Hepatocellular expression of glucose-6-phosphatase is unaltered during hepatic regeneration

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2Division of Gastroenterology and Hepatology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22901; and 3Section of Digestive and Liver Diseases, University of Illinois at Chicago College of Medicine and Chicago Veterans Affairs Medical Center-Westside Division, Chicago, Illinois 60612-7323

Zakko, Wsam F., Carl L. Berg, John L. Gollan, and Richard M. Green. Hepatocellular expression of glucose-6-phosphatase is unaltered during hepatic regeneration. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G717–G722, 1998.—Glucose-6-phosphatase and glycogenolysis are essential hepatic functions required for glucose homeostasis. During the initial phase of hepatic regeneration, the immediate-early genes (IEG) are rapidly expressed, and the IEG RL-1 encodes for glucose-6-phosphatase (G-6-Pase). G-6-Pase is a microsomal enzyme essential for gluconeogenesis and glycogenolysis. This study employs a partial-hepatectomy model to examine the expression and activity of G-6-Pase. After partial hepatectomy, rat hepatic G-6-Pase gene expression is transcriptionally regulated, and mRNA levels are increased ~30-fold. However, in contrast to this rapid gene induction, microsomal enzyme activity is unchanged after partial hepatectomy. Western blotting demonstrates that microsomal G-6-Pase protein expression is also unchanged after partial hepatectomy, and similar results are also noted in whole liver homogenate. Thus, despite marked induction in gene expression of the IEG G-6-Pase after partial hepatectomy, protein expression and enzyme activity remain unchanged. These data indicate that, although this hepatocyte IEG is transcriptionally regulated, the physiologically important level of regulation is posttranscriptional. This highlights the importance of correlating gene expression of IEG with protein expression and physiological function.

METHODS

G-6-Pase is a hepatic microsomal enzyme critical for gluconeogenesis, and several metabolic diseases of glucose metabolism result from defects in the G-6-Pase system (5, 19, 20, 25). In addition, hypoglycemia remains a potentially life-threatening complication of severe hepatic failure, a pathophysiological state where hepatic regeneration is critical for maintaining hepatic function. Therefore, the regulation of G-6-Pase activity during disease states associated with hepatic regeneration remains extremely important for the viability of the organism.

A two-thirds partial hepatectomy is a well-established model for the hepatic regeneration occurring in response to hepatocellular injury. In the rat, 24 h after two-thirds hepatectomy, the liver returns to one-half of its original mass, and complete regeneration occurs after 7–10 days. The partial-hepatectomy model has been employed to characterize the gene expression of IEG, and it has been previously demonstrated that steady-state mRNA levels of G-6-Pase increase ~25-fold within 30 min of partial hepatectomy (11, 13). However, protein expression and enzyme activity in the endoplasmic reticulum, the cellular compartment where G-6-Pase is critical for glucose metabolism, have not been previously examined.

In this study, we demonstrate that, although G6PT gene transcription and mRNA levels increase dramatically after partial hepatectomy, protein expression and enzyme activity remain unchanged. This occurs in both hepatic microsomes enriched in endoplasmic reticulum and in liver homogenate and with both detergent-treated and latent vesicles. This marked increase in gene expression of the IEG G-6-Pase, with the concomitant lack of change in both protein expression and enzyme activity of this metabolic protein, demonstrates the importance of correlating gene expression of the IEG with the physiological functions for which these genes encode. In addition, these findings have important implications for the glucose metabolism occurring during pathophysiological states in which hepatic regeneration occurs and for the regulation of this essential hepatic enzyme.

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dylate were obtained from Fisher Chemicals (Springfield, NJ). Bradford assay kit for protein determination was obtained from Bio-Rad Laboratories (Richmond, CA). Percoll and density marker beads were purchased from Pharmacia (Milwaukee, WI). Ascorbic acid was obtained from Aldrich (Milwaukee, WI). Oligo(dt) cellulose, type 3, was purchased from Collaborative Biomedical Products (Bedford, MA). Polyclonal sheep anti-G-6-Pase was a gift from Dr. Anne Burchell (University of Dundee, Scotland, UK), and horseradish peroxidase-linked rabbit anti-sheep IgG antibody was obtained from Pierce (Rockford, IL).

