Published in final edited form as:

Arch Biochem Biophys. 2019 March 15; 663: 199–213. doi:10.1016/j.abb.2019.01.011.

Molecular and Cellular Regulation of Human Glucokinase

Shawn M. Sternisha¹ and Brian G. Miller^{1,*}

¹Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306, USA

Abstract

Glucose metabolism in humans is tightly controlled by the activity of glucokinase (GCK). GCK is predominantly produced in the pancreas, where it catalyzes the rate-limiting step of insulin secretion, and in hepatocytes, where it participates in glycogen synthesis. A multitude of disease-causing mutations within the *gck* gene have been identified. Activating mutations manifest themselves in the clinic as congenital hyperinsulinism, while loss-of-function mutations produce several diabetic conditions. Indeed, pharmaceutical companies have shown great interest in developing GCK-associated treatments for diabetic patients. Due to its essential role in maintaining whole-body glucose homeostasis, GCK activity is extensively regulated at multiple levels. GCK possesses a unique ability to self-regulate its own activity via slow conformational dynamics, which allows for a cooperative response to glucose. GCK is also subject to a number of protein-protein interactions and post-translational modification events that produce a broad range of physiological consequences. While significant advances in our understanding of these individual regulatory mechanisms have been recently achieved, how these strategies are integrated and coordinated within the cell is less clear. This review serves to synthesize the relevant findings and offer insights into the connections between molecular and cellular control of GCK.

Keywords

Glucokinase; Diabetes; Conformational dynamics; Protein-protein interaction; Regulation

Introduction

Glucokinase (GCK, hexokinase IV) is a monomeric enzyme that catalyzes the ATP-dependent conversion of glucose to glucose 6-phosphate, the first and rate-limiting step of glycolysis in the liver and pancreas.[1,2] GCK was first discovered in the early 1960's, and shortly thereafter it became the subject of intense study due to its unique sigmoidal kinetic response to glucose and its essential role in glucose metabolism.[3–5] Although it shares extensive sequence identity with the three other human hexokinase isozymes, GCK is

*Corresponding author: 4005 Chemical Sciences Lab, Department of Chemistry and Biochemistry, Florida State University, Tallahassee FL 32303; miller@chem.fsu.edu; 850-645-6570.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

considered the body's primary glucose sensor because small fluctuations in its activity alter the threshold for glucose-stimulated insulin secretion (GSIS) from pancreatic β -cells.[6–8] GCK's midpoint of glucose responsiveness ($K_{0.5}$) is ~30-fold lower than that of homologous isozymes (7 mM for GCK vs. ~0.2 mM for hexokinases I-III) and this value closely matches physiological, circulatory glucose concentrations.[9] Unlike the other hexokinases, GCK is not susceptible to feedback inhibition by physiological concentrations of its product glucose 6-phosphate.[5,9,10]

In humans, GCK is primarily produced in pancreatic β -cells and liver hepatoparenchymal cells. Within pancreatic β -cells, GCK acts to maintain glucose homeostasis by governing the rate of insulin secretion, while in the liver GCK participates in glycogen synthesis.[2,11] The importance of precise control over GCK activity in both tissues is emphasized by several disease phenotypes that result from mutations in the human *gck* locus (Figure 1). Maturity onset diabetes of the young type 2 (MODY2) and the more serious permanent neonatal diabetes mellitus (PNDM) are caused by heterozygous inactivating *gck* mutations.[12–14] By contrast, gain-of-function, activating *gck* mutations produce persistent hyperinsulinemic hypoglycemia of infancy (PHHI), the severity of which directly correlates with the level of enzyme activation.[12] The importance of GCK in glucose metabolism and disease has stimulated much interest from the pharmaceutical industry to develop activators of the enzyme.[15–18] A variety of lead molecules that stimulate GCK *in vivo*, either directly or indirectly, have been identified. To date, however, a viable therapeutic agent has yet to emerge from these efforts.

Given the significance of GCK to glucose homeostatic maintenance, it is not surprising that considerable efforts have been made to characterize the full suite of biochemical processes that regulate its activity. GCK regulation is complex and a number of unique regulatory strategies have been discovered. Alternative and tissue-specific promoters drive GCK transcription and gene expression to varying degrees.[3,19–22] In addition, several hormones and metabolites, including insulin and glucose, regulate GCK expression at the transcriptional level.[5,23–26] The hormonal, metabolic and transcriptional basis of GCK regulation has been recently reviewed elsewhere, so these topics will not be discussed here.[27] Instead, the focus of this review is to discuss our current understanding of GCK regulation, with a special emphasis on bridging the gap between regulation of GCK at the molecular and the cellular levels. We begin with a brief update of our current knowledge regarding the mechanistic origin of GCK's unique kinetic cooperativity, which has advanced significantly in recent years as a result of many diverse biochemical and biophysical studies of the isolated enzyme. We then turn our attention to the regulation of GCK within the cellular context, which appears to be dominated by interactions with a number of established and putative binding partners. Finally, we conclude with a look to the future, emphasizing the need for continued studies aimed at providing a comprehensive understanding of how GCK's dynamic conformational landscape is linked to regulation within the cell.

1. Regulation of GCK by Intrinsic Conformational Dynamics

GCK is a 465-residue, 52-kDa enzyme comprised of two domains, hereafter referred to as the large domain and the small domain, that are separated by a flexible hinge region. As with

other proteins that adopt the hexokinase fold, substrates bind at a cleft between the large and small domains and induce a conformational change to a more compact form of the enzyme. [1] A single crystal structure of unliganded, apo GCK has been determined.[28] This structure reveals a unique “super-open” conformation not previously observed in other hexokinases, which is characterized by a large opening angle between the large and small domains and an invisible, disordered loop comprised of residues 151–180 (Figure 2). Multiple structures of GCK bound to both glucose and various synthetic allosteric activators have also been determined.[29] In these structures, GCK adopts a “closed” conformation, in which the large and small domains are separated by a small opening angle and the disordered loop is fully observable. The binding site for allosteric activators is located within the small domain, near the hinge region, at a site that is ~20 Å away from the substrate binding site.[28] Interestingly, many activating mutations responsible for PHHI can also be traced to residues in close proximity to the allosteric site.[30]

In the closed structure, the N-terminus of the 151–180 loop folds into a β -hairpin, allowing residues near the C-terminus of the loop to contact glucose. The closed conformation is also observed in crystal structures of GCK in complex with glucose and a non-hydrolyzable ATP analog.[31] A second, glucose- and activator-bound crystal structure of GCK has also been reported, which has been termed the “open” structure due to the fact that it reveals a conformation that is intermediary between the unliganded and closed structures.[29] In this structure, residues 154–178 are not observed, consistent with the retention of disorder in the loop despite the occupancy of glucose in the binding site. The open structure appears to be stabilized by an iodine atom, present in the crystallization buffer, which occupies a discrete site near the domain interface. Importantly, small angle X-ray scattering experiments support the existence of all three crystallographically observed structures in solution.[31]

GCK displays mild positive kinetic cooperativity toward glucose, a regulatory process that is derived from substrate-induced conformational changes.[28,32] The enzyme contains only a single glucose binding site and it does not oligomerize, thus the allosteric properties of GCK are not explicable by traditional two-state allosteric models. Two prominent theoretical mechanisms to describe GCK’s allosteric-like behavior, the ligand-induced slow transition (LIST) model and the mnemonic model, have been developed.[33,34] Both models explain cooperativity in terms of the view that unliganded GCK exists as a multi-state system that undergoes millisecond timescale conformational dynamics. A key difference between the models is the number of distinct conformations sampled by the unliganded enzyme. The LIST model describes GCK as existing in two structurally discrete states that are slowly interconverting, and which display varying affinities for glucose. The mnemonic model hypothesizes that unliganded GCK exists exclusively in a low-affinity conformation, which reorganizes to the high-affinity state upon binding to glucose. This high-affinity state then binds ATP, catalysis occurs, and products are released. If glucose concentrations are high, another molecule of glucose can bind to the high-affinity state to facilitate rapid turnover; however, if glucose concentrations are low, the enzyme has sufficient time to relax to the low-affinity conformation and sigmoidal steady-state kinetics result. Notably, both models indicate that cooperativity is only produced when the conformational changes occur on a timescale comparable with k_{cat} .

Given the proposed importance of conformational dynamics to GCK's allosteric regulation, a number of biophysical methods have been utilized to probe the enzyme's dynamic structural landscape. Investigations into the mechanism of glucose binding, using changes in the enzyme's intrinsic fluorescence to report on structure, confirmed that GCK experiences slow dynamics.[35] Two different transient-state stopped-flow studies provided compelling evidence in support of the LIST mechanism by demonstrating the existence of multiple unliganded enzyme conformations.[36,37] Carr-Purcell-Meiboom-Gill (CPMG) NMR experiments provided an estimate for the rate constant of conformational exchange between a putative ground and "excited" conformation of the enzyme, verifying a temporal equivalency between conformational exchange and enzymatic turnover.[38] Pulsed proteolysis studies demonstrated that the flexible active site loop is the primary site of disorder in unliganded GCK, consistent with NMR and crystallographic data indicating the small domain retains a high degree of order in the absence of glucose.[38] NMR studies also showed that glucose binding promotes folding of the flexible active site loop, quenches the millisecond dynamics of the small domain, and substantially narrows the protein's conformational ensemble.[39] Together this information provides a detailed atomistic description of the structural and dynamic origins of GCK's allosteric regulation by glucose.

The molecular picture of GCK emerging from the studies summarized above provides a framework for understanding the mechanistic basis of hyperactive, PHHI-associated GCK variants that lack cooperativity. Based on these studies, loss of cooperativity is expected to occur from alterations in the rate constants dictating turnover (k_{cat}) and/or conformational exchange (k_{ex}). In line with that expectation, two functionally distinct GCK activation mechanisms were recently identified by characterizing genetically engineered GCK mutants: $\langle$ - and @-type activation.[40] Specific mutations in GCK's α 13 helix give rise to a variant that symbolizes α -type activation. β -type activation, on the other hand, is exemplified by β -hairpin GCK, which contains targeted alterations in the mobile loop region. Although both activation schemes are operational in clinically identified mutants, the $\langle$ 13 helix and @-hairpin GCK variants have shown substantial utility in developing a molecular understanding of each activation process. Solution NMR, viscosity variation, transient chemical quench-flow kinetics and hydrogen-deuterium exchange mass spectrometry (HDX-MS) were recently used to elucidate the mechanism of action of both variants.[40] These combined efforts demonstrate that unliganded α -activated GCK is characterized by a shift in the conformational ensemble toward a structure that resembles the glucose-bound enzyme. Consequently, α -activation is associated with an alteration in the value of the conformational exchange rate constant, k_{ex} . This shift is accompanied by an increase in glucose affinity and decreased proteolytic susceptibility within the flexible loop. β -activated GCK, however, appears to be structurally identical to unliganded wild type GCK and activation results from an accelerated product release step. This mutant also displays enhanced proteolytic susceptibility of the active site mobile loop,[38] suggesting that swift product release is facilitated by increased dynamics of the mobile loop. Interestingly, GKAs that have been explored as potential MODY therapeutics appear to operate via the α -type activation mechanism.[41]

Despite recent biochemical and biophysical investigations uncovering a wealth of information regarding GCK intramolecular dynamics, a number of important details remain

obscure. For example, we still lack a complete description of the detailed kinetic mechanism of the enzymatic process, including quantification of the microscopic rate constants associated with glucose binding, ATP binding, chemistry and product release. The multitude of conformational changes occurring during the reaction coordinate, and the lack of unique spectroscopic reporters within the protein, hinder progress on this front. A detailed structural description of the “excited” state detected in NMR studies is also lacking, although it seems plausible that this structure may resemble the crystallographic open structure described above. Elucidation of an atomic-level structure of this and other meta-stable states along the pathway could prove useful to target with therapeutics to achieve partial activation. Finally, understanding how intrinsic dynamics, protein-protein interactions and post-translational modifications are coordinated to provide stringent regulation at the cellular level is essential. Such knowledge promises to provide novel mechanisms for disease treatment and drive therapeutic innovation.

