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Mini-review

Glycerol-3-phosphate transporter of *Escherichia coli*: Structure, function and regulation

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Abstract

Glycerol-3-phosphate (G3P) plays a major role in glycolysis and phospholipid biosynthesis in the cell. *Escherichia coli* uses a secondary membrane transporter protein, GlpT, to uptake G3P into the cytoplasm. The crystal structure of the protein was recently determined to 3.3 Å resolution. The protein consists of an N- and a C-terminal domain, each formed by a compact bundle of six transmembrane α-helices. The substrate-translocation pore is found at the domain interface and faces the cytoplasm. At the closed end of the pore is the substrate binding site, which is formed by two arginine residues. In combination with biochemical data, the crystal structure suggests a single binding site, alternating access mechanism for substrate translocation, namely, the substrate bound at the N- and C-terminal domain interface is transported across the membrane via a rocker-switch type of movement of the domains. Furthermore, GlpT may serve as a structural and mechanistic paradigm for other secondary active membrane transporters.

Keywords: Glycerol-3-phosphate transporter; Glycerol-3-phosphate metabolism; Membrane transport mechanism; Secondary active membrane transporter; Membrane protein structure; *Escherichia coli*

1. Introduction

Like every prokaryotic or eukaryotic cell, *Escherichia coli* uses membrane transporter proteins for nutrient uptake and waste export. Such transporters catalyze the translocation of solutes across the hydrophilic barrier imposed by the inner membrane, and are essential for the cell's growth and metabolism. In the outer membrane, transport proteins mostly display low or no substrate specificity, with the best-known examples being porins [31]. In the inner membrane, however, transport proteins show both high substrate specificity and enormous structural and functional diversity. The *E. coli* genome contains more than 350 transport proteins, which can be grouped into 60 families or superfamilies [36]. Among them, transporters driven by ATP hydrolysis are classified as primary active transporters, whereas those driven by the electrochemical gradient are referred to as secondary active transporters. The largest family among them is the major facilitator superfamily (MFS), a group of second active membrane transporters with 70 members identified in the *E. coli* genome [34]. One of the MFS proteins is GlpT, the glycerol-3-phosphate (G3P) transporter.

Prior to 1964, it was thought that membrane was impermeable to G3P. A study by Lin and co-workers that year, however, established that *E. coli* strains were able to grow on media with G3P as the sole carbon source [20]. Dissimilation of G3P was ruled out since *E. coli* growth occurred in strains lacking glycerol kinase but not in strains lacking the dehydrogenase. Accumulation of the radioactive substrate confirmed the existence of a specific membrane-embedded transporter for G3P. Almost two decades later, the gene and its protein product responsible for G3P transport were identified in the laboratory of Boos [24,39]. Using subcellular fractionation, the protein product was localized to the inner membrane of *E. coli*. Biochemical and transport assays showed that the import of G3P transport was driven by the efflux of inorganic phosphate (P_i) [14]. More detailed studies

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on GlpT transport in a reconstituted system by Maloney and co-workers distinguished the phosphate transport via GlpT from others such as the Pit and Pst systems [4].

While many transporters, including GlpT, have been studied biochemically, the lack of structural information has limited our understanding of their molecular mechanism. The recent structure determination of GlpT at 3.3 Å has shed light on its mechanism for substrate translocation [23,26]. In this review, we will discuss the structure, mechanism, function and regulation of the G3P transporter in *E. coli*.

2. Function of GlpT

2.1. Role of G3P in the cell

Glycerol-3-phosphate is an important intermediate both in glycolysis and in phospholipid biosynthesis (Fig. 1). *E. coli* can grow using G3P as the sole carbon source. Wild-type *E. coli* cells swim towards G3P with a threshold concentration of 10^{-4} M, whereas they show no chemotaxis to glycerol even at 0.1 M [2]. In the cell, G3P molecules are reduced by aerobic or anaerobic G3P dehydrogenase into dihydroxyacetone phosphate (DHAP). DHAP is further converted into fructose-1,6-diphosphate (FDP) or glyceraldehyde-3-phosphate (GAP), which enter the glycolysis pathway.

