Islets 4:1, 1–9; January/February 2012; © 2012 Landes Bioscience

Overcoming the spatial barriers of the stimulus secretion cascade in pancreatic β-cells

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Keywords: islets, insulin secretion, metabolism, mitochondria, carriers

The ability of the pancreatic β-cells to adapt the rate of insulin release in accordance to changes in circulating glucose levels is essential for glucose homeostasis. Two spatial barriers imposed by the plasma membrane and inner mitochondrial membrane need to be overcome in order to achieve stringent coupling between the different steps in the stimulus-secretion cascade.

The first spatial barrier is overcome by the presence of a glucose transporter (GLUT) in the plasma membrane, whereas a low affinity hexokinase IV (glucokinase, GK) in the cytosol conveys glucose availability into a metabolic flux that triggers and accelerates insulin release. The mitochondrial inner membrane comprises a second spatial barrier that compartmentalizes glucose metabolism into glycolysis (cytosol) and tricarboxylate (TCA) cycle (mitochondrial matrix).

The exchange of metabolites between cytosol and mitochondrial matrix is mediated via a set of mitochondrial carriers, including the aspartate-glutamate carrier (aralar1), α-ketoglutarate carrier (OGC), ATP/ADP carrier (AAC), glutamate carrier (GC1), dicarboxylate carrier (DIC) and citrate/isocitrate carrier (CIC). The scope of this review is to provide an overview of the role these carriers play in stimulus-secretion coupling and discuss the importance of these findings in the context of the exquisite glucose responsive state of the pancreatic β-cell.

Insulin Secretion from the Pancreatic β-cell

The consensus model of glucose-stimulated insulin secretion (GSIS) holds that an increase in glucose metabolism results in a rise of the cytosolic ATP/ADP ratio (**Fig. 1A**), which promotes closure of the ATP-sensitive K^+ (K_{ATP}) channels and triggers plasma membrane depolarization. This depolarization event opens voltage-gated Ca^{2+} channels (VDCC) that facilitate the influx of Ca^{2+} which accelerates insulin release.[1-4] This K_{ATP} channel-dependent pathway appears to be particularly important in the first acute triggering phase, whereas other metabolites have been suggested to play a role in the second and more sustained phase of insulin release.[5] Strong evidence for the K_{ATP} channel-independent pathways of GSIS has been provided by studies showing that glucose can still augment insulin secretion in islets from mice that lack functional K_{ATP} channels or in conditions where the K_{ATP} channels are held open by application of diazoxide and membrane depolarization is evoked by high K^+.[6,7] These observations led to the adaptation of the original model, taking into account that ATP may target alternate modalities in the stimulus-secretion cascade or that other metabolic signals may promote insulin release via a pathway that does not involve the K_{ATP}-channel. Several metabolites, including GTP,[8,9] malonyl-CoA,[10] long chain acyl (LC)-CoAs,[11] glutamate[12] and NADPH[13,14] have been proposed to act as metabolic coupling factors in the K_{ATP}-channel-independent pathways of GSIS.

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Submitted: 09/05/11; Revised: 10/06/11; Accepted: 10/07/11
http://dx.doi.org/10.4161/isl.18338

The Proximal Glucose Sensor

Considering that glucose metabolism is essential for GSIS, both the high K_m glucose transporter GLUT2 (SLC2A2) and glucose phosphorylating enzyme GK have been suggested to mediate a pivotal role as a proximal glucose sensor.[15,16] Parallel loss of GLUT2 and GSIS has been reported in various rodent models of diabetes,[17,18] indicating that inadequate glucose transport in β-cells may be involved in the etiology of the metabolic disease.[19,20] However, comparative studies between rat and human β-cells have revealed a remarkable difference, as human β-cells abundantly express GLUT1 (SLC2A1), whereas the inverse holds true for rat β-cells.[21] These interspecies differences have challenged the view that GLUT2 is an obligatory component of the β-cell glucose sensor and this notion has been supported by various lines of investigation. First, GLUT2 protein levels are at least 90% lower in islets obtained from transgenic mice that express the human [Val^{12}]HRAS oncoprotein under control of the insulin promoter.[22] Nevertheless, these animals remain glucose tolerant for several months. Second, parallel measurements of glucose transport and glucose utilization in the glucose competent βTC3 and βHC9 cell lines have indicated that elevated GLUT1 levels are sufficient to provide sufficient glucose over the plasma membrane to support glycolysis.[23] Consistent with this idea, glucose transport was determined to occur at least one order of magnitude in excess of glycolytic flux in rodent islets and FACS-enriched rat β-cells.[23,24] In human islets, glucose transport also exceeds glycolytic flux at least 4- to 5-fold,[21] indicating that glucose uptake is not the rate limiting step in glucose metabolism, and therefore, does not affect the degree of glucose sensitivity in β-cells. Finally, the idea that GLUT1 can compensate for the complete lack of GLUT2 was evidenced in vivo as β-cell specific expression of GLUT1 in GLUT2 deficient

