Glucose Regulates EF-2 Phosphorylation and Protein Translation by a Protein Phosphatase-2A-dependent Mechanism in INS-1-derived 832/13 Cells*

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The role of elongation factor (EF)-2 phosphorylation in the regulation of pancreatic β-cell protein synthesis by glucose was investigated in the INS-1-derived cell line 832/13. Incubation of cells in media containing 1 mM glucose resulted in a progressive increase in EF-2 phosphorylation that was maximal by 1–2 h. Readdition of 10 mM glucose promoted a rapid dephosphorylation of EF-2 that was complete in 10 min and maintained over the ensuing 2 h. Similar results were obtained using primary rat islets or Min-6 insulinoma cells. The glucose effect in 832/13 cells was replicated by addition of pyruvate or α-ketocaproate, but not 2-deoxyglucose, suggesting that mitochondrial metabolism was required. Accordingly, glucose-mediated dephosphorylation of EF-2 was completely blocked by the mitochondrial respiratory antagonists antymycin A and oligomycin. The hypoglycemic effect was not mimicked by incubation of cells in 100 nM insulin, 30 mM potassium chloride, or 0.25 mM diazoxide, indicating that insulin secretion and/or depolarization of β cells was not required. The locus of the high glucose effect appeared to be protein phosphatase-2A, the principal phosphatase acting on EF-2. Protein phosphatase-2A activity was stimulated by glucose addition to 832/13 cells, but neither protein phosphatase-1 nor calmodulin kinase III (EF-2 kinase) activity was affected under these conditions. The slower dephosphorylation of EF-2 during the transition from high to low glucose may involve effects on EF-2 kinase activity. Addition of 5-aminoimidazole-4-carboxamide 1-β-o-ribofuranoside in high glucose led to a marked stimulation of EF-2 phosphorylation, consistent with the possibility that increased AMP kinase activity in low glucose stimulates EF-2 kinase. In parallel with the effects on EF-2 dephosphorylation, addition of high glucose to 832/13 cells markedly increased the incorporation of [35S]methionine into total protein. Taken together, these results suggest that modulation of extracellular glucose impacts protein translation rate in β cells at least in part through regulation of the elongation step, via phosphorylation/dephosphorylation of EF-2.

Glucose exerts many effects on pancreatic islets in addition to the familiar enhancement of insulin secretion. Several studies have shown that hyperglycemia results in both transcriptional and translational activation in β cells or their derivative cell lines (e.g. Refs. 1–5). For example, proinsulin production is controlled by glucose at both levels, and acetyl-CoA carboxylase gene transcription is activated. Measures of total protein synthesis using [35S]methionine labeling and two-dimensional SDS-PAGE reveal substantial increases in many proteins after glucose stimulation of isolated islets, particularly the components of secretory granules (6, 7). Mechanistic studies of the glucose effect on protein synthesis have been ambiguous. Early studies in isolated islets suggested that the initiation step was primarily affected (1), but subsequent studies proposed that both initiation and elongation were involved (2). However, attempts to identify the specific initiation and/or elongation factors that might contribute to changes in translational control have been inconclusive. The situation is complicated by the fact that glucose causes insulin secretion, and insulin itself can exert profound effects on protein synthesis in responsive cells, including β cells themselves. Thus, some of the effects of glucose on translation in β cells may well be mediated in an autocrine manner (8).

Regulation at the level of initiation is often dependent on the phosphorylation of the limiting accessory factors eIF-2B and eIF4E-BP (4E-BP, Phas-1) (for review, see Ref. 9). Whereas insulin is known to alter the activity of 4E-BP via the phosphatidylinositol 3-kinase-Akt-mTOR pathway in many target cells, glucose appears not to directly affect this system in β cells (10) but can induce 4E-BP phosphorylation via the autocrine pathway, provided that amino acids are present (8). Glucose does appear to increase the activity of eIF-2B, but not by altering the phosphorylation state of the eIF-2α factor commonly found to be involved in translational initiation control (10). Protein translation can also be regulated at the level of elongation, principally by secondary modification of both elongation factors 1 and 2. EF-1 is a substrate for several protein kinases, and its activity can be modulated by insulin in target cells (11). Phosphorylation of EF-2 by the enzyme Ca2+/CaM-dependent protein kinase III (EF-2 kinase), primarily at Thr-56, blocks the ability of this factor to participate in protein synthesis (12, 13). EF-2 phosphorylation state has subsequently been shown to vary in a wide variety of cell types in response to diverse stimuli (for review, see Ref. 14).