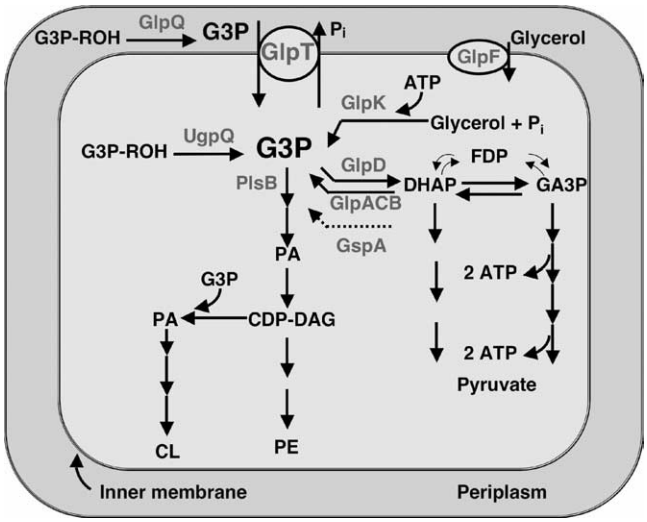

Fig. 1. Metabolic pathways for sn-glycerol-3-phosphate (G3P) in *E. coli* (adapted from [37]). G3P plays a major role in lipid biosynthesis in addition to acting as a carbon source in glycolysis. Gene products directly involved in producing or metabolizing G3P are highlighted. Abbreviations: ATP, adenosine triphosphate; CDP-DAG, cytidine triphosphate diacylglycerol; CL, cardiolipin; DHAP, dihydroxyacetone phosphate; FDP, fructose-1,6-diphosphate; G3P, glycerol-3-phosphate; G3P-ROH, glycerophosphodiester; GA3P, glyceraldehyde-3-phosphate; GlpA, anaerobic sn-glycerol-3-phosphate dehydrogenase; GlpD, aerobic sn-glycerol-3-phosphate dehydrogenase; GlpF, glycerol facilitator; GlpK, glycerol kinase; GlpQ, periplasmic glycerophosphodiester phosphodiesterase; GlpT, sn-glycerol-3-phosphate transporter; PA, phosphatydic acid; PE, phosphatidylethanolamine; PlsB, sn-glycerol-3-phosphate acyltransferase; P_i, inorganic phosphate; UgpQ, cytoplasmic glycerophosphodiester phosphodiesterase.

Phospholipid is the major component of many biological membranes. G3P forms the backbone of all phospholipid molecules and the polar groups of phosphatidylglycerol and cardiolipin [12]. When it is present in the growth medium, G3P is taken up from the medium, catalyzed by the G3P transporter (Fig. 1). Growth on G3P elevates its cellular concentration by about 20 times and thus shuts down the endogenous synthesis of the compound [27]. In the absence of G3P in the growth medium, however, it is produced by phosphorylating glycerol, catalyzed by glycerol kinase, or by reducing dihydroxyacetone phosphate with NADH, catalyzed by G3P dehydrogenase [12]. For phospholipid biosynthesis, the first acyl chain is added to position 1 of G3P by G3P acyltransferase, whereas the second fatty acid is added by 1-acyl-G3P acyltransferase, followed by the addition of the head group.

2.2. GlpT as a G3P/P_i antiporter

The membrane transporter in *E. coli* for G3P uptake, GlpT, is an antiporter that exchanges a phosphate ion for a G3P molecule. GlpT can uptake P_i, with a K_m of 0.24 mM at 37 °C, whereas its K_m for G3P is 12 μM [27]. Incubation of *E. coli* cells in 2 μM ^{14}C-G3P at 30 °C resulted in a 1000-fold concentration of the substrate within 10 min.

The transport activity of GlpT has been extensively characterized [14]. In reconstituted proteoliposomes, its maximal transport rate was measured to be 130 nmol/min/mg GlpT [5]. In addition to the G3P–P_i exchange, GlpT has been shown to be able to catalyze P_i–P_i exchange across the membrane [29]. Two other substrates of GlpT are arsenate and fosfomycin [44], the latter being the only known phosphate-based antibiotic [21]. Using *E. coli* cell's resistance to the antibiotic, many GlpT-negative point mutations have been generated [28,44]. While few mutagenesis studies have been performed on GlpT, extensive characterization have been carried out on its closest homolog, the glucose-6-phosphate transporter (UhpT) [18,19].