Animals and experimental preparations. Male Sprague-Dawley rats weighing 250–350 g, obtained from Charles River Farms (Wilmington, MA), were subjected either to partial hepatectomy or sham laparotomy. Animals were allowed free access to standard laboratory chow and water preoperatively and laboratory chow and a 10% solution of glucose in water postoperatively. Rats were maintained in a constant-temperature environment under controlled light conditions. Care of the animals was under the supervision of the Harvard Medical School Animal Management Program in compliance with National Institutes of Health guidelines. Surgery was performed under ether anesthesia, and when sham operations were performed, the animals were exteriorized and manipulated but not resected. Partial hepatectomy was performed as previously described (15, 27). After surgery, the animals received 10 ml of sterile 0.9% saline subcutaneously and were warmed under a heat lamp until they regained consciousness. Additional experiments documented that results were similar whether supplemental oral glucose or subcutaneous saline injection was either administered or omitted postoperatively. All microsomal preparations were carried out at 4°C. The animals were killed by decapitation, and microsomes were prepared using Percoll centrifugation as previously described (27). Experiments were performed either on freshly prepared microsomes or homogenate or within 48 h of storage at −70°C. Protein was determined according to the Bradford technique using bovine serum albumin as a standard.

Assay of G-6-Pase activity. G-6-Pase activity was measured spectrophotometrically in intact and detergent-treated microsomes and homogenate (2). Detergent treatment was carried out by incubating microsomes and homogenate with 0.1% Triton X-100 for 20 min on ice. The concentration of Triton X-100 was selected after experiments were performed with different dilutions of detergent, because it was efficacious for complete membrane lysis, yet did not interfere with enzyme assays. The assay medium (100 μl) contains 50 mM sodium cacodylate, pH 6.5, 2 mM EDTA, and 6 mM G-6-Pase. The reaction was initiated by adding 20 μl of microsomal protein or homogenate. The reaction time was 10 min at 30°C, after which the reaction was stopped by adding 0.9 ml of stop solution (6 vol of 0.42% ammonium molybdate in 1 N H2SO4, 2 vol of 10% SDS solution, and 1 vol of 10% ascorbic acid). The reaction time was determined after preliminary experiments demonstrated linear activity at 0–15 min. After an additional incubation for 1 h at 37°C, absorbance was measured at 820 nm, and enzyme activity was then converted to nanomoles of Pi per milligram protein per minute. All measurements were performed in triplicate for each membrane preparation, with a minimum of three preparations for each time point assayed.

Western and Northern blotting. In Western blotting experiments, equal quantities of microsomal protein or cellular homogenate isolated from the partial hepatectomy and sham laparotomy rats were loaded per lane, and protein electrophoresis was performed using a 10% polyacrylamide gel. Proteins were then electrophoretically transferred to nitrocellulose paper for 2 h. Protein transfer was verified using ponceau S staining, and the nitrocellulose blots were then blocked overnight at 4°C in a solution of Tris-buffered saline (TBS) with 5% nonfat milk and 0.05% bovine serum albumin. After the blots were washed with TBS, the blots were then incubated with the polyclonal sheep anti-G-6-Pase antibody (1:300) for 1 h at 25°C. The blots were then washed, reblocked in TBS with 5% fetal calf serum, and, after repeat washing, incubated with the horseradish peroxidase-labeled secondary antibody (1:5,000). Enhanced chemiluminescence (Amerham, Arlington Heights, IL) was then performed according to the manufacturer’s instructions. In vitro translation of polyadenylated RNA was performed using a rabbit reticulocyte lysate in vitro translation kit (Promega, Madison, WI) according to the directions of the manufacturer. Northern blotting was performed as previously described (10), employing a probe derived from a cDNA encoding for murine G-6-Pase. Polyadenylated RNA was isolated and purified using an oligo(dt) cellulose column purification. The blots were subsequently stripped and reprobed using a probe derived from a ubiquitin (which remains unchanged after partial hepatectomy (11)) cDNA to confirm the RNA integrity and verify equal loading.