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**EXPERIMENTAL PROCEDURES**

**Materials**—Cell culture reagents were supplied by Meditech, Inc., except for cysteine/methionine-free media, which was from Invitrogen. All chemicals were purchased from Sigma, with the exception of AICAR (Toronto Research Chemicals) and rapamycin, PD98059, and wortmannin (Calbiochem). Trun[^35S]label (1000 Ci/mmol) and [γ-[^32P]]ATP (4500 Ci/mmol) were obtained from ICN. Anti-EF-2 and anti-phospho-EF-2 antibodies were generated as described previously (16); the phosphoacetyl-CoA carboxylase antibody was from Upstate Cell Signaling Solutions. Horseradish peroxidase-conjugated goat anti-rabbit and goat anti-mouse IgG was from Bio-Rad, whereas ECL reagent was obtained from Amersham Biosciences.

**Cell Culture and Experimental Treatment**—The INS-1-derived cell lines 832/1 and 832/13 were generously provided by Dr. C. Newgard (Duke University) and cultured as described previously (15). Multiwell cultures (75–90% confluent) were rinsed twice with phosphate-buffered saline and incubated for 2 h in Krebs-Ringer buffer supplemented with 25 mM Hepes (pH 7.4) and 0.5% bovine serum albumin (KRBBH) and either 1 or 10 mM glucose. Sample wells were then switched to KRBBH containing the same or different glucose concentrations for various periods as indicated in the figures. Cells were then rapidly washed three times on ice with phosphate-buffered saline, scraped into lysis buffer (50 mM Hepes (pH 7.4), 150 mM NaCl, 10 mM NaF, 10 mM EDTA, 10% glycerol, 0.5% Triton X-100, and protease inhibitors), and centrifuged at 14,000 g for 10 min at 4 °C. Supernatants were removed, and equal amounts of lysate protein were analyzed by SDS-PAGE and immunoblotting. Rat islets were prepared as described previously (17), and treated samples were kindly provided by Drs. J. Corbett and P. Hansen (St. Louis University).

**Enzyme Assays**—Ca^2+/-CaM-dependent EF-2 kinase activity was measured as described previously (18), using purified rat liver EF-2 as substrate. Reactions contained 5 µg of lysate protein, 3 µg of EF-2, and 25 µM [γ-[^32P]]ATP. Reactions were terminated by the addition of SDS sample buffer and boiling. [γ-[^32P]]EF-2 was resolved by SDS-PAGE, identified by autoradiography, and counted by liquid scintillation. Protein phosphatase assays were performed as described previously (19). After treatment, cells were collected in phosphatase homogenization buffer (50 mM Hepes (pH 7.4), 2 mM EDTA, 2 mg/ml glycogen, 0.2% β-mercaptoethanol, 0.5% Triton X-100, and protease inhibitors). Samples were then centrifuged at 14,000 × g for 10 min at 4 °C, and ~5 µg of supernatant protein was used for assays. [γ-[^32P]]Phosphatase was used as substrate and prepared as described previously (19), with the modification that recombinant phospholamban kinase catalytic subunit was used. Phosphatase activity inhibited by addition of 3 mM okadaic acid is defined as PP2A, whereas phosphatase activity lost between 3 and 500 nM okadaic acid is defined as protein phosphatase-1. Addition of 500 nM okadaic acid completely inhibited all cellular phosphatase activity measured under these conditions.

[^35S]Methionine/Cysteine Incorporation into Total Protein—832/13 cells were plated in 12-well dishes and serum-starved as described above. Cells were pretreated in triplicate for 10 min as indicated, and then 10 µCi of Tran[^35S]-label was added to all wells. After a 10-min incubation at room temperature, the dishes were washed thrice with ice-cold 10% trichloroacetic acid; precipitated proteins were then dissolved in 0.5 ml of 0.1 M NaOH and transferred to scintillation vials. The samples were neutralized by addition of 10 µl of 10% acetic acid, and[^35S] incorporation into proteins was measured by scintillation counting. In parallel, a replicate well for each condition was washed three times with phosphate-buffered saline, and protein lysates were analyzed by SDS-PAGE and autoradiography.

