



Is 14-3-3 the Combination to Unlock New Pathways to Improve Metabolic Homeostasis and β -Cell Function?

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Since their discovery nearly five decades ago, molecular scaffolds belonging to the 14-3-3 protein family have been recognized as pleiotropic regulators of diverse cellular and physiological functions. With their ability to bind to proteins harboring specific serine and threonine phosphorylation motifs, 14-3-3 proteins can interact with and influence the function of docking proteins, enzymes, transcription factors, and transporters that have essential roles in metabolism and glucose homeostasis. Here, we will discuss the regulatory functions of 14-3-3 proteins that will be of great interest to the fields of metabolism, pancreatic β -cell biology, and diabetes. We first describe how 14-3-3 proteins play a central role in glucose and lipid homeostasis by modulating key pathways of glucose uptake, glycolysis, oxidative phosphorylation, and adipogenesis. This is followed by a discussion of the contributions of 14-3-3 proteins to calcium-dependent exocytosis and how this relates to insulin secretion from β -cells. As 14-3-3 proteins are major modulators of apoptosis and cell cycle progression, we will explore if 14-3-3 proteins represent a viable target for promoting β -cell regeneration and discuss the feasibility of targeting 14-3-3 proteins to treat metabolic diseases such as diabetes.

Molecular scaffold proteins belonging to the 14-3-3 protein family are widely conserved among eukaryotes (1–3), and in mammals, they consist of seven ubiquitously expressed isoforms, namely, β , γ , ζ , η , θ , σ , and ϵ (4). 14-3-3 proteins are 30-kDa acidic proteins that exist as homodimers or heterodimers, and they interact with client proteins through the recognition of specific binding motifs created by phosphorylated serine (Ser) or threonine (Thr) residues. These binding motifs are generally defined by RSXpS/TXP and RXXXpS/TXP, where pS/T represents a phosphorylated serine or

ARTICLE HIGHLIGHTS

- 14-3-3 proteins are ubiquitously expressed scaffolds with multiple roles in glucose homeostasis and metabolism.
- 14-3-3 ζ regulates adipogenesis via distinct mechanisms and is required for postnatal adiposity and adipocyte function.
- 14-3-3 ζ controls glucose-stimulated insulin secretion from pancreatic β -cells by regulating mitochondrial function and ATP synthesis as well as facilitating cross talk between β -cells and α -cells.

threonine residue (5). However, they can also interact with nonphosphorylated proteins (6) or those with other post-translational modifications, such as O-GlcNAcylation (7). Additionally, 14-3-3 proteins are also regulated by phosphorylation, which promotes their dissociation from client proteins (8,9), the destabilization of 14-3-3 protein homo- or heterodimers (10), or ubiquitination and subsequent degradation (11).

14-3-3 proteins bind to a diverse array of client proteins (enzymes, transcription factors, transporters, etc.) to regulate their compartmentalization or activity, and this results in 14-3-3 proteins being able to influence a broad variety of cellular pathways. This perspective will focus on pathways related to metabolism and glucose homeostasis, calcium-dependent exocytosis, cell cycle regulation and proliferation, and apoptosis and cell survival (Fig. 1). Emphasis will be placed on the relevance of 14-3-3 protein-dependent actions on lipid and glucose metabolism as well as for pancreatic β -cell function and survival. Lastly, the potential or likelihood of 14-3-3 ζ to serve as a molecular target for the

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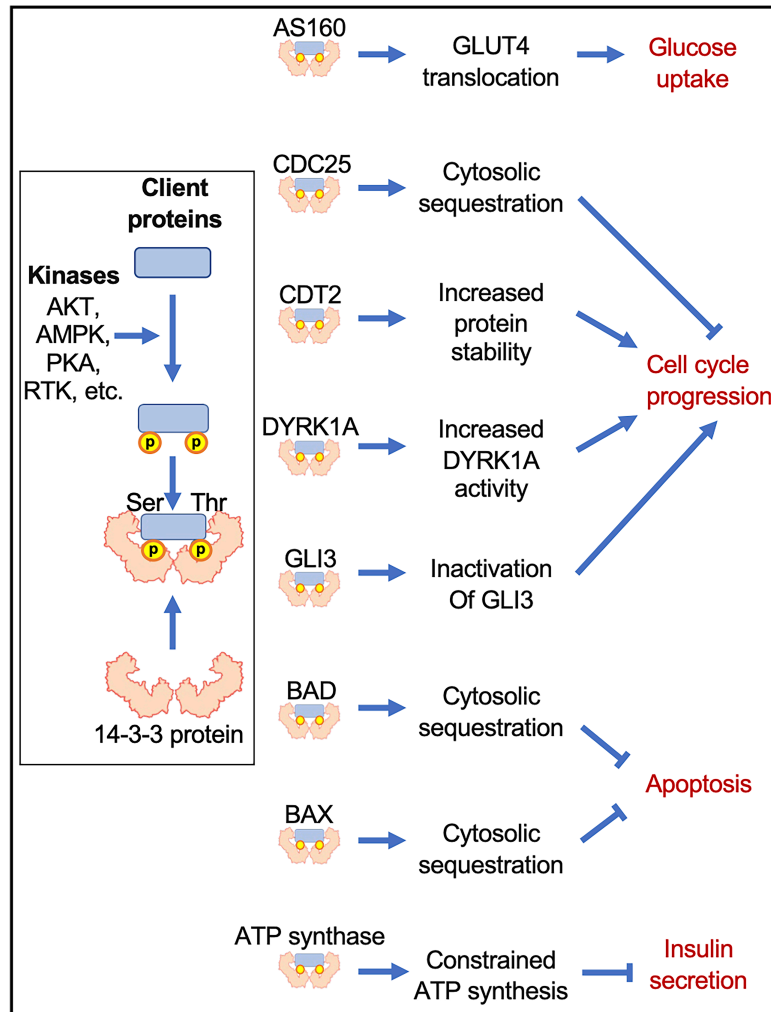