In vitro transcriptional (nuclear runoff) assays. Nuclear runoff assays were performed as previously described (9). Rat livers were Dounce homogenized 10 times in 10 ml of 0.32 M sucrose-3 mM MgCl2-1 mM HEPES-5 mM dithiothreitol (DTT), pH 6.8. After centrifugation for 10 min in a J-A20.1 at 3,000 revolutions/min at 4°C, the pellet was resuspended in 7 ml of 1.65 M sucrose-5 mM MgCl2-1 mM HEPES-5 mM DTT, pH 6.8, layered over 4 ml of 2.1 M sucrose-3 mM MgCl2-1 mM HEPES-5 mM DTT, pH 6.8, and centrifuged for 1 h at 70,000 g at 4°C. The nuclei were resuspended in 200 μl of storage buffer [40% (vol/vol) glycerol-5 mM MgCl2-0.1 mM EDTA-0.5 M Tris-Cl, pH 8.3] and 200 μl of 5 mM MgCl2-0.3 M KCl-100 μM RNasin-10 mM Tris-Cl, pH 8.0, and 10 μl of [α32P]UTP (3,000 Ci/ml) were immediately added. After incubation for 30 min at 30°C, 600 μl of 0.5 M NaCl-50 mM MgCl2-2 mM CaCl2-10 mM Tris-Cl, pH 7.4, with 24 μg of RNAse-free DNAse was added and incubated 5 min at 30°C. Next, 200 μl of 5% (wt/vol) SDS-0.125 M EDTA-0.5 M Tris-Cl, pH 7.4, containing 5 μg of yeast RNA and 100 μg/ml of proteinase K were added and incubated for 30 min at 42°C. RNA was extracted with phenol-chloroform-isomyl alcohol (25:24:1) and ethanol precipitated, the pellet was resuspended in 500 μl of diethyl pyrocarbonate-H2O and denatured at 70°C for 5 min, and an aliquot containing 107 counts/min was added to 2 ml of hybridization solution. The filters were hybridized 72 h, washed for 20 min in 2× saline sodium citrate (SSC) at 42°C, treated 5 min with 10 μg/ml of RNase A at 37°C, and rewarmed two times in 2× SSC, followed by autoradiography.

Statistical analysis. Values are expressed as means ± SD. Student’s t-test was used for statistical comparisons, and P values < 0.05 were deemed as statistically significant.

RESULTS

To document that hepatic mRNA levels of G6PT, the gene encoding for G-6-Pase, rapidly increase after partial hepatectomy, we initially performed two-thirds hepatectomies and sham laparotomies on rats, isolated total liver mRNA, and measured G6PT mRNA levels using Northern blot analysis. G6PT mRNA levels increased ~30-fold within 30 min after partial hepatectomy and remained markedly elevated for 24 h (Fig. 1A). These findings are similar to recently published
data (11, 13). Similar results were noted when polyadenylated liver RNA was isolated from rats subjected to partial hepatectomy or sham laparotomy (Fig. 1A). To determine whether this rapid increase in G6PT expression was transcriptionally regulated, in vitro transcription assays were performed. Consistent with the Northern analysis, gene transcription of G6PT was markedly increased after partial hepatectomy (Fig. 2), indicating that the increased gene expression is transcriptionally regulated.

Having confirmed that G6PT mRNA levels increase ∼30-fold after partial hepatectomy, we examined the effect of hepatic regeneration on microsomal protein expression and enzyme activity. After partial hepatectomy or sham laparotomy, rat hepatic microsomes were isolated using sequential sucrose- and Percoll-gradient centrifugation (1). We have recently demonstrated (employing a Percoll-gradient centrifugation after a standard sucrose-gradient centrifugation) that highly latent microsomes can be isolated from rats subjected to partial hepatectomy which have an endoplasmic reticulum enzyme enrichment similar to those isolated from control rats subjected to sham laparotomy (27).

