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Structure and function of prokaryotic UDP-glucose pyrophosphorylase, a drug target candidate

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Abbreviations used: UDP: uridine diphosphate; UTP: uridine triphosphate; UGP: UDP-glucose pyrophosphorylase; LPS: lipopolysaccharide; ITC: Isothermal titration calorimetry.

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Abstract

UDP-glucose is an essential metabolite for a variety of processes in the cell physiology in all organisms. In prokaryotes, it is involved in the synthesis of trehalose, an osmoprotectant, in galactose utilization via the Leloir pathway and it plays a key role in the synthesis of the components of the bacterial envelope, particularly the lipopolysaccharide and the capsule, which represent necessary virulence factors of many bacterial pathogens. UDP-glucose is synthesized in bacteria by the prokaryotic UDP-glucose pyrophosphorylase (UGP, EC 2.7.7.9), an enzyme belonging to the family of sugar:nucleotidyl transferases. Despite the ubiquitous distribution of UGP activity in all domains of life, prokaryotic UGPs are evolutionarily unrelated to their eukaryotic counterparts. Taken together, these features make of bacterial UGP an attractive target candidate for the discovery and development of new generation antibiotics. This review summarizes the current knowledge on structure and function of bacterial UGPs, underlying their potential as drug target candidates.

1. INTRODUCTION

The last decades have witnessed an increasing interest in the study of carbohydrates and glycoconjugates, given the pivotal roles they play in a number of processes, including infection, fertility, cell size control, cell-cell and cell-matrix interactions, and many others, through their recognition by lectins [1] and other molecular receptors, such as antibodies [2] and carbohydrate-processing enzymes [3-4].

Glycosyl phosphoesters of nucleoside pyrophosphates, more often referred to as sugar-nucleotides, occupy a relevant place in carbohydrate metabolism as the activated forms serving as glycosyl donors for the synthesis of complex carbohydrates and the sugar moieties of glycoconjugates. Of these, UDP-glucose is a highly multifunctional metabolite, being essential for a rich variety of processes and a crossroad point in carbohydrate metabolism. In animal and fungal metabolism, UDP-glucose is the sugar donor for glycogen synthesis and, as in all eukaryotes, in the formation of the carbohydrate moieties of glycolipids, glycoproteins and proteoglycans [5], and is required for galactose utilization [6]. In plants, UDP-glucose is used for the synthesis of sucrose and cellulose and is involved in starch metabolism [7]. In prokaryotes, perhaps, the most distinguishing role of UDP-glucose is its participation in the synthesis of different components of the bacterial envelope, particularly the lipopolysaccharide (LPS) and the capsule, structures that represent necessary virulence factors of many microorganisms.

UTP:α-D-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9), commonly referred to as UDP-glucose pyrophosphorylase, and abbreviated as UGP or by the capitalization of its coding gene in *E. coli* (GalU), catalyzes the reversible formation of UDP-glucose and pyrophosphate (PPi) from glucose-1-phosphate and UTP (fig. 1). UGP activity is ubiquitous to all domains of life, given the functional importance of its product. However, and interestingly, prokaryotic UGPs are evolutionary unrelated to their eukaryotic counterparts [8].

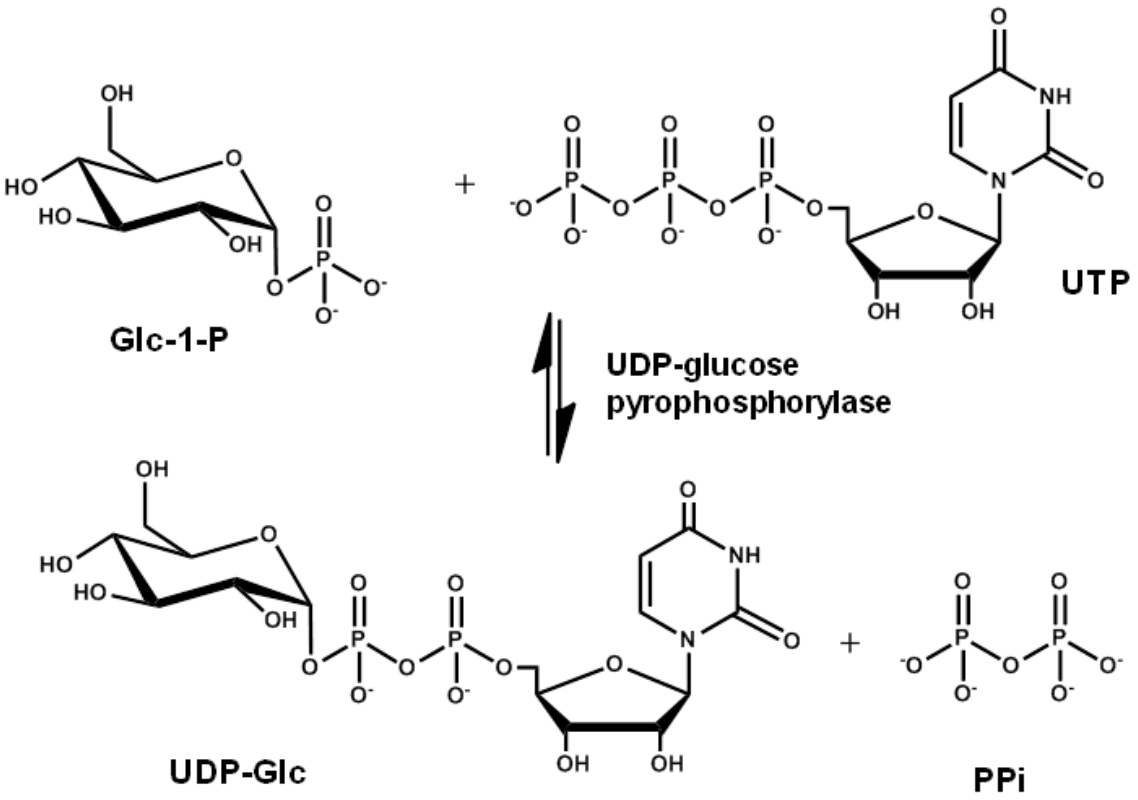

Figure 1: Enzymatic conversion catalyzed by UDP-glucose pyrophosphorylase.

The aim of this review is to summarize the current knowledge on structure, activity and function of prokaryotic UGP, and a special emphasis in its potential use as an antimicrobial target is made. Comprehensive information on eukaryotic UGPs can be found elsewhere [7, 9].

