Hepatic Glycogen Synthesis in the Absence of Glucokinase
THE CASE OF EMBRYONIC LIVER

Received for publication, August 1, 2007, and in revised form, December 20, 2007 Published, JBC Papers in Press, December 28, 2007, DOI 10.1074/jbc.M706334200

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Glucokinase (GK, hexokinase type IV) is required for the accumulation of glycogen in adult liver and hepatoma cells. Paradoxically, mammalian embryonic livers store glycogen successfully in the absence of GK. Here we address how mammalian embryonic livers, but not adult livers or hepatoma cells, manage to accumulate glycogen in the absence of this enzyme. Hexokinase type I or II (HKI, HKII) substitutes for GK in hepatomas and in embryonic livers. We engineered FTO2B cells, a hepatoma cell line in which GK is not expressed, to unveil the modifications required to allow them to accumulate glycogen. In the light of these results, we then examined glycogen metabolism in embryonic liver. Glycogen accumulation in FTO2B cells can be triggered through elevated expression of HKI or either of the protein phosphatase 1 regulatory subunits, namely PTG or GL.

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During gestation, the liver of the fetus is the main source of energy at birth, from maternal starvation. The embryonic liver safeguards its glycogen deposits, required as the main source of energy at birth, from maternal starvation.

In adult mammalian liver, the expression of the liver isoform of glycogen synthase (LGS) (EC 2.4.1.11) is present. The muscle isoform (MGS) is most abundant in this tissue but can be found in a variety of organs. In contrast, the liver isoform (LGS) is restricted to hepatocytes. Both isoenzymes are regulated by phosphorylation at multiple sites and are active when in a hypophosphorylated state. The mechanism of GS dephosphorylation, which leads to the conformational activation of GS, involves the targeting of protein phosphatase 1 to glycogen by the adaptor proteins PTG (protein targeting to glycogen) (9) and Gf (10). Glc-6-P, which is a powerful activator of GS, enhances the dephosphorylation of this enzyme, probably by inducing a conformational change that converts GS into a better substrate for protein phosphatase 1 (11). GK produces Glc-6-P in adult liver, thereby exerting a strong positive control on hepatic glycogen deposition (12). In re-fed rats, hepatic Glc-6-P levels must reach a threshold concentration before LGS is activated (13). The key role of GK in hepatic glycogen deposition is most apparent when GK gene dosage is altered in vivo. It has been shown that an increase in the GK levels in livers of healthy animals leads to augmented hepatic glycogen deposition and a subsequent reduction in glycemia (14). On the contrary, ablation of GK in liver leads to reduced hepatic glycogen stores and hyperglycemia (15). Similarly, mice lacking GK regulatory protein show decreased glycogen accumulation (6). However, in mouse models of diabetes mellitus in which glycogen deposition is impaired, transgenic overexpression of GK in liver enhances glycogen synthesis and normalizes blood glucose levels (16, 17). Similarly, overexpression of GK in hepatocytes from Zucker diabetic fatty rats restores hepatic glycogen metabolism (18). In contrast, overex-
pression of brain HKI in these hepatocytes does not increase glycogen accumulation. A similar scenario is observed in FTO2B cells (19), a rat hepatoma cell line (20) that lacks GK but expresses HKI (21). This cell line does not therefore accumulate glycogen, although it presents an elevated rate of glycolysis, the hallmark of hepatoma cells. This increased rate has been related to the greater HK activity of these cells compared with normal hepatocytes (22, 23). Either GK or MGS expression triggers glycogen deposition in FTO2B cells (19). The lack of effect of HK on promoting hepatic glycogen synthesis has been interpreted in terms of channeling or compartmentalization of Glc-6-P (19, 24). Surprisingly, mammalian embryonic livers accumulate glycogen despite the absence of GK expression. During development, rat liver expresses HKI and HKII (25). It is when pups are weaned that the first solid carbohydrate-rich ingestion triggers an insulin peak, which stimulates the insulin-dependent promoter of GK in liver (26).