Figure 1—Pathways regulated by 14-3-3 proteins that influence cell survival and development. 14-3-3 proteins primarily interact with client proteins through the recognition of high-affinity, specific binding motifs created by phosphorylated serine (Ser) or threonine (Thr) residues. This permits 14-3-3 proteins to directly modulate the activity, subcellular localization, and/or stability of a wide range of enzymes, transcription factors, and molecular scaffolds. The latter are key effectors of essential cellular events such as glucose metabolism, cell cycle, apoptosis, or insulin secretion.

treatment of metabolic disorders, such as obesity and diabetes, will be discussed.

FUNDAMENTAL ROLES OF 14-3-3 PROTEINS IN THE REGULATION OF GLUCOSE HOMEOSTASIS

While generally underappreciated in the context of metabolic regulation, the involvement of 14-3-3 proteins in the control of glucose homeostasis has been documented, as they participate in signal transduction pathways that facilitate glucose uptake. For example, insulin-stimulated phosphorylation of Thr649 on the Rab GTPase-activating protein (RabGAP), AKT substrate of 160 kDa (AS160/TBC1D4), promotes its binding to 14-3-3 proteins, which inhibits the RabGAP activity of AS160 and permits GLUT4 translocation to the plasma membrane (Fig. 1) (12). The importance of this interaction was shown in mice overexpressing an AS160 variant with a Thr649Ala mutation, as impaired glucose uptake, glucose

clearance, and insulin sensitivity were observed (12). A similar mechanism in skeletal muscle in response to insulin or contraction has been observed whereby binding of 14-3-3 proteins to GARNL1/RalGAP α 1 controls Ral-A-mediated GLUT4 translocation to the plasma membrane (13). Another RabGAP, TBC1D1, governs GLUT4 translocation once phosphorylated by AMP-activated protein kinase (AMPK) on Ser231, and as with Thr649 on AS160, this serine residue is a critical binding site for 14-3-3 proteins (14). The importance of Ser231 in AMPK-mediated glucose uptake was seen in animals expressing a Ser231Ala TBC1D1 mutant, which led to impaired muscle- and whole-body glucose clearance in response to AICAR, a potent activator of AMPK, and loss of GLUT4 translocation in isolated skeletal muscle (15).

Another potential mechanism by which 14-3-3 proteins may affect glucose homeostasis is through their binding to Insulin Receptor Substrate 1 and 2 (IRS1 and IRS2) (16). 14-3-3 proteins bind to IRS1 within its phosphotyrosine

binding domain, which attenuates its activation by the insulin receptor (16). 14-3-3 proteins also control the stability of IRS2 through Ser1137- and Ser1138-dependent binding, as observed in HEK293 cells and primary murine hepatocytes (17). In vivo evidence of a role of 14-3-3 proteins in glucose homeostasis was seen through our use of systemic 14-3-3 ζ knockout mice, which displayed decreased insulin sensitivity in response to an insulin bolus (18). In a similar vein, decreased 14-3-3 ζ abundance in human skeletal muscle biopsies is associated with decreased insulin sensitivity (19). Altogether, these observations demonstrate contributions of 14-3-3 proteins to insulin signaling and glucose homeostasis.

14-3-3 ζ IS CRUCIAL FOR ADIPOCYTE FATE

To date, we have explored the critical roles of 14-3-3 ζ in the regulation of whole-body adiposity, adipogenesis, and adipocyte function. Using in vitro and in vivo models, we identified 14-3-3 ζ as an essential regulator of adipogenesis (18). Systemic deletion of 14-3-3 ζ in mice significantly reduced visceral white adiposity, and glucose intolerance and insulin resistance were observed (18). In contrast, transgenic overexpression of 14-3-3 ζ enhanced age- and high-fat-diet-induced weight gain and fat accumulation (18). Mechanistically, 14-3-3 ζ was required for mitotic clonal expansion of murine preadipocytes by controlling the abundance of the cyclin-dependent kinase inhibitor CDKN1B/p27^{Kip1} in addition to stabilizing the abundance of the early adipogenic transcription factor C/EBP- δ during the initial stages of adipogenesis (18).