2.3. Regulation of GlpT expression and function

The expression of GlpT in *E. coli* is induced by the presence of G3P in the growth medium [11]. Because of the importance of G3P in the cell's metabolism, its transport across the inner membrane of prokaryotes provides a control point for both growth and metabolism (Fig. 1). This task is achieved via a group of overlapped control mechanisms for GlpT expression [51]. The first point of control is via the *glp* regulon, which regulates the overall rate of glycerol/G3P metabolism. There are five operons on the *glp* regulon, and the *glpT* gene is located on one consisting of *glpC,B,A,T,Q*. Genes in the operon may be linked at the transcriptional level due to the presence of multiple promoters which often

exist in such systems. The *glpT* gene is repressed by the GlpR repressor encoded by the *glpEGR* operon (Fig. 1). By binding to the operators near the *glpT* promotor, GlpR blocks its expression. Mutation in the *glpR* gene can activate the transcription of *glpT*, indicating the regulatory role of the GlpR in *glpT* transcription [51]. The interaction of G3P with GlpR protein has been examined by Larson et al., who showed that G3P interacts with GlpR with a K_d of 20–50 μM affinity [25]. While glycerol-phosphate analogs also bind to GlpR, glycerol does not. The association of G3P reduces affinity of GlpR for the operators. GlpR can bind to three different genes in the *glp* regulon: *glpT/A*, *glpD* and *glpK* [25]. The *glpT* and *glpD* operators bind the repressor more tightly than the *glpK* operator. Interaction of GlpR with G3P prevents its association with the operator of *glpK*, and glycerol kinase, but not *glpT*. This is not unexpected since high intracellular G3P levels would reduce the need for the kinase to generate G3P.

End products of lipid biosynthesis and glycolysis may provide the second point for *glpT* inhibition. Indirect evidence suggests that GlpT expression may be regulated by a byproduct from the glycerolipid biosynthetic pathway [27]. Removal of anaerobic consumption of G3P does not interfere with the activation of *glpT* in cells grown in G3P. This suggests that a metabolite from the glycerolipid biosynthetic pathway may be responsible for the inhibition of repression of GlpT [37]. There is also evidence that high levels of internal phosphate signal the uptake of external G3P in order to maintain a homeostatic P_i balance [50]. Indeed, there are many additional points along the catabolic pathway of G3P that may influence the expression or function of GlpT.

2.4. Role of GlpT in antibiotic resistance

Besides nutrient and ion uptake, GlpT plays an important role in resistance to the antibiotic fosfomycin. Fosfomycin inhibits UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA), a cytoplasmic enzyme that is involved in the first step of the biosynthesis of cell wall peptidoglycan, thus preventing bacterial cell division [52]. The inhibition occurs via a covalent linkage and hydrogen bonding between MurA and fosfomycin [40].

Fosfomycin is widely used to treat urinary tract and pediatric gastrointestinal infections of *E. coli* and other bacteria. This phosphate-based antibiotic enters cells via the GlpT and UhpT transporters, and impaired function of GlpT is one mechanism for fosfomycin resistance. Although such a mechanism is the reverse of antibiotic resistance by expressing an efflux pump [48], the physiological consequence is the same. It results in cytosolic fosfomycin concentrations too low to interfere with cell wall synthesis. Impairment of fosfomycin accumulation in Shiga-like toxin-producing *E. coli* cells suggests that malfunction of GlpT may be responsible in some cases of resistance [22]. In addition, a percentage of urinary tract isolates with resistance to fosfomycin have alterations on their GlpT transporter [7,32].

Dozens of point mutations have been generated using the *E. coli* cell's resistance to fosfomycin [28,44]. In spite of the high mutation frequency of GlpT in vivo, only a few fosfomycin-resistant strains have been isolated clinically [32]. Interestingly, those vitro-selected mutants grew more slowly both in the presence and absence of fosfomycin. Because GlpT is involved in the cell's metabolism, Nilsson and colleagues attribute the low clinical occurrence of the fosfomycin-resistant strains in the bladder to the high cost of such mutations to the metabolism of the bacteria [32]. Given its low toxicity and strong inhibition to the growth of both Gram-positive and Gram-negative species [42], such a low occurrence of resistance would suggest a wider application for fosfomycin.

