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The *Helicobacter pylori ureC* Gene Codes for a Phosphoglucosamine Mutase

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The function of UreC, the product of a 1,335-bp-long open reading frame upstream from the urease structural genes (*ureAB*) of *Helicobacter pylori*, was investigated. We present data showing that the *ureC* gene product is a phosphoglucosamine mutase. D. Mengin-Lecreulx and J. van Heijenoort (J. Biol. Chem. 271:32–39, 1996) observed that UreC is similar (43% identity) to the GlmM protein of *Escherichia coli*. Those authors showed that GlmM is a phosphoglucosamine mutase catalyzing interconversion of glucosamine-6-phosphate into glucosamine-1-phosphate, which is subsequently transformed into UDP-*N*-acetylglucosamine. The latter product is one of the main cytoplasmic precursors of cell wall peptidoglycan and outer membrane lipopolysaccharides. The present paper reports that, like its *E. coli* homolog *glmM*, the *H. pylori ureC* gene is essential for cell growth. It was known that growth of a lethal conditional *glmM* mutant of *E. coli* at a nonpermissive temperature can be restored in the presence of the *ureC* gene. We showed that complete complementation of the *glmM* mutant can be obtained with a plasmid overproducing UreC. The peptidoglycan content and the specific phosphoglucosamine mutase activity of such a complemented strain were measured; these results demonstrated that the *ureC* gene product functions as a phosphoglucosamine mutase. Homologs of the UreC and GlmM proteins were identified in *Haemophilus influenzae*, *Mycobacterium leprae*, *Clostridium perfringens*, *Synechocystis* sp. strain PCC6803, and *Methanococcus jannaschii*. Significant conservation of the amino acid sequence of these proteins in such diverse organisms suggests a very ancient common ancestor for the genes and defines a consensus motif for the phosphoglucosamine mutase active site. We propose renaming the *H. pylori ureC* gene the *glmM* gene.

Helicobacter pylori is a microaerophilic, gram-negative bacterium which is an etiologic agent of chronic gastritis; it is associated with peptic ulceration and some cases of gastric carcinoma (15). Various virulence factors have been identified, but the pathogenic mechanisms of *H. pylori* are still poorly understood (7). One of the identified factors, a very active urease, is common to all *H. pylori* isolates. The genetic organization of a large *H. pylori* DNA region comprising the urease structural (*ureAB*) and accessory (*ureIEFGH*) genes has been determined (2, 8) (see Fig. 1). The presence of a 2.2-kb-long DNA region immediately upstream from the *ureAB* genes is required to confer urease activity on a *Campylobacter jejuni* recipient strain (8). However, nothing was known about the importance of this region containing two open reading frames (ORFs), designated *ureC* and *ureD*, for urease production in *H. pylori*. No role has been attributed to the *ureD* ORF and, similarly, no function has been defined for the product of the ORF *ureC* (1,335 bp), which maps 820 bp upstream from the start codon of *ureA* and is transcribed in the same direction (Fig. 1). Downstream from the *ureC* gene, there is a palindromic sequence followed by a run of eight T residues showing all of the characteristics of the *Escherichia coli* rho-independent terminators, suggesting that *ureC* and *ureA* are not cotranscribed. Sixty *H. pylori* isolates have been investigated by PCR analysis and DNA sequencing for the presence of *ureC*; the gene was found in every isolate, and the UreC protein encoded is highly conserved (reference 6 and unpublished results).

A previously published (13) search of protein databases for homologs of the *E. coli* phosphoglucosamine mutase GlmM identified a protein with 43% identity: the *ureC* gene product. Herein, we present data showing that the *ureC* gene product is a phosphoglucosamine mutase, an enzyme which catalyzes the interconversion of GlcN-6-phosphate (GlcN-6-P) and GlcN-1-P isomers (13). In *E. coli*, GlcN-1-P is acetylated to GlcNAc-1-P and then UDP ribosylated to produce UDP-GlcNAc. UDP-GlcNAc is one of the main cytoplasmic precursors of bacterial cell wall peptidoglycan (20) and in *E. coli* (and other gram-negative bacteria) is also a precursor of outer membrane lipopolysaccharide (16).

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The *E. coli* strains used in this work are listed in Table 1. pILL594, carrying a 5.1-kb DNA fragment from *H. pylori* including the *ureC* gene, has been previously described (8), and the p*Trc*99A vector was purchased from Pharmacia (Uppsala, Sweden). Unless indicated otherwise, 2YT, a rich medium, was used to grow cells (14). Growth was monitored by measuring optical density at 600 nm (OD_{600}) with a spectrophotometer (model 240; Gilford Instrument Laboratories, Inc., Oberlin, Ohio). For strains carrying drug resistance genes, antibiotics were used at the following final concentrations (in micrograms per milliliter): ampicillin, 100; spectinomycin, 100; kanamycin, 30; chloramphenicol, 25.

General DNA techniques, *E. coli* cell transformation, and complementation of the *E. coli glmM* thermosensitive mutant. The alkaline lysis method was used for both small- and large-scale plasmid preparations. Plasmid DNA was further purified on cesium chloride-ethidium bromide gradients (18). Standard procedures for endonuclease digestions, ligation, and agarose electrophoresis were used (18). *E. coli* cells were made competent and transformed with plasmid DNA either by the method of Dagert and Ehrlich (3) or by electroporation.

Thermosensitive mutant strain GPM83 was transformed with the plasmids to be tested as follows. Competent cells (300 μl), mixed with plasmid DNA, were kept on ice for 3 h and then heated for 3 min at 42°C. A 400-μl volume of 2YT

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TABLE 1. *E. coli* strains used in this study

Strain	Genotype	Source or reference
JM83	F$^-$ *ara* Δ(*lac-proAB*) *rpsL* *thi* [Φ80 d*lac*Δ(*lacZ*)*M15*]	21
GPM83	JM83 *glmM*::*kan*(pGMM)	13
URE831	JM83 *glmM*::*kan*(pILL594)	This work
URE832	JM83 *glmM*::*kan*(pMLD106)	This work

medium was then added, and the cells were incubated at 30°C for over 2 h to allow expression of plasmid-encoded genes. One-hundred-microliter aliquots of the cell suspension were plated onto two prewarmed 2YT-antibiotic plates, one incubated at 30°C and the other incubated at 42°C. Growth was observed after 24 h of incubation.

Construction of plasmids. A plasmid suitable for high-level overproduction of UreC was constructed as follows. PCR primers were designed to incorporate a *Bsp*HI site (in boldface) 5′ at the initiation codon (underlined) of *ureC* (5′-TA TAA**TCATGA**AAATTTTTGGGACT-3′) and a *Pst*I site (in boldface) 3′ to the gene after the stop codon (5′-TTAG**CTGCAG**TTAGCACAAATGCCCTTC-3′). These primers were used to amplify the *ureC* gene from plasmid pILL594. The isolated DNA was then treated with *Bsp*HI and *Pst*I, and the two resulting fragments (*ureC* contains an internal *Bsp*HI site) were then ligated together between the compatible *Nco*I and *Pst*I sites of vector p*Trc*99A. The ligation mixture was used to transform (by electroporation) strain GPM83, and transformants were selected for both ampicillin resistance and growth at 42°C. About 20 such transformants were isolated, all carrying the desired construct, named pMLD106, allowing expression of the reconstituted *ureC* gene under the control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *trc* promoter.

Isolation of sacculi and quantification of peptidoglycan. JM83, URE831, or URE832 cells (0.5-liter cultures) were grown exponentially at 30 or 42°C in 2YT medium to an OD_{600} of 0.7 (250 mg of bacteria [dry weight] per liter of culture). GPM83 cells were grown first at 30°C, and at a cell density of $6 \times 10^4 \cdot ml^{-1}$, the temperature of the culture was either maintained at 30°C or increased to 42°C. Incubation was then continued until the OD_{600} of the culture at 42°C reached a plateau of 0.6 to 0.7, about 5 h later. The cells were then rapidly chilled to 0°C, harvested in the cold, washed with a cold 0.85% NaCl solution, and centrifuged. The bacterial pellet was then rapidly suspended by vigorous stirring in 40 ml of a hot (95 to 100°C), aqueous 4% sodium dodecyl sulfate (SDS) solution for 30 min. The suspensions were left to stand overnight at room temperature and centrifuged for 30 min at 200,000 × *g*, and the pellets were washed several times with water. Each pellet was suspended in 5 ml of water and homogenized by brief sonication. Aliquots were hydrolyzed (6 M HCl, 95°C, 16 h) and analyzed with an amino acid analyzer (LC-2000; Biotronik, Frankfurt, Germany) equipped with a column of DC-6A (Dionex, Sunnyvale, Calif.) and a Spectra-Glo fluorometer (Gilson, Villiers-le-Bel, France). *o*-Phthalaldehyde–β-mercaptoethanol was used as the postcolumn derivatization reagent. The peptidoglycan content of the sacculi is expressed in terms of muramic acid content (11).

Preparation of crude enzyme. Cells (0.5-liter cultures) were grown exponentially at 30, 37, or 42°C in 2YT medium as described above. When required, 1 mM IPTG was added at an OD_{600} of 0.1 and growth was continued for approximately 2 h. In all cases, cells were harvested in the cold when the OD_{600} reached 0.7 and were washed with 40 ml of cold 0.02 M potassium phosphate buffer (pH 7.4) containing 0.3 mM $MgCl_2$ and 0.1% β-mercaptoethanol. The wet cell pellet was suspended in 5 ml of the same buffer and disrupted by sonication (Sonicator 150; T. S. Ultrasons, Annemasse, France) for 10 min with cooling. The resulting suspension was centrifuged at 4°C for 30 min at 200,000 × *g* in a Beckman TL100 centrifuge. The supernatant was dialyzed overnight at 4°C against 100 volumes of the same phosphate buffer, and the resulting solution (5 ml, 10 to 12 mg of protein $\cdot ml^{-1}$) designated the crude enzyme was stored at −20°C. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) as previously described (9), with 13% polyacrylamide gels. Protein concentrations were determined by the method of Lowry et al. (10) with bovine serum albumin as the standard.

Assay for phosphoglucosamine mutase activity. A coupled assay was routinely used in which the GlcN-1-P synthesized from GlcN-6-P by the mutase was quantitatively converted to UDP-GlcNAc in the presence of the purified bifunctional GlmU enzyme (12). The standard assay mixture contained 50 mM Tris-HCl buffer (pH 8.0), 3 mM $MgCl_2$, 1 mM GlcN-6-P, 0.4 mM [^{14}C]acetyl coenzyme A (acetyl-CoA) (700 Bq), 10 mM UTP, 0.7 mM Glc-1,6-diP, 1 μg of the pure GlmU enzyme, and the crude enzyme (0.1 to 10 μg of protein, depending on overexpression and purification factors) in a final volume of 100 μl. Mixtures were incubated at 37°C for 30 min, and reactions were terminated by addition of 10 μl of acetic acid. Reaction products were separated by high-voltage electrophoresis on Whatman 3MM filter paper in 2% formic acid (pH 1.9) for 90 min at 40 V/cm with an LT36 apparatus (Savant Instruments, Hicksville, N.Y.). The only two radioactive spots (acetyl-CoA and UDP-GlcNAc) were located by overnight autoradiography with type R2 films (3M, St. Paul, Minn.) or with a radioactivity scanner (Multi-Tracermaster LB285; Berthold, France, Elancourt, France). Spots were cut out and counted in an Intertechnique SL 30 liquid scintillation spectrophotometer with a solvent system consisting of 2 ml of water and 13 ml of Aqualyte mixture (J. T. Baker Chemicals, Deventer, The Netherlands). One unit of enzyme activity was defined as the amount which catalyzed the synthesis of 1 μmol GlcN-1-P per min.

Chemicals. [^{14}C]acetyl-CoA (1.85 GBq $\cdot$ mmol^{-1}) was purchased from ICN (Irvine, Calif.). GlcN-1-P, GlcN-6-P, Glc-1,6-diP, UTP, and UDP-GlcNAc were from Sigma Chemical Co. (St. Louis, Mo.).

RESULTS AND DISCUSSION

Complementation of an *E. coli glmM* conditional mutant by the *H. pylori ureC* gene. In *E. coli*, the *glmM* gene, like the other genes involved in peptidoglycan synthesis, is essential for bacterial growth. Attempts to inactivate *ureC* on the *H. pylori* chromosome by allelic exchange with a copy of the gene interrupted by a kanamycin resistance cassette were repeatedly unsuccessful, indicating that *ureC* is also an essential gene in *H. pylori*.

To test whether a GlmM deficiency in *E. coli* could be compensated by the *H. pylori* UreC protein, we used the *E. coli glmM* lethal conditional mutant GPM83 (13). This strain is a JM83 derivative and contains (i) an inactivated *glmM* copy on the chromosome and (ii) a wild-type *glmM* allele on plasmid pGMM, a derivative of vector pMAK705 which bears a thermosensitive replicon (Table 1). At a nonpermissive temperature for plasmid replication (42°C), GPM83 failed to grow on 2YT plates because of the loss of plasmid-borne *glmM*. At a permissive temperature (30°C), JM83 and GPM83 were identical in growth rate and cell morphology. Strain GPM83 was transformed with pILL594 (Fig. 1), a pILL570 derivative (itself derived from pBR322) carrying part of the urease region and the entire *ureC* gene under control of its own promoter (8). As previously described (13), this strain, designated URE831 (Table 1), grew on plates at the nonpermissive temperature (42°C). However, strain URE831 formed smaller colonies than did strain GPM83 harboring a pUC18 derivative, pMLD99, carrying *glmM* (13) under the same growth conditions. The loss of thermosensitive plasmid pGMM in strain URE831 was verified by screening for loss of chloramphenicol resistance conferred by pGMM. During liquid growth at 42°C, the URE831 cells bulged slightly and were ovoid in shape as assessed by phase-contrast microscopy, suggesting some defect in cell wall synthesis. The complementation of the *E. coli glmM* mutant by *ureC* on pILL594 was presumably, therefore, only partial. Consequently, we constructed a plasmid overproducing UreC in *E. coli*.

Construction of an *E. coli* strain overexpressing the *H. pylori ureC* gene. To overproduce UreC in *E. coli*, the *ureC* gene was put under control of the IPTG-inducible *trc* promoter of vector p*Trc*99A to give pMLD106 (see Materials and Methods and Fig. 1). Introduction of pMLD106 into *E. coli glmM* mutant GPM83 resulted in strain URE832 (Table 1). Strain URE832 formed normal colonies (i.e., comparable to those of strain GPM83 carrying pMLD99) at the nonpermissive temperature, even in the absence of IPTG, indicating that the repression by the plasmid *lacI*^{q} gene was not complete.

Strain URE832 was grown in the presence of 1 mM IPTG at the nonpermissive temperature. The *ureC* gene was strongly overexpressed. SDS-PAGE was used to analyze crude extracts (prepared as detailed in Materials and Methods) of strain URE832 carrying grown in the absence or presence of 1 mM IPTG (lanes B and C of Fig. 2, respectively) and of parental strain JM83 carrying the vector p*Trc*99A as a control (lane A of Fig. 2). A large amount of an approximately 50-kDa protein was detected in strain URE832 grown in the presence of IPTG. This protein corresponds to UreC; its molecular mass is in

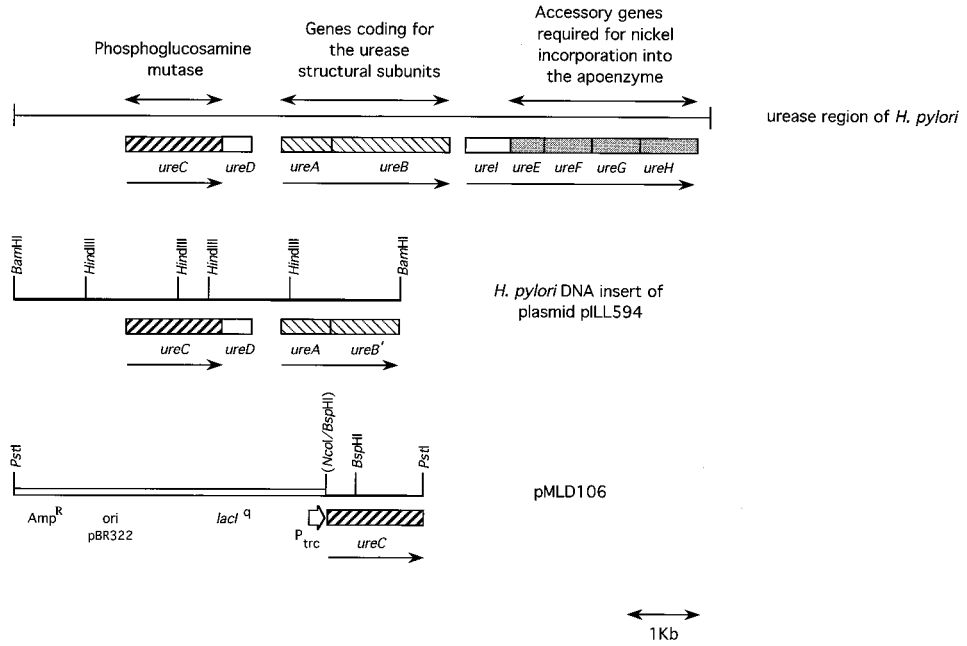

FIG. 1. Localization of the *ureC* gene near the urease region of *H. pylori* and restriction maps of the *H. pylori* DNA insert of pILL594 (8) and the entire pMLD106 plasmid constructed to overexpress the *ureC* gene. The genes are shown as boxes with arrows indicating the direction of their transcription. The boxed region of pMDL106 corresponds to the vector p*Trc*99A with the IPTG-inducible promoter P_{trc}, the *lacI*^{q} gene, the pBR322 origin of replication, and the gene conferring ampicillin resistance. *ureB′* indicates that this gene is truncated in pILL594.

accordance with the 49,191 Da calculated from the deduced amino acid sequence of *ureC*.

In strain URE832 grown with IPTG, about two-thirds of the UreC produced appeared to aggregate and was thus not retained in the soluble fraction obtained after sonication. Aggregates, presumably of UreC, were also detected by microscopic examination of these cells as intracellular refringent nodules. In addition, these cells presented an unusual morphology: they were curved and presented a clear defect in cell septation.

Peptidoglycan content of the *E. coli glmM* conditional mutant complemented by the *ureC* or *glmM* gene product. When a culture of strain GPM83 was shifted from exponential growth at 30°C (at a cell density of approximately $6 \times 10^4 \cdot ml^{-1}$) to the nonpermissive temperature of 42°C, the growth rate rapidly slowed and finally stopped 5 h later (after approximately 12 doubling times) at a cell density of approximately $3 \times 10^8 \cdot ml^{-1}$. At that stage, cells entered into a prococious stationary phase and progressively lysed, as shown by a slow decrease of the culture OD_{600}. In addition, the cell morphology progressively changed from rods to enlarged ovoids whereas that of parental cells grown under the same conditions was unaltered. This altered morphology was a consequence of progressive depletion of the GlmM protein, an enzyme essential for peptidoglycan synthesis, which was itself due to the dilution of plasmid pGMM by cell division (13). To evaluate the effects on peptidoglycan synthesis of the *glmM* mutation alone or complemented by the wild-type *glmM* allele or by the *H. pylori ureC* gene, the murein contents of sacculi of various *E. coli* transformants were measured (Table 2 and Materials and Methods).

The peptidoglycan concentration in strain GPM83 was 9,000 nmol · g (dry weight) of bacteria^{-1} during growth at 30°C. At 42°C, it reached the critical value of 5,500 nmol · g^{-1}, at which the cells stop growing and start to lyse (Table 2). Strain URE831, grown at 42°C, contained only 7,000 nmol · g^{-1}, confirming the partial complementation by the *ureC* gene expressed from plasmid pILL594 (Table 2). However, this peptidoglycan level was sufficient to allow growth. It is thus possible to reduce the *E. coli* peptidoglycan content by about 30 to 40% without seriously affecting cell wall integrity. This suggested that about 50% of the total murein content of the cell is in excess, possibly allowing maintenance of cell integrity if growth conditions change.

In strain URE832 grown at 42°C with or without IPTG, sufficient amounts of UreC protein were produced to fully replace the *E. coli* GlmM protein for peptidoglycan synthesis since the murein level was equivalent to that of a wild-type strain (Table 2).

Levels of phosphoglucosamine mutase of the *E. coli glmM* conditional mutant complemented by the *ureC* or *glmM* gene product. To measure phosphoglucosamine mutase activity, a coupled assