MINIREVIEW

The Biochemistry and Molecular Biology of the Glucose-6-Phosphatase System[1]

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Progress has continued to be made over the past 4 years in our understanding of the glucose-6-phosphatase (G6Pase) system. The gene for a second component of the system, the putative glucose-6-P transporter (G6PT), was cloned, and mutations in this gene were found in patients diagnosed with glycogen storage disease type 1b. The functional characterization of this putative G6PT has been initiated, and the relationship between substrate transport via the G6PT and catalysis by the system's catalytic subunit continues to be explored. A lively debate over the feasibility of various aspects of the two proposed models of the G6Pase system persists, and the functional/structural relationships of the individual components of the system remain a hot topic of interest in G6Pase research. New evidence supportive of physiologic roles for the biosynthetic functions of the G6Pase system *in vivo* also has emerged over the past 4 years.

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We last reviewed recent advances in the field of glucose-6-phosphatase (G6Pase) research for this journal in 1997 (1). Since then, work on the G6Pase system has continued to intensify due to the identification of the gene for the putative glucose-6-P transporter (G6PT) (2) component of the system as well as the earlier identification of the gene for the catalytic unit (3). We recently have reviewed the system, focusing on its components and their acute and long-term regulation with regard to the control of glucose production by the liver (4). A voluminous literature, which is beyond the scope of this Minireview, now exists regarding the regulation of the expression of the genes for the catalytic unit and G6PT of the system and the metabolic effect of the overexpression of these components via recombinant adenoviral vectors (5). Gérald van de Werve and his collogues (6) recently have authored a comprehensive review that covers much of this material as well as other aspects of the G6Pase system, including its components and their gene structure and inborn errors of the system.

The identification of the genes for the catalytic unit and G6PT components of the system has allowed researchers to identify several mutations that underlie glycogen storage disease type 1 (GSD-1). Rake *et al.* (7) published an article that summarizes the mutations that are reported in the literature, and provides a flowchart for the diagnosis of GSD-1a and GSD-1b. Janice Chou's group (8) recently reported the functional characterization of 48 missense mutations and one deletion mutation in the G6Pase catalytic unit that were grouped as active site, helical, and nonhelical mutations (Fig. 1). The G6Pase catalytic unit continues to be a therapeutic target for the treatment of type 2 diabetes, and now the G6PT component of the system (Fig. 2) has become a therapeutic target as well. Madsen and Westergaard (10) have reviewed several compounds that exert inhibitory action on the G6Pase system. These compounds have surfaced

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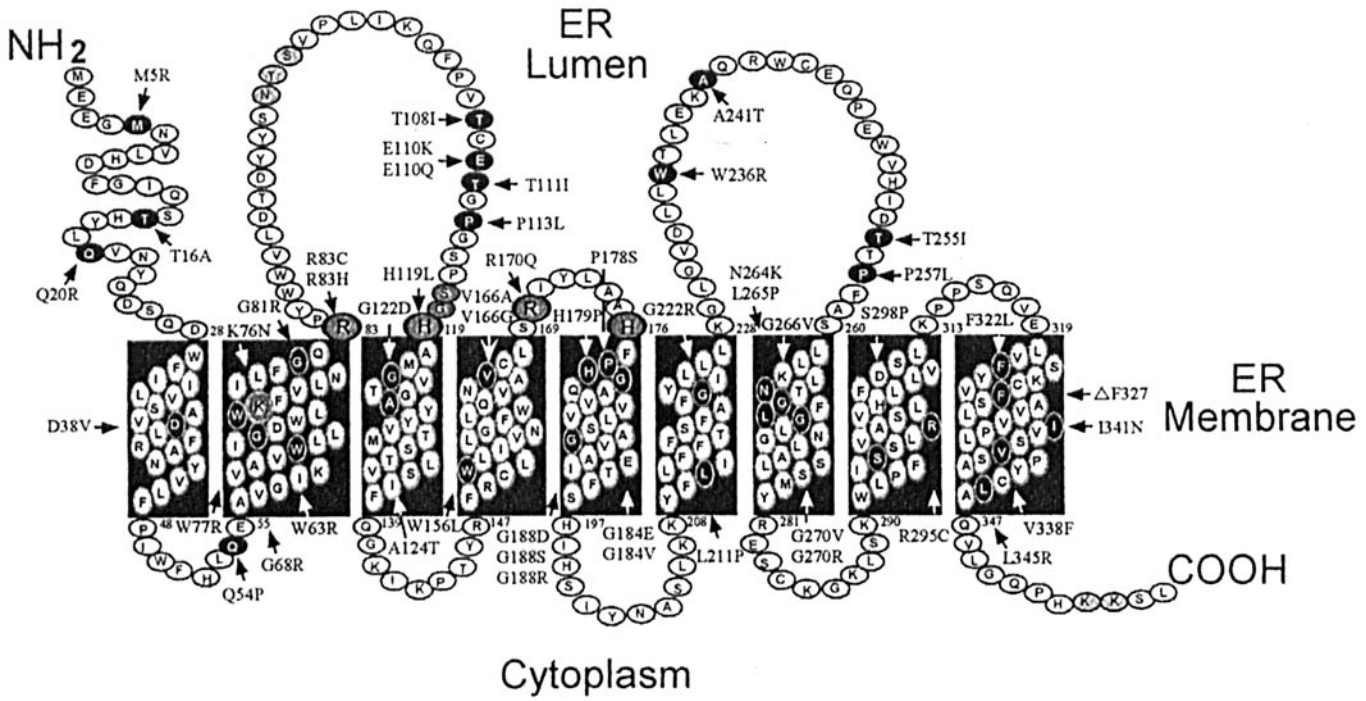

Figure 1. The predicted nine-transmembrane helical structure of human G6Pase catalytic unit showing the location of missense and codon deletion mutations identified in GSD-1a patients. The mutations are indicated and shown in black. Amino acid residues comprising the phosphatase signature motif are denoted by large shaded circles. Reproduced with permission from Ref. 8.

as a result of a substantial effort to develop a therapeutic treatment for type 2 diabetes.

