Regulation of Both Glycogen Synthase and PHAS-I by Insulin in Rat Skeletal Muscle Involves Mitogen-activated Protein Kinase-independent and Rapamycin-sensitive Pathways*

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Incubating rat diaphragm muscles with insulin increased the glycogen synthase activity ratio (minus glucose 6-phosphate/plus glucose 6-phosphate) by approximately 2-fold. Insulin increased the activities of mitogen-activated protein (MAP) kinase and the Mkp = 90,000 isoform of ribosomal protein S6 kinase (Rsk) by approximately 1.5-2.0-fold. Epidermal growth factor (EGF) was more effective than insulin in increasing MAP kinase and Rsk activity, but in contrast to insulin, EGF did not affect glycogen synthase activity. The activation of both MAP kinase and Rsk by insulin was abolished by incubating muscles with the MAP kinase kinase (MEK) inhibitor, PD 098059; however, the MEK inhibitor did not significantly reduce the effect of insulin on activating glycogen synthase. Incubating muscles with concentrations of rapamycin that inhibited activation of p70S6k abolished the activation of glycogen synthase. Insulin also increased the phosphorylation of PHAS-I (phosphorylated heat- and acid-stable protein) and promoted the dissociation of the PHAS-I-eIF-4E complex. Increasing MAP kinase activity with EGF did not mimic the effect of insulin on PHAS-I phosphorylation, and the effect of insulin on increasing MAP kinase could be abolished with the MEK inhibitor without decreasing the effect of insulin on PHAS-I. The effects of insulin on PHAS-I were attenuated by rapamycin. Thus, activation of the MAP kinase/Rsk signaling pathway appears to be neither necessary nor sufficient for insulin action on glycogen synthase and PHAS-I in rat skeletal muscle. The results indicate that the effects of insulin on increasing the synthesis of glycogen and protein in skeletal muscle, two of the most important actions of the hormone, involve a rapamycin-sensitive mechanism that may include elements of the p70S6K signaling pathway.

Glycogen synthesis in skeletal muscle has a key role in the control of blood glucose levels by insulin. The large majority of postprandial glucose uptake occurs in skeletal muscle (1, 2), and most of the glucose that enters muscle fibers in response to insulin is converted to glycogen (3). This hormonal effect involves activation of glycogen synthase, the enzyme that catalyzes the rate-limiting step in the conversion of intracellular glucose to glycogen (4, 5). Insulin activates synthase by promoting dephosphorylation of sites in the COOH- and NH2-terminal regions of the enzyme (4, 5). The pattern of dephosphorylation is consistent with the hypothesis that insulin activates PP1G, the glycogen-bound form of type I protein phosphatase, as this phosphatase is able to dephosphorylate multiple sites in the synthase subunit (6). PP1G is controlled by phosphorylation of sites in its regulatory subunit (6, 7), designated RGL (8). Phosphorylation of site 1 increases phosphatase activity toward glycogen synthase. This site is readily phosphorylated in vitro by Rsk-2 (9–11), a kinase that is phosphorylated and activated by MAP1 kinase when cells or tissues are incubated with insulin (12, 13). Injecting insulin into rabbits has been reported to increase phosphorylation of site 1 in RGL (9), and it is widely believed that the activation of glycogen synthase by insulin in skeletal muscle involves the sequential activation of MAP kinase, Rsk-2, and PP1G.

Insulin also stimulates protein synthesis in many cells, but again skeletal muscle is of particular importance as this tissue is the largest reservoir of body protein (14). Muscle wasting is a hallmark of untreated diabetes mellitus in humans, and inducing diabetes in rats decreases by half the rate of protein synthesis in skeletal muscle (15). Experimental diabetes also causes dispersion of polysomes and accumulation of free ribosomal subunits (16, 17). Within 2 h of treating diabetic rats with insulin, the polysome profile returns to the prediabetic state. These effects of insulin and diabetes are indicative of regulation of translation initiation, which is generally the rate-limiting phase of protein synthesis (18–20). Initiation involves recognition of capped mRNA, melting of secondary structure in the 5′-nontranslated region of the mRNA, and binding of the 40S ribosomal subunit. Initiation is mediated by several factors, the least abundant of which is the mRNA cap-binding protein, eIF-4E. Several lines of evidence indicate that eIF-4E activity is limiting for initiation (18–20).

Insulin increases eIF-4E activity in adipocytes by stimulating the phosphorylation of the translational regulator, PHAS-I (21–24). PHAS-I cDNA was originally cloned from a rat skeletal muscle library, and PHAS-I mRNA was found in highest levels in skeletal muscle and fat (25). Nonphosphorylated PHAS-I binds tightly to eIF-4E and inhibits translation (24), probably by preventing the association of eIF-4E with eIF-4A.

1 The abbreviations used are: MAP kinase, mitogen-activated protein kinase; CHO, Chinese hamster ovary; EGF, epidermal growth factor; eIF, eukaryotic initiation factor; MEK, MAP kinase kinase; MBP, myelin basic protein; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PHAS, phosphorylated heat- and acid-stable protein; TOR, target of rapamycin.

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(26). When PHAS-I is phosphorylated in response to insulin, the PHAS-I-elf-4E complex dissociates (21–24), allowing elf-4E to participate in translation initiation. PHAS-I is an excellent substrate for MAP kinase in vitro, and the major site (Ser\textsuperscript{64}) phosphorylated by MAP kinase in vitro is phosphorylated in response to insulin in adipocytes (27). Moreover, essentially all of the insulin-stimulated PHAS-I kinase activity in adipocyte extracts is accounted for by the ERK-1 and ERK-2 isoforms of MAP kinase (23). Based on these results, MAP kinase was proposed to mediate the phosphorylation of PHAS-I by insulin in adipocytes. Thus far, all studies of the regulation of PHAS-I by insulin have been confined to adipocytes.

Recent findings indicate that activation of MAP kinase is neither necessary nor sufficient for the effects of insulin on glycogen synthase in fat cells (28–32). EGF and other agents that are as effective as insulin in activating MAP kinase and Rsk did not activate glycogen synthase in either primary (29, 31) or 3T3-L1 adipocytes (28, 32). MAP kinase activation by insulin in 3T3-L1 adipocytes was blocked by a novel inhibitor of MEK, PD 098059, without inhibiting the effect of insulin on glycogen synthase (30). PHAS-I phosphorylation is increased by agonists that activate MAP kinase; however, the phosphorylation of PHAS-I by insulin was not attenuated by inhibition of MAP kinase activation with PD 098059 (22). In contrast, the effect of insulin on increasing PHAS-I phosphorylation and promoting the dissociation of the PHAS-I-elf-4E complex was markedly inhibited by rapamycin (21, 22). Rapamycin acts to inhibit transduction through a pathway that is distinct from the Ras-MAP kinase pathway and that leads to the activation of p70\textsuperscript{S6K} (33–36). Rapamycin was without effect on the activation of glycogen synthase by insulin in rat adipocytes (29, 31) but markedly inhibited the activation of synthase in 3T3-L1 adipocytes (37). Multiple pathways had been previously shown to exist for the activation of glycogen synthase by insulin in rat adipocytes (38). Different cell types might utilize different transduction pathways to activate synthase, and it has been suggested that the mechanism of activation in skeletal muscle differs from that in adipocytes.

In view of the importance of skeletal muscle in the regulation of glycogen and protein metabolism by insulin, it is important to determine which signaling pathways are utilized by insulin in this tissue. In the present experiments, we have investigated the roles of the MAP kinase and rapamycin-sensitive pathways in mediating the effects of insulin on glycogen synthase and PHAS-I in rat diaphragm.

**Experimental Procedures**

**Incubation of Muscle in Vitro**—Male rats (60–80 g, Sprague-Dawley, Sasco) were fed ad libitum before diaphragms with the surrounding ribs were removed as described by Goldberg et al. (39). Incubations were conducted essentially as described previously (40). Briefly, to remove endogenous hormones, muscles were incubated at 37 °C in Dulbecco’s modified Eagle’s medium for 45 min. For treatments, muscles were conducted essentially as described previously (40). Briefly, to remove endogenous hormones, muscles were incubated at 37 °C in Dulbecco’s modified Eagle’s medium for 45 min. For treatments, muscles were incubated with beads (10 mg, dry weight) of individual fibers.

**Ribosomal Protein S6 Kinase Activities**—Rsk activity was measured by using [γ\textsuperscript{32}P]ATP and MBP as substrates (48). The construction of the reaction mixture and the incubation and the incubation were exactly as described previously (29). An immune complex assay was used to assess the activity of the ERK-1 isoform of MAP kinase. Except for the use of diaphragm extracts (20 μg of protein), this assay was performed exactly as described previously for measuring ERK-1 activity in adipocyte extracts (44). Activation of ERK-1 and ERK-2 was also assessed by using the “in-gel” assay described by Wang and Erikson (49). In some experiments, single muscle fibers were manually dissected from freeze-dried specimens of control and EGF-treated diaphragms and dissolved in SDS sample buffer (50). The “in-gel” assay was used to assess ERK-1 and ERK-2 activities in samples (20 μg, dry weight) of individual fibers.
subjected to SDS-PAGE (52), and the relative amounts of $^{32}$P incorporated into \([\text{His}_6]^{\text{RGL}}\) were determined by using a phosphorimager (Molecular Dynamics).

To measure p70$^\text{S6K}$ activities, immune complexes were incubated as described for measuring Rsk activities, except that 40 S ribosomes (2 mg/ml final concentration) were used as substrate. The ribosomes were purified on rat liver as described by Krieg et al. (53). The protein kinase reaction was terminated by adding SDS sample buffer, and samples were subjected to SDS-PAGE (52). The relative amounts of $^{32}$P incorporated into ribosomal protein S6 were determined by phosphorimaging.

Preparation of \([\text{His}_6]^{\text{RGL}}\).—The 1329-base pair Ncol-Ncol fragment was excised from pET-8c plasmid (8), blunt-ended, and then inserted into blunt-ended Xhol and Bpu1102 sites in pET-15b (Novagen). This construct encodes the first 443 amino acid residues of RGL plus 6 NH$_2$-terminal His residues and 4 additional amino acids (Val-Ser-Asn-Asp) at the COOH terminus. The orientation and reading frame of the insert were determined to be correct by restriction mapping and nucleotide sequencing. The resulting plasmid, \(\Delta\text{C}-\text{pET}\), was transformed into Escherichia coli (BL21(DE3)), and after induction with isopropyl-1-thio-$\beta$-D-galactopyranoside, \([\text{His}_6]^{\text{RGL}}\) was extracted and purified by chromatography using Ni$^{2+}$-NTA-agarose (Qiagen). After SDS-PAGE and staining with Coomassie Blue, the recombinant protein appeared as a species of apparent M, \(65,000\). This is somewhat higher than the M, \(52,000\) expected of the protein encoded by the cDNA, but intact RGL also exhibits lower electrophoretic mobility than would be expected of its actual M, \(8\). The purified protein was identified as \([\text{His}_6]^{\text{RGL}}\) by amino acid sequencing of the NH$_2$-terminal residues and by immunoblotting with RGL antibodies (8).

Immunoblotting.—Samples were subjected to SDS-PAGE (52) before proteins were electrophoretically transferred to nylon membranes (Immobilon, Millipore), which were then incubated in a solution of PBS containing 5% milk (Carnation) and 1% Triton X-100. Membranes were then incubated for 2 h with 1000-fold dilutions of Rsk, p70$^\text{S6K}$, or PHAS-I antisera or 0.1 mg/ml affinity-purified glycogen synthase antibodies. After washing for six times with 1% Triton X-100 in PBS, the membranes were incubated with either protein A conjugated to alkaline phosphatase (5000-fold dilution) or with rabbit anti-chicken antibodies conjugated to horseradish peroxidase (3000-fold dilution). Membranes were then washed six times with 1% Triton X-100 in PBS before antibody binding was detected by using enhanced chemiluminesence kits containing reagents for the alkaline phosphatase reaction (Tropix) or horseradish peroxidase (Amersham).

Purification of PHAS-I-of F-4E Complexes—Muscle extracts (100 $\mu$g of protein), prepared as described above for the extraction of glycogen synthase, were mixed with 10 $\mu$l of mGTP-Sepharose (Pharmacia) and incubated for 30 min at 21°C. The resin was washed three times (0.5 ml/wash) with kinase homogenization buffer. Proteins were eluted with SDS sample buffer and subjected to SDS-PAGE (52). PHAS-I that copurified with eF-4E was determined by immunoblotting.

Other Materials—UDP-[U-$^{14}$C]glucose was prepared as described by Thomas et al. (42) by using [U-$^{14}$C]glucose from ICN. [y-$^{32}$P]ATP was obtained from DuPont NEN. Porphine insulin (27 units/mg) and EGF were from Eli Lilly and United Biotechnology International, respectively. PD 098059 was provided by Parke-Davis. Microcystin and okadaic acid were from LC Labs, and rapamycin was purchased from Calbiochem.

RESULTS

Effects of Insulin and EGF on Glycogen Synthase and MAP Kinase Activities—Incubating rat diaphragm muscles for increasing times with insulin resulted in a rapid activation of glycogen synthase (Fig. 1). The activity ratio in control muscles prior to incubation with the hormone was 0.25, and the ratio did not significantly change during the subsequent incubation period. After 30 min of incubation with insulin, the activity increased to approximately 0.44. Phosphorylation of certain sites in purified glycogen synthase has been shown to decrease the electrophoretic mobility of the protein in SDS-PAGE (54). The synthase subunit was readily detected by immunoblotting after extract samples were subjected to SDS-PAGE (Fig. 1, inset). Neither insulin nor EGF changed the amount of synthase detected, consistent with the observation that total glycogen synthase activity was changed by another agent (Table I). Insulin promoted a shift in the electrophoretic mobility of the synthase to forms of higher electrophoretic mobility (Fig. 1, inset), consistent with its well established action to promote dephosphorylation of the protein (4, 5). In contrast to insulin, EGF was without effect on the synthase activity ratio or the electrophoretic mobility of the synthase subunit.

EGF was much more effective than insulin in activating MAP kinase. In Fig. 2, the ERK-1 and ERK-2 isoforms of MAP kinase in extracts of insulin- and EGF-treated muscles were resolved by SDS-PAGE before kinase activities were measured after renaturation by using an in-gel assay (49). Bands of activity corresponding to species of apparent M, \(44,000\) and \(42,000\) were detected (Fig. 2A, for example). These species had exactly the same electrophoretic mobilities as the ERK-1 and ERK-2 isoforms from adipocytes. There was no indication of sequential activation (ERK-1 before ERK-2) of the isoforms as was reported to occur in bimini muscle when rats were injected with insulin (55). Insulin increased the activities of ERK-1 (Fig. 2A) and ERK-2 (Fig. 2B) by only 50–70%.

| TABLE I | Failure of the MEK inhibitor, PD 098059, to block activation of glycogen synthase by insulin |
|---------|-----------------------------------------------------------------------------------------|
| Treatment | Glycogen synthase Synthase Activity ratio of G6P to G1P | Total activity |
|----------|-------------------------------------------------|---------------|
| None     | 0.20 ± 0.02                                    | 34 ± 1        |
| Insulin, 20 nm | 0.32 ± 0.02                                      | 30 ± 3        |
| EGF, 100 nm | 0.22 ± 0.01                                      | 29 ± 5        |
| PD 098059, 25 μm | 0.22 ± 0.02                                    | 30 ± 5        |
| PD 098059, 25 μm plus insulin, 20 nm | 0.29 ± 0.03                                      | 31 ± 3        |
| PD 098059, 25 μm plus EGF, 100 nm | 0.19 ± 0.02                                    | 32 ± 5        |

2 I. Azpiazu and J. C. Lawrence, Jr., unpublished observations.
comparison, EGF increased the activities of the ERK-1 and ERK-2 by approximately 3- and 5-fold, respectively.

The finding that both isoforms of MAP kinase were markedly increased by EGF under conditions in which glycogen synthase activity was not changed indicates that MAP kinase activation is not sufficient for the activation of glycogen synthase. Because of the important implications of the findings, control experiments were performed to verify that EGF was more effective than insulin in activating MAP kinase. EGF produced a larger increase than insulin when total MAP kinase activity was measured in nonfractionated extracts or when the activity of the ERK-1 isoform was measured in immune complex assays (Fig. 3D). Thus, the difference between the effectiveness of EGF and insulin was not dependent on the method used to assay MAP kinase. MAP kinase activity was found to be increased in single muscle fibers that had been manually dissected from control and EGF-treated muscles, indicating that the effect of EGF was not due to effects on other cell types present in skeletal muscle.

Failure of the MEK Inhibitor, PD 098059, to Prevent Activation of Glycogen Synthase by Insulin—PD 098059 acts noncompetitively with respect to ATP to inhibit activation of MEK1 and MEK 2 in vitro (56). The MEK inhibitor appears to block transduction through the MAP kinase pathway without inhibiting other protein or lipid kinases (22, 30, 56, 57). The inhibitor markedly decreased activation of MAP kinase by EGF in diaphragms, and it abolished the effects of insulin on MAP kinase when ERK-1 or ERK-2 activities were measured by using the in-gel assay (Fig. 3A-C) or when ERK-1 activity was assessed after immunoprecipitation (Fig. 3D). In the same muscles, the MEK inhibitor was without significant effect on glycogen synthase activity in either the absence or presence of EGF or insulin (Table I).

Inhibiting MAP kinase activation with the MEK inhibitor was associated with marked inhibition of Rsk (Fig. 4). As with MAP kinase activity, Rsk activity measured in an immune complex assay using a large S6 peptide as substrate was increased much more by EGF than by insulin. Treating muscles with PD 098059 abolished activation of Rsk by insulin and decreased the activity observed in the presence of EGF by approximately 80%. Rsk immunoprecipitated from muscle extracts readily phosphorylated [His6]eIF-4E when PHAS-I was present as the nonphosphorylated (a) form, RGL, a truncated form of PHAS-I (Fig. 5), was also detected (Fig. 5). Previous experiments have established that phosphorylation of the appropriate sites retards the electrophoretic mobility of PHAS-I in SDS-PAGE (23). In extracts of rat diaphragm, a relatively smaller amount of a fourth more slowly migrating species, denoted (b), was also detected (Fig. 5). Previous experiments have established that phosphorylation of the appropriate sites retards the electrophoretic mobility of PHAS-I in SDS-PAGE (23, 25, 27). Thus, the different bands represent protein phosphorylated to differing extents, and a shift to species of higher apparent M, is indicative of increased phosphorylation. In muscles incubated without insulin or EGF, approximately 25% of the PHAS-I was present as the nonphosphorylated (a) form, which binds tightly to eIF-4E. As previously observed (21–24), PHAS-I(a) and (b) were the predominant forms found bound to eIF-4E when PHAS-I-eIF-4E complexes were isolated from extracts incubated without insulin or EGF.
electrophoretic mobility of PHAS-I produced by insulin. The PHAS-I bound to eIF-4E. Inhibiting the activation of MAP kinase by PD 098059 markedly decreased PHAS-I binding to eIF-4E (Fig. 5). Moreover, MAP kinase was markedly increased by EGF without activating glycogen synthase (Table I), and EGF did not mimic the effects of insulin on PHAS-I (Fig. 5). Consequently, it is unlikely that a small amount of insulin-stimulated MAP kinase activity, as might have been undetected in experiments with the MEK inhibitor, could account for the effects of insulin on PHAS-I and glycogen synthase.

The activation of Rsk by insulin was also abolished by the MEK inhibitor (Fig. 4). This was expected as Rsk is phosphorylated and activated by MAP kinase (12, 59). However, the findings that EGF activated Rsk without activating glycogen synthase and that inhibiting Rsk activation with the MEK inhibitor did not abolish synthase activation by insulin (Fig. 4, Table I) were not predicted by the model proposed by Dent et al.
Control of Glycogen Synthase and PHAS-I

The finding that the effects of insulin on activating glycogen synthase and p70S6K were inhibited by similar concentrations of rapamycin is suggestive of a role of the p70S6K pathway in the control of synthase (Fig. 7, A and B). Rapamycin is believed to act by binding FKBP-12, an M, = 12,000 protein that is also the receptor for the immunosuppressant, FK-506 (61, 62). FKBP-12 possesses peptidyl-prolyl isomerase activity that is inhibited by both rapamycin and FK-506. This action does not appear to explain the effect of rapamycin to attenuate insulin signaling, as FK-506 blocked the activation of neither p70S6K (21, 36) nor glycogen synthase2 by insulin. Insensitivity to FK-506 would also seem to exclude the calcium-sensitive phosphatase, calcineurin, in the activation of synthase, as the FKBP-12-FK-506 complex binds to and inhibits calcineurin (61, 62). The FKBP-12-rapamycin complex does not inhibit calcineurin but instead binds to TOR (target of rapamycin), a protein originally identified in yeast and more recently cloned from mammalian cells (63–65). The intervening steps between TOR and p70S6K have not been identified.

Somewhat higher concentrations of rapamycin were needed to inhibit synthase or p70S6K in the diaphragm than to inhibit p70S6K in suspended or cultured cells (21, 22, 33, 34, 36), where most previous studies of rapamycin sensitivity have been performed. The possibility that rapamycin acts nonspecifically to inhibit activation of p70S6K and glycogen synthase cannot be excluded. However, to achieve effective concentrations in fibers within the intact muscle, rapamycin must cross diffusion barriers that are not present in cultured cells. This may be why higher concentrations of rapamycin were needed to inhibit p70S6K in muscle. Alternatively, TOR or other elements in the signal transduction pathways leading to the activation of p70S6K in skeletal muscle may be less sensitive to rapamycin than those in other cells.

Under conditions in which MAP kinase activity was increased severalfold by EGF, neither the electrophoretic mobility (Fig. 6) nor the activity of p70S6K was significantly changed. Thus, the failure of EGF to activate synthase is not inconsistent with a role of the p70S6K pathway in regulating synthase. One potential mechanism by which activation of p70S6K could lead to synthase activation involves GSK-3, the only protein kinase known to phosphorylate site 3(a–b–c) in synthase (4, 66). GSK-3 can be phosphorylated and inactivated by p70S6K in vitro (67), and insulin has been shown to inhibit GSK-3 activity in adipocytes (68), L6 muscle cells (69), and Chinese hamster ovary cells overexpressing the human insulin receptor (CHO.T cells) (70). Inhibition of GSK-3 alone would be insufficient to account for dephosphorylation of synthase in rat skeletal muscle, where insulin promotes dephosphorylation not only of site 3(a–b–c) but also of site 2(a–b) (5, 71), which is not phosphorylated by GSK-3 (4, 66). Moreover, there is reason to believe that GSK-3 inhibition is not involved in the rapamycin-sensitive regulation of glycogen synthase, as rapamycin did not block the effect of insulin on inhibiting GSK-3 activity by insulin in CHO.T cells (70) or in L6 cells (69).

The effect of rapamycin on glycogen synthase activity in skeletal muscle is similar to that observed by Shepherd et al. (37) in 3T3-L1 cells but differs from findings in rat adipocytes (29, 31) and CHO cells (72), where rapamycin did not block the activation of synthase by insulin. Thus, different cells may utilize different pathways for activation of synthase. There is other evidence to support this view. In rat adipocytes glucose potentiates activation of synthase by insulin (38), whereas in skeletal muscle (5) or 3T3-L1 adipocytes (28) glucose has little if any effect on activating the enzyme. In Swiss mouse 3T3

FIG. 7. Rapamycin attenuates the effects of insulin on p70S6K, glycogen synthase, and PHAS-I. Diaphragms were incubated at 37°C for 30 min with increasing concentrations of rapamycin. Incubations were then continued for 30 min without (circles) or with (squares) 20 nM insulin. A, p70S6K was immunoprecipitated from extracts, and kinase activity was measured by using 80 S ribosomes and [γ-32P]ATP as substrate. Activities are expressed as percentages of maximum kinase activity, which in all experiments was observed in extracts of muscles that had been incubated with insulin in the absence of rapamycin. The inset is an immunoblot showing p70S6K after immunoprecipitation from extracts of muscles that had been incubated without additions (CON), with 25 nM rapamycin (RAP), with 20 nM insulin (INS), or with insulin plus rapamycin (INS + RAP). B, glycogen activity ratios were measured in muscle extracts, and mean values ± S.E. are presented. Total synthase activities (nmol/min/mg extract protein) were as follows: control, 33 ± 7; 25 nM rapamycin, 39 ± 4; 100 nM rapamycin, 35 ± 4; insulin, 30 ± 3; 25 nM rapamycin plus insulin, 34 ± 4; and 100 nM rapamycin plus insulin, 27 ± 6. C, Extracts were subjected to SDS-PAGE, and the relative amounts of the α and β forms of PHAS-I were determined after immunoblotting (see inset). The results are expressed as percentages of the total PHAS-I and are mean values ± S.E. of three experiments. Error bars not shown fall within the symbol.

(9) in which synthase activation is mediated by IQPK, the rabbit equivalent of Rsk1 (10, 11, 60). Rsk immunoprecipitated from diaphragm extracts phosphorylated [His6]PRGL, indicating that the antibodies recognize forms of the kinase that are capable of phosphorylating PP1L (Fig. 4). Our interpretation of the results is that Rsk can phosphorylate RGL in vitro, but Rsk does not mediate the activation of glycogen synthase by insulin in intact skeletal muscle. It should not be inferred that synthase activation does not involve phosphorylation of PP1L, although experiments are needed to confirm that site 1 is phosphorylated in response to insulin in skeletal muscle of rats and other species in which the hormone activates glycogen synthase.
cells, EGF and platelet-derived growth factor activated glycogen synthase (73, 74), but EGF did not increase synthase activity in adipocytes (28, 29, 32) or skeletal muscle (Fig. 1). As skeletal muscle is the most important site of insulin-stimulated glycogen deposition (3), it will be most important to determine the mechanism of synthase activation by insulin in this tissue.

The present study demonstrates that insulin increases the phosphorylation of PHAS-I and dissociation of the PHAS-I-eIF-4E complex in skeletal muscle. As PHAS-I inhibits eIF-4E (24), dissociation would be expected to increase rates of initiation. This is consistent with the increased PHAS-I phosphorylation in skeletal muscle (14). The phosphorylation of PHAS-I in response to insulin in skeletal muscle involves a rapamycin-sensitive pathway (Fig. 7); however, as observed in adipocytes, rapamycin did not completely inhibit insulin-stimulated phosphorylation of PHAS-I (21, 22). Consequently, the control of phosphorylation of PHAS-I by insulin in skeletal muscle also involves a rapamycin-insensitive pathway.

The kinase responsible for the rapamycin-sensitive phosphorylation of PHAS-I has not been identified. It is likely that the PHAS-I kinase is p70RSK, as purified p70RSK did not directly phosphorylate recombinant PHAS-I (27). This does not exclude the possibility that TOR itself may signal as a pro-kinase.

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Regulation of Both Glycogen Synthase and PHAS-I by Insulin in Rat Skeletal Muscle Involves Mitogen-activated Protein Kinase-independent and Rapamycin-sensitive Pathways

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