Review

MOLECULAR METABOLISM

Friend and foe: β-cell Ca^{2+} signaling and the development of diabetes

Paul V. Sabatini [1,2,3,4], **Thilo Speckmann** [1,2,3], **Francis C. Lynn** [1,2,3,*]

ABSTRACT

Background: The divalent cation Calcium (Ca^{2+}) regulates a wide range of processes in disparate cell types. Within insulin-producing β-cells, increases in cytosolic Ca^{2+} directly stimulate insulin vesicle exocytosis, but also initiate multiple signaling pathways. Mediated through activation of downstream kinases and transcription factors, Ca^{2+}-regulated signaling pathways leverage substantial influence on a number of critical cellular processes within the β-cell. Additionally, there is evidence that prolonged activation of these same pathways is detrimental to β-cell health and may contribute to Type 2 Diabetes pathogenesis.

Scope of review: This review aims to briefly highlight canonical Ca^{2+} signaling pathways in β-cells and how β-cells regulate the movement of Ca^{2+} across numerous organelles and microdomains. As a main focus, this review synthesizes experimental data from *in vitro* and *in vivo* models on both the beneficial and detrimental effects of Ca^{2+} signaling pathways for β-cell function and health.

Major conclusions: Acute increases in intracellular Ca^{2+} stimulate a number of signaling cascades, resulting in (de-)phosphorylation events and activation of downstream transcription factors. The short-term stimulation of these Ca^{2+} signaling pathways promotes numerous cellular processes critical to β-cell function, including increased viability, replication, and insulin production and secretion. Conversely, chronic stimulation of Ca^{2+} signaling pathways increases β-cell ER stress and results in the loss of β-cell differentiation status. Together, decades of study demonstrate that Ca^{2+} movement is tightly regulated within the β-cell, which is at least partially due to its dual roles as a potent signaling molecule.

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Keywords β-cells; Diabetes; Ca^{2+}; CREB; NFAT; Calmodulin; Calcineurin; CaMK

1. INTRODUCTION

Elevated cytosolic Ca^{2+} (Ca^{2+}_i) initiates a broad range of physiological responses in excitatory cells, from promoting exocytosis in endocrine cells and neurons to muscle contraction in myocytes. These processes are triggered within microseconds of Ca^{2+} influx into the cytosol [1]. Elevations in Ca^{2+}_i that persist for seconds to minutes produce long-term responses, dependent on the activation of downstream signaling pathways [1]. Dysregulation of the Ca^{2+} signaling cascade contributes to the dysfunction of multiple tissues and cell types in metabolic disorders [2–4].

Within insulin-producing β-cells, increased Ca^{2+}_i causes insulin granule exocytosis, but Ca^{2+}-mediated signaling pathways also have critical roles in promoting the function, survival, and proliferation of these cells. This review aims to highlight sources of Ca^{2+}_i, important mediators of β-cell Ca^{2+} signaling and their relevance to β-cell biology and type 2 diabetes (T2D).

2. Ca^{2+} HANDLING IN β-CELLS

β-cells regulate the systemic response to hyperglycemia through the production and secretion of the hormone insulin. Given the detrimental effects of either impaired or elevated insulin release, the increase in Ca^{2+}_i that effectively stimulates insulin exocytosis from the β-cell must be closely regulated. This tight control requires the cooperation between multiple Ca^{2+} exchangers, pumps, and channels [5].

In the postprandial state, glucose elicits the influx of Ca^{2+} through L-type voltage-gated Ca^{2+} channels (L-VGCCs). Mediated via glucose metabolism and ATP production, shifts in the ratio of ATP to ADP (ATP:ADP) within the β-cell result in the closure of ATP-sensitive potassium (K_{ATP}) channels and membrane depolarization. In human β-cells, L-VGCCs are activated at a membrane potential of −40 mV and, in concert with T-type and P/Q-type Ca^{2+} channels, allow Ca^{2+} influx to elicit insulin exocytosis [6]. Ca^{2+} flux across the β-cell plasma membrane is further regulated by a number of metabolites and nutrients including free fatty

[1]Diabetes Research Group, BC Children's Hospital Research Institute, Vancouver, British Columbia, Canada [2]Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada [3]Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada [4]Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

*Corresponding author. 950 28th Ave W, Vancouver, BC, V5Z 4H4, Canada. Fax: +604 875 2373. E-mails: francis.lynn@ubc.ca, @nictitate (F.C. Lynn).

URL: http://www.betacell.ca

Received September 6, 2018 • Revision received December 3, 2018 • Accepted December 19, 2018 • Available online 24 December 2018

https://doi.org/10.1016/j.molmet.2018.12.007

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acid signaling and cAMP [7], likely through the activation of PKA and subsequent phosphorylation of voltage gated Ca^{2+} channels [8]. Furthermore, hormones including leptin [9] and ghrelin [10] and classical neurotransmitters [11,12] also regulate Ca^{2+} influx.

In addition to influx of extracellular Ca^{2+}, there are multiple membrane-bound organelles that regulate Ca^{2+}_i levels, including the nucleus, endoplasmic reticulum (ER), mitochondria, Golgi, as well as vesicles and granules [13,14]. Intracellular Ca^{2+} stores are distinguished based on their sensitivity to inositol-1,4,5-trisphosphate (IP_3), nicotinic acid adenine dinucleotide phosphate (NAADP), or ryanodine (summarized in Figure 1). Additionally, intracellular Ca^{2+} stores are responsive to circulating signals, including insulin [15,16], circulating fatty acids [17], IL-6 [18], and incretin hormones [19–23].

Ca^{2+} release from IP_3-sensitive pools occurs through activation of the IP_3 receptor (IP_3R), which is expressed on the ER membrane [24], insulin granules, and Golgi [25,26]. IP_3 is generated downstream of certain $G\alpha_q$-associated G protein coupled receptors, such as the free fatty acid receptor GPR40 (FFAR1), which signals through phospholipase C (PLC) [27]. PLC then converts phosphatidylinositol-4,5-bisphosphateinositol (PIP2) to IP_3. Alternatively, PLC is also activated by an increase in Ca^{2+}_i; suggesting other sources of Ca^{2+} (i.e. extracellular, NAADH-responsive) can trigger release of Ca^{2+} from IP_3R-responsive stores [28,29].

A second source of Ca^{2+}_i is the NAADP-responsive pool. Within β-cells, NAADP acts as a second messenger of glucose metabolism, as elevated glucose exposure rapidly increases β-cell NAADP content [30]. NAADP is generated from NADP through ADP-ribosyl cyclases (ARC) such as CD38 [31] and mediates Ca^{2+} release from acidic vesicles such as lysosomes and insulin granules [32] through two pore channel 1 [15,33,34]. The NAADP-sensitive stores are required for glucose-stimulated elevations in Ca^{2+}_i, as their inhibition is sufficient to impair glucose-stimulated insulin secretion [35,36].

The third source for Ca^{2+}_i is the ryanodine-sensitive pool [37]. The ryanodine receptors (RyRs) are homotetramers with a combined molecular mass of ~2.3 MDa [38]. While controversy has persisted as to which RyR family members are expressed in β-cells [32,39], this may be due to the naturally low expression of RyRs, differences in cell type (immortalized cell line or primary tissue) or detection method (less sensitive western blot or PCR), as well as possible differences in splicing. More recently, examination of multiple exons within all three RyR family members in human islets demonstrated detectable expression of all family members [40]. RyRs have been proposed to exist on the β-cell ER [41], insulin granules [32], early endosomes [37] and the plasma membrane [42]. Functionally, RyR channels can be activated by ATP, cAMP and long chain acyl CoA [43], as well as the second messenger cyclic ADP ribose (cADPR), which is produced from NAD^+ by ARC enzymes, including CD38 [44]. Activation of RyRs promotes glucose-independent insulin release [37]. Additionally, RyRs contribute to glucose-stimulated insulin secretion by mediating the process of Ca^{2+}-induced Ca^{2+} release (CICR) [32] through several possible mechanisms, depending on which Ca^{2+} store expresses RyRs. With ER-localized RyRs, CICR is thought to increase Ca^{2+}_i in close proximity to mitochondria and maintain high rates of ATP generation. Similarly, RyRs expressed on the insulin granule increase Ca^{2+}_i in the immediate proximity of the insulin granule and facilitate Ca^{2+}-dependent vesicle docking and fusion [43].

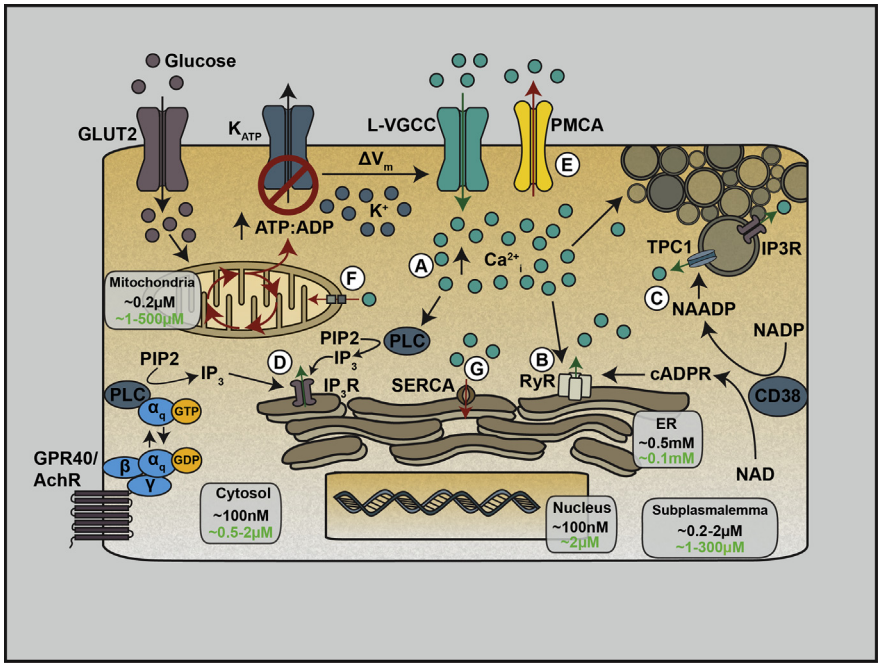

Figure 1: Schematic of β-cell Ca^{2+} homeostasis pathways. Extracellular Ca^{2+} influx in β-cells is triggered by the uptake of glucose through glucose transporters (GLUT2 in rodents; GLUT1 in humans) and subsequent metabolism. This shifts the ratio of ATP to ADP, which closes the ATP-sensitive potassium channel (K_{ATP}) and opens L-type voltage-gated Ca^{2+} channels (L-VGCCs) (A). There are also intracellular Ca^{2+} pools which contribute to the increase in cytosolic Ca^{2+} (Ca^{2+}_i), including through the ryanodine receptor (RyR) on the ER membrane, through a process termed "Ca^{2+}-induced Ca^{2+} release" (B). Additionally, Ca^{2+} is released following glucose metabolism and production of NAADP by CD38, which acts through two pore channel 1 (TPC1) found on acidic vesicles including insulin granules (C). Finally, intracellular Ca^{2+} can be released through the activation of IP_3 receptors (IP_3R) found on the ER membrane and on insulin granules. IP_3Rs are stimulated by the production of IP_3 from PIP2 following activation of phospholipase C (PLC) by increased Ca^{2+}_i or by $G\alpha_q$-coupled G-protein receptors including the free fatty acid receptor 1 (FFAR1/GPR40) and acetylcholine receptor (AchR) (D). Following the rise in Ca^{2+}_i levels, the plasma membrane Ca^{2+} ATPase (PMCA) pumps Ca^{2+} out of the cell (E). Ca^{2+} is also sequestered in the mitochondria by voltage-dependent anion channels and the mitochondrial Ca^{2+} uniporter (F) and the ER through the actions of the sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA) (G). Ca^{2+} concentrations within different cellular compartments are shown (black: basal; green: stimulated).

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Beside the regulation of these Ca^{2+}-sensitive stores, mitochondria are additional β-cell organelles in which Ca^{2+} handling is tightly regulated and critical for function [45]. Ca^{2+} is exported from mitochondria via the Na^{+}/Ca^{2+} exchanger (NCLX) [46], while Ca^{2+} influx into the mitochondrial matrix is achieved through voltage-dependent anion channels (VDACs) in the outer mitochondrial membrane, and the mitochondrial Ca^{2+} uniporter (MCU) complex in the inner mitochondrial membrane [47,48]. Regulation of MCU by mitochondrial Ca^{2+} uptake 1 (MICU1) is critical for mitochondrial function and β-cell function, as knockdown of MICU1 in INS1 cells reduces Ca^{2+} influx into mitochondria, resulting in reduced glucose-stimulated mitochondrial respiration, ATP production, and insulin secretion [49,50]. Notably, mitochondrial Ca^{2+} entry from the cytosol is limited by the low affinity of the MCU, but microdomains between the ER and mitochondria (mitochondria-associated membranes; MAMs), tethered through GRP75 and mitofusin 1 and 2, facilitate the rapid transport of large quantities of Ca^{2+} from the ER into mitochondria following IP_3R- or RyR2-mediated ER Ca^{2+} release [47,48,51]. Functionally, Ca^{2+} influx into the mitochondria during periods of high metabolic demands ensures adequate ATP production to maintain insulin secretion by increasing the availability of metabolic substrates and stimulating the TCA cycle (possibly through activation of 2-oxoglutarate dehydrogenase and isocitrate dehydrogenase) [45]. Together, these studies demonstrate the importance of tightly regulated mitochondrial Ca^{2+} levels.

β-cells maintain tight control of $Ca^{2+}{}_i$ levels through the regulation of extracellular Ca^{2+} influx and the movement of Ca^{2+} within intracellular depots. The degree of this complexity is illustrated through Ca^{2+} microdomains. Basal levels of free intracellular Ca^{2+} are approximately 100 nM, 20,000× lower than free extracellular Ca^{2+}. Following stimulation, whole cell $Ca^{2+}{}_i$ increases to 300–1000 nM, but more responsive Ca^{2+} microdomains exist within multiple subcellular locales including dense core vesicles, ER, mitochondria, subplasmalemma, and within the nucleus [52].

Each of these microdomains have functional consequences. The increase in nuclear Ca^{2+} is required for activation of cAMP response element binding (CREB) [53,54]. Ca^{2+} microdomains surrounding dense core vesicles have been postulated to amplify insulin secretion by increasing Ca^{2+} concentrations in close proximity to Ca^{2+}-dependent synaptic proteins [55], and the Ca^{2+} microdomains formed within the mitochondria following high glucose exposure are required for mitochondrial function and second phase insulin secretion [56].

The regulation of Ca^{2+} handling is highly complex, requiring multiple receptors and channels on multiple organelles and the plasma membrane. The potency of Ca^{2+} as a signaling molecule is a major reason for this degree of intricacy.

3. Ca^{2+} SIGNALING PATHWAYS

Once elevated, $Ca^{2+}{}_i$ initiates multiple signaling cascades by binding to and activating the Ca^{2+} sensor protein Calmodulin (CaM). CaM then undergoes a conformational change, allowing it to activate numerous downstream targets [57]. Interaction between CaM and its partners is highly diverse; certain proteins are nearly continuously bound to CaM, while others interact with CaM specifically under either low or high $Ca^{2+}{}_i$ conditions [58]. CaM-mediated activation can occur through facilitated dimerization, remodeling of active sites, or removal of autoinhibition [59].

The Ca^{2+}/Calmodulin-dependent protein kinases (CaMK) are one class of proteins activated by Ca^{2+}-bound CaM. Of the CaMK isoforms [60], CaMKK1 [61], CaMKK2 [62], traces of CaMKI isoforms (α, γ, δ) [63–65], all CaMKII isoforms (α, β, γ, δ) [66], and CaMKIV [61] have been detected in β-cells. Targets of the CaMKs include the transcription factor CREB. Under low Ca^{2+} conditions, inactive CREB is bound to consensus sites (TGACGTCA) [67], whereas increases in $Ca^{2+}{}_i$ result in CREB activation through a CaMK-dependent pathway [68,69]. Phosphorylated CREB then interacts with its co-factors CREB regulated transcription coactivator 2 (CRTC2) and CREB binding protein (CBP) to promote target gene transcription [70]. Besides $Ca^{2+}{}_i$-mediated phosphorylation of CREB, Ca^{2+} signaling pathways also increase CREB activity via CRTC2. Activation of the phosphatase Calcineurin (CaN) results in the dephosphorylation of cytoplasmic CRTC2, which subsequently dissociates from cytoplasmic 14-3-3 chaperone proteins and translocates to the nucleus, where it increases CREB transcriptional activity [71]. CRTC2 is exported from the nucleus following re-phosphorylation by microtubule affinity regulating kinase 2 (MARK2) [72] and salt inducible kinase 2 (SIK2) [71].

Independent of the CaMK/CREB pathway, CaM also activates a separate signaling cascade through CaN. CaN has many target proteins, including nuclear factor of activated T cells (NFAT) [73] and myocyte enhancer factor-2 (MEF2) [74] family members. CaN-mediated dephosphorylation results in NFAT nuclear translocation and transcriptional activation [73]. NFAT proteins are exported from the nucleus via re-phosphorylation by the kinases DYRK1A and GSK3β [75].

In addition to CaMK and CaN pathways, increased $Ca^{2+}{}_i$ in β-cells activates other proteins and signaling cascades, including the MAP kinase pathway. This is mediated through the activation of Ras-GEF and B-Raf via CaM [76] and CaN [77–79], respectively, and results in activation of p42/44 (ERK1/2) [80]. Additionally, both p38 MAPK [81] and NF-κB [82] are activated by elevated $Ca^{2+}{}_i$ in β-cells. CaMKII mediates the activation of NF-κB activation in β-cells through the phosphorylation of IκBα [82], a known target of CaMKII in neurons [83] (Figure 2). The temporal dynamics and sensitivity to $Ca^{2+}{}_i$ of each of these pathways are not well defined in β-cells. While computational modeling predicts that increasing frequency of $Ca^{2+}{}_i$ oscillations preferentially activates CaMKII over CaN [84], experimental data generated in β-cells are needed.

The disparate pathways active by elevated $Ca^{2+}{}_i$ suggest that Ca^{2+} is a central mediator of many different cellular processes within the β-cell. Indeed, the study of the mediators and effectors of these Ca^{2+} signaling pathways demonstrates their importance in maintaining β-cell function and glucose homeostasis.

4. THE ROLE OF Ca^{2+} IN INSULIN PRODUCTION AND SECRETION

During periods of elevated metabolic demand, β-cells must increase the production of insulin to ensure adequate insulin stores are maintained. As such, high glucose exposure increases insulin production in rat islets [85]. Influx of extracellular Ca^{2+} is critical for this process, as blocking L-VGCCs with verapamil ameliorates glucose-mediated insulin transcription [86]. The Ca^{2+}-mediated promotion of insulin transcription can be separated into NFAT- and CaMK-dependent pathways. The rat insulin 1 promoter contains multiple NFAT binding sites [87], and NFATC2 is enriched at the insulin promoter following high glucose exposure in MIN6 cells and human islets [88]. Please note, immortalized β-cell lines have abnormal rates of apoptosis and replication and likely have abnormal activation of Ca^{2+} signaling pathways; therefore, conclusions derived from cell lines should be verified in primary cells. Furthermore, inhibiting NFAT with the CaN inhibitor tacrolimus (FK-506) abrogates the glucose-mediated increase in insulin promoter activity in INS-1 cells [87]. NFAT proteins are also

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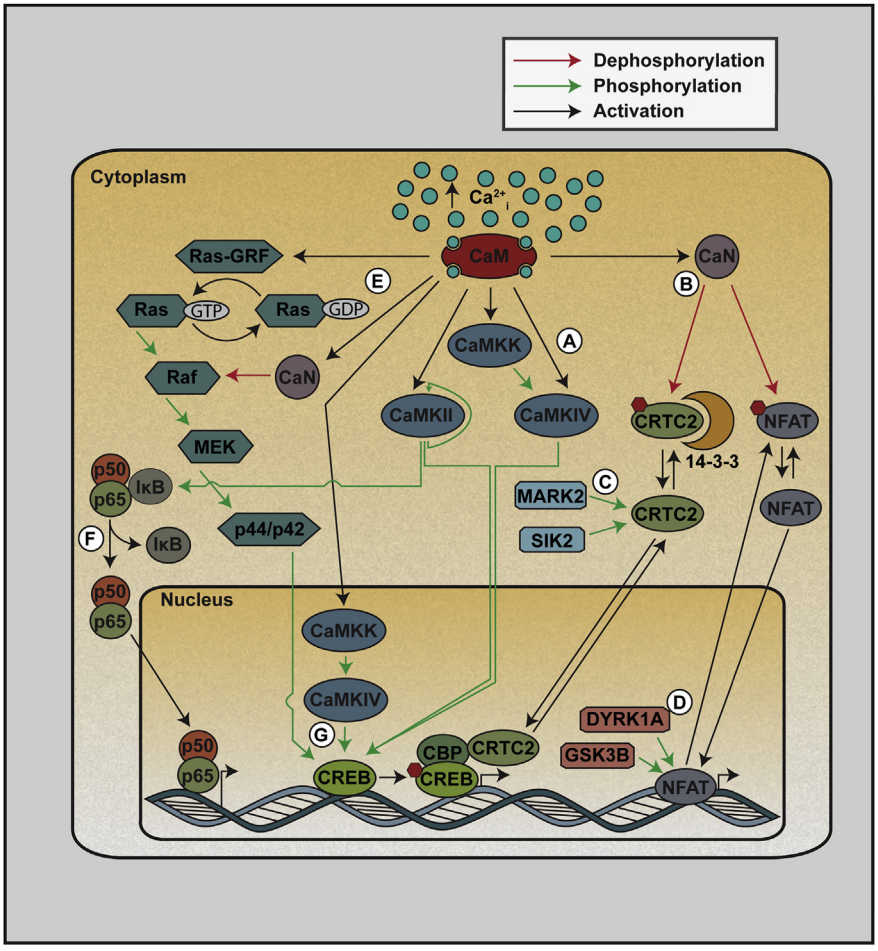