Hepatic microsomes were isolated from rats 30 min, 2 h, and 24 h after partial hepatectomy or sham laparotomy. After protein quantitation and membrane solubilization in Lamelli buffer, equal amounts of microsomal protein were loaded per lane and subjected to SDS-PAGE. After electrophoretic transfer to nitrocellulose membranes, Western blotting was performed using an antibody directed against G-6-Pase. Despite dramatic increases of G-6-Pase mRNA levels after partial hepatectomy, microsomal protein expression was unchanged 2, 24, and 48 h after partial hepatectomy, compared with sham laparotomy controls (n = 4; Fig. 3). In vitro translation of polyadenylated liver RNA, followed by immunodetection with anti-G-6-Pase antibody, failed to detect synthesized protein in either the experimental or sham groups.

To further confirm that microsomal G-6-Pase levels are unaffected by hepatic regeneration, enzyme activity was assayed in both latent and detergent-treated microsomes. Microsomal enzyme activity in latent microsomes was similar in animals subjected to partial hepatectomy or sham laparotomy (219 ± 65, 205 ± 23, and 271 ± 22 nmol P_i · mg protein −1 · min −1 2, 24, and 48 h after partial hepatectomy vs. 238 ± 47, 261 ± 46, and 234 ± 52 nmol P_i · mg protein −1 · min −1 2, 24, and 48 h after sham laparotomy, respectively). The data suggest that the increased G-6-Pase mRNA levels after partial hepatectomy are not translated into increased microsomal protein expression.

Fig. 1. Northern blot of glucose-6-phosphatase (G-6-Pase) gene expression after partial hepatectomy. Rats were subjected to a two-thirds hepatectomy, and total liver mRNA was isolated after 0.5, 2, or 24 h. Each lane was loaded with 15 µg of total mRNA, and Northern blotting was performed using a radiolabeled cDNA probe for G-6-Pase. The blots were stripped and reprobed with ubiquitin cDNA (A). In similar experiments, 16 h after partial hepatectomy (PH) or sham laparotomy (SL), polyadenylated RNA was isolated, and 1 µg was used for Northern blot analysis (B). G-6-Pase mRNA levels rapidly increase 30-fold within 0.5 h of partial hepatectomy and remain elevated for 24 h.

Fig. 2. In vitro transcription of G6PT after partial hepatectomy. Two hours after partial hepatectomy or sham laparotomy, in vitro transcription assays were performed. G6PT or ubiquitin cDNA in vector Bluescript and vector Bluescript alone (5 µg) were loaded onto nylon membranes and hybridized for 72 h with transcribed RNA. This figure is a representative experiment demonstrating that G6PT transcription is increased in nuclei isolated 2 h after partial hepatectomy compared with sham laparotomy controls (n = 3). Similar results were detected 30 min after partial hepatectomy.
partial hepatectomy and sham laparotomy groups. Somal enzyme activity was again similar in both the partial hepatectomy or sham laparotomy, membranes were solubilized in Laemmli buffer, and equal quantities of protein were loaded in each lane. After SDS-PAGE, Western immunoblotting was performed using a polyclonal antibody directed against G-6-Pase. This representative experiment demonstrates that hepatic microsomal protein expression of G-6-Pase is unchanged 24 and 48 h after partial hepatectomy. Similar results were noted 2 h after surgery.

234 ± 54 nmol Pi · mg protein−1 · min−1 after sham laparotomy; Fig. 4; n = 3–4). Detergent treatment increases G-6-Pase enzyme activity, likely because it alleviates the requirement for transmembrane transport of enzyme substrates and end products (2). When microsomes were detergent treated with Triton X-100, G-6-Pase enzyme activity increased. However, microsomal enzyme activity was again similar in both the partial hepatectomy and sham laparotomy groups (399 ± 55, 342 ± 29, and 408 ± 36 nmol Pi · mg protein−1 · min−1 2, 24, and 48 h after partial hepatectomy vs. 389 ± 26, 401 ± 34, and 405 ± 35 nmol Pi · mg protein−1 · min−1 after sham laparotomy; Fig. 4; n = 4). Therefore, despite the marked increase in G-6-Pase mRNA levels after partial hepatectomy, microsomal enzyme activity remains unchanged. These data are consistent with the levels of protein expression detected on Western blot analysis.