2. Inhibition of GCK by the Glucokinase Regulatory Protein

The earliest identified, and to date best characterized GCK interaction partner is the liver-specific glucokinase regulatory protein (GKRP, GRP). First discovered by VanSchaftingen nearly 30 years ago[42,43], GKRP has been extensively studied using a variety of biochemical, biophysical, structural and cellular methods. Importantly, this chapter discusses hepatic GCK regulation exclusively, as this interaction occurs only in liver hepatocytes and not in pancreatic β -cells. Below we provide a brief summary of the salient features of the GCK-GKRP interaction with a special focus on studies conducted since 2013, when crystal structures of the mammalian and xenopus GCK-GKRP complexes first appeared.[44,45] For a more in depth treatment of the subject, the reader is referred to several excellent recent reviews.[27,46,47]

GKRP binds to the inactive, super-open conformation of GCK, acting as a competitive inhibitor of glucose association with the enzyme (Figure 3).[45] Upon formation of the inhibitory complex with GKRP, GCK is sequestered into the hepatocyte nucleus.[48] The detailed mechanism by which GKRP mediates translocation of GCK into the nucleus is not fully understood. GKRP is predominantly localized to the hepatocyte nucleus under a variety of conditions,[48,49] but the regulatory protein has also been detected in the cytosol and in a mitochondrial complex with GCK.[50] GKRP appears to recruit GCK to the nucleus via a “piggy-back” mechanism,[48] however direct nuclear localization of GCK via a signaling sequence remains possible, since a nuclear localization signal (NLS) has been identified in pancreatic GCK and this region is conserved in the liver isoform.[48,51] Nevertheless, numerous studies have indicated that nuclear localization of liver GCK is fully dependent upon a functional GKRP.

GKRP-mediated inhibition is modulated by several phosphorylated carbohydrates that bind to the protein’s ancestral etherase active site. Fructose 6-phosphate and sorbitol 6-phosphate promote interaction of GKRP with GCK, whereas fructose 1-phosphate weakens the interaction.[42,52,53] The GCK-GKRP interaction is also disrupted by synthetic drugs that bind to a distinct site on GKRP that is located near the carbohydrate binding site.[54] Recent kinetic and mutational work suggest that the differential effects of these ligands upon

complex stability are dictated by the conformational state of the flexible N-terminus.[55,56] Ligands that favor an extended N-terminus poise GKR for optimal coulombic interactions with residues located near the hinge region of GCK. Conversely, ligands that favor a more compact N-terminus disrupt these stabilizing interactions and weaken the protein-protein interaction.

GKR-mediated inhibition of GCK is also modulated by several post-translational processes in the liver. GKR promoted nuclear translocation of GCK may be impaired by GCK SUMOylation, a modification that also serves to stabilize and activate the enzyme.[51] GKR itself is acetylated near the N-terminus by the p300 acetyltransferase, which prolongs the protein's lifespan and enhances its inhibitory potential.[57] Sirtuin 2, an NAD⁺-dependent deacetylase reverses this modification and also deacetylates Lys126, which reportedly promotes hepatic glucose uptake.[58] The existence of several layers of control over the GCK-GKR complex demonstrates the physiological importance of regulating GCK activity via this protein-protein interaction.

Although much is known about the GCK-GKR interaction, several questions remain. The known, natural carbohydrate ligands of GKR bind with rather low affinity, suggesting that they may not be physiologically relevant. Indeed, one of the main unresolved issues is whether other natural ligands might be important for governing the GCK-GKR interaction in cells. Another area of uncertainty centers around potential additional, yet-to-be described functions of GKR that could be independent of its GCK inhibitory function. Although GKR does not retain detectable enzymatic activity characteristic of its ancestor[59], it could interact with other proteins and/or fulfill additional functions within the hepatocyte nucleus. Genome-wide association studies have linked mutations in the *gkr* locus to hyperlipidemia and cardiovascular disease, however the link between these diseases and the GCK inhibitory activity of GKR is murky.[60,61] Finally, as mentioned above, GKR has been observed to be mitochondrially associated in primary hepatocytes, and its role in localizing GCK to this (or other) cellular locations is unclear.[50]

3. Activation of GCK by Phosphofructokinase-2/Fructose Bisphosphatase 2

Phosphofructokinase-2/fructose bisphosphatase-2 (PFK-2/FBPase-2) is an enzyme responsible for the synthesis and degradation of fructose-2,6-bisphosphate, an important allosteric regulator of glycolysis and gluconeogenesis. Phage display of a peptide library identified a GCK binding epitope of sequence SLKVWT, which matches a conserved stretch within the FBPase-2 domain of the bifunctional liver enzyme.[62] Subsequent studies demonstrated that the phosphatase domain alone is sufficient for interaction with GCK.[62,63] As PFK-2/FBPase-2 is expressed in both liver hepatocytes and pancreatic β -cells, it contributes to GCK regulation in both organs. This chapter, therefore, will discuss the interaction in the context of both organs. *In vitro* studies using recombinant human pancreatic GCK and rat liver PFK-2/FBPase-2 indicate the proteins can interact directly, albeit weakly, with a 1:1 stoichiometry (Figure 4).[63] The V_{max} value of GCK is modestly enhanced by exposure to 5–10 fold molar excess of recombinant PFK-2/FBPase-2, in a

manner that is independent of activation by known synthetic allosteric activators.[63,64] Notably, interaction with PFK-2/FBPase-2 does not alter GCK's responsiveness to glucose, as reflected by the enzyme's $K_{0.5}$ value[64], however one report suggests that the interaction perturbs GCK's hallmark cooperativity.[65] In addition to direct activation of GCK, PFK-2/FBPase-2 appears to stabilize GCK, in part by protecting the protein against oxidative damage.[66] GCK contains 13 reduced cysteine residues that are essential for proper activity and glucose responsiveness.[67] Thus, an interaction that provides protection against reactive oxygen species likely serves a significant physiological role.

A majority of investigations of the GCK-PFK-2/FBPase-2 interaction have taken place within cells. Immunofluorescence studies in several fixed cell lines indicate that GCK colocalizes with PFK-2/FBPase-2 in the cytoplasm following overexpression.[68–70] Live cell imaging conducted on cells overexpressing fluorescent fusion proteins support colocalization of GCK and PFK-2/FBPase in a glucose-dependent manner.[71] In these studies, colocalization was most notable following treatment with elevated glucose levels (10–25 mM), whereas the interaction was less apparent in cells exposed to 3 mM glucose. Together with the observation that PFK-2/FBPase-2 stimulates the V_{max} value of GCK, these observations support a model in which PFK-2/FBPase interacts preferentially with the closed, glucose-bound conformation of GCK.[71] This stands in contrast to the interaction of GCK with GKRP, which is specific to the super-open conformation.

Association with PFK-2/FBPase-2 produces modest increases of GCK activity in insulin-producing cells following adenoviral overexpression.[64] Interestingly, this activation was shown to be sensitive to forskolin, a cAMP stimulator, when using the liver PFK-2/FBPase-2 isozyme, but not the islet PFK-2/FBPase-2 isozyme.[64] This observation is consistent with a role for protein kinase A mediated phosphorylation in regulating the GCK-PFK-2/FBPase-2 interaction. The activity of liver PFK-2/FBPase-2 is regulated by phosphorylation of Ser32, a modification that is stimulated by treatment with glucagon. Phosphorylation of Ser32 is also stimulated by adenosine monophosphate (AMP), via the action of AMP-activated protein kinase (AMPK).[72] Glucagon inhibits glucose-stimulated translocation of GCK from the nucleus to the cytoplasm in liver cells and a phosphorylation inactive variant of liver PFK-2/FBPase-2 reverses this effect.[73] These results suggest that unphosphorylated PFK-2/FBPase-2 interacts with GCK to stimulate the active closed form of the enzyme and promote cytoplasmic localization. Glucagon stimulates protein kinase A activity and AMP stimulates AMPK activity, both of which promote PFK-2/FBPase-2 phosphorylation, impairing the interaction with GCK and facilitating GKRP-dependent nuclear sequestration of GCK.[70] It is important to note, however, that one study failed to detect a quantitative difference in the nuclear-to-cytoplasmic ratio of GCK that is dependent upon the phosphorylation state of PFK-2/FBPase-2.[74]

Several important aspects of the GCK-PFK-2/FBPase-2 interaction remain unresolved. Several lines of evidence suggest that while the GCK-PFK-2/FBPase-2 interaction is significantly weaker than that of GCK-GKRP interaction, it still represents the strongest interaction of GCK with a protein activator.[63,71] Notably, reconstitution of the complex using purified proteins has yet to be achieved.[63,71] Thus, the regions of GCK responsible for the PFK-2/FBPase-2 interaction are unknown and the extent to which these might

overlap with sites of naturally occurring GCK activating mutations is unclear. Uncertainly also centers around whether the interaction involves other, yet-to-be determined factors that could enhance the strength of the complex.[63] Finally, there is incomplete knowledge regarding how this interaction is modulated by metabolic cues beyond glucose, AMP and glucagon, and how the interaction is coordinated with other potential binding partners at other locations with the cell. Despite these questions, there is strong evidence from a number of independent investigations that the GCK-PFK-2/FBPase-2 interaction plays an important role in liver and pancreatic β -cell function[68–70] and further studies are clearly warranted.

4. Regulation of GCK via Transient Interaction Partners

4.1. Formation of a heteropentameric complex at the mitochondrial membrane

In a seminal report in 2003, Danial and coworkers provided evidence that GCK resides in a mitochondrial-associated complex (Figure 5) containing BCL 2-associated death promoter (BAD), protein kinase A (PKA, cAMP-dependent protein kinase), protein phosphatase 1 (PP1, dual-specificity serine/threonine phosphatase), and Wiskott-Aldrich family member (WAVE1).[75] BAD was postulated to function as a scaffold for organizing these components and integrating glycolysis and apoptosis.[75,76] The absence of BAD protects hepatocytes from apoptosis during glucose deprivation, highlighting its critical role as an apoptotic guardian.[77] Phosphorylation of S155 of BAD acts as a molecular switch that promotes GCK activation, enhancing glucose metabolism and GSIS in β -cells.[76,78] BAD can also be phosphorylated at two other residues and this process is strongly regulated by intracellular levels of glucose and insulin.[78] Mice expressing a non-phosphorylatable S155A variant display impaired GSIS.[75,76] In addition to these metabolic enhancements, phosphorylation also impedes BAD's interaction with a number of pro-survival proteins and attenuates its pro-apoptotic function.[76,78,79] Hormones and nutrients specific to fed and fasted states seemingly determine the BAD-mediated cellular fate (i.e. metabolic vs. apoptotic progression).[78] Interestingly, the β -cells in type 2 diabetic patients fail to produce enough insulin to supply the body's demand, largely due to insulin resistance-induced β -cell insensitivity to the physiological stimulus glucose, and to loss of β -cell mass via apoptosis, further intertwining these two seemingly divergent pathways.[80]

Interaction between BAD and GCK has been detected in both human and mouse hepatocytes and pancreatic β -cells, indicating conservation and potential clinical significance.[81] BAD knockdown or ablation results in similar phenotypes as does GCK knockdown, including perturbation of GSIS in β -cells and improper glucose sensing in hepatocytes.[75,76,82] Knockdown of hepatic BAD or inhibition of hepatic BAD phosphorylation also leads to reduced glycolysis and increased gluconeogenesis, despite the absence of detectable alterations in GCK mRNA and protein levels.[82] Furthermore, the introduction of BAD S155D, which mimics phosphorylated BAD, in obese and diabetic (*ob/ob*) mouse models restores proper gluconeogenic regulation.[82] A β -cell specific insulin receptor knockout has also been shown to alter the interaction stoichiometry of BAD and GCK and reduce GCK activity.[81] However, reintroduction of the receptor in knockout cells largely restores proper interaction and mitochondrial function.[81] Moreover, the BAD-GCK complex is disrupted in islets from type 2 diabetic humans, indicating a pathological significance and potential as

a therapeutic target.[81] In addition, altered insulin signaling is associated with mitochondrial dysfunction, seemingly stemming from reduced ATP levels and glucose-mediated alterations in mitochondrial membrane potential.[81] However, these defects could completely result from, or at least be exacerbated by, the production of reactive oxygen species, which is an additional effect upon glucose stimulation in insulin receptor knockout cells.[80,81,83] Glucose-mediated aerobic respiration occurring in the mitochondria is also perturbed in BAD knockout hepatocytes and mice.[76] Mitochondrial localization of GCK could serve to coerce GCK into preferentially utilizing ATP from oxidative phosphorylation. The ADP produced by GCK can then activate mitochondrial respiration.[11,50,78] Seemingly, these unique metabolic and survival functions of BAD could be selectively targeted by therapeutics.[76,79,82]

Most studies of the GCK-BAD complex have used only the BH3 domain of BAD, which has been sufficient for influencing GCK activity and is easier to produce than full-length, properly folded BAD.[76,79,84,85] A hydrocarbon-stapled BAD BH3 analog augments GCK's $V_{\max}$ value without affecting its Hill coefficient or $K_{0.5}$, characteristics that are unique compared to all other small molecule GCK activators.[79] Allosteric activators typically increase the risk of hypoglycemia due to their ability to lower the glucose $K_{0.5}$ value. The BAD BH3 mimetics appear to associate with GCK near the active site rather than the allosteric site.[79] While the mechanism of action is not known, BAD BH3 domains might stabilize specific conformations of GCK or accelerate product release, which has been observed in some activating GCK mutations.[40] This new interaction site could be exploited for future drug development. In fact, stabilized BAD BH3 domain helices have already shown promising therapeutic utility as they restore proper β -cell function and insulin secretion in BAD-deficient β -cells.[79]

Despite several experiments supporting direct interaction of BAD and GCK *in vitro*, including transcription/translation[76] and chemical cross-linking[79], a recent study demonstrated that highly purified full-length BAD is unable to interact with GCK *in vitro* using several techniques.[84] Recombinant BAD displayed known and expected functional properties, including association with Bcl-xL, but it did not interact with GCK nor stimulate its activity to a measurable extent.[84] This suggests that additional factors are necessary for the interaction. Identifying such factors remains an important objective toward understanding this interaction in detail. Another remaining question is whether BAD influences the activities of hexokinases 1–3, which are operative in other tissues and bind glucose with a much greater affinity.[1,11] BAD has also been shown to interact with phosphofructokinase-1 (PFK-1).[86] This is intriguing because PFK-1 is allosterically regulated by fructose-2,6-bisphosphate, which is the product of the cytosolic GCK binding partner PFK-2/FBPase-2. Whether this critical metabolic signaling pathway is mediated by the BAD-GCK interaction remains to be seen.

