, 503–512
23. Hang, Y. *et al.* (2014) The MafA transcription factor becomes essential to islet beta-cells soon after birth. *Diabetes* 63, 1994–2005
24. Nishimura, W. *et al.* (2015) MafA is critical for maintenance of the mature beta cell phenotype in mice. *Diabetologia* 58, 566–574
25. Aguayo-Mazzucato, C. *et al.* (2013) Thyroid hormone promotes postnatal rat pancreatic beta-cell development and glucose-responsive insulin secretion through MAFA. *Diabetes* 62, 1569–1580
26. Scoville, D.W. *et al.* (2015) MLL3 and MLL4 methyltransferases bind to the MAFA and MAFB transcription factors to regulate islet beta-cell function. *Diabetes* 64, 3772–3783
27. Van de Velde, S. *et al.* (2019) CREB promotes beta cell gene expression by targeting its coactivators to tissue-specific enhancers. *Mol. Cell. Biol.* 39, e00200–e00219
28. Pasquali, L. *et al.* (2014) Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. *Nat. Genet.* 46, 136–143
29. Avrahami, D. *et al.* (2015) Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved beta cell function. *Cell Metab.* 22, 619–632
30. Yoshihara, E. *et al.* (2016) ERRgamma Is required for the metabolic maturation of therapeutically functional glucose-responsive beta cells. *Cell Metab.* 23, 622–634
31. Arda, H.E. *et al.* (2016) Age-dependent pancreatic gene regulation reveals mechanisms governing human beta cell function. *Cell Metab.* 23, 909–920
32. Wortham, M. *et al.* (2018) Integrated in vivo quantitative proteomics and nutrient tracing reveals age-related metabolic rewiring of pancreatic beta cell function. *Cell Rep.* 25, 2904–2918.e2908
33. Jaafar, R. *et al.* (2019) mTORC1 to AMPK switching underlies beta-cell metabolic plasticity during maturation and diabetes. *J. Clin. Invest.* 129, 4124–4137
34. Devarakonda, S. *et al.* (2011) Disorder-to-order transition underlies the structural basis for the assembly of a transcriptionally active PGC-1alpha/ERRgamma complex. *Proc. Natl. Acad. Sci. U. S. A.* 108, 18678–18683
35. Jia, S. *et al.* (2015) Insm1 cooperates with Neurod1 and Foxa2 to maintain mature pancreatic beta-cell function. *EMBO J.* 34, 1417–1433
36. Velazco-Cruz, L. *et al.* (2020) SIX2 regulates human beta cell differentiation from stem cells and functional maturation in vitro. *Cell Rep.* 31, 107687
37. Bevacqua, R.J. *et al.* (2021) SIX2 and SIX3 coordinately regulate functional maturity and fate of human pancreatic beta cells. *Genes Dev.* 35, 234–239
38. Rakshit, K. *et al.* (2018) Postnatal ontogenesis of the islet circadian clock plays a contributory role in beta-cell maturation process. *Diabetes* 67, 911–922
39. Zhang, Y. *et al.* (2016) HNF6 and Rev-erbalpha integrate hepatic lipid metabolism by overlapping and distinct transcriptional mechanisms. *Genes Dev.* 30, 1636–1644
40. Zhang, Y. *et al.* (2015) Gene regulation. Discrete functions of nuclear receptor Rev-erbalpha couple metabolism to the clock. *Science* 348, 1488–1492

41. Beytebiere, J.R. *et al.* (2019) Tissue-specific BMAL1 cistromes reveal that rhythmic transcription is associated with rhythmic enhancer-enhancer interactions. *Genes Dev.* 33, 294–309
42. Perelis, M. *et al.* (2015) Pancreatic beta cell enhancers regulate rhythmic transcription of genes controlling insulin secretion. *Science* 350, aac4250
43. Yoshihara, E. *et al.* (2020) Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* 586, 606–611
44. Aylward, A. *et al.* (2020) Glucocorticoid signaling in pancreatic islets modulates gene regulatory programs and genetic risk of type 2 diabetes. *bioRxiv* https://doi.org/10.1101/2020.05.15.038679
45. Nammo, T. *et al.* (2018) Genome-wide profiling of histone H3K27 acetylation featured fatty acid signalling in pancreatic beta cells in diet-induced obesity in mice. *Diabetologia* 61, 2608–2620
46. Kuo, T. *et al.* (2019) Identification of C2CD4A as a human diabetes susceptibility gene with a role in beta cell insulin secretion. *Proc. Natl. Acad. Sci. U. S. A.* 116, 20033–20042
47. Gupta, D. *et al.* (2013) Peroxisome proliferator-activated receptor gamma (PPARgamma) and its target genes are downstream effectors of FoxO1 protein in islet beta-cells: mechanism of beta-cell compensation and failure. *J. Biol. Chem.* 288, 25440–25449
48. Kitamura, Y.I. *et al.* (2005) FoxO1 protects against pancreatic beta cell failure through NeuroD and MafA induction. *Cell Metab.* 2, 153–163
49. Gupta, D. *et al.* (2017) Temporal characterization of beta cell-adaptive and -maladaptive mechanisms during chronic high-fat feeding in C57BL/6NTac mice. *J. Biol. Chem.* 292, 12449–12459
50. Zhou, Y.P. *et al.* (1999) Basal insulin hypersecretion in insulin-resistant Zucker diabetic and Zucker fatty rats: role of enhanced fuel metabolism. *Metabolism* 48, 857–864
51. Liu, Y.Q. *et al.* (2002) Beta-cell adaptation to insulin resistance. Increased pyruvate carboxylase and malate-pyruvate shuttle activity in islets of nondiabetic Zucker fatty rats. *J. Biol. Chem.* 277, 39163–39168
52. Briand, O. *et al.* (2012) The nuclear orphan receptor Nur77 is a lipotoxicity sensor regulating glucose-induced insulin secretion in pancreatic beta-cells. *Mol. Endocrinol.* 26, 399–413
53. Reynolds, M.S. *et al.* (2016) Beta-cell deletion of Nr4a1 and Nr4a3 nuclear receptors impedes mitochondrial respiration and insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* 311, E186–E201
54. Tang, T. *et al.* (2013) Desnutrin/ATGL activates PPARdelta to promote mitochondrial function for insulin secretion in islet beta cells. *Cell Metab.* 18, 883–895
55. Park, S. *et al.* (2006) Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. *J. Biol. Chem.* 281, 1159–1168
56. Blanchet, E. *et al.* (2015) Feedback inhibition of CREB signaling promotes beta cell dysfunction in insulin resistance. *Cell Rep.* 10, 1149–1157
57. Veres, A. *et al.* (2019) Charting cellular identity during human in vitro beta-cell differentiation. *Nature* 569, 368–373
58. Nair, G.G. *et al.* (2019) Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived beta cells. *Nat. Cell Biol.* 21, 263–274
59. Velazco-Cruz, L. *et al.* (2019) Acquisition of dynamic function in human stem cell-derived beta cells. *Stem Cell Rep.* 12, 351–365
60. Zeng, C. *et al.* (2017) Pseudotemporal ordering of single cells reveals metabolic control of postnatal beta cell proliferation. *Cell Metab.* 25, 1160–1175.e1111
61. Wortham, M. *et al.* (2019) Nutrient regulation of the islet epigenome controls adaptive insulin secretion. *BioRxiv* https://doi.org/10.1101/742403
62. Buenrostro, J.D. *et al.* (2015) Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature* 523, 486–490
63. Ramos-Rodriguez, M. *et al.* (2019) The impact of proinflammatory cytokines on the beta-cell regulatory landscape provides insights into the genetics of type 1 diabetes. *Nat. Genet.* 51, 1588–1595
64. Zhang, E. *et al.* (2019) Preserving insulin secretion in diabetes by inhibiting VDAC1 overexpression and surface translocation in beta cells. *Cell Metab.* 29, 64–77.e66
65. Montemurro, C. *et al.* (2019) IAPP toxicity activates HIF1alpha/PFKFB3 signaling delaying beta-cell loss at the expense of beta-cell function. *Nat. Commun.* 10, 2679
66. Guo, S. *et al.* (2013) Inactivation of specific beta cell transcription factors in type 2 diabetes. *J. Clin. Invest.* 123, 3305–3316
67. Sachs, S. *et al.* (2020) Targeted pharmacological therapy restores beta-cell function for diabetes remission. *Nat. Metab.* 2, 192–209
68. Chung, K.M. *et al.* (2020) Endocrine-exocrine signaling drives obesity-associated pancreatic ductal adenocarcinoma. *Cell* 181, 832–847.e818
69. Swinstead, E.E. *et al.* (2016) Steroid receptors reprogram FoxA1 occupancy through dynamic chromatin transitions. *Cell* 165, 593–605
70. Skene, P.J. and Henikoff, S. (2017) An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. *eLife* 6, e21856
71. Taylor, B.L. *et al.* (2013) Nkx6.1 is essential for maintaining the functional state of pancreatic beta cells. *Cell Rep.* 4, 1262–1275
72. Shen, Y. *et al.* (2012) A map of the cis-regulatory sequences in the mouse genome. *Nature* 488, 116–120
73. Wang, H. *et al.* (2007) MAFA controls genes implicated in insulin biosynthesis and secretion. *Diabetologia* 50, 348–358
74. Puri, S. *et al.* (2018) Replication confers beta cell immaturity. *Nat. Commun.* 9, 485
75. Helman, A. *et al.* (2020) A nutrient-sensing transition at birth triggers glucose-responsive insulin secretion. *Cell Metab.* 31, 1004–1016.e1005
76. Piccand, J. *et al.* (2014) Rfx6 maintains the functional identity of adult pancreatic beta cells. *Cell Rep.* 9, 2219–2232
77. Lemaire, K. *et al.* (2016) Disallowed and allowed gene expression: two faces of mature islet beta cells. *Annu. Rev. Nutr.* 36, 45–71
78. Gao, N. *et al.* (2007) Foxa2 controls vesicle docking and insulin secretion in mature Beta cells. *Cell Metab.* 6, 267–279
79. Gupta, R.K. *et al.* (2005) The MODY1 gene HNF-4alpha regulates selected genes involved in insulin secretion. *J. Clin. Invest.* 115, 1006–1015
80. Gu, C. *et al.* (2010) Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. *Cell Metab.* 11, 298–310
81. Gutierrez, G.D. *et al.* (2017) Pancreatic beta cell identity requires continual repression of non-beta cell programs. *J. Clin. Invest.* 127, 244–259
82. Gao, T. *et al.* (2014) Pdx1 maintains beta cell identity and function by repressing an alpha cell program. *Cell Metab.* 19, 259–271
83. Zmuda, E.J. *et al.* (2010) The roles of ATF3, an adaptive-response gene, in high-fat-diet-induced diabetes and pancreatic beta-cell dysfunction. *Mol. Endocrinol.* 24, 1423–1433
84. Juliana, C.A. *et al.* (2018) A PDX1-ATF transcriptional complex governs beta cell survival during stress. *Mol. Metab.* 17, 39–48
85. Cheong, M.W. *et al.* (2015) Loss of Egr-1 sensitizes pancreatic beta-cells to palmitate-induced ER stress and apoptosis. *J. Mol. Med.* 93, 807–818
86. Ray, J.D. *et al.* (2016) Nkx6.1-mediated insulin secretion and beta-cell proliferation is dependent on upregulation of c-Fos. *FEBS Lett.* 590, 1791–1803
87. Kim-Muller, J.Y. *et al.* (2014) Metabolic inflexibility impairs insulin secretion and results in MODY-like diabetes in triple FoxO-deficient mice. *Cell Metab.* 20, 593–602
88. Rosselot, C. *et al.* (2019) Myc Is required for adaptive beta-cell replication in young mice but is not sufficient in one-year-old mice fed with a high-fat diet. *Diabetes* 68, 1934–1949
89. Goodyer, W.R. *et al.* (2012) Neonatal beta cell development in mice and humans is regulated by calcineurin/NFAT. *Dev. Cell* 23, 21–34
90. Heit, J.J. *et al.* (2006) Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 443, 345–349
91. Keller, M.P. *et al.* (2016) The transcription factor Nfatc2 regulates beta-cell proliferation and genes associated with type 2 diabetes in mouse and human islets. *PLoS Genet.* 12, e1006466
92. Li, X. *et al.* (2016) The MDM2-p53-pyruvate carboxylase signalling axis couples mitochondrial metabolism to glucose-

stimulated insulin secretion in pancreatic beta-cells. *Nat. Commun.* 7, 11740
93. Vieira, E. *et al.* (2012) The clock gene Rev-erbalpha regulates pancreatic beta-cell function: modulation by leptin and high-fat diet. *Endocrinology* 153, 592–601
94. Smart, N.G. *et al.* (2006) Conditional expression of Smad7 in pancreatic beta cells disrupts TGF-beta signaling and induces reversible diabetes mellitus. *PLoS Biol.* 4, e39
95. Tennant, B.R. *et al.* (2013) Identification and analysis of murine pancreatic islet enhancers. *Diabetologia* 56, 542–552