REVIEW ARTICLE

The glucose-6-phosphatase system

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Glucose-6-phosphatase (G6Pase), an enzyme found mainly in the liver and the kidneys, plays the important role of providing glucose during starvation. Unlike most phosphatases acting on water-soluble compounds, it is a membrane-bound enzyme, being associated with the endoplasmic reticulum. In 1975, W. Arion and co-workers proposed a model according to which G6Pase was thought to be a rather unspecific phosphatase, with its catalytic site oriented towards the lumen of the endoplasmic reticulum [Arion, Wallin, Lange and Ballas (1975) Mol. Cell. Biochem. **6**, 75–83]. Substrate would be provided to this enzyme by a translocase that is specific for glucose 6-phosphate, thereby accounting for the specificity of the phosphatase for glucose 6-phosphate in intact microsomes. Distinct transporters would allow inorganic phosphate and glucose to leave the vesicles. At variance with this substrate-transport model, other models propose that conformational changes play an important role in the properties of G6Pase. The last 10 years have witnessed important progress in our knowledge of the glucose 6-phosphate hydrolysis system. The genes encoding G6Pase and the glucose 6-phosphate translocase have been cloned and shown to be mutated in glycogen storage disease type Ia and type Ib respectively. The gene encoding a G6Pase-related protein, expressed specifically in pancreatic islets, has also been cloned. Specific potent inhibitors of G6Pase and of the glucose 6-phosphate translocase have been synthesized or isolated from micro-organisms. These as well as other findings support the model initially proposed by Arion. Much progress has also been made with regard to the regulation of the expression of G6Pase by insulin, glucocorticoids, cAMP and glucose.

Key words: gluconeogenesis, glucose 6-phosphate, glycogen, glycogen storage disease, transporter.

INTRODUCTION

One of the important functions of the liver and, to a lesser extent, of the kidney cortex is to provide glucose during conditions of starvation. Glucose is formed from gluconeogenic precursors in both tissues, and in the liver also from glycogen. Both gluconeogenesis and glycogenolysis result in the formation of glucose 6-phosphate (Glc-6-*P*), which has to be hydrolysed by glucose-6-phosphatase (G6Pase) before being liberated as glucose into the circulation. G6Pase plays thus a critical role in blood glucose homoeostasis.

The work of the Coris [1] showed that glycogen is degraded by phosphorolysis to glucose 1-phosphate, implying that the phosphate must be removed at a later stage to form free glucose. de Duve and co-workers [2] showed that the liver contains a phosphatase that can be partially purified by precipitation at pH 5 and acts specifically on Glc-6-*P*. Cell fractionation studies later showed that G6Pase is associated with the endoplasmic reticulum [3]. Further major developments were the finding that G6Pase deficiency is responsible for glycogen storage disease type I (GSD I) [4] and the hypothesis, put forward in 1975 by Arion and co-workers [5], that G6Pase has its catalytic site oriented towards the lumen of the endoplasmic reticulum, and that it requires transporters for Glc-6-*P*, glucose and P_i. Lack of Glc-6-*P* transport was then shown to be the cause of a variant of GSD I called GSD Ib [6].

Since then, important progress has been made in our knowledge of the G6Pase system, in particular with the cloning of two of its constituents and the identification of specific inhibitors. Our purpose is to review the present state of knowledge of this enzymic system, with particular emphasis on the progress made during the last 10 years. For other recent reviews on the subject, see [7,8].

G6Pase

Kinetic properties

Reactions catalysed and effect of detergents

The properties of G6Pase depend on the integrity of the microsomal membrane. In intact microsomes, it catalyses almost specifically the hydrolysis of Glc-6-*P* [9], its activity on this phosphate ester being more than 10-fold higher than that on mannose 6-phosphate (Man-6-*P*) [10]. Treatment with detergents, whether anionic, cationic or neutral, modestly stimulates the hydrolysis of Glc-6-*P*, but considerably (by 10–15-fold) increases the phosphatase activity on other substrates, such as Man-6-*P*, glucosamine 6-phosphate and 2-deoxyglucose 6-phosphate, which become as good substrates as Glc-6-*P* [10]. Two divergent interpretations have been given for this effect, based either on the existence of a separate specific Glc-6-*P* transporter or on detergent-induced conformational changes in G6Pase itself (see below).

G6Pase also hydrolyses substrates other than sugar phosphates, such as PP_i and carbamoyl phosphate [11]. The pyrophosphatase activity is increased approx. 2-fold by detergents, and is

Abbreviations used: AICAriboside, 5-amino-4-imidazolecarboxamide riboside; AICAR, 5-amino-4-imidazolecarboxamide ribotide; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulphonate; Glc-6-*P*, glucose 6-phosphate; G6Pase, glucose-6-phosphatase; GSD, glycogen storage disease; HNF, hepatocyte nuclear factor; Man-6-*P*, mannose 6-phosphate.

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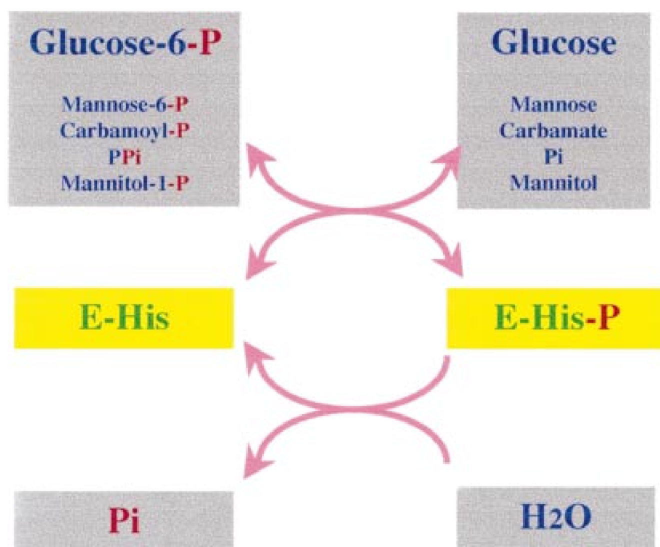

Figure 1 Reaction mechanism of G6Pase

In the presence of an adequate phosphate donor, the enzyme (E) forms a phosphoenzyme, which can be hydrolysed or can serve to phosphorylate another substrate. Under physiological conditions, only Glc-6-*P* serves as a substrate and the phosphoenzyme is hydrolysed.

maximal at a more acidic pH (5.5) than the G6Pase activity (6.5) [12]. Because of its reaction mechanism (Figure 1), which involves a phosphoenzyme intermediate [13] (see below), G6Pase also displays phosphotransferase activity, being able to synthesize Glc-6-*P* from glucose and various phosphate donors such as PP_i, Man-6-*P* [11] and carbamoyl phosphate [14]. These activities are more apparent in microsomes treated with detergents [12]. Due to this phosphotransferase activity, it has been proposed that G6Pase may also participate in the phosphorylation of glucose, by using carbamoyl phosphate as a phosphate donor, when the sugar concentration is elevated, as in diabetes [15]. However, the observations that G6Pase deficiency leads to profound hypoglycaemia and that its overexpression results in glucose intolerance indicate that the physiological role of this enzyme is in glucose production (see below). This conclusion is consistent with a study on isolated hepatocytes showing that the rate of glucose phosphorylation correlates with the activity of glucokinase [16].

Reaction mechanism

The observation that G6Pase catalyses an exchange reaction between glucose and Glc-6-*P* provided the first proof that this enzyme forms a phosphoenzyme intermediate [13], a mechanism that is also consistent with the multiple phosphotransferase activities. Incorporation of ^{32}P into microsomal protein upon incubation with [^{32}P]Glc-6-*P* was demonstrated, and was shown to be inhibited by unlabelled Glc-6-*P* and by PP_i, indicating that it is related to G6Pase [17,18]. The phosphoamino acid was identified as a phosphohistidine [18]. The apparent size of the labelled protein is 36.5 kDa [19].

Inhibitors

Glucose behaves as a non-competitive inhibitor of G6Pase, irrespective of the presence of detergents, with a K_i amounting to 50–200 mM [20,21]. Inhibition by P_i is non-competitive in intact microsomes, but competitive in the presence of detergents [22], which has been taken as an indication for the presence of a P_i transporter (see below).

Vanadate, a well known inhibitor of phosphatases that form a phosphoenzyme intermediate [23], inhibits the phosphohydrolase and phosphotransferase activities of G6Pase. It is more potent in detergent-treated than in intact microsomes, with K_i values of 2.2 and 5.6 μM respectively [24]. Its effect is suppressed by metal chelators such as EDTA (E. Van Schaftingen and I. Gerin, unpublished work), presumably due to the formation of an EDTA–vanadate complex [25]. Tungstate is also a potent inhibitor of G6Pase, with a K_i of 10–25 μM in intact microsomes and of 1–7 μM in detergent-treated preparations [26].

G6Pase is inhibited by several amphiphilic compounds, such as fatty acids [27] and acyl-CoAs [28,29]. The latter are inhibitory at low concentrations, but result in activation at higher concentrations, probably due to disruption of the microsomal membrane. Phosphoinositides such as diphosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 4,5-bisphosphate and phosphatidylinositol 3,4-bisphosphate are also inhibitors, displaying K_is in the micromolar range [30]. The inhibitory effect of phosphoinositides is less marked, although present in cholate-treated microsomes, and is of the competitive type, which suggests binding to the catalytic site [30]. Phosphoinositide concentrations needed to observe inhibition are relatively high (up to approx. 30 %) when expressed as a proportion of the total phospholipid content of microsomes. The physiological significance of these effects therefore deserves further investigation.

Potent inhibitors of G6Pase, with K_i values as low as 0.17 μM, have recently been synthesized [31,32]. Compounds that inhibit G6Pase by acting on the Glc-6-*P* translocase are discussed later in the review.

Purification

Many attempts have been made to purify G6Pase. This turned out to be a very difficult task, owing to the instability of the enzyme after its extraction from membranes [33–35] and to the fact that G6Pase represents only approx. 0.1 % of total liver microsomal protein. The most successful purification was based on the observation that treatment of microsomes with pyridoxal 5′-phosphate, followed by reduction with sodium borohydride, rendered G6Pase more stable after its extraction from membranes [36]. Starting from microsomes treated in this way, rat liver G6Pase was purified approx. 700-fold by combining several chromatographic steps. Although not completely homogeneous after the last step, G6Pase could be identified as a 35 kDa polypeptide that co-eluted with the enzymic activity [36].

cDNA and gene

The cloning of the cDNA encoding G6Pase took advantage of the low level of expression of this enzyme in mice homozygous for the radiation-induced albino mutation, which causes profound hypoglycaemia in the first hours after birth. The activities of some other hepatic enzymes, such as phosphoenolpyruvate carboxykinase and glutamine synthetase, are also lowered in these mice [37,38]. A differential screening of cDNA libraries led to the isolation of several clones that are weakly expressed in the livers of mice homozygous for the lethal albino mutation [39]. One of these was shown [40] to encode a 357-residue protein which was particularly hydrophobic and possessed two lysine residues in positions −3 and −4 with respect to the C-terminus, i.e. a retention signal for the endoplasmic reticulum [41]. Expression of the cDNA in COS cells showed that it indeed coded for G6Pase [40].

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GPHs MEEGMNVLHDFGIQSTHYLQVNYQDSQDWFILVSVIADLRNAFYVLFPIWFH  52
GPSa ----MDLLHSWGVELAVHLQTTYSSYEGLFGLASTVADLHTTFFFFFPFWFH  48
RPMm ----MDFLHRSGVLIIHHLQEDYRTYYGFLNFMSNVGDPRNIFSIYFPLWFQ  48

                                                *
GPHs LQEAVGIKLLWVAVIGDWLNLVFKWILFGQRPYWWVLDTDYYSNTSVPLIKQ 104
GPSa LRRDTGLRLIWVAVLGDWLNLVLKWVLFGERPYWWVHETRFYGAAPAPALRQ 100
RPMm LNQNVGTKMIWVAVIGDWFNLIFKWILFGHRPYWWIQETEIYPNHSSPCLEQ 100
CPO                      353 KWEFEFWRP 361

GPHs FPVTCETGPGSPSGHAMGTAGVYYVMVTSTLS-IFQGKIKPTYRFRCLN-VI 154
GPSa FPITCETGPGSPSGHAMGAAGVWYVMVTALLA-TATEKRCPPLLYRFLQ-LG 150
RPMm FPTTCETGPGSPSGHAMGSSCVWYVMVTAALSYTISRMEESSVTLHRLTWSF 152
CPO       398 AYPSGHATF 406
                         P
GPHs LWLGFWAVQLNVCLSRIYLAAHFPHQVVAGVLSGIAVTETFSHIHSIYNASL 206
GPSa LWALMGLLVLVVCMSRVYMAAHFPHQVIAGVITGVLVAEVVSKEKWIYDASM 202
RPMm LWSVFWLIQISVCISRVFIATHFPHQVILGVIGGMLVAEAFEHTPGVHMASL 204
CPO     482 LMFENAISRIFLGVHWRFD 500

GPHs KKYFLITFFLFSFAIGFYLLLKGLGVDLLWTLEKAQRWCEQPEWVHIDTTPF 258
GPSa RKYFHTTLSLTSLAVGFYLLLRVLGVDLLWTMEKAQKWCVNPEWVHLDSTPF 254
RPMm SVYLKTNVFLFLFALGFYLLLRLFGIDLLWSVPIAKKWCANPDWIHIDSTPF 256

GPHs ASLLKNLGTLFGLGLALNSSMYRESCKGKLSKWLPFRLSSIVASLVLLHVFD 310
GPSa ASLLRNMGTLFGLGLGLHSPLNSETKNTSTS----FKTGCIIVSLFLLHLLD 302
RPMm AGLVRNLGVLFGLGFAINSEMFLRSCQGENGTKPSFRLLCALTSLTTMQLYR 308

GPHs SLKPPSQVELVFYVLSFCKSAVVPLASVSVIPYCLAQVLGQPHKKSL      357
GPSa GWTFSSENLTTFYFLSFGKSAVALLIPTTLVPFALCWIRPGKTEDKNL     350
RPMm FIKIPTHAEPLFYLLSFCKSASIPLMVVALIPYCVHMLMRPGDKKTK      355
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Figure 2 Alignment of human G6Pase (GPHs) with *Sparus aurata* (gilthead sea bream) G6Pase (GPSa) and mouse islet G6Pase-related protein (RPMm)

The predicted transmembrane domains are highlighted in yellow, the cytosolic loops in green and the luminal loops in cyan. The predicted phosphorylation site is indicated by 'P' (red), and the glycosylation site by an asterisk. Parts of the sequence of *Curvularia inaequalis* vanadium-dependent chloroperoxidase (CPO) are also shown.

This cDNA allowed the isolation of the human cDNA, which encodes a protein (Figure 2) of the same size as mouse G6Pase, and of the human [42] and mouse [40] genes. Both are 10–12 kb long and comprise five exons. The human gene is localized in 17q21 [43]. The cDNA sequences from other species, including rat [44], dog [45] and two fish species [46] (Figure 2), have also been described.

Screening of a mouse insulinoma library yielded a cDNA encoding a protein sharing 49 % identity with liver G6Pase [47] (Figure 2). This protein is also very hydrophobic and possesses a retention signal for the endoplasmic reticulum. Northern blots indicate that it is expressed exclusively in the endocrine pancreas. The coding sequence has been inserted in various expression vectors, which were transfected in various cell types. These attempts have, however, failed to demonstrate that the encoded protein has a phosphatase or a phosphotransferase activity, suggesting that this G6Pase-related protein is not responsible for the G6Pase activity observed in islets. The mouse [48], human and rat [49] genes corresponding to this cDNA have been identified and their structures determined. The mouse and human genes are both localized on chromosome 2, and have the same five-exon structure as the G6Pase gene. The rat gene appears to be a pseudogene, since exon 4 is missing, and exons 1 and 5 are interrupted by frame-shifting mutations [49]. The role (if any) of the product encoded by this gene in humans and mice is still elusive.

Sequence comparisons and membrane topology

Sequence comparisons indicate that G6Pase shares three conserved motifs (KXXXXXXRP, PSGH and SRXXXXXHXXXQ/D) with several membrane phosphatases acting on lipids, such as phosphatidic acid phosphatase and sphingosine-1-phosphatase, as well as with a soluble, vanadate-dependent chloroperoxidase (Figure 2) [50–52]. The three-dimensional structure of the latter enzyme indicates that vanadate is bound to the extremely conserved histidine residue in the third motif [53]. This chloroperoxidase also has a minor phosphatase activity [52]. These data suggest that the phosphorylatable residue in human G6Pase is His^{176} [50–52]. Replacement of this residue indeed results in a complete loss of activity. Site-directed mutagenesis studies have confirmed that the conserved His^{119} residue in the second motif, and Arg^{83} in the first motif, play critical roles, the former most probably as an acid–base catalyst and the latter by forming an ion pair with the phosphate group [54].

Although the three-dimensional structure of G6Pase is not known, its membrane topology has been studied. A model with six transmembrane domains was first proposed [55], but has been superseded by a model with nine transmembrane domains [54,56]. In the latter (Figure 2), the conserved residues predicted to play a role in catalysis (Arg^{83}, His^{119} and His^{176}) are on the same side of the membrane, whereas they are distributed on both sides of the membrane in the other model. Furthermore, only the model

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with nine transmembrane domains is compatible with studies showing that, in intact microsomes, the N-terminus is protected against attack by proteases, but the C-terminus is not [54]. Mutagenesis studies and *in vitro* translation experiments indicate that there is only one glycosylation site, at Asn^{96} [57], which is compatible with both models.

Short-term regulation

The K_m of G6Pase (2–3 mM) is higher than the intracellular concentration of Glc-6-*P* (0.05–1 mM). The physiological activity of this enzyme is thus regulated by substrate concentration. The latter increases several-fold when glycogen degradation and/or gluconeogenesis are stimulated by adrenergic hormones or by glucagon, accounting for their stimulatory effect on glucose production (reviewed in [58]). There is indeed a nice correlation between glucose production and the intracellular concentration of Glc-6-*P* in hepatocytes incubated with gluconeogenic precursors with or without glucagon [59]. (Note that hepatocyte suspensions often contain extracellular Glc-6-*P* resulting from cell breakage [60].)

At variance with these results, Ichai et al. [61] observed that glucagon increased glucose production from dihydroxyacetone, but decreased the intracellular concentration of Glc-6-*P*, in perifused hepatocytes. Similarly, infusion of glucagon to anaesthetized rats caused a 1.8-fold increase in hepatic glucose production, but a 2.8-fold decrease in the Glc-6-*P* level measured after 3 h, again suggesting activation of G6Pase. A potential explanation for the *in vivo* results is that the effect of glucagon decreases progressively with time, despite sustained infusion, due to desensitization of its receptor [62]. The Glc-6-*P* level measured at the end of the experiment therefore may not be representative of what it was during the whole infusion time. This explanation does not apply to the experiments performed with perifused hepatocytes, because hepatocytes were sampled repeatedly. This decrease in Glc-6-*P* level induced by glucagon contrasts, however, with many other reports showing the opposite effect in perfused liver [63,64], suspensions of isolated hepatocytes [59] and perifused hepatocytes [65]. There is presently no simple explanation for this contradiction.

The inhibition exerted by glucose on G6Pase is probably of little physiological significance, since the K_i value (≈ 0.1 M) is more than one order of magnitude above glycaemia. It is noteworthy, nevertheless, that glucose stimulates glycogen synthesis and, at least under some conditions, decreases the Glc-6-*P* concentration, hence slowing down glucose formation by G6Pase (reviewed in [66]).

The physiological significance of the inhibition exerted by acyl-CoAs is questionable, since the liver contains a fatty-acyl-CoA binding protein [67], which may well prevent this effect. Purified glucokinase is also inhibited by long-chain acyl-CoAs [68], but flux measurements in hepatocytes do not indicate that these inhibitors play a role in the control of this enzyme activity in the intact cell [69]. The fact that phosphatidylinositol 3-kinase is translocated to the liver endoplasmic reticulum upon refeeding makes the participation of inhibition by phosphoinositides in the control of G6Pase an attractive hypothesis [70].

Tissue distribution

G6Pase is expressed mainly in the liver and in the kidney cortex [71], the two most important gluconeogenic tissues. It is also expressed in the β-cells of pancreatic islets [72] and in the intestinal mucosa of the human, mouse [73] and rat [74], most particularly in the starved and diabetic states [75]. Isotope dilution experiments indicate that the intestine may contribute approx. 20 % of total glucose production in starved or diabetic rats [76,77]. Low G6Pase activities have also been described in skeletal muscle [78] and in astrocytes [79]. These activities represent, at most, a few per cent of the activity found in the liver, and probably have little physiological significance [80]. The presence of G6Pase has also been detected histochemically in the syncytiotrophoblasts of the placenta [81]. Dilution of isotopically labelled glucose in the umbilical vein of the fetus as compared with maternal blood indicates production of glucose by the placenta [82].

Northern blots indicate that the G6Pase mRNA is expressed principally in liver and kidney, but so in lower amounts in the small intestine [40,74,83]. Lower levels of expression have been detected in other tissues by reverse transcription–PCR [84].

Regulation of expression in the liver

Perinatal period

G6Pase activity greatly increases in rat liver around birth [85], i.e. when the animal must become autonomous in terms of glucose production. The increase in G6Pase activity is preceded by an increase in the concentration of its mRNA, which is highest at parturition [86]. G6Pase activity appears to be less specific in extracts of fetal than of adult liver. Furthermore, the fetal liver enzyme is inhibited by an antibody raised against the loop containing the catalytic site, whereas the adult enzyme is not. This suggests an extravesicular orientation of the catalytic site in a significant fraction of microsomes derived from fetal liver [87], possibly because of the presence of vesicles with an inverted orientation. The physiological relevance of these observations is unknown. In the kidneys, both the mRNA and the activity of G6Pase appear during the first days of extrauterine life, reaching a maximum after 2–3 weeks [74,86].

Starvation, diabetes and insulin

Starvation and diabetes cause a 2–3-fold increase in G6Pase activity in the liver [88–90]. Northern blots have shown that these conditions are associated with a 2–4-fold increase in G6Pase mRNA [91–93]. These effects are probably due to the reciprocal changes in insulinaemia and glucagonaemia. Insulin causes a decrease in the activity of G6Pase in the liver *in vivo* [94,95]. It also decreases G6Pase mRNA both *in vivo* and in hepatoma cells, where the effect appears to be transcriptional [91,96]. An insulin response element has been identified, which comprises two regions. Region A (-231 to -199), an accessory region, binds hepatocyte nuclear factor 1 (HNF1), which enhances insulin signalling [97]. Region B (-198 to -159), which contains three insulin response sequences, is able to confer insulin sensitivity to a heterologous promoter [97]. The forkhead transcription factor FKHR bind(s) to the insulin response sequence [98], stimulates the expression of G6Pase [99] and may mediate the effect of insulin [100]. FKHR is phosphorylated by protein kinase B and is then translocated out of the nucleus. Evidence has been provided for the involvement of this protein kinase [99,100], as well as of phosphatidylinositol 3-kinase [101], in the effect of insulin (reviewed in [102]).

Glucocorticoids

Injection of glucocorticoids *in vivo* increases to a modest extent (by about 40 %) the activity of G6Pase in the livers of control and adrenalectomized rats [94,103]. Dexamethasone causes a

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larger (up to 10-fold) increase in G6Pase activity [104] and in the level of its mRNA [44,105] in cultured hepatoma cells. The latter effect is completely suppressed by 0.1 nM insulin [44]. The promoter of the G6Pase gene contains two potential glucocorticoid response elements, but only one of them (between -178 and -164) is required for glucorticoid action. Binding of HNF1 to its cognate site (between -226 and -212) plays an essential role in the effect of glucocorticoids [106].

cAMP

The effect of cAMP to increase the expression of G6Pase is a transcriptional effect [74], involving several *cis*-acting sequences in the promoter. One of them, in the region between -114 and -99, binds HNF6, which is phosphorylated by cAMP-dependent protein kinase [107]. Another sequence, located between -161 and -152, is a cAMP response element that partially overlaps the most proximal insulin response sequence in region B and binds the cAMP response element binding protein [108,109]. As for glucocorticoids and insulin, HNF1 appears to play the role of an accessory factor to enhance the effect of cAMP [109].

Glucose

An elevated concentration of glucose (27.5 mM) causes a 3-fold increase in G6Pase mRNA in Fao hepatoma cells, and a 20-fold increase in cultured hepatocytes [110]. Glucose increases the mRNA by up to 20-fold in hepatoma cells overexpressing glucokinase. The G6Pase mRNA is increased by approx. 6-fold and its activity by approx. 2-fold when the intracellular concentration of fructose 2,6-bisphosphate is increased through expression of a 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase mutant lacking fructose-2,6-bisphosphatase activity [110]. These observations indicate that a metabolite of the glycolytic/gluconeogenic pathway could play an important role in the expression of G6Pase. Accordingly, the effect of glucose is mimicked by substrates (xylitol, fructose, and to a lesser extent mannose and glycerol) that enter glycolysis/gluconeogenesis at different levels [111]. The glucose effect is mediated partly through a transcriptional effect and partly through stabilization of the mRNA [111].

The role played by glucose *in vivo* has been studied in rats made diabetic by subtotal pancreatectomy. The $\approx$ 5-fold increase in G6Pase mRNA observed under this condition can be corrected by normalizing glycaemia not only with insulin but also with phloridzin, which increases the urinary excretion of glucose by blocking tubular reabsorption. These results indicate that glucose may play a direct role as a stimulator of G6Pase expression [112]. Little effect of glucose on G6Pase mRNA has, however, been observed in dogs clamped at two different concentrations of glucose and fixed concentrations of insulin [96].

Other effects

Up-regulation of G6Pase expression is observed when animals are treated with an inhibitor of the Glc-6-*P* translocase [113,114]. This effect is not suppressed when hypoglycaemia is prevented by a glucose clamp [113], indicating that it is not mediated through hormonal changes, but possibly through changes in the concentration of an intracellular metabolite. Inhibition of the Glc-6-*P* translocase causes a marked (more than 4-fold) increase in the concentration of Glc-6-*P* [114], and probably also in the concentrations of fructose 2,6-bisphosphate and some glycolytic intermediates.

Long-chain fatty acids increase the amount of G6Pase mRNA in cultured fetal hepatocytes [74] and in adult rat liver [115]. PMA [116] and AICAriboside (5-amino-4-imidazolecarboxamide riboside) [117], which is phosphorylated in the cell to AICAR (5-amino-4-imidazolecarboxamide ribotide; also known as ZMP), a stimulator of AMP-activated protein kinase, inhibit the expression of G6Pase in hepatoma cells, suggesting that protein kinase C and AMP-activated protein kinase participate in the control of the expression of this enzyme. It should be kept in mind that AICAR is also an inhibitor of fructose-1,6-bisphosphatase [118]. Therefore the effect of AICAriboside may be mediated by a glycolytic intermediate.

ARION'S SUBSTRATE-TRANSPORT MODEL

In 1975, Arion et al. [5] proposed a model (Figure 3), later termed the 'substrate-transport model', to account for the properties of G6Pase. According to this model, G6Pase is a phosphatase with a rather broad specificity, which has its catalytic site oriented towards the lumen of the endoplasmic reticulum. Transfer of Glc-6-*P* from the cytosolic face to the lumen is carried out by a (reversible) transporter (Glc-6-*P* translocase; sometimes called T1). The membrane of the endoplasmic reticulum is also proposed to contain structures permitting the exit of P_i (P_i translocase; T2) and of glucose (T3).

If one takes into account the fact that the lumen of the endoplasmic reticulum corresponds to the lumen of microsomes, and its cytosolic face to the external face of microsomes, this model provides an explanation for the following observations. (1) G6Pase is almost specific for Glc-6-*P* in intact microsomes, but behaves as a phosphatase with broad specificity when the microsomal membrane is disrupted. Thus Man-6-*P*, the C-2 epimer of Glc-6-*P*, is hydrolysed at a rate of $\approx 5\%$ of that of Glc-6-*P* in intact microsomes, but at almost the same rate in detergent-treated microsomes. According to Arion's model, this is due to the fact that the Glc-6-*P* transporter is unable to transport Man-6-*P*, therefore imposing its specificity to the phosphatase in intact microsomes. Thus determination of mannose-6-phosphatase activity can serve to determine the degree of membrane integrity. Mannose-6-phosphatase latency is defined as the proportion of the total phosphatase activity that is revealed by detergents [119]. (2) G6Pase activity is partially latent in intact microsomes, with detergents decreasing the K_m by approx. 2-fold. This indicates that the transporter is not sufficiently rapid to allow equilibration of Glc-6-*P* across the microsomal membrane [5]. (3) Some thiol reagents inhibit G6Pase activity much more in intact than in disrupted microsomes [120,121]. Such inhibitors are presumed to act at lower concentrations on the Glc-6-*P* transporter than on the hydrolase. (4) The activation energy of G6Pase is approx. 40% higher in intact microsomes than in disrupted preparations [10], suggesting the presence of an additional step (substrate transport) in intact microsomes. (5) Cytochemical studies show that a lead phosphate precipitate forms inside the cisternae of the endoplasmic reticulum when liver preparations are incubated with Glc-6-*P* and Pb^{2+} [122,123], suggesting luminal orientation of the catalytic site.

A nice proof for the substrate-transport model would have been to purify its constituents and demonstrate that each of them has the expected function. However, only G6Pase could be purified until now, and none of the transporters could be studied through reconstitution experiments.

As reviewed in the following sections of the present paper, Arion's model has received additional support over the years. This includes: (1) experiments confirming the existence of an independent Glc-6-*P* transporter; (2) the identification of

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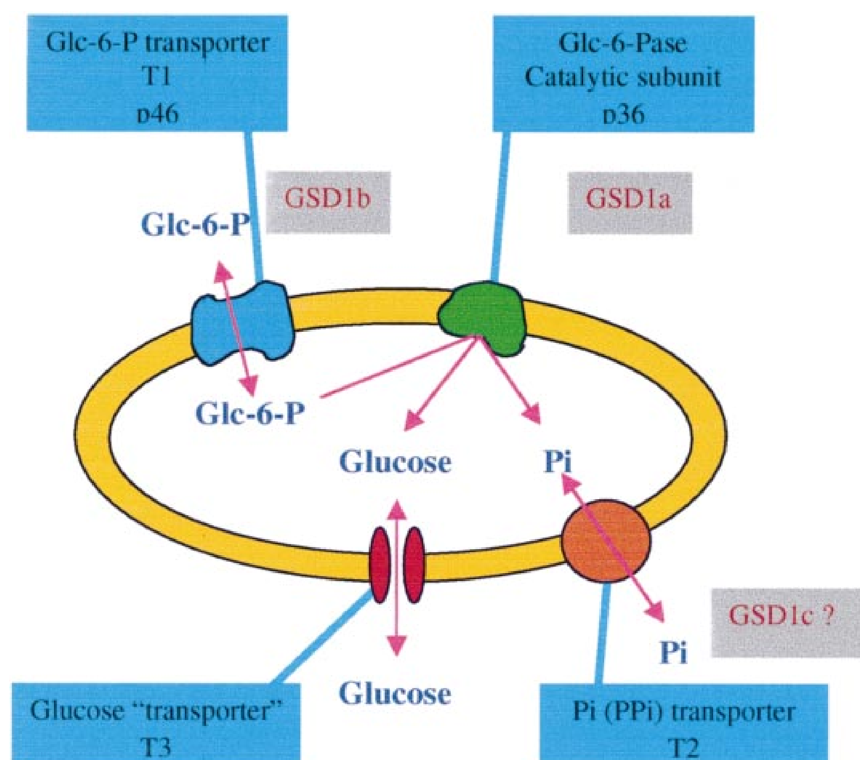

Figure 3 Substrate-transport model (Arion's model)

The boxes show the synonyms used to denote the four components of the G6Pase system. Also shown are the different forms of GSD caused by a deficiency in one of these constituents.

inhibitors acting specifically on this translocase; (3) the identification of a variant form of GSD (GSD Ib) that is due to a defect in Glc-6-*P* transport; (4) the cloning of the gene mutated in this disease, which codes for a protein belonging to the family of organophosphate transporters; and (5) the demonstration that the products of the reaction catalysed by G6Pase are first liberated in the lumen of microsomes.

Nevertheless, a few experimental observations are, according to some authors, difficult to reconcile with the substrate-transport model, and alternative models have been proposed (see below).

THE Glc-6-*P* TRANSLOCASE

Evidence for Glc-6-*P* transport

To check for the presence of a specific Glc-6-*P* transporter in the endoplasmic reticulum, Ballas and Arion [124] incubated rat liver microsomes with Glc-6-*P* or Man-6-*P* and centrifuged the preparation through a silicone layer in a medium containing $HClO_4$. From measurements of the hexose 6-phosphate present in the $HClO_4$ extract, they concluded that Glc-6-*P* was more concentrated than Man-6-*P* inside microsomes. The difficulty with such a technique is that the extravesicular water space is quite large ($\approx 80\%$) compared with the total water space in the $HClO_4$ layer, leading to large errors in the calculation of concentrations (a few negative values were computed).

Glc-6-*P* transport in microsomes has most often been measured by a filtration technique: microsomes are incubated with ^{32}P- or ^{14}C-labelled substrates and spotted on to a filter, which is washed as rapidly as possible (usually in approx. 20 s). This technique shows that Glc-6-*P* enters liver microsomes, whereas Man-6-*P* does not, and that the entry of Glc-6-*P* is deficient in GSD type Ib [125,126] (see below). What accumulates inside microsomes is essentially glucose and P_i [127,128], which is not surprising considering the high G6Pase activity in the microsomal lumen and the fact that this enzyme catalyses a highly exergonic reaction. Thus glucose and P_i can reach much higher concentrations in the lumen than Glc-6-*P*, the concentration of which is at most equal to that in the medium (and probably much less, because it can escape during the filtration step). This feature explains how the intramicrosomal accumulation of radioactivity can be inhibited by vanadate and in G6Pase deficiency [129,130]. Some authors have even failed to demonstrate any uptake of Glc-6-*P* in the presence of vanadate [131], probably due to leakage during the filtration step.

Although these observations have been interpreted as indicating that Glc-6-*P* cannot enter without being hydrolysed [130], there is ample evidence that entry of Glc-6-*P* into microsomes is not dependent on G6Pase. In fact, Banhegyi et al. [128] have shown that the size of the intramicrosomal Glc-6-*P* pool is inversely correlated with G6Pase activity in microsomes incubated with Glc-6-*P*, providing evidence that this intramicrosomal pool is metabolically active. More proof for the independence of Glc-6-*P* entry from G6Pase activity comes from the observation that Glc-6-*P* can be utilized by phosphoglucose isomerase (as indicated by the release of tritiated water from [2-^{3}H]Glc-6-*P*) when this enzyme has been artificially entrapped in liver microsomes. This utilization by phosphoglucose isomerase is not inhibited by vanadate, but rather is stimulated by it, indicating that intravesicular phosphoglucose isomerase competes with G6Pase for its substrate [132].

Another – at first sight – puzzling observation with the filtration technique described above is that intravesicular glucose and

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P_i account at most for only ≈ 10 % of the Glc-6-*P* that has been hydrolysed, even after short incubation times (< 10 s) [133,134]. In our view, this is due to the fact that glucose and P_i escape quickly (in a few seconds or less) from most of the vesicles, which are equipped with a rapid transport mechanism for glucose or P_i, but that they remain for a longer time in the minority of vesicles that are devoid of such rapid transport mechanisms (Figure 4). As mentioned below, the characteristics of glucose transport in microsomes are compatible with this interpretation. Furthermore, ≈ 80 % of P_i is still intravesicular after 5 min of incubation when Glc-6-*P* hydrolysis takes place in the presence of Pb^{2+} [132]. In such experiments, the formation of a lead phosphate complex prevents the rapid efflux of P_i.

The transport of Glc-6-*P* and other solutes can also be determined by light-scattering, which monitors modifications in the size and the shape of vesicles resulting from osmotic changes in the medium. When a non-permeant solute is added at a sufficiently high concentration, the vesicles shrink rapidly, due to water loss, and the light-scattering signal changes rapidly to reach a new plateau. When a permeant solute is added, the vesicles first shrink, then re-expand slowly due to entry of solute and water. This results in a biphasic signal, the rapid change in one direction being followed by a slow change in the other direction [135]. This technique showed that Glc-6-*P* enters microsomes more rapidly than Man-6-*P*, and that its entry is not affected by vanadate, indicating that the transporter functions independently from the phosphatase [136]. The problem with the light-scattering technique is that it is not possible to quantify the rate of solute entry in microsomes.

Inhibitors of Glc-6-*P* transport

Inhibitors of the Glc-6-*P* translocase are expected to inhibit G6Pase in intact but not in disrupted microsomes. Furthermore, they should not inhibit the pyrophosphatase activity of G6Pase. The existence of compounds with such properties is an important argument in favour of a Glc-6-*P* transporter [137].

One such compound is DIDS (4,4′-di-isothiocyanostilbene-2,2′-disulphonate) [138], a well known inhibitor of anion transporters: it inhibits G6Pase with a K_i of 35 μM, more than one order of magnitude lower than the K_i for the pyrophosphatase activity. Incubation of [^{3}H]DIDS with rat liver microsomes resulted in its covalent binding to a protein of 54 kDa. This binding was inhibited slightly more by Glc-6-*P* than by Man-6-*P*, suggesting that the labelled protein was the Glc-6-*P* translocase [139]. However, as will be discussed below, the Glc-6-*P* translocase has a predicted mass of 46 kDa and migrates in SDS/PAGE with an apparent mass of ≈ 37 kDa [140].

3-Mercaptopicolinic acid, a well known inhibitor of phosphoenolpyruvate carboxykinase, appears to inhibit Glc-6-*P* translocase at millimolar concentrations [141]. Tosyl-lysylchloromethane ('TLCK') and tosylphenylalanylchloromethane ('TPCK'), two protease inhibitors, were also reported to inhibit G6Pase by blocking entry of Glc-6-*P* into microsomes [142].

More specific inhibitors of the Glc-6-*P* translocase have been identified or synthesized by the pharmaceutical industry as potential new therapeutic agents for diabetes. Starting from chlorogenic acid, a weak (K_i 0.5 mM) inhibitor of G6Pase activity in intact microsomes [143], potent derivatives, with K_i values lower than 1 μM, have been synthesized [144,145]. They decrease glucose production in isolated hepatocytes and *in vivo*, and cause glycogen accumulation in liver and kidney [144,146–148]. Further proof for the fact that this class of compounds acts on the Glc-6-*P* translocase came with the observation that one of them (S3483) inhibits phosphoglucose isomerase when this enzyme is entrapped in liver microsomes, but has no effect on the free enzyme [132]. Furthermore, S3483 causes the intravesicular accumulation of Glc-6-*P* in microsomes incubated with glucose and carbamoyl phosphate (I. Gerin and E. Van Schaftingen, unpublished work). Of interest is the fact that S3483 does not inhibit G6Pase activity in extracts of islets of Langerhans, indicating the presence of a different Glc-6-*P* translocase [149].

Binding of a radiolabelled chlorogenic acid derivative (S5627) to liver microsomal membranes was shown to be competed by Glc-6-*P*, but not by Man-6-*P*, as expected for a ligand of the Glc-6-*P* translocase [137]. A photoaffinity label derived from chlorogenic acid (S0957) binds covalently to several proteins of rat and human liver microsomes. Among these, a 50 kDa protein was tentatively identified as the rat Glc-6-*P* translocase, and a 55 kDa protein as the human Glc-6-*P* translocase [150].

Inhibitors of Glc-6-*P* translocase from two other families have recently been identified. Kodaistatin A and C, isolated from *Aspergillus terreus*, inhibit with K_i values of 80 nM and 130 nM respectively [151], whereas mumbaistatin, isolated from *Streptomyces* sp., acts with a K_i of 5 nM and is therefore the most potent compound known hitherto [152].

Cloning of the cDNA and the gene encoding the Glc-6-*P* translocase

The cDNA encoding this protein was identified owing to its homology with bacterial transporters. Besides the endoplasmic reticulum of animal cells, two other types of membranes are known to contain Glc-6-*P* transporters: (1) the inner membrane of bacteria, such as *Escherichia coli*, which are able to use Glc-6-*P* as a substrate (reviewed in [153]); and (2) the membrane of plant amyloplasts, which exchange hexose phosphates for P_i with the cytoplasm [154]. The bacterial transporter for hexose phosphates, known as UhpT, belongs to a family of organophosphate transporters. This family also contains GlpT, the transporter for glycerol 3-phosphate [155], and PgtP, the transporter for phosphoglycerate, as well as UhpC, a putative Glc-6-*P* receptor controlling the expression of UhpT [156,157]. UhpT and GlpT function as antiporters that exchange phosphate esters against one or two molecules of P_i, depending on pH [158]. The hexose-phosphate transporters of plant amyloplasts belong to a different family, which comprises also the triose-phosphate/P_i exchangers of the chloroplast membrane.

A search in EST (expressed sequence tag) databanks allowed the identification of mouse and human cDNA sequences showing identity with the bacterial hexose-phosphate translocase [159]. This permitted the cloning of a full-length cDNA encoding a 429-residue hydrophobic protein displaying sequence identity of 26 % with UhpC, 25 % with GlpT and 20 % with UhpT. This protein possesses a retention signal for the endoplasmic reticulum. Northern blots showed that the corresponding mRNA was 2.2 kb long and was more abundant in liver and kidney than in other tissues, as expected for a protein involved in gluconeogenesis. Proof that this cDNA encoded the Glc-6-*P* translocase of the endoplasmic reticulum came from the finding that it was mutated in two patients with GSD type Ib [159] (see also below).

The sequences of cDNAs encoding mouse and rat Glc-6-*P* translocases were reported later [83]. They encode proteins sharing 93 % identity with their human counterpart. BLAST searches with the human sequence indicated the presence of homologous cDNAs encoding proteins with 90–95 % identity in the pig and the cow, ≈ 80 % in the chicken, ≈ 75 % in the zebrafish and ≈ 50 % in *Trichinella spiralis* (a roundworm) and *Ambliomma americanum* (a tick) (E. Van Schaftingen and I. Gerin,

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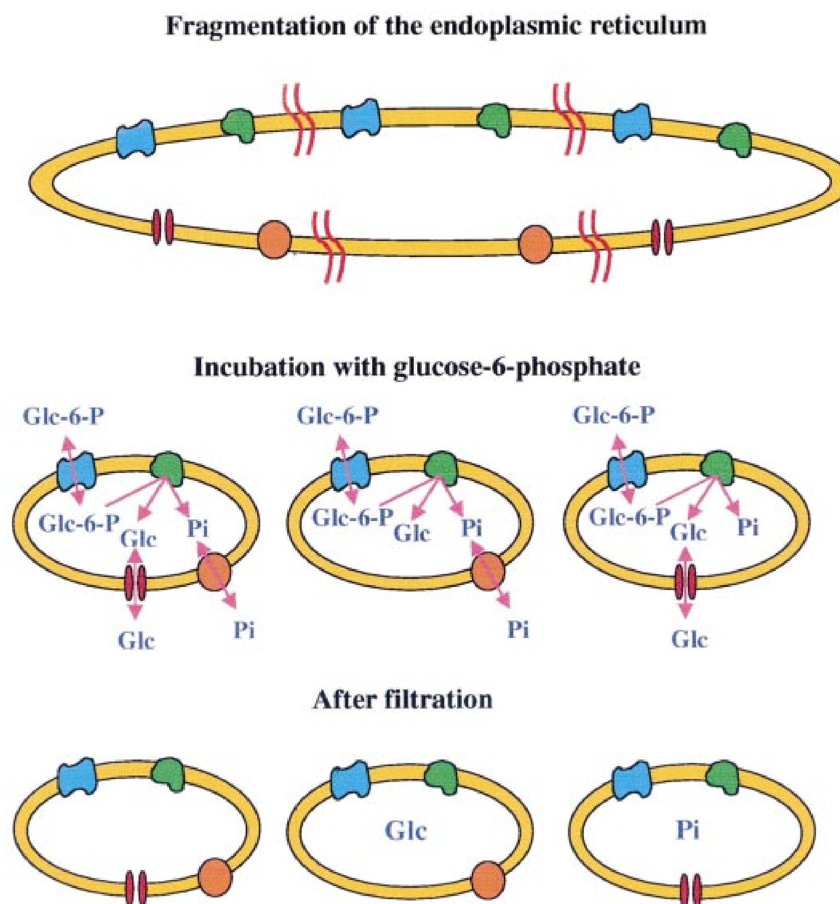

Figure 4 Implications of microsomal heterogeneity for the interpretation of uptake measurements

Fragmentation of the endoplasmic reticulum during homogenization means that portions of the microsomal vesicles lack the glucose 'transporter' (centre) or the P_i transporter (right), whereas other vesicles (left) contain all four constituents. Glc-6-*P* enters the three types of microsomes and is hydrolysed in the lumen, and the products escape rapidly if the appropriate transporter is present. Upon filtration, intact Glc-6-*P* is lost rapidly from the three types of vesicles; P_i and glucose also escape unless the vesicles are devoid of the appropriate transporter. Other vesicles (not shown) may lack the Glc-6-*P* translocase (not shown), accounting for partial latency of G6Pase.

unpublished work). By contrast, the genomes of *Saccharomyces cerevisiae* and *Drosophila melanogaster* do not seem to contain homologous proteins. The closest bacterial transporters to human Glc-6-*P* translocase are sequences from various *Chlamydia* species, which show only 34% identity with the human protein. These observations do not support the proposal that Glc-6-*P* translocase has been imported from a bacterial genome through lateral transfer [160]. The sequence of human Glc-6-*P* translocase shows internal identity between amino acids 89–186 and 317–414, suggesting an ancestral gene duplication event. A similar internal identity has been identified in glucose transporters [161].

The gene encoding human Glc-6-*P* translocase comprises nine exons, with the initiator ATG and the stop codon in the first and last exon respectively [162–166]. Exon 7, which comprises 66 nucleotides in the human and bovine sequences and 63 nucleotides in the mouse sequence, is present in mRNAs of brain, heart and skeletal muscle, but not of liver, kidney or leucocytes [164,167–170]. The amino acid sequence encoded by this exon is relatively conserved (> 90% identity between the human and mouse sequences). The role of this exon is still unknown (see below), although it does not appear to be related to G6Pase, as indicated by the difference in tissue distribution.

5′-Rapid amplification of cDNA ends and RNase protection assays performed on RNAs from human liver, kidney and leucocytes indicated that the same two main regions of transcription start, at approx. −200 and −100 bp with respect to the initiator ATG, were used in all three tissues or cells [164].

Functional studies and membrane topology

The human Glc-6-*P* translocase has been overexpressed successfully in COS cells [165]. The fusion protein with a FLAG tag migrates in SDS/PAGE gels with an apparent molecular mass of ≈ 37 kDa [171], lower than the expected mass (46 kDa), possibly because of the hydrophobic character of the protein. Overexpression of Glc-6-*P* translocase alone modestly (less than 2-fold) stimulates the uptake of Glc-6-*P* in microsomes, but has a larger effect (at least 5-fold) when G6Pase is co-expressed [165]; the mechanism of this apparent synergism between G6Pase and the translocase has been discussed above. The transport induced by overexpression of the Glc-6-*P* translocase shows the same sensitivity to inhibition by chlorogenic acid as the transporter found in liver microsomes [165]. Furthermore, the mutations found in GSD type Ib lead to a loss of activity of this protein (see below). These results are consistent with the con-

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clusion that the cloned cDNA encodes the chlorogenic acid-sensitive Glc-6-*P* translocase that is mutated in GSD type Ib.

As mentioned above, the Glc-6-*P* translocase is homologous to bacterial transporters for hexose 6-phosphates. These belong to the same superfamily of transmembrane facilitators as do hexose transporters, which classically contain 12 transmembrane helices [172]. Accordingly, a first model proposed for the human Glc-6-*P* translocase contained 12 helices [159]. However, structure predictions based on a recent algorithm indicate that it has only 10 transmembrane helices [171]. Both models agree with the finding that the two extremities of the protein are on the cytosolic side of the membrane, as indicated by the sensitivity to protease of constructs containing an N- or C-terminal FLAG peptide linked to the Glc-6-*P* translocase sequence by a consensus cleavage site for factor Xa. Both models also agree with the fact that the protein is not glycosylated, the only potential glycosylation site being on the cytosolic side of the membrane [171]. To discriminate between the two models, Pan et al. [171] introduced, by site-directed mutagenesis, two glycosylation consensus sequences at the level of a loop predicted to be in the lumen of the endoplasmic reticulum in the 10-transmembrane-helix domain model, but on the cytosolic side in the 12-transmembrane domain model. Expression of the mutant protein in COS cells indicated that it was glycosylated, in support of the 10-helix model.

Expression studies in COS cells have also shown that the form of the translocase containing exon 7 transports Glc-6-*P* as well as the shorter form does [170]. Topological predictions indicate that the peptide encoded by exon 7 is inserted in a luminal loop between helices 7 and 8 in the 10-helix model [171]. Such a localization could allow the interaction of the long form of the translocase with a luminal protein.

Taken together, these studies show that the putative mammalian Glc-6-*P* translocase indeed transports Glc-6-*P*. Based on its closer similarity to UhpC, the putative bacterial Glc-6-*P* receptor, than to UhpT, the bacterial Glc-6-*P* translocase (26% compared with 21% sequence identity), it has been proposed that mammalian transporter might also function as a Glc-6-*P* receptor [173]. However, the fact that the transporter shows 25% sequence identity with the bacterial transporter for glycerol 3-phosphate (i.e. more than with UhpT) illustrates that it is not possible to predict the precise function of a protein on the sole basis of low degrees of identity. There is therefore presently no reason to believe that this transporter plays the role of a receptor.

Tissue distribution and regulation of expression

The mRNA encoding the Glc-6-*P* translocase is most abundant in the liver and in the kidneys, but is also present at lower levels in all other tissues or cells examined, including neutrophils [83,159,164,169,174]. As mentioned above, the form including exon 7 is expressed in brain, heart and skeletal muscle. Low amounts of other splice variants have been identified [169], but their physiological significance is unknown.

The mRNA encoding Glc-6-*P* translocase is present in mouse liver on the 16th day of intrauterine life. Its concentration increases, reaching a maximum during the 3 days before birth, and decreases slightly after birth [86]. Western blots using an anti-peptide antibody indicate that the Glc-6-*P* translocase, also called p46 by some authors, is present at day 20 of intrauterine life, and that its concentration does not vary to a significant extent afterwards [175]. Light-scattering measurements indicate that Glc-6-*P* transport is active before birth [87]. However, filtration assays measuring the uptake of [^{14}C]Glc-6-*P* indicate that Glc-6-*P* transport appears only after birth [175] and reaches the adult level after 4 weeks. The disagreement between these results is probably due to the fact that the second type of assay, but not the first, depends on the presence of an active G6Pase (see above), which appears in the liver around birth. The mRNA encoding Glc-6-*P* translocase is detectable in the kidneys on the 19th day of intrauterine life, and its concentration increases in the first days of extrauterine life, whereas G6Pase activity increases progressively during the first 3 weeks after birth [86].

Streptozotocin-induced diabetes increases the concentration of Glc-6-*P* translocase mRNA by 2–3-fold in rat liver and kidney. Western blots indicate that the amount of protein is increased approx. 2-fold in the livers of diabetic rats as compared with control animals [176]. Starvation increases the level of the mRNA by $\approx 30\%$, without significantly changing the concentration of the protein [177]. Insulin decreases the amount of translocase mRNA in hepatoma cells [176,178] and in dog liver [96] to a lesser extent than its effect on the G6Pase mRNA. In HepG2 hepatoma cells, an elevated glucose concentration (25 mM), known to stimulate the expression of G6Pase, increases the amount of the translocase mRNA by $\approx$ 5-fold and also, to a lesser extent, the amount of the protein [178]. cAMP does not appear to stimulate the expression of the translocase in hepatoma cells [96,178].

The administration of a phosphate-poor diet, known to stimulate the expression of some P_i transporters [179], increases by $\approx$ 2-fold the concentrations of the mRNAs encoding G6Pase and Glc6P translocase in rat liver. An $\approx$ 2-fold increase in the amount of immunoreactive G6Pase is also observed, but there is no significant change in the amount of immunoreactive translocase [177].

Transactivation studies indicate that HNF1α is required for the transcription of the Glc-6-*P* translocase gene, accounting for the low level of expression of this constituent of the G6Pase system in HNF1α null mice [180].

Existence of a distinct, less specific, transporter

Transport of Glc-6-*P*, and also of glucose 1-phosphate, into microsomes derived from fibroblasts has been demonstrated. This transport is not inhibited by chlorogenic acid and is not deficient in fibroblasts derived from patients with GSD type Ib, indicating that it is not carried out by the specific Glc-6-*P* translocase [181]. Due to the existence of such a non-specific transporter, mannose 6-phosphatase latency must be interpreted with caution, since part of the hydrolysis of Man-6-*P* may be carried out by intact microsomes into which Man-6-*P* is imported by this non-specific carrier. This interpretation agrees with the finding that the substrate saturation curve of mannose-6-phosphatase activity is biphasic in untreated microsomes [182].

TRANSPORT OF P_i

Independence from Glc-6-*P* transport

The fact that the mammalian Glc-6-*P* translocase belongs to the same family as UhpT and GlpT, which act as exchangers, makes it particularly relevant to determine whether Glc-6-*P* and P_i are transported by the same carrier in the endoplasmic reticulum.

Kinetic studies carried out on liver microsomes have indicated the existence of a common transporter for P_i, PP_i and carbamoyl phosphate, which is distinct from the Glc-6-*P* transporter [22]. Thus, in disrupted microsomes, P_i, PP_i and carbamoyl phosphate act as competitive inhibitors of G6Pase activity, whereas they behave as non-competitive inhibitors in intact microsomes. Again in intact microsomes, the hydrolysis of PP_i is competitively inhibited by P_i and carbamoyl phosphate, but

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non-competitively by Glc-6-*P* [22]. These results indicate that P_i, PP_i and carbamoyl phosphate compete for a common step, distinct from the hydrolysis by G6Pase.

Assuming that P_i and PP_i use the same transport system, the fact that the pyrophosphatase activity of microsomes is not affected by the specific inhibitors of Glc-6-*P* transport confirms the existence of a distinct transporter for P_i and PP_i [143]. P_i entry into microsomes has also been demonstrated by the light-scattering technique, showing that the transport of this ion is not affected in GSD Ib, a condition in which Glc-6-*P* transport is abolished [183].

Taken together, all of these data argue against the Glc-6-*P* translocase playing the role of a Glc-6-P/P_i exchanger, which may seem surprising, since the homologous proteins, UhpT and GlpT, behave as exchangers. However, all of these proteins belong to a superfamily comprising uniporters (e.g. Glut1 etc.), symporters (e.g. the inositol–Na^+ symporter) and antiporters (UhpT and GlpT) [172], which suggests that it is not possible at this stage to predict the physiological behaviour of a transporter on the sole basis of its sequence.

Recent experiments using a filtration technique indicate a slow uptake of P_i, with a half-life of 20–25 s and a low activation energy, suggesting intervention of a pore rather than a transporter [132]. Furthermore, P_i release from microsomes (whether after loading with Glc-6-*P* or with P_i) takes place with the same time constant as its entry, irrespective of the presence of Glc-6-*P* [131]. We have mentioned above that substantial liberation of P_i from Glc-6-*P* in the luminal compartment of intact microsomes can only be demonstrated in the presence of Pb^{2+}, which forms a complex with P_i and so prevents its exit from microsomes. This indicates that P_i efflux from most of the vesicles is likely to occur with a half-life much shorter than 20–25 s, probably of the order of 1 s. This rapid transport cannot be investigated by the filtration technique.

Molecular identity

The identity of the protein(s) involved in P_i transport in microsomes is still elusive. Mammalian cells contain several P_i transporters. The one present in the internal membranes of the mitochondria is a 34 kDa protein [184] that carries out the exchange of monobasic phosphate for OH^-. P_i transporters of the plasma membrane carry out Na^+-dependent transport, and belong to at least three different families: NaP_i-I (also called NPT-1), NaP_i-II and NaP_i-III [185].

It has been reported that microsomal fractions from human liver contain a protein that cross-reacts with antibodies directed against the mitochondrial P_i transporter, and is the same size as this protein [186]. Since this protein was absent from microsomes derived from a patient with GSD type Ic (supposed to be deficient in P_i transport; see below), the conclusion was reached that microsomal P_i transport is effected by the same protein as mitochondrial P_i transport. This conclusion has to be taken with caution, however, because most patients with GSD type Ic have later proved to be deficient in the Glc-6-*P* translocase [168,187–189]. The results of Waddell and co-workers [186] are probably explained by variable degrees of contamination of the microsomal fraction by submitochondrial particles.

THE GLUCOSE ‘TRANSPORTER’ OF THE ENDOPLASMIC RETICULUM

Transport of glucose in animal cells

The facilitated transport of glucose across the plasma membrane is effected by a family of proteins known as the Glut proteins, of which at least eight members are known: Glut1–5 (reviewed in [190,191]), Glut8, Glut9 and Glut10 [192–195]. These transporters are stereospecific, and most of them are inhibited by cytochalasin B and phlorizin. They differ from each other by their kinetic properties, specificity and tissue distribution, and by the fact that some of them (Glut4, Glut 8) can shuttle from an intracellular membrane pool to the plasma membrane in response to insulin.

In liver and kidneys, the two organs in which G6Pase is most active, transport of glucose across the basolateral plasma membrane is carried out by Glut2 [196]. This transporter, which has a relatively high K_m for glucose ($\approx$ 15–20 mM; [197]), is sufficiently active to maintain the intracellular concentration of glucose at the same level as in blood. Histochemical studies have shown that Glut2 is found mainly in the plasma membrane, but some authors have described its association with intracellular membranes [198]. Based on this observation, a cDNA was isolated which encoded a protein (called Glut7) sharing substantial sequence identity with Glut2, but possessing a retention signal for the endoplasmic reticulum [199]; however, this work has been retracted [200].

Measurement of glucose transport in microsomes

Measurement of glucose uptake by the filtration technique is hampered by the fact that radioactive glucose that has entered vesicles can escape rapidly during filtration, particularly because there is no simple means of blocking this outflow. To circumvent this difficulty, Meissner and Allen [201] studied the exit of glucose and other solutes from microsomes preincubated for 1 h with radioactive compounds. This incubation time allows equilibration of all compounds, including sucrose, across the membrane. The microsomes were then diluted 500-fold in an isotonic medium and filtered for different times (30 s to 4 min) thereafter, and the retained radioactivity was counted. These experiments showed that glucose and other solutes of the same size (including L-glucose), but not sucrose, crossed the microsomal membrane of 50–75 % of the vesicles rapidly (half-life < 10 s), and crossed that of the remaining vesicles more slowly (half-life ≈ 3 min). The presence of a rapid transport mechanism was confirmed by the finding that microsomes loaded with sucrose burst (and lose therefore radiolabelled sucrose) when they are diluted in a hypotonic medium, or in a medium made isotonic with a permeant molecule, whereas they remain intact if they are diluted in a medium made isotonic with a non-permeant molecule. Taken together, these experiments showed that microsomal membranes are permeable to a number of neutral or cationic compounds, probably due to a pore-like structure with an internal diameter of 7–8 Å (0.7–0.8 nm). The fact that L-glucose is transported as well as D-glucose argues strongly against the intervention of one of the transporters of the Glut family. The pore-like structure would not be particularly abundant, explaining why some of the microsomal vesicles are devoid of it, but of course this would not apply to the *in vivo* situation, where the endoplasmic reticulum is a continuous structure (Figure 4).

Banhegyi and co-workers [202] confirmed the existence of two phases in the outflow of glucose from pre-loaded vesicles. The slow phase is inhibited neither by phloretin nor by cytochalasin B, indicating that it is not mediated by a member of the Glut family. Glucose exit in this phase is proportional to the intravesicular glucose concentration and has a similar time constant as the outflow of glucose from vesicles that have been incubated with [^{14}C]Glc-6-*P* [202].

Glucose entry into microsomes has also been studied by the light-scattering method [203]. It takes place with a half-life of ≈ 4 s at 22 °C, displays a very high K_m (> 100 mM) and is partly

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inhibited by cytochalasin B and by pentamidine. This method confirmed that several other solutes (L-glucose, 2-deoxyglucose, mannitol) enter microsomes with similar kinetics as glucose, supporting the pore-like nature of the structure involved in this process.

Glucose uptake into liver microsomes has also been detected with the direct uptake method [134,202], but it should be kept in mind that this method is not able to detect the rapid transport mechanism, which is the physiologically most relevant. A very high K_m value (> 100 mM [202]) and a low activation energy {≈ 33.5 kJ (≈ 8 kcal)/mol [134]} have been noted. The failure to detect glucose entry into liver microsomes by some authors [204] is most probably due to the utilization of mannitol as a marker for the extravesicular space; mannitol indeed penetrates liver microsomes [201].

Vesicle-mediated transport of the glucose released by G6Pase?

It is generally accepted that glucose formed by G6Pase is first transferred to the cytosol before being transported out of the liver by Glut2. However, Glut2 homozygous knockout mice have nearly normal liver glucose production [205]. This observation led Thorens and co-workers [205] to propose that the glucose formed by G6Pase bypasses Glut2 and is secreted by way of a vesicular transport mechanism. Accordingly, glucose production by hepatocytes from Glut2$^{-/-}$ mice was inhibited by progesterone, which is known to slow down exocytosis.

Such a mechanism would require an elevated intravesicular glucose concentration and substantial vesicular traffic: if the luminal concentration were 100 mM, glucose production of 2 μmol · min · g^{-1} wet weight would be associated with a traffic corresponding to 2 μmol · min^{-1} · g^{-1}/100 μmol · ml^{-1}, i.e. ≈ 2 % of the cell volume per min! Furthermore, the velocity with which glucose exits microsomes *in vitro*, and therefore the endoplasmic reticulum *in vivo*, is a major objection against such a mechanism. In addition, vesicular transport would not explain the glycogen accumulation found in the livers of patients with Fanconi–Bickel syndrome, a disease due to Glut2 deficiency [206]. By contrast, the classical secretion mechanism implies that glucose, a well known precursor and activator of glycogen synthesis [66], should accumulate in the cytosol of patients with this condition. It is more likely that glucose transport in Glut2$^{-/-}$ mice involves one of the more recently described members of the Glut family [192–194].

OTHER PROCESSES INVOLVING THE TRANSPORTERS

Hexose-6-phosphate dehydrogenase

Hexose-6-phosphate dehydrogenase is another enzyme that utilizes Glc-6-*P* in the endoplasmic reticulum. It is less specific than cytosolic Glc-6-*P* dehydrogenase, since it acts not only on Glc-6-*P*, but also on Man-6-*P*, deoxyglucose 6-phosphate, galactose 6-phosphate and even, at elevated concentrations, glucose [207–209]. Hexose-6-phosphate dehydrogenase is apparently present in all tissues, but is particularly active in the liver and adrenal gland [210]. It is an ≈ 800-amino-acid protein that contains an N-terminal domain homologous to Glc-6-*P* dehydrogenase and a C-terminal domain homologous to 6-phosphogluconolactonase, indicating that it catalyses the first two reactions of the pentose phosphate pathway [211–213]. The role of hexose-6-phosphate dehydrogenase is to provide the reducing equivalents needed for several reductases present in the lumen of the endoplasmic reticulum, including 11β-hydroxysteroid dehydrogenase-1 [214], metyrapone reductase [215], thioredoxin reductase [216] and glutathione reductase [217].

Hexose-6-phosphate dehydrogenase is latent in liver microsomes, probably due to poor penetration of NADP [209]. Oxidation of Glc-6-*P* in intact liver microsomes is, however, stimulated by electron acceptors and inhibited by the anion transport inhibitor DIDS [217] and by the chlorogenic acid derivative S3483 (I. Gerin and E. Van Schaftingen, unpublished work), indicating that Glc-6-*P* must be transported before being utilized by the enzyme. The wide tissue distribution of hexose-6-phosphate dehydrogenase is therefore consistent with the presence of the mRNA encoding Glc-6-*P* translocase in all tissues.

Stimulation of Ca^{2+} uptake by Glc-6-*P*

Glc-6-*P* increases the uptake of Ca^{2+} in liver or kidney microsomes incubated with Mg-ATP [218,219], probably due to the trapping of Ca^{2+} as a complex with the P_i formed from Glc-6-*P*. Accordingly, the stimulation is not observed in the presence of vanadate or in microsomes derived from hepatoma cells, which contain very little G6Pase [220].

Stimulation of the uptake of Ca^{2+} by 2–5 mM Glc-6-*P* has also been observed in brain and heart microsomes [221]. Since the microsomes from these tissues have very little, if any, G6Pase, the explanation proposed for the liver does not apply. One possibility is that the rather elevated concentrations of Glc-6-*P* that were used are able to form a complex with Ca^{2+} in the microsomal lumen when this ion is taken up and concentrated by Ca-ATPase. This is consistent with the finding that the intramicrosomal Glc-6-*P* concentration exceeds its concentration in the medium in the presence of ATP and Ca^{2+} [221]. The physiological significance of this regulation of Ca^{2+} uptake by Glc-6-*P* is not known.

Glycoprotein synthesis

The transport of P_i and glucose out of the endoplasmic reticulum must probably also take place in relation to protein N-glycosylation. This process involves the assembly of an oligosaccharide on a dolichol pyrophosphate molecule, the transfer of the oligosaccharide to its acceptor protein, and the trimming and remodelling of the protein-bound oligosaccharide (reviewed in [222]). The first steps in the formation of the dolichol pyrophosphate oligosaccharide take place on the outer leaflet of the endoplasmic reticulum membrane, whereas the last seven steps, i.e. the transfer of glucose and mannose from dolichol phosphate precursors, occur on the luminal side. The transfer of the oligosaccharide to the acceptor protein also takes place in the lumen. In order to be re-utilized, dolichol pyrophosphate and dolichol phosphate have to flip their hydrophilic head back on the cytosolic face of the membrane. It is thought that this process necessitates dephosphorylation by dolichol phosphate phosphatase on the luminal face of the membrane [223], thus liberating P_i.

It is well established that trimming of the protein-bound oligosaccharides releases three molecules of glucose into the lumen of the endoplasmic reticulum. The latter most probably have to get out of the organelle. Since the steps of N-glycosylation that occur in the endoplasmic reticulum are conserved among eukaryotes, the proteins that are involved in the transport of P_i and of glucose out of this organelle may be conserved as well, which could be of great help for their identification.

DEFECTS IN THE G6Pase SYSTEM

GSD type I (or von Gierke disease) is an autosomal recessive disorder that is caused by deficient G6Pase activity (reviewed in

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[224]). It is characterized by the association of hepatomegaly and nephromegaly, due to the accumulation of large amounts of glycogen in these organs, with hypoglycaemia and lactic acidosis. Hyperuricaemia and hyperlipidaemia are also commonly found. Two main forms of this disease have been described.

GSD type Ia

This is the most frequent form, accounting for about 80 % of the cases. As shown initially by Cori and Cori in 1952 [4], it is caused by a lack of G6Pase activity, which is easily demonstrated by measuring the activity of this enzyme in a liver biopsy specimen.

The deficiency of G6Pase activity is caused by mutations in the gene encoding this enzyme [42,189,225–227]. In their 1999 review paper [226], Chou and Mansfield mentioned 31 distinct mutations identified in a total of 128 patients. Most of them (20) are missense mutations; others include nonsense mutations, insertions/deletions with or without a shift in the reading frame, or splice site mutations. The missense mutations are clustered in the two large luminal loops and in the transmembrane helices in the topological model of G6Pase, suggesting that these structures are critical for G6Pase activity. No mutation has been identified in the four cytoplasmic loops. Transient expression in COS cells has shown that most of the missense mutations abolish or greatly reduce G6Pase activity [42,43,55].

A mouse model of G6Pase deficiency has been generated by homologous recombination [130]. $G6Pase^{-/-}$ mice show essentially the same symptoms as human patients with GSD Ia, including liver and kidney enlargement, hypoglycaemia, growth retardation, hyperlipidaemia and hyperuricaemia. Blood lactate is, however, not increased. Glycogen and lipid accumulation is observed in the liver and, as expected, G6Pase activity is not detectable in liver microsomes. Little transport of [^{14}C]Glc-6-*P* can be demonstrated with the filtration technique [130], but, as discussed above, this is no proof that the Glc-6-*P* transporter is deficient. Correction of GSD Ia by adenovirus-mediated expression of murine G6Pase has been reported [228].

G6Pase deficiency was also described in two Maltese puppies with hepatomegaly and growth retardation. cDNA analysis revealed a homozygous mutation in the G6Pase gene leading to replacement of Met^{121} by an isoleucine. Transient expression analysis has shown that this mutation decreases G6Pase activity by approx. 15-fold [45]. A canine model of GSD Ia has been produced by cross-breeding dogs with this mutation [229].

GSD type Ib

In the late 1950s, a number of patients were identified with the typical symptoms of GSD type I, but normal liver G6Pase activity. The term GSD type Ib was coined [230], to distinguish this new form of GSD I from the classical form, now termed type Ia. Soon after the seminal publication by Arion on the transporter model [5], Narisawa and co-workers [6] proposed that GSD type Ib is due to a lack of Glc-6-*P* transport in the endoplasmic reticulum. In support of this, G6Pase activity was shown to be abnormally latent in extracts from fresh liver, although not in extracts from frozen liver (which was mostly used until then for practical reasons) [6,231]. Narisawa's group later showed that the uptake of [^{14}C]Glc-6-*P* is reduced in microsomes from patients with GSD type Ib [125].

The identification of the cDNA mutated in GSD type Ib and the finding that it encodes a protein of the organophosphate transporter family [159] fully confirmed the view that this disease is due to a lack of Glc-6-*P* transport. The availability of the gene structure allowed the analysis of more than 80 patients with GSD Ib [163,166,168,188–190,203,232,233]. Around 60 different mutations have been identified. Most of them are 'severe' mutations (splice site mutations, frame-shifting mutations, substitutions of a highly conserved residue), likely to result in a complete loss of function. Chen et al. [140] studied the effects of 16 substitutions found in GSD type Ib on the transport activity of Glc-6-*P* translocase by expressing the mutated proteins together with G6Pase in COS cells. They found that 15 of these mutations abolished Glc-6-*P* transport, the only exception being a substitution ($Asn^{198} \rightarrow Ile$) thought to be a polymorphism rather than a disease-causing mutation [168]. Among the 15 inactivating mutations, only three resulted in destabilization of the protein, indicating that at least 12 of the other substitutions result in an intrinsic loss of transport activity.

Chen et al. [140] have studied the effects of nonsense mutations near the C-terminal end. Mutation W393X, which truncates the protein in the middle of helix 10, abolishes the expression of the protein. By contrast, R415X, which removes the last cytosolic part of the protein, reduces expression by about 70 %, while keeping significant transport activity. Stop codons introduced beyond residue 415 have no effect on the expression of the protein or on its transport activity [140]. Since these proteins are devoid of their two C-terminal lysines, this result suggests that other signals exist to retain the Glc-6-*P* translocase in the endoplasmic reticulum.

Neutropenia in GSD type Ib

One distinguishing clinical feature between GSD Ia and GSD Ib is that most patients with the latter condition are more susceptible to bacterial infections, owing to neutropenia and neutrophil dysfunction. Polymorphonuclear neutrophils phagocytose and kill bacteria and other micro-organisms. For this purpose they are able to produce oxygen derivatives with bactericidal action (superoxide anion, OH radical, H_2O_2, ClO^-). Superoxide anions are produced from O_2 and cytosolic NADPH by NADPH oxidase. Its production can be stimulated *in vitro* by opsonized yeast particles (zymosan), by *N*-formylmethionyl-leucylphenylalanine (a chemotactic peptide) and by PMA, which causes a significant increase in O_2 consumption known as 'respiratory burst' (reviewed in [234]). Most [235,236] patients with GSD Ib have neutropenia, which is apparently not due to a defect in production by the bone marrow, since there appears to be increased myelopoietic activity [237]. Neutrophil dysfunction, which is also present in almost all patients with GSD Ib [235,236,238], consists of a decrease in the respiratory burst and motility in response to stimuli. In addition, neutrophils from patients with GSD Ib show a lower rise in cytosolic Ca^{2+} [239] and in glucose consumption [240–242] in response to stimuli. A few patients do not suffer from neutropenia. One of them was shown to have mutation R415X [233], which only partially affects the activity of the transporter [140].

The link between this variety of perturbations and the absence of functional Glc-6-*P* translocase is not clear. G6Pase is not present in leucocytes, and there is no evidence of problems with neutrophils in patients with GSD Ia (with the exception of one reported case [243]). This indicates that an enzyme different from G6Pase has to be supplied with Glc-6-*P* in the endoplasmic reticulum of neutrophils. This enzyme is most probably hexose-6-phosphate dehydrogenase, which serves to produce NADPH in the endoplasmic reticulum and therefore permits the regeneration of reduced glutathione. The latter is likely to play an important role by protecting the endoplasmic reticulum against damage by reactive oxygen species. Our hypothesis is that this lack of protection results in premature cell death through apoptosis.

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Other forms of GSD type I?

Nordlie and co-workers [244] described the case of an 11-year-old patient, with Type I (insulin-dependent) diabetes, who suffered from frequent episodes of hypoglycaemia and had liver glycogenosis. The levels of several enzymes of glycogen metabolism (debranching enzyme, lysosomal α-glucosidase) were normal. Total G6Pase activity in the liver was in the normal range, but its degree of latency was very high ($\approx 75\%$), although lower than in patients with GSD type Ib. The carbamoyl-phosphate: glucose phosphotransferase, pyrophosphate:glucose phosphotransferase and pyrophosphatase activities were totally latent, indicating a defect in the P_i transporter. The authors termed this new metabolic disorder 'GSD type Ic'.

The conclusion that this disease is due to a deficiency in P_i translocase must, however, be treated with caution. In the absence of a P_i transporter, the increase in G6Pase latency must be due to the intramicrosomal accumulation of P_i, an inhibitor of the reaction. It is expected, therefore, that the formation of product should fall off progressively in the course of the incubation, which was not observed [244,245]. Furthermore, no complete clinical description of this case has been published, and the hypoglycaemic episodes may well be the result of insulin overdosage. A deficiency in phosphorylase kinase, which could explain glycogen accumulation in the liver and the hypoglycaemic episodes, was apparently not ruled out. Recent work has indicated that this patient has no mutation in the genes encoding G6Pase and the Glc-6-*P* translocase [246,247].

Since this publication, GSD type Ic has been diagnosed in other cases, based on abnormal latency of G6Pase and inorganic pyrophosphatase in liver microsomes [245,248–250]. Clinically, these cases do not seem to be distinguishable from GSD type Ib, although no systematic study has been published. It has been established that these patients have mutations in the Glc-6-*P* translocase [168,187–189], a finding which agrees with the observation that the loci for GSD Ib [251] and GSD Ic [252] are located in the same region (q23) of chromosome 11. Since there is ample evidence that Glc-6-*P* and P_i (as well as PP_i) are transported by distinct mechanisms, these patients have been concluded to suffer from GSD type Ib [168,187,253].

The Glc-6-*P* translocase gene is also mutated [187] in a patient thought to be deficient in the microsomal glucose transporter (GSD Id) [254]. A mutation in G6Pase gene has been found [246] in a patient reported to be deficient in a G6Pase-stabilizing protein (GSD IaSP) [255].

Taken together, these data indicate that mutations in the G6Pase gene (GSD Ia) and in the Glc-6-*P* translocase gene (GSD Ib) account for most, if not all, typical cases of GSD type I [253]. The existence of other forms of GSD I remains to be substantiated.

Overexpression studies

Overexpression of G6Pase in hepatocytes using recombinant adenovirus causes a marked decrease in the concentrations of Glc-6-*P* and glycogen, and a several-fold increase in the rate of glucose formation, as assessed by the incorporation of ^3H_2O into this sugar [256,257]. It also decreases glucose usage, as determined by the rate of formation of ^3H_2O from 3-[^{3}H]glucose, probably by diverting [3-^{3}H]Glc-6-*P* towards hydrolysis. It does not, however, change the level of UDP-glucose, indicating that the effect on glycogen is mediated through a decrease in the stimulation of glycogen synthase by Glc-6-*P* [256]. Overexpression of the Glc-6-*P* translocase, in contrast, has a much smaller effect on glycogen concentration and no detectable effect on glycolysis [258]. These results indicate that G6Pase, rather than the translocase, is rate-limiting. Surprisingly, overexpression of the translocase enhanced hydrolysis of glucose 1-phosphate in intact microsomes [258]. Further work is needed to confirm this effect and understand its mechanism.

Overexpression of G6Pase in the liver *in vivo* induces several of the abnormalities that are associated with Type II (non-insulin-dependent) diabetes mellitus, including glucose intolerance, hyperinsulinaemia, decreased hepatic glycogen content and increased muscle triacylglycerol stores. However, it also causes a decrease in circulating levels of fatty acids and triacylglycerols, probably as a result of increased insulinaemia [259].

CONFORMATIONAL MODELS

Before the cloning of the cDNA encoding the Glc-6-*P* translocase and the identification of inhibitors acting on this protein (which strongly supported the substrate-transport model), other models were proposed to account for properties of the enzyme that were apparently difficult to reconcile with Arion's model. All of them assume that G6Pase can undergo conformational changes that explain its change of specificity in the presence of detergents. The arguments supporting these models are reviewed in the following paragraphs.

Combined conformational-flexibility substrate-transport model

According to this model [260], G6Pase would be deeply embedded in the microsomal membrane and in contact with a channel allowing access of Glc-6-*P* from the medium to the catalytic site and diffusion of the reaction products to the lumen. This model is based on the observation that an agarose-bound organomercurial inhibits G6Pase in intact microsomes, indicating that the catalytic site is not deeper in the membrane than the total length of the free portion of the inhibitor (18 Å, compared with a membrane thickness of 60 Å) and therefore not on the luminal side. Furthermore, polyclonal antibodies raised against microsomes inhibit G6Pase in detergent-treated microsomes, but not in sonicated or intact microsomes, indicating that the enzyme is buried in intact microsomes. The channel is required to allow access of the substrate to the catalytic site and to account for the observation that at least some of the products are released into the microsomal lumen.

This model is not easy to reconcile with the topological model of G6Pase (see above). The latter clearly indicates the presence of several hydrophilic loops that most probably reside alternatively on the cytosolic and luminal faces of the membrane. Furthermore, the fact that the Glc-6-*P* transporter and G6Pase are separate entities is now well established. It is likely that the agarose-bound organomercurial inhibits G6Pase not by binding directly to its catalytic site, but by binding to one of the transmembrane helices, and that detergents permit an easier access of the catalytic site to antibodies than sonication.

This model is thought also to account for the fact that only a small proportion of the products appears to be released into the lumen of the microsomes, the rest being apparently discharged directly into the medium [133]. The best explanation for such results is that glucose and P_i escape rapidly from most of the vesicles (see above).

Apparent coupling of transport and hydrolysis

We have already mentioned the fact that the uptake of [^{14}C]Glc-6-*P* is apparently inhibited if G6Pase is inhibited or inactive. These results formed the basis of a model in which the transport and hydrolysis of Glc-6-*P* are coupled to each other [129,130]. However, we have explained in an earlier section how such

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results can be reconciled with the substrate-transport model. The latter is strongly supported by experiments showing that the entry and the hydrolysis of Glc-6-*P* are independent from each other.

Modification of the properties of G6Pase during the reaction

In Arion's substrate-transport model, Glc-6-*P* must enter the microsomes before being hydrolysed by G6Pase. There should therefore be an initial short lag period during which the reaction accelerates before reaching a steady state. Using a rapid sampling apparatus, Berteloot and co-workers [127] measured the formation of P_i and glucose at times ranging from 0.5 to 60 s after initiation of the reaction. However, they did not observe the expected 'lag' kinetics, but rather there was a 40 % decline in reaction rate (not explained by substrate exhaustion) that occurred progressively after approx. 10 s ('burst'-type kinetics). No decrease in the rate was observed in detergent-treated microsomes. From the absence of a lag phase the authors concluded that Glc-6-*P* does not have to enter the microsomes to be hydrolysed by G6Pase, and from the burst-type kinetics that the enzyme undergoes a conformational change.

Other intriguing observations have been made with respect to the kinetic properties of G6Pase in the first few seconds of the reaction. Man-6-*P* exerts up to 40 % inhibition in this initial burst phase, but only 15 % inhibition at later times [261]. Furthermore, G6Pase appears to be less specific in the initial phase than later, being able to hydrolyse Man-6-*P* as quickly as Glc-6-*P* [262,263]. All these results have been interpreted as indicating a progressive conformational change of G6Pase.

With respect to the absence of the lag phase expected from Arion's model, one may wonder if such a lag could really be detected in the experimental conditions of Berteloot et al. [127]. From their data, and assuming an intravesicular space of 3 μl/mg of protein [203], it can be calculated that the intravesicular pool of Glc-6-*P* must be renewed at least once every 0.6 s, whereas the first sample was taken after 0.5 s. Another critique concerns the medium (0.15 M $ZnSO_4$) used to stop the reaction, which is probably not harsh enough to cause instantaneous inhibition of G6Pase, therefore obscuring any lag [137].

The burst phenomenon and the rapid increase in specificity are more difficult to interpret. We do not know any example of an enzyme whose specificity changes upon incubation with its substrate. Even if such a change really occurs, it may involve the translocase as well as the phosphatase, and these observations do not really support one model rather than the other. We rather favour the hypothesis that the permeability of the microsomal membrane is increased artefactually during the first few seconds of the reaction, possibly in relation to the change in the composition of the medium or to mixing.

Loss of specificity induced by histones

Histones H2A cause an approx. 2-fold increase in the G6Pase and phosphotransferase activities of microsomes [264,265], but stimulate to a much greater extent the mannose-6-phosphatase activity, which may reach approx. 75 % of the G6Pase activity [266]. The histones do not act simply as detergents, since they do not cause any decrease, but rather a slight increase, in the intravesicular accumulation of [^{14}C]glucose from [^{14}C]Glc-6-*P*. Intriguingly, histones do not stimulate any accumulation of [^{14}C]mannose from [^{14}C]Man-6-*P*. From these observations it was concluded that the histones act by causing a conformational change in G6Pase [266]. It is difficult to understand, however, how a conformational change can cause a situation whereby G6Pase no longer discriminates between Glc-6-*P* and Man-6-*P* in the hydrolytic reaction, but still discriminates well with regard to the direction in which it 'sends' the products of the reaction. Again this intriguing observation does not really allow one to discriminate between conformational models and the substrate-transport model.

In the context of the substrate-transport model, a potential explanation might be that histones H2A interact with the pores through which glucose diffuses rapidly, altering their selectivity in such a way that Man-6-*P* can enter the vesicles. The hydrolysis of Man-6-*P* would only occur in vesicles equipped with pores, and would therefore never result in the accumulation of [^{14}C] mannose. By contrast, hydrolysis of [^{14}C]Glc-6-*P* would also occur in vesicles lacking pores, and would therefore be associated with the accumulation of [^{14}C]glucose.

CONCLUSION AND PERSPECTIVES

Our understanding of the G6Pase system has progressed greatly during the last 10 years. The cDNAs of its two principal constituents have been cloned, and mutations in these two constituents are now known to be responsible for the two main, and maybe the two sole, forms of GSD I. Other evidence has been obtained in support of the substrate-transport model, and data that would apparently disprove this model can be reconciled with the model if account is taken of the complexity of the experimental material.

Further work is needed to determine the molecular identity of the proteins (transporter or pore) responsible for the efflux of P_i and glucose, to improve our understanding of the long-term regulation of the enzymic system, and to establish the existence of short-term regulation other than that exerted by Glc-6-*P*. Another unsolved problem is whether G6Pase, the Glc-6-*P* translocase and other components of the system form a supramolecular assembly. Although no definitive answer can be given to this question at this stage, we think that the Glc-6-*P* translocase is not bound to G6Pase, because it serves to 'feed' another enzyme, hexose-6-phosphate dehydrogenase, and because it is expressed in tissues where G6Pase is not expressed.

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