2. A HISTORIC OVERVIEW

In 1950, as part of their studies on galactose metabolism in yeast, L. F. Leloir and his co-workers discovered a compound which was needed for a step of the reaction pathway now known as the Leloir pathway. Presence of uridine, phosphate and glucose in this compound, as

well as the correct linkage of the different components were ascertained, thus identifying it as UDP-glucose, the first sugar nucleotide to be discovered, and described as a «*coenzyme of the galactose phosphate-glucose phosphate transformation*» [10]. Later, Kalckar *et al*. detected UDPG:PP activity in yeast samples [11]. Specifically, they found out that dialyzed yeast maceration juice, when in the presence of UDP-glucose, promoted the formation of inorganic pyrophosphate and UTP, hence discovering the reverse reaction. As a result, the newly discovered enzyme adopted the name of "UDP-glucose pyrophosphorylase" (UGP), a denomination which has prevailed ever since, although the name "UTP:glucose-1-P uridilyltransferase" is also widely used. In addition, other authors have historically employed different denominations, such as "UDP-glucose synthetase", coined by Sundararajan *et al*. in the early 1960s to stress the biosynthetic role of this enzyme [12].

The importance of UGP activity in bacterial biochemical pathways was soon realized, while further investigations during the genomic era brought to the realization that no phylogenetic relation existed between the prokaryotic enzyme and its counterpart of eukaryotic species [13]. This prompted interest in targeting bacterial UGP, as a means of disrupting a key enzymatic activity of prokaryotic pathogens without disrupting the host metabolism. With this objective in mind, several research groups provided crystallographic structures of UGPs from different bacterial sources [14-17].

3. PHYLOGENETIC CONTEXT

UDP-glucose phosphorylase belongs to the superfamily of the NTP:sugar nucleotidyltransferases (nucleoside diphosphate sugar pyrophosphorylases), a related group of proteins that exhibit a similar folding [17] and certain conserved sequence elements, most notably the N-terminal motif GXGTRXLPXT [18], carrying a putative catalytic arginine residue [17, 19]. Apart from the mentioned features, it is characteristic of bacterial UGPs the presence of the signature motif VEKP, which carries essential residues involved in interaction with the glucosyl part of its substrates [15, 17] (fig. 2).

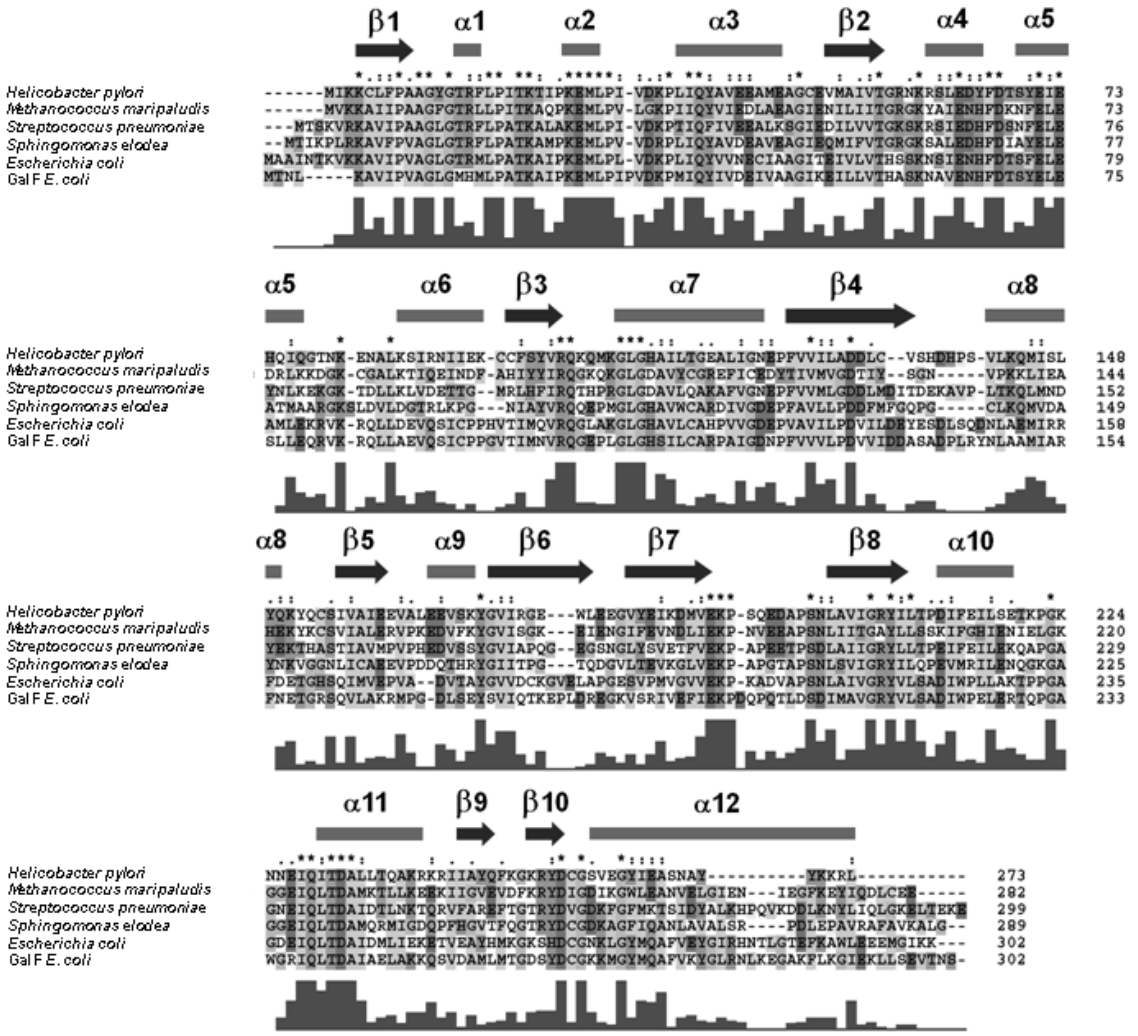

Figure 2: ClustalX (v. 2.0.12) multiple sequence alignment of UGPs of selected prokaryotic organisms. The alignment shows the great conservation of the UGP sequence throughout the prokaryotic lineage. The *E. coli* GalF regulatory subunit still keeps a high degree of conservation, but with a catalytic arginine residue in helix α1 which is necessary for UGP activity being replaced by a histidine.

Given its essential activity for the cell metabolism, UDP-glucose pyrophosphorylases are ubiquitously distributed throughout all domains of life. UGPs are, in addition, very well conserved proteins: for instance, the sequence of the *Escherichia coli* UGP bears a 29% identity with that from the archean *Sulfolobus*, and the human and barley enzymes share a 55% identity. However, and interestingly, sequence similarities between UGPs of prokaryotic origin and their eukaryotic counterparts lie around 8%, which is considered non-significant. For comparison, human UDP-galactose-4-epimerase, another enzyme rendering UDP-glucose as product, shares an identity of 51% with its *E. coli* homologue [20]. Thus, the UGPs of prokaryotic and of eukaryotic origin are believed to be evolutionarily unrelated [8, 17]. Fittingly, Hartman and Fedorov classified the eukaryotic UGP as a "eukaryotic signature protein", being itself one of

only a handful enzymes in a list of 347 proteins that were considered to be genuine eukaryotic inventions [13].

In light of this data, a number of authors have underlined the attractiveness of bacterial UGPs as potential antimicrobial targets, as a means of selectively inhibit the pathogen enzyme without disrupting the host metabolism [8, 17, 21].

Nonetheless, eukaryotic organisms do posses other nucleotidyltransferases sharing certain homology with the members of the bacterial superfamily, notably the eukaryotic GDP-mannose pyrophosphorylases (GMPPs) (fig. 3), but also plant sucrose synthase (SuSy, which in fact employs UDP-glucose as a substrate, too) and others [9].

Interestingly, bacterial UGPs share some sequence similarity with some other eukaryotic proteins not involved in sugar metabolism. In eukaryotic genomes, the sequences most similar to those of prokaryotic UGP and GMPPs, belong to the γ-subunit of the eukaryotic translation initiation factor eIF2B (fig. 3), a multisubunit complex that is required for protein synthesis initiation and its regulation in eukaryotic cells. The eIF2B-γ subunit binds GTP [22] and it shows, in fact, a strong sequence similarity with GMPPs, suggesting that evolution might have taken advantage of the binding capabilities of a hypothetical ancestor related to GMPPs to tailor this protein.

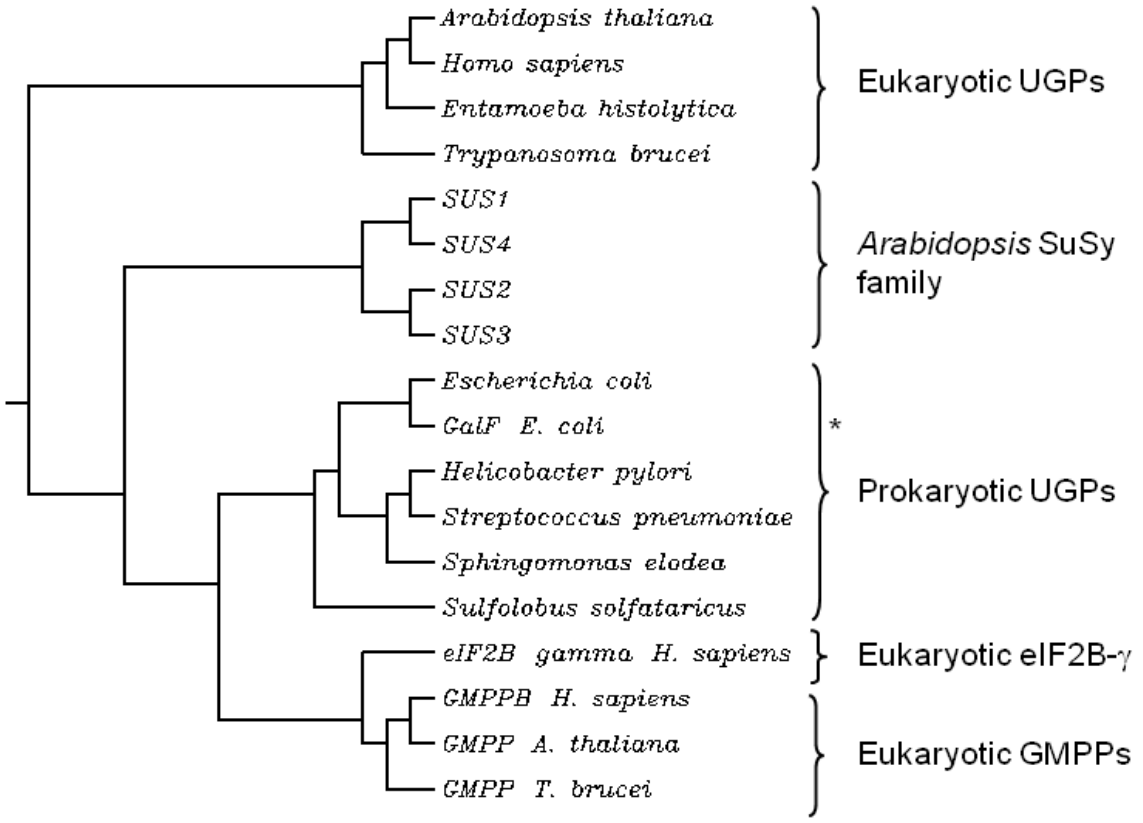

Figure 3: Dendrogram showing phylogenetic relations of a selection of prokaryotic and eukaryotic UGPs, eukaryotic GTP-mannose pyrophosphorylases, members of the *Arabidopsis* sucrose synthase (SuSy) family and the gamma subunit of the human eukaryotic initiation factor 2B (eIF2B-γ). A multiple sequence alignment was generated with ClustalW (http://www.genome.jp/tools/clustalw/) using the BLOSUM matrix. An asterisk indicates the position of the non-catalytic GalF protein from *E. coli*.

In short, prokaryotic UGPs belong to a cluster of phylogenetically related proteins, which include the eukaryotic eIF2B-γ and GMP, but not the eukaryotic UGP. In spite of the phylogenetic relations between bacterial UGPs and the latter eukaryotic proteins, the lack of homologous eukaryotic protein acting on the same substrates gives support to the attractiveness of bacterial UGPs as novel target candidates.

4. STRUCTURE AND MECHANISM OF ACTION

Up to date, crystallographic structures of four bacterial UGPs have been reported, specifically those from *E. coli* (PDB code: 2E3D) [14], *Corynebacterium glutamicum* (PDB code: 2PA4) [15], *Sphingomonas elodea* (PDB code: 2UX8) [16] and *Helicobacter pylori* (PDB code 3JUJ and 3JUK) [17], all of which share a very similar structure. Here, the structure of the *H. pylori* enzyme is discussed as representative of the group.

4.1. Overall structure

All four UGP structures were crystallized as tetramers (fig. 4). Each monomer, of approximately 30-35 kDa, presents a characteristic folding, dominated by a central mixed β-sheet, reminiscent of the nucleotide-binding Rossmann fold, but including nine β-strands, surrounded by eleven α-helices and two additional β-strands (fig 5). The overall quaternary structure can be understood as a dimer of dimers, with 'tight' dimers resulting of extensive interactions established between two adjacent subunits, and 'loose' dimers arisen from weaker packing interactions between the alternate pairs of subunits (fig. 4). In the *H. pylori* UGP, the 'tight' dimers result from interactions made by residues located mainly in the α1, α4 and α12 helices and the α2-α3 loop, between subunits A and B, and C and D, respectively. In other UGPs (e.g. that of *S. elodea*), an additional helix exists at the C-terminus, which gives rise, together with the preceding helix, to a V-shaped domain that further stabilizes the dimer by interacting with its homologous in the other monomer. The 'loose' dimers arise from contacts between the β3 strands from each alternate subunit A and C, and B and D, respectively.

A very similar folding in the monomers is found in other NDP:sugar pyrophosphorylases [19, 23-25], even though the amino acid sequence homology among the members of this family is often very small, or none. By contrast, the quaternary structure greatly varies within the family, existing trimers (N-acetylglucosamine-1-P uridylyltransferases [26]), hexamers (glucose-1-P cytidylyltransferases [24]), homodimers (GDP-mannose pyrophosphorylases [25]) and heterodimers (plant ADP-glucose pyrophosphorylases [23]), among other forms of arrangement.

4.2. Active center

As in other pyrophosphorylases, such as thymidylyltransferases, each bacterial UGP subunit carries on an active site made up by residues located in the same polypeptide chain [19]. This is in contrast with cytidylyltransferases, in which active sites are formed by residues located in different subunits [15]. In UGPs, the active center is located in a deep cleft delimited by β1, β4, β8, α9, α7, α11 and nearby loops (fig. 5). Residues involved in binding and catalysis are extremely conserved among UGPs of different species. These residues are located mainly in loops facing the active site cleft, rather than in the neighboring α-helices or β-strands.

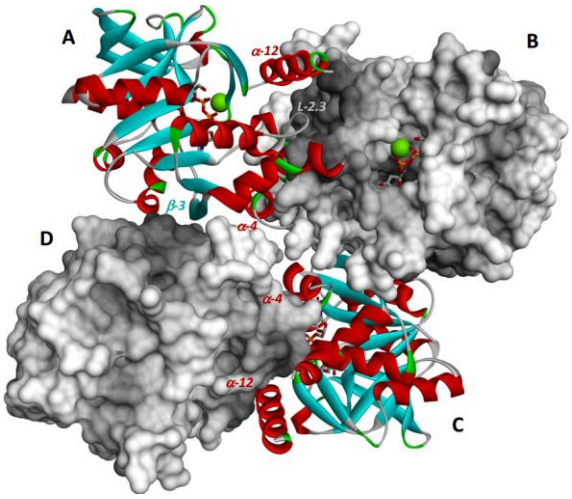

Figure 4: Tetrameric structure of *H. pylori* UGP (PDB code: 3JUK) [17]. Subunits A and C (secondary structure ribbon representation) and B and D (solvent accesibility surface representation). Subunit pairs A/B and C/D interact within a region including three α-helices (α-4 and α-12 are highlighted in subunits A and C) and a loop between α-2 and α-3 (highlighted in A), forming the respective 'tight dimers'. In addition, subunits A and D, and B and C are packed, in a weaker manner, with participation of residues located in their respective β-3 strands.

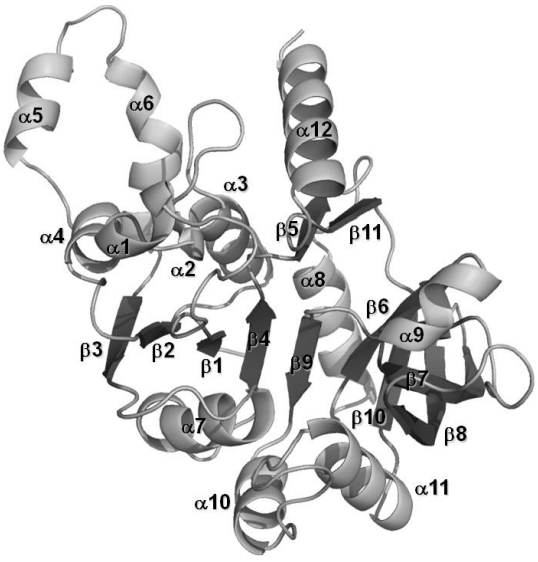

Figure 5: *H. pylori* UGPase in its apo form (PDB code: 3JUJ), with labels indicating the identity of the individual alpha helices and beta strands.

The available crystal structures of prokaryotic UGPs include the apo-enzyme (3JUJ, 2E3D), the binary complex UGP/UDP-glucose (3JUK) and the binary complex UGP/UTP (2UX8). In all cases, the tertiary structure of the enzyme, and the interactions that it establishes with the nucleotide moiety of the substrates are essentially similar. Fig. 6 shows UDP-glucose anchored to the *H. pylori* UGPase active site, showing key contacts between the enzyme atoms and those of its product. In the bound state, both the nucleoside and sugar moieties are buried inside the active site pocket, unlike the phosphate groups, which remain fully accessible to the solvent. The nucleoside moiety of UDP-glucose interacts with the Ala10 and Gly11 backbone NHs from the conserved N-terminal motif, at the level of the O2 and O2' atoms, respectively. In addition, the Gln102 ε-amide and the Gly107 NH interact with O4 of the uracyl. Negatively charged phosphate groups are docked to the enzyme by electrostatic interactions with a pair of conserved lysine residues. The first phosphate interacts with the Lys191 side-chain from the VEKP signature motif, whereas the second phosphate contacts the Lys25 side-chain. Two phosphoryl oxygens from the phosphate pair are coordinated with a Mg^{2+} ion, whose coordination sphere is

completed by the Glu130 carboxylate and three ordered water molecules. Gly171 can hydrogen-bond to any of both the 3'- and 4'-hydroxyl groups of the glucose moiety, Asp131 contacts the 6'-hydroxyl group, and Glu190 carboxyl oxygens from the VEKP motif interact with the sugar 2' and 3'-hydroxyl groups, a bidentate interaction commonly found in other glucose-protein interactions, such as those found in lectins [27]. Finally, a conserved arginine residue (Arg15) is located apart from the ligand and is not involved in binding, since mutation of this residue has no significant effects on the affinity of the enzyme towards its substrates [17]. Instead, it plays an important role in the catalytic activity.