Fully differentiated white adipocytes also require 14-3-3 ζ for their optimal function. Indeed, we discovered that depletion of 14-3-3 ζ in mature adipocytes impaired β -adrenergic receptor-mediated lipolysis, one of the principal metabolic functions of adipose tissue (20). This is consistent with previous findings reporting cAMP-dependent protein kinase (PKA)-dependent phosphorylation of Ser660 and Ser406 of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), respectively, both of which represent 14-3-3 binding sites (21,22). Phosphorylated HSL and ATGL, together with monoacylglycerol lipase (MGL), catalyze the sequential hydrolysis of triglycerides stored within lipid droplets into diglycerides and free fatty acids and glycerol during lipolysis (23). Depletion of 14-3-3 ζ also decreased the expression of peroxisome proliferator-activated receptor γ 2 (PPAR γ 2), which is often considered the master regulator of adipogenesis (20). We also discovered that 14-3-3 ζ overexpression enhanced cold-induced beiging of inguinal white adipose tissue, as seen by increased expression of the thermogenic protein Uncoupling Protein 1 (UCP1) (24).

Recently, we identified an alternative mechanism of 14-3-3 ζ -dependent adipogenesis (25). In mouse embryonic fibroblasts undergoing adipogenesis, the interactome of a tandem affinity purification (TAP) epitope-tagged 14-3-3 ζ was identified by mass spectrometry. Several RNA-splicing factors, such as HNRPF, DDX6, and SFPQ, were enriched in the

TAP-14-3-3 ζ interactome during adipogenesis, and their silencing via siRNA impeded adipogenesis by preventing the generation of pro-adipogenic mRNA splice variants (25).

Taken together, these findings demonstrate important physiological roles of 14-3-3 ζ in adipocyte development and function. In-depth studies aimed at defining the adipocyte-specific contributions of 14-3-3 ζ to age- and high-fat-diet-associated weight gain and obesity are still required, and these studies would aid in fully understanding the importance of 14-3-3 ζ to adipocyte biology.

THE IMPORTANCE OF 14-3-3 PROTEINS FOR β -CELL FUNCTION, SURVIVAL, AND PROLIFERATION

The importance of the pancreatic β -cell in the regulation of glucose homeostasis is undeniable, as it is the predominant cell type that produces insulin (Fig. 2A). Impaired insulin secretion and β -cell apoptosis are key determinants in the development of diabetes, which further underscores the importance of the pancreatic β -cell (26). While 14-3-3 proteins are important for regulating metabolic signaling pathways that underpin glucose or lipid homeostasis in various cell types, an important question is whether 14-3-3 ζ and its related isoforms similarly influence important aspects of β -cell function, namely, insulin secretion and proliferation.

14-3-3 Proteins and Cell Cycle Progression Control

In multiple cell types, 14-3-3 proteins are known to regulate the eukaryotic cell cycle (Fig. 1), consisting of growth or Gap1 (G₁), DNA synthesis (S), G₂, and mitosis (M) phases (27). The transition from each phase is strictly controlled by cyclins (Cyc) and serine/threonine cyclin-dependent kinases (CDKs). Mitogenic stimuli trigger the expression of CycD during G₁, which binds to CDKs like CDK4 and CDK6. These CycD-CDK complexes alleviate the inhibition of E2F transcription factors by pocket proteins, thereby promoting the expression of genes involved in S phase. The transitions from S to G₂ phases, and then from G₂ to M, are driven by CycA-CDK2 and CycB-CDK1 complexes, respectively (27). At key transitions of the cell cycle, Cyc-CDK complexes can be inhibited by CDK inhibitors such as CDKN1B/p27^{Kip1}. Tumor suppressors, such as p53, DNA damage, starvation, or differentiation can induce the expression of various CDK inhibitors, for instance, CDKN1B/p27^{Kip1}, to limit cell cycle progression (18,27).

One of the first pieces of evidence of a regulatory role of 14-3-3 proteins on the cell cycle was reported in colorectal cancer cells and yeast (28). 14-3-3 σ , the expression of which is induced by p53, was shown to bind to and sequester the phosphatase Cdc25C in the cytosol, thereby preventing the dephosphorylation of CycB-CDK1 complexes and permitting the transition from G₂ to M phase (Fig. 1) (28). The importance of 14-3-3 proteins in cell cycle progression was further described by pan-inhibition or isoform-specific overexpression, which caused aberrant cell cycle progression or blocked proliferation, respectively (29,30). Some 14-3-3 protein isoforms,

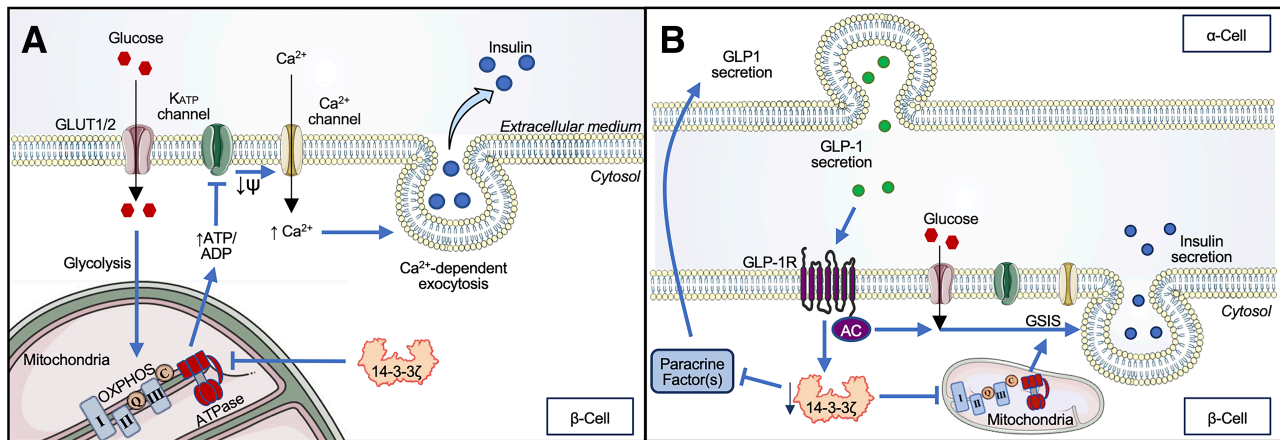


Figure 2—Role of 14-3-3 ζ proteins in exocytosis and insulin secretion. **A:** The localization of 14-3-3 ζ in mitochondria may directly affect ATP synthesis, as evidenced by the ability of 14-3-3 ζ to restrict mitochondrial OXPHOS. This ultimately constrains ATP-dependent insulin secretion. **B:** In parallel, β -cell 14-3-3 ζ may regulate cross talk between β -cells and α -cells. Secretion of the insulinotropic peptide GLP-1 by α -cells leads to activation of the GLP-1R in β -cells, leading to reductions in 14-3-3 ζ expression and the liberation of β -cell-derived paracrine factors that enhance β -cell function.

such as 14-3-3 σ , bind to CDK2, CDK4, and various cyclins to inhibit their function and impair cell cycle entry and proliferation (30). Deletion of 14-3-3 ϵ downregulates CycE1 expression, which promotes CDKN1B/p27^{Kip1} accumulation and prevents progression from G₀/G₁ to G₂/M (31). We have similarly discovered that 14-3-3 ζ is essential to the cell cycle during adipogenesis in vitro, as 14-3-3 ζ depletion activated a novel GLI3–CDKN1B/p27^{Kip1} axis to block cell cycle progression in 3T3-L1 preadipocytes (Fig. 1) (18). Alternatively, 14-3-3 ϵ and 14-3-3 γ isoforms interact with chromatin licensing and DNA replication factor-2 (CDT2) in a Thr464 phosphorylation–dependent manner to protect CDT2 against E3 ubiquitin ligase–triggered proteasomal degradation (32). This conserves the ability of CDT2 to promote G₂/M phase progression (Fig. 1) (32).

We found that systemic 14-3-3 ζ deletion in mice increased β -cell area (33); however, this increase in β -cell area could have been a compensatory response to decreased whole-body insulin sensitivity (18). Recently, we found that treatment of dispersed mouse and human islet preparations with pan-14-3-3 protein inhibitors significantly increased β -cell proliferation ex vivo, demonstrating a regulatory role of 14-3-3 proteins in β -cell proliferation (34). Moreover, we also reported that deletion of 14-3-3 ζ in murine β -cells in vivo increased β -cell proliferation, as measured by the expression of proliferative markers (Ki-67 and PCNA), but surprisingly, this was not associated with a corresponding increase in β -cell mass (34). It is unclear why β -cell mass remained unchanged despite increased numbers of proliferating β -cells. One possibility is that 14-3-3 ζ functions at specific checkpoints in the cell cycle and that other factors, including other 14-3-3 protein isoforms, are still needed to facilitate mitosis in β -cells (28). Additional studies are required to explore this further, as this would increase our understanding of the mechanisms that control β -cell proliferation.

Relationship Between 14-3-3 Proteins and DYRK1A-Mediated Proliferation

A proposed approach to treat type 1 and type 2 diabetes (T1D and T2D, respectively) is to induce the proliferation of β -cells to increase functional β -cell mass; however, whether this can be applied to the clinical setting is unclear. In humans, β -cell proliferation primarily occurs during the first few years of life, starting around birth and reaching its highest rate at the first postnatal year before drastically declining during early childhood (35). Adult human β -cells are generally refractory to proliferative stimuli and fail to undergo cell cycle progression and proliferation, at least in part due to a failure of cyclins and CDKs to undergo nuclear translocation (35,36).

Among identified regulators of β -cell proliferation, DYRK1A represents an intermediate that may link 14-3-3 protein activity with cell cycle progression in β -cells. DYRK1A modulates a broad range of biological functions, including mRNA splicing, apoptosis, cell division, and differentiation (37). DYRK1A inhibitors, such as harmine, promote murine and human β -cell proliferation in vitro and improve glucose homeostasis when administered to diabetic mice (37–39). Mechanistically, DYRK1A inhibition decreases CDKN1B/p27^{Kip1} stability, which permits cell cycle entry of human β -cells when assessed in vitro or in human islet transplantation studies (38). Direct interactions between 14-3-3 proteins and DYRK1A were discovered by yeast two-hybrid screening and coimmunoprecipitation approaches, and disrupting interactions between 14-3-3 proteins and DYRK1A inhibits DYRK1A kinase activity (40). Lastly, 14-3-3 β has also been shown to interact with DYRK1A via phosphorylated Ser520, thereby increasing its kinase activity (Fig. 1) (41). Given the established role of DYRK1A in β -cell proliferation, these observations highlight the need to further explore the relationship between DYRK1A and 14-3-3 proteins in β -cells, especially in the context of proliferation.

Roles of 14-3-3 Proteins in Hormone Secretion

Glucose-stimulated insulin secretion (GSIS) from pancreatic β -cells occurs in response to elevated glycemia and is characterized by glucose entry, closure of K_{ATP} channels, opening of voltage-gated Ca^{2+} channels, and exocytosis of insulin-containing granules (Fig. 2A) (42). One of the first studies that implicated 14-3-3 proteins in the control of Ca^{2+} -dependent hormone secretion was performed in adrenal chromaffin cells (43). Morgan and Burgoyne (43) identified a 30-kDa cytosolic protein, previously identified as EXO1, that potentiated protein kinase C-mediated Ca^{2+} -dependent release of catecholamines. It was later determined that EXO1 shared sequence homology with 14-3-3 proteins.

We recently discovered a novel role for 14-3-3 ζ and its related isoforms in the regulation of ATP-dependent insulin secretion (Fig. 2A). β -Cell-specific deletion of 14-3-3 ζ in mice, as well as in vitro treatment of mouse and human islets with pan-14-3-3 protein inhibitors, enhanced GSIS without negatively impacting cell survival or growth. This was due to increased mitochondrial function, potentiated ATP synthesis, and upregulated expression of genes involved in oxidative phosphorylation (OXPHOS) (34). Moreover, in human islets from donors with T2D and isolated islets from *db/db* mice, pan-inhibition of 14-3-3 proteins also significantly potentiated GSIS, mitochondrial function, and ATP synthesis (34). Collectively, these observations demonstrate that 14-3-3 ζ and its related isoforms restrain the full secretory potential of β -cells (Fig. 2A). As 14-3-3 proteins are known regulators of ATP synthase activity in mitochondria and chloroplasts in plants, a similar role of 14-3-3 ζ may exist in mammalian cells, including pancreatic β -cells (44). Indeed, we have previously detected several ATP synthase subunits in the interactome of 14-3-3 ζ in differentiating preadipocytes (25), and this warrants further studies to examine the relationship between 14-3-3 ζ and ATP synthase complex formation and function in β -cells in the context of insulin secretion.

14-3-3 ζ , Major Hub for the Regulation of Apoptosis and Cell Survival

One of the initial functions assigned to 14-3-3 proteins was the ability to regulate apoptosis (45), as it was shown that an inactive mutant of 14-3-3 ζ markedly increased p38 mitogen-activated protein kinase-dependent apoptosis (45). The intrinsic apoptotic pathway is mediated by the BCL2 antagonist of cell death, BAD, and all seven mammalian isoforms of 14-3-3 proteins interact with BAD to promote cell survival (Fig. 1) (46). Human BAD binds to 14-3-3 proteins in a phosphorylation-dependent manner, with Ser74 and Ser75 serving as noncanonical and canonical 14-3-3 binding sites, respectively (47). In murine BAD, phosphorylated Ser112, Ser136, and Ser155 constitute canonical binding sites for 14-3-3 proteins (48). When apoptosis is initiated, dephosphorylation of BAD promotes its dissociation from 14-3-3 proteins and permits BAD translocation to mitochondria to inhibit the antiapoptotic BCL2-related protein long isoform (BCL-XL) (49). Another major effector of apoptosis, BCL2-

associated X protein (BAX), is sequestered in the cytosol by 14-3-3 θ in resting cells, and proapoptotic stimuli can promote caspase-dependent and -independent cleavage of 14-3-3 θ to promote BAX mitochondrial translocation and the release of cytochrome c (Fig. 1) (50). We previously reported that 14-3-3 ζ regulates MIN6 insulinoma cell survival, as its overexpression and depletion were associated with increased cytoplasmic sequestration of BAD and decreased cytoplasmic sequestration of BAX (51).

Taken together, these findings demonstrate important roles of 14-3-3 proteins in the regulation of cell survival, proliferation, and exocytosis, all of which are critical for optimal pancreatic β -cell function and glucose homeostasis.

HOW DOES 14-3-3 ζ FIT INTO ALTERNATIVE METABOLIC PATHWAYS THAT REGULATE β -CELL FUNCTION?

The recent discovery of various alternative pathways that regulate insulin secretion has changed our perspective on how insulin release from the β -cell is controlled. Whether 14-3-3 proteins are involved in these pathways is not known, and below we discuss whether 14-3-3 proteins contribute to these pathways as well as newly evidenced cellular cross talk within pancreatic islets.

The Good Part of BAD

Although BAD is an established regulator of apoptosis, a series of seminal studies have also demonstrated its metabolic activity in mitochondria (52–54). Indeed, an enzymatic complex consisting of phosphorylated BAD (Ser112, Ser136, and Ser155) and glucokinase has been identified (52,53), whereby phosphorylated BAD is required for stabilizing the enzymatic complex and promoting optimal glucokinase-dependent mitochondrial respiration (54). Whole-body deletion of BAD in vivo, as well as transgenic overexpression of a nonphosphorylatable BAD mutant (Ser112/136/155A), resulted in impaired glucose tolerance in mice (54). Further examination of systemic BAD knockout mice and isolated islets revealed defective GSIS and impaired expansion of β -cell mass in response to high-fat diet feeding (52).

Of the three 14-3-3 protein binding sites in murine BAD, Ser112 and Ser136 are essential in the regulation of apoptosis (52–54). To date, the contributions of Ser155 to β -cell survival are not well-established, but phosphorylation of Ser155 in the BH3 domain of BAD has been found to shift the function of BAD from regulating apoptosis toward a metabolic role by promoting the enzymatic activity of glucokinase in mitochondria as well as positively influencing ATP-dependent GSIS. When phosphorylated BAD BH3 domain mimetics were administered in vitro and in vivo, β -cells were protected from inflammation-, hypoxia-, and nitric oxide-related apoptosis, along with enhanced GSIS, in a glucokinase-dependent manner. Moreover, these phosphorylated BAD BH3 domain mimetics were also able to prolong the survival and enhance the function of healthy mouse

islets transplanted into diabetic, streptozotocin-treated mice to restore glucose homeostasis (53).

Given our finding that 14-3-3 ζ has important roles in regulating insulin secretion, 14-3-3 ζ may have dual roles in regulating ATP synthase activity (34) while also influencing the mitochondrial BAD-glucokinase complex, depending on the bioenergetic status of the β -cell. Depending on the phosphorylation status of BAD, 14-3-3 ζ may have a critical role in balancing BAD's apoptotic functions by limiting discrete pools of BAD to translocate to mitochondria to participate in the BAD-glucokinase complex (52,53) while sequestering the majority of BAD in the cytoplasm to prevent apoptosis (51). This suggests a novel area of study to further increase our understanding of GSIS and its regulation by BAD.

Is There Any Place for 14-3-3 ζ in the New *Mito_{cat}-Mito_{ox}* Model?

A novel paradigm that challenges the canonical model of GSIS has been proposed, but whether 14-3-3 ζ and its related isoforms are involved is unclear (55–57). According to this model, termed *Mito_{cat}-Mito_{ox}*, activation of membrane-associated pyruvate kinase (PK) leads to a localized increase in the ATP-to-ADP ratio to promote K_{ATP} channel closure, the subsequent rise in intracellular Ca^{2+} , and insulin secretion (55–57). Energy consumption during this secretory process increases levels of ADP, which serves as a substrate for OXPHOS-fueled mitochondrial ATP synthesis necessary for sustained K_{ATP} channel closure and insulin secretion. It has been proposed that this cycle underlies glucose-induced oscillatory release of insulin (55–57). Given the scaffolding functions of 14-3-3 ζ and its ability to compartmentalize proteins in a cell (51,58), it would be interesting to assess if, where, and how it regulates the activities and localization of key effectors of the *Mito_{cat}-Mito_{ox}* model. Moreover, how our recent finding of 14-3-3 ζ -dependent regulation of ATP synthesis fits into this model will be of great interest to those who study insulin secretion. Collectively, these studies would increase our understanding of the pathways that facilitate GSIS.

Contributions of 14-3-3 ζ in the Regulation of GSIS via Islet Cells Cross Talk

The α -cell has long been established as a cell type that is critically involved in the counterregulatory response to hypoglycemia, as it secretes glucagon to stimulate hepatic glucose output. However, it is increasingly evident that α -cells play an important role in the potentiation of GSIS. Accumulating evidence demonstrates that the proglucagon-derived peptides, glucagon and glucagon-like peptide-1 (GLP-1), contribute to GSIS (59,60). α -Cells exhibit heterogeneity in their hormone profile. While they typically produce glucagon, under certain circumstances, such as obesity and diabetes, some α -cells produce active GLP-1 (59,61). The regulation of this heterogeneity in α -cell hormone production is poorly understood.

We have found that enhanced β -cell GLP-1 receptor (GLP-1R) signaling, via surgical intervention or pharmacological activation, activates α -cell GLP-1 production in mouse islets both in vivo and in vitro and in human islets in vitro (60,62,63). We do not find an effect of GLP-1R agonist treatment of cultured α -TC1-6-cells to induce α -cell GLP-1 production, which is consistent with the literature reporting that, at most, only a small population of α -cells expresses the GLP-1R (64). Instead, the effect of β -cell GLP-1R signaling to activate α -cell GLP-1 production is mediated by a paracrine protein factor (60). While we have not yet identified the secreted factor that mediates this effect, our efforts to do so have identified downregulation of β -cell 14-3-3 ζ expression as a key downstream regulator of this pathway (Fig. 2B) (60). Specifically, we found that GLP-1R activation downregulates β -cell 14-3-3 ζ expression in human islets in vitro and mouse islets in vivo, but it does not do so in mouse islets with the β -cell GLP-1R knockout. Further, addition of a pan-inhibitor of 14-3-3 proteins increased active GLP-1 production and secretion in both mouse and human islets in vitro. Finally, addition of recombinant 14-3-3 ζ ablated the ability of a GLP-1R agonist to activate mediators of α -cell GLP-1 production in mouse islets in vitro. Future work is needed to define the β -cell GLP-1R signaling cascade that regulates 14-3-3 ζ expression and the proteins that interact with 14-3-3 ζ within the β -cell that may become disinhibited in response to 14-3-3 ζ downregulation. Such studies are of importance, as they will increase our understanding of how 14-3-3 ζ mediates intraislet cross talk and how it acts in a paracrine manner to activate α -cell GLP-1 production.

These observations align with our previous finding where whole-body 14-3-3 ζ ablation improved glucose tolerance in a GLP-1R-dependent manner (Fig. 2B) (33). In a similar vein, three 14-3-3 isoforms, β , ϵ , and θ , have also been associated with the protective effects of GLP-1R and glucokinase pathways against β -cell toxicity (65). Together, these findings provide compelling evidence that 14-3-3 ζ regulates cross talk between β -cells and α -cells and supports a role for β -cell 14-3-3 ζ as a negative regulator of GSIS in human and mouse islets (Fig. 2B).

“14-3-3”: A Secret Code to Enhance β -Cell Function and Proliferation?

One of the main goals in developing treatments for diabetes is to safely increase functional β -cell mass. Our findings that 14-3-3 ζ has critical roles in the regulation of β -cell survival and function (33,34,51,60) raise the question of whether 14-3-3 ζ represents a novel therapeutic target for the treatment of diabetes and other metabolic diseases.

Changes in the expression profiles of 14-3-3 protein isoforms have been confirmed in the context of T2D, which suggests that altered expression influences β -cell function. For example, recent single-cell RNA sequencing-based analyses of isolated islets from human subjects with T2D or mice exposed to cellular stresses in vitro showed

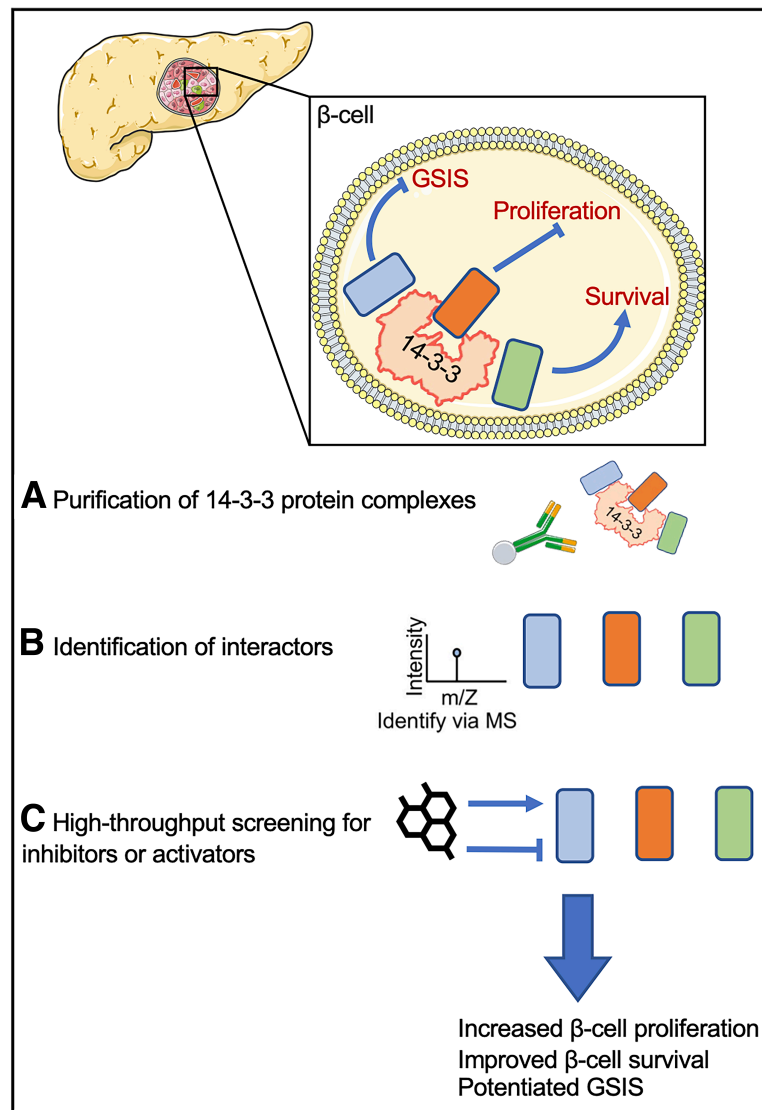


Figure 3—Is the 14-3-3 protein interactome a pharmacological gold mine for protecting β -cells? 14-3-3 ζ can interact with proteins harboring specific binding motifs created by phosphorylated serine (Ser) or threonine (Thr) residues, and this results in a dynamic interactome that can be elucidated for identifying novel regulators of β -cell function, proliferation, or survival. For example, using cadaveric human islets from healthy donors or those from donors with type 2 diabetes, 14-3-3 ζ -anchored protein complexes can be purified (A) and subjected to mass spectrometry (MS) (B) to elucidate the interactome of 14-3-3 ζ , and the contributions of identified interactors could then be determined by high-throughput screening assays or genomic approaches to assess their roles in β -cell survival, proliferation, and insulin secretion (C).

that most 14-3-3 isoforms are upregulated in β -cells (66,67). We have also confirmed in samples of pancreatic islets from T2D human donors and *db/db* mice that *Ywhaz/YWHAZ* mRNA levels were increased compared with those of healthy, nondiabetic control samples (34). Further support for this notion was our finding that overexpression of 14-3-3 ζ in mouse islets impaired β -cell function in vitro and in vivo (33,34). Of special interest was the discovery of single nucleotide polymorphisms associated with *YWHAZ*, the gene coding for 14-3-3 γ , that contribute to increased risk of islet dysfunction associated with T2D (68). Collectively, these observations suggest potential pathogenic effects of elevated expression of 14-3-3 ζ and its related isoforms in β -cells.

To date, there are no approved therapies that have been specifically designed or developed to directly target 14-3-3 ζ or its related isoforms. Fingolimod (FTY720) was initially developed as a treatment for multiple sclerosis and acts by decreasing the activity of the sphingosine-1-phosphate receptor, S1PR1 (69), and it has been shown to inactivate all 14-3-3 proteins by promoting their phosphorylation and causing the dissociation of 14-3-3 protein dimers (9). While it is unclear if fingolimod's actions are due to inhibition of 14-3-3 proteins, administration of Fingolimod has beneficial effects on multiple aspects of glucose homeostasis and β -cell function and survival. For example, prolonged administration of fingolimod to diabetic *db/db* mice restores normoglycemia, glucose tolerance, and

insulin sensitivity, in addition to promoting the growth and proliferation of pancreatic β -cells (70). These beneficial effects of fingolimod on β -cell function and proliferation are also maintained in spontaneously diabetic nonhuman primates (71). As an immunosuppressant, fingolimod protects grafted organs, including pancreatic islets, against autoimmune-mediated rejection by suppressing the infiltration of peripheral cytotoxic CD8⁺ T cells without affecting systemic immunity (72–75). Lastly, fingolimod is not reported to have detrimental effects on human islet function (76).

In-depth studies will be required to determine if it is possible to develop fingolimod-based approaches to target 14-3-3 ζ and its related isoforms, which we have established are critical for β -cell function, proliferation, and survival (33,34,51,60). The ubiquitous expression of 14-3-3 proteins highlights the complexity of specifically targeting β -cells, and this potentially can be circumvented by elucidating the interactomes of 14-3-3 ζ and its related isoforms in specific contexts, such as β -cell proliferation or GSIS (Fig. 3). This would permit the identification of specific mediators that function downstream of 14-3-3 ζ in β -cells and that may represent alternative targets for the treatment of diabetes and obesity (Fig. 3). Additional studies in this domain are required before targeting 14-3-3 ζ for the treatment of diabetes can come to fruition.

CONCLUSION

Although they were initially described as regulators of apoptosis and neurotransmitter synthesis and secretion, members of the 14-3-3 protein family have now been recognized as crucial regulators of metabolic signaling pathways. Moreover, their associations with the pathogenesis of chronic metabolic diseases, including diabetes and obesity, have become apparent. Among the seven mammalian 14-3-3 protein isoforms, we have identified 14-3-3 ζ as a critical regulator of cell growth, proliferation, and apoptosis; glucose and lipid homeostasis; and islet and β -cell function. These ascribed functions of 14-3-3 ζ are of particular interest, as they highlight the therapeutic potential of targeting 14-3-3 ζ to treat metabolic diseases. In the context of diabetes, this could lead to the restoration of functional β -cell mass, which is a key determinant in the pathogenesis of both predominant forms of diabetes; however, it is important to note that this notion is very much in its infancy, and a large body of work will be required to comprehensively evaluate this possibility.

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