Here we address the occurrence of hepatic glycogen synthesis in the absence of GK. We show that the GK/LGS tandem expressed in adult liver provides the optimal conditions for synthesizing glycogen in this organ when blood glucose concentration is elevated. However, the substitution of HKI for GK in hepatoma and embryonic liver forms the unusual tandem HKI/LGS. The HK-for-GK switch serves to guarantee their capacity to exploit glucose at any concentration of the sugar. Furthermore, the embryonic liver, by expressing huge amounts of HK, is also able to accumulate glycogen, which is safeguarded from changes in the mother’s blood glucose.

**EXPERIMENTAL PROCEDURES**

**Animals**—OF1 pregnant mice (Charles River Laboratories) were used for the metabolic studies. The day at which a vaginal plug was detected was considered embryonic day 0. The experiments were performed in accordance with the European Council Directive and the National Institutes of Health guidelines for the care and use of laboratory animals.

**Preparation of Recombinant Adenoviruses**—AdCMV-GK and AdCMV-HKI (27), AdCMV-LGS (12), AdCMV-green fluorescence protein (GFP), and AdCMV-MGS (19) were described previously. In the metabolic studies, FTO2B cells infected with AdCMV-GFP were used as control.

**Plasmid Construction and Transfection**—The cDNA of HxKII, an ancient HK from Saccharomyces cerevisiae, was courtesy of F. Moreno ( Universidad de Oviedo). The coding region of HxKII was cloned in-frame in pEGFP-C2 (Clontech) between EcoRI and BamHI restriction sites. pEGFP/PTG and pEGFP/G1 were kindly provided by J. C. Ferrer (Universitat de Barcelona). Cells were transfected at 90% of confluence by electroporation. Briefly, for each plate, cells were detached, washed with phosphate-buffered saline (PBS), and resuspended to a final volume of 800 μl in Dulbecco’s modified Eagle’s medium (DMEM) without antibiotics but with 100 μg of salmon sperm DNA (Stratagene) and 100 μg of the desired plasmid constructs. Samples were transferred to a Gene Pulser cuvette (Bio-Rad) with an electrode gap of 0.4 cm and electroporated in a Gene Pulser II device (Bio-Rad) at 200 V and 950 microfarads. Cells were then diluted in an appropriate volume of DMEM and plated.

**FTO2B Cell Culture Conditions and Treatment with Recombinant Adenoviruses**—FTO2B rat hepatoma cells were cultured in 100-mm diameter plates. They were kept in DMEM supplemented with 25 mM glucose, 10% (v/v) fetal bovine serum, and penicillin/streptomycin. Cells were treated for 2 h with an appropriate amount of adenovirus, previously titrated either by Glc-6-P or HK activity determinations. Infection media were then replaced with DMEM containing 25 mM glucose, and cells were incubated for 24 h at 37°C in humidified 5% CO2 and 95% air. Media were then replaced by DMEM without glucose, and another incubation of 14–16 h was carried out. Cells were then incubated in DMEM with 10 or 25 mM glucose, as indicated, for 4 h at 37°C.

**Metabolite Determinations**—To measure glycogen content, cell monolayers from 100-mm diameter plates were scraped using 200 μl of 30% (v/v) KOH, and the extract was then boiled for 15 min and centrifuged at 5000 × g for 15 min. Glycogen was measured in the cleared supernatants as described in Ref. 28. To determine the intracellular concentration of Glc-6-P, at the end of incubations cell culture plates were flash-frozen in liquid N2 without rinsing with PBS to prevent Glc-6-P consumption. Cell monolayers from 100-mm diameter plates were scraped with 200 μl of perchloric acid, and the extract was sonicated and centrifugated at 10,000 × g for 15 min. The pH of the cleared supernatants was adjusted to neutrality, and Glc-6-P quantification was performed by a spectrophotometric assay (29). A fraction of these perchloric extracts was assayed for nucleotide concentration by high pressure liquid chromatography. Briefly, 20 μl of the sample was injected in a reverse-phase column (Excel 120 ODS b, 3 μm, 20 × 0.46) with a pressure of 1900 p.s.i and eluted with a gradient of 0.1 M KH2PO4 + 0.5 mM tetrabutylammonium, pH 6.4, and 30% methanol at a constant flow of 0.6 ml/min. Nucleotides were detected with a Waters 2996 PDA detector set at λ = 260 nm.