4.2. Direct ubiquitination and interaction with free ubiquitin chains and ubiquitin-like domain (ULD) containing proteins

Regulation of GCK by various protein interaction partners has been a topic of great discovery in the last two decades.[3,48,62,75,87,88] However, much less is known about

regulation of GCK via post-translational modification (PTM) events. The ubiquitin-proteasome system (UPS) has been shown to influence glucose-induced insulin synthesis and secretion, as well as the expression and function of ATP-sensitive K^+ channels, all of which are critical steps of whole-body glucose maintenance and homeostasis.[88–92] These results, and the observation that GCK contains a canonical ubiquitin interacting motif, led to several reports of GCK ubiquitination, the first of which relied on a rabbit reticulocyte lysate system for *in vitro* ubiquitination analysis (Figure 6).[88] In this report, radiolabeling and western blotting revealed that both the pancreatic and liver isoforms of GCK are ubiquitinated in the *in vitro* system. In addition, transfection of MIN6 β -cells and HepG2 human liver carcinoma cells resulted in high levels of ubiquitin (Ub)-GCK. A glutathione-S-transferase-based holdup assay was also leveraged in these experiments to demonstrate that the C-terminal $\alpha 13$ helix of GCK binds to free-pentaubiquitin chains (Ub₅). This interaction was found to stimulate GCK activity by a modest 1.4-fold.[88] The first step of the Ub conjugation cascade is the adenylation of the Ub C-terminal carboxyl group, which effectively activates Ub for subsequent transfer and conjugation to a target protein.[93,94] Importantly, the (E1) enzymes that catalyze this activation are completely dependent on ATP.[93] Thus, one possibility is that the glucose-stimulated increase in intracellular [ATP] could promote Ub₅ chain formation and subsequent catalytic activation of GCK. This would facilitate rapid glucose catabolism and expedite glucose clearance and energy production in the fed state.

Ubiquitin-like domain (ULD) containing proteins are those that possess a domain strongly resembling ubiquitin. Such proteins are often involved in the regulation of metabolic enzymes via cooperation with other players in the ubiquitin-proteasome system (UPS).[95–97] Upregulation of genes encoding ULD containing proteins in pancreatic islets at low glucose concentrations has been reported.[92,95] Förster resonance energy transfer (FRET) studies of recombinant GCK and several ULD containing proteins revealed interaction and cytoplasmic localization in a number of cells and tissues at low glucose concentrations (3 mM).[95] Furthermore, association of ULD containing proteins with GCK resulted in reduced GCK activity and, although cell viability and GCK expression remained unaffected, GSIS in MIN6 β -cells was significantly reduced upon individual overexpression of ULD containing proteins. Taken together, these complementary results suggest that GCK activity is partially regulated by expression of, and interaction with, ULD containing proteins. Although a physiological significance has been demonstrated, identifying the molecular roles of specific ULD containing proteins and how they operate within the broader scope of the UPS will be critical for fully understanding GCK regulation.

The role of the UPS in marking and targeting improperly folded proteins for degradation has been extensively investigated.[98–101] One such study has provided several lines of evidence demonstrating the importance of the UPS in the context of GCK regulation.[102] The addition of the proteasome inhibitor MG132 promoted clustering of recombinant GCK in MIN6 cells, which colocalized with an aggresome marker. This observation was also verified in mouse islets and hepatocytes. Additionally, GCK was conjugated to a photoswitchable fluorescent protein and a temporal analysis revealed that GCK underwent gradual cytoplasmic aggregation in the presence of MG132. These data support the postulate

that GCK has a relatively high flexibility and susceptibility to misfolding and, thus, requires sufficient control by the UPS for proper physiological function.

Identifying specific conditions such as metabolic signals or amino acid mutations which promote or inhibit GCK aggregation and interaction with ULD containing proteins would be beneficial for developing clinical interventions. For example, the introduction of small molecules that promote association between GCK and ULD containing proteins would selectively reduce GCK activity and could potentially be used as a treatment for hyperinsulinemic patients. Clearly, ubiquitin-based processes (interaction with Ub5 chains, interaction with ULD containing proteins and direct ubiquitination) represent important strategies for regulating GCK. It is unclear at this point, however, how balance between these related ubiquitin-based processes is achieved. Elucidating links between each of these processes and how they are each coordinated with other regulatory processes in the cell will remain a critical aspect of future studies.

4.3. Conjugation of the small ubiquitin-like modifier protein (SUMO-1)

A variety of essential cellular processes are regulated via post-translational modification by small ubiquitin-like modifier (SUMO) proteins.[103–110] Improper SUMOylation of target proteins has been directly linked to diabetes-associated cardiovascular complications. [103,111–113] A 2013 study demonstrated that pancreatic and liver GCK were SUMOylated *in vitro*, with the pancreatic isoform being the preferred substrate (Figure 7).[114] The authors demonstrated that SUMOylated pancreatic GCK could be immunoprecipitated from transfected MIN6 cells. 2D-PAGE also revealed that endogenous GCK is SUMOylated in INS-1 cells. Therefore, GCK SUMOylation does not appear to be a cell-line specific phenomenon. Several lysine-to-arginine mutants of GCK showed either a substantial reduction or complete loss of SUMOylation, which indicates that SUMOylation of GCK occurs via the standard lysine amino group-mediated conjugation pathway. The authors also stated that SUMOylation-deficient GCK mutants displayed wild-type-like ubiquitination patterns, suggesting that the two PTMs do not compete for the same lysine residues. There are approximately 15 lysine based mutations in GCK that have been implicated in diabetes, some of which result in thermally unstable proteins.[12,115,116] Whether protein conjugation at these lysine residues serves as a mechanism for regulating GCK cellular stability remains to be investigated.

SUMOylation increases GCK's catalytic activity and overall stability. Typically, only a small percentage (< 10%) of a target protein's overall pool is SUMOylated at any given moment *in vivo*. Although the authors observed a 1.4-fold increase in GCK activity, they estimated SUMOylation of only ~8% of total cellular GCK. The fact that this low SUMOylation frequency was sufficient for producing an increase in GCK activity demonstrates the potential of SUMOylation for regulating GCK. The stimulation in GCK's V_{max} was observed at ~80 nM SUMO-1. Interestingly, this is the same concentration range for which Ub5 and GKAs increase GCK activity.[88,117] A subsequent study sought to investigate the impact of SUMOylation on GCK nuclear translocation.[51] PredictNLS software identified residues 30–36 of pancreatic GCK, which are conserved among human, rat and mouse isoforms, as a NLS.[51] Several MODY2-causing mutations can be traced to

this region. Fractionation and immunostaining revealed a substantial amount of GCK in the nucleus of MIN6 β -cells, as well as in human and mouse pancreatic tissue sections where GCK partly colocalized with insulin.[51] Mutation of lysine residues within the NLS resulted in decreased nuclear localization of transfected GCK, whereas co-transfection of GCK and SUMO machinery promoted nuclear localization. Shiota and coworkers identified a leucine-rich nuclear export signal (NES) in GCK spanning residues 300–310.[48] This NES is in close spatial proximity to the lysine residues associated with SUMOylation, suggesting that SUMOylation might serve to mask the NES and prevent GCK nuclear export. How this process is coordinated with nuclear localization of GCK via GGRP remains an important question.

We recently reported that activating mutations in GCK can result in global conformational alterations.[38,40] It is possible that SUMOylation also alters the conformational dynamics of GCK, as has been observed for other proteins[107,118–121], but an investigation into the structural consequences of GCK SUMOylation has yet to be pursued. In particular, it remains to be determined which enzyme conformations of GCK are amenable to SUMOylation. The increased $V_{\max}$ observed upon SUMO-conjugation indicates that the closed conformation of GCK is involved in the SUMOylation process. However, it's unclear whether SUMO-1 is attached to the closed conformation initially or whether GCK undergoes a conformational change to the ordered, closed conformation upon SUMOylation. It is also not clear how cellular metabolites, which alter the conformation of GCK, might impact SUMOylation. As both GGRP- and SUMO-mediated interaction trigger nuclear localization of GCK, establishing connections between both regulatory events will be essential for a generating cellular or systemic description of GCK regulation. Finally, although SUMO-1 overexpression has been shown to impair GSIS[122], the specific impact that SUMOylation of GCK has on glucose metabolism and insulin signaling dynamics remains unknown.

4.4. Sequestration at the periphery of insulin granules and S-nitrosylation

In addition to cytosolic, nuclear, and mitochondrial localization, localization of GCK with insulin and somatostatin secretory granules has also been observed in several pancreatic cell lines via cell microscopy (Figure 8).[68,123–130] This granular localization pattern has been observed by several research groups, but the conditions that promote such organization remain somewhat unclear. Gradient fractionation experiments revealed that GCK cofractionated with phogrin, an insulin granule membrane marker and not insulin itself [129], suggesting that GCK is actually associated with the outer structure rather than the granule core. With a few exceptions, most reports indicate that this particular GCK distribution is not mediated by glucose.[68,123–125,129] Alternatively, a regulatory feedback mechanism involving insulin has been postulated and discussed in the literature. [127] When cells are incubated with inhibitors of insulin secretion, granular localization of GCK is abolished. Release of GCK from the granules is accompanied by an increase in catalytic activity and an apparent conformational change.[127] Subsequent insulin doping of the cells restores localization with insulin-containing granules, suggesting that insulin post-translationally modulates GCK activity and association with secretory granules.

Although colocalization between GCK and insulin has been reported, a direct interaction between the two molecules has not been established. Moreover, the mechanism by which insulin modulates granular GCK localization remains uncertain. Because GCK contains many reduced cysteine residues that are essential for proper activity and physiology [131], post-translational S-nitrosylation was postulated as key regulatory step in GCK-insulin granule dynamics. Interestingly, inhibition of nitric oxide synthase (NOS), which catalyzes the S-nitrosylation of cellular proteins, blocks the stimulatory effects of insulin on granular GCK localization. [128] Several experiments have demonstrated that association with NOS dimers facilitates binding of GCK to insulin secretory granules and that S-nitrosylation of GCK reverses this localization [126,128], indicating that both physical interaction and chemical modification contribute to regulating GCK localization to secretory granules. Expression of GCK and NOS mutants that promote interaction lead to increased GCK protein levels, while the expression of mutants that hamper interaction results in reduced cellular GCK content. [126] Moreover, the magnitude of association of GCK with insulin granules is directly correlated to the relative concentrations of both GCK and NOS. [126] One explanation of these data is that granule-bound GCK simply serves as a reservoir for transient storage of GCK molecules. UPS-mediated proteasomal degradation of GCK is likely to proceed via its cytoplasmic pool, thus, tethering of GCK to insulin granules would effectively reduce cytoplasmic GCK content and allow for proteasomal evasion. Since granular association of GCK is mediated by NOS dimers and S-nitrosylation reverses granular association, perhaps this mechanism provides the ability to rapidly mobilize substantial quantities of cytoplasmic GCK in response to metabolic status, which would undoubtedly be accomplished more rapidly than *de novo* ribosomal synthesis and translocation of new GCK molecules. However, denitrosylation of GCK does not appear to be fast enough for rapid cycling between active, cytoplasmic GCK and insulin granule-bound GCK. [132] Identifying the exact mechanism responsible for GCK denitrosylation is a logical next step toward understanding the physiological significance of NOS- and insulin granule-based regulation of GCK.

Amino acid mutations in GCK's S-nitrosylation motif have proven useful for elucidating the regulatory nature of this post-translational modification. A series of biochemical experiments revealed C371 of GCK as the primary target for S-nitrosylation. [126,128] Substitution of C371 with serine eliminates granular localization of GCK *in vivo* and increases GCK's K_m and decreases its V_{max} *in vitro*. [128] Several MODY2-causing mutants of GCK, including V367M and R369P, are near the C371 S-nitrosylation site and display significantly reduced S-nitrosylation profiles. [128] In addition, S-nitrosylation of GCK and subsequent release from insulin granules is accompanied by an increased cellular GCK activity. [126] These observations demonstrate that granule association and S-nitrosylation likely play vital roles in regulating glycolysis and GSIS. Intriguingly, GCK activity and S-nitrosylation both appear to be upregulated by the incretin hormone glucagon-like peptide 1 (GLP-1). [133] As GLP-1 is known to potentiate GSIS at physiologically intermediate (8–11 mM) glucose concentrations [134], it seems likely that this insulinotropic effect is achieved, in part, by promoting S-nitrosylation of GCK. [133] A recent report also demonstrated that chemically-mediated S-nitrosylation activates GCK by promoting the highly active closed GCK conformation [135], much like α -type activating mutations [40], small molecule

GKAs [15,16,117], and high glucose concentrations. Despite these results, the direct influence that granular association and S-nitrosylation have on GCK activity remain incompletely understood. It is unclear whether the increased cellular GCK activity detected upon S-nitrosylation and granule release is caused by the S-nitrosylation-mediated shift of GCK toward the closed conformation, or the inherent increase in number of active cytosolic GCK molecules. Perhaps the observed increase is caused by a combination of the two mechanisms. Rigorous kinetic and structural investigations of S-nitrosylated GCK have been challenging to accomplish due to the presence of highly reducing reagents that are necessary for stabilizing recombinantly expressed GCK *in vitro*. [128] However, such investigations are critical for generating a comprehensive molecular description of diabetes-causing GCK mutants that are unsusceptible to S-nitrosylation and display perturbed association with secretory granules. Clearly, further cellular-based investigations of this GCK regulatory mechanism are also necessary to identify additional players and obtain a deeper understanding of the links between insulin granule association, S-nitrosylation and cellular physiology.

5. Subordinate interaction partners

GCK has been reported to colocalize with actin filaments. [136] Immunofluorescence staining revealed a mesh-like distribution of endogenous GCK in the cytoplasm of rat hepatocytes, which appeared to coincide with actin staining. Additionally, COS-7 cells, which are adherent and behave like fibroblasts, were transiently transfected with either liver or pancreatic GCK. In these dissimilar cells, colocalization with actin, independent of glucose concentrations, was observed in both cases. [136] This pattern was not observed in MIN6 β -cells, however. Tissue-specific promoters result in GCK expression levels that are significantly lower in pancreatic β -cells than hepatocytes [137], suggesting that an improved detection limit might be required to observe GCK-actin colocalization in these cells. Although the initial report of GCK-actin colocalization by Murata and coworkers represents the only evidence of this phenomenon, the interaction remains intriguing for two reasons: (1) *Arabidopsis* hexokinase associates with actin to modulate actin polymerization rate and glucose signaling [138–140] and (2) GCK has been reported to colocalize with other motor proteins including α -tubulin, β -tubulin and kinesin. [68] Perhaps GCK associates with motor proteins to facilitate rapid movement in response to metabolic stimuli and/or to alter its localization to discrete sites within the cell.

Yeast two-hybrid screening studies have provided the foundation for countless investigations of protein-protein interactions. [141–143] To identify additional hepatic proteins similar to GKRP that are implicated in GCK regulation, a group of researchers conducted a two-hybrid study using rat liver GCK cDNA. [144] Their screen revealed both GKRP and a 37 kDa tyrosine phosphatase as interaction partners. A BLAST search of the coding sequence identifies this protein as dual specificity protein phosphatase 12, which shares 83% sequence identity to the homologous human isoform. *In vitro* assays revealed that this protein was able to dephosphorylate GCK and increase GCK's activity in a dose-dependent manner and at different stoichiometries. [144] These results, while being the only published report of this interaction, offer unique insights into the regulatory mechanisms that govern cellular GCK. As the interplay between phosphorylation and proteolysis has been well described [145], it

seems likely that the phosphorylation state of GCK might contribute to its stability and intracellular concentration. The physiological consequences of direct interaction with this tyrosine phosphatase, and those of the dephosphorylation it catalyzes, are still poorly understood. Another yeast two-hybrid screen revealed interaction between propionyl-CoA carboxylase β subunit (p β PCCase) and both the liver and pancreatic isoforms of GCK.[146] The interaction was confirmed in an immobilized binding assay and the authors observed an increase in GCK's $V_{\max}$ and an improved glucose $K_{0.5}$ value in a p β PCCase concentration-dependent manner.[146] Interestingly, the product of p β PCCase, (S)-methylmalonyl CoA, is a precursor for gluconeogenic intermediates,[147] indicating a seemingly contradictory role between GCK and p β PCCase. It is unclear how these seemingly subsidiary interactions of GCK cooperate with the more established regulatory mechanisms within the broader context of the cell. However, continued investigation surrounding the aforementioned GCK interaction partners and explorations aimed at discovering latent GCK regulatory schemes are critical to future therapeutic strategies.

6. Concluding Remarks

Biochemical, biophysical and cellular investigations performed over the last decade have resulted in tremendous advances in our molecular understanding of GCK regulation. These studies have yielded a semi-quantitative model for the enzyme's kinetic cooperativity and have uncovered two distinct mechanisms by which the enzyme is activated in hyperinsulinemia-associated variants. Molecular and cellular studies have also identified a variety of interaction partners and post-translational events capable of regulating GCK activity. Taken together, these data support the view that GCK regulation is best represented as a network of processes that operate in close coordination with the metabolic state of the cell. Several perspective and review articles have integrated known cellular GCK regulatory strategies into a holistic understanding of pancreatic β -cell and liver hepatocyte function. [6,46,148] The next major step in understanding global GCK regulation is to incorporate our understanding of molecular and cellular regulatory mechanisms into a unified model for GCK regulation *in vivo*. Several considerations, outlined below, suggest that undertaking such a task is both feasible and close at hand.

The enzyme's intrinsic ability to sample discrete conformations, a characteristic that drives the allosteric response toward glucose, can be modulated by known post-translational modifications. For example, S-nitrosylation of GCK was recently shown to promote the glucose bound, closed conformation. Similarly, SUMOylation of GCK increases the catalytic activity of the enzyme, suggestive of a potential for this modification to shift the conformational ensemble toward a more active state. GCK's interaction with free pentaubiquitin chains involves the C-terminal $\alpha 13$ helix, a region of the protein that has highly varying degrees of accessibility depending upon the conformational state of the enzyme. In the inactive, super-open conformation this helix is completely exposed to solvent, whereas in the closed conformation this helix is nearly completely excluded into the protein's interior. It seems likely that the molecular details of the enzyme structure dictate its ability to participate in ubiquitin-mediated regulation. These findings provide impetus to investigate how conformational heterogeneity is modulated by specific post-translational events in response to changes in metabolism within living cells.

GCK's conformational heterogeneity also appears correlated with subcellular localization of the enzyme, a characteristic that is conferred by GCK interaction partners. Nuclear localization of GCK in hepatocytes requires binding to GKRP and only occurs when the enzyme adopts the inactive, super-open state. Conversely, current evidence suggests that recognition by PFK-2/FBPase-2 involves the glucose-bound state, an interaction that is believed to be predominantly cytosolic in nature. Moreover, PFK-2/FBPase-2-mediated regulation of GCK is more favorable in pancreatic β -cells than in liver hepatocytes because it is not hindered by the presence of the liver-specific GKRP. The third known site of GCK localization, the mitochondria, is likely to involve interactions with multiple components of the 5-membered BAD-containing complex. No information is available regarding the conformation(s) involved in tethering GCK at the mitochondria, emphasizing the need for further study. Combining recent advances in super-resolution microscopy with the ability to engineer conformationally restricted variants of GCK could provide new insights into the link between cellular localization and enzyme conformation. Live-cell imaging studies could also establish the extent to which GCK can participate in higher order multienzyme complexes similar to the recently described purinosome and glucosome.[149,150] Clearly, more investigations of GCK structure and dynamics within the complex cellular environment are warranted, and the information gleaned from such investigations promises to drive the field of GCK regulation into new, unexplored avenues.

Acknowledgements

The authors thank Malcolm McCray for helpful discussions and input.

Funding Sources

This work was supported, in part, by NIH GM115388 to BGM.

References

- [1]. Wilson JE, Hexokinases, *Rev Physiol Biochem Pharmacol.* 126 (1995) 65–198. <http://www.biomednet.com/db/medline/95192617>. [PubMed: 7886381]
- [2]. Ferre T, Riu E, Bosch F, Valera A, Evidence from transgenic mice that glucokinase is rate limiting for glucose utilization in the liver., *FASEB J.* 10 (1996) 1213–8. <http://www.ncbi.nlm.nih.gov/pubmed/8751724>. [PubMed: 8751724]
- [3]. Iynedjian PB, Molecular physiology of mammalian glucokinase, *Cell. Mol. Life Sci* 66 (2009) 27–42. doi:10.1007/s00018-008-8322-9. [PubMed: 18726182]
- [4]. Printz R, Magnuson M, Granner D, Mammalian glucokinase, *Annu. Rev. Nutr* (1993). doi: 10.1146/annurev.nu.13.070193.002335.
- [5]. Iynedjian PB, Mammalian glucokinase and its gene., *Biochem. J* 293 (1993) 1–13. doi:10.1042/bj2930001. [PubMed: 8392329]
- [6]. Matschinsky FM, Glucokinase as glucose sensor and metabolic signal generator in pancreatic β -cells and hepatocytes, *Diabetes.* 39 (1990) 647–652. doi:10.2337/diabetes.39.6.647. [PubMed: 2189759]
- [7]. Kawai S, Mukai T, Mori S, Mikami B, Murata K, Hypothesis: Structures, evolution, and ancestor of glucose kinases in the hexokinase family, *J. Biosci. Bioeng* (2005). doi:10.1263/jbb.99.320.
- [8]. Cárdenas ML, Cornish-Bowden A, Ureta T, Evolution and regulatory role of the hexokinases, *Biochim. Biophys. Acta - Mol. Cell Res* 1401 (1998) 242–264. doi:10.1016/S0167-4889(97)00150-X.
- [9]. Nelson DL, Cox MM, *Lehninger Principles of Biochemistry* 6th ed., 2013. doi:10.1016/j.jse.2011.03.016.

- [10]. Aiston S, Trinh KY, Lange AJ, Newgard CB, Agius L, Glucose-6-phosphatase overexpression lowers glucose 6-phosphate and inhibits glycogen synthesis and glycolysis in hepatocytes without affecting glucokinase translocation. Evidence against feedback inhibition of glucokinase, *J. Biol. Chem* (1999). doi:10.1074/jbc.274.35.24559.
- [11]. Wilson JE, Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function, *J. Exp. Biol* 206 (2003) 2049–2057. doi:10.1242/jeb.00241. [PubMed: 12756287]
- [12]. Osbak KK, Colclough K, Saint-Martin C, Beer NL, Bellanné-Chantelot C, Ellard S, Gloyn AL, Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia, *Hum. Mutat* 30 (2009) 1512–1526. doi:10.1002/humu.21110. [PubMed: 19790256]
- [13]. Bell GI, Polonsky KS, Diabetes mellitus and genetically programmed defects in beta-cell function., *Nature*. 414 (2001) 788–791. doi:10.1038/414788a. [PubMed: 11742410]
- [14]. Kim SH, Maturity-Onset Diabetes of the Young: What Do Clinicians Need to Know?, *Diabetes Metab. J* 39 (2015) 468–77. doi:10.4093/dmj.2015.39.6.468. [PubMed: 26706916]
- [15]. Matschinsky FM, Zelen B, Doliba N, Li C, Vanderkooi JM, Naji A, Sarabu R, Grimsby J, Glucokinase activators for diabetes therapy: May 2010 status report, *Diabetes Care*. (2011). doi: 10.2337/dc11-s236.
- [16]. Bowler JM, Hervert KL, Kearley ML, Miller BG, Small-molecule allosteric activation of human glucokinase in the absence of glucose, *ACS Med. Chem. Lett* (2013). doi:10.1021/ml400061x.
- [17]. Grewal A, Sekhon B, Lather V, Recent Updates on Glucokinase Activators for the Treatment of Type 2 Diabetes Mellitus, *Mini-Reviews Med. Chem* (2014). doi: 10.2174/1389557514666140722082713.
- [18]. Xiao Q, Jackson JJ, Basak A, Bowler JM, Miller BG, Zakarian A, Enantioselective synthesis of tatanans A-C and reinvestigation of their glucokinase-activating properties, *Nat. Chem* (2013). doi:10.1038/nchem.1597.
- [19]. Shelton KD, Franklin AJ, Khoor A, Beechem J, Magnuson MA, Multiple elements in the upstream glucokinase promoter contribute to transcription in insulinoma cells, *Mol Cell Biol*. (1992). doi:10.1128/MCB.12.10.4578.Updated.
- [20]. Jetton TL, Liang Y, Pettepher CC, Zimmerman EC, Cox FG, Horvath K, Matschinsky FM, Magnuson MA, Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut, *J. Biol. Chem* (1994).
- [21]. Moates JM, Magnuson MA, The Pal elements in the upstream glucokinase promoter exhibit dyad symmetry and display cell-specific enhancer activity when multimerised, *Diabetologia*. (2004). doi:10.1007/s00125-004-1497-1.
- [22]. Moates JM, Nanda S, Cissell MA, Tsai MJ, Stein R, BETA2 activates transcription from the upstream glucokinase gene promoter in islet β -cells and gut endocrine cells, *Diabetes*. (2003). doi:10.2337/diabetes.52.2.403.
- [23]. Iynedjian PB, Gjinovci A, Renold AE, Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats, *J. Biol. Chem* (1988).
- [24]. Wu C, Okar DA, Stoeckman AK, Peng LJ, Herrera AH, Herrera JE, Towle HC, Lange AJ, A Potential Role for Fructose-2,6-Bisphosphate in the Stimulation of Hepatic Glucokinase Gene Expression, *Endocrinology*. (2004). doi:10.1210/en.2003-1290.
- [25]. Roth U, Curth K, Unterman TG, Kietzmann T, The Transcription Factors HIF-1 and HNF-4 and the Coactivator p300 Are Involved in Insulin-regulated Glucokinase Gene Expression via the Phosphatidylinositol 3-Kinase/Protein Kinase B Pathway, *J. Biol. Chem* (2004). doi:10.1074/jbc.M308391200.
- [26]. Iynedjian PB, Marie S, Gjinovci A, Genin B, Deng SP, Buhler L, Morel P, Mentha G, Glucokinase and cytosolic phosphoenolpyruvate carboxykinase (GTP) in the human liver. Regulation of gene expression in cultured hepatocytes., *J. Clin. Invest* 95 (1995) 1966–73. doi: 10.1172/JCI117880. [PubMed: 7738162]
- [27]. Agius L, Hormonal and Metabolite Regulation of Hepatic Glucokinase, *Annu. Rev. Nutr* 36 (2016) 389–415. doi:10.1146/annurev-nutr-071715-051145. [PubMed: 27146014]

- [28]. Kamata K, Mitsuya M, Nishimura T, Eiki JI, Nagata Y, Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase, *Structure*. 12 (2004) 429–438. doi: 10.1016/j.str.2004.02.005. [PubMed: 15016359]
- [29]. Nishimura T, Iino T, Mitsuya M, Bamba M, Watanabe H, Tsukahara D, Kamata K, Sasaki K, Ohyama S, Hosaka H, Futamura M, Nagata Y, ichi Eiki J, Identification of novel and potent 2-amino benzamide derivatives as allosteric glucokinase activators, *Bioorganic Med. Chem. Lett* 19 (2009) 1357–1360. doi:10.1016/j.bmcl.2009.01.053.
- [30]. Pal P, Miller BG, Activating mutations in the human glucokinase gene revealed by genetic selection, *Biochemistry*. 48 (2009) 814–816. doi:10.1021/bi802142q. [PubMed: 19146401]
- [31]. Liu S, Ammirati MJ, Song X, Knafels JD, Zhang J, Greasley SE, Pfefferkorn JA, Qiu X, Insights into mechanism of glucokinase activation: Observation of multiple distinct protein conformations, *J. Biol. Chem* (2012). doi:10.1074/jbc.M111.274126.
- [32]. Matschinsky FM, Regulation of pancreatic β -cell glucokinase: From basics to therapeutics, in: *Diabetes*, 2002. doi:10.2337/diabetes.51.2007.S394.
- [33]. RICARD J, C MEUNIER J, BUC J, Regulatory Behavior of Monomeric Enzymes: 1. The Mnemonical Enzyme Concept, *Eur. J. Biochem* (1974). doi:10.1111/j.1432-1033.1974.tb03825.x.
- [34]. Ainslie GR, Shill JP, Neet KE, Transients and cooperativity. A slow transition model for relating transients and cooperative kinetics of enzymes., *J. Biol. Chem* (1972).
- [35]. Neet KE, Keenan RP, Tippet PS, Observation of a Kinetic Slow Transition in Monomeric Glucokinase, *Biochemistry*. (1990). doi:10.1021/bi00455a026.
- [36]. Heredia VV, Carlson TJ, Garcia E, Sun S, Biochemical basis of glucokinase activation and the regulation by glucokinase regulatory protein in naturally occurring mutations, *J. Biol. Chem* 281 (2006) 40201–40207. doi:10.1074/jbc.M607987200. [PubMed: 17082186]
- [37]. Antoine M, Boutin JA, Ferry G, Binding kinetics of glucose and allosteric activators to human glucokinase reveal multiple conformational states, *Biochemistry*. (2009). doi:10.1021/bi900374c.
- [38]. Whittington AC, Ramsey KM, Larion M, Brüscheweiler R, Bowler JM, Miller BG, Dual allosteric activation mechanisms in monomeric human glucokinase, *Proc. Natl. Acad. Sci. U. S. A* 112 (2015) 11553–11558. doi:10.1073/pnas.1506664112. [PubMed: 26283387]
- [39]. Larion M, Salinas RK, Bruschweiler-Li L, Miller BG, Brüscheweiler R, Order-Disorder Transitions Govern Kinetic Cooperativity and Allostery of Monomeric Human Glucokinase, *PLoS Biol.* 10 (2012). doi:10.1371/journal.pbio.1001452.
- [40]. Sternisha SM, Liu P, Marshall AG, Miller BG, Mechanistic Origins of Enzyme Activation in Human Glucokinase Variants Associated with Congenital Hyperinsulinism, *Biochemistry*. (2018). doi:10.1021/acs.biochem.8b00022.
- [41]. Bowler JM, Hervert KL, Kearley ML, Miller BG, Small-molecule allosteric activation of human glucokinase in the absence of glucose, *ACS Med. Chem. Lett* 4 (2013) 580–584. doi:10.1021/ml400061x.
- [42]. Van Schaftingen E, A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6 phosphate and fructose 1 phosphate, *Eur. J. Biochem* 179 (1989) 179–184. doi:10.1111/j.1432-1033.1989.tb14538.x. [PubMed: 2917560]
- [43]. Van Schaftingen E, Vandercammen A, Detheux M, Davies DR, The regulatory protein of liver glucokinase, *Adv. Enzyme Regul.* 32 (1992) 133–148. doi:10.1016/0065-2571(92)90013-P. [PubMed: 1496915]
- [44]. Choi JM, Seo M-H, Kyeong H-H, Kim E, Kim H-S, Molecular basis for the role of glucokinase regulatory protein as the allosteric switch for glucokinase., *Proc. Natl. Acad. Sci. U. S. A* 110 (2013) 10171–6. doi:10.1073/pnas.1300457110. [PubMed: 23733961]
- [45]. Beck T, Miller BG, Structural basis for regulation of human glucokinase by glucokinase regulatory protein, *Biochemistry*. 52 (2013) 6232–6239. doi:10.1021/bi400838t. [PubMed: 23957911]
- [46]. Lenzen S, A fresh view of glycolysis and glucokinase regulation: History and current status, *J. Biol. Chem* 289 (2014) 12189–12194. doi:10.1074/jbc.R114.557314. [PubMed: 24637025]

- [47]. Raimondo A, Rees MG, Gloyn AL, Glucokinase regulatory protein: Complexity at the crossroads of triglyceride and glucose metabolism, *Curr. Opin. Lipidol* 26 (2015) 88–95. doi:10.1097/MOL.000000000000155. [PubMed: 25692341]
- [48]. Shiota C, Coffey J, Grimsby J, Grippio JF, Magnuson MA, Nuclear import of hepatic glucokinase depends upon glucokinase regulatory protein, whereas export is due to a nuclear export signal sequence in glucokinase, *J. Biol. Chem* 274 (1999) 37125–37130. doi:10.1074/jbc.274.52.37125. [PubMed: 10601273]
- [49]. Agius L, Glucokinase and molecular aspects of liver glycogen metabolism, *Biochem. J* 414 (2008) 1–18. doi:10.1042/BJ20080595. [PubMed: 18651836]
- [50]. Arden C, Baltrusch S, Agius L, Glucokinase regulatory protein is associated with mitochondria in hepatocytes, *FEBS Lett.* 580 (2006) 2065–2070. doi:10.1016/j.febslet.2006.03.009. [PubMed: 16542652]
- [51]. Johansson BB, Fjeld K, Solheim MH, Shirakawa J, Zhang E, Keindl M, Hu J, Lindqvist A, Døskeland A, Mellgren G, Flatmark T, Njølstad PR, Kulkarni RN, Wierup N, Aukrust I, Bjørkhaug L, Nuclear import of glucokinase in pancreatic beta-cells is mediated by a nuclear localization signal and modulated by SUMOylation, *Mol. Cell. Endocrinol* 454 (2017) 146–157. doi:10.1016/j.mce.2017.06.020. [PubMed: 28648619]
- [52]. Anderka O, Boyken J, Aschenbach U, Batzer A, Boscheinen O, Schmoll D, Biophysical characterization of the interaction between hepatic glucokinase and its regulatory protein: Impact of physiological and pharmacological effectors, *J. Biol. Chem* 283 (2008) 31333–31340. doi:10.1074/jbc.M805434200. [PubMed: 18809676]
- [53]. Pautsch A, Stadler N, Löhle A, Rist W, Berg A, Nar H, Reinert D, Lenter M, Heckel A, Schnapp G, Stefan G, Supporting Information Crystal structure of glucokinase regulatory protein, *Biochemistry.* 52 (2013) 1–7. [PubMed: 23256782]
- [54]. Lloyd DJ, St Jean DJ, Kurzeja RJM, Wahl RC, Michelsen K, Cupples R, Chen M, Wu J, Sivits G, Helmering J, Komorowski R, Ashton KS, Pennington LD, Fotsch C, Vazir M, Chen K, Chmait S, Zhang J, Liu L, Norman MH, Andrews KL, Bartberger MD, Van G, Galbreath EJ, Vonderfecht SL, Wang M, Jordan SR, Véniant MM, Hale C, Antidiabetic effects of glucokinase regulatory protein small-molecule disruptors, *Nature.* 504 (2013) 437–440. doi:10.1038/nature12724. [PubMed: 24226772]
- [55]. Casey AK, Miller BG, Kinetic Basis of Carbohydrate-Mediated Inhibition of Human Glucokinase by the Glucokinase Regulatory Protein, *Biochemistry.* (2016). doi:10.1021/acs.biochem.6b00349.
- [56]. Martinez JA, Xiao Q, Zakarian A, Miller BG, Antidiabetic Disruptors of the Glucokinase-Glucokinase Regulatory Protein Complex Reorganize a Coulombic Interface, *Biochemistry.* (2017). doi:10.1021/acs.biochem.7b00377.
- [57]. Park JM, Kim TH, Jo SH, Kim MY, Ahn YH, Acetylation of glucokinase regulatory protein decreases glucose metabolism by suppressing glucokinase activity, *Sci. Rep* 5 (2015) 1–13. doi:10.1038/srep17395.
- [58]. Watanabe H, Inaba Y, Kimura K, Matsumoto M, Kaneko S, Kasuga M, Inoue H, Sirt2 facilitates hepatic glucose uptake by deacetylating glucokinase regulatory protein, *Nat. Commun* 9 (2018) 1–14. doi:10.1038/s41467-017-02537-6. [PubMed: 29317637]
- [59]. Veiga-Da-Cunha M, Sokolova T, Oppendoes F, Van Schaftingen E, Evolution of vertebrate glucokinase regulatory protein from a bacterial N-acetylmuramate 6-phosphate etherase, *Biochem J.* 423 (2009) 323–332. [PubMed: 19671048]
- [60]. Orho-Melander M, Melander O, Guiducci C, Perez-martinez P, Corella D, Roos C, Tewhey R, Rieder MJ, Hall J, Abecasis G, Tai ES, Welch C, Arnett DK, Lyssenko V, Lindholm E, Saxena R, De Bakker PIW, Burt N, Voight BF, Hirschhorn JN, Tucker KL, Hedner T, Tuomi T, Isomaa B, Eriksson K, Taskinen M, Hughes TE, Parnell LD, Havulinna AS, Salomaa V, Nilsson P, Groop L, Altshuler D, Ordovas JM, Kathiresan S, Common Missense Variant in the Glucokinase Regulatory Protein Gene Is Associated With Increased Plasma Triglyceride and C-Reactive Protein but Lower Fasting Glucose Concentrations, *Stroke.* 57 (2008) 3112–3121. doi:10.2337/db08-0516.
- [61]. Sparsø T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, Nielsen AL, Albrechtsen A, Rasmussen SS, Jørgensen T, Borch-Johnsen K, Sandbaek A, Lauritzen T, Madsbad S, Hansen T,

- Pedersen O, The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes, *Diabetologia*. 51 (2008) 70–75. doi:10.1007/s00125-007-0865-z. [PubMed: 18008060]
- [62]. Baltrusch S, Lenzen S, Okar DA, Lange AJ, Tiedge M, Characterization of glucokinase-binding protein epitopes by a phage-displayed peptide library: Identification of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase as a novel interaction partner, *J. Biol. Chem* 276 (2001) 43915–43923. doi:10.1074/jbc.M105470200. [PubMed: 11522786]
- [63]. Smith WE, Langer S, Wu C, Baltrusch S, Okar DA, Molecular Coordination of Hepatic Glucose Metabolism by the 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase:Glucokinase Complex, *Mol. Endocrinol* 21 (2007) 1478–1487. doi:10.1210/me.2006-0356. [PubMed: 17374851]
- [64]. Baltrusch S, Schmitt H, Brix A, Langer S, Lenzen S, Additive activation of glucokinase by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase and the chemical activator LY2121260, *Biochem. Pharmacol* 83 (2012) 1300–1306. doi:10.1016/j.bcp.2012.01.020. [PubMed: 22305745]
- [65]. Massa L, Baltrusch S, Okar DA, Lange AJ, Lenzen S, Tiedge M, Interaction of 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase (PFK-2/FBPase-2) with Glucokinase Activates Glucose Phosphorylation and Glucose Metabolism in Insulin-Producing Cells, *Diabetes*. 53 (2004) 1020–1029. doi:10.2337/diabetes.53.4.1020. [PubMed: 15047617]
- [66]. Cullen KS, Matschinsky FM, Agius L, Arden C, Susceptibility of glucokinase-MODY mutants to inactivation by oxidative stress in pancreatic β -cells, *Diabetes*. (2011). doi:10.2337/db11-0423.
- [67]. Tiedge M, Richter T, Lenzen S, Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase., *Arch. Biochem. Biophys* 375 (2000) 251–260. doi:10.1006/abbi.1999.1666. [PubMed: 10700381]
- [68]. Baltrusch S, Lenzen S, Novel insights into the regulation of the bound and diffusible glucokinase in MIN6 β -cells, *Diabetes*. 56 (2007) 1305–1315. doi:10.2337/db06-0894. [PubMed: 17287461]
- [69]. Payne VA, Arden C, Lange AJ, Agius L, Contributions of glucokinase and phosphofructokinase-2/fructose bisphosphatase-2 to the elevated glycolysis in hepatocytes from Zucker fa/fa rats., *Am. J. Physiol. Regul. Integr. Comp. Physiol* 293 (2007) R618–25. doi: 10.1152/ajpregu.00061.2007. [PubMed: 17553851]
- [70]. Cullen KS, Al-Oanzi ZH, O'Harte FPM, Agius L, Arden C, Glucagon induces translocation of glucokinase from the cytoplasm to the nucleus of hepatocytes by transfer between 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase-2 and the glucokinase regulatory protein, *Biochim. Biophys. Acta - Mol. Cell Res* 1843 (2014) 1123–1134. doi:10.1016/j.bbamcr.2014.02.006.
- [71]. Langer S, Kaminski MT, Lenzen S, Baltrusch S, Endogenous activation of glucokinase by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase is glucose dependent., *Mol. Endocrinol* 24 (2010) 1988–97. doi:10.1210/me.2010-0115. [PubMed: 20702580]
- [72]. Mukhtar MH, Payne VA, Arden C, Harbottle A, Khan S, Lange AJ, Agius L, Inhibition of glucokinase translocation by AMP-activated protein kinase is associated with phosphorylation of both GKRP and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, *AJP Regul. Integr. Comp. Physiol* (2008). doi:10.1152/ajpregu.00593.2007.
- [73]. Payne VA, Arden C, Wu C, Lange AJ, Agius L, Dual role of phosphofructokinase-2/fructose bisphosphatase-2 in regulating the compartmentation and expression of glucokinase in hepatocytes, *Diabetes*. 54 (2005) 1949–1957. doi:10.2337/diabetes.54.7.1949. [PubMed: 15983194]
- [74]. Watanabe F, Furuya E, Quantitative image analysis reveals that phosphorylation of liver-type isozyme of fructose-6-phosphate 2-kinase/fructose-2,6-bisphosphatase does not affect nuclear translocation of glucokinase in rat primary hepatocytes, *J. Biochem* 148 (2010) 713–719. doi: 10.1093/jb/mvq107. [PubMed: 20843823]
- [75]. Danial NN, Gramm CF, Scorrano L, Zhang C-Y, Krauss S, Ranger AM, Datta SR, Greenberg ME, Licklider LJ, Lowell BB, Gygi SP, Korsmeyer SJ, BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis, *Nature*. 424 (2003) 952–956. doi:10.1038/nature01825. [PubMed: 12931191]

- [76]. Danial NN, Walensky LD, Zhang CY, Choi CS, Fisher JK, Molina AJA, Datta SR, Pitter KL, Bird GH, Wikstrom JD, Deeney JT, Robertson K, Morash J, Kulkarni A, Neschen S, Kim S, Greenberg ME, Corkey BE, Shirihai OS, Shulman GI, Lowell BB, Korsmeyer SJ, Dual role of proapoptotic BAD in insulin secretion and beta cell survival, *Nat. Med* 14 (2008) 144–153. doi: 10.1038/nm1717. [PubMed: 18223655]
- [77]. Yang J, Li JH, Wang J, Zhang CY, Molecular modeling of BAD complex resided in a mitochondrion integrating glycolysis and apoptosis, *J. Theor. Biol* 266 (2010) 231–241. doi: 10.1016/j.jtbi.2010.06.009. [PubMed: 20540951]
- [78]. Danial NN, Bad: Undertaker by night, candyman by day, *Oncogene*. 27 (2009). doi:10.1038/onc.2009.44.
- [79]. Szlyk B, Braun CR, Ljubcic S, Patton E, Bird GH, Osundiji MA, Matschinsky FM, Walensky LD, Danial NN, A phospho-BAD BH3 helix activates glucokinase by a mechanism distinct from that of allosteric activators, *Nat. Struct. Mol. Biol* 21 (2014) 36–42. doi:10.1038/nsmb.2717. [PubMed: 24317490]
- [80]. Lee JW, Kim WH, Lim JH, Song EH, Song J, Choi KY, Jung MH, Mitochondrial dysfunction: Glucokinase downregulation lowers interaction of glucokinase with mitochondria, resulting in apoptosis of pancreatic β -cells, *Cell. Signal* 21 (2009) 69–78. doi:10.1016/j.cellsig.2008.09.015. [PubMed: 18940247]
- [81]. Liu S, Okada T, Assmann A, Soto J, Liew CW, Bugger H, Shirihai OS, Abel ED, Kulkarni RN, Insulin signaling regulates mitochondrial function in pancreatic beta-cells, *PLoS One*. 4 (2009) e7983. doi:10.1371/journal.pone.0007983. [PubMed: 19956695]
- [82]. Giménez-Cassina A, Garcia-Haro L, Choi CS, Osundiji MA, Lane EA, Huang H, Yildirim MA, Szlyk B, Fisher JK, Polak K, Patton E, Wiwczar J, Godes M, Lee DH, Robertson K, Kim S, Kulkarni A, Distefano A, Samuel V, Cline G, Kim YB, Shulman GI, Danial NN, Regulation of hepatic energy metabolism and gluconeogenesis by BAD, *Cell Metab*. 19 (2014) 272–284. doi: 10.1016/j.cmet.2013.12.001. [PubMed: 24506868]
- [83]. Boudina S, Bugger H, Sena S, O'Neill BT, Zaha VG, Ilkun O, Wright JJ, Mazumder PK, Palfreyman E, Tidwell TJ, Theobald H, Khalimonchuk O, Wayment B, Sheng X, Rodnick KJ, Centini R, Chen D, Litwin SE, Weimer BE, Abel ED, Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart, *Circulation*. 119 (2009) 1272–1283. doi:10.1161/CIRCULATIONAHA.108.792101. [PubMed: 19237663]
- [84]. Rexford A, Zorio DAR, Miller BG, Biochemical and biophysical investigations of the interaction between human glucokinase and pro-apoptotic BAD, *PLoS One*. 12 (2017). doi:10.1371/journal.pone.0171587.
- [85]. Zhang H, Nimmer P, Rosenberg SH, Ng SC, Joseph M, Development of a high-throughput fluorescence polarization assay for Bcl-xL, *Anal. Biochem* 307 (2002) 70–75. doi:10.1016/S0003-2697(02)00028-3. [PubMed: 12137781]
- [86]. Yu C, Minemoto Y, Zhang J, Liu J, Tang F, Bui TN, Xiang J, Lin A, JNK Suppresses Apoptosis via Phosphorylation of the Proapoptotic Bcl-2 Family Protein BAD, *Mol. Cell* 13 (2004) 329–340. doi:10.1016/S1097-2765(04)00028-0. [PubMed: 14967141]
- [87]. Grimsby J, Coffey JW, Dvornozniak MT, Magram J, Li G, Matschinsky FM, Shiota C, Kaur S, Magnuson MA, Grippo JF, Characterization of glucokinase regulatory protein-deficient mice, *J. Biol. Chem* 275 (2000) 7826–7831. doi:10.1074/jbc.275.11.7826. [PubMed: 10713097]
- [88]. Bjørkhaug L, Molnes J, Sjøvik O, Njølstad PR, Flatmark T, Allosteric activation of human glucokinase by free polyubiquitin chains and its ubiquitin-dependent cotranslational proteasomal degradation, *J. Biol. Chem* 282 (2007) 22757–22764. doi:10.1074/jbc.M700517200. [PubMed: 17561510]
- [89]. Yan F-F, Lin C-W, a Cartier E, Shyng S-L, Role of ubiquitin-proteasome degradation pathway in biogenesis efficiency of β -cell ATP-sensitive potassium channels., *Am. J. Physiol. Cell Physiol* 289 (2005) C1351–C1359. doi:10.1152/ajpcell.00240.2005. [PubMed: 15987767]
- [90]. Kitiphongspattana K, Mathews CE, Leiter EH, Gaskins HR, Proteasome inhibition alters glucose-stimulated (pro)insulin secretion and turnover in pancreatic β -cells, *J. Biol. Chem* 280 (2005) 15727–15734. doi:10.1074/jbc.M410876200. [PubMed: 15705591]