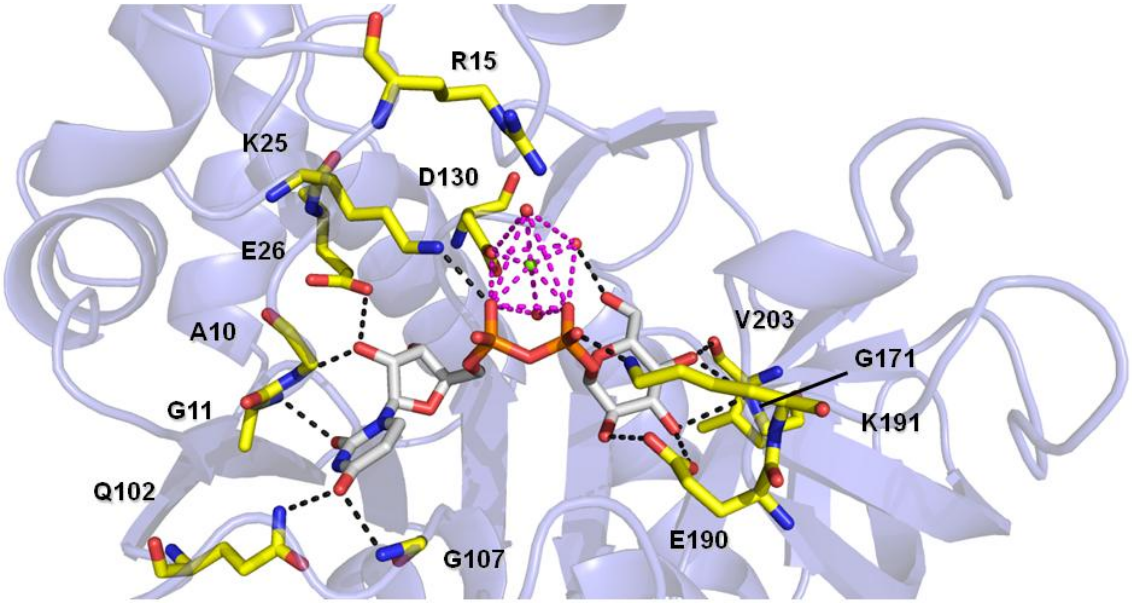

Figure 6: Diagram of the *H. pylori* UGP active center, in complex with UDP-glucose [17]. The protein residues participating in substrate binding are depicted in yellow, and a magnesium ion, coordinated with Glu130, two phosphoryl groups and three water molecules, is shown in green. Black dotted lines represent the inferred interactions implicated in substrate binding.

4.3. Specificity

The specificity of UGP for its substrates is determined by the architecture of its active site. Substrates incorporating sugars other than lactose are not processed by this enzyme. Galactose binding, although sterically possible, is less favored than glucose binding by the loss of a hydrogen bond with Gly171. Binding of purine bases is sterically impeded by residues from β3, α7 and loops β1-α1 and β3-α7. Cytosine binding is prevented by the lack of specific hydrogen bonds [17]. However, promiscuity towards dTTP has been documented for UGPases from different sources, such as *Salmonella enterica* [28], *S. elodea* and *E. coli* [29-30], while the

enzyme from other organisms, like *H. pylori* [17], has been shown to be strictly specific to uracyl nucleotides. In the *H. pylori* UGP, accommodation of thymine instead of uracyl is impeded because of steric clashes between the extra methyl group of thymine and the bulky Met105 side-chain on the β3-β4 loop. In the *S. elodea* enzyme, however, this methionine is changed to a proline (Pro108), leaving enough space for a thymine base. Similarly, an alanine occupies the same position in the *E. coli* enzyme (fig. 2), which could account for its promiscuity towards dTTP. Being the residues within this loop less conserved than other sequence elements (fig. 2), this region could be differently sculpted, depending on the organism's requirements, to acquire the desired specificity.

4.4. Reaction mechanism

The catalytic mechanism followed by NDP-sugar pyrophosphorylases has historically been a subject of controversy. Early insights into the mode of action of a member of the family suggested a sequential ordered Bi-Bi mechanism, in which the substrates enter the active site in an orderly fashion, react, and depart from the enzyme in a precise order. Nonetheless, it was speculated that the phosphorolysis reaction could operate also through a ping-pong mechanism, via a covalent intermediate between the nucleotide and the enzyme [31], a possibility that was discarded by the absence of ^{14}C incorporation to the enzyme from the labelled sugar-phosphate. Speculation persisted, nonetheless, if the second substrate binds and simultaneously reacts (the so-called 'hit and run' mechanism) or binds, and then reacts [19].

Isothermal titration calorimetry (ITC) measurements, together with crystallographic data for a number of pyrophosphorylases [17, 19], including UGP, point to a sequential ordered Bi-Bi catalytic mechanism, in which the nucleoside triphosphate binds to the enzyme first, followed by the sugar-phosphate binding to the enzyme/nucleotide complex.

In UDP-glucose pyrophosphorylases, the reaction is initiated by a nucleophilic attack of the glucose-1-P phosphoryl oxygen on the phosphorous atom of the UTP α-phosphate. It is thought that a Mg^{2+} ion participates in the catalysis by both stabilizing the excess of negative charge and properly positioning the phosphoryl oxygen of glucose-1-phosphate for its nucleophilic attack [32]. Indeed, the presence of magnesium has been shown to be absolutely required for the catalysis [33], as well as enhancing the binding affinity of UGP towards UTP and UDP-glucose [17]. After the reaction takes place, the β- and γ-phosphates are displaced and leave the enzyme/UDP-glucose complex as inorganic pyrophosphate, followed by dissociation of UDP-glucose (Fig. 7). In the reverse reaction, pyrophosphate nucleophilically attacks UDP-glucose and glucose-1-P is displaced, followed by release of UTP.

Kinetic parameters for UGP of different prokaryotic sources are available, showing some variation among species. In *E. coli*, Michaelis-Menten constant (K_M) values calculated are 0.71 mM for UDP-Glc, 0.53 mM for PPi, 0.15 mM for Glc-1-P, and 0.23 for UTP [34], which are similar to the calculated K_M values for the *S. pneumoniae* enzyme: 0.40 mM for UDP-Glc, 0.26 mM for PPi, 0.19 mM for Glc-1-P, and 0.24 for UTP [33]. In contrast, the UGP of *Sphingomonas paucimobilis* shows a very low K_M value for UDP-Glc (7.5 μM) [18], whereas the *Acetobacter xylinum* enzyme shows a higher K_M value of 3.2 mM [35].

Catalyst rate constant (k_{cat}) values are usually higher for UDP-Glc formation, from 40 s^{-1} in *Xanthomonas* [36] to 148 s^{-1} in *Streptomyces coelicolor* [37], than for UDP-Glc pyrophosphorolysis (0.55 s^{-1} and 5.7 s^{-1} in *Streptomyces* and *Xhantomonas*, respectively). As can be inferred, enzyme efficiencies, calculated as k_{cat}/K_M, are higher for UDP-Glc synthesis than UDP-Glc pyrophosphorolysis.

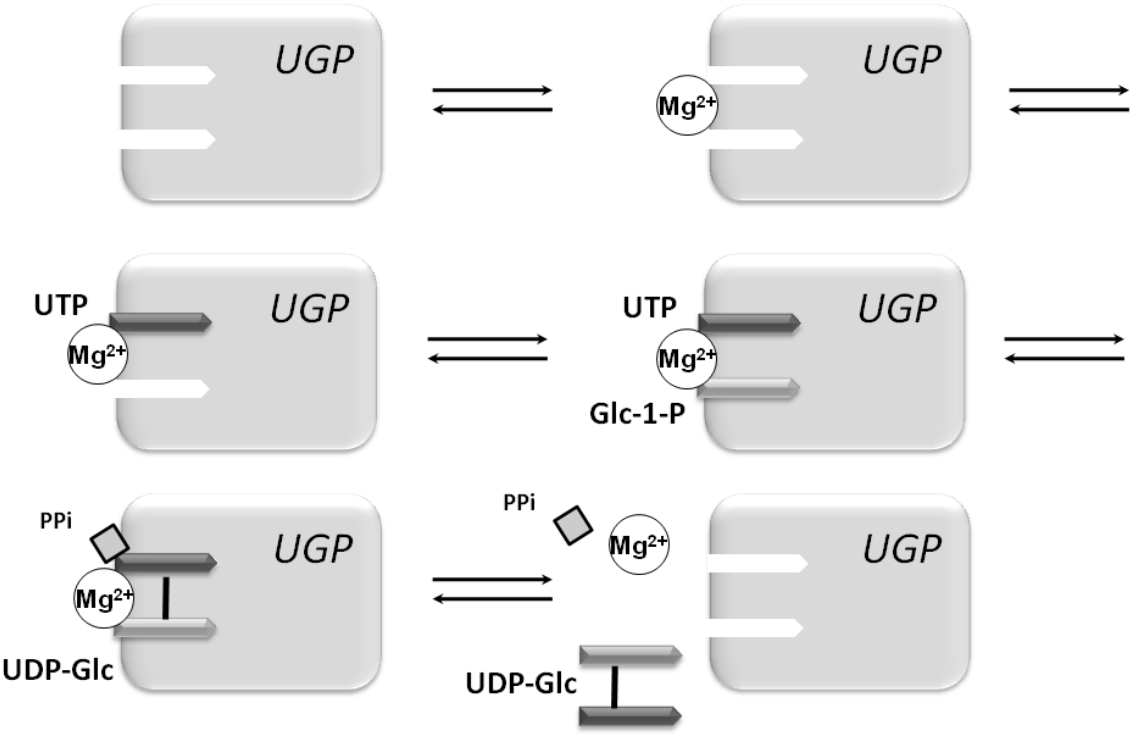

Figure 7: Scheme of the reaction mechanism for the synthesis of UDP-glucose followed by prokaryotic UGPs.

5. FUNCTION AND REGULATION

The relevance of UGPs for bacterial cell physiology is determined by the pivotal role of its product UDP-glucose in the bacterial metabolic pathways (Fig. 8). Occupying this central position in the bacterial metabolism, UDP-glucose serves both as a signaling molecule and as a building block for the synthesis of complex polysaccharide structures. As noted above, one of the main functions of UDP-glucose, in prokaryotes is to serve as glucosyl donor for carbohydrate biosynthesis. UDP-glucose is required for the synthesis of LPS, cell wall sugar moieties, capsular polysaccharides and membrane-derived oligosaccharides, as well as exopolysaccharides, either directly or via UDP-galactose. In addition, production of UDP-glucose has been linked with osmotolerance and the complex coordination of cell size and the control of the bacterial cell cycle.

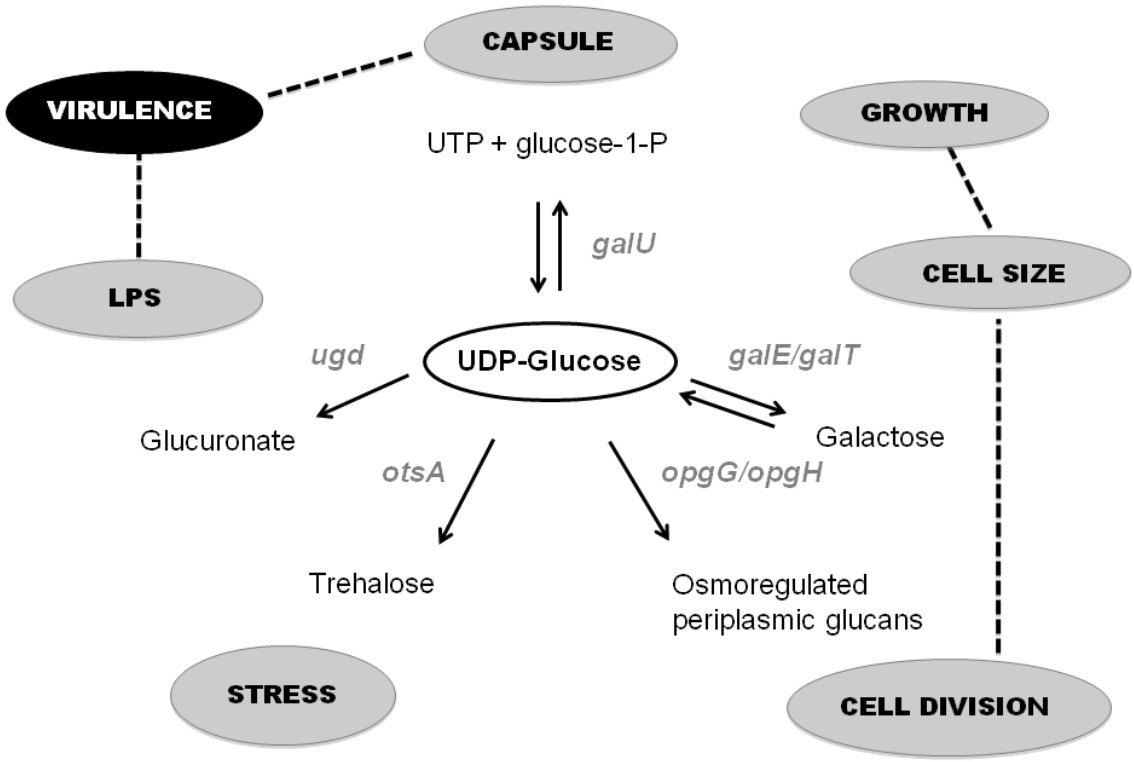

Figure 8: Diagram showing the central position of UDP-glucose in the bacterial biochemical pathways. Arrows indicate the enzymatic transformations of UDP-glucose into several metabolites in *E. coli*, catalyzed by enzymes denoted by codes in gray. Gray ovals represent bacterial functions associated with those enzymatic activities. These functions, especially the presence of capsule and/or LPS in the bacterial envelope, are related with bacterial virulence (represented by a black oval).

5.1. UDP-glucuronate synthesis

UDP-glucose is a substrate of UDP-glucose-6-dehydrogenase (product of the *ugd* gene), which catalyzes the conversion of UDP-glucose into UDP-glucuronic acid. This sugar nucleotide is an important component of many bacterial virulence factors. In many gram negative bacteria, UDP-glucuronate is a precursor for the synthesis of the O-antigen, an important component of the lipopolysaccharide (LPS). In some gram-positive bacteria, such as *Streptococcus pneumoniae*, UDP-glucuronate is used as a donor for the addition of glucuronic acid to the capsular polysaccharide, considered an essential virulence factor of this microorganism [38-39]. Furthermore, in certain organisms, UDP-glucuronic acid is a precursor of UDP-4-amino-4-deoxy-L-arabinose (UDP-Ara4N), which is utilized for the incorporation of Ara4N to lipid A of virulent strains of prominence, such as *Pseudomonas* isolated from cystic fibrosis patients [40]. This modification is a well-known defense mechanism against cationic antimicrobial peptides [41].

5.2. Galactose metabolism

In most bacteria, UDP-glucose is required for the utilization of galactose, via the Leloir pathway [42]. In the first step of this pathway, β-D-galactose is epimerized to α-D-galactose by action of galactose mutarotase. Then, α-D-galactose is phosphorylated in position 1 by galactokinase, consuming ATP. Next, UDP is transferred from UDP-glucose to galactose-1-P by action of galactose-1-P uridyltransferase (*galT*), hereby generating UDP-galactose and glucose-1-P. UDP-galactose yields back UDP-glucose by action of UDP-galactose-4'-epimerase (*galE*). This means of transforming β-D-galactose into the more metabolically useful glucose derivatives permits organisms using this pathway to develop in cultures with galactose as the sole source of carbon, and bacteria lacking any of the implicated enzymes fail to grow in such media [43]. Similarly, defects in the human genes coding for enzymes of this pathway lead to a pathology known collectively as galactosemia [44]. Importantly, as UDP-glucose is involved in the pathway, UGP defective strains are unable to utilize galactose as a carbon and energy source.

In addition, UDP-galactose and UDP-galacturonate are utilized as sugar donors of galactose and galacturonate, respectively, for the synthesis of complex bacterial polysaccharides, such as the LPS and the gram-positive capsule polysaccharide [45-46]. This way, the lack of UGP activity shows detrimental effects for the synthesis of these bacterial structures, not only directly at the level of its glucose and glucuronic units, but also indirectly on the incorporation of galactose and galacturonic acid. In *S. pneumoniae*, at least one of these four sugars is present in the capsule of all virulent serotypes [47].

5.3. Trehalose synthesis and response to stress

In most bacterial organisms, UDP-glucose is a precursor of trehalose, a dissacharide composed of two glucose units linked in an α,α-1,1-glycosidic linkage. The most widely found biosynthetic pathway for trehalose synthesis involves the transfer of glucose from UDP-glucose to glucose-6-phosphate to form trehalose-6-P, a reaction catalyzed by trehalose-P synthase (coded by *otsA* gene in *E. coli*), followed by trehalose-6-P to trehalose conversion by trehalose-P phosphatase (coded by *otsB* gene in *E. coli*) [48]. The so-obtained trehalose can be utilized in bacteria as a protectant and stabilizer of proteins and membranes upon a variety of stressful conditions. Trehalose confers bacterial resistance to desiccation [34-35], through inhibition of lipid fusion and phase transition of lipid bilayers occurring upon dehydration [48]. It also protects the bacterial cell against cold: an *E. coli* strain defective in the trehalose synthesis died much faster at 4 ºC, and its susceptibility was reverted after transformation with *otsA*/*otsB*; it was also shown that trehalose content in the bacterial cell increased eight-fold after exposure to 16 ºC of cultures maintained at 37 ºC [49]. In addition, trehalose is a well-known osmoprotectant metabolite in *E. coli* and other bacteria, and its accumulation in the prokaryotic cell in response to salt stress has been documented [50-51]; notably, trehalose is synthesized at high levels in cells of *Desulfohalobium retbaense*, a microorganism able to grow in media containing NaCl concentrations up to 24 % [52]. *E. coli* strains defective in UGP are unable to synthesize trehalose and become osmotically sensitive [53].

Interestingly, some data suggest that the protective effects of trehalose might be elicited by UDP-glucose itself, acting as an intracellular signaling metabolite. In *E. coli* cells, *galU* mutation caused overexpression of the σ^S subunit of RNA polymerase, a factor which is involved in response to stress through the regulation of σ^S-dependent genes, which include *otsA* and *otsB* [54].

In addition to its protectant role, some organisms, such as mycobacteria and corynebacteria, incorporate trehalose as a structural component of their cell wall [48]. Importantly, the *M. tuberculosis* cell wall contains trehalose-dimycolate, a glycolipid composed of the fatty acid mycolic acid, esterified to the 6-hydroxyl group of both glucose units of trehalose. This glycolipid is considered among the major toxic components of the mycobacterial envelope, and a factor favoring its low permeability, a cause for the ample resistance of *M. tuberculosis* to many known antibiotics [55].

5.4. Coupling cell size with nutrient availability

Recently, the participation of UDP-glucose as a sensing molecule involved in the control of the complex coordination that regulates cell size and division has begun to be unveiled in *Escherichia coli* [56]. As an intracellular proxy for nutrient status, UDP-glucose links nutrient availability with cell division. Nutrient-rich media permit UDP-glucose activation of OpgH, an inner-membrane glycosyltransferase involved in the biogenesis of the bacterial envelope, leading to the inhibition of FtsZ ring formation. Direct protein-protein interaction with FtsZ by the N-terminus domain of OpgH is dependent of UDP-glucose, endowing this glycosyltranferase with an inhibitory role on cell division. In addition, OpgH governs the synthesis of osmoregulated periplasmic glucans (OPG), a family of polyglucose backbones which are important components of the envelope of gram negative bacteria that play a role in their interaction with eukaryotic hosts [57] OPGs are linear β(1→2)glucose polymers with β(1→6) branches, that can be further modified with phosphoglycerol, phosphoethanolamine and succinic esther. In many proteobacteria, such as *Erwinia* and *Pseudomonas*, they are involved in osmoprotection, biofilm formation and resistance to antibiotics [58]. Nevertheless, the regulation of cell size by OpgH is independent of its role in OPG synthesis.

5.5. GalF: a regulatory subunit?

The function of UGPs of some organisms has been reported to be modulated by the protein encoded by the gene *galF*. This gene was found immediately upstream to the operon *rfb*, which codes for proteins involved in the biosynthesis of the sugar-nucleotide dTDP-rhamnose in *E. coli*. The *galF* gene product showed high sequence homology with that of *galU*, in such way that both genes were thought to code for isoenzymes [59]. However, Marolda and Valvano demonstrated the lack of UGP activity of the *galF* gene product, GalF, and showed that GalF and UGP physically interacted in vivo. GalF-UGP interaction was shown to decrease the rate of phosphorolysis, thus maintaining UDP-glucose levels high. Furthermore, in the same study, they found that GalF binding enhanced UGP thermostability. The authors concluded that GalF might be a modulating, non-catalytic subunit of the UGP complex *in vivo* [60]. Since the tetrameric nature of the complex *in vivo* is generally well accepted, it is conceivable that UGP, jointly with GalF, could be active in the form of heterotetramers in the bacterial cell of GalF-having organisms.

The absence of pyrophosphorylase activity in GalF is likely due to mutation of critical amino acids, despite a very well-conserved primary sequence homology with UGP. Notably, the catalytic arginine in UGP is substituted by a histidine in GalF (fig. 2).