**Enzyme Activity Assays**—Frozen cell monolayers from 100-mm diameter plates were scraped using 300 μl of homogenization buffer, which consisted of 10 mM Tris–HCl (pH 7.4), 150 mM KF, 15 mM EDTA, 15 mM 2-mercaptoethanol, protease inhibitor mixture, and 0.5% (v/v) Tween 20. Thawing plus Polytron disruption caused cell bursting. Fresh tissue samples (100 mg) were homogenized in 1 ml of homogenization buffer. Protein concentration was measured following the method of Bradford (30) using a Bio-Rad assay reagent. Glucose phosphorlyating activity was measured spectrophotometrically in the supernatant fraction of cell or tissue extracts centrifuged at 10,000 × g for 15 min using 0, 0.5, or 100 mM glucose to differentiate between hexokinases of low (GK) and high (HKI and HxKII) affinity for glucose. Activity was measured at 30°C with the Glc-6-P dehydrogenase coupled assay, as described previously (31). The activity measured at 0 mM glucose was used to set the base line. HK activity is expressed as milliunits/mg of protein, where 1 milliunit is the amount of enzyme phosphorlyating 1 nmol of glucose/min at 37°C. M100 of GS for Glc-6-P was measured in glucose-depleted FTO2B cells infected with adenoviruses expressing either LGS or MGS. Cell monolayers were homogenized as described above in homogenization buffer without Tween 20. GS activity in these homogenates was measured at a constant UDP-
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glucose concentration (0.2 mm) but with several increasing Glc-6-P concentrations ranging from 0 to 7.1 mm, as described in Ref. 32.

RNA Purification and Retrotranscription—Total RNA was isolated from mouse tissue as follows. First, 100 µg of the sample tissue was homogenated in 1 ml of TRIzol reagent with a Dounce homogenizer. After centrifugation at 12,000 × g for 5 min, 0.2 ml of chloroform was added to the supernatant, and it was then centrifuged again at 12,000 × g for 15 min at 4 °C to separate it into two phases. Total RNA was then precipitated by adding 0.5 ml of isopropyl alcohol to the aqueous phase. After an incubation of 10 min at room temperature, samples were centrifuged at 12,000 × g for 10 min at 4 °C. Pellets were washed with 1 ml of 75% ethanol and centrifuged at 7500 × g for 5 min at 4 °C. The desiccated pellets were resuspended in 100 µl of RNase-free water. Total RNA from tissue was repurified at this step with RNeasy minicolumns (RNeasy total RNA isolation kit, Qiagen) following the manufacturer’s instructions. Also total RNA isolation from plate-cultured cells was started at this step using RNeasy columns. Up to 5 µg of total RNA from each sample was reverse-transcribed for 50 min at 42 °C in a 15-ml reaction volume using 200 units of SuperScript III reverse transcriptase (SuperScript First-strand Synthesis System for RT-PCR, Invitrogen) in the presence of 50 ng of random hexamers.

Quantitative Real-time PCR—PCR tests were performed following the standard real-time PCR protocol of the ABI Prism 7700 Detection System together with the appropriate ready-made TaqMan primer/probe sets (Applied Biosystems) at the Genomic Unit core facility of the Serveis Cientificotècnics-Universitat de Barcelona. Each sample was analyzed in three replicate wells with 30 ng of first-strand cDNA in a total reaction volume of 20 µl. The temperature profile consisted of 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Data were analyzed with the 2−ΔΔCt method using 18 S rRNA as endogenous control.