Figure 2: Ca^{2+} signaling pathways in β-cells. Following Ca^{2+} binding to Calmodulin (CaM), multiple downstream pathways are activated. CaM activates the Ca^{2+}/Calmodulin-dependent protein kinase kinase (CaMKK) as well as members of the Ca^{2+}/Calmodulin-dependent protein kinases (CaMK). Ca^{2+}/CaM-bound CaMKK can phosphorylate and activate CaMKIV (A). Ca^{2+}-bound CaM also activates the phosphatase Calcineurin (CaN) (B), which removes phosphate groups from CREB regulated transcription coactivator 2 (CRTC2) and nuclear factor of activated T cells (NFAT) proteins, resulting in their nuclear localization. CRTC2 can be inactivated through phosphorylation by microtubule affinity regulating kinase 2 (MARK2) and salt inducible kinase 2 (SIK2) (C), and NFAT can be inactivated by the kinases GSK3B and DYRK1A (D). Ca^{2+} results in the activation of p44/p42 (ERK1/2) or the MAP kinase pathway through the stimulation of Ras-GRF by CaM and the dephosphorylation of Raf by CaN (E). The NF-κB pathway can also be activated by Ca^{2+} in β-cells through the phosphorylation of IκB, which releases the p50 and p65 subunits (F). The upstream activation of various CaMKs and members of the MAP kinase pathway result in the phosphorylation and activation of cAMP response element binding (CREB) (G).

sufficient to increase insulin gene expression, since a β-cell specific doxycycline-responsive constitutively active NFATC2 significantly increases *Ins1* and *Ins2* gene expression *in vivo* [89].

Besides NFAT, CaMKIV is also required to induce insulin expression, as shown by transfection of INS-1 cells with a kinase-dead CaMKIV, which blocks glucose-mediated elevations in insulin promoter activity [61]. Conversely, overexpression of constitutively active CaMKIV significantly increases insulin gene expression in INS-1 cells [61]. CaMKIV may promote insulin expression through the actions of the transcription factors ATF2 (CREB2) and EGR1. Both ATF2 and EGR1 are positively regulated by Ca^{2+} in CaMKIV- and SRF-dependent manners, respectively, and overexpression of either factor is sufficient to increase insulin promoter activity [90–92]. The promotion of insulin production downstream of NFAT and CaMK pathway activation creates a system wherein Ca^{2+}, acting as a stimulus for insulin secretion and also a signal to increase insulin synthesis, ensures adequate insulin levels during prolonged stimulation. In addition to the transcriptional regulation of insulin by members of Ca^{2+} signaling pathways, elevated glucose also increases rates of insulin mRNA translation [93] and stabilizes insulin mRNA [94]. However, the role of Ca^{2+} signaling pathway members in these processes is unknown.

While increases in $Ca^{2+}{}_i$ are required for insulin granule fusion to the plasma membrane, activation of Ca^{2+} signaling pathways also promotes insulin secretion through CaMK- and CaN-dependent pathways. The importance of Ca^{2+} signaling pathways in promoting insulin secretion is observed in mouse models wherein diminished activity or expression of CaMKII [95], CREB [96], CaM [97] or CRTC2 [98] in mouse β-cells impairs insulin secretion and systemic glucose homeostasis. Furthermore, pharmacological inhibition of CaN with either FK-506 or cyclosporin A decreases insulin secretion in human islets [99,100], while overexpression of NFATC1 and NFATC2 increases glucose- and KCl-stimulated insulin secretion in mouse islets [101]. These *in vitro* and *in vivo* models all support a critical role for members of Ca^{2+} signaling pathways in the promotion of insulin secretion.

One mechanism through which Ca^{2+} signaling promotes insulin secretion is through the formation β-cell "metabolic memory", wherein repeated exposure to elevated glucose primes β-cells to significantly increase insulin secretion during an ensuing high glucose

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exposure [102]. Inhibiting CaMKII activity with KN93 abrogates the augmentation of insulin secretion during the secondary glucose challenge, suggesting a critical role for this kinase in the formation of a metabolic memory [102]. While the precise mediators which form the β-cell metabolic memory have not been elucidated, repeated high glucose exposure increases the expression of glucokinase, SNAP25, and MAFA. Additionally, phosphorylation levels of Synapsin I, a direct target of CaMKII, are increased following repeated high glucose exposure [103].

Ca^{2+} signaling may also promote insulin secretion by elevating mitochondrial activity through a process termed "Ca^{2+}-metabolic coupling". Periods of elevated insulin secretion require increased mitochondrial activity to replenish the ATP stores that sustain ATP-mediated membrane depolarization and insulin release. Influx of Ca^{2+} and downstream activation of CaMKs is required for this prolonged elevation in mitochondrial function, as inhibiting L-VGCCs or CaMKs blocks increased oxygen consumption rate (OCR; a measure of mitochondrial function) [104–106]. Furthermore, directly stimulating L-VGCCs with BayK8644 increases β-cell OCR, demonstrating the tight coupling of $Ca^{2+}{}_i$ with mitochondrial function [105].

These studies establish that, in addition to Ca^{2+}-mediated insulin vesicle fusion, activation of CaN/NFAT and CaMK also promote insulin secretion by increasing mitochondrial respiration and priming the β-cell under repeated high glucose exposures.

5. THE ROLE OF Ca^{2+} IN β-CELL REPLICATION

Increased rates of β-cell proliferation are one adaptive mechanism β-cells employ to compensate for elevated metabolic demand and ensure euglycemia is maintained. Both *in vitro* [107] and *in vivo* studies [108,109] have observed that increased β-cell proliferation in response to elevated glucose concentrations and Ca^{2+} signaling is critical for this process. Pharmacologic stimulation of glucokinase also increases β-cell replication [110,111], which can be blocked by inhibiting membrane depolarization with diazoxide [110], suggesting that Ca^{2+} influx, as opposed to glucose metabolism alone, is necessary. Furthermore, increasing $Ca^{2+}{}_i$ with the L-VGCC agonist, BayK8644, induces rat β-cell proliferation [112,113], providing additional support for the role of Ca^{2+} signaling pathways in promoting β-cell proliferation.

Both CaMK- and NFAT-dependent mechanisms mediate the mitogenic effects of elevated $Ca^{2+}{}_i$ in β-cells. Blocking CaMK activity with KN62 abrogates the glucose-mediated increase in β-cell proliferation [114]. Additionally, overexpression of constitutively active CaMKIV or dominant-negative CaMKIV significantly elevates or diminishes β-cell proliferative rates, respectively [114]. Downstream of CaMKIV, CREB activity is also required, as co-expression of a dominant-negative CREB can abrogate the mitogenic effects of CaMKIV overexpression and the CREB targets *Irs2* and *Nr4a1* promote β-cell proliferation [69,107,114–117]. In sum, these data suggest that the CaMKIV/CREB/*Irs2* and *Nr4a1* pathway is one mechanism by which elevations in $Ca^{2+}{}_i$ promote β-cell replication.

NFAT proteins also promote β-cell replication. Islets from juveniles (age 0.5 to nine years old) have higher proliferation rates associated with higher expression of *NFATC1*, *NFATC2*, and *NFATC4* than islets from adults (20 years or older) [118]. Additionally, the expression of a doxycycline-mediated constitutively nuclear NFATC2 in mice increases β-cell proliferation rates 2-fold *in vivo* [89]. Within cultured human islets, overexpression of constitutively active NFATC1 or NFATC2 increases proliferation rates by 2- and 3-fold, respectively [101]. In support of the proliferative role of NFAT proteins in β-cells, two unbiased small molecule screens identified β-cell mitogens that act by inhibiting the NFAT kinases DYRK1A and GSK3β, thus increasing NFAT activity [112,119]. These small molecule screens have been validated by an independent study, which found that the small molecule 5-iodotubercidin inhibits multiple DYRK family members and induces human β-cell proliferation through a CaN-dependent pathway [120]. Finally, increases in CaN activity may mediate the proliferative effects of the GLP-1 receptor agonist, exendin-4, on β-cells. Exendin-4-treated human islets have a 2-fold increase in proliferation rates and an associated significant increase in *NFATC1*, *NFATC3*, and *NFATC4* expression. Inhibition of CaN with FK-506 abrogated exendin-4-mediated increases in NFAT gene expression level and β-cell proliferation rates [118]. Mechanistically, NFAT proteins transcriptionally regulate a large number of cell cycle and mitogenic genes in β-cells [101], including direct induction of *Irs2* [121,122], *Ccdn1*, and *Cdk4* [89], which may all promote β-cell proliferation.

Similar to the positive effect of Ca^{2+} signaling pathways on insulin production, elevated β-cell proliferation rates during periods of increased systemic insulin demand allow for appropriate β-cell compensation and ensure appropriate β-cell functional capacity to maintain euglycemia.

6. THE ROLE OF Ca^{2+} IN β-CELL SURVIVAL

Ca^{2+} signaling pathways also promote β-cell viability and survival. MIN6 cells incubated for 24 h in high glucose (25 mM) have significantly reduced rates of apoptosis compared to MIN6 incubated in low glucose (5 mM) concentrations [123]. The cytoprotective effects of elevated glucose are blocked by inhibiting depolarization with diazoxide or Ca^{2+} influx with nifedipine [123]. Both CaN- and CaMK-dependent pathways have been suggested to mediate the pro-survival effects of Ca^{2+}.

Inhibition of CaN with either FK-506 or cyclosporine A induces β-cell apoptosis in human islets *in vitro* [100], and FK-506 treatment of diabetic mice transplanted with human islets impairs graft function and glucose homeostasis [100,124]. Examination of pancreatic biopsies from individuals receiving either cyclosporine A or FK-506 as an immunosuppressant display cellular evidence of β-cell apoptosis [125]. Finally, use of CaN inhibitors FK-506, cyclosporine A, and sirolimus as immunosuppressants in solid organ transplant is associated with the development of impaired glucose homeostasis and diabetes [126,127]. These results suggest that CaN activity is required for β-cell survival.

In addition to the role of NFAT proteins, the CaMKIV/CREB pathway also promotes β-cell viability. MIN6 cells incubated in 12.5 mM glucose have significantly reduced caspase-3 activity compared to MIN6 incubated in 2.5 mM glucose [114]. CaMKIV may mediate these effects, as expression of a constitutively active CaMKIV reduces β-cell apoptosis rates and co-expression of a dominant-negative CREB is sufficient to block the cytoprotective effects of increased CaMKIV activity [114]. Supporting the role of CREB in promoting β-cell viability, *in vivo* studies show that transgenic expression of a dominant-negative CREB (A-CREB) in β-cells increases apoptosis and results in diabetes in mice [68], and *in vitro* studies demonstrate knockdown of CREB in INS-1 cells increased levels of cleaved caspase-3 [128]. CREB may promote β-cell viability through induction of cytoprotective factors *Irs2* and *Npas4*, which both protect β-cells from stress and cell death [68,129,130].

In addition to the ability of elevated $Ca^{2+}{}_i$ to promote β-cell viability, decreased $Ca^{2+}{}_i$ also adversely impacts β-cell survival by impairing ER

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and mitochondrial Ca^{2+} handling. Depletion of ER Ca^{2+} results in ER stress and β-cell apoptosis [131—133]. Mechanistically, during states of low β-cell $Ca^{2+}{}_i$, such as low glucose exposure, ER Ca^{2+} depletion occurs due to inactivation of the sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA) and the ensuing lack of ER Ca^{2+} uptake [134]. $Ca^{2+}{}_i$ is also intricately connected to mitochondrial function, as the activity of several mitochondrial enzymes depends on Ca^{2+} [135]. Mitochondrial Ca^{2+} uptake follows depolarization-dependent increases in $Ca^{2+}{}_i$, and ATP production increases as a consequence [136,137]. Thus, decreases in $Ca^{2+}{}_i$ are predicted to decrease mitochondrial activity, ATP production and SERCA action; and in so doing promote ER Ca^{2+} depletion, ER stress and β-cell death.

7. Ca^{2+} SIGNALING PATHWAYS IN T2D

As outlined above, Ca^{2+} signaling pathways have critical roles in regulating β-cell function, proliferation and viability; all processes that fail during the development of T2D. Despite this importance, only few members of Ca^{2+} signaling pathways have appeared as susceptibility loci in T2D GWAS studies [138], including the likely causal *CDC123/CAMK1D* locus [139,140] and *CAMKK2* variants [141]. However, a recent analysis of regulatory elements upstream of T2D susceptibility genes identified *NFATC2* as a regulatory factor for 40% of genes identified through GWAS [101]. Furthermore, overexpression of NFATC1 or NFATC2 in human islets significantly alters the expression of a number of T2D susceptibility genes including *KLF11*, *HHEX*, and *PROX1* [101]. While the genetic link between Ca^{2+} signaling pathways and T2D requires further examination, research using animal models and clinical data support a role for impaired Ca^{2+} signaling in β-cell failure during T2D pathogenesis.

The prediabetic milieu, characterized by increased glucose and fatty acids levels, results in increased β-cell depolarization, Ca^{2+} influx, and insulin secretion to maintain euglycemia. Short-term stimulation of Ca^{2+} signaling pathways yields positive effects for the β-cell (insulin production, secretion, replication and viability). In contrast, chronic stimulation of these pathways has deleterious effects. This is observed in multiple rodent models in which members of Ca^{2+} signaling pathways are overexpressed. For instance, overexpression of a constitutively active CaN increases β-cell apoptosis, decreases proliferation, and results in glucose intolerance [142]. Similarly, mice that overexpress a constitutively active CaMKIIα in β-cells also develop diabetes associated with decreased β-cell mass [143]. CaM overexpression in mouse β-cells also leads to diabetes [144]. In this CaM overexpression model, there is also a loss of insulin-expressing cells with a concomitant increase in islet cells expressing glucagon, perhaps due to β-cell transdifferentiation into α-cells [144]. The observations from mouse models suggest chronic activation of Ca^{2+} signaling pathways impairs β-cell function, which is supported by human studies in which individuals with T2D are treated with diazoxide to inhibit β-cell depolarization. After a multi-day treatment period, insulin secretion is improved [145,146]. This model of pathogenic Ca^{2+} flux may also explain why sulphonylureas initially improve, but ultimately worsen, glycemic control in individuals with T2D [147].

Chronically elevated $Ca^{2+}{}_i$ may drive β-cell dysfunction and failure by exacerbating ER stress and β-cell differentiation. β-cell ER stress is sufficient to cause diabetes in mice [148], has been observed in individuals with T2D [149], and relies on activation of Ca^{2+} signaling pathways [4]. Treatment with a combination of high glucose and palmitate results in stark activation of ER stress and increases rates of β-cell apoptosis in both immortalized β-cell lines and primary islets [150,151]. However, blocking depolarization with diazoxide [150] or Ca^{2+} influx with nifedipine [151] protects against the induction of ER stress and subsequent apoptosis of β-cells.

In addition to exacerbation of ER stress, chronically active Ca^{2+} signaling also results in loss of β-cell maturation. Models in which β-cells are exposed to chronically elevated glucose levels and increased $Ca^{2+}{}_i$ result in the loss of β-cell maturation; such as the *db/db* or Akita mouse, a diphtheria toxin-mediated β-cell ablation model, insulin receptor antagonism [152], or genetic removal of insulin genes from β-cells [153]. Furthermore, inhibiting β-cell depolarization in the *db/db* mouse, by crossing it to a β-cell specific constitutively active *Kir6.2* mutant, significantly reduces rates of β-cell transdifferentiation into gastrin-expressing cells compared to *db/db* controls, despite no improvement in glucose handling [152]. This experiment strongly suggests that β-cell depolarization, and not hyperglycemia alone, is required to drive β-cell dedifferentiation. This hypothesis is supported by an *in vitro* transdifferentiation model in which mouse islets are cultured at either 5 mM or 25 mM glucose. After 2 days in culture, islets exposed to high glucose have a significant increase in gastrin expression, which could be abrogated by co-culture with either diazoxide, nifedipine or FK-506 [152], demonstrating that Ca^{2+} influx and CaN activity are also required for this process. Importantly, islets from individuals with T2D have significantly increased numbers of gastrin-expressing cells; although it remains unknown whether aberrant Ca^{2+} signaling is the cause [152].

The role of Ca^{2+} signaling in driving β-cell transdifferentiation is further supported by data from the β-cell *Abcc8* knockout mouse. Deletion of *Abcc8*, a subunit of the K_{ATP} channel, from β-cells increases intracellular Ca^{2+} most notably under low glucose conditions, but also under high glucose exposure [154]. This is accompanied by the loss of β-cell maturation status and transdifferentiation into PP-cells, despite an absence of frank hyperglycemia [154]. Additionally, expression of the dedifferentiation marker *Aldh1a3* is significantly increased in the *Abcc8* null mouse and can be largely normalized by blocking Ca^{2+} influx with verapamil [154]. In contrast to other dedifferentiation models, which present with profound hyperglycemia, this *Abcc8* null model decouples hyperglycemia from increased Ca^{2+} influx and elegantly demonstrates that chronically active Ca^{2+} signaling pathways are sufficient to promote β-cell dedifferentiation.

These studies define a clear role for Ca^{2+} signaling pathways in driving β-cell dedifferentiation and transdifferentiation and support a model in which chronic activation of Ca^{2+} signaling pathways results in increased stress and a loss of β-cell maturation status that contributes to β-cell failure in T2D. Less certain, however, is the role of altered Ca^{2+} handling in the development of type 1 diabetes (T1D). *In vitro* models of cytokine treatment show impaired β-cell Ca^{2+} handling following exposure to proinflammatory cytokines, including reduced oscillatory Ca^{2+} fluctuations [155] and impaired glucose-stimulated Ca^{2+} influx [156]. Additionally, blockage of L-VGCCs protects mice from diabetes and β-cell loss in low-dose STZ-induced diabetes [157]. However, further assessments in T1D models such as the non-obese diabetic mouse may bring further illumination as to the role of Ca^{2+}-regulated cell death in T1D.

8. CONCLUSIONS AND FUTURE DIRECTIONS

In the postprandial state, β-cells undergo waves of depolarization and Ca^{2+} influx, which activates multiple downstream signaling pathways. Stimulation of these pathways promotes insulin production and secretion, proliferation, and viability. The importance of Ca^{2+} signaling in β-cells is evidenced by the β-cell dysfunction and impairment in systemic glucose homeostasis that results from inhibiting the activity

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Table 1 — Mouse models of Ca^{2+}-related diabetes.

Target gene	Model	Phenotype	Reference(s)
Models of decreased expression/activity			
Calm1	Transgenic OE of inactive *Calm1* (CaM-8)	Reduced insulin secretion resulting in diabetes	[97] Ribar et al., 1995
Camk2 (a,b,d,g)	Tetracycline-mediated OE of *Camk2* pseudosubstrate inhibitory peptide (EAC3I)	Reduced insulin secretion and impaired glucose tolerance	[95] Dadi et al., 2014
Creb1	Transgenic OE of DN *Creb1* (A-CREB)	Increased apoptosis resulting in diabetes	[68] Jhala et al., 2003
	Pdx1-CreER^{Tg}-mediated deletion	Females on HFD glucose intolerant (not observed in males)	[96] Shin et al., 2014
Crtc2	MIP-CreER-mediated deletion	Reduced insulin secretion and glucose intolerance	[98] Blanchet et al., 2015
Ppp3r1	Ins2-Cre^{Tg}-mediated deletion of calcineurin b1	Diabetes after 10 weeks	[89] Heit et al., 2006
Nr4a1	Germline deletion	Reduced β-cell proliferation	[117] Tessem et al., 2014
Abcc8	Ins2-Cre^{Tg}-mediated deletion	β-cell transdifferentation into PP-cells	[154] Stancill et al., 2017
Models of increased expression/activity			
Calm1	Transgenic OE of chicken *Calm1* in β-cells	Increased apoptosis, possible transdifferentiation resulting in diabetes	[144] Epstein et al., 1989 [158] Yu et al., 2002
Camk2a	Transgenic OE in β-cells	Increased apoptosis, decreased proliferation resulting in diabetes	[143] Kato et al., 2008
Nfatc1	Doxycycline-inducible transgenic OE of constitutively active *Nfatc1* (Nfatc1^{nuc})	Increased Pdx1, Glut2, and β-cell proliferation	[89] Heit et al., 2006
Ppp3ca	Transgenic OE of constitutively active calcineurin A in β-cells	Reduced proliferation/increased apoptosis resulting in diabetes	[142] Bernal-Mizrachi et al., 2010

Summary of mouse models of diabetes or β-cell dysfunction following overexpression (OE) or deletion of Ca^{2+}-related genes. DN = dominant-negative; HFD = high fat diet; Tg = transgenic.

of various members of the Ca^{2+} signaling cascade, including CREB [68,96], CaN [89], and CaMKII [95]. Conversely, overstimulation of these pathways (a summary of mouse models in Table 1), as is observed under chronic hyperglycemia, also results in β-cell dysfunction and loss of β-cell differentiation status. This is in line with observations from mouse models that specifically overexpress CaN [142], CaMKII [143], or CaM [144,158], which impairs β-cell function, maturation status, and viability. Together, these studies underscore the requirement for tight control over $Ca^{2+}{}_i$ and the downstream pathways it regulates in β-cells.

While current studies have observed disrupted β-cell Ca^{2+} handling in mouse models [159,160] and in humans with T2D [125–127,161], there are several avenues of research which offer greater understanding of the pathogenic role of altered Ca^{2+} signaling in the β-cell. These include a further characterization of how altered Ca^{2+} signaling impacts β-cell transcriptomics, ER and mitochondrial function, and defining the nature of the altered Ca^{2+} handling by the β-cell under pathologic conditions, particularly by important Ca^{2+} stores such as ER and mitochondria. Finally, it will be important to determine if and how Ca^{2+} signaling pathways are impaired in β-cells from individuals with T2D and whether these pathways can be therapeutically targeted.

ACKNOWLEDGEMENTS

F.C.L., P.V.S. and T.S. wrote and edited the manuscript.

This work was supported by an operating grant to F.C.L. from the CIHR (MOP 142222). Salary support to F.C.L. was provided by the Michael Smith Foundation for Health Research (#5238 BIOM), the Diabetes Canada, and the BC Children's Hospital Research Institute. Fellowship support was provided by the CIHR-BC Transplantation Trainee Program (T.S.), UBC 4YF (P.V.S.) and the Diabetes Canada (P.V.S.). We thank members of the Lynn Lab for critical reading of the manuscript and peer reviewers for their insightful feedback. While every effort was made to make this review as comprehensive as possible, there undoubtedly is excellent research that was excluded due to length constraints.

CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Berridge, M.J., Bootman, M.D., Roderick, H.L., 2003. Calcium signalling: dynamics, homeostasis and remodelling. Nature Reviews Molecular Cell Biology 4:517–529.

[2] Swami, M., 2012. Metabolism: calcium-mediated control. Nature Medicine 18, 670–670.

[3] Ozcan, L., Tabas, I., 2014. CaMKII in cardiometabolic disease. Aging (Albany NY) 6:430–431.

[4] Timmins, J.M., Ozcan, L., Seimon, T.A., Li, G., Malagelada, C., Backs, J., et al., 2009. Calcium/calmodulin-dependent protein kinase II links ER stress with Fas and mitochondrial apoptosis pathways. Journal of Clinical Investigation 119:2925–2941.

[5] Gilon, P., Chae, H.Y., Rutter, G.A., Ravier, M.A., 2014. Calcium signaling in pancreatic beta-cells in health and in type 2 diabetes. Cell Calcium 56:340–361.

[6] Braun, M., Ramracheya, R., Bengtsson, M., Zhang, Q., Karanauskaite, J., Partridge, C., et al., 2008. Voltage-gated ion channels in human pancreatic beta-cells: electrophysiological characterization and role in insulin secretion. Diabetes 57:1618–1628.

[7] Prentki, M., Matschinsky, F.M., Madiraju, S.R., 2013. Metabolic signaling in fuel-induced insulin secretion. Cell Metabolism 18:162–185.

[8] Fuller, M.D., Emrick, M.A., Sadilek, M., Scheuer, T., Catterall, W.A., 2010. Molecular mechanism of calcium channel regulation in the fight-or-flight response. Science Signaling 3:ra70.

[9] Wu, Y., Shyng, S.L., Chen, P.C., 2015. Concerted trafficking regulation of Kv2.1 and KATP channels by leptin in pancreatic beta-cells. Journal of Biological Chemistry 290:29676–29690.

[10] Dezaki, K., Hosoda, H., Kakei, M., Hashiguchi, S., Watanabe, M., Kangawa, K., et al., 2004. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca^{2+} signaling in beta-cells: implication in the glycemic control in rodents. Diabetes 53:3142–3151.

[11] Gibson, T.B., Lawrence, M.C., Gibson, C.J., Vanderbilt, C.A., McGlynn, K., Arnette, D., et al., 2006. Inhibition of glucose-stimulated activation of extracellular signal-regulated protein kinases 1 and 2 by epinephrine in pancreatic beta-cells. Diabetes 55:1066–1073.

[12] Gilon, P., Henquin, J.C., 2001. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. Endocrine Reviews 22:565–604.

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[13] Prins, D., Michalak, M., 2011. Organellar calcium buffers. Cold Spring Harbor Perspectives in Biology 3.
[14] Contreras, L., Drago, I., Zampese, E., Pozzan, T., 2010. Mitochondria: the calcium connection. Biochimica et Biophysica Acta 1797:607–618.
[15] Johnson, J.D., Misler, S., 2002. Nicotinic acid-adenine dinucleotide phosphate-sensitive calcium stores initiate insulin signaling in human beta cells. Proceedings of the National Academy of Sciences of the United States of America 99:14566–14571.
[16] Borge, P.D., Moibi, J., Greene, S.R., Trucco, M., Young, R.A., Gao, Z., et al., 2002. Insulin receptor signaling and sarco/endoplasmic reticulum calcium ATPase in beta-cells. Diabetes 51(Suppl 3):S427–S433.
[17] Mancini, A.D., Poitout, V., 2013. The fatty acid receptor FFA1/GPR40 a decade later: how much do we know? Trends in Endocrinology and Metabolism 24:398–407.
[18] Suzuki, T., Imai, J., Yamada, T., Ishigaki, Y., Kaneko, K., Uno, K., et al., 2011. Interleukin-6 enhances glucose-stimulated insulin secretion from pancreatic beta-cells: potential involvement of the PLC-IP3-dependent pathway. Diabetes 60:537–547.
[19] Sasaki, S., Nakagaki, I., Kondo, H., Hori, S., 2002. Involvement of the ryanodine-sensitive Ca2+ store in GLP-1-induced Ca2+ oscillations in insulin-secreting HIT cells. Pflügers Archiv 445:342–351.
[20] Lu, M., Wheeler, M.B., Leng, X.H., Boyd 3rd, A.E., 1993. The role of the free cytosolic calcium level in beta-cell signal transduction by gastric inhibitory polypeptide and glucagon-like peptide I(7-37). Endocrinology 132:94–100.
[21] Gromada, J., Dissing, S., Bokvist, K., Renstrom, E., Frokjaer-Jensen, J., Wulff, B.S., et al., 1995. Glucagon-like peptide I increases cytoplasmic calcium in insulin-secreting beta TC3-cells by enhancement of intracellular calcium mobilization. Diabetes 44:767–774.
[22] Kim, B.J., Park, K.H., Yim, C.Y., Takasawa, S., Okamoto, H., Im, M.J., et al., 2008. Generation of nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose by glucagon-like peptide-1 evokes Ca2+ signal that is essential for insulin secretion in mouse pancreatic islets. Diabetes 57:868–878.
[23] MacDonald, P.E., El-Kholy, W., Riedel, M.J., Salapatek, A.M., Light, P.E., Wheeler, M.B., 2002. The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. Diabetes 51(Suppl 3):S434–S442.
[24] Ross, C.A., Meldolesi, J., Milner, T.A., Satoh, T., Supattapone, S., Snyder, S.H., 1989. Inositol 1,4,5-trisphosphate receptor localized to endoplasmic reticulum in cerebellar Purkinje neurons. Nature 339:468–470.
[25] Micaroni, M., Mironov, A.A., 2010. Roles of Ca and secretory pathway Ca-ATPase pump type 1 (SPCA1) in intra-Golgi transport. Communicative & Integrative Biology 3:504–507.
[26] Pinton, P., Pozzan, T., Rizzuto, R., 1998. The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca2+ store, with functional properties distinct from those of the endoplasmic reticulum. The EMBO Journal 17:5298–5308.
[27] Burant, C.F., 2013. Activation of GPR40 as a therapeutic target for the treatment of type 2 diabetes. Diabetes Care 36(Suppl 2):S175–S179.
[28] Thore, S., Dyachok, O., Tengholm, A., 2004. Oscillations of phospholipase C activity triggered by depolarization and Ca2+ influx in insulin-secreting cells. Journal of Biological Chemistry 279:19396–19400.
[29] Thore, S., Dyachok, O., Gylfe, E., Tengholm, A., 2005. Feedback activation of phospholipase C via intracellular mobilization and store-operated influx of Ca2+ in insulin-secreting beta-cells. Journal of Cell Science 118:4463–4471.
[30] Masgrau, R., Churchill, G.C., Morgan, A.J., Ashcroft, S.J., Galione, A., 2003. NAADP: a new second messenger for glucose-induced Ca2+ responses in clonal pancreatic beta cells. Current Biology 13:247–251.
[31] Cosker, F., Cheviron, N., Yamasaki, M., Menteyne, A., Lund, F.E., Moutin, M.J., et al., 2010. The ecto-enzyme CD38 is a nicotinic acid adenine dinucleotide phosphate (NAADP) synthase that couples receptor activation to Ca2+ mobilization from lysosomes in pancreatic acinar cells. Journal of Biological Chemistry 285:38251–38259.
[32] Mitchell, K.J., Lai, F.A., Rutter, G.A., 2003. Ryanodine receptor type I and nicotinic acid adenine dinucleotide phosphate receptors mediate Ca2+ release from insulin-containing vesicles in living pancreatic beta-cells (MIN6). Journal of Biological Chemistry 278:11057–11064.
[33] Calcraft, P.J., Ruas, M., Pan, Z., Cheng, X., Arredouani, A., Hao, X., et al., 2009. NAADP mobilizes calcium from acidic organelles through two-pore channels. Nature 459:596–600.
[34] Cane, M.C., Parrington, J., Rorsman, P., Galione, A., Rutter, G.A., 2016. The two pore channel TPC2 is dispensable in pancreatic beta-cells for normal Ca(2)(+) dynamics and insulin secretion. Cell Calcium 59:32–40.
[35] Arredouani, A., Ruas, M., Collins, S.C., Parkesh, R., Clough, F., Pillinger, T., et al., 2015. Nicotinic acid adenine dinucleotide phosphate (NAADP) and endolysosomal two-pore channels modulate membrane excitability and stimulus-secretion coupling in mouse pancreatic beta cells. Journal of Biological Chemistry 290:21376–21392.
[36] Kato, I., Yamamoto, Y., Fujimura, M., Noguchi, N., Takasawa, S., Okamoto, H., 1999. CD38 disruption impairs glucose-induced increases in cyclic ADP-ribose, [Ca2+]i, and insulin secretion. Journal of Biological Chemistry 274:1869–1872.
[37] Johnson, J.D., Kuang, S., Misler, S., Polonsky, K.S., 2004. Ryanodine receptors in human pancreatic beta cells: localization and effects on insulin secretion. The FASEB Journal 18:878–880.
[38] Lanner, J.T., Georgiou, D.K., Joshi, A.D., Hamilton, S.L., 2010. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. Cold Spring Harbor Perspectives in Biology 2:a003996.
[39] Dixit, S.S., Wang, T., Manzano, E.J., Yoo, S., Lee, J., Chiang, D.Y., et al., 2013. Effects of CaMKII-mediated phosphorylation of ryanodine receptor type 2 on islet calcium handling, insulin secretion, and glucose tolerance. PLoS One 8:e58655.
[40] Dror, V., Kalynyak, T.B., Bychkivska, Y., Frey, M.H., Tee, M., Jeffrey, K.D., et al., 2008. Glucose and endoplasmic reticulum calcium channels regulate HIF-1beta via presenilin in pancreatic beta-cells. Journal of Biological Chemistry 283:9909–9916.
[41] Varadi, A., Rutter, G.A., 2002. Dynamic imaging of endoplasmic reticulum Ca2+ concentration in insulin-secreting MIN6 Cells using recombinant targeted cameleons: roles of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA)-2 and ryanodine receptors. Diabetes 51(Suppl 1):S190–S201.
[42] Rosker, C., Meur, G., Taylor, E.J., Taylor, C.W., 2009. Functional ryanodine receptors in the plasma membrane of RINm5F pancreatic beta-cells. Journal of Biological Chemistry 284:5186–5194.
[43] Islam, M.S., 2002. The ryanodine receptor calcium channel of beta-cells: molecular regulation and physiological significance. Diabetes 51:1299–1309.
[44] Lee, H.C., 2012. Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. Journal of Biological Chemistry 287:31633–31640.
[45] Wiederkehr, A., Wollheim, C.B., 2008. Impact of mitochondrial calcium on the coupling of metabolism to insulin secretion in the pancreatic beta-cell. Cell Calcium 44:64–76.
[46] Palty, R., Silverman, W.F., Hershfinkel, M., Caporale, T., Sensi, S.L., Parnis, J., et al., 2010. NCLX is an essential component of mitochondrial Na+/Ca2+ exchange. Proceedings of the National Academy of Sciences of the United States of America 107:436–441.
[47] Raffaello, A., Mammucari, C., Gherardi, G., Rizzuto, R., 2016. Calcium at the center of cell signaling: interplay between endoplasmic reticulum, mitochondria, and lysosomes. Trends in Biochemical Sciences 41:1035–1049.
[48] Decuypere, J.P., Monaco, G., Bultynck, G., Missiaen, L., De Smedt, H., Parys, J.B., 2011. The IP(3) receptor-mitochondria connection in apoptosis and autophagy. Biochimica et Biophysica Acta 1813:1003–1013.

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[49] Alam, M.R., Groschner, L.N., Parichatikanond, W., Kuo, L., Bondarenko, A.I., Rost, R., et al., 2012. Mitochondrial Ca2+ uptake 1 (MICU1) and mitochondrial Ca2+ uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic beta-cells. Journal of Biological Chemistry 287:34445–34454.

[50] Quan, X., Nguyen, T.T., Choi, S.K., Xu, S., Das, R., Cha, S.K., et al., 2015. Essential role of mitochondrial Ca2+ uniporter in the generation of mitochondrial pH gradient and metabolism-secretion coupling in insulin-releasing cells. Journal of Biological Chemistry 290:4086–4096.

[51] Johnson, J.D., Bround, M.J., White, S.A., Luciani, D.S., 2012. Nanospaces between endoplasmic reticulum and mitochondria as control centres of pancreatic beta-cell metabolism and survival. Protoplasma 249(Suppl 1):S49–S58.

[52] Laude, A.J., Simpson, A.W., 2009. Compartmentalized signalling: Ca2+ compartments, microdomains and the many facets of Ca2+ signalling. FEBS Journal 276:1800–1816.

[53] Quesada, I., Rovira, J.M., Martin, F., Roche, E., Nadal, A., Soria, B., 2002. Nuclear KATP channels trigger nuclear Ca(2+) transients that modulate nuclear function. Proceedings of the National Academy of Sciences of the United States of America 99:9544–9549.

[54] Hardingham, G.E., Arnold, F.J., Bading, H., 2001. Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. Nature Neuroscience 4:261–267.

[55] Rutter, G.A., Tsuboi, T., Ravier, M.A., 2006. Ca2+ microdomains and the control of insulin secretion. Cell Calcium 40:539–551.

[56] Wiederkehr, A., Szanda, G., Akhmedov, D., Mataki, C., Heizmann, C.W., Schoonjans, K., et al., 2011. Mitochondrial matrix calcium is an activating signal for hormone secretion. Cell Metabolism 13:601–611.

[57] Shen, X., Valencia, C.A., Szostak, J.W., Dong, B., Liu, R., 2005. Scanning the human proteome for calmodulin-binding proteins. Proceedings of the National Academy of Sciences of the United States of America 102:5969–5974.

[58] Chin, D., Means, A.R., 2000. Calmodulin: a prototypical calcium sensor. Trends in Cell Biology 10:322–328.

[59] Hoeflich, K.P., Ikura, M., 2002. Calmodulin in action: diversity in target recognition and activation mechanisms. Cell 108:739–742.

[60] Wayman, G.A., Tokumitsu, H., Davare, M.A., Soderling, T.R., 2011. Analysis of CaM-kinase signaling in cells. Cell Calcium 50:1–8.

[61] Yu, X., Murao, K., Sayo, Y., Imachi, H., Cao, W.M., Ohtsuka, S., et al., 2004. The role of calcium/calmodulin-dependent protein kinase cascade in glucose upregulation of insulin gene expression. Diabetes 53:1475–1481.

[62] Marcelo, K.L., Ribar, T., Means, C.R., Tsimelzon, A., Stevens, R.D., Ilkayeva, O., et al., 2016. Research resource: roles for calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) in systems metabolism. Molecular Endocrinology 30:557–572.

[63] Fogarty, M.P., Cannon, M.E., Vadlamudi, S., Gaulton, K.J., Mohlke, K.L., 2014. Identification of a regulatory variant that binds FOXA1 and FOXA2 at the CDC123/CAMK1D type 2 diabetes GWAS locus. PLoS Genetics 10: e1004633.

[64] Segerstolpe, A., Palasantza, A., Eliasson, P., Andersson, E.M., Andreasson, A.C., Sun, X., et al., 2016. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. Cell Metabolism 24:593–607.

[65] Wang, X., Li, H., De Leo, D., Guo, W., Koshkin, V., Fantus, I.G., et al., 2004. Gene and protein kinase expression profiling of reactive oxygen species-associated lipotoxicity in the pancreatic beta-cell line MIN6. Diabetes 53: 129–140.

[66] Easom, R.A., 1999. CaM kinase II: a protein kinase with extraordinary talents germane to insulin exocytosis. Diabetes 48:675–684.

[67] Kim, T.K., Hemberg, M., Gray, J.M., Costa, A.M., Bear, D.M., Wu, J., et al., 2010. Widespread transcription at neuronal activity-regulated enhancers. Nature 465:182–187.

[68] Jhala, U.S., Canettieri, G., Screaton, R.A., Kulkarni, R.N., Krajewski, S., Reed, J., et al., 2003. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. Genes & Development 17:1575–1580.

[69] Persaud, S.J., Liu, B., Sampaio, H.B., Jones, P.M., Muller, D.S., 2011. Calcium/calmodulin-dependent kinase IV controls glucose-induced Irs2 expression in mouse beta cells via activation of cAMP response element-binding protein. Diabetologia 54:1109–1120.

[70] Dalle, S., Quoyer, J., Varin, E., Costes, S., 2011. Roles and regulation of the transcription factor CREB in pancreatic beta-cells. Current Molecular Pharmacology 4:187–195.

[71] Screaton, R.A., Conkright, M.D., Katoh, Y., Best, J.L., Canettieri, G., Jeffries, S., et al., 2004. The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell 119:61–74.

[72] Jansson, D., Ng, A.C., Fu, A., Depatie, C., Al Azzabi, M., Screaton, R.A., 2008. Glucose controls CREB activity in islet cells via regulated phosphorylation of TORC2. Proceedings of the National Academy of Sciences of the United States of America 105:10161–10166.

[73] Macian, F., 2005. NFAT proteins: key regulators of T-cell development and function. Nature Reviews Immunology 5:472–484.

[74] Wu, H., Rothermel, B., Kanatous, S., Rosenberg, P., Naya, F.J., Shelton, J.M., et al., 2001. Activation of MEF2 by muscle activity is mediated through a calcineurin-dependent pathway. The EMBO Journal 20:6414–6423.

[75] Crabtree, G.R., Schreiber, S.L., 2009. SnapShot: Ca2+-calcineurin-NFAT signaling. Cell 138, 210, 210 e211.

[76] Farnsworth, C.L., Freshney, N.W., Rosen, L.B., Ghosh, A., Greenberg, M.E., Feig, L.A., 1995. Calcium activation of Ras mediated by neuronal exchange factor Ras-GRF. Nature 376:524–527.

[77] Briaud, I., Lingohr, M.K., Dickson, L.M., Wrede, C.E., Rhodes, C.J., 2003. Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic beta-cells. Diabetes 52:974–983.

[78] Duan, L., Cobb, M.H., 2010. Calcineurin increases glucose activation of ERK1/2 by reversing negative feedback. Proceedings of the National Academy of Sciences of the United States of America 107:22314–22319.

[79] Lawrence, M.C., McGlynn, K., Park, B.H., Cobb, M.H., 2005. ERK1/2-dependent activation of transcription factors required for acute and chronic effects of glucose on the insulin gene promoter. Journal of Biological Chemistry 280:26751–26759.

[80] Frodin, M., Sekine, N., Roche, E., Filloux, C., Prentki, M., Wollheim, C.B., et al., 1995. Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting beta-cell line, INS-1. Journal of Biological Chemistry 270:7882–7889.

[81] Macfarlane, W.M., Smith, S.B., James, R.F., Clifton, A.D., Doza, Y.N., Cohen, P., et al., 1997. The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic beta-cells. Journal of Biological Chemistry 272: 20936–20944.

[82] Bernal-Mizrachi, E., Wen, W., Shornick, M., Permutt, M.A., 2002. Activation of nuclear factor-kappaB by depolarization and Ca(2+) influx in MIN6 insulinoma cells. Diabetes 51(Suppl 3):S484–S488.

[83] Meffert, M.K., Chang, J.M., Wiltgen, B.J., Fanselow, M.S., Baltimore, D., 2003. NF-kappa B functions in synaptic signaling and behavior. Nature Neuroscience 6:1072–1078.

[84] Li, L., Stefan, M.I., Le Novere, N., 2012. Calcium input frequency, duration and amplitude differentially modulate the relative activation of calcineurin and CaMKII. PLoS One 7:e43810.

[85] Alarcon, C., Verchere, C.B., Rhodes, C.J., 2012. Translational control of glucose-induced islet amyloid polypeptide production in pancreatic islets. Endocrinology 153:2082–2087.

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[86] German, M.S., Moss, L.G., Rutter, W.J., 1990. Regulation of insulin gene expression by glucose and calcium in transfected primary islet cultures. Journal of Biological Chemistry 265:22063–22066.
[87] Lawrence, M.C., Bhatt, H.S., Easom, R.A., 2002. NFAT regulates insulin gene promoter activity in response to synergistic pathways induced by glucose and glucagon-like peptide-1. Diabetes 51:691–698.
[88] Lawrence, M.C., Borenstein-Auerbach, N., McGlynn, K., Kunnathodi, F., Shahbazov, R., Syed, I., et al., 2015. NFAT targets signaling molecules to gene promoters in pancreatic beta-cells. Molecular Endocrinology 29:274–288.
[89] Heit, J.J., Apelqvist, A.A., Gu, X., Winslow, M.M., Neilson, J.R., Crabtree, G.R., et al., 2006. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. Nature 443:345–349.
[90] Ban, N., Yamada, Y., Someya, Y., Ihara, Y., Adachi, T., Kubota, A., et al., 2000. Activating transcription factor-2 is a positive regulator in CaM kinase IV-induced human insulin gene expression. Diabetes 49:1142–1148.
[91] Eto, K., Kaur, V., Thomas, M.K., 2006. Regulation of insulin gene transcription by the immediate-early growth response gene Egr-1. Endocrinology 147: 2923–2935.
[92] Bernal-Mizrachi, E., Wice, B., Inoue, H., Permutt, M.A., 2000. Activation of serum response factor in the depolarization induction of Egr-1 transcription in pancreatic islet beta-cells. Journal of Biological Chemistry 275:25681–25689.
[93] Welsh, M., Brunstedt, J., Hellerstrom, C., 1986. Effects of D-glucose, L-leucine, and 2-ketoisocaproate on insulin mRNA levels in mouse pancreatic islets. Diabetes 35:228–231.
[94] Welsh, M., Nielsen, D.A., MacKrell, A.J., Steiner, D.F., 1985. Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. Journal of Biological Chemistry 260:13590–13594.
[95] Dadi, P.K., Vierra, N.C., Ustione, A., Piston, D.W., Colbran, R.J., Jacobson, D.A., 2014. Inhibition of pancreatic beta-cell Ca2+/calmodulin-dependent protein kinase II reduces glucose-stimulated calcium influx and insulin secretion, impairing glucose tolerance. Journal of Biological Chemistry 289:12435–12445.
[96] Shin, S., Le Lay, J., Everett, L.J., Gupta, R., Rafiq, K., Kaestner, K.H., 2014. CREB mediates the insulinotropic and anti-apoptotic effects of GLP-1 signaling in adult mouse beta-cells. Molecular Metabolism 3: 803–812.
[97] Ribar, T.J., Epstein, P.N., Overbeek, P.A., Means, A.R., 1995. Targeted overexpression of an inactive calmodulin that binds Ca2+ to the mouse pancreatic beta-cell results in impaired secretion and chronic hyperglycemia. Endocrinology 136:106–115.
[98] Blanchet, E., Van de Velde, S., Matsumura, S., Hao, E., LeLay, J., Kaestner, K., et al., 2015. Feedback inhibition of CREB signaling promotes beta cell dysfunction in insulin resistance. Cell Reports 10:1149–1157.
[99] Nielsen, J.H., Mandrup-Poulsen, T., Nerup, J., 1986. Direct effects of cyclosporin A on human pancreatic beta-cells. Diabetes 35:1049–1052.
[100] Johnson, J.D., Ao, Z., Ao, P., Li, H., Dai, L.J., He, Z., et al., 2009. Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets. Cell Transplantation 18:833–845.
[101] Keller, M.P., Paul, P.K., Rabaglia, M.E., Stapleton, D.S., Schueler, K.L., Broman, A.T., et al., 2016. The transcription factor Nfatc2 regulates beta-cell proliferation and genes associated with type 2 diabetes in mouse and human islets. PLoS Genetics 12:e1006466.
[102] Santos, G.J., Ferreira, S.M., Ortis, F., Rezende, L.F., Li, C., Naji, A., et al., 2014. Metabolic memory of beta-cells controls insulin secretion and is mediated by CaMKII. Molecular Metabolism 3:484–489.
[103] Krueger, K.A., Ings, E.I., Brun, A.M., Landt, M., Easom, R.A., 1999. Site-specific phosphorylation of synapsin I by Ca2+/calmodulin-dependent protein kinase II in pancreatic betaTC3 cells: synapsin I is not associated with insulin secretory granules. Diabetes 48:499–506.
[104] Sweet, I.R., Gilbert, M., 2006. Contribution of calcium influx in mediating glucose-stimulated oxygen consumption in pancreatic islets. Diabetes 55: 3509–3519.
[105] Jung, S.R., Reed, B.J., Sweet, I.R., 2009. A highly energetic process couples calcium influx through L-type calcium channels to insulin secretion in pancreatic beta-cells. American Journal of Physiology. Endocrinology and Metabolism 297:E717–E727.
[106] De Marchi, U., Thevenet, J., Hermant, A., Dioum, E., Wiederkehr, A., 2014. Calcium co-regulates oxidative metabolism and ATP synthase-dependent respiration in pancreatic beta cells. Journal of Biological Chemistry 289: 9182–9194.
[107] Stamateris, R.E., Sharma, R.B., Kong, Y., Ebrahimpour, P., Panday, D., Ranganath, P., et al., 2016. Glucose induces mouse beta-cell proliferation via IRS2, MTOR, and cyclin D2 but not the insulin receptor. Diabetes 65:981–995.
[108] Alonso, L.C., Yokoe, T., Zhang, P., Scott, D.K., Kim, S.K., O'Donnell, C.P., et al., 2007. Glucose infusion in mice: a new model to induce beta-cell replication. Diabetes 56:1792–1801.
[109] Levitt, H.E., Cyphert, T.J., Pascoe, J.L., Hollern, D.A., Abraham, N., Lundell, R.J., et al., 2011. Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. Diabetologia 54:572–582.
[110] Porat, S., Weinberg-Corem, N., Tornovsky-Babaey, S., Schyr-Ben-Haroush, R., Hija, A., Stolovich-Rain, M., et al., 2011. Control of pancreatic beta cell regeneration by glucose metabolism. Cell Metabolism 13:440–449.
[111] Hija, A., Salpeter, S., Klochendler, A., Grimsby, J., Brandeis, M., Glaser, B., et al., 2014. G0-G1 transition and the restriction point in pancreatic beta-cells in vivo. Diabetes 63:578–584.
[112] Shen, W., Taylor, B., Jin, Q., Nguyen-Tran, V., Meeusen, S., Zhang, Y.Q., et al., 2015. Inhibition of DYRK1A and GSK3B induces human beta-cell proliferation. Nature Communications 6:8372.
[113] Wang, W., Walker, J.R., Wang, X., Tremblay, M.S., Lee, J.W., Wu, X., et al., 2009. Identification of small-molecule inducers of pancreatic beta-cell expansion. Proceedings of the National Academy of Sciences of the United States of America 106:1427–1432.
[114] Liu, B., Barbosa-Sampaio, H., Jones, P.M., Persaud, S.J., Muller, D.S., 2012. The CaMK4/CREB/IRS-2 cascade stimulates proliferation and inhibits apoptosis of beta-cells. PLoS One 7:e45711.
[115] Roche, E., Buteau, J., Aniento, I., Reig, J.A., Soria, B., Prentki, M., 1999. Palmitate and oleate induce the immediate-early response genes c-fos and nur-77 in the pancreatic beta-cell line INS-1. Diabetes 48:2007–2014.
[116] Impey, S., McCorkle, S.R., Cha-Molstad, H., Dwyer, J.M., Yochum, G.S., Boss, J.M., et al., 2004. Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. Cell 119:1041–1054.
[117] Tessem, J.S., Moss, L.G., Chao, L.C., Arlotto, M., Lu, D., Jensen, M.V., et al., 2014. Nkx6.1 regulates islet beta-cell proliferation via Nr4a1 and Nr4a3 nuclear receptors. Proceedings of the National Academy of Sciences of the United States of America 111:5242–5247.
[118] Dai, C., Hang, Y., Shostak, A., Poffenberger, G., Hart, N., Prasad, N., et al., 2017. Age-dependent human beta cell proliferation induced by glucagon-like peptide 1 and calcineurin signaling. Journal of Clinical Investigation 127: 3835–3844.
[119] Wang, P., Alvarez-Perez, J.C., Felsenfeld, D.P., Liu, H., Sivendran, S., Bender, A., et al., 2015. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. Nature Medicine 21:383–388.
[120] Dirice, E., Walpita, D., Vetere, A., Meier, B.C., Kahraman, S., Hu, J., et al., 2016. Inhibition of DYRK1A stimulates human beta-cell proliferation. Diabetes 65:1660–1671.

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[121] Demozay, D., Tsunekawa, S., Briaud, I., Shah, R., Rhodes, C.J., 2011. Specific glucose-induced control of insulin receptor substrate-2 expression is mediated via Ca2+-dependent calcineurin/NFAT signaling in primary pancreatic islet beta-cells. Diabetes 60:2892–2902.

[122] Soleimanpour, S.A., Crutchlow, M.F., Ferrari, A.M., Raum, J.C., Groff, D.N., Rankin, M.M., et al., 2010. Calcineurin signaling regulates human islet beta-cell survival. Journal of Biological Chemistry 285:40050–40059.

[123] Srinivasan, S., Bernal-Mizrachi, E., Ohsugi, M., Permutt, M.A., 2002. Glucose promotes pancreatic islet beta-cell survival through a PI 3-kinase/Akt-signaling pathway. American Journal of Physiology. Endocrinology and Metabolism 283:E784–E793.

[124] Ricordi, C., Zeng, Y.J., Alejandro, R., Tzakis, A., Venkataramanan, R., Fung, J., et al., 1991. In vivo effect of FK506 on human pancreatic islets. Transplantation 52:519–522.

[125] Drachenberg, C.B., Klassen, D.K., Weir, M.R., Wiland, A., Fink, J.C., Bartlett, S.T., et al., 1999. Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. Transplantation 68:396–402.

[126] Johnston, O., Rose, C.L., Webster, A.C., Gill, J.S., 2008. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. Journal of the American Society of Nephrology 19:1411–1418.

[127] Krentz, A.J., Dousset, B., Mayer, D., McMaster, P., Buckels, J., Cramb, R., et al., 1993. Metabolic effects of cyclosporin A and FK 506 in liver transplant recipients. Diabetes 42:1753–1759.

[128] Costes, S., Vandewalle, B., Tourrel-Cuzin, C., Broca, C., Linck, N., Bertrand, G., et al., 2009. Degradation of cAMP-responsive element-binding protein by the ubiquitin-proteasome pathway contributes to glucotoxicity in beta-cells and human pancreatic islets. Diabetes 58:1105–1115.

[129] Sabatini, P.V., Krentz, N.A., Zarrouki, B., Westwell-Roper, C.Y., Nian, C., Uy, R.A., et al., 2013. Npas4 is a novel activity-regulated cytoprotective factor in pancreatic beta-cells. Diabetes 62:2808–2820.

[130] Speckmann, T., Sabatini, P.V., Nian, C., Smith, R.G., Lynn, F.C., 2016. Npas4 transcription factor expression is regulated by calcium signaling pathways and prevents tacrolimus-induced cytotoxicity in pancreatic beta cells. Journal of Biological Chemistry 291:2682–2695.

[131] Oyadomari, S., Takeda, K., Takiguchi, M., Gotoh, T., Matsumoto, M., Wada, I., et al., 2001. Nitric oxide-induced apoptosis in pancreatic beta cells is mediated by the endoplasmic reticulum stress pathway. Proceedings of the National Academy of Sciences of the United States of America 98:10845–10850.

[132] Gwiazda, K.S., Yang, T.L., Lin, Y., Johnson, J.D., 2009. Effects of palmitate on ER and cytosolic Ca2+ homeostasis in beta-cells. American Journal of Physiology. Endocrinology and Metabolism 296:E690–E701.

[133] Hara, T., Mahadevan, J., Kanekura, K., Hara, M., Lu, S., Urano, F., 2014. Calcium efflux from the endoplasmic reticulum leads to beta-cell death. Endocrinology 155:758–768.

[134] Moore, C.E., Omikorede, O., Gomez, E., Willars, G.B., Herbert, T.P., 2011. PERK activation at low glucose concentration is mediated by SERCA pump inhibition and confers preemptive cytoprotection to pancreatic beta-cells. Molecular Endocrinology 25:315–326.

[135] Rutter, G.A., Hodson, D.J., Chabosseau, P., Haythorne, E., Pullen, T.J., Leclerc, I., 2017. Local and regional control of calcium dynamics in the pancreatic islet. Diabetes, Obesity and Metabolism 19(Suppl 1):30–41.

[136] Tarasov, A.I., Semplici, F., Ravier, M.A., Bellomo, E.A., Pullen, T.J., Gilon, P., et al., 2012. The mitochondrial Ca2+ uniporter MCU is essential for glucose-induced ATP increases in pancreatic beta-cells. PLoS One 7:e39722.

[137] Tarasov, A.I., Semplici, F., Li, D., Rizzuto, R., Ravier, M.A., Gilon, P., et al., 2013. Frequency-dependent mitochondrial Ca(2+) accumulation regulates ATP synthesis in pancreatic beta cells. Pflügers Archiv 465:543–554.

[138] Bonnefond, A., Froguel, P., 2015. Rare and common genetic events in type 2 diabetes: what should biologists know? Cell Metabolism 21:357–368.

[139] Zeggini, E., Scott, L.J., Saxena, R., Voight, B.F., Marchini, J.L., Hu, T., et al., 2008. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nature Genetics 40:638–645.

[140] Thurner, M., van de Bunt, M., Torres, J.M., Mahajan, A., Nylander, V., Bennett, A.J., et al., 2018. Integration of human pancreatic islet genomic data refines regulatory mechanisms at type 2 diabetes susceptibility loci. Elife 7.

[141] Bonas-Guarch, S., Guindo-Martinez, M., Miguel-Escalada, I., Grarup, N., Sebastian, D., Rodriguez-Fos, E., et al., 2018. Re-analysis of public genetic data reveals a rare X-chromosomal variant associated with type 2 diabetes. Nature Communications 9:321.

[142] Bernal-Mizrachi, E., Cras-Meneur, C., Ye, B.R., Johnson, J.D., Permutt, M.A., 2010. Transgenic overexpression of active calcineurin in beta-cells results in decreased beta-cell mass and hyperglycemia. PLoS One 5:e11969.

[143] Kato, I., Oya, T., Suzuki, H., Takasawa, K., Ichsan, A.M., Nakada, S., et al., 2008. A novel model of insulin-dependent diabetes with renal and retinal lesions by transgenic expression of CaMKIIalpha (Thr286Asp) in pancreatic beta-cells. Diabetes or Metabolism Research and Reviews 24:486–497.

[144] Epstein, P.N., Overbeek, P.A., Means, A.R., 1989. Calmodulin-induced early-onset diabetes in transgenic mice. Cell 58:1067–1073.

[145] Greenwood, R.H., Mahler, R.F., Hales, C.N., 1976. Improvement in insulin secretion in diabetes after diazoxide. Lancet 1:444–447.

[146] Guldstrand, M., Grill, V., Bjorklund, A., Lins, P.E., Adamson, U., 2002. Improved beta cell function after short-term treatment with diazoxide in obese subjects with type 2 diabetes. Diabetes & Metabolism 28:448–456.

[147] Defronzo, R.A., 2009. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58:773–795.

[148] Wang, J., Takeuchi, T., Tanaka, S., Kubo, S.K., Kayo, T., Lu, D., et al., 1999. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. Journal of Clinical Investigation 103:27–37.

[149] Huang, C.J., Lin, C.Y., Haataja, L., Gurlo, T., Butler, A.E., Rizza, R.A., et al., 2007. High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. Diabetes 56:2016–2027.

[150] Sargsyan, E., Ortsater, H., Thorn, K., Bergsten, P., 2008. Diazoxide-induced beta-cell rest reduces endoplasmic reticulum stress in lipotoxic beta-cells. Journal of Endocrinology 199:41–50.

[151] Zhou, Y., Sun, P., Wang, T., Chen, K., Zhu, W., Wang, H., 2015. Inhibition of calcium influx reduces dysfunction and apoptosis in lipotoxic pancreatic beta-cells via regulation of endoplasmic reticulum stress. PLoS One 10:e0132411.

[152] Dahan, T., Ziv, O., Horwitz, E., Zemmour, H., Lavi, J., Swisa, A., et al., 2017. Pancreatic beta-cells express the fetal islet hormone gastrin in rodent and human diabetes. Diabetes 66:426–436.

[153] Szabat, M., Page, M.M., Panzhinskiy, E., Skovso, S., Mojibian, M., Fernandez-Tajes, J., et al., 2016. Reduced insulin production relieves endoplasmic reticulum stress and induces beta cell proliferation. Cell Metabolism 23:179–193.

[154] Stancill, J.S., Cartailler, J.P., Clayton, H.W., O'Connor, J.T., Dickerson, M.T., Dadi, P.K., et al., 2017. Chronic beta-cell depolarization impairs beta-cell identity by disrupting a network of Ca(2+)-regulated genes. Diabetes 66:2175–2187.

[155] Dula, S.B., Jecmenica, M., Wu, R., Jahanshahi, P., Verrilli, G.M., Carter, J.D., et al., 2010. Evidence that low-grade systemic inflammation can induce islet dysfunction as measured by impaired calcium handling. Cell Calcium 48:133–142.

[156] Ramadan, J.W., Steiner, S.R., O'Neill, C.M., Nunemaker, C.S., 2011. The central role of calcium in the effects of cytokines on beta-cell function: implications for type 1 and type 2 diabetes. Cell Calcium 50:481–490.

[157] Xu, G., Chen, J., Jing, G., Shalev, A., 2012. Preventing beta-cell loss and diabetes with calcium channel blockers. Diabetes 61:848–856.

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[158] Yu, W., Niwa, T., Miura, Y., Horio, F., Teradaira, S., Ribar, T.J., et al., 2002. Calmodulin overexpression causes Ca(2+)-dependent apoptosis of pancreatic beta cells, which can be prevented by inhibition of nitric oxide synthase. Laboratory Investigation 82:1229–1239.

[159] Do, O.H., Low, J.T., Gaisano, H.Y., Thorn, P., 2014. The secretory deficit in islets from db/db mice is mainly due to a loss of responding beta cells. Diabetologia 57:1400–1409.

[160] Rose, T., Efendic, S., Rupnik, M., 2007. Ca2+-secretion coupling is impaired in diabetic Goto Kakizaki rats. The Journal of General Physiology 129:493–508.

[161] Antonelli, A., Ferrannini, E., 2004. CD38 autoimmunity: recent advances and relevance to human diabetes. Journal of Endocrinological Investigation 27: 695–707.

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