The G6Pase field, like many others in science, is not without strong differences in opinion and accompanying controversies, as is reflected in the various reviews, including the present, which communicate the differing points of view of the authors. Here, we have restricted ourselves to developments since 1997 in selected areas of research in the G6Pase field that we believe are of greatest interest and impact, and will continue to be promising and fruitful areas of progress in the quest to understand the complex G6Pase system and the interrelationships of its components. In discussing these selected areas, we will encounter points of contention, which we will attempt to illuminate with our point of view.

The Putative G6PT

Over the past 20 to 25 years, the organizational composition of the G6Pase system has been an area of great debate and contention. Currently, there are two proposed concepts of structure-function relationships for G6Pase (see Refs. 1 and 6, and refs. therein). Briefly, according to the substrate transport-catalytic unit concept (Fig. 3), G6Pase is a muticomponent system consisting of a fairly nonspecific catalytic unit with its active site located on the lumenal side of the endoplasmic reticulum (ER), and at least three transmembrane-spanning translocases that confer specificity to this system by allowing selective substrates/products access to, or egress from, the sequestered catalytic unit In contrast, according to the combined conformational flexibility-substrate transport concept (Fig. 4), G6Pase is a multifunctional enzyme embedded within the ER membrane that possesses both catalytic and substrate/product transport activities. In the latter concept, G6Pase is depicted as a single protein that must confer specificity for all of the various substrates and products of the multiple activities catalyzed by the enzyme.

A multitude of kinetic studies over the years (13–18) involving a combination of substrates and inhibitors, and more recently, a variety of genetic studies (2, 3, 19–22) involving variants of GSD-1 have provided strong evidence that G6Pase is a multicomponent system. Recently, the gene for a putative G6PT was cloned (2), and mutations in this gene were found in patients diagnosed with GSD type 1b (2, 23). The initial report identified a human cDNA sequence encoding a 46-kDa protein that has homology with the bacterial uptake of hexose phosphates (Uhp) gene (2). The identification of this gene provided evidence, in addition to many previous studies (13–15, 24), that a gene product in addition to the 36-kDa G6Pase catalytic unit is involved in G6Pase action.

The human recombinant G6PT's function was characterized after expression in COS-1 cells (22). Microsomes derived from G6PT-transfected COS-1 cells demonstrated a low level of ^{14}C radiolabel accumulation in the presence of exogenous [^{14}C]G6P, and this radiolabel accumulation was greatly increased in microsomes derived from COS-1 cells that were co-transfected with the G6Pase catalytic unit cDNA and G6PT (22). However, G6P uptake *per se* was not measured in this study; the accumulation of ^{14}C radiolabel