**RESULTS**

**Glucose Increases Protein Translation in 832/13 Cells**—Shifts in extracellular glucose markedly affect insulin secretion and protein biosynthesis in β-cell lines and primary islets (1–5). To confirm that glucose levels also regulate protein synthesis in the INS-1-derived 832/13 cells, we preincubated cells for 2 h in 1 mM glucose and then added back either glucose (3 or 10 mM), pyruvate (5 mM), or insulin (100 nM) for 10 min and measured incorporation of [[^35S]methionine into total cellular protein. Addition of glucose caused a ~4-fold increase in total incorporation (Fig. 1A), and analysis of labeled proteins by SDS-PAGE showed that this effect involved numerous species (Fig. 1B). The effect was reproduced by pyruvate, but insulin in

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**Fig. 1. Stimulation of protein synthesis in INS-1-derived 832/13 cells by glucose.** 832/13 cells were maintained in KRBH/10 mM glucose (con) or glucose-restricted in KRBH/1 mM glucose for 2 h. Duplicate wells kept in low glucose were stimulated with glucose (3 or 10 mM), 5 mM pyruvate, or 100 nM insulin (in 1 mM glucose) for 10 min, and then all samples were pulse-labeled in [[^35S]methionine-containing medium for an additional 10 min. Samples were collected for measurement of total protein synthesis by trichloroacetic acid precipitation (A) or for analysis of synthesized proteins by SDS-7.5% PAGE and autoradiography (B). Results in A are a summary of three experiments, each point performed in duplicate (data are means ± S.D.). B: lane 1, 1 mM glucose; lane 2, 3 mM glucose; lane 3, 10 mM glucose; lane 4, 5 mM pyruvate; lane 5, 100 nM insulin. Autoradiograph in B is representative of three independent experiments.
were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with rabbit polyclonal anti-EF-2P IgG (0.5 μg/ml), followed by secondary antibody and development using ECL. After exposure, blots were stripped and reprobed with anti-EF-2 IgG as a loading control (bottom panels; signals varied <10% by densitometric analysis).

In four studies (for review, see Ref. 14), we investigated whether ambient glucose affected EF-2P levels in the 832/13 cells. When samples were preincubated with the mitochondrial inhibitors antimycin A or oligomycin, either of which blocked the effect of glucose, EF-2P levels declined within 10 min to the level found in cells maintained in low glucose (Fig. 3A). By contrast, addition of 5 mM of either pyruvate or a-ketocaproic acid, both of which can enter mitochondria and be directly metabolized to produce ATP, could substitute for glucose in mediating EF-2 dephosphorylation (Fig. 3B). Further evidence for mitochondrial involvement came from the use of respiratory chain inhibitors such as antimycin A or oligomycin, either of which blocked the effect of glucose on EF-2 dephosphorylation (Fig. 3C). These data indicate that production of ATP or some catabolite derived from glucose may be an important intermediary in promoting EF-2 dephosphorylation.

Glucose-stimulated EF-2 Dephosphorylation Is Not Mediated by Insulin Secretion or by Insulin-activated Signaling Pathways—INS-1 cells and islet β cells themselves have insulin receptors, and it is likely that some effects of glucose are mediated by released insulin, acting in an autocrine or paracrine manner (20). Moreover, insulin treatment of some other cell types has been reported to reduce EF-2P levels via inactivation of EF-2 kinase (e.g. see Ref. 21). To investigate the potential role of insulin release/signaling on EF-2P levels, 832/13 cells were preincubated for 2 h in buffer containing 1 mM glucose. Insulin-dependent effects on EF-2P were esti-
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To investigate potential signaling pathways mediating the regulation of EF-2P levels by extracellular glucose, we characterized kinase inhibitors were used. 832/13 cells were glucose-deprived for 2 h (Fig. 4A) but was unable to mimic the glucose effect, even after 2 h (data not shown). Additionally, stimulation of endogenous insulin release from 832/13 cells by addition of 100 µM tolbutamide or 30 mM KCl had no effect on EF-2P levels (Fig. 4B; data not shown). EF-2 dephosphorylation resulting from glucose addition was also unaffected by inclusion of 0.25 mM diazoxide, an inhibitor of insulin exocytosis (data not shown). Finally, the glucose effect was evinced in 832/1 cells (Fig. 4B), INS-1-derived cells that exhibit meager glucose-stimulated insulin secretion (15).