- [91]. Kawaguchi M, Minami K, Nagashima K, Seino S, Essential role of ubiquitin-proteasome system in normal regulation of insulin secretion, *J. Biol. Chem* 281 (2006) 13015–13020. doi:10.1074/jbc.M601228200. [PubMed: 16543239]
- [92]. López-Avalos MD, Duvivier-Kali VF, Xu G, Bonner-Weir S, Sharma A, Weir GC, Evidence for a role of the ubiquitin-proteasome pathway in pancreatic islets, *Diabetes*. 55 (2006) 1223–1231. doi:10.2337/db05-0450. [PubMed: 16644676]
- [93]. Callis J, The Ubiquitination Machinery of the Ubiquitin System, *Arab. B* 12 (2014) e0174. doi: 10.1199/tab.0174.
- [94]. Komander D, Rape M, The Ubiquitin Code, *Annu. Rev. Biochem* 81 (2012) 203–229. doi: 10.1146/annurev-biochem-060310-170328. [PubMed: 22524316]
- [95]. Hofmeister-Brix A, Kollmann K, Langer S, Schultz J, Lenzen S, Baltrusch S, Identification of the ubiquitin-like domain of midnolin as a new glucokinase interaction partner, *J. Biol. Chem* 288 (2013) 35824–35839. doi:10.1074/jbc.M113.526632. [PubMed: 24187134]
- [96]. Grabbe C, Dikic I, Functional roles of ubiquitin-like domain (ULD) and ubiquitin-binding domain (UBD) containing proteins, *Chem. Rev* (2009). doi:10.1021/cr800413p.
- [97]. Walters KJ, Goh AM, Wang Q, Wagner G, Howley PM, Ubiquitin family proteins and their relationship to the proteasome: A structural perspective, *Biochim. Biophys. Acta - Mol. Cell Res* (2004). doi:10.1016/j.bbamcr.2004.10.005.
- [98]. Nandi D, Tahiliani P, Kumar A, Chandu D, The Ubiquitin-Proteasome System, *J. Biosci* 31 (2006) 137–155. doi:10.1007/2789.
- [99]. Ravid T, Hochstrasser M, Diversity of degradation signals in the ubiquitin-proteasome system, *Nat. Rev. Mol. Cell Biol* 9 (2008) 679–689. doi:10.1038/nrm2468. [PubMed: 18698327]
- [100]. Kravtsova-Ivantsiv Y, Ciechanover A, Non-canonical ubiquitin-based signals for proteasomal degradation, *J. Cell Sci* 125 (2012) 539–548. doi:10.1242/jcs.093567. [PubMed: 22389393]
- [101]. Muratani M, Tansey WP, How the ubiquitin-proteasome system controls transcription, *Nat. Rev. Mol. Cell Biol* 4 (2003) 192–201. doi:10.1038/nrm1049. [PubMed: 12612638]
- [102]. Hofmeister-Brix A, Lenzen S, Baltrusch S, The ubiquitin-proteasome system regulates the stability and activity of the glucose sensor glucokinase in pancreatic β -cells., *Biochem. J* 456 (2013) 173–84. doi:10.1042/BJ20130262. [PubMed: 24028089]
- [103]. Flotho A, Melchior F, Sumoylation: a regulatory protein modification in health and disease., *Annu. Rev. Biochem* 82 (2013) 357–85. doi:10.1146/annurev-biochem-061909-093311. [PubMed: 23746258]
- [104]. Bologna S, Ferrari S, It takes two to tango: Ubiquitin and SUMO in the DNA damage response, *Front. Genet* 4 (2013). doi:10.3389/fgene.2013.00106.
- [105]. Jackson SP, Durocher D, Regulation of DNA Damage Responses by Ubiquitin and SUMO, *Mol. Cell* 49 (2013) 795–807. doi:10.1016/j.molcel.2013.01.017. [PubMed: 23416108]
- [106]. Johnson ES, Protein Modification by SUMO, *Annu. Rev. Biochem* 73 (2004) 355–382. doi: 10.1146/annurev.biochem.73.011303.074118. [PubMed: 15189146]
- [107]. Gareau JR, Lima CD, The SUMO pathway: Emerging mechanisms that shape specificity, conjugation and recognition, *Nat. Rev. Mol. Cell Biol* 11 (2010) 861–871. doi:10.1038/nrm3011. [PubMed: 21102611]
- [108]. Geiss-friedlander R, Melchior F, Concepts in sumoylation: a decade on, *Nat. Rev. Mol. Cell Biol* 8 (2007) 947–956. doi:10.1038/nrm2293. [PubMed: 18000527]
- [109]. Miura K, Jin JB, Hasegawa PM, Zhu J-K, Shimamoto K, Sumoylation, a post-translational regulatory process in plants This review comes from a themed issue on Cell Signalling and Gene Regulation Edited, *Curr. Opin. Plant Biol* 10 (2007) 495–502. doi:10.1016/j.pbi.2007.07.002. [PubMed: 17720613]
- [110]. Wilkinson K. a., Hensley JM, Mechanisms, regulation and consequences of protein SUMOylation., *Biochem. J* 428 (2012) 133–145. doi:10.1042/BJ20100158.Mechanisms.
- [111]. Kanakousaki K, Gibson MC, A differential requirement for SUMOylation in proliferating and non-proliferating cells during *Drosophila* development, *Development*. 139 (2012) 2751–2762. doi:10.1242/dev.082974. [PubMed: 22745316]

- [112]. Bawa-Khalfe T, Yeh ETH, SUMO Losing Balance: SUMO Proteases Disrupt SUMO Homeostasis to Facilitate Cancer Development and Progression, *Genes Cancer*. 1 (2010) 748–752. doi:10.1177/1947601910382555. [PubMed: 21152235]
- [113]. Wang J, Feng XH, Schwartz RJ, SUMO-1 modification activated GATA4-dependent cardiogenic gene activity, *J. Biol. Chem* 279 (2004) 49091–49098. doi:10.1074/jbc.M407494200. [PubMed: 15337742]
- [114]. Aukrust I, Bjorkhaug L, Negahdar M, Molnes J, Johansson BB, Müller Y, Haas W, Gygi SP, Sovik O, Flatmark T, Kulkarni RN, Njolstad PR, SUMOylation of pancreatic glucokinase regulates its cellular stability and activity, *J. Biol. Chem* 288 (2013) 5951–5962. doi:10.1074/jbc.M112.393769. [PubMed: 23297408]
- [115]. Galán M, Vincent O, Roncero I, Azriel S, Boix-Pallares P, Delgado-Alvarez E, Díaz-Cadorniga F, Blázquez E, Navas M-A, Effects of novel maturity-onset diabetes of the young (MODY)-associated mutations on glucokinase activity and protein stability., *Biochem. J* 393 (2006) 389–96. doi:10.1042/BJ20051137. [PubMed: 16173921]
- [116]. V Burke C, Buettger CW, a Davis E, McClane SJ, Matschinsky FM, Raper SE, Cell-biological assessment of human glucokinase mutants causing maturity-onset diabetes of the young type 2 (MODY-2) or glucokinase-linked hyperinsulinaemia (GK-HI)., *Biochem. J* 342 (1999) 345–352. doi:10.1042/0264-6021:3420345. [PubMed: 10455021]
- [117]. Grimsby J, Sarabu R, Corbett WL, Haynes N-E, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Tengi J, Magnuson MA, Chu CA, Dvornozniak MT, Matschinsky FM, Grippo JF, Allosteric activators of glucokinase: potential role in diabetes therapy., *Science*. 301 (2003) 370–3. doi:10.1126/science.1084073. [PubMed: 12869762]
- [118]. Caron D, Maaroufi H, Michaud S, Tanguay RM, Faure RL, Annexin A1 is regulated by domains cross-talk through post-translational phosphorylation and SUMOylation, *Cell. Signal* 25 (2013) 1962–1969. doi:10.1016/j.cellsig.2013.05.028. [PubMed: 23727357]
- [119]. Jongjitwimol J, Baldock RA, Morley SJ, Watts FZ, Sumoylation of eIF4A2 affects stress granule formation, *J. Cell Sci* 129 (2016) 2407–2415. doi:10.1242/jcs.184614. [PubMed: 27160682]
- [120]. Ulrich HD, SUMO modification: Wrestling with protein conformation, *Curr. Biol* 15 (2005). doi:10.1016/j.cub.2005.03.021.
- [121]. a Campbell L, Faivre EJ, Show MD, Ingraham JG, Flinders J, Gross JD, a Ingraham H, Decreased recognition of SUMO-sensitive target genes following modification of SF-1 (NR5A1)., *Mol. Cell. Biol* 28 (2008) 7476–86. doi:10.1128/MCB.00103-08. [PubMed: 18838537]
- [122]. Dai XQ, Plummer G, Casimir M, Kang Y, Hajmrle C, Gaisano HY, Manning Fox JE, MacDonald PE, SUMOylation regulates insulin exocytosis downstream of secretory granule docking in rodents and humans, *Diabetes*. 60 (2011) 838–847. doi:10.2337/db10-0440. [PubMed: 21266332]
- [123]. Toyoda Y, Yoshie S, Fujita T, Ito Y, Nonogaki T, Miwa I, Glucokinase is located in secretory granules of pancreatic D-cells, *FEBS Lett.* 415 (1997) 281–284. doi:10.1016/S0014-5793(97)01139-3. [PubMed: 9357983]
- [124]. Toyoda Y, Shironoguchi H, Miwa I, Yoshie S, Glucokinase is concentrated in insulin-secretory granules of pancreatic B-cells, *Histochem. Cell Biol* 112 (1999) 35–40. doi:10.1007/s004180050389. [PubMed: 10461810]
- [125]. Stubbs M, Aiston S, Agius L, Subcellular localization, mobility, and kinetic activity of glucokinase in glucose-responsive insulin-secreting cells, *Diabetes*. 49 (2000) 2048–2055. doi: 10.2337/diabetes.49.12.2048. [PubMed: 11118006]
- [126]. Markwardt ML, Nkobena A, Ding S-Y, a Rizzo M, Association with nitric oxide synthase on insulin secretory granules regulates glucokinase protein levels., *Mol. Endocrinol* 26 (2012) 1617–29. doi:10.1210/me.2012-1183. [PubMed: 22771492]
- [127]. Rizzo MA, Magnuson MA, Drain PF, Piston DW, A functional link between glucokinase binding to insulin granules and conformational alterations in response to glucose and insulin, *J. Biol. Chem* 277 (2002) 34168–34175. doi:10.1074/jbc.M112478200. [PubMed: 12101177]

- [128]. Rizzo MA, Piston DW, Regulation of β cell glucokinase by S-nitrosylation and association with nitric oxide synthase, *J. Cell Biol* 161 (2003) 243–248. doi:10.1083/jcb.200301063. [PubMed: 12707306]
- [129]. Arden C, Harboottle A, Baltrusch S, Tiedge M, Agius L, Glucokinase is an integral component of the insulin granules in glucose-responsive insulin secretory cells and does not translocate during glucose stimulation, *Diabetes*. 53 (2004) 2346–2352. doi:10.2337/diabetes.53.9.2346. [PubMed: 15331544]
- [130]. Ding S-Y, Tribble ND, a Kraft C, Markwardt M, Gloyn AL, a Rizzo M, Naturally occurring glucokinase mutations are associated with defects in posttranslational S-nitrosylation., *Mol. Endocrinol* 24 (2010) 171–7. doi:10.1210/me.2009-0138. [PubMed: 19934346]
- [131]. Tiedge M, Richter T, Lenzen S, Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase., *Arch. Biochem. Biophys* (2000). doi: 10.1006/abbi.1999.1666.
- [132]. Markwardt ML, Seckinger KM, Rizzo MA, Regulation of glucokinase by intracellular calcium levels in pancreatic β cells, *J. Biol. Chem* (2016). doi:10.1074/jbc.M115.692160.
- [133]. Ding SY, Nkobena A, Kraft CA, Markwardt ML, Rizzo MA, Glucagon-like peptide 1 stimulates post-translational activation of glucokinase in pancreatic β cells, *J. Biol. Chem* (2011). doi: 10.1074/jbc.M110.192799.
- [134]. Weir GC, Mojsov S, Hendrick GK, Habener JF, Glucagonlike peptide I (7–37) actions on endocrine pancreas, *Diabetes*. (1989). doi:10.2337/diab.38.3.338.
- [135]. Seckinger KM, Rao VP, Snell NE, Mancini AE, Markwardt ML, Rizzo MA, Nitric Oxide Activates β -Cell Glucokinase by Promoting Formation of the “glucose-Activated” State, *Biochemistry*. 57 (2018) 5136–5144. doi:10.1021/acs.biochem.8b00333. [PubMed: 30053375]
- [136]. Murata T, Katagiri H, Ishihara H, Shibasaki Y, Asano T, Toyoda Y, Pekiner B, Pekiner C, Miwa I, Oka Y, Co-localization of glucokinase with actin filaments, *FEBS Lett*. 406 (1997) 109–113. doi:10.1016/S0014-5793(97)00253-6. [PubMed: 9109397]
- [137]. Magnuson MA, Tissue-specific regulation of glucokinase gene expression, *J. Cell. Biochem* 48 (1992) 115–121. doi:10.1002/jcb.240480202. [PubMed: 1618925]
- [138]. Balasubramanian R, Karve A, Kandasamy M, Meagher RB, Moore B. d., A Role for F-Actin in Hexokinase-Mediated Glucose Signaling, *PLANT Physiol*. 145 (2007) 1423–1434. doi: 10.1104/pp.107.108704. [PubMed: 17965176]
- [139]. Balasubramanian R, Karve A, Moore BD, Actin-based cellular framework for glucose signaling by Arabidopsis hexokinase1, *Plant Signal. Behav* 3 (2008) 322–324. doi:10.4161/psb.3.5.5319. [PubMed: 19841659]
- [140]. Wagner O, Zinke J, Dancker P, Grill W, Bereiter-Hahn J, Viscoelastic Properties of f-actin, Microtubules, f-actin/ α -actinin, and f-actin/Hexokinase Determined in Microliter Volumes with a Novel Nondestructive Method, *Biophys. J* 76 (1999) 2784–2796. doi:10.1016/S0006-3495(99)77432-1. [PubMed: 10233094]
- [141]. Fields S, Song O, A novel genetic system to detect protein-protein interactions. [Yeast two hybrid], *Nature*. 340 (1989) 245–246. doi:10.1038/340245a0. [PubMed: 2547163]
- [142]. Fields S, Sternglanz R, The two-hybrid system: an assay for protein-protein interactions, *Trends Genet*. 10 (1994) 286–292. doi:10.1016/0168-9525(90)90012-U. [PubMed: 7940758]
- [143]. Stynen B, Tournu H, Tavernier J, Van Dijck P, Diversity in Genetic In Vivo Methods for Protein-Protein Interaction Studies: from the Yeast Two-Hybrid System to the Mammalian Split-Luciferase System, *Microbiol. Mol. Biol. Rev* 76 (2012) 331–382. doi:10.1128/MMBR.05021-11. [PubMed: 22688816]
- [144]. Muñoz-Alonso MJ, Guillemain G, Kassis N, Girard J, Burnol AF, Leturque A, A novel cytosolic dual specificity phosphatase, interacting with glucokinase, increases glucose phosphorylation rate, *J. Biol. Chem* 275 (2000) 32406–32412. doi:10.1074/jbc.M000841200. [PubMed: 10913113]
- [145]. Renatus M, Farady CJ, Phosphorylation meets proteolysis, *Structure*. 20 (2012) 570–571. doi: 10.1016/j.str.2012.03.006. [PubMed: 22483104]
- [146]. Shiraishi A, Yamada Y, Tsuura Y, Fijimoto S, Tsukiyama K, Mukai E, Toyoda Y, Miwa I, Seino Y, A novel glucokinase regulator in pancreatic β cells. Precursor of propionyl-CoA carboxylase β

subunit interacts with glucokinase and augments its activity, *J. Biol. Chem* 276 (2001) 2325–2328. doi:10.1074/jbc.C000530200. [PubMed: 11085976]

- [147]. V Bhagavan N, Ha C-E, *Essentials of Medical Biochemistry with Clinical Cases*, *Essentials Med. Biochem* (2013) 151–168. doi:10.1016/B978-0-12-095461-2.00014-X.
- [148]. Baltrusch S, Tiedge M, Glucokinase regulatory network in pancreatic β -cells and liver, *Diabetes*. (2006). doi:10.2337/db06-S008.
- [149]. Schmitt DL, An S, Spatial Organization of Metabolic Enzyme Complexes in Cells, *Biochemistry*. (2017). doi:10.1021/acs.biochem.7b00249.
- [150]. Kohnhorst CL, Kyoung M, Jeon M, Schmitt DL, Kennedy EL, Ramirez J, Bracey SM, Luu BT, Russell SJ, An S, Identification of a multienzyme complex for glucose metabolism in living cells, *J. Biol. Chem* (2017). doi:10.1074/jbc.M117.783050.
- [151]. Baltrusch S, Langer S, Massa L, Tiedge M, Lenzen S, Improved metabolic stimulus for glucose-induced insulin secretion through GK and PFK-2/FBPase-2 coexpression in insulin-producing RINm5F cells, *Endocrinology*. 147 (2006) 5768–5776. doi:10.1210/en.2006-0694. [PubMed: 16980436]
- [152]. Arden C, Hampson LJ, Huang GC, Shaw JAM, Aldibbiat A, Holliman G, Manas D, Khan S, Lange AJ, Agius L, A role for PFK-2/FBPase-2, as distinct from fructose 2,6-bisphosphate, in regulation of insulin secretion in pancreatic beta-cells., *Biochem. J* 411 (2008) 41–51. doi: 10.1042/BJ20070962. [PubMed: 18039179]

Highlights

- Cooperativity allows glucokinase to serve as the body's glucose sensor
- Intrinsic conformational dynamics regulate glucokinase at the molecular level
- Protein-protein interactions and posttranslational modifications regulate glucokinase in the cell
- Establishing links between regulatory mechanisms is essential for treating disease
- This review integrates results from molecular and cellular regulation studies

Figure 1.

Regulation of GCK activity is essential for glucose homeostasis. Under physiological conditions (black), GCK displays a sigmoidal kinetic response to glucose that is characterized by a Hill coefficient = 1.7. The midpoint of GCK's glucose responsiveness ($K_{0.5} = 7$ mM) correlates with circulating glucose concentrations and the threshold for glucose-stimulated insulin secretion, providing maximum sensitivity in this region. Activated PHHI-causing GCK variants (red) display lower cooperativity and require lower glucose for insulin secretion. MODY-2 associated, reduced activity GCK variants require higher glucose levels to induce insulin release.

Figure 2.

GCK undergoes large-scale conformational changes. In the absence of glucose, GCK exists as an ensemble of conformations. X-ray crystallography has been used to elucidate one of these conformations (left), which has been coined the super-open conformation due to the sizable opening angle between the small domain (gold) and large domain (gray). In this conformation, the intrinsically disordered active site loop displays no electron density. Glucose (green) binding promotes folding of the small domain towards the large domain and the interdomain angle is reduced to 65° (middle). In this intermediary open structure, the active site mobile loop is still invisible and the C-terminal $\alpha 13$ helix (red) shifts from a solvent exposed position to a more structurally compact conformation between both domains. The closed conformation (right) is the most compact conformation and is characterized by a small opening angle of 40°. In this conformation, the $\alpha 13$ helix is buried and the mobile loop forms an antiparallel β -hairpin (cyan) and folds over the bound glucose molecule.

Figure 3.

GCRP regulates GCR by localizing it in the hepatocyte nucleus. GCRP binds to the super-open conformation, thereby locking GCR in an inactive state and reducing glycolysis. This interaction is inhibited by fructose 1-phosphate (F1P) and promoted by fructose 6-phosphate (F6P). Glucose levels strongly influence nuclear-to-cytosolic translocation of the GCR-GCRP complex. GCRP can be acetylated near its N-terminus by p300, which enhances its inhibitory and nuclear localization potential. Conversely, Sirtuin 2 deacetylates GCRP, subsequently promoting glucose uptake via glucose transporter 2 and favoring cytoplasmic GK-GCRP localization. SUMO-1 conjugation to GCR stabilizes and activates the enzyme and impairs GCRP-mediated nuclear sequestration of GCR. Finally, mitochondrial association of GCRP has been observed, but the influence this has on GCR physiology has yet to be determined.

Figure 4.

GCK is activated and stabilized by interaction with the bifunctional enzyme PFK-2/FBPase-2. PFK-2/FBPase-2 is tightly regulated by phosphorylation of S32, which is promoted by glucagon, AMP and several kinases. Phosphorylated PFK-2/FBPase-2 contributes to high glycolytic activity and low gluconeogenic activity. Insulin modulates liver hepatocyte metabolism by activating protein phosphatases that dephosphorylate PFK-2/FBPase-2, thereby increasing the ratio of glycolysis to gluconeogenesis. A similar regulatory strategy exists in pancreatic β -cells but is instead modulated by glucose. It should be noted however, that gluconeogenesis is of minimal importance in β -cells as they store virtually no glycogen. Data suggest that the phosphatase domain of PFK-2/FBPase-2 weakly interacts with the closed conformation of GCK, a process which correlates with intracellular glucose levels. This interaction increases GCK's V_{max} and impedes nuclear localization of GCK by GKRP. In addition, a stabilizing role for the interaction has been described by which PFK-2/FBPase-2 protects GCK from oxidative stress (reactive oxygen species, ROS). PFK-2/FBPase-2 interconverts fructose 6-phosphate and fructose-2,6-bisphosphate, the latter of which promotes phosphofructokinase-1 (PFK-1) activity. PFK-1 has been observed to interact with BAD, but the impact this has on mitochondrial GCK association remains to be seen.

Figure 5.

BAD integrates glycolysis and apoptosis by anchoring GCR to the mitochondrial membrane. The mitochondrial complex consists of BAD, GCR, PP1, Wave-1 and PKA. Phosphorylation of BAD promotes activation of GCR and enhances glycolysis and GSIS. Glucose and insulin promote BAD phosphorylation and BAD-GCR interaction via feedback mechanisms. Additionally, phosphorylation of BAD serves to reduce its interaction with apoptotic proteins and promote β -cell survival. β -cell specific insulin receptor knockout impedes the GCR-BAD interaction and results in a reduction of GCR activity. The BAD-GCR interaction is disrupted in patients with type 2 diabetes, indicating a need for drugs that promote the interaction. Finally, BAD has been shown to interact with PFK-1. Another interaction partner of GCR, PFK-2/FBPase-2, produces the primary regulatory molecule of PFK-1, suggesting a link exists between these important metabolic interactions.

Figure 6.

GCR activity is influenced by the UPS. The proteins of the UPS act as molecular guardians when newly synthesized GCR is misfolded or when GCR aggregates form over time. Ubiquitin (Ub) is covalently linked to the improperly folded or aggregated GCR polypeptides to mark them for degradation, which is carried out by other UPS proteins. MG132 inhibits the UPS and results in an increase in cytoplasmic GCR aggregation. E1 proteins, which are part of the UPS, also link Ub molecules together. Ub₅ chains interact with GCR to significantly increase its activity. Presumably, the increase in GCR activity would result in glycolytic ATP production, which in turn, would allow for further Ub chain formation, thereby enhancing GCR activity to an even greater extent.

Figure 7.

SUMOylation augments GSK's activity and stability. SUMOylation of GSK occurs primarily at lysine residues that are in close spatial proximity to the six-residue NES. SUMOylation serves to mask the NES and promote nuclear translocation of GSK, in contrast to GKR-mediated nuclear sequestration of GSK, which impedes GSK activity. It is not clear how these two nuclear localization interactions are connected, but metabolic cues such as glucose or insulin likely determine the fate of SUMOylation, as seen for GKR-mediated GSK regulation.

Figure 8. Localization of GCK to insulin granules results in reduced activity and is partially mediated by interaction with NOS dimers. In addition to interaction with GCK, NOS can S-nitrosylate C371 of GCK, which serves two primary functions. It improves GCK's activity and promotes release of GCK from granules. In addition, a model in which insulin post-translationally modulates GCK granular localization and activity has been postulated. Inhibitors of insulin secretion impede GCK granular localization and the synthesis, packaging and secretion of insulin likely significantly influences granular GCK localization.

Table 1.

Functional characteristics of known GCK binding partners.

Interaction Partner	Cell Lines	Cellular Location	GCK conformation	Functionality	Primary Methods of Observation	References
Glucokinase regulatory protein (GKRP)	Human and rat hepatocytes; HeLa; HepG2; mouse and rat models	Nucleus; cytoplasm; mitochondria	Super-open	Sequesters GCK in the nucleus and reduces its activity in the fasting state; modulated by sugar phosphates; mitochondrial role unknown	Fluorescence microscopy; X-ray crystallography; western blotting	[27,42–50,52–61]
Phosphofructokinase-2/fructose biphosphatase-2 (PFK-2/FBPase-2)	Human, rat, and mouse islets; rat hepatocytes; MIN-6; RIN-m5f; INS-1; COS; mouse and rat models	Cytoplasm	Closed	Increases GCK activity and stability in a glucose dependent manner; regulated by insulin and phosphorylation	Fluorescence Microscopy; two-hybrid screening; western blotting	[62–66,68–74,151,152]
Bel-2-associated death promoter (BAD) and pentameric complex components	Human and mouse hepatocytes and islets; mouse and rat models	Mitochondrial membrane	Closed	Modestly increases GCK activity; increases GSIS; regulated by various metabolites and phosphorylation; seemingly integrates glycolysis and apoptosis; additional factors are necessary for interaction	Western blotting	[75–86]
Ubiquitin and free pentaubiquitin chains	INS-1; MIN-6; HepG2; HeLa; COS; RINm5F; MH-777A; mouse islets and hepatocytes	Cytoplasm	?	Ubiquitin conjugation promotes GCK degradation; free pentaubiquitin chains moderately increase GCK activity;	Two-hybrid screening; western blotting; <i>in vitro</i> transcription/translation	[88,95,102]
Small ubiquitin-like modifier protein 1 (SUMO-1)	MIN-6; INS-1; human and mouse islets	Cytoplasm; nucleus	?	Increases GCK activity and stability; modulates nuclear localization of GCK;	Fluorescence microscopy; western blotting	[51,114]
Nitric oxide synthase (NOS)	MIN-6; INS-1; β TC3; β TC-tet; Rat pancreatic islets	Insulin granules (outer membrane)	?	Reversibly inactivates GCK; promotes granular localization of GCK via direct interaction and S-nitrosylation; modulated by insulin	Fluorescence microscopy; fractionation; western blotting	[68,123–130]
Actin (and other motor proteins)	COS; rat hepatocytes	Cytoplasm	?	?	Fluorescence microscopy	[68,136]
Dual specificity protein phosphatase 12	mhAT3F	?	?	Dose-dependency increases GCK activity; dephosphorylates GCK	Two-hybrid screening; western blotting	[144]
Propionyl-CoA Carboxylase β subunit (p β PCase)	HIT-T15	Cytosol; mitochondria	?	Dose-dependency increases GCK activity	Two-hybrid screening; fractionation; western blotting	[146]