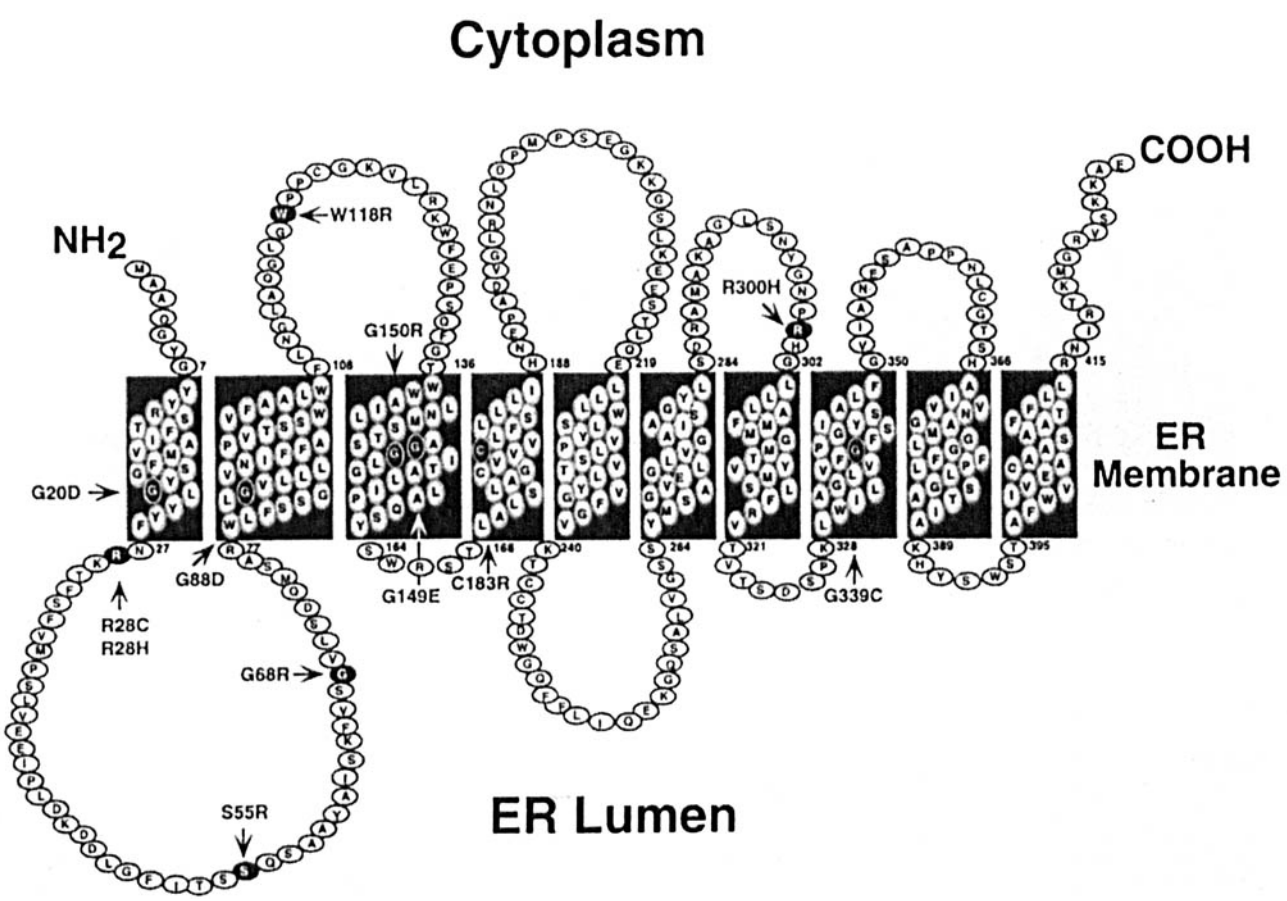

Figure 2. The predicted 10-transmembrane helical structure of human G6PT showing the location of 12 missense mutations identified in the G6PT gene of GSD-1b patients. The mutations are indicated and shown in black. Reproduced with permission from Ref. 9.

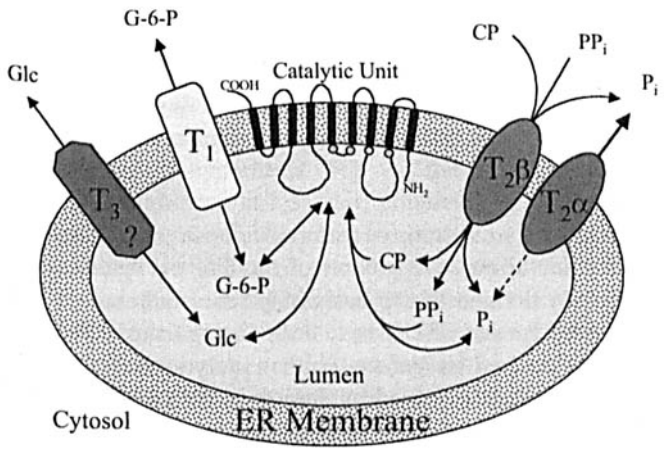

Figure 3. Structure-function relationship of the G6Pase system according to the substrate translocase-catalytic unit hypothesis. A cross-section of the ER is depicted: T_1 (G6PT), $T_2\alpha$, $T_2\beta$, and T_3 are substrate/product transporters and/or auxiliary proteins with the indicated specificity; Catalytic Unit is G6Pase (EC 3.1.3.9) embedded within the ER membrane with nine-transmembrane-spanning helical regions. Circles on the inner loops of the Catalytic Unit indicate amino acid residues comprising the phosphatase signature motif (8). Modified from Wallert *et al.* (Ref. 11).

in microsomes, which contain G6Pase hydrolytic activity, represents a combination of [^{14}C]glucose plus [^{14}C]G6P. As G6P enters the microsome and binds at the G6Pase catalytic unit's active site, G6P is rapidly hydrolyzed to glucose and P_i when G6Pase hydrolytic activity is present. To truly measure microsomal G6P accumulation, it is necessary either to perform the uptake analysis in the absence of hydrolytic activity or to separate [^{14}C]G6P from [^{14}C]glucose in the uptake analysis. Because this was not done in this initial characterization of the putative G6PT (22), it is difficult to assess the transport function of G6PT in the presence of G6Pase catalytic unit and hydrolytic activity.

When expressed alone in COS-1 cells that contain relatively low levels of hydrolytic activity, G6PT appears to have only a low level of G6P transport function that is sensitive to inhibition by chlorogenic acid (CHA), a specific inhibitor of the G6P translocase. However, this study (22) and other previous studies (25, 26) suggest that a tight coupling of some sort between microsomal G6P uptake and G6Pase activity exists.

Recently, we (27) examined microsomal G6PT function in relation to G6Pase hydrolytic activity. In this study, we measured microsomal [^{14}C]G6P accumulation *per se* by separating [^{14}C]G6P from [^{14}C]glucose in the uptake analysis. We demonstrated that microsomal G6P accumulation varies directly with the level of G6Pase hydrolytic activity. When G6Pase activity is diminished greatly (e.g., in microsomes derived from Ehrlich ascites tumor-bearing mice, or in the presence 4 m*M* vanadate), there is a concomitant

