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PROSPECT

The Role of Protein Translocation in the Regulation of Glycogen Metabolism

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Abstract Early biochemical analyses of metabolic pathways assumed that the free diffusion of substrates and enzymes in an evenly mixed cellular space provided the interactions that enabled reactions to proceed. Metabolic complexes have since been shown to assemble and disassemble in response to changes in cellular conditions, and in turn, to channel metabolic intermediates within discreet cellular compartments, allowing for the efficient use or storage of energy. A fundamental component to the formation of metabolic complexes and the channeling of metabolites is the translocation of enzymes in response to specific extra- and intracellular signals. These generalities play an important role in the metabolism of glucose to glycogen within skeletal muscle and liver. In this review, the similarities and differences in skeletal muscle and liver glucose metabolism with regards to glucose transport and intracellular processing will be addressed during the fasted to fed transition. More specifically, the importance of isoform expression and protein translocation in the tissue specific control of glucose homeostasis will be covered. J. Cell. Biochem. 104: 435–443, 2008.

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Some of the earliest theories regarding the internal environment of the cell described an elemental mixed bag of molecules, where diffusion and random interactions drove cellular events, including the metabolism of glucose. Paul Srere was one of the first to argue that free intracellular space was most likely extremely restricted based on the number and size of proteins known to be expressed within a cell [Srere, 1967]. The idea of a crowded intracellular environment led to the hypothesis that enzymes in a common pathway might act as multiprotein complexes, interacting with one another in an organized fashion and channeling metabolites in an ordered manner, allowing for the observation that measured rates of reactions often exceed the upper limits of diffusion [Gaertner and Cole, 1977]. Evidence of metabolic enzyme complexes has since accumulated [Robinson and Srere, 1985] and furthermore, led to the idea that metabolic events occur within distinct compartments within the cell [Ovadi and Saks, 2004]. For example, localized production of ATP due to compartmentalized glycolysis outside of mitochondria has been described in both cardiac and skeletal muscle [Weiss and Lamp, 1987; Han et al., 1992]. So-called microdomains, as described in these studies, provide a model for understanding how the cell works by reducing global cellular events into smaller functional units that operate throughout the cell. In addition to glycolysis, insulin-stimulated glucose uptake, metabolism and storage are tightly controlled through changes in the subcellular localization of signaling proteins, intermediate metabolites, and rate-limiting enzymes. Thus, protein translocation provides a powerful control mechanism over glucose metabolism through the regulated, transient formation of multimeric complexes and the shuttling of metabolites within distinct locations in the cell.

Whole body glucose homeostasis is achieved through coordination of events in distinct tissue types via hormonal, neuronal, and nutritional signals. In the fed state, elevated blood glucose

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stimulates the secretion of insulin from pancreatic β-cells, which in turn promotes the disposal of glucose as glycogen primarily in skeletal muscle myocytes and to a lesser degree in hepatocytes. In the fasted state, the insulin to glucagon ratio declines, promoting increased expression of gluconeogenic enzymes in liver and augmented hepatic glucose output to maintain blood glucose levels, while simultaneously reducing skeletal muscle blood glucose extraction. As two of the primary tissues regulating plasma glucose levels, skeletal muscle and liver exert powerful control over global glucose homeostasis. In this review, the regulation of skeletal muscle and hepatic glucose uptake, metabolism and storage will be examined during the fasted to fed transition period. In particular, the critical role of protein translocation in response to changes in extracellular insulin and/or glucose will be addressed, while comparing the similarities and differences in mechanisms governing protein translocation induced by covalent modifications versus changes in intracellular metabolites.

GLYCOGEN METABOLISM IN MUSCLE

Insulin Signaling

Glucose storage by skeletal muscle requires both an increase in glucose transport and glycogen synthetic rates. These events are coordinated in large part through stimulation of the insulin receptor and the subsequent assembly of metabolic complexes. Although many of the individual signaling events in insulin action have been extensively reviewed [Taniguchi et al., 2006], the overall regulation of muscle glucose metabolism by "protein translocation cascades" induced by both insulin and subsequent elevation of intracellular glucose metabolites has received less attention. Autophosphorylation of the insulin receptor following ligand binding results in the association of insulin receptor substrate (IRS) proteins though direct binding of their PTB domain to the NPEpY motif on the insulin receptor. This interaction is further stabilized by the PH domain of IRS proteins binding to phospholipids on the plasma membrane. The colocalization of the activated insulin receptor with IRS proteins promotes tyrosine phosphorylation on multiple sites. The resulting phospho-tyrosine residues on IRS proteins generate docking sites for a wide variety of enzymatic proteins and adaptor molecules that contain either SH2 and/or PTB domains [White, 1998], which then propagate and diversify the initial ligand binding signal (for a comprehensive review, see [Taniguchi et al., 2006]).

