Control of Liver Glycogen Synthase Activity and Intracellular Distribution by Phosphorylation*

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Eukaryotic glycogen synthase activity is regulated by reversible phosphorylation at multiple sites. Of the two GS isoforms found in mammals, the muscle enzyme (muscle glycogen synthase) has received more attention and the relative importance of every known phosphorylation site in the control of its activity and intracellular distribution has been previously addressed. We have analyzed the impact of the dephosphorylation at the homologous sites of the glycogen synthase liver (LGS) isoform. Serine residues at these sites were replaced by non-phosphorylatable alanine residues, singly or in pairs, and the resultant LGS variants were expressed in cultured cells using adenoviral vectors. The sole mutation at site 2 (Ser7) yielded an enzyme that was almost fully active and able to induce glycogen deposition in primary hepatocytes incubated in the absence of glucose and in FTO2B cells, a cell line that does not normally synthesize glycogen. Mutation at site 2 was also sufficient to trigger the aggregation and translocation of LGS from the cytoplasm to the hepatocyte cell cortex in the absence of glucose. However, this redistribution was not observed in hepatocytes incubated without glucose when an additional mutation (E509A), which renders the enzyme inactive, was introduced. This result suggests that LGS translocation is strictly dependent on glycogen synthesis.

The rate-limiting enzyme for glycogen synthesis is glycogen synthase (GS), which catalyzes the addition of α1,4-linked glucose units from UDP-glucose to a growing glycogen chain. The activity of the enzyme is tightly regulated by phosphorylation at multiple sites and by allosteric effectors, such that phosphorylation tends to inactivate the enzyme, but even the most phosphorylated forms become fully active in the presence of glucose-6-P (Glc-6-P).

In mammals there are two GS isoforms, liver glycogen synthase (LGS), whose expression is tissue-specific, and muscle glycogen synthase (MGS), which is expressed in almost all tissues (1). Although the two isoforms are 70% identical, the NH2- and COOH-terminal extremes are only 50% homologous (2), and the LGS COOH-terminal domain is shorter (3). The regulatory sites of MGS that undergo reversible phosphorylation have been identified: two of them, sites 2 and 2a, are located near the NH2 terminus, whereas the remaining seven sites, 3a, 3b, 3c, 4, 5, 1a, and 1b, are found within the COOH-terminal 100 residues (Fig. 1). The relative importance of each site in the regulation of MGS activity has been documented (4–6). A similar type of analysis has not been performed with LGS, in which homologues of seven of the nine MGS phosphorylation sites are present (Fig. 1). Only one mutant form of LGS, which contains six Ser to Ala substitutions at sites 2, 3a, 3b, 3c, 4, and 5, has been studied. This mutant is constitutively active and causes a large increase in glycogen accumulation when expressed in rat primary hepatocytes (7). LGS is phosphorylated at multiple sites when hepatocytes are incubated with glycogenolytic agents such as adrenaline, glucagon, or phorbol esters (8, 9). Furthermore, LGS can be phosphorylated in vitro by several kinases, including cAMP-dependent protein kinase, protein kinase C, casein kinase 1 and 2, AMP-stimulated protein kinase, phosphorylase kinase, and glycogen synthase kinase 3 (GSK-3) (10, 11).

The muscle and liver isoforms of GS also differ in their intracellular distribution. When glycogen stores are depleted in cells incubated in the absence of glucose, MGS concentrates in the nucleus, but moves to the cytoplasm, where glycogen deposition occurs, when levels of the monosaccharide increase (12, 13). An arginine-rich cluster, located near the COOH terminus and involved in the MGS allosteric activation by Glc-6-P (3), is also required for the nuclear accumulation of the enzyme (14). However, the regulation of the nucleocytoplasmic shuttling does not reside in any of the known MGS phosphorylation sites that control its activity (14). In sharp contrast, LGS translocates from a diffuse cytoplasmic distribution to the cell cortex in response to glucose (15, 16). Consequently, in hepatocytes, glycogen synthesis is initiated at the periphery and progresses toward the cellular interior (17).