Although G-6-Pase is known to be an enzyme primarily localized in the endoplasmic reticulum (3, 7, 16, 24, 25), discrepancies between hepatic expression of mRNA and microsomal protein could potentially be due to an altered targeting of G-6-Pase to other cellular compartments during hepatic regeneration. It is possible that hepatic protein expression of this enzyme could increase after partial hepatectomy, yet the amount of G-6-Pase in the endoplasmic reticulum would remain unchanged. Therefore, we subsequently measured G-6-Pase protein expression and enzyme activity in whole liver homogenate. By assaying levels in hepatic homogenate, any significant protein expression that may be targeted to cellular compartments other than the endoplasmic reticulum would be detected.

Hepatocellular protein expression and enzyme activity of G-6-Pase was also unchanged after partial hepatectomy. Western blot analysis revealed that G-6-Pase expression in hepatic homogenate was comparable 2, 24, and 48 h after partial hepatectomy compared with sham laparotomy controls (Fig. 5). Consistent with this finding, G-6-Pase enzyme activity remained unchanged during hepatic regeneration in both intact and detergent-treated liver homogenate. Enzyme activity in liver homogenate was 73 ± 13, 69 ± 7, and 58 ± 2 nmol Pi · mg protein−1 · min−1 2, 24, and 48 h after partial hepatectomy vs. 63 ± 6, 69 ± 12, and 61 ± 5 nmol Pi · mg protein−1 · min−1 after sham laparotomy (Fig. 6). After Triton X-100 treatment, enzyme activity in liver homogenate was 95 ± 15, 96 ± 13, and 96 ± 18 nmol Pi · mg protein−1 · min−1 2, 24, and 48 h after partial hepatectomy vs. 90 ± 16, 93 ± 9, and 103 ± 14 nmol Pi · mg protein−1 · min−1 after sham laparotomy (Fig. 6). Therefore, the dramatically elevated G-6-Pase mRNA levels that are induced during hepatic regeneration do not result in an increase in either hepatic protein expression or functional activity of the enzyme.

DISCUSSION

The liver plays a central role in regulating blood glucose levels, and G-6-Pase is an endoplasmic reticulum protein that is essential for both gluconeogenesis and glycogenolysis (6, 20). The regulation of this microsomal enzyme is critical for both normal physiology and in pathophysiological states such as hepatic failure where hypoglycemia may be a potentially life-threatening complication. Partial hepatectomy is a well-

![Fig. 3. Western immunoblotting of microsomal protein expression of G-6-Pase after partial hepatectomy or sham laparotomy.](Image)

![Fig. 4. Hepatic microsomal G-6-Pase enzyme activity after partial hepatectomy or sham laparotomy.](Image)

![Fig. 5. Western immunoblotting of G-6-Pase protein expression in liver homogenate after partial hepatectomy or sham laparotomy.](Image)
characterized model of the hepatic regeneration that occurs in response to hepatic injury. It provides an animal model in which the normally quiescent hepatocytes proliferate and express a large number of growth-related genes. However, the liver must maintain many metabolic and synthetic functions, including glucose homeostasis, while it regenerates. After partial hepatectomy, portal vein levels of gluconon rapidly increase, and insulin levels fall. These hormonal changes help enable the remaining liver to maintain relative euglycemia despite removal of two-thirds of the hepatic mass.

Increased expression and function of the hepatic endoplasmic reticulum enzyme G-6-Pase after partial hepatectomy could provide a potential mechanism to allow the liver to maintain adequate serum glucose levels and avoid hypoglycemia. In addition, because the mRNA levels of this gene are rapidly induced 30-fold after two-thirds hepatectomy, this gene has been identified to be an IEG expressed during hepatic regeneration (12, 13, 22). Therefore, we have investigated the effect of partial hepatectomy on microsomal protein expression and enzyme activity of G-6-Pase.

We have demonstrated in the rat that, 30 min after partial hepatectomy, G6PT mRNA levels are dramatically increased, and this increase in gene expression persists for 24 h. These findings are in agreement with previously published data (12, 13) and are consistent with the hypothesis that G6PT is a hepatic IEG. This increased gene expression is transcriptionally regulated, similar to other IEG. Because this putative IEG encodes for an endoplasmic reticulum protein, we subsequently investigated the effect of partial hepatectomy on microsomal protein expression. In sharp contrast to the rapid rise of steady-state mRNA levels, Western blots revealed that microsomal protein expression was unchanged. Although mRNA levels dramatically increased within 30 min after partial hepatectomy, protein levels were unaffected, even at time points extending to 48 h.