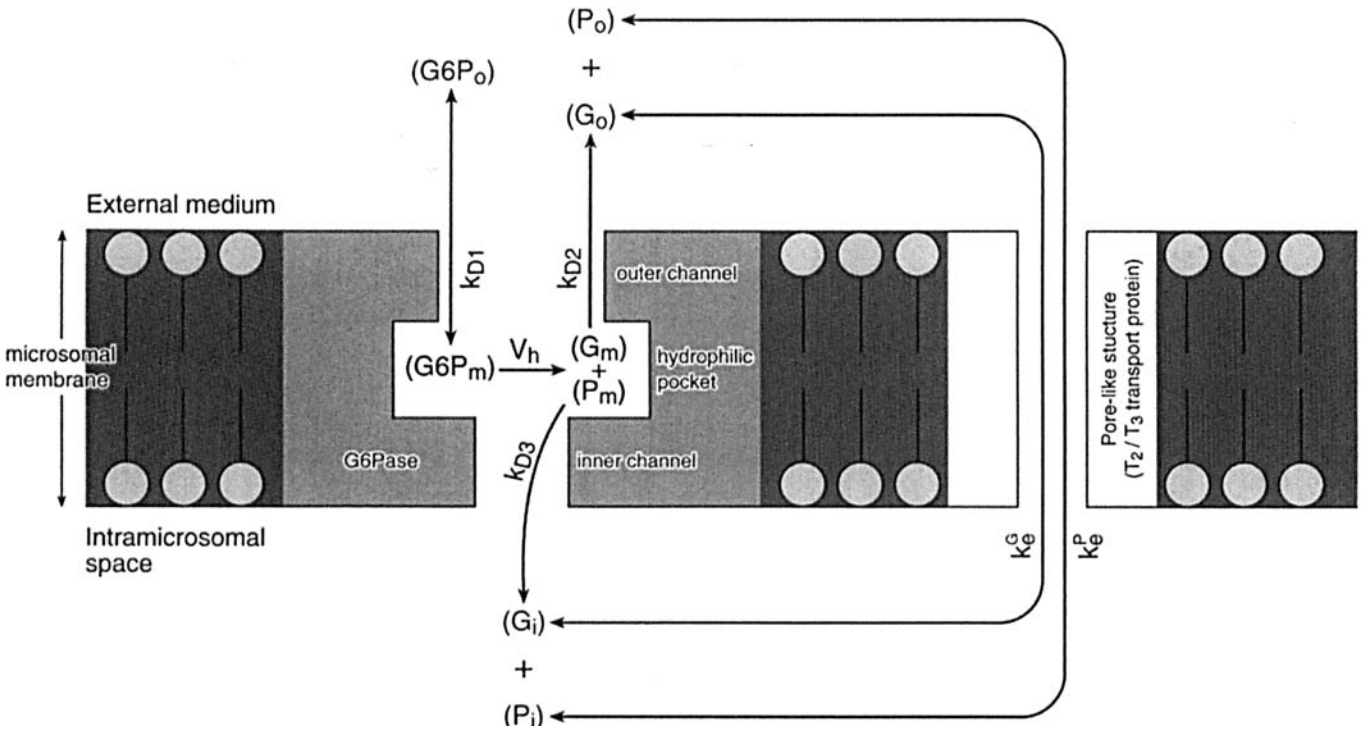

Figure 4. Revised version of the combined conformational flexibility-substrate transport model. Microsomal G6Pase is depicted as a transmembrane protein with the catalytic site lying in a hydrophilic pocket deep inside the protein where G6P hydrolysis occurs. Access to and exit from the hydrophilic pocket are controlled by outer and inner channels with different intrinsic permeabilities to G6P, glucose, and phosphate. Glucose (G) and phosphate (P) exchange between the extra- and intravesicular spaces is made possible through a pore-like structure that would account for most (if not all) of the glucose and phosphate transport functions usually attributed to the putative T_3 and T_2 translocases in intact microsomes. The figure was provided through the courtesy of Dr. Alfred Berteloot and is reproduced with permission from Ref. 12.

decrease in microsomal G6P accumulation (27). Although the extent of microsomal G6P accumulation is dependent on G6Pase hydrolytic activity, we also confirmed in this study (27) that G6PT function is absolutely necessary for microsomal G6P accumulation by demonstrating that the accumulation of G6P, as well as glucose, is abolished in microsomes pretreated with the time-dependent irreversible G6PT inhibitor *N*-bromoacetlyethanolamine phosphate (BAEP). Additional previous studies (28–31) also indicate that G6PT function is a necessity for full G6Pase activity in an intact system.

Our G6P uptake studies (27) and those reported previously by others (22, 25, 26) suggest that there is a tight coupling between G6P uptake and G6Pase hydrolytic activity. If G6PT action is blocked in microsomes by the presence of compounds such as CHA and BAEP, G6P uptake as well as G6P hydrolysis is greatly reduced or blocked, depending on the potency of the inhibitor. This inhibition of G6P hydrolysis by blocking G6PT function can be overcome by disrupting the integrity of the microsomal membrane where presumably G6P now has greater access to the active site of the G6Pase catalytic unit. In addition, the inhibition of G6Pase hydrolytic activity with the use of inhibitors such as vanadate or in systems where G6Pase catalytic unit is absent or low also results in the reduction of microsomal G6P uptake and accumulation. This close association between G6Pase catalytic unit and G6PT may indicate the potential for a protein-protein interaction and/or channeling between the two proteins. The examination of the relationship and potential interaction between the G6Pase catalytic unit and the G6PT of the system should provide an exciting but challenging new dimension in the study of G6Pase.

GSDs-1

GSDs-1 are a group of metabolic disorders arising from defects in various components of the G6Pase system (20). Originally defined on the basis of kinetic studies, four different subtypes of GSD-1 have been distinguished: GSD-1a, which is caused by defects in the catalytic unit (Fig. 1); GSD-1b, which is caused by defects in the G6PT (Fig. 2); GSD-1c, which is caused by deficient activity of the phosphate/pyrophosphate translocase; and GSD-1d, which is proposed to be caused by deficient activity of a putative glucose translocase. Molecular genetic evidence has unequivocally demonstrated that GSD-1a and GSD-1b are caused by mutations in the genes for the G6Pase catalytic unit (3) and the G6PT (2, 23), respectively (Figs. 1 and 2). However, many cases of GSD type 1-non-a have been misdiagnosed (32, 33). For example, a patient originally diagnosed with GSD 1aSP was shown subsequently to be homozygous for a common mutation (R83C) in the gene encoding the G6Pase catalytic unit. In addition, many patients originally diagnosed with GSD-1c were found subsequently

to have mutations in the gene for the G6PT. An exception to this is a GSD-1c case reported by Nordlie *et al.* (34). After a thorough kinetic analysis that included the measurement of both hydrolytic and synthetic activities of the G6Pase system, it was concluded that this patient exhibited characteristics predictive of a deficiency in the pyrophosphate/phosphate transporter. This GSD-1c patient did not manifest neutropenia, which commonly is seen with GSD-1b patients. In addition, both the G6Pase catalytic unit and G6PT genes of this patient were found later to be intact (21). This evidence strongly suggests the existence of a separate gene locus for GSD-1c and supports the existence of a component in addition to the catalytic subunit and the G6PT that participates in G6Pase function. Previously, a detailed kinetic analysis (13–18) suggested the existence of components in addition to the catalytic unit of the system, which has proven to be the case with the identification of the gene for the G6PT of the system and seems likely to be the case for a phosphate/pyrophosphate translocase, in light of recent molecular genetic results (21). At this point, it seems premature to divide GSD into only two subtypes (type 1a and type 1-non a), as some have begun to implement (6, 32, 33). However, it is still unclear how these components work together in an organized system.

Glucose and Phosphate Transport in the G6Pase System

There has been a long and ongoing debate over the extent of glucose and phosphate transport in microsomal vesicles. Several discrepancies exist in the results reported by different groups using rapid-sampling radiotracer and light scattering techniques. It is not clear if these discrepancies are the result of differing methodologies or of difficulties in the interpretation of the kinetic data from a complicated multicomponent system. According to the substrate-transport hypothesis (Fig. 3), the products of G6P hydrolysis (glucose and P_i) are produced on the lumenal side of the microsomal membrane and require some path by which they are delivered to the cytoplasmic side of the membrane. Previous kinetic studies (13) and the identification of a microsomal protein with the use of antibodies raised against a mitochondrial phosphate/hydroxyl ion antiport protein (35) have indicated the existence of a pyrophosphate/phosphate transporter that is distinct from the G6PT in the G6Pase system. In addition, the recent genetic evidence that both the G6Pase catalytic unit and G6PT genes of a GSD type 1c patient were intact (21) strongly suggests the existence of an additional component (i.e., the pyrophosphate/phosphate translocase) of the system. Berteloot and co-workers (12) recently have proposed a revised version of the combined conformational flexibility-substrate transport model (Fig. 4). In this modified model, only small portions (less than 10%) of the P_i and glucose produced by G6P hydrolysis are released into the lumen of the microsome, whereas the remainder is released directly into the external medium. Intravesicular glucose and P_i are proposed to exchange with the extravesicular space through a pore-like structure (12). This pore is suggested to account for most if not all of the glucose and P_i transport functions usually attributed to the putative T_3 and T_2 translocases of the substrate-transport model.

Van Schaftingen and co-workers (36) recently investigated the site of release of P_i generated from G6P hydrolysis. In this study, microsomes were incubated with Pb^{2+}, which forms an insoluble complex with P_i and prevents its rapid exit from microsomes (37–39). After a 5-min incubation of microsomes with G6P, approximately 80% of the P_i that was formed was intramicrosomal in contrast to less than 10% P_i that was intramicrosomal in the absence of Pb^{2+}. This finding is in direct disagreement with that of Berteloot and van de Werve (12) whose kinetic data indicated that less than 10% of the glucose and P_i produced by G6P hydrolysis is released into the lumen of the microsome, whereas the remainder (greater than 90%) is released directly into the external medium. This discrepancy is suggested (36) to be the result of a very rapid export of glucose and P_i from the microsomal vesicle that is not detected unless there is an efficient way of blocking this export (e.g., Pb^{2+} in the case of P_i).

In addition to investigating the site of glucose and P_i release, Van Schaftingen and co-workers (36) have used a novel strategy to demonstrate the existence of an intramicrosomal G6P pool. Microsomes preloaded with yeast phosphoglucose isomerase (PGI) catalyzed the detritiation of [2-^{3}H]G6P, and this reaction was inhibited by up to 90% by the G6PT inhibitor, S3483. Detritiation by intramicrosomal PGI was stimulated 2-fold in the presence of 1 m*M* vanadate, a potent phosphatase inhibitor. These observations indicate that G6Pase catalytic unit and PGI compete for the same intravesicular pool. Bánhegyi *et al.* (40) likewise have demonstrated a metabolically active G6P pool in the lumen of liver microsomal vesicles. The competition of G6Pase catalytic unit and lumenal PGI for a common G6P pool seems contrary to several lines of evidence that indicate G6P transport and hydrolysis are tightly coupled (see above). It would be interesting to see if intravesicularly trapped PGI could use a G6P pool in microsomes in which only the G6PT component is expressed and G6Pase catalytic unit is not present.

In 1992, Burchell and co-workers (41) reported the cloning and expression of a hepatic microsomal glucose transport protein, which was termed GLUT 7. This discovery supported the existence of a glucose transporter proposed under the substrate-transport hypothesis that was part of the G6Pase system. Recently, however, the Burchell laboratory (42) has reevaluated the GLUT 7 clone and has determined that it was a cloning artifact. This finding places in doubt the existence of such a transporter with similarities to the other glucose transporters (GLUTs). However, Thorens and co-workers (43, 44) recently reported the possible existence of an alternate pathway for the release of glucose from hepatocytes that is based on a membrane trafficking

mechanism. These studies (43, 44) demonstrated that hepatic glucose output was normal in GLUT2 knockout mice with no detectable overexpression of any known GLUT isoforms in the livers of these animals (44). In the absence of GLUT2, only very low levels of facilitated diffusion of glucose across the hepatocytes plasma membrane were detectable, and the remaining pathway for glucose output was sensitive to low temperature. Although glucose production was quantitatively normal in the livers from these GLUT2 knockout animals, the mobilization of glycogen stores was strongly impaired and correlated with enduring elevated G6P levels in the fed-to-fasted state transition (44). It was concluded in this second study (44) that GLUT2 is not required for normal glucose output but is necessary to equilibrate cytosolic glucose with the extracellular space. The role of the G6Pase system in the context of a pathway for the release of glucose from hepatocytes that is membrane-based seems very plausible considering the membranous nature of the ER and its association with other cellular organelles (see below and Refs. 45-47). Nordlie *et al.* (48) earlier have suggested such a role for the G6Pase system based on kinetic evidence.

Multiple Activities of the G6Pase System

The multiple activities of the G6Pase system, both hydrolytic and synthetic, were described in detail previously (1, 15, 49). In our two earlier reviews for this journal (1, 15), we reported several lines of evidence that have accumulated and are supportive of the participation of phosphotransferase activity of the G6Pase system in hepatic glucose phosphorylation, perhaps supplementary to, or in place of, insulin-dependent glucokinase.

Recently, electron microscopic tomography has provided novel information about mitochondrial interactions with other cellular components. Mannella and co-workers (45–47) have shown mitochondria in close apposition with at least one ER membrane. The attachment of small membrane vesicles to mitochondria is not uncommon in isolates from rat liver, and these attachments likely are pieces of ER firmly attached to the mitochondria surface (47). This association would explain the presence of significant amounts of G6Pase activities reported in mitochondrial preparations (50).

The association of ER with the outer membrane of mitochondria is supportive of the participation of carbamyl-P-glucose phosphotransferase activity of the G6Pase system in hepatic glucose phosphorylation. Carbamyl-P synthase I is present in mitochondria and may supply the substrate carbamyl-P to the G6Pase system that is found in the ER firmly attached to the mitochondria surface. Lueck and Nordlie (51) demonstrated that a reconstituted system, consisting of hepatic mitochondria and microsomes (ER), synthesized G6P when provided with glucose and the substrates and activator for carbamyl-P synthase I. Isolated perfusion studies support such G6P biosynthetic activity in intact livers (52, 53).

Regardless of one's position on the physiologic significance of the synthetic activities of the G6Pase system, these activities do exist and are useful tools in the examination of the complex G6Pase system and the two prominent models of the system that have been put forth (1, 6). In a recent review, van de Werve and colleagues (6) stated that "the synthetic capacity of the G6Pase system mostly results from the reversibility of a number of steps involved in the phosphohydrolase reaction, such that any mechanism should be able to incorporate these features." This is an inaccurate statement because orthophosphate is not incorporated into G6P by reversal of the phosphohydrolase reaction (54, 55). Phosphoryl donors such as carbamyl-P, inorganic pyrophosphate (PP_i), or hexose-P are required to produce a phosphoryl-enzyme intermediate, which in turn can produce G6P in the presence of glucose. In addition, it has been demonstrated by kinetic analysis that at low, physiologic substrate concentrations, the several activities of the enzyme behave almost completely as though they were catalyzed by several enzymes, each distinct for an individual activity (15, 56). Therefore, any proposed model of the system should attempt to incorporate both the synthetic and hydrolytic activities of the system. Attempts of this nature may in turn stimulate new ideas and lead to experiments that answer several questions that have been raised over the years regarding the differences in the two proposed working models of the G6Pase system.

Finally, we would reiterate our view here that many controversies regarding G6Pase structure/function may relate to the routine use of an artifact, isolated fragments of the ER ("microsomes") as the model for experimentation. We would urge that thought be redirected back to the use of permeablized hepatocytes wherein ER are intact, as employed by Jorgenson and Nordlie in the early 1980s (57, 58).

1. Foster JD, Pederson BA, Nordlie RC. Glucose-6-phosphatase structure, regulation, and function: an update. Proc Soc Exp Biol Med **215:** 314–332, 1997.
2. Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, Van Schaftingen E. Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type 1b. FEBS Lett **419:**235–238, 1997.
3. Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY. Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. Science **262:**580–583, 1993.
4. Nordlie RC, Foster JD, Lange AJ. Regulation of glucose production by the liver. Annu Rev Nutr **19:**379–406, 1999.
5. Antinozzi PA, Berman HK, O'Doherty RM, Newgard CB. Metabolic engineering with recombinant adenoviruses. Annu Rev Nutr **19:**511–544, 1999.
6. van de Werve G, Lange A, Newgard C, Mechin MC, Li Y, Berteloot A. New lessons in the regulation of glucose metabolism taught by the glucose 6-phosphatase system. Eur J Biochem **267:**1533–1549, 2000.
7. Rake JP, ten Berge AM, Visser G, Verlind E, Niezen-Koning KE, Buys CH, Smit GP, Scheffer H. Glycogen storage disease type 1a: recent experience with mutation analysis, a summary of mutations reported in the literature and a newly developed diagnostic flow chart. Eur J Pediatr **159:**322–330, 2000.
8. Shieh JJ, Terzioglu M, Hiraiwa H, Marsh J, Pan CJ, Chen LY, Chou