Here we evaluated the relative potency of each of the LGS phosphorylation sites in the regulation of its activity. We also analyzed how the phosphorylation status of these sites affects the intracellular distribution of the liver enzyme. Our results indicate that, in contrast to what occurs with MGS, whose activation requires dephosphorylation of site 2, at the NH2 termi-
nus, and site 3a or 3b, at the COOH terminus (4), a single Ser to Ala mutation at site 2 of LGS is sufficient to render an essentially fully active form of the liver enzyme.

EXPERIMENTAL PROCEDURES

Construction of Recombinant Adenoviruses—Recombinant adenovirus coding for wild-type liver GS (LGS WT) has been described (18). Ser to Ala mutations were introduced into pACCMV-RnLGS plasmid at sites 2, 2a, 3a, 3b, 3c, 4, and 5 by site-directed mutagenesis using the following primers: site 2 forward primer, CTCAGGGGCCGCTCCTTGGCTGTGAC and reverse primer, GTCAAGGCTGCAGGCGGCTCGCTGAG; site 2a forward primer, CTGTGACGGCCCTTGGG and reverse primer, GTCACAGCCAAGGAGCGGCCCCTGAG; site 3a forward primer, CTTTAAGTATCCCAGGCCCTCCGAGTACCA and reverse primer, TGGTACTGCGGAGGGCCTGGGATACTTAAAG; site 3b forward primer, 5’-CAGTCACACTGCCCATCAGGG and reverse primer, 5’-CCTGA-TGCGGCGAGGTGTTACTCT; site 3c forward primer, CCCACTAGCTGCCCAGCTCAGTG and reverse primer, GCTCTGAGGAGCTGAAGTCTGGGATCCTGATGG; and site 5 forward primer, CTCAAGGCGGATGTTGAGAACGAAG and reverse primer, CTTGTTTTCCACATCCGGGCTCTCTGAG. The adenoviruses coding for mutated LGS in these sites or in combinations of two were constructed as described (19). The E509A mutation was introduced into pACCMV-RnLGS and pACCMV-RnLGS 2 + 3 plasmids using the forward primer (cctatacactatgcgccctggggttacacg) and reverse primer (CGTGTAAACCCCAGGGCGCATAGTATGG). A virus coding for the bacterial enzyme β-galactosidase, was used as a negative control in the metabolic impact studies. The DNAs encoding the recombinant proteins were sequenced using the ABI-PRISM DNA sequencing kit and an ABI-PRISM 377 automatic DNA sequencer (Applied Biosystems).

Hepatocyte Isolation, Culture, and Treatment with Recombinant Adenoviruses—Collagenase perfusion was used to isolate hepatocytes from male Wistar rats (180–225 g) fasted for 24 h, as described previously (20). Cells were suspended in Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10 mM glucose, 10% (v/v) fetal bovine serum (FBS), 100 nM insulin (Sigma), and 100 nM dexamethasone (Sigma), and then seeded onto plastic plates of 60-mm diameter treated with 0.001% (w/v) collagen solution (Sigma) at a final density of $8 \times 10^5$ cells/cm². Media were replaced with fresh DMEM containing 25 mM glucose and 1% (v/v) FBS, and cells were treated for 2 h with adenoviruses, at a similar multiplicity of infection. Media were then replaced by DMEM without glucose or FBS, and another incubation of 12–14 h was carried out. Cells were then incubated in DMEM in the absence or presence of 25 mM glucose, as described in the text and figure legends. At the end of each manipulation, cell monolayers were flash-frozen in liquid N2 and stored at −80 °C until analysis.

**FT02B Culture and Treatment with Recombinant Adenoviruses**—FT02B rat hepatoma cells (21) were cultured in 60-mm plates and kept in DMEM supplemented with 25 mM glucose and 10% (v/v) FBS. Media were replaced with fresh DMEM containing 25 mM glucose and 1% (v/v) FBS, and cells were treated for 2 h with adenoviruses at...
the same multiplicity of infection. After this time, media containing adenoviruses were replaced with fresh media containing 25 mM glucose and 1% (v/v) FBS, and cells were incubated for 24 h at 37 °C. Media were then replaced by DMEM without glucose or FBS, and another incubation of 12–14 h was performed. Cells were incubated in DMEM plus 25 mM glucose, as described in the text and figure legends. At the end of each manipulation, cell monolayers were frozen in liquid N2 and stored at −80 °C until analysis.