A central pathway for the regulation of glucose uptake and storage by insulin is the sequential translocation and binding of phosphatidylinositol 3-kinase (PI3K) to IRS proteins, resulting in enzymatic activation and generation of phosphotidylinositol-tris-phosphate (PIP_3) on the inner surface of the plasma membrane. The protein kinase Akt is localized in the cytosol in the basal state and translocates to the plasma membrane upon insulin stimulation, an event mediated by the binding of the PH domain of Akt to the PIP_3 generated by PI3K activation. Akt is then phosphorylated on two critical residues, resulting in enzymatic activation and release from the plasma membrane. Akt has been implicated in both the activation of glycogen synthase (via inactivation of glycogen synthase kinase-3) as well as the translocation of GLUT4 glucose transporter containing vesicles to the cell surface that mediates enhanced glucose uptake by myocytes. Akt exists as three isoforms; however, several lines of evidence have specifically implicated Akt2 as being an essential component of the insulin signaling cascade leading to glucose transport [Calera et al., 1998; Hill et al., 1999; Cho et al., 2001; Watson and Pessin, 2006].

The control of GLUT4 localization and translocation by hormones and exercise is a highly regulated process that has been extensively reviewed [Richter et al., 2004; Huang and Czech, 2007]. Despite several unanswered questions regarding the interface between insulin signaling, muscle contraction based signaling and the GLUT4 storage compartment, the importance of transporter translocation in skeletal muscle for the maintenance of glucose homeostasis is widely accepted. Under hyperglycemic, hyperinsulinemic clamp conditions, nuclear magnetic resonance spectroscopy identified the defect in skeletal muscle glucose disposal in patients with type 2 diabetes to be at the level of glucose transport [Rothman et al., 1992]. This result and later studies led to the hypothesis that glucose transport and phosphorylation to glucose-6-phosphate (G6P) by hexokinase is the rate-limiting step in skeletal muscle glycogen synthesis [Shulman et al., 1995], and thus a principle regulator of global

glucose homeostasis since skeletal muscle is the principal site of glucose storage in vivo.

Thus, insulin-stimulated glucose uptake into skeletal muscle results from the orchestration of sequential activation of tyrosine, lipid and serine/threonine kinases. The resulting increase in phosphorylation of substrates generates binding sites recognized by a variety of protein motifs that mediate the movement of multiple enzymes and signaling molecules including IRS proteins, PI3K, Akt, and subsequently GLUT4 from intracellular retention sites to the cell surface. As discussed below, the subsequent increase in glucose uptake and its metabolism in turn results in the translocation of metabolic enzymes including hexokinase II, glycogen synthase and phosphorylase. Thus, activation of glycogen synthesis by insulin in muscle involves sequential changes in the subcellular localization from the insulin receptor to the glycogen particle.

Hexokinase Localization and Regulation

Glucose entering cells is rapidly phosphorylated by a family of enzymes termed hexokinases to form G6P. The resulting G6P molecule can then either enter the glycolytic pathway to generate ATP or be metabolized for storage as glycogen. Hexokinases exist as four known isoforms (HKI-IV), two of which (I and II) are expressed in skeletal muscle. HKI possesses an N-terminal regulatory domain that contains both a G6P and latent glucose binding domain, as well as a catalytic C-terminal domain that binds glucose, ATP and G6P. In contrast to HKI, both the N-terminal and C-terminal domains of HKII possess catalytic activity [Pastorino and Hoek, 2003]. Both HKI and II are potently inhibited by their product G6P, in part due to interdomain interactions resulting from the latent G6P and glucose binding domains in the C-terminal and N-terminal regions, respectively. Inhibition of HK isoforms by G6P prevents excessive energy uptake and storage that would be damaging to the muscle cell. HKI is constitutively targeted to mitochondria by an N-terminal sequence that interacts with mitochondrial porins, also known as voltage-dependent anion channels (VDACs) [Fiek et al., 1982; Linden et al., 1982]. VDACs allow ATP produced by the mitochondria to be released into the cytosol and it has been postulated that association of HK with VDAC provides the enzyme preferential access to ATP generated by mitochondria [Wilson, 2003]. By pairing the initial step of glycolysis with the end point in mitochondrial ATP production, the cell is able to match glycolytic flux with oxidative phosphorylation and ensure proper energy provision, while also reducing accumulation of metabolic intermediates such as lactate. Interestingly, inhibition of HKI by G6P is attenuated by low Pi [Ellison et al., 1974, 1975; Tsai and Wilson, 1995], where low Pi and high G6P reflects periods of energy flux/use by the working muscle. HKI, therefore, functions primarily in a catabolic capacity by generating G6P for utilization by the glycolytic pathway. In contrast, basal HKII activity is primarily localized in the cytosolic fraction, but translocates to mitochondria in response to insulin [Chen-Zion et al., 1992; Vogt et al., 1998]. The translocation of HKII provides the necessary delay in access to mitochondrial derived ATP such that HKII appears to function more as an anabolic enzyme by directing glucose toward glycogen storage. The definitive signal responsible for HKII translocation to the mitochondria is not known, but changes in cellular glucose and G6P levels resulting in HK conformational changes most likely play a role. In support of this idea, crystallization studies of HKI bound by glucose and G6P, as well as modeling studies of HKII, suggest that both isoforms dimerize and bind mitochondria in pairs, forming a tetramer with VDAC [Wilson, 1995; Mulichak et al., 1998]. Such a conformation is dependent upon extensive N-terminal and C-terminal interactions resulting from changes in metabolite availability.

Glycogen Synthase Distribution and Activation

Following uptake and phosphorylation, glucose that is stored as glycogen is first metabolized to UDP-glucose. The conversion of UDP-glucose to glycogen is catalyzed by the enzyme glycogen synthase, an 84 kDa protein that is activated covalently via dephosphorylation and allosterically by increased G6P levels. Nine phosphorylation sites have been identified on glycogen synthase and patterns of hierarchical phosphorylation motifs, which recruit additional kinases resulting in synthase inactivation, have been described (for a thorough review, see [Roach, 1991]). Mutational analyses have shown that phosphorylation at sites 2, 2a, 3a, and 3b are most important in the regulation of glycogen synthase activity [Skurat et al.,

1994], however, inactivation due to phosphorylation can be overcome via binding of G6P, which also makes synthase a better substrate for phosphatase activity [Villar-Palasi, 1991].

In a resting muscle, the majority of glycogen synthase is associated with glycogen, and glycogen depletion in response to exercise results in the translocation of glycogen synthase from the glycogen-enriched fraction to a cytoskeletal fraction [Nielsen et al., 2001]. The redistribution of glycogen synthase to this cytoskeletal fraction is important since glycogenin, the enzyme responsible for the initiation of glycogen chain formation, has been detected in the same cytoskeletal fraction [Nielsen et al., 2001]. This colocalization of glycogen synthase and glycogenin enables the efficient initiation of glycogen synthesis following conclusion of exercise and provision of extracellular glucose. Furthermore, when glycogen levels are low, basal and insulin-stimulated glycogen synthase activity have been shown to be greater than when glycogen levels are high, demonstrating that the localization of glycogen synthase also may play an important role in the regulation of synthase activity [Nielsen et al., 2001].

Glycogen synthase translocates in response to a variety of stimuli, including changes in intracellular glucose and treatment with insulin [Ferrer et al., 1997; Ou et al., 2005]. It has been recently shown that phosphorylation may also regulate the translocation of glycogen synthase in skeletal muscle [Prats et al., 2005]. Using a combination of immunofluorescence and transmission electron microscopy imaging of intact rabbit skeletal muscle fibers after contraction, a redistribution of glycogen synthase was observed that occurred at a newly formed and previously undocumented spherical structure following glycogen depletion [Prats et al., 2005]. Further study is necessary to establish the identity of these structures, but the presence of β- and α-actinin within the structures suggests that they may be the result of actin cytoskeletal remodeling. Given previous observations that glycogenin is associated with cytoskeletal elements following glycogen depletion [Nielsen et al., 2001], the observations made here suggest that the newly formed spherical structures may be important for the initiation of glycogen re-synthesis. Glycogen synthase detected at the spherical structures was dephosphorylated specifically at sites 1b and 2 + 2a, indicating that synthase associated with the novel structures was highly activated, and furthermore, that dephosphorylation at these sites may regulate glycogen synthase translocation [Prats et al., 2005]. Interestingly, the enzyme responsible for glycogen breakdown, glycogen phosphorylase, colocalized with glycogen synthase at the same spherical structures following glycogen depletion, and furthermore, the activity of phosphorylase at these structures was decreased. Together, the above observations suggest a mechanism for the control of glycogen re-synthesis following contraction. Translocation in response to glycogen depletion puts glycogen synthase and phosphorylase in association with glycogenin and other cellular structures that may regulate synthase and phosphorylase activity, increasing and decreasing them, respectively, allowing net glycogen accumulation and replacement.

GLYCOGEN METABOLISM IN LIVER

In contrast to skeletal muscle, glucose is both taken up and released into the bloodstream by the liver. During fasting conditions, the liver mobilizes glycogen stores and synthesizes glucose from three carbon precursors to ensure that plasma glucose levels remain near the physiological set point of 5 mM. During the fasted to fed transition, the liver must rapidly switch from glucose production and release to glucose uptake and utilization. These changes occur on many levels including hormonal signaling, gene transcription as well as protein translocation driven by glucose and its metabolites. The liver expresses several distinct isoforms from muscle which enable hepatocytes to rapidly switch between gluconeogenesis and glycogen synthesis. In liver hepatocytes, glucose enters and exits the cell through the bidirectional GLUT2 transporter isoform. In contrast to GLUT4, GLUT2 is constitutively localized in the plasma membrane in an insulin-independent manner, allowing the liver to provide glucose generated by gluconeogenesis to the circulation during hypoglycemic episodes when insulin levels are low. GLUT2 has a high Km for glucose of 15–20 mM [Craik and Elliott, 1979], enabling glucose transport rates via GLUT2 to change directly in proportion to fluctuations in circulating glucose levels. Therefore, the rise in blood glucose following a meal causes intracellular glucose levels to rise, and this facilitates the transition from glucose production to storage by

promoting the translocation of a number of key enzymes involved in hepatic glucose metabolism.

Glucokinase

Following GLUT2 mediated glucose transport into the hepatocyte, glucose is phosphorylated to G6P by type IV hexokinase, also known as glucokinase (GK). GK is distinct from HK I-III in that GK has a lower affinity for glucose, is approximately half the size of HK I-III, is insensitive to changes in G6P levels and exists in a free-state or bound to a regulatory protein termed glucokinase regulatory binding protein (GKRP) [Vandercammen and Van Schaftingen, 1990]. GKRP plays a critical role in the regulated subcellular localization and translocation of GK in response to changes in extracellular glucose [Brown et al., 1997; Baltrusch and Tiedge, 2006]. Evidence that GKRP is responsible for localizing GK to the nuclear compartment comes from studies conducted in COS-1 cells, which express no endogenous GK or GKRP. Transfection of cells with either a fluorescent-tagged GK or GKRP alone resulted in a strictly cytosolic or mostly nuclear localization of fluorescence, respectively. However, cotransfection of cells with GK and GKRP resulted in a redistribution of GK-associated fluorescence to a primarily nuclear localization under low ambient glucose [de la Iglesia et al., 1999]. GK contains a nuclear export signal that is thought to be involved in GK export from the nucleus following release from GKRP [Shiota et al., 1999], corroborating the finding in COS-1 cells that GK-associated fluorescence is strictly localized to the cytoplasm in the absence of GKRP. Several independent investigations have demonstrated that GK translocates from the nucleus to the cytosol in response to increases in blood and hepatic glucose concentration [Toyoda et al., 1994; Brown et al., 1997; Chu et al., 2004]. Additionally, fructose treatment of hepatocytes and fructose-1-phosphate has also been shown to promote the dissociation of GK and GKRP [Agius et al., 1995], in turn stimulating G6P production [Davies et al., 1990]. The importance of GK translocation in hepatic glucose metabolism during the fasted to fed transition is demonstrated by the observation that glycogen synthesis rates increase sharply in parallel with free cytosolic GK activity [Agius et al., 1996]. Thus, the sequestration of GK in the nucleus prevents the inappropriate phosphorylation of glucose destined for hepatic glucose output during hypoglycemic episodes, while translocation of GK into the cytosol upon elevation of plasma glucose levels facilitates the hepatic transition from glucose production to glucose storage.

Further evidence for the physiological importance of GK/GKRP interaction in the control of hepatic glucose metabolism comes from two independent in vivo studies where GKRP was knocked out in mice [Farrelly et al., 1999; Grimsby et al., 2000]. GKRP knockout resulted in GK exclusion from the nucleus and interestingly, reduced total GK protein levels and activity by approximately 50%. No change in GK mRNA was detected, suggesting that binding to GKRP stabilized GK levels by reducing GK protein turnover. Fed and fasted blood glucose and insulin were unaffected in the knockout mice but glucose tolerance was modestly impaired, while dysregulation of circulating insulin and glucose levels upon high sucrose/high fat feeding was exaggerated in knockout animals [Farrelly et al., 1999]. Furthermore, liver glycogen levels were reduced by 30% in knockout animals fed a chow diet in one study [Farrelly et al., 1999], while no change was reported in the second [Grimsby et al., 2000], potentially due to temporal differences in when the tissues were harvested and analyzed. Cumulatively, these data indicate that reversible GK translocation between the nucleus and cytosol contributes to hepatic glucose uptake and storage, although firm conclusions are clouded by the concurrent reduction in GK protein levels in the GKRP knockout mice and incomplete metabolic analysis of the animals. Regardless, these data implicate GKRP in GK nuclear sequestering and potentially protein stabilization in vivo, and are suggestive of a role for this protein complex in the regulation of hepatic glucose metabolism.

In addition to the release and activation of GK in liver, the opposing enzyme glucose-6-phosphatase (G6Pase) must also be inactivated during the fasted to fed transition for proper hepatic glucose disposal. G6Pase dephosphorylates G6P produced by hepatic gluconeogenesis, enabling its release from the hepatocyte via GLUT2 into the bloodstream. During prolonged fasts, G6Pase gene transcription is increased via a cAMP/protein kinase A-dependent pathway [Gautier-Stein et al., 2005]. G6Pase gene expression has been shown to be regulated by a

number of transcription factors, including the peroxisome proliferative activated receptor-γ coactivator 1α (PGC1α), CAAT/enhancer-binding protein-α (C/EBP-α), the cAMP response element-binding protein (CREB), hepatocyte nuclear factor 4α (HNF4α), as well as the forkhead transcription factor, FOXO1 [Wang et al., 1995; Nakae et al., 2001; Boustead et al., 2003; Puigserver et al., 2003; Gautier-Stein et al., 2005; Schilling et al., 2006]. Insulin inhibits G6Pase expression by promoting the phosphorylation of FOXO1 [Nakae et al., 2001], resulting in its translocation and exclusion from the nucleus [Puigserver et al., 2003]. However, there is a temporal delay in the reduction of G6Pase protein expression that occurs in the fasted to fed transition, and during the interim the presence of both GK and G6Pase could result in futile cycling of glucose to G6P and back. To prevent this scenario, it has been observed that liver G6Pase activity was lowered by approximately 30% after re-feeding in fasted rats, and that insulin-regulated translocation of PI3K to a microsomal fraction played a significant role in G6Pase inhibition [Daniele et al., 1999]. Thus, during the fasted to fed transition, increased blood glucose results in the translocation and activation of GK, while concomitant increases in portal insulin activate the PI3K signaling cascade and inhibit G6Pase activity and transcription, reducing the release of glucose from the liver into the bloodstream.

Glycogen Synthase and Phosphorylase

Like skeletal muscle, synthesis of glycogen from glucose in liver is catalyzed by the enzyme glycogen synthase. Under low glucose conditions, the majority of glycogen synthase is observed in the cytoplasm [Garcia-Rocha et al., 2001]. In the presence of glucose, however, glycogen synthase localization is observed to shift to the cell periphery [Garcia-Rocha et al., 2001] and colocalize with actin filaments [Fernandez-Novell et al., 1997]. Interestingly, the pattern of glycogen synthase redistribution under conditions favoring glycogen accumulation seems to follow the growth of the glycogen chain [Fernandez-Novell et al., 2002]. This result suggests that glycogen synthase translocates to the site of elongation on the growing glycogen chain and increases the efficiency of the synthetic process. Furthermore, glycogen synthase redistribution to glycogen in primary hepatocytes has been shown to be regulated by G6P levels, as enzyme translocation corresponded linearly with cellular G6P levels [Fernandez-Novell et al., 1996], and occurred in the presence of 2-deoxy-glucose [Fernandez-Novell et al., 1992], which is not metabolized efficiently following generation of 2-deoxy-glucose-6P. Together, these observations suggest the presence of a glucose-induced translocation cascade that is an essential mechanism in the ordered regulation of hepatic glycogen synthesis.

In addition to regulating the cellular distribution of glycogen synthase, G6P levels also regulate the activity and localization of glycogen phosphorylase [Aiston et al., 2003, 2004], the principal enzyme responsible for glycogen breakdown. Glycogen phosphorylase is activated by phosphorylation and is allosterically inhibited by glucose, which concomitantly makes the enzyme a better substrate for dephosphorylation [Ferrer et al., 2003]. Increasing hepatic G6P levels by overexpressing GK has been shown to increase the amount of phosphorylase found in the glycogen enriched, particulate fraction of the cell [Aiston et al., 2003, 2004]. The same effect has also been observed by increasing hepatic glycogen levels via overexpression of protein phosphatase-1 (PP1) glycogen targeting subunits [Green et al., 2004]. Conversely, inhibition of GK resulting in decreased hepatic G6P levels has been shown to reduce the amount of phosphorylase detected in the pellet fraction [Aiston et al., 2003, 2004]. These data suggest a model whereby glycogen synthase and phosphorylase are released from glycogen during glycogenolysis since both proteins contain glycogen-binding domains. The movement of glycogen synthase would prevent futile cycling when energy mobilization is required, while glycogen phosphorylase release would help prevent complete consumption of the limited glycogen stores. During the fasted to fed transition, tandem translocation of both glycogen synthase and phosphorylase in hepatocytes mediated by increased G6P levels also contributes to the regulation of glycogen metabolism. Importantly, PP1 activity bound to glycogen targeting subunits has been shown to coordinately activate glycogen synthase and deactivate phosphorylase [Newgard et al., 2000]. Thus, mechanisms targeting glycogen synthase and phosphorylase to the glycogen compartment during net glycogen synthesis

could help ensure the proper activation and deactivation of synthase and phosphorylase, respectively, by colocalizing these enzymes together with phosphatase activity in the same cellular compartment. The simultaneous translocation of glycogen synthase and phosphorylase also underlies the future, rapid transition from glycogen synthesis to glycogenolysis, as glycogen-bound phosphorylase would be readily activated by glycogenolytic signals. Further, active glycogen phosphorylase is a potent inhibitor of glycogen synthase phosphatase [Doherty et al., 1995], which would further promote glycogen synthase inactivation and the efficient mobilization of glucose from glycogen.

CONCLUSION

Translocation of enzymes involved in glycogen synthesis has emerged as an important mechanism in the regulation of skeletal muscle and hepatic glucose metabolism over the last decade. Movement of IRS proteins, PI3K and Akt during insulin signaling in skeletal muscle allows for the redistribution of GLUT4 from the cytosol to the plasma membrane and the clearance of increased blood glucose in the fed state. In liver, insulin-independent entry of glucose via the GLUT2 transporter results in the release of GK from the nucleus and the production of G6P. Changes in G6P within the hepatocyte then communicate the rise in blood glucose levels to the cell, resulting in the redistribution of glycogen synthase and phosphorylase to the growing glycogen chain, and the coordinate activation of glycogen synthase and inhibition of phosphorylase. In muscle, activation of HK also allows for the temporal control of metabolism, enabling the immediate shunting of G6P toward glycolysis by mitochondrial associated HKI and the delayed provision of G6P for glycogen synthesis following translocation of HKII to the mitochondria. Finally, in both skeletal muscle and liver, glycogen synthase translocation in the fed state allows for the efficient transfer of glycosyl residues to the growing glycogen chain.

Several important questions remain unanswered in our understanding of the mechanism(s) and importance of protein translocation in the regulation of glycogen metabolism. Proximal insulin receptor signaling events have been extensively studied, and the role of defined protein domains such as SH2, PTP, and PH in the recognition of specific phospho-residues and—lipids provides a molecular basis for protein translocation and changes in protein/protein association following receptor activation. The molecular mechanisms by which glucose and its metabolites promote changes in the subcellular distribution of metabolic enzymes have received far less attention. Presumably, binding of these factors induces conformational changes in target proteins thus facilitating their redistribution following increased cellular glucose uptake in skeletal muscle or liver. However, the structural basis for these intracellular movements requires future study. In contrast to defined protein motifs which recognize changes in phosphorylation states of binding partners, the specific regions on hexokinase, glycogen synthase, and phosphorylase that mediate differential subcellular localization during different nutritional states have not been definitively defined. Additionally, the potential role of additional binding proteins in the subcellular redistribution of these enzymes by glucose metabolites has not been fully explored. Finally, the fundamental importance of protein translocation in the regulation of intermediary metabolism has not been fully defined. The potential identification and mutation of specific regions on metabolic enzymes that would prevent protein translocation without affecting enzymatic function would be an important step in elucidating many of these unresolved issues.

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