2.5. GlpT homologs in other organisms

GlpT belongs to the organophosphate:phosphate antiporter (OPA) family of the major facilitator superfamily. Homologs to GlpT have been identified and studied in various bacterial species, including *Bacillus subtilis* [33], *Shigella flexneri* [43], *Haemophilus influenzae* [41] and *Rickettsia prowazekii* [6]. In humans, the GlpT homolog G3P permease (G3PP) plays a role in oxidative phosphorylation by transporting G3P into the mitochondria for the glycerol-phosphate shuttle [9]. Interestingly, the G3PP gene is located on chromosome 21 at a locus associated with non-syndromic autosomal recessive deafness [9]. It is yet to be determined whether G3PP plays a role in Down's syndrome. A defect in G3PP may have similar phenotypes to glycerol kinase deficiency-like syndrome characterized by hyperglycerolemia and glyceroluria. Other phenotypes of G3PP deficiency may include decreased energy metabolism, seizures, respiratory distress and moderate developmental delay. Another human homolog of GlpT is the microsomal glucose-6-phosphate transporter, G6PT, from the lung and kidney. Various mutations in the *G6PT* gene, including 28 missense mutations, have been identified that cause the glycogen storage disease type Ib [3,10]. While there is little homology between different families in the major facilitator superfamily, proteins within the OPA family share significant sequence homology (Fig. 2)

3. Structure of GlpT

Hydrophobicity analysis based on amino acid sequence first suggested that GlpT has twelve transmembrane α-helices [13]. The N- and C-terminal halves show weak sequence homology, indicating a gene duplication event in the evolutionary history of MSF proteins. This topology of GlpT was experimentally confirmed using GlpT-alkaline phosphatase and β-galactosidase fusion experiments [17].

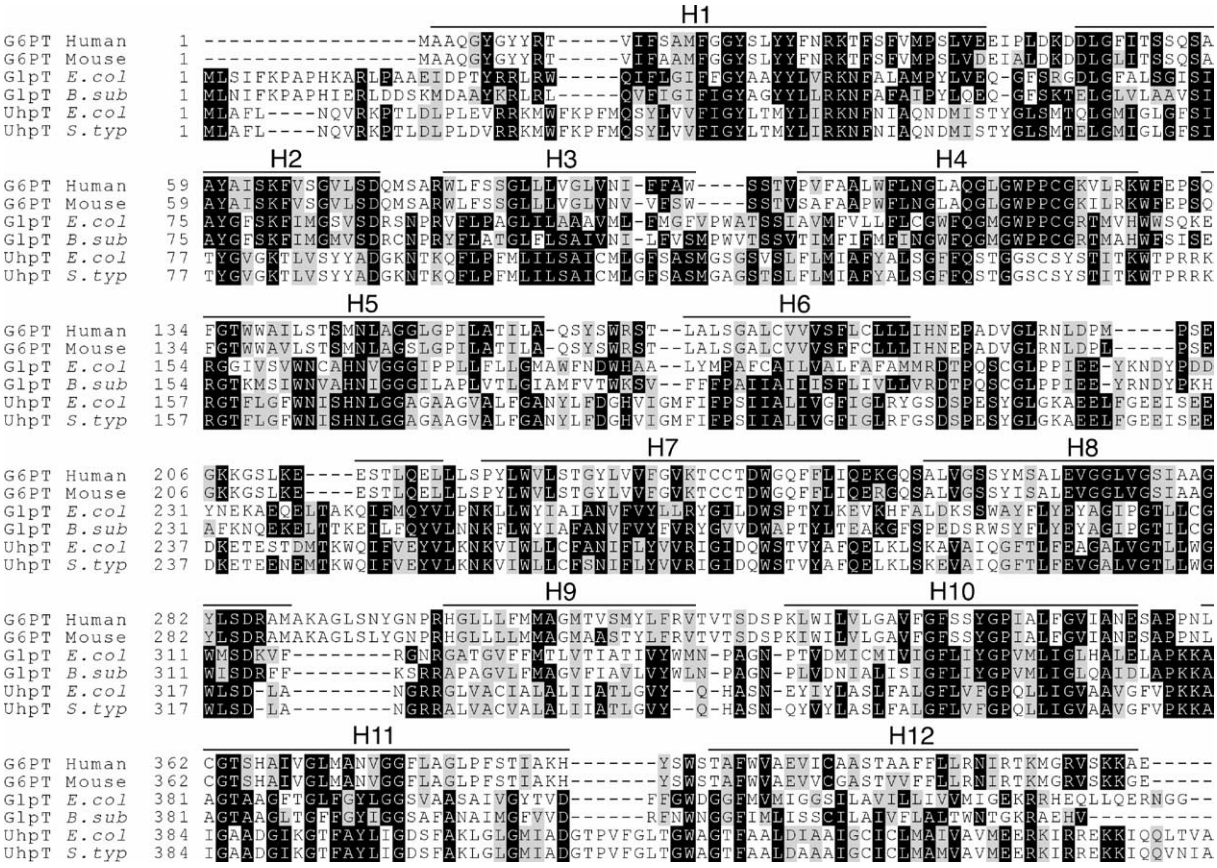

Fig. 2. Multiple sequence alignment for members of the organophosphate:phosphate antiporter family of the major facilitator superfamily [3]. Residues conserved in at least half of the proteins are highlighted in black (identical matches) or gray (similar matches). The positions of the twelve transmembrane α-helices in GlpT are indicated. *E.col—Escherichia coli*; *B.sub—Bacillus subtilis*; *S.typ—Salmonella typhimurium*.

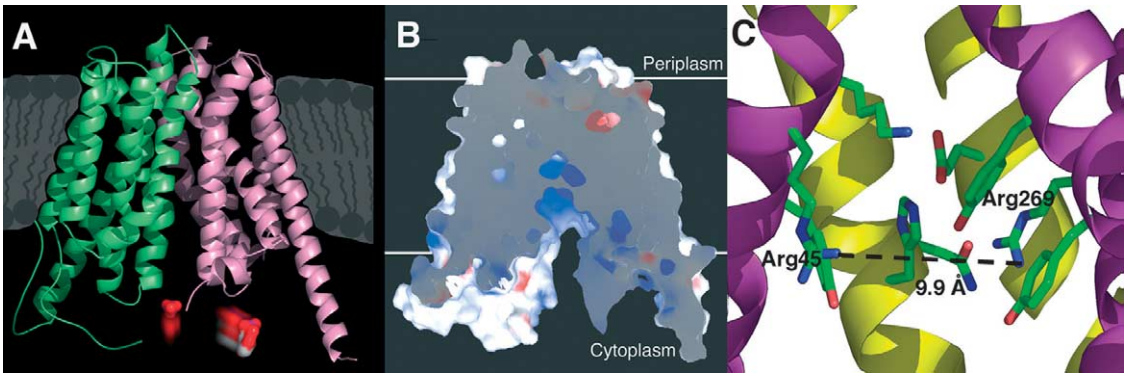

Fig. 3. Structure of GlpT (adapted from [23]). (A) Ribbon representation of the structure viewed from within the membrane, with one G3P molecule leaving and one P_i molecule (space-filling models) entering the cytoplasmic pore. (B) Substrate-translocation pathway and periplasmic barrier. Electrostatic surface potential of GlpT is shown with the front part of the molecule removed for clarity. Blue indicates positive. The pathway is located in the interface between the N- and C-terminal domains. It opens in the middle of the membrane to the cytoplasmic side but is closed off to the periplasm. The electrostatic surface potential is highly positive at the closed end of the translocation pathway. (C) Substrate binding site. The shortest distance between Arg45 from the first helix and Arg269 from the 7th helix is 9.9 Å.

Recently, our lab has crystallized GlpT and determined its three-dimensional structure to 3.3 Å using X-ray diffraction (Fig. 3) [23,26]. The structure of GlpT has the shape of a Mayan temple. The protein is wider at the cytoplasmic side and constricted at the periplasmic end. Twelve transmembrane α-helices are present in the structure, with varied lengths and tilt angles from the membrane normal (Fig. 3A). The positions of the short helices at the outer perimeter of the molecule most likely represent the boundary for the hydrophobic portion of the lipid bilayer. Only a few of the helices in the structure are oriented perfectly perpendicular to the plane of the lipid bilayer. The twelve helices pack

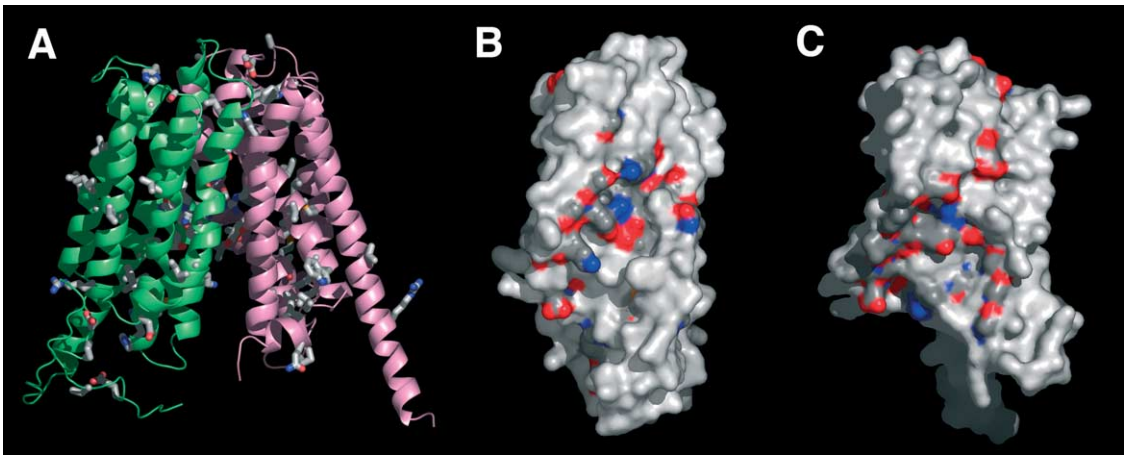

Fig. 4. Mapping at the N- and C-terminal domain interface of residues conserved among members of the organophosphate:phosphate antiporter family of the major facilitator superfamily. (A) Structure of GlpT shown together with conserved residues. (B) Surface representation of the N-terminal domain with conserved residues colored, viewed from the C-terminal domain within the membrane plane. (C) Surface representation of the C-terminal domain with conserved residues colored, viewed from the N-terminal domain within the membrane plane.

into two six-helix domains, the N- and C-terminal domains. Whereas the helices at the two ends of the protein tend to be straight, those at the N-/C-terminal domain interface are highly curved. A pseudo twofold symmetry exists between the N-terminal and C-terminal halves, agreeing with the weak sequence homology of the two halves of the protein [13]. The two halves of the protein are connected by a long cytoplasmic loop. Although there are no salt bridges and few hydrogen bonds between the two domains, extensive van der Waals interactions are present at the periplasmic side of the protein.

Importantly, a pore exists between the N- and C-terminal domains on the cytoplasmic side of the protein, which constricts toward the center of the molecule (Fig. 3B). This represents the substrate-translocation pore. Interestingly, there are bulky side chain residues on the N-terminal side of the pore that fit into pockets on the C-terminal side of the pore. Electrostatic surface potential of GlpT shows an electroneutral pore surface, except at the closed end of the pore in the middle of the membrane, where it is positive. This strong positively-charged area is attributed to two conserved arginine residues, Arg45 from helix 1 and Arg269 from helix 7 (Fig. 3C) [23]. These two arginines have also been shown to be essential for the activity in the *E. coli* UhpT protein [15]. They have therefore been proposed to form the substrate binding site, to bind to the phosphate moiety of a substrate [23]. Similarly, the substrate binding site in the lactose transporter (LacY) is found to be at the same position [1].

In addition to these two arginines, a number of other residues are also conserved around the substrate binding site among members of the OPA family (Figs. 2 and 4). They include both charged and hydrophobic residues (Table 1). Interestingly, they are located on both the cytoplasmic and the periplasmic sides of residues Arg45 and Arg269, and may therefore be involved substrate binding in the C_i or C_o conformation.