To investigate potential signaling pathways mediating the regulation of EF-2P levels by glucose, a variety of well-characterized kinase inhibitors were used. 832/13 cells were glucose-restricted for 2 h in KRHB containing 1 mM glucose and preincubated for 15 min with the indicated compounds, and then 10 mM glucose was added for a final 10-min period. Pretreatment of 832/13 cells with 100 nM of the phosphatidylinositol 3-kinase inhibitor wortmannin had no effect on the glucose-dependent dephosphorylation of EF-2, whereas the modest insulin effect on EF-2P levels was completely blocked by the drug (Fig. 4A). Neither the mitogen-activated protein kinase/extracellular signal-regulated kinase inhibitor PD98059 (20 µM) nor the mTOR inhibitor rapamycin (100 µM), alone, in combination, or together with wortmannin, had any effect on the glucose-mediated reduction in EF-2P levels (Fig. 4C; data not shown). The efficacy of these compounds on their respective signaling pathways was confirmed in parallel experiments using insulin-stimulated 3T3-L1 adipocytes (data not shown).

Cumulatively, these results indicate that the regulation of EF-2P levels by extracellular glucose occurs independently of insulin secretion/signaling and activation of several well-characterized kinase signaling cascades.

Fig. 4. The glucose effect on EF-2P does not involve insulin-dependent signal transduction pathways. A, 832/13 cells were maintained in KRHB/1 mM glucose (lo) for 2 h to elevate EF-2P. Half the samples were preincubated for 15 min with 100 nM wortmannin; 10 mM glucose (hi) or 100 mM insulin (lo + Ins) was then added for 10 min. EF-2P levels were analyzed by immunoblotting; loading controls using total EF-2 immunoblotting showed <10% variation between lanes (data not shown). B, left panel, cells were glucose-deprived for 2 h (lo) and then pretreated for 15 min without or with 10 µM tolbutamide (T). 10 mM glucose (hi) was then added to half the wells for 10 min before preparation of cell lysates. Right panel, a variant INS-1-derived cell line (832/1), selected for poor insulin secretory responses to glucose, was treated as described in the Fig. 2 legend. Note that an increase in glucose also lowers EF-2P in these cells. C, inhibitors of the Ras-mitogen-activated protein kinase and phosphatidylinositol 3-kinase-mTOR pathways do not block glucose-induced dephosphorylation of EF-2P. After 2 h of glucose restriction, 832/13 cells were maintained in either 1 mM glucose (lo; lanes 1–5) or 10 mM glucose (hi; lanes 1’–5’) for 10 min. Lanes 1, control; lanes 2, PD98059 (20 µM); lanes 3, rapamycin (100 nM); lanes 4, wortmannin (200 nM); lanes 5, PD98059 + wortmannin. All drugs were preincubated with cells for 15 min before shift to high glucose. EF-2P levels were analyzed by immunoblotting. Bottom panel, reprobe of blot with EF-2 IgG as loading control. All results are representative of three independent experiments.

Stimulation of Protein Phosphatase-2A Plays a Major Role in the High Glucose Effect on EF-2 Dephosphorylation—The regulation of EF-2P levels by extracellular glucose must involve alteration of kinase and/or phosphatase activities. EF-2 kinase is the major and perhaps only kinase that phosphorylates EF-2, whereas PP2A is the most active cellular phosphatase acting on EF-2P (12, 14, 18, 22). Direct assays of kinase activity in cell lysates derived from cells shifted from low to high glucose revealed no significant difference in either basal or Ca2+/CaM-stimulated activity (using activity in 1 mM glucose as 100%, cells maintained in 10 mM glucose had 113% of this activity, whereas cells shifted for 10 min from 1 to 10 mM glucose had 97% of this activity), suggesting that this enzyme is not the primary target of the hyperglycemic effect. In parallel, both protein phosphatase-1 and PP2A activities were measured in cellular lysates using [32P]phosphorylase as substrate. Extracts from cells incubated with 10 mM glucose contained a high level of PP2A activity (Fig. 5A). Reducing extracellular glucose to 1 mM for 2 h reduced PP2A activity by ~2-fold. Conversely, raising extracellular glucose from 1 to 10 mM caused a substantial activation of PP2A activity within 10 min; by contrast, protein phosphatase-1 activity measured in the same lysate remained constant during these transitions (Fig. 5A). If stimulation of PP2A is involved in the effect of high glucose, then the response should be sensitive to permissive inhibitors of this enzyme. Accordingly, preincubation of 832/13 cells with calyculin A (50 nM), an inhibitor of both protein phosphatase-1 and PP2A but not of protein phosphatase-2B or protein phosphatase-2C, markedly impaired EF-2 dephosphorylation in response to glucose addition (Fig. 5, B and C). Taken together, these data suggest that the hyperglycemic reduction in EF-2 phosphorylation state appears to be mediated primarily by an increase in PP2A rather than a decrease in EF-2 kinase activity.

Phosphorylation of EF-2 in Low Glucose May Involve AMP Kinase—When cells were shifted from high to low glucose media, there was a relatively slow reappearance of EF-2P (Fig. 6A). The marked kinetic difference between dephosphorylation in high glucose and rephosphorylation in low glucose suggested that the two processes might be mediated by distinct mechanisms. Indeed, whereas calyculin A attenuated the dephosphorylation of EF-2 on glucose addition, the phosphatase inhibitor did not substantially raise the already low EF-2P levels of 832/13 cells maintained in high glucose (e.g. see Fig. 5B; data not shown). Thus, a simple reversal of PP2A stimulation cannot account for the effect of glucose restriction. This result implies that stimulation of EF-2 kinase, rather than a decrease in PP2A activity with no change in EF-2 kinase, is somehow involved in the phosphorylation phenomenon. It seemed highly
unlikely that such a stimulation could occur via changes in intracellular Ca$^{2+}$ because low glucose hyperpolarizes β cells, resulting in the closure of plasma membrane Ca$^{2+}$ channels and a consequent lowering of cytoplasmic [Ca]. Additionally, a 15-min treatment with 10 μM forskolin caused only a slight increase in EF-2 phosphorylation, suggesting that changes in cAMP levels were not involved in the phosphorylation response to low glucose (Fig. 6).

Shifts in extracellular glucose have been reported to alter the ATP/AMP ratio in β cells, and it has been proposed that AMP kinase might act as a sensor to transmit signals from metabolism to various effector pathways (23). Accordingly, we tested whether AICAR, a nucleotide analog activator of AMP kinase (24), would affect EF-2 phosphorylation in 832/13 cells. AICAR treatment of cells maintained in high glucose led to an increase in EF-2P that peaked at 30 min (Fig. 6B). The effects of the nucleotide were not additive with glucose restriction (data not shown), suggesting that AMP kinase may play a role in the calcium-independent activation of EF-2 kinase in glucose-restricted cells. This conclusion was bolstered by the observations that glucose restriction increased the phosphorylation of the AMP kinase substrate acetyl-CoA carboxylase (25), with a time course similar to that of EF-2 (Fig. 6, compare A with C), and that AICAR stimulation of EF-2 and acetyl-CoA carboxylase phosphorylation was also temporally correlated (Fig. 6, compare C with B).

**DISCUSSION**

The acceleration of protein synthesis by glucose in pancreatic β cells was established many years ago, yet the mechanisms underlying the increase in translational capacity are still obscure. Initiation is the rate-limiting step in translation under many circumstances, thus it had been anticipated that glucose stimulation would primarily impact this step. One report does
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FIG. 7. Model of translational regulation in β cells. Extracellular glucose impacts protein synthesis by a variety of mechanisms (dashed lines indicate undefined mechanisms, whereas solid lines represent established pathways). Increased glucose uptake and metabolism alter translational initiation through modulation of eIF-2B activity (10). As shown here, extracellular glucose levels also regulate protein translation rate through the bidirectional modulation of EF-2 phosphorylation. Elevation of metabolites in high glucose causes the PP2A-mediated dephosphorylation of EF-2, contributing to increased protein translation rate. Conversely, reduced metabolites in low glucose favor EF-2 phosphorylation, potentially through an AMP kinase (AMPk)-EF-2 kinase (EF-2k) link, which contributes to the suppression of protein synthesis. Additionally, glucose stimulates insulin secretion and autocrine signaling to protein synthesis through the phosphatidylinositol 3-kinase-mTOR pathway (8).