**Metabolite Determinations and Enzyme Activity Assays**—To measure GS activity in cell culture assays, cell culture plates frozen in liquid nitrogen were scrapped using 100 μl of ice-cold homogenization buffer consisting of 10 mM Tris-HCl, pH 7.4, 150 mM potassium fluoride, 15 mM EDTA, 15 mM 2-mercaptoethanol, 0.6 M sucrose, 1 mM benzamidine, 1 mM phenylmethylsulfonl fluoride, 25 mM okadaic acid, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 μg/ml pepstatin. The extracts were passed 10 times through a 25-gauge needle. Protein concentration was measured following the method of Bradford (22) using a Bio-Rad assay reagent. GS activity was measured in homogenates or in the supernatant and pellet fraction of hepatocyte extracts centrifuged at 10,000 × g for 15 min, in the presence or absence of 6.6 mM Glc-6-P (23). GS activity measured in the presence of Glc-6-P informs on the total amount of enzyme, whereas measurement in the absence of Glc-6-P is an indication of the active GS form(s). The −/+/ Glc-6-P activity ratio is an estimation of the activation state of the enzyme. Glycogen content was determined using an amyloglucosidase-based assay, as described elsewhere (24).

**Electrophoresis and Immunoblotting**—15 μg of protein of the GS activity homogenates was resolved by 10% SDS-PAGE, transferred onto a nitrocellulose membrane, and probed with the following antibodies: a rabbit antibody against rat LGS (16), a rabbit antibody against GS phosphorylated on Ser640 (PGS Ser640, Cell Signaling), or a mouse antibody against glyceraldehyde-3-phosphate dehydrogenase (Sigma). We also used a polyclonal affinity purified antibody generated against GS phosphorylated on site 2. The antibody was raised in rabbit against the peptide LGRGSLpS-VTSLGGL, which resembles residues 1–14 of rat LGS (PGS Ser7, Davids Biotechnologie). Secondary antibodies conjugated to horseradish peroxidase against rabbit (GE Healthcare) or mouse (Dako Cytomation) immunoglobulins were used. Immunoreactive bands were visualized using an ECL Plus kit (GE Healthcare), following the manufacturer’s instructions. Signals were quantified using Image J Software (NIH).

**Immunocytochemistry**—Hepatocytes seeded on collagen-coated coverslips (Sigma) were fixed for 30 min in phosphate-buffered saline containing 4% (v/v) paraformaldehyde. After fixation, cells were incubated with 1 mg/ml NaBH4 for 10 min and permeabilized for 20 min with phosphate-buffered saline containing 0.2% (v/v) Triton X-100. After 10 min of blocking
with phosphate-buffered saline containing 3% bovine serum albumin (w/v), incubation with the primary and secondary antibodies was carried out. Coverslips were washed, air-dried, and mounted onto glass slides using Mowiol (Sigma). We used primary antibodies against rat total LGS (16) and a monoclonal antibody against glycogen (25) (a gift from O. Baba, Tokyo Medical and Dental University). The secondary antibodies used were red dye-conjugated donkey anti-rabbit IgG (Anti-rabbit Texas Red, Jackson) and tetramethylrhodamine (TRITC)-conjugated goat anti-mouse IgM (Chemicon).

Statistical Analysis—Data are expressed as the mean ± S.E. Statistical significance was determined by the unpaired Student’s t test using Microsoft Excel (version XP; Microsoft Corp., Redmond, WA). Statistical significance was assumed at \( p \leq 0.05 \).

RESULTS

Effect of the Mutation of the Regulatory Sites on LGS Activity—To identify the key phosphorylation sites that regulate LGS activity, we generated seven adenoviral constructs coding for mutant forms of rat LGS. In each mutant one of the seven Ser residues homologous to the MGS phosphorylation sites (Fig. 1) was replaced by an Ala residue, thereby mimicking dephosphorylation. The mutated residues were Ser\(^7\) (site 2), Ser\(^{10}\) (site 2a), Ser\(^{640}\) (site 3a), Ser\(^{644}\) (site 3b), Ser\(^{648}\) (site 3c), Ser\(^{652}\) (site 4), and Ser\(^{656}\) (site 5) and the corresponding mutant forms are referred to as LGS 2, 2a, 3a, 3b, 3c, 4, or 5, respectively, and combinations thereof, when appropriate.