Table 1
Conserved residues among OPA proteins around the substrate binding site

Helix	Periplasmic side	Middle	Cytoplasmic side
N-terminal domain			
H1	Lys46	Arg45	Tyr38, Tyr42
H2	Ser73		Tyr76, Gly77, Lys80
H4	Gln134		Gly137
H5	Asn166, Gly169		
C-terminal domain			
H7	Asp274	Arg269	Tyr266
H8	Glu299, Ile303		Thr306
H10			Try362
H11			Gly389, Gly392

4. Mechanism of substrate translocation of GlpT

The GlpT crystal structure [23] and biochemical data on GlpT [4,8,14,20] and on UhpT [15,16,30] suggest that GlpT operates via a single binding site, alternating access mechanism, which is realized via a rocker-switch type of movement of the N- and C-terminal domains. Originally suggested based on kinetic studies and thermodynamic considerations [35,46,49], such a mechanism requires that the transporter exist in two major types of conformations with the substrate binding site facing opposite sides of the membrane, the inward facing C_i and the outward facing C_o. Based on the crystal structure of GlpT, we were able to propose a more detailed model that can be tested experimentally [23]. In the inward facing conformation of GlpT, the shortest distance between Arg45 and Arg269 at the substrate binding site is about 9.9 Å (Fig. 3C). This is 1.4 Å longer than required for a bound phosphate to form optimal hydrogen bonds simultaneously with both arginines. We proposed that P_i binding pulls these two arginines and, thus, the N- and C-terminal domains closer and narrows the cytoplasmic pore (Fig. 5). The substrate binding also

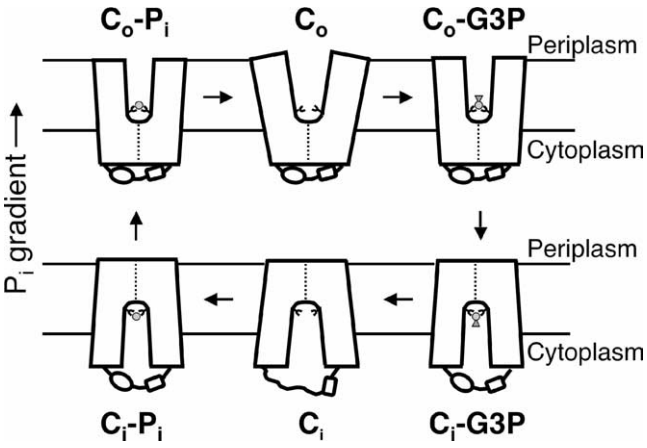

Fig. 5. Single-binding-site, alternating-access mechanism with a rocker-switch type of movement of the N- and C-terminal domains [23]. Positions of Arg45 and Arg269 are indicated. P_i is represented by a small disk, and the G3P molecule a small disk and a triangle.

destabilizes the interface between the N- and C-terminal domains on the periplasmic side and allows further tilting of the two domains to yield the C_o conformation. In the periplasm the lower affinity of the protein for P_i allows its replacement by G3P, whereas in the cytoplasm P_i replaces G3P at the binding site due to its much higher cytosolic concentration.

5. Final considerations

E. coli employs both primary and secondary active transporters for solute translocation across the inner membrane. The largest group of transporters in the cell is the major facilitator (70 members), followed by the ATP binding cassette superfamily (ABC, 67 members) [36]. Both groups of transporters have very diverse substrate specificity. Given that it takes the translocation of 3–4 protons to generate one ATP molecule by F_oF_1–ATP synthase [47] and a primary transporter typically needs one ATP to translocate one substrate, it is mostly the case that a secondary transporter consumes a lot less energy than a primary transporter for every substrate transported. On the other hand, a secondary active membrane transporter may not be able to drive against as steep a gradient of substrate across the membrane as a primary transporter can. As a cell's genome often contains both a primary and a secondary transporter protein for the same substrate, it can express the proper transporter depending on both its needs and the environment. Primary and secondary membrane transporters are connected in more than one way. Recently, a truncated form of the ABC transporter LmrA in *Lactococcus lactis* lacking the ATP binding domain was found to function as a proton-driven symporter without the requirement for ATP [45]. This validates Saier's hypothesis that primary active membrane transporters are descendants of secondary transporters [38].

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