The failure to find an obvious effect of glucose addition on EF-2 kinase activity raised the possibility that the high glucose control of EF-2P resided primarily on the phosphatase arm of the cycle. Hyperglycemic conditions evoke an activation of protein phosphatase activity in 832/13 cells, confirming a previous preliminary report in islets (26). Differential measurement of protein phosphatase activities revealed that PP2A was the likely locus of the effect in 832/13 cells. With respect to EF-2 dephosphorylation, this makes sense because PP2A is the most potent phosphatase acting on EF-2P (12, 22). Dephosphorylation of EF-2P upon glucose addition was attenuated by calyculin A, supporting the notion that control is exercised at the phosphatase level in the glucose signaling pathway. PP2A is a key member of the serine/threonine-protein phosphatase family and is responsible for a substantial fraction of the dephosphorylating activity present in all mammalian cells. In this group of enzymes, a conserved catalytic subunit (C) is linked to variable anchoring (A) and regulatory subunits (B) to form a variety of heterotrimeric enzymes, although AC dimers may also exist in cells (27). A novel regulatory mechanism involving the TAP42/α4 protein may also play an important role in PP2A specificity. TAP42 is a yeast protein implicated in the TOR pathway, and its mammalian equivalent (α4) has been found in association with the PP2A catalytic subunit in vivo. Indeed, overexpression of α4 has been reported to affect EF-2P levels in COS-7 cells without influencing other phosphoproteins relevant to protein synthesis (28). It is possible that the effects of glucose in β cells will be mediated by some factor that modulates the association of PP2A with a specific regulatory subunit. Molecular identification of PP2A subtypes in β cells is clearly warranted and will be required before the details of the glucose signaling pathway can be elucidated. Our data are not consistent with a recent report claiming that glucose might exert some of its effects in β cells through the inhibition of protein phosphatase activity (29). In that study, several metabolites of glucose such as phoshoenolpyruvate were found to inhibit total protein serine/threonine phosphatase activity in β-cell extracts. However, very high concentrations of these intermediates were used, and no attempt was made either to discriminate between different phosphatases or to show that enzyme activity was modulated in intact cells. Glucose has also been reported to increase protein phosphatase-2B (calcineurin) activity in islets through a calcium-dependent mechanism, and this may be involved in transcriptional and secretory control in β cells (30). Protein phosphatase-2B is unlikely to be involved in the glucose phosphorylation because depolarization had no effect on EF-2P state, and protein phosphatase-2B has minimal activity against EF-2P in vitro.

EF-2 kinase is a member of an atypical family of protein kinases that differ in the primary structure of the catalytic domain from the "conventional" protein kinases, such as protein kinase A or CaM kinase I (31, 32). The enzyme is conserved from Caenorhabditis elegans to man and presumably plays a critical role in cell behavior because it is expressed in most tissues of the body. The purified enzyme is activated by Ca²⁺/CaM, but other calcium-independent mechanisms of activation as well as inhibition of activity have been demonstrated. For example, as pointed out above, insulin appears to inhibit the enzyme in some cells, leading to a decrease in EF-2P (21). The mechanism of this effect is still poorly defined but may involve mTOR-dependent phosphorylation of EF-2 kinase because it is reportedly blocked by rapamycin (33). In the present study, we did find a small effect of exogenous insulin on EF-2P levels, consistent with an inhibition of EF-2 kinase, and this was blocked by wortmannin and rapamycin. In addition, the stress-activated pathway initiated by treatments such as protein synthesis inhibitors or ligands such as tumor necrosis factor α can also significantly inhibit EF-2 kinase in epithelial cells (34). This effect is mediated by direct phosphorylation of the enzyme on serine residues by stress-activated protein kinase/p38 (35). Our results suggest that glucose addition does not lead to an acute inhibition of EF-2 kinase activity that would contribute significantly to the dephosphorylation of EF-2. Nevertheless, it seems likely that the increase in phosphorylation of EF-2 during glucose restriction involves stimulatory effects on kinase activity, rather than just a reversal of PP2A stimulation, because inhibition of PP2A activity by calyculin A in high glucose did not substantially raise EF-2P levels. An attractive possibility is that AMP kinase is responsible for the indirect stimulation of EF-2 kinase under hypoglycemic conditions. Although
further work is needed on this question, the fact that AICAR treatment resulted in elevated EF-2P levels in cells maintained in high glucose suggests that a connection between AMP kinase and EF-2 phosphorylation exists. Indeed, low glucose levels do increase AMP kinase in islets (23), and EF-2 phosphorylation levels are sensitive to AMP kinase activation in other cell types (36). Although it is too early to rule out an effect of AMP kinase on EF-2 phosphorylation by a more circuitous route in β cells (e.g., via inhibition of PP2A), an AMP kinase stimulation of EF-2 kinase and a consequent increase in EF-2P have been proposed to account for the rapid, reversible shut-off in protein synthesis found in hepatocytes under hypoxic conditions (36). Evidently, the complex regulation of EF-2 phosphorylation at both the kinase and phosphatase levels expands the options available in β cells for modulating protein synthetic capacity in response to a variety of inputs (see Fig. 7 for summary).

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