JY. The molecular basis of glycogen storage disease type 1a: structure and function analysis of mutations in glucose-6-phosphatase. J Biol Chem **277:**5047–5053, 2002.
9. Pan CJ, Lin B, Chou JY. Transmembrane topology of human glucose 6-phosphate transporter. J Biol Chem **274:**13865–13869, 1999.
10. Madsen P, Westergaard N. Glucose-6-phosphatase inhibitors for the treatment of type 2 diabetes. Expert Opin Ther Patents **11:**1429–1441, 2001.
11. Wallert MA, Foster JD, Scholnick DA, Olmschenk SM, Kuehn BJ, Provost JJ. Kinetic analysis of glucose-6-phosphatase: an investigative approach to carbohydrate metabolism and kinetics. Biochem Mol Biol **29:**199–203, 2001.
12. Xie W, van de Werve G, Berteloot A. An integrated view of the kinetics of glucose and phosphate transport, and of glucose 6-phosphate transport and hydrolysis in intact rat liver microsomes. J Membr Biol **179:**113–126, 2001.
13. Arion WJ, Lange AJ, Walls HE, Ballas LM. Evidence for the participation of independent translocation for phosphate and glucose 6-phosphate in the microsomal glucose-6- phosphatase system: interactions of the system with orthophosphate, inorganic pyrophosphate, and carbamyl phosphate. J Biol Chem **255:**10396–10406, 1980.
14. Arion WJ, Wallin BK, Lange AJ, Ballas LM. On the involvement of a glucose 6-phosphate transport system in the function of microsomal glucose 6-phosphatase. Mol Cell Biochem **6:**75–83, 1975.
15. Nordlie RC, Bode AM, Foster JD. Recent advances in hepatic glucose 6-phosphatase regulation and function. Proc Soc Exp Biol Med **203:**274–285, 1993.
16. Sukalski KA, Nordlie RC. Glucose-6-phosphatase: two concepts of membrane-function relationship. In: Meister A, Ed. Advances in Enzymology and Related Areas of Molecular Biology. New York: John Wiley and Sons, Inc., pp93–117, 1989.
17. Burchell A, Waddell ID. The molecular basis of the hepatic microsomal glucose-6-phosphatase system. Biochim Biophys Acta **1092:**129–137, 1991.
18. Burchell A, Allan BB, Hume R. Glucose-6-phosphatase proteins of the endoplasmic reticulum. Mol Membr Biol **11:**217–227, 1994.
19. Burchell A. The molecular pathology of glucose-6-phosphatase. FASEB J **4:**2978–2988, 1990.
20. Chen Y-T. Glycogen storage diseases. In: Sciver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler KW, Vogelstein B, Eds. The Metabolic and Molecular Bases of Inherited Disease, 8th edition. New York: McGraw-Hill, pp1521–1551, 2001.
21. Lin B, Hiraiwa H, Pan CJ, Nordlie RC, Chou JY. Type-1c glycogen storage disease is not caused by mutations in the glucose-6-phosphate transporter gene. Hum Genet **105:**515–517, 1999.
22. Hiraiwa H, Pan CJ, Lin B, Moses SW, Chou JY. Inactivation of the glucose 6-phosphate transporter causes glycogen storage disease type 1b. J Biol Chem **274:**5532–5536, 1999.
23. Veiga-da-Cunha M, Gerin I, Chen YT, de Barsy T, de Lonlay P, Dionisi-Vici C, Fenske CD, Lee PJ, Leonard JV, Maire I, McConkie-Rosell A, Schweitzer S, Vikkula M, Van Schaftingen E. A gene on chromosome 11q23 coding for a putative glucose- 6-phosphate translocase is mutated in glycogen-storage disease types 1b and 1c. Am J Hum Genet **63:**976–983, 1998.
24. Lange AJ, Arion WJ, Beaudet AL. Type 1b glycogen storage disease is caused by a defect in the glucose-6- phosphate translocase of the microsomal glucose-6-phosphatase system. J Biol Chem **255:**8381–8384, 1980.
25. Lei KJ, Chen H, Pan CJ, Ward JM, Mosinger B Jr, Lee EJ, Westphal H, Mansfield BC, Chou JY. Glucose-6-phosphatase dependent substrate transport in the glycogen storage disease type-1a mouse. Nat Genet **13:**203–209, 1996.
26. Berteloot A, Vidal H, van de Werve G. Rapid kinetics of liver microsomal glucose-6-phosphatase: evidence for tight-coupling between glucose-6-phosphate transport and phosphohydrolase activity. J Biol Chem **266:**5497–5507, 1991.
27. Foster JD, Wiedemann JM, Pan CJ, Chou JY, Nordlie RC. Discriminant responses of the catalytic unit and glucose 6-phosphate transporter components of the hepatic glucose-6-phosphatase system in Ehrlich ascites-tumor-bearing mice. Arch Biochem Biophys **393:**117–122, 2001.
28. Pan CJ, Lei KJ, Chen H, Ward JM, Chou JY. Ontogeny of the murine glucose-6-phosphatase system. Arch Biochem Biophys **358:**17–24, 1998.
29. Mechin MC, Annabi B, Pegorier JP, van de Werve G. Ontogeny of the catalytic subunit and putative glucose-6-phosphate transporter proteins of the rat microsomal liver glucose-6-phosphatase system. Metabolism **49:**1200–1203, 2000.
30. Foster JD, Stevens AL, Nordlie RC. *N*-Bromoacetylethanolamine phosphate as a probe for the identification of a liver microsomal glucose-6-phosphate transporter peptide in rats and Ehrlich ascites tumor-bearing mice. Arch Biochem Biophys **377:**115–121, 2000.
31. Caraco C, Aloj L, Chen LY, Chou JY, Eckelman WC. Cellular release of [^{18}F]2-fluoro-2-deoxyglucose as a function of the glucose-6-phosphatase enzyme system. J Biol Chem **275:**18489–18494, 2000.
32. Veiga-da-Cunha M, Gerin I, Van Schaftingen E. How many forms of glycogen storage disease type 1? Eur J Pediatr **159:**314–318, 2000.
33. Veiga-da-Cunha M, Gerin I, Chen YT, Lee PJ, Leonard JV, Maire I, Wendel U, Vikkula M, Van Schaftingen E. The putative glucose 6-phosphate translocase gene is mutated in essentially all cases of glycogen storage disease type 1 non-a. Eur J Hum Genet **7:**717–723, 1999.
34. Nordlie RC, Sukalski KA, Munoz JM, Baldwin JJ. Type 1c, a novel glycogenosis: underlying mechanism. J Biol Chem **258:**9739–9744, 1983.
35. Waddell ID, Lindsay JG, Burchell A. The identification of T2: the phosphate/pyrophosphate transport protein of the hepatic microsomal glucose-6-phosphatase system. FEBS Lett **229:**179–182, 1988.
36. Gerin I, Noel G, Van Schaftingen E. Novel arguments in favor of the substrate-transport model of glucose-6- phosphatase. Diabetes **50:**1531–1538, 2001.
37. Pederson BA, Foster JD, Nordlie RC. Low-Km mannose-6-phosphatase as a criterion for microsomal integrity. Biochem Cell Biol **76:**115–124, 1998.
38. Arion WJ, Ballas LM, Lange AJ, Wallin BK. Microsomal membrane permeability and the hepatic glucose-6-phosphatase system. Interactions of the system with D-mannose 6-phosphate and D-mannose. J Biol Chem **251:**4891–4897, 1976.
39. Leskes A, Siekevitz P, Palade GE. Differentiation of endoplasmic reticulum in hepatocytes. II. Glucose-6-phosphatase in rough microsomes. J Cell Biol **49:**288–302, 1971.
40. Banhegyi G, Marcolongo P, Fulceri R, Hinds C, Burchell A, Benedetti A. Demonstration of a metabolically active glucose-6-phosphate pool in the lumen of liver microsomal vesicles. J Biol Chem **272:**13584–13590, 1997.
41. Waddell ID, Zomerschoe AG, Voice MW, Burchell A. Cloning and expression of a hepatic microsomal glucose transport protein: comparison with liver plasma-membrane glucose-transport protein glut 2. Biochem J **286:**173–177, 1992.
42. Burchell A. A re-evaluation of glut 7. Biochem J **331:**973, 1998.
43. Guillam MT, Burcelin R, Thorens B. Normal hepatic glucose production in the absence of glut2 reveals an alternative pathway for glucose release from hepatocytes. Proc Natl Acad Sci U S A **95:**12317–12321, 1998.
44. Burcelin R, del Carmen Munoz M, Guillam MT, Thorens B. Liver hyperplasia and paradoxical regulation of glycogen metabolism and glucose-sensitive gene expression in glut2-null hepatocytes: Further evidence for the existence of a membrane-based glucose release pathway. J Biol Chem **275:**10930–10936, 2000.
45. Mannella CA. Introduction: our changing views of mitochondria. J Bioenerg Biomembr **32:**1–4, 2000.
46. Mannella CA, Buttle K, Rath BK, Marko M. Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. Biofactors **8:**225–228, 1998.