Because regulation of the enzyme activity of G-6-Pase is critical for glucose metabolism during disease states where hepatic regeneration occurs, we also measured hepatic microsomal G-6-Pase enzyme activity after partial hepatectomy or sham laparotomy. Enzyme activity of G-6-Pase was unchanged during hepatic regeneration. Activity was similar in latent hepatic microsomes isolated from both rats subjected to partial hepatectomy or sham laparotomy. Because the transmembrane transport of substrates or end products can effect enzyme activity (4), we also measured G-6-Pase activity in detergent-treated microsomes. Enzyme activity was again similar in the partial hepatectomy and control groups. These data on enzyme activity support our findings of Western blot analysis in which microsomal G-6-Pase protein expression was unaffected.

Finally, because dramatically elevated mRNA levels of G6PT could result in a markedly increased synthesis of hepatic protein, which could potentially be targeted to cellular compartments other than the endoplasmic reticulum, we also examined the effect of partial hepatectomy on protein expression and enzyme activity in whole liver homogenate. As was demonstrated in experiments performed on hepatic microsomes, neither protein expression nor enzyme activity in latent and detergent-treated liver homogenate was altered after partial hepatectomy. These data indicate that, after partial hepatectomy, the dramatic increase in steady-state G6PT mRNA levels does not result in an increase of either protein expression or enzyme activity. The discrepancy in protein and gene expression could potentially be due to inefficient translation or increased protein turnover. Unfortunately, attempts to measure in vitro protein translation using polyadenylated hepatic RNA and rabbit reticulocyte lysate, with immunodetection with Western blotting, failed to detect G-6-Pase (either pre- or posthepatectomy), whereas control translation reactions were detectable. The inability to detect G-6-P protein translation after hepatectomy is consistent with the lack of increased G-6-Pase protein expression, despite abundant expression of G6PT mRNA after partial hepatectomy.

G-6-Pase is an important enzyme for glucose homeostasis and for the prevention of hypoglycemia. Six to twelve hours after two-thirds hepatectomy, there is a statistically significant decrease in serum glucose, although the decrease is mild and hypoglycemia does not occur (21, 26). In contrast, hypoglycemia is present 6–24 h after subtotal (80%) hepatectomy (26). Because our study employed a two-thirds hepatectomy model, it is unlikely that the increased G6PT gene expression was caused by hypoglycemia. Previous studies have demonstrated that hepatic glycogen stores are reduced after partial hepatectomy, an effect that can be prevented by glucose infusion (14, 23). Regulation of G-6-Pase may potentially be important in maintenance of glycogen stores under these conditions. However, our data indicate that, despite the changes in G6PT gene expression, G-6-Pase enzyme activity was unchanged.
in both rat liver microsomes and homogenate after two-thirds hepatectomy.

These findings are in contrast to other recently published data (11, 13). In agreement with these studies, partial hepatectomy induces a dramatic rise of steady-state mRNA. However, in contrast to these studies, we have examined the protein expression and enzyme activity of hepatic microsomes enriched in endoplasmic reticulum (the cellular compartment where G-6-Pase is physiologically active (4, 7, 16, 19, 24)) and liver homogenate. Based on our findings, we conclude that the increased gene transcription and expression do not result in an increase in protein expression in the endoplasmic reticulum, and therefore, during hepatic regeneration, gene expression of G-6-Pase is not an important level of regulation.

In summary, these data indicate that, although hepatic gene expression of G-6-Pase is markedly increased after partial hepatectomy, protein expression and enzyme activity remain unchanged. These findings have important implications for glucose metabolism in pathophysiological states such as fulminant hepatic failure, where hepatic regeneration is essential for the maintenance of adequate hepatic function, and hypoglycemia may be a potentially life-threatening complication. These data also indicate that this hepatocyte IEG may be regulated in a posttranscriptional manner and highlights the importance of correlating gene expression of IEGs with protein expression and physiological function.

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