Rat primary hepatocytes were infected with these adenoviruses or with control adenoviruses encoding β-galactosidase from Escherichia coli (β-galactosidase) or wild-type rat LGS (LGS WT). Because glucose induces the dephosphorylation and activation of LGS (26–28), all assays were performed in the absence of the monosaccharide to prevent dephosphorylation of the Ser residues that were not mutated. By analyzing total GS activity in cell homogenates, we first checked that similar amounts of the LGS WT and mutant forms were expressed in all cases (Fig. 2A). We then measured the GS activity ratio in the supernatant and pellet fractions of the hepatocyte extracts expressing the various constructs (Fig. 2B). The endogenous total GS activity of the hepatocytes, as determined in the control cells that overexpress β-galactosidase (Fig. 2A), is negligible compared with the total activity found in the hepatocyte extracts overexpressing LGS WT or the phosphorylation mutants. Therefore, the activity ratios measured can be directly assigned to each mutant without any further correction.

We examined whether the activation induced by constitutive dephosphorylation of site 2 could be increased by the simultaneous dephosphorylation of a second site.
that combine Ser to Ala substitutions at site 2 plus an additional site showed somewhat higher activity ratios, although the differences were not statistically significant when compared with LGS 2.

Expression of LGS Phosphorylation Mutants in FTO2B Cells—Among hepatoma cell lines, FTO2B cells show the most consistent hepatocyte-like phenotype (21). Although this cell line expresses LGS, the enzyme is maintained in a highly phosphorylated inactive form, and these cells are unable to synthesize glycogen when incubated with glucose. Glycogen deposition can be restored through the expression of glucokinase (29, 30). The increased intracellular levels of Glc-6-P promote LGS allosteric activation but also its dephosphorylation by endogenous phosphatases (31). Given its lack of glucokinase, the FTO2B cell line is an adequate model to study the activation status of the LGS phosphorylation mutants and their capacity to drive glycogen deposition in the presence of glucose, without promoting the dephosphorylation of other LGS sites.

FTO2B cells were infected with adenoviruses encoding LGS WT or the mutant forms and were incubated in the presence of glucose. Determination of total GS activity showed that LGS WT and the mutant forms were expressed to similar levels (Fig. 3A). The activity ratios determined in the homogenates of FTO2B cells expressing endogenous LGS (cells infected with the β-galactosidase adenovirus), LGS WT, or the LGS 3a, 3b, or 3c variants were about 0.1 or below, whereas the extracts of cells expressing LGS 2, or the double mutants at this site plus one additional site, showed activity ratios above 0.5 (Fig. 3B). As in the experiments performed with hepatocytes, the differences between the activity ratios of the single LGS 2 mutant and the double mutants were not statistically significant. It must be noted that, because endogenous GS activity of the FTO2B cells was not negligible compared with the activity of the cells overexpressing LGS WT or the mutant forms (it represented from −1/5 to 1/3 of the total GS activity; Fig. 3A), the activity ratios determined in the FTO2B homogenates are an underestimation of the true values of the mutant forms with activity ratios higher than 0.1. Assuming that the activity ratio of 0.1, determined for endogenous LGS in control FTO2B cells infected with the β-galactosidase adenovirus (Fig. 3B), does not change in cells expressing LGS 2, the corrected figure for this mutant is 0.6.

These ratios nicely correlated with the capacity of the corresponding LGS phosphorylation mutants to trigger glycogen deposition in FTO2B cells: LGS WT and the 3a, 3b, and 3c variants produced very low levels of glycogen, similar to those found in control cells overexpressing β-galactosidase, whereas the single and double mutants containing the Ser to Ala mutation at site 2 yielded significantly higher levels of the polysaccharide (Fig. 3C).