Electrophoresis and Immunoblotting—Electrotransfer of proteins from the gel to the nitrocellulose was performed at 150 V (constant) at room temperature using a Bio-Rad miniature transfer apparatus, as described previously (33). The nitrocellulose blot was incubated overnight at 4 °C in blocking buffer (3% bovine serum albumin in PBS). The blot was then incubated for 1 h at room temperature with a rabbit antibody against LGS (34), MGS (35), or GK (4) or a goat antibody against HKII (Santa Cruz Biotechnology). It was then washed and incubated for 1 h with an anti-rabbit (Amersham Biosciences) or anti-goat (Jackson ImmunoResearch Laboratories) secondary antibody conjugated to horseradish peroxidase. Immunoreactive bands were visualized using an ECL kit (Amersham Biosciences) following the manufacturer’s instructions.

Immunocytochemistry—Cells were fixed with paraformaldehyde diluted 4% in PBS. Coverslips with the cell monolayer were rinsed three times with PBS, permeabilized with PBS containing 0.2% (v/v) Triton X-100 (Sigma) for 30 min, and blocked with the previous mixture supplemented with 3% (w/v) bovine serum albumin (Sigma) for 10 min. Primary mouse IgM antibody raised against glycogen (kindly provided by O. Baba, Tokyo Medical and Dental University) was diluted in PBS/bovine serum albumin (3%, 1:20) and applied to the cells for 45 min at room temperature. Coverslips were then washed several times with PBS and subjected to incubation with TRITC-conjugated goat anti-mouse IgM secondary antibody (Chemicon) diluted 1:200 in PBS/bovine serum albumin for 30 min at room temperature. Finally, coverslips were washed, air-dried, and mounted onto glass slides using Mowiol mounting medium. Fluorescence images were obtained with a Leica SPII spectral microscope and 63× (numerical aperture of 1.3, oil) Leitz Plan-Apo objective. The light source was an argon/krypton laser (75 milliwatts).

RESULTS

Glycogen Accumulation in Embryonic Liver and Hepatoma Cells—Mouse livers from E16 had active glycogen metabolism because they accumulated Glc-6-P (0.61 ± 0.07 nmol of Glc-6-P/mg of tissue) and showed glycogen levels (13.7 ± 2.5 mg of Glc/g of tissue) comparable with those in adult livers of animals fed ad libitum (0.47 ± 0.07 nmol of Glc-6-P/mg of tissue and 12.6 ± 1.7 mg of Glc/g of tissue). However, as reported for rats (25), these embryos did not express GK (Fig. 1). Moreover, Western blot analysis revealed that LGS, but not MGS, is the isofrom expressed in embryonic liver (Fig. 1). Similarly, FTO2B cells, a rat hepatoma cell line, express LGS and lack GK (Fig. 1). Instead, this cell line expresses HKI (21). However, unlike embryonic liver, FTO2B cells do not accumulate glycogen even in the presence of high concentrations of glucose in the culture medium (Fig. 2A) (19).

HK-triggered Glycogen Deposition in FTO2B Cells—We engineered FTO2B cells to test whether they can be induced to accumulate this polysaccharide. We first overexpressed rat HKI or rat GK by infecting FTO2B cells with recombinant adenoviruses at the same multiplicity of infection (m.o.i.). After incubation with 10 mM glucose, we measured glycogen concentrations. As reported previously (36), cells expressing GK but not HKI accumulated glycogen (Fig. 2A).

Because increased AMP levels activate LGS (37, 38), we tested whether the distinct cellular localization of HKI and GK exerts a differential effect on AMP pool and hence on LGS activation. For this purpose, we determined ATP, ADP, and AMP levels in adenovirus-infected FTO2B cells incubated with glucose. An increase in the AMP levels of both HKI- and GK-expressing cells was detected with respect to controls (Fig. 2B).
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The activity of HKI and GK was assayed with 0.5 mM glucose in the supernatant and in the pellet (mitochondrial) fraction of cell extracts. S.E. for eight independent experiments.

**Levels of the FTO2B cells infected with either AdCMV-GK (HKI) or AdCMV-HKI (GK) at m.o.i. of 5 and incubated 16 h in DMEM without glucose followed by incubation with 10 mM glucose for 4 h. Data represent the mean ± S.E. for eight independent experiments. *, p ≤ 0.05. C, hexokinase activity in FTO2B cells incubated with 25 mM Glc and with or without 20 μM clotrimazole (CTZ) for 4 h after 16 h without glucose. High affinity hexokinase activity was assayed with 0.5 mM glucose in the supernatant and in the pellet (mitochondrial) fraction of cell extracts. **, p ≤ 0.01 with respect to pellet + Glc; #, p < 0.01 with respect to supernatant (Super) + Glc. Data represent the mean ± S.E. for four independent experiments. Statistical comparisons were made by Student’s t test for unpaired samples. ctrl, control.

Whereas the other nucleotides remained unchanged. However, because these two isoenzymes raised AMP concentration to the same extent, this increase cannot account for the differences in glycogen deposition observed between HKI- and GK-expressing cells.

Because GK is soluble but HKI is bound to mitochondria, we next tested the hypothesis that HKI mitochondrial localization affects the capacity of this enzyme to trigger glycogen synthesis. To this end, we treated FTO2B cells with clotrimazole, a compound that releases HKI from the mitochondria (39). Although incubation with 20 μM clotrimazole enriched the cytosolic fraction of FTO2B cell extracts (Fig. 2C), we did not detect glycogen accumulation (data not shown). This observation suggests that the binding of HKI to mitochondria is not the cause of impaired glycogen synthesis.

We next addressed whether the kinetic features of HKI and GK were responsible for the differences in glycogen deposition. To explore this, we used HxkII, an ancestral hexokinase from S. cerevisiae with mixed kinetic features. Like HKI, this enzyme shows affinity for glucose in the submillimolar range, but like GK, it is not inhibited by Glc-6-P (40, 41). Thus, we compared the capacity of HKI, GK, and HxkII to trigger glycogen synthesis in FTO2B cells upon incubation with glucose. We expressed rat GK in these cells at levels similar to those found in liver and then adjusted the expression of yeast HxkII to achieve similar levels of HK activity (when measured in vitro) (Fig. 3A). Both GK and HxkII expression increased basal HK activity 3-fold and effectively raised both Glc-6-P and glycogen pools (Fig. 3, B and C). In contrast, cells overexpressing HKI activity 7-fold over endogenous basal HK activity did not increase their Glc-6-P or glycogen levels. These results indicate that the affinity of HKI for glucose is not a key factor in this process and that HKI performs poorly in promoting glycogen synthesis because retinoinhibition by Glc-6-P limits its capacity to efficiently increase Glc-6-P levels in vivo.

To further extend these observations, we overexpressed HKI in FTO2B cells until, upon incubation with 10 mM glucose, we achieved Glc-6-P levels 8-fold higher than in control cells (Fig. 3D). In these conditions, HKI induced glycogen accumulation (Fig. 3F). It is important to stress that to reach these Glc-6-P levels, a 120-fold increase in HKI activity over the basal HK activity was required (Fig. 3E).

These observations indicate that HKI promotes glycogen deposition in FTO2B cells under conditions where it can increase Glc-6-P above a threshold concentration; however, to do so, huge amounts of enzyme are required. In this regard, at high glucose concentrations, GK is far more efficient than HKI in producing the levels of Glc-6-P required to trigger hepatic glycogen synthesis.

PTG- and G_{i,2} triggered Glycogen Deposition in FTO2B Cells—An alternative mechanism to activate LGS without raising Glc-6-P concentration is to increase the amount of PTG or G_{i,2}, the regulatory subunits of protein phosphatase 1 in hepatocytes. Overexpression of PTG in isolated rat hepatocytes enhances glycogen deposition (42). However, we questioned whether this occurs in cells that do not express GK. To address this point, we overexpressed the GFP fusions of PTG and G_{i,2} in FTO2B cells. After incubation of these cells with glucose, we assayed glycogen accumulation. In contrast to GFP-expressing control cells, both PTG- and G_{i,2}-overexpressing cells accumulated glycogen (data not shown). Furthermore, by immunocy-
tochemistry, we observed that only PTG- and G1-overexpressing cells accumulated the polysaccharide (Fig. 4) and that PTG and G1 colocalized with the growing glycogen deposits. These results indicate that an increase in the protein phosphatase regulatory subunits also has the capacity to trigger glycogen accumulation in these cells.

**Affinity of LGS and MGS for Glc-6-P** — In a previous report we showed that FTO2B cells induced to express MGS accumulate glycogen with only the endogenous HKs, whereas LGS-overexpressing cells do not (19). This observation suggests that MGS is activated even at the low Glc-6-P concentrations and PTG/G1 levels found in unmodified FTO2B cells. Because Glc-6-P makes GS a better substrate for protein phosphatase 1, we checked whether this distinct behavior could be a consequence of the differing affinity of the two GS isoenzymes for Glc-6-P.

To this end, we determined the $M_{0.5}$ for Glc-6-P, the kinetic constant that stands for the Glc-6-P concentration required to achieve half-maximum activation of GS, in extracts from LGS- and MGS-overexpressing FTO2B cells incubated in the absence of glucose. The $M_{0.5}$ values for LGS and MGS were 2.6 and 0.6 mM, respectively (Fig. 5). These results show that in these conditions, LGS has lower affinity for Glc-6-P than MGS and therefore requires higher levels of Glc-6-P to be activated.

**Enzymatic Profile of the Embryonic Liver** — Having shown that embryonic livers do not express MGS (Fig. 1), we tested whether these organs fulfill one of the two situations in which we have shown that glycogen is accumulated in the absence of GK. Determination by quantitative real-time PCR of the levels of PTG and G1 in embryonic liver showed that neither of these subunits was increased in comparison with adult liver (Fig. 6A).
glycogen. We next analyzed the mobilization of glycogen activity (Fig. 6). In contrast, huge HKI and HKII mRNA levels were detected in fetal and adult livers under conditions in which blood glucose levels are not elevated. After fasting pregnant mice overnight, maternal hepatic glycogen deposits were reduced to a minimum (Fig. 6E), whereas hepatic glycogen stores in embryos were safeguarded.

**DISCUSSION**

In the metabolic network of the cell, branch-point metabolites are substrates of multiple enzymes, which can divert metabolic flux toward distinct cellular processes. This is the case of Glc-6-P, which can be used as a precursor for a variety of catabolic and anabolic pathways. Glc-6-P is diverted toward glycogen metabolism only when GS is activated, a process that is controlled in turn by Glc-6-P itself. An increase in the Glc-6-P produced by HKs results in the dephosphorylation of GS and therefore in its activation (push effect). This increase in active GS in turn pulls the substrate into glycogen. This tight link between HK and GS is revealed by the exquisitely tailored complementation of the GK/LGS functional tandem in liver and the HKI-II/MGS tandem in brain and muscle.

In a previous study, we found that GK showed a greater capacity to activate LGS than did HK. To explain this behavior, we hypothesized the compartmentalization of Glc-6-P in at least two pools in such a way that only the pool replenished by GK or gluconeogenesis would be accessible to LGS (19, 24). In the present study, we have demonstrated that by greatly overexpressing HKI to levels similar to those found in embryonic liver, HKI can indeed trigger hepatic glycogen accumulation. In light of the new results, it is not necessary to postulate the presence of two pools of Glc-6-P to account for the distinct capacity of HKI and GK on LGS. The data can be explained by a model of a unique Glc-6-P pool. Following this simple model, the diversion of Glc-6-P toward glycolysis or glycogen synthesis would rely on the kinetic characteristics of the isoenzymes of HK and GS present in the tissue. Thus, the differential affinity of these enzymes for activators, inhibitors, and substrates may explain their distinct capacity to direct glucose into glycogen. Moreover, this model further strengthens the physiological significance of the GK/LGS and HKI/MGS tandems.

HKI has a high affinity for glucose, but given that it is retroinhibited by Glc-6-P, its capacity to build up substantial Glc-6-P concentrations is impaired. Despite having a much higher $S_{0.5}$ for glucose, GK is far more efficient than HKI in building up high levels of Glc-6-P because it is not retroinhibited by its product, although this is only possible when glucose concentration in the media is high. Because activation of LGS requires higher concentrations of Glc-6-P than MGS, the combination of GK and LGS is much more efficient than that of HKI and LGS provided that the glucose concentration is sufficiently elevated. HKI or HKII would not normally have the capacity to build up high enough levels of Glc-6-P to activate LGS. On the contrary, the activation of MGS is not impeded at the lower concentrations of Glc-6-P that may accumulate in tissues expressing retroinhibited HKs. Therefore, our observations suggest that the biochemical features of the distinct HK and GS isoforms are adapted to each other and create functional tandems that suit specific tissue requirements.
 Nonetheless, our studies reveal a third scenario in which these canonical tandems of isoenzymes are redefined. Mammalian embryonic liver expresses a combination of hepatic (LGS), muscle (HKII), and brain (HKI) isoforms of glycogen metabolism enzymes. This is the only scenario reported in which these tissue-specific isoforms are merged in healthy animals.

A similar strategy is also followed by many hepatocarcinomas, which, like developing embryos, have a high energy demand to fuel their growth and are hosted in an environment with changing glucose levels. In a healthy adult liver, expression and post-transcriptional regulation of GK are fine-tuned to induce glycogen deposition only when glucose is in excess. Insulin regulates GK expression (43); furthermore, glucose triggers changes in GK intracellular localization and activity through the release of GK from GK regulatory protein (2, 5). This tight control over GK preserves glucose for peripheral tissues when the blood levels are low. In contrast, hepatoma cells and embryonic liver are avid for glucose and thus express HKI and HKII. These high affinity HKs allow these cells to exploit blood glucose in all conditions, even when concentrations are low. However, there is a crucial difference between the embryonic liver and tumor cells, namely, the latter do not require the accumulation of glycogen deposits as an energy backup because glucose is always available. Therefore, moderately elevated levels of high affinity HKs are sufficient for these tumor cells to guarantee their capacity to use glucose regardless of the concentration of this sugar. In contrast, when pups are born they depend on their glycogen reserves to survive as independent organisms. In embryonic livers, LGS is coexpressed with very high (200-fold over adult liver) levels of HKI and HKII. With this combination of isoenzymes, embryos accumulate hepatic glycogen efficiently despite a lack of GK expression because by heavily expressing these enzymes their retroinhibition by Glc-6-P is sufficiently overcome to attain the levels of Glc-6-P required to activate LGS. This observation raises the question of the advantages of replacing GK by much higher amounts of HKI, an isoform that is much more inefficient at building up a Glc-6-P pool.

Our results in fasted pregnant mice provide a teleological explanation for the HK reorganization in embryonic liver. Glycogen plays a key role during embryonic development as it ensures pup survival in the space of time between birth and first birth.
receiving their mother’s milk. Pups use glycogen deposits as a ready source of energy at the moment of birth and need to build their glycogen reserves during development in such a way that they do not depend on their mother’s feeding state. In this scenario, by greatly increasing the expression of HKI and HKII, embryos not only ensure their capacity to use glucose in all circumstances, but at the same time they may produce enough Glc-6-P to ensure hepatic glycogen accumulation even if the mother is fasting and blood glucose levels decrease. By this mechanism, embryos safeguard their liver glycogen stores and thereby provide a crucial advantage at the moment of birth.

Acknowledgments—We thank Anna Adrover for excellent technical assistance and Jorge Domínguez for critical reading of the manuscript. We also thank Tanya Yates for correcting the English manuscript.

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