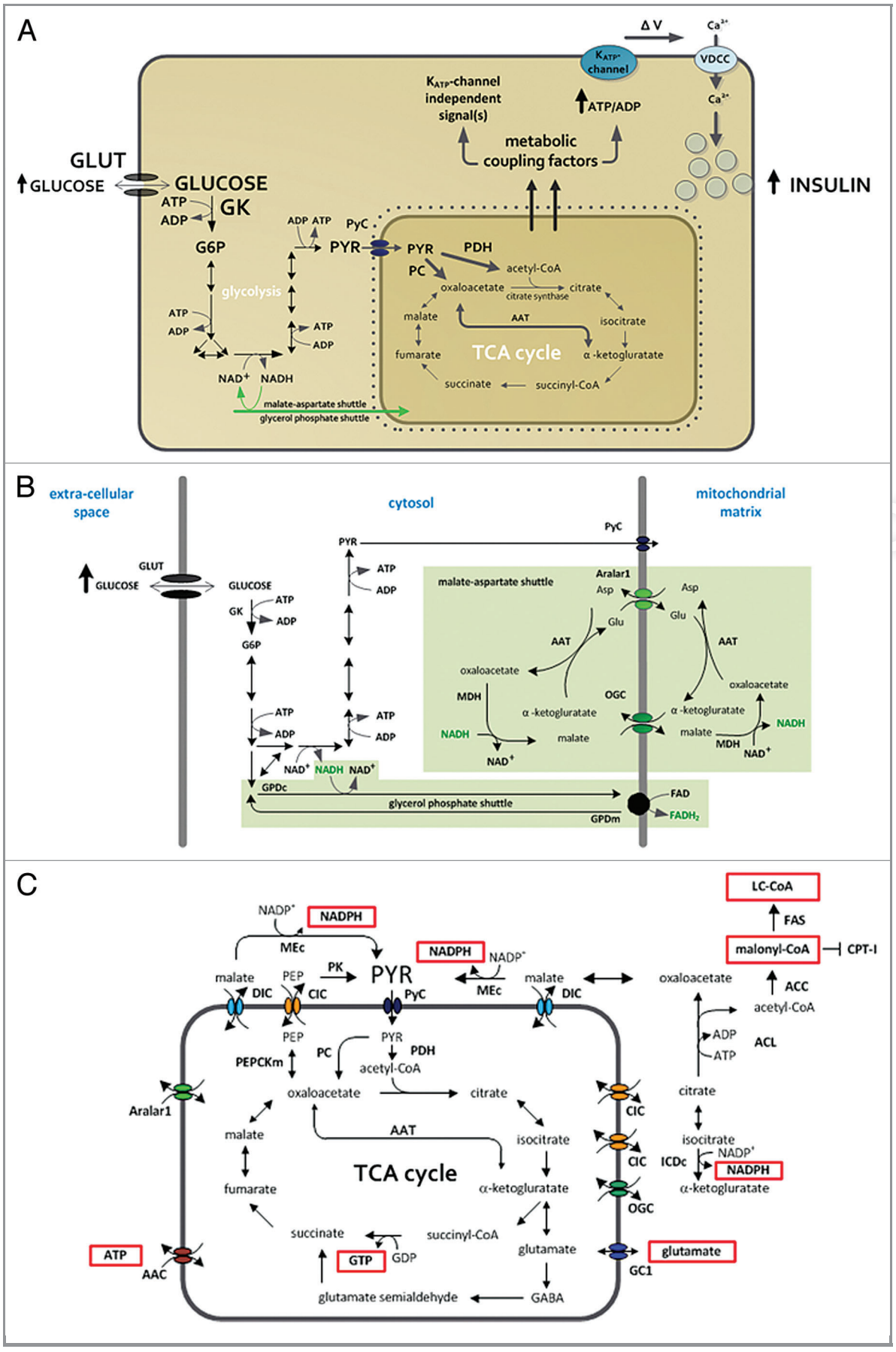

Figure 1 (See opposite page). The stimulus-secretion cascade in pancreatic β-cells. (A) Overview of the spatial barriers in the stimulus-secretion cascade. Two spatial barriers, imposed by the plasma membrane and mitochondrial inner membrane, need to be overcome in order to achieve efficient coupling between the different steps in the stimulus-secretion cascade. The first spatial barrier is surmounted by the presence of a glucose transporter (GLUT) in the plasma membrane, whereas a low affinity hexokinase IV, also known as glucokinase (GK), sets the glycolytic pace in accordance to changes in cellular glucose availability. Together, these proximal components play an essential role in the stimulus-secretion cascade as they convey extracellular glucose availability into a metabolic flux. The second spatial barrier in the stimulus-secretion cascade, imposed by the mitochondrial inner membrane is overcome by a set of mitochondrial carrier proteins that not only ensures tight metabolic coupling between glycolysis and TCA cycle via the efficient import of glycolytic end products into the mitochondrial matrix, but also facilitates the export of mitochondrial signals that promote insulin release via K_{ATP}channel-dependent and -independent pathways. The mitochondrial import of pyruvate occurs via the pyruvate carrier (PyC) after which the glycolytic end product enters the TCA cycle in roughly equal proportion via oxidative (pyruvate dehydrogenase, PDH) and anaplerotic (pyruvate carboxylase, PC) pathways. Reducing equivalents captured during glycolysis are transferred into the mitochondrial matrix for ATP production via the glycerol phosphate and malate-aspartate shuttle, both of which warrant the continuation of glycolysis by replenishing cytosolic NAD^+ levels [indicated in green; more detailed overview in (B)]. (B) Role of the mitochondrial carrier proteins in the NADH shuttles. The transfer of electrons from cytosolic NADH into the mitochondrial matrix is mediated via the malate-aspartate shuttle and critically depends on the presence of the aspartateglutamate carrier (aralar1) and α-ketoglutarate carrier (OGC). The glycerol phosphate shuttle, on the other hand, transports the electrons directly in the respiratory chain via the existence of a cytosolic and membrane bound isoform of glycerol-3-phosphate dehydrogenase. (C) Role of the mitochondrial carrier proteins in the transport or production of cytosolic coupling factors. The efficient exchange of metabolites between cytosol and mitochondrial