Analysis of the Interaction between Phosphorylation Sites—We analyzed whether the Ser to Ala mutations of the other regulatory sites had any influence on the phosphorylation status of sites 2 and 3a of LGS expressed in hepatocytes. Using antibodies that recognize total LGS or LGS phosphorylated site 2 (Ser(Ph)3), we performed Western blot analysis of hepatocytes expressing LGS WT or the LGS single point mutants. Constitutive dephosphorylation of any of the other sites did not cause a significant reduction in the phosphate content of site 2 (Fig. 4A). A similar analysis was performed using an antibody that recognizes LGS only when site 3a is phosphorylated (Ser(Ph)640). In this case, for the LGS 3b variant there was a measurable decrease in phosphorylation at site 3a, whereas the remaining LGS mutants were all effectively phosphorylated at this site (Fig. 4B). Obviously, LGS 2 and LGS 3a were not detected by the
antibodies that recognize the corresponding phosphoserines 7 and 640, respectively.

As also observed for MGS (4), the LGS electrophoretic mobility in SDS-PAGE gels increased when sites 3a and/or 3b were dephosphorylated. Only the bands of LGS variants in which sites 3a or 3b were mutated presented larger mobility (Fig. 4). This observation corroborates the previous finding that Ser to Ala mutations at the other NH2- or COOH-terminal regulatory sites of LGS do not prevent phosphorylation of sites 3a and 3b.

**Effect of the Mutation of the Regulatory Sites on the Intracellular Distribution of LGS**—Incubation of isolated hepatocytes with glucose activates LGS and also causes its translocation from a homogeneous cytoplasmic distribution to the cell periphery (15–17). To evaluate the impact of the phosphorylation status of the regulatory sites on LGS subcellular distribution, we performed immunofluorescence analyses on hepatocytes expressing the LGS variants. Using the control hepatocytes infected with the β-galactosidase adenovirus, confocal laser intensity was adjusted so that endogenous LGS signal was not observable. Hepatocytes overexpressing LGS WT showed an enzyme uniformly distributed throughout the cytoplasm in the absence of glucose, as described for endogenous LGS (15, 16). However, LGS 2 presented the characteristic speckled pattern of a translocated enzyme at the initial stages of glycogen deposition, even when the hepatocytes were incubated in the absence of glucose. The remaining single-site LGS mutants behaved like LGS WT (Fig. 5). The double Ser to Ala mutations at site 2 plus one of the other six regulatory sites also produced enzymes that, in the absence of glucose, concentrated in the form of speckles near the hepatocyte cortex (data not shown). Because the pattern shown by the LGS 2 mutant was characteristic of hepatocytes synthesizing glycogen, we checked whether this mutant was able to drive the deposition of the polysaccharide in hepatocytes incubated without glucose. Immunofluorescence analysis with an antiglycogen antibody (25) showed that whereas hepatocytes expressing LGS WT or any of the other mutants did not accumulate glycogen in the absence of glucose, cells overexpressing LGS 2 (Fig. 6) or LGS double mutants (site 2 plus another site; data not shown) did synthesize glycogen, even in these conditions.

Finally, we examined whether translocation could be dissociated from glycogen synthesis. To this end, we generated adenovirus coding for the catalytically inactive variants LGS E509A and LGS 2 + 3b E509A. We have previously shown that in MGS the replacement of Glu509 by an Ala residue yields an inactive enzyme (13). Hepatocytes infected with these adenoviruses expressed similar amount of the corresponding mutant enzymes, as revealed by Western blot analysis (Fig. 7A). The extracts of control hepatocytes infected with the β-galactosidase adenovirus and those of cells overexpressing LGS E509A and LGS 2 + 3b E509A showed identical total GS activity (Fig. 7B), thereby confirming that the catalytic mechanism of LGS was impaired by the mutation. Accordingly, no changes were found in the glycogen content of LGS E509A, LGS 2 + 3b E509A, and β-galactosidase overexpressing cells (Fig. 7C). Confocal microscopy analysis of the intracellular distribution of LGS E509A and LGS 2 + 3b E509A in cultured hepatocytes revealed that, in both the presence and absence of glucose, the two catalytically inactive mutants exhibited a distribution identical to the wild-type enzyme (Fig. 8). In the absence of glucose they uniformly distributed throughout the cytoplasm, but concentrated in the cell cortex when the monosaccharide was added, probably binding to glycogen produced by the endogenous enzyme.

**DISCUSSION**

Although the activity of the two mammalian isoforms of glycogen synthase, MGS and LGS, is controlled by reversible phosphorylation and allosteric effectors, the fine details of the regulation differ between the two enzymes. For MGS, the acti-
vation state of Ser to Ala variants at the phosphorylation sites was not significantly different to that of the wild-type enzyme, when mutagenesis was restricted to either the NH2 or COOH terminus (4). An MGS mutant at site 2 showed an activity ratio of only 0.03, and an additional Ser to Ala mutation at site 3a or 3b was required to substantially increase this value (4). In contrast, the sole dephosphorylation of LGS at site 2 drastically raised its activity ratio up to about 0.6, both in hepatocytes and FTO2B cells. This value is very close to that previously found for an LGS mutant containing six Ser to Ala substitutions at sites 2, 3a, 3b, 3c, 4, and 5, which showed an activity ratio of 0.65 (7). Thus, it appears that the single LGS 2 mutant is essentially fully active and that most of the control exerted by phosphorylation on LGS activity resides in site 2 (Ser7). The equivalent MGS variant with six Ser to Ala substitutions at the homologous sites possesses an activity ratio of ~0.8 (5). This observation further highlights the differences between MGS and LGS, as it shows that the capacity of Glc-6-P to further increase the activity of a fully dephosphorylated enzyme is greater in the liver than in the muscle isoform of GS. The lack of a model that adequately explains at the molecular level how phosphorylation and Glc-6-P regulate the activity of these enzymes makes it impossible to address these differences at this time.

An orderly mechanism for the phosphorylation of the COOH-terminal sites of GS has been proposed previously (10, 11, 32). According to this hypothesis, phosphorylation at sites 3a, 3b, 3c, 4, and 5 proceeds in a hierarchical fashion, beginning with the introduction of a phosphate moiety at site 5 followed by the sequential action of GSK-3 at the four adjacent sites. Our results do not support this hypothesis and show that mutation of COOH-terminal sites 3c, 4, or 5 does not prevent phosphorylation at site 3a of the corresponding LGS mutants expressed in cultured hepatocytes. Only the removal of site 3b has some influence on the extent to which site 3a is phosphorylated. Similar results were obtained for MGS, which can be efficiently phosphorylated at sites 3a and 3b in COS cells or Rat1 fibroblasts, even when the GSK-3 recognition motif is disrupted by Ser to Ala mutations at sites 3c, 4, or 5 (4–6, 33). We conclude that alternative and/or additional mechanisms, involving protein kinases other than GSK-3, operate in hepatocytes for the COOH-terminal phosphorylation of LGS.

Our results also raise a question about the function of the phosphorylation sites located at the COOH terminus of GS, because they apparently exert little or no effect on the activation state of this enzyme. One explanation may be that these sites have functions other than the regulation of GS activity that have not yet been uncovered. However, it must be noted that the approach used in this work and previous studies with MGS (4, 6) to dissect the role of each regulatory site, namely the replacement of Ser residues by non-phosphorylatable Ala residues, introduces radical changes in the capacity of the enzyme to incorporate phosphate units. The true situation is likely to be
Signal (right column). Laser intensity was adjusted so that endogenous LGS activity in response to distinct stimuli, the phosphorylation and activation or inactivation of LGS is the result of more subtle variations in the overall phosphate content of the molecules, the molar phosphate content of a given site may not change all the way from one to zero. As many in vivo studies have shown (34–37), these changes are almost invariably fractional and activation or inactivation of LGS is the result of more subtle variations in the overall phosphate content of the enzyme. In this scenario, which allows for a better fine tuning of LGS activity in response to distinct stimuli, the phosphorylation sites at the COOH terminus may indeed be relevant in the control of the LGS activation state. The observations concerning the effect of LiCl, a potent inhibitor of GSK-3, point in this direction (38). Incubation of cultured hepatocytes with lithium ions provokes a concentration- and time-dependent increase in the LGS activity ratio (39), which, however, reaches a maximum value of 0.3 after 16 h of treatment (16).

Another relevant finding of this study is that the constitutive activation of LGS, through a Ser to Ala mutation at site 2, yields an enzyme that is able to cause net glycogen deposition in hepatocytes, in the absence of glucose, and in FTO2B cells. The hepatoma cell line possesses all the machinery to synthesize glycogen, with the only exception glucokinase. This glucose-phosphorylating enzyme is necessary to produce Glc-6-P at the required levels or in the adequate compartment (30, 40) to active LGS and, therefore, trigger glycogen synthesis in response to glucose (41). In FTO2B cells, the LGS 2 mutant bypasses this requirement by means of its constitutively high activation state. The explanation for the cultured hepatocytes is more complex, because they accumulate glycogen even when they are incubated in the absence of glucose. The glucose units incorporated into the glycogen molecule must therefore arise from gluconeogenic precursors present in the culture medium. DMEM contains 4 mM glutamine and a total amino acid concentration of 10.7 mM. Hepatocytes expressing the LGS variant with six Ser to Ala mutations at sites 2, 3a, 3b, 3c, 4, and 5 were shown to accumulate large amounts of glycogen, preferentially using the “indirect pathway” rather than extracellular glucose via the “direct pathway,” even when incubated in the presence of 5.5 mM glucose (7). The capacity of cultured hepatocytes (42) and livers of live rats (43) to accumulate glycogen was also greatly increased when they overexpressed the protein targeting to glycogen (PTG). Also, in these cases the indirect pathway was the preferred route for the synthesis of the polysaccharide. One apparent difference, however, resides in the observation that PTG overexpression has a direct impact on the glycogen metabolizing machinery as a whole and for example, apart from increasing the activation state of LGS, it also produces a 40% decrease in glycogen phosphorylase activity (42). In contrast, LGS 2 and the double mutants cause net glycogen accumulation in a situation in which liver glycogen phosphorylase should presumably be active, because of the absence of glucose in the culture medium.

When hepatocytes are incubated with glucose, LGS moves from the cytoplasm to the cell cortex, where it forms aggregates, the size of which increases with time (15, 16). Although glycogen content is measurable, LGS intracellular distribution closely resembles that of the polysaccharide, thereby indicating that LGS remains bound to its substrate and product in the interior of the hepatocyte (16). We have also shown that when glycogen stores are low, the glycogenenogenic sites in which synthesis of the polysaccharide begins are located in the hepatocyte periphery. However, as glycogen deposition progresses, more glycogenenogenic sites are generated from the cortex to the hepatocyte interior (17). Here we report that dephosphorylation at site 2 of LGS causes the accumulation of the enzyme at the hepatocyte periphery, even in the absence of glucose. In contrast, the inactive LGS 2 + 3b E509A variant only translocated in the presence of the monosaccharide. We believe that this behavior is simply a reflection of the capacity of (activated) LGS...
to bind glycogen rather than the dephosphorylation of site 2 being a signal for the translocation of the enzyme. Thus, the constitutively active LGS 2 mutant would bind to the peripheral glycogenenogenic sites and, even in the absence of glucose, initiate the synthesis of glycogen, which in turn could bind more LGS molecules, thereby leading to the aggregation and accumulation of the enzyme observed at the hepatocyte cortex. The inactive LGS 2 + 3b E509A mutant also binds glycogen produced by the endogenous LGS in the presence of glucose. However, because this variant cannot catalyze glycogen deposition, it does not generate new glycogenogenic sites to which other LGS molecules can bind when glucose is absent from the culture medium. Relevant in this context is the observation that the LiCl-induced activation of LGS, although probably involving phosphorylation sites other than site 2, does not promote net glycogen synthesis nor LGS translocation in hepatocytes incubated without glucose (16, 39). This result also indicates that, as long as there is no glycogen deposition, covalent activation of LGS does not lead to its translocation.

In conclusion, site 2 of LGS (Ser1) is the most potent regulatory site of the activity of the enzyme. Its dephosphorylation, which yields an almost fully active enzyme, is not sufficient to trigger LGS translocation to the hepatocyte cortex, and net glycogen accumulation is also required to observe this phenomenon.

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REFERENCES

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