47. Frey TG, Mannella CA. The internal structure of mitochondria. Trends Biochem Sci **25:**319–324, 2000.
48. Nordlie RC, Stepanik PA, Traxinger RR. Comparative reactivity of carbamyl phosphate and glucose-6-phosphate with the glucose-6-phosphatase of intact microsomes. Biochim Biophys Acta **881:**300–304, 1986.
49. Nordlie RC. Fine tuning of blood glucose concentrations. Trends Biochem Sci **10:**70–75, 1985.
50. Stetten MR. Metabolism of inorganic pyrophosphate. I. Microsomal inorganic pyrophosphate phosphotransferase of rat liver. J Biol Chem **239:**3576–3583, 1964.
51. Lueck JD, Nordlie RC. The utilization of intramitochondrially generated carbamyl phosphate for microsomal glucose 6-phosphate biosynthesis. FEBS Lett. **20:**195–198, 1972.
52. Bode AM, Nordlie RC. Reciprocal effects of proline and glutamine on glycogenesis from glucose and ureagenesis in isolated, perfused rat livers. J Biol Chem **268:**16298–16301, 1993.
53. Bode AM, Foster JD, Nordlie RC. Glycogenesis from glucose and ureagenesis in isolated perfused rat livers influence of ammonium ion, norvaline, and ethoxyzolamide. J Biol Chem **269:**7879–7886, 1994.
54. Hass LF, Byrne WL. The mechanism of glucose-6-phosphatase. Science **82:**947–954, 1960.
55. Nordlie RC. Glucose-6-phosphatase, hydrolytic and synthetic activities. In: Boyer PD, Ed. The Enzymes. New York: Academic Press, Vol. **4:**pp543–609, 1971.
56. Nordlie RC, Sukalski KA, Robbins BL. Some unique kinetic aspects of multifunctional glucose-6-phosphatase. Fed Proc **43:**1960, 1984.
57. Jorgenson RA, Nordlie RC. Multifunctional glucose-6-phosphatase studied in permeable isolated hepatocytes. J Biol Chem **255:**5907–5915, 1980.
58. Nordlie RC, Jorgenson RA. Latency and inhibitability by metabolites of glucose-6-phosphatase of permeable hepatocytes from fasted and fed rats. J Biol Chem **256:**4768–4771, 1981.