Most bacterial groups lack GalF homologues, being it apparently restricted to enterobacteria. In fact, enterobacterial GalF sequences aligned with those of UPG of various organisms cluster with enterobacterial UPGs sequences, indicating that GalF originated in a recent divergence within this group (fig. 3). In addition, GalF seems not to be absolutely required for cell viability, nor it is absolutely required for normal UGP activity in vivo, as *galF* mutation did not affect growth and the LPS profile in *Yersinia* [61]. In any case, considerable investigation is still needed to elucidate the precise role of GalF on the modulation of the UGP activity.

6. VALUE AS A THERAPEUTIC TARGET

The central position of UDP-glucose in the bacterial biochemical pathways, very prominently those involved in the synthesis of envelope structures, determines that strains defective for this enzyme show markedly impaired pathogenic phenotypes. Not unexpectedly, the gene *galU* can be found as an essential gene for one third of all bacteria taxa included in the database of essential genes [62].

In *S. pneumoniae*, at least 90 capsular types have been described [63], and the organization of the gene cluster responsible for the biosynthesis of several capsular types has been analyzed [47, 63-64]. UDP-glucose is needed for the addition of glucose, galactose (via the epimerization of the activated sugar), glucuronic and galacturonic acid residues of the pneumococcal capsule. Since at least one of these four sugars is found in all capsular polysaccharides, pharmacological inhibition of UGP would affect the biosynthesis of the pneumococcal capsule, considered a *sine qua non* component of pathogenicity of this microorganism [21, 33]. Figure 9C shows the unencapsulated phenotype of *galU* defective pneumococci, which is correlated with its apathogenicity.

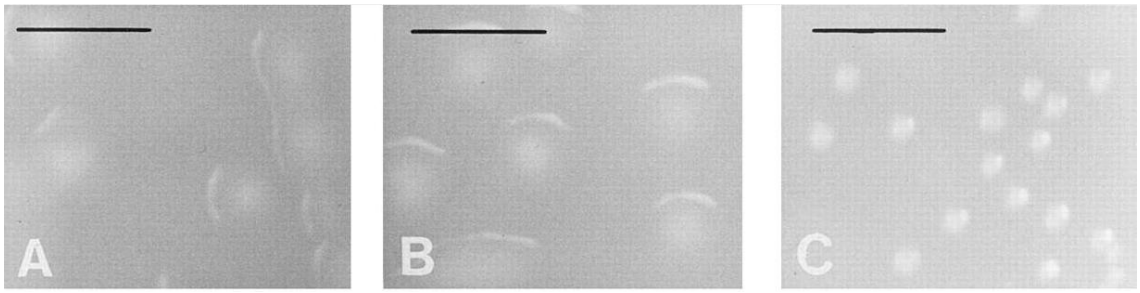

Figure 9: Capsule phenotypes for three pneumococcal strains: A: M23, B: M23c, and C: M23g (GalU), showing the unencapsulated phenotype of the *galU* mutant (C). Bar, 1 mm. Reproduced under the

In addition to *S. pneumoniae*, it has been shown that *galU* mutation produces aberrant envelope structures in a large number of organisms. Consequently, the GalU UDPG:PP enzymatic activity may represent an important target in fighting bacterial infectious diseases [33]. In *E. coli*, such mutants were reported to produce incomplete LPS, containing only heptose and 3-deoxy-D-manooctulosonic acid as sugar moieties. Moreover, these mutants showed motility impairment due to lack of functional flagella formation [65]. Loss of motility has been also shown to occur in other pathogenic organisms, such as *Pseudomonas*, upon *galU* mutation [66]. In addition, mutations in genes coding for UGPs have been shown to lead to decreased virulence of a number of diverse bacterial pathogens. In *Klebsiella pulmoniae*, *galU* mutation led to loss of mucoid colony phenotype and virulence in mice and high sensitiveness in human serum [67]. *galU Vibrio colerae* was defective in colonization and lost its capability to synthesize an exopolysaccharide biofilm involved in the formation of a resistant rugose variant [68]. Similarly, cornea infecting and systemic spreading capacities of *Pseudomonas aeruginosa* were impeded upon *galU* mutation [69], while the plant pathogen *Xanthomonas citri* was shown to require UGP activity for *in planta* growth and pathogenicity [70].

Considering all above, and the tight control of nutrient availability, cell size, growth rate and central metabolism exerted by UDP-glucose, the advent of new molecules targeted against the activity of bacterial UGPs will obviously open new ways for broad applications in biotechnology and biomedicine. Specifically, inhibitors of this enzyme would be of great value for the treatment of diseases caused by pathogens that are resistant to the current therapeutic arsenal. Antimicrobial resistances to traditional drugs may take the global healthcare systems back to pre-antibiotic era in many aspects, making necessary a radical change of resource allocation and assessing interventions to research strategies that enable the identification of new targets. However, although pharma investments have been huge, it is surprising that the last decades of rational-based discovery have yet to be translated into a greater number of new medicines [71]. Rapid advances in Structural Biology, combined with Pharmacology, will not only enable the search for new targets, but also facilitate the study of existing targets for finding clues to new target identification and validation, and for probing the molecular mechanisms of drug actions and adverse reactions for both novel and existing drugs. Therefore, molecular understanding of protein-ligand binding will significantly impact and enhance Drug Discovery, from the *in silico* design to preclinical and clinical development.

In this context, and although no inhibitors of prokaryotic UGPs have been reported to date, their promising profile as target candidates has been referred to by many authors, given its participation in key metabolic pathways and the synthesis of some of the most important bacterial virulence factors, together with the fact that there is no relation, aside from the catalytic activity, between bacterial UGPs and their eukaryotic counterparts. This last feature very well suits bacterial UGPs to provide the required specificity to avoid undesired toxicities, therefore possibly accelerating its development and speeding up its process to market.

CONCLUDING REMARKS

Antimicrobial resistance is not a recent phenomenon, but it constitutes a critical issue for healthcare systems worldwide. There is an urgent need to deeply investigate new alternatives to overcome the disease burden caused by infections. In this regard, bacterial UGPs are highly conserved enzymes which are involved in a number of key bacterial processes, including the synthesis of different elements of the bacterial envelope, considered necessary virulence factors of many pathogens.

Detailed knowledge on the three-dimensional structure of UGPs at atomic scale, as well as their mechanism of action, are key to the process. Today, ample structural and mechanistic information is available to aid at drug discovery programs targeted to these enzymes. In addition, current understanding of the function of UGPs in the context of bacterial pathogenicity provides a sound base supporting a good perspective for potential drugs inhibiting UGPs.

Taken together, all these characteristics make of bacterial UGPs an attractive target for alternative discovery programs and the potential development of new antibacterial agents with novel mechanisms of action.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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