matrix is mediated by a set of mitochondrial carriers, including the ATP/ADP carrier (AAC), the aspartate-glutamate carrier (aralar1), α-ketoglutarate carrier (OGC), citrate/isocitrate carrier (CIC), dicarboxylate carrier (DIC) and glutamate carrier (GC1). The exchange carrier AAC mediates the transport of ADP and ATP over the mitochondrial inner membrane. Aralar1 catalyzes the electrogenic exchange of aspartate for glutamate and a H^+, whereas OGC mediates the electroneutral exchange of α-ketoglutarate for some other dicarboxylates, of which malate is bound with the highest affinity. CIC catalyzes the electroneutral exchange of one of three tricarboxylic acids (citrate, isocitrate, cis-aconitate) plus a proton, for another tricarboxylate-H^+, a dicarboxylate (malate or succinate) or phosphoenolpyruvate, whereas DIC catalyzes the electroneutral exchange of certain dicarboxylates (e.g., malate and succinate) for inorganic phosphate or sulfur-containing compounds such as, sulphite, sulfate or thiosulphate. GC1, on the other hand, catalyzes the transport of glutamate across the inner mitochondrial membrane either by proton co-transport or in exchange for hydroxyl ions and is, therefore, depicted as a unilateral transporter. These metabolite transporters are structural components in pathways that either ensure a certain degree of metabolic coupling between glycolysis and TCA cycle (**B**), provide anaplerotic substrate into the TCA cycle, or facilitate the export of mitochondrial signals into the cytosol. Candidate coupling factors proposed to augment GSIS either via K_{ATP}-channel-dependent or -independent pathways are depicted in a red box and include: ATP, GTP, glutamate, malonyl-CoA, long chain acyl (LC)-CoA's and NADPH. (Abbreviations used in the figure: AAC, ATP/ADP carrier; AAT, aspartate aminotransferase; ACC, acetyl-CoA carboxylase; ACL, ATP-dependent citrate lyase; Aralar1, aspartate/glutamate carrier; Asp, aspartate; CIC, citrate/isocitrate carrier; CPTI, carnitine palmitoyltransferase I; DIC, dicarboxylate carrier; FAS, fatty acid synthase; GC1, glutamate carrier; GK, glucokinase; Glu, glutamate; GLUT, glucose transporter; G6P, glucose-6-phosphate; GPDc, cytosolic glycerol-3-phosphate dehydrogenase; GPDm, membrane-bound glycerol-3-phosphate dehydrogenase; ICDc, cytoplasmic isocitrate dehydrogenase; MDH, malate dehydrogenase; MEc, cytoplasmic malic enzyme; OGC, 2-oxoglutarate carrier; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PEPCKm, mitochondrial phosphoenolpyruvate carboxykinase; PyC, pyruvate carrier; PYR, pyruvate).

mice (*GLUT2*$^{-/-}$) was sufficient to restore glucose tolerance.[25] Introduction of high affinity, low capacity components to compete with the low affinity, high capacity components of the proximal glucose sensor has provided more support for the idea that GLUT2 is not an essential component of the glucose sensor as introduction of GLUT1 did not affect GSIS, whereas the introduction of hexokinase I severely diminishes glucose responsiveness.[26] These findings indicate that GK is a more likely candidate glucose sensor protein as its activity is more tightly correlated with glycolytic flux.[21,22] The idea that the phosphorylation of glucose constitutes the rate-determining step in glycolysis is supported by several approaches.

First, mild upregulation of GK increases the glucose responsive state of β-cells, whereas more substantial levels of overexpression (> 4-fold) tend to abolish glucose responsiveness, and more distal steps in glucose metabolism acquire rate limiting properties.[27,28] Second, differences in GK expression levels seem to account for the heterogeneity that exists among individual β-cells as GK levels in high responsive β-cells are more elevated than those observed in low responsive β-cells.[29] The idea that GK is an essential component of the β-cell glucose sensor is furthermore supported by the observation that β-cell specific heterozygous GK deficiency leads to moderate hyperglycemia, whereas homozygous deficient animals die within a few days after birth from severe diabetes.[30] Studies in humans with specific GK gene mutations have led to similar conclusions as mutations that increase GK activity cause persistent hypoglycemic hyperinsulinemia,[31,32] whereas mutations that reduce its catalytic activity or negatively affect protein stability have been associated with the occurrence of maturity-onset diabetes of the young type 2. (MODY2).[33-35]

The Metabolic Fate of Glucose in Glycolysis and Mitochondrial Glucose Metabolism

Biochemical measurements on rodent β-cells have indicated that the metabolic fate of glucose is almost entirely directed toward entry into the TCA cycle as the metabolic flux via the pentose phosphate pathway is low and glycogen synthesis accounts for only a minor fraction of total glucose utilization.[36,37] In addition, β-cells have been shown to express very low levels of lactate dehydrogenase, suggesting that shunting of pyruvate away from mitochondrial entry is not likely to occur.[38] When added to the culture medium, pyruvate appears to be a poor stimulus for insulin secretion and this so-called "pyruvate paradox" was resolved after it was demonstrated that the monocarboxylate transporter 1 protein (MCT1, SLC16A1) is scarcely present in the plasma membrane of primary β-cells.[38,39] As such, these cells appear to be unable to transport pyruvate efficiently over the plasma membrane and this point was elegantly made when forced MCT1 overexpression did render primary β-cells in a pyruvate

responsive state.[40] Silencing of the MCT1 gene appears to be crucial for normal β-cell function as mutations that induce MCT1 gene expression cause hyperinsulinemia during exercise and other catabolic states.[41,42]

In conditions where cytosolic pyruvate levels are elevated, the glycolytic end product enters the TCA cycle via pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) in roughly equal proportion feeding acetyl-CoA and oxaloacetate into the oxidative and anaplerotic branches of the TCA cycle.[37,43] The PC-mediated replenishment of oxaloacetate (anaplerosis) seems to be of critical importance for β-cell glucose competence as C^{13}-NMR isotopomer analysis revealed that glucose responsiveness is more stringently associated with anaplerotic substrate flow than with oxidative metabolism.[44] The notion that anaplerosis might be an important determinant of glucose responsiveness has furthermore been strengthened by several independent studies that focus on the inhibition of PC activity either via pharmacologic compounds or siRNAmediated knockdown.[45-48] These studies have clearly established that efficient inhibition of PC activity (by 50% or more) renders clonal β-cells and primary rat islets in a glucose unresponsive state that is furthermore characterized by a significant drop in glucose-stimulated NADPH/$NADP^+$ and ATP/ADP levels despite normal glucose oxidation rates.

Mitochondrial Carrier Proteins

Most of the key mitochondrial carrier proteins that facilitate the transport of important metabolites, nucleotides and cofactors across the mitochondrial membrane belong to the solute carrier family 25 (SLC25).[49] A generally accepted model of these mitochondrial transporter proteins is that they exist as homodimers with the C- and N-terminus of each monomer being directed toward the intermembrane space. Each monomer consists of six hydrophobic transmembrane segments that traverse the mitochondrial inner membrane as α-helices and form a gateway for the transport of specific metabolites over the mitochondrial inner membrane. This review focuses on a role for the 2-oxoglutarate carrier (OGC; SLC25A11), aspartate-glutamate carrier aralar1 (AGC1; SLC25A12), glutamate carrier (GC1; SLC25A22), dicarboxylate carrier (DIC; SLC25A10) and citrate/isocitrate carrier (CIC; SLC25A1) in pancreatic β-cells.

A Role for the Mitochondrial Carrier Proteins in Metabolic Coupling between Cytosolic and Mitochondrial Glucose Metabolism

Although the mitochondrial pyruvate carrier (PyC) still awaits identification, it is evident that the transport of pyruvate over the mitochondrial inner membrane remains an obligatory step in order to achieve stringent metabolic coupling between glycolysis and mitochondrial glucose metabolism. But pyruvate is not the only glycolytic end product as electrons captured during the conversion of NAD^+ to NADH by glyceraldehyde phosphate dehydrogenase (GAPDH) need to be transferred to the respiratory chain in order to yield ATP. The glycerol-phosphate and malate-aspartate shuttles not only provide a way to transfer reducing equivalents from the cytosol into the electron transport chain for ATP production, but also allow the continuation of glycolysis by the replenishment of cytosolic NAD^+ levels (**Fig. 1A and B**).[50,51] It was demonstrated that NADH shuttle activity is enhanced by a cytosolic rise in Ca^{2+} levels, indicating that a more efficient state of metabolic coupling may be attained during GSIS.[52,53]

The glycerol-phosphate shuttle does not require mitochondrial carriers and transfers electrons to the electron transport chain via a series of metabolic reactions catalyzed by cytosolic and mitochondrial glycerol-3-phosphate dehydrogenase. The functional state of the malate-aspartate shuttle, on the other hand, critically depends on the presence of OGC and aralar1. Overexpression of aralar1 in a clonal β-cell line or rat islets markedly enhances glucose competence characterized by improved glucose oxidation, NAD(P)H yield and ATP production.[54,55]

Studies aimed at evaluating the importance of these NADH shuttle systems initially raised the possibility that a certain degree of redundancy may exist as their combined inhibition is required to render β-cells in a glucose unresponsive state.[56,57] However, more recent studies have shown that siRNA-mediated knockdown of aralar1 negatively affected GSIS by a moderate 25% in INS-1, but not in rat islets.[58] Knockdown of OGC, however, did significantly lower the glucose responsive state of INS-1 cells and rat islets, indicating that the mitochondrial carrier may be involved in other pathways in addition to the malate-aspartate shuttle.[59]

A Role for the Mitochondrial Carrier Proteins in the Rise of Cytosolic Coupling Factors that Enhance GSIS

ATP is considered to be a pivotal signal for the initiation of GSIS as the rise in ATP/ADP levels was shown to precede the influx of Ca^{2+} ions which accelerates insulin release.[60] However, ATP does not seem to be the only relevant signal for GSIS as some nutrients raise the ATP/ADP ratio, but do not augment the rate of insulin release.[61] Other metabolites, such as succinate, on the other hand possess the ability to augment insulin release even in conditions where cytosolic ATP and Ca^{2+} levels are clamped at maximal levels.[62] These observations suggest that metabolic factors can enhance GSIS without affecting ATP or Ca^{2+} levels.

Role for the GTP/GDP Ratio in GSIS

GTP was proposed to act as a coupling factor in rodent islets after it was established that pharmacologic inhibition of de novo cytosolic GTP production significantly lowers GSIS.[8] The idea that the GTP/GDP ratio is a more relevant signal for insulin release followed from the observation that the insulinotropic actions of GTP-γ-S can be counterbalanced by the addition of GDP-β-S.[63,64] GTP regained attention as a potential coupling factor after mitochondrial GTP production was lowered by siRNA-mediated knockdown of GTP-specific succinyl-CoA synthetase (GTP-SCS) in INS-1 cells and rat islets.[9] Under these conditions, a marked reduction in oxygen consumption, cytosolic

Ca^{2+} levels and GSIS was noted. Conversely, when the rate of mitochondrial GTP synthesis was raised by knockdown of ATP-specific succinyl-CoA synthetase (ATP-SCS) a marked reduction in ATP production was observed despite increased oxygen consumption, cytosolic Ca^{2+} levels and insulin release.[9] In the cytosol, GTP could serve as a substrate for several GTPases that have been implicated in cytoskeletal remodeling and insulin granule fusion, but it remains to be established how GTP produced in the mitochondria can be conveyed into the cytosol.[65,66]

One possibility is that GTP is transported across the mitochondrial membrane either directly via a mitochondrial GTP/GDP carrier as observed in yeast,[67] or indirectly via prior conversion into ATP by mitochondrial nucleoside diphosphate kinases (NDK's) and export via the ADP/ATP carrier AAC followed by the reconversion into GTP by cytosolic NDK's. Evidence for the latter possibility was presented as NDPK-D forms a complex with GTP-SCS[68] and was reported to be associated with AAC on both sides of the inner mitochondrial membrane.[69] Another possibility is that GTP directly stimulates the activity of a mitochondrial enzyme, whose product conveys the signal into the cytosol. GTP-dependent mitochondrial PEPCK (PEPCKm) was suggested to perform such a role via the production of phosphoenolpyruvate (PEP), which can traverse the mitochondrial membrane via CIC and re-enter the TCA cycle after its conversion to pyruvate by pyruvate kinase (**Fig. 1C**).[70] A valid alternative to the proposed PEP cycle arises from the possibility that PEP itself acts as an insulinotropic metabolite by suppressing okadaic acid-sensitive Ser/Thr protein phosphatase activity,[71] an event that has been shown to augment Ca^{2+} influx in RINm5F cells.[72]

Role for Glutamate in GSIS

Glutamate was originally proposed as a potential coupling factor in GSIS by Wollheim and Maechler.[12] Findings by several other groups, however, do not support the idea that a causal relationship exists between a rise in cellular glutamate levels and insulin release.[73,74] When added as a stimulus, the insulinotropic action of glutamate appears to be insensitive to the ATP synthase inhibitor, oligomycin, indicating that glutamate may act in a K_{ATP}-independent fashion, most likely by providing anaplerotic input into the TCA cycle.[12] Glutamate can enter the mitochondrial matrix either via aralar1 or GC1, but considering that glutamate transported by aralar1 is mainly utilized for transamination in the malate-aspartate shuttle, it has been proposed that most of the glutamate consumed in other glutaminolytic reactions enters the mitochondrial matrix via GC1 (**Fig. 1C**).[75] The best studied glutaminolytic pathways in pancreatic β-cells are the deamination of glutamate by glutamate dehydrogenase (GDH) to yield α-ketoglutarate and the oxidative decarboxylation of glutamate by glutamate decarboxylase (GAD) to generate gamma-aminobutyric acid (GABA).[76,77] Considering that the production of GABA is enhanced by glutamine and taking into account that α-ketoglutarate promotes GABA conversion to succinate, it seems plausible that these two glutaminolytic pathways work in concert to provide anaplerotic input into the TCA cycle. This idea is not only supported by earlier studies demonstrating the insulinotropic potential of α-ketoglutarate and succinate,[78,79] but is also in agreement with the observation that glutamine does not exert a significant insulinotropic impact unless GDH is allosterically activated by leucine or 2-norbornane carboxylic acid (BCH).[73,80]

Role for Malonyl-CoA and LC-CoA's in GSIS

A role for intermediates of lipogenic pathways as potential coupling factor in GSIS was first proposed by Barbara Corkey and Marc Prentki, showing that the intracellular levels of citrate, malonyl-CoA and LC-CoA's rise rapidly when clonal β-cells or rodent islets are exposed to elevated glucose concentrations.[10,11] The malonyl-CoA/LC-CoA hypothesis states that PC-mediated replenishment of oxaloacetate in the TCA cycle facilitates the accumulation and escape of citrate from the mitochondrial matrix into the cytosol, where it serves as a donor of acetyl-CoA in the production of lipogenic coupling factors (**Fig. 1C**).

The mitochondrial export of citrate is mediated via CIC, possibly in conjunction with DIC, as the latter was shown to provide sufficient cytosolic malate levels in order to support CIC function.[81-83] Evidence supporting a role for CIC in β-cell glucose responsiveness is quite strong as pharmacological inhibition or siRNA-mediated knockdown of this mitochondrial carrier protein renders INS-1 832/13 cells and rat islets in a glucose-unresponsive state that is furthermore characterized by a 20–30% lower NADPH/$NADP^+$ ratio, yet normal glucose utilization, glucose oxidation and ATP production rates.[81,82]

In the cytosol, ATP-dependent citrate lyase (ACL) utilizes citrate as donor of acetyl-CoA for lipogenesis and protein acetylation (**Fig. 1C**). Pharmacological inhibition of ACL, which liberates acetyl-CoA from citrate, was initially reported to inhibit GSIS[84,85] but this may have resulted from an excess of salt that accrues during the preparation of the CL inhibitor hydroxycitrate.[86] The role of ACL in GSIS remains controversal as shRNAmediated suppression of ACL was reported to inhibit GSIS in one study,[81] whereas effective knockdown of ACL did not affect GSIS in other studies.[86-88] Downstream of ACL, the flux of glucose carbon entering the malonylCoA/LC-CoA pathway is predominantly controlled by acetyl-CoA carboxylase 1 (ACC1) as ACC2 is poorly expressed in INS-1 and rat islets.[89] ACC1 catalyzes the irreversible carboxylation of acetyl-CoA to generate malonyl-CoA which acts as an allosteric inhibitor of carnitine palmitoyltransferase-I (CPT-I) and subsequently inhibits fatty acid oxidation when glucose is readily available.[85,90,91] Consistent with this concept, overexpression of a malonyl-CoA insensitive mutant of CPT-I reduced metabolic flexibility and glucose competence in INS-1 832/13 cells.[92] However, forced overexpression of malonyl-CoA decarboxylase (MCD), which effectively opposes the glucose-induced rise in malonyl-CoA levels had no negative impact on GSIS[93] and later it was shown that the presence of exogenous fatty acids is required in order to observe the negative impact of MCD overexpression on GSIS.[94]

Knockdown of ACC1 using small interfering RNA duplexes was reported to diminish GSIS as a consequence of lower GK

levels, glycolytic flux, glucose oxidation, ATP production and pyruvate cycling rates,[95] whereas siRNA mediated knockdown of fatty acid synthase (FAS) also significantly lowered glucose carbon incorporation into lipids, but did not affect GSIS.[86] Interestingly, application of the ACC1 inhibitor TOFA mimicked the inhibitory actions of ACC1 knockdown on GSIS when applied for 72 h, but not after 2 h, suggesting that long-term inhibition of ACC1 is required to negatively affect the glucose responsive state of the β-cell.[95]

Finally, it is noteworthy to mention that patch-clamp and whole-cell studies have demonstrated that LC-CoA's actually increase K_{ATP} channel activity, an effect that cannot be reconciled with its proposed role as a coupling factor.[96-99]

Role for the NADPH/NADP+ Ratio in GSIS

The insulinotropic actions of NADPH were first described by Watkins et al. in islets of toadfish.[13,100-102] Studies aimed at unveiling the mechanism by which NADPH facilitates insulin release have indicated that its reductive power may be transferred to proteins present in the plasma membrane or secretory granules.[99-101] Both Kv2.1 channels and the thiol disulfide oxido-reductase glutaredoxin-1 (GRX-1) have been proposed as potential targets in the redox control of insulin secretion.[103-105] The existence of aldose reductase-like motifs in the regulatory β-subunits of the voltage-gated K^+ channels (Kv) is particularly intriguing as increments in the NADPH/$NADP^+$ ratio accelerate Kv2.1 channel inactivation, an event that ultimately renders primary β-cells in a more excitable, glucose competent state.[105] GRX1 is a cytosolic enzyme localized near to the plasma membrane that catalyzes the reduction of disulphide bonds in its target proteins by harnessing the reductive power of glutathione (GSH). Oxidized glutathione (GSSG) is converted back to GSH by glutathione reductase at the expense of NADPH. Together, these proteins provide a link between glucose-induced increments in the NADPH/$NADP^+$ ratio and the cellular redox state, in particular, the reduction of disulfide bridges in proteins targeted by GRX1. The involvement of GRX1 in the stimulus-secretion cascade was demonstrated by a 40% increase in glucose-stimulated insulin output after GRX1 overexpression in INS-1 and rat islets, whereas siRNA-mediated knockdown of GRX1 renders INS-1 832/13 cells insensitive to the stimulatory actions of NADPH.[103,104] Cytosolic NADPH levels rise rapidly in response to several metabolic fuels, such as glucose or leucine[106,107] and when the cytosolic rise in the NADPH/$NADP^+$ ratio is blunted by the addition of $NADP^+$ or menadione, a poor glucose responsive state is attained.[104,108,109] Most of the glucose-induced rise in NADPH levels originates from the cytosolic isoforms of malic enzyme (MEc) and isocitrate dehydrogenase (ICDCc), which are operative in the pyruvate-malate, pyruvate-citrate and pyruvate-isocitrate pathway[14] (**Fig. 1C**). Knockdown of MEc by transfection with specific siRNA sequences was reported to inhibit GSIS by approximately 40% in INS-1 832/13,[81,110] but not when MEc is suppressed chronically.[111] Silencing of MEc in primary mouse or rat islets yielded conflicting results as MEc knockdown in rat islets does not affect GSIS despite a 30% reduction in the NADPH/$NADP^+$ levels,[112] whereas a similar reduction of the NADPH/$NADP^+$ levels in mouse islets significantly lowered GSIS.[113] Effective siRNA-mediated knockdown of ICDc, on the other hand, was reported to inhibit GSIS by approximately 60% in INS-1 832/13 and rat islets despite a moderate, yet significant, reduction in the NADPH/$NADP^+$ levels by 18%.[114] Silencing or pharmacological inhibition of the mitochondrial carrier proteins CIC, DIC and OGC, all of which are indispensable structural components of the pyruvate cycling pathways, rendered β-cells in a glucose unresponsive state accompanied with a 20–40% reduction in the NADPH/$NADP^+$ levels at stimulatory glucose concentrations, yet normal glucose utilization and oxidation rates.[59,81-83] Considering that OGC is a structural component in both the malate-aspartate shuttle (**Fig. 1B**) and pyruvate-isocitrate cycle (**Fig. 1C**), it is noteworthy to mention that the observed loss in glucose competence after siRNAmediated silencing of OGC occurs in conjunction with a significant 23% reduction in the NADPH/$NADP^+$ ratio at stimulatory glucose concentrations, but did not affect the glucose utilization rate or glucose-induced changes in the ATP/ADP ratio.[59] Therefore, the observed loss in glucose competence after a reduction in OGC protein levels by approximately 50% appears to result primarily from its role as a structural component in pyruvate cycling, rather than from its involvement in the malate-aspartate shuttle.

Conclusion

The proximal glucose sensor is an essential component of the β-cell stimulus secretion cascade as it conveys extracellular glucose availability into a metabolic flux over the first spatial barrier imposed by the plasma membrane (**Fig. 1A-C**). Downstream of the glucose sensor, the mitochondrial inner membrane constitutes a second spatial barrier which separates cytosolic (glycolysis) and mitochondrial glucose metabolism (TCA cycle). The existence of several mitochondrial carrier proteins, including the aspartateglutamate carrier (aralar1), α-ketoglutarate carrier (OGC), ATP/ADP carrier (AAC), glutamate carrier (GC1), dicarboxylate carrier (DIC) and citrate/isocitrate carrier (CIC), are indispensable in the stimulus secretion cascade as these metabolic gateways enhance β-cell glucose responsiveness on at least three levels as: (1) these metabolic carriers not only ensure efficient metabolic coupling between glycolysis and the TCA cycle, but also (2) provide an effective way to enhance anaplerotic substrate flux and (3) facilitate the mitochondrial export of metabolic signals that are required to trigger and enhance GSIS via both K_{ATP}-channel-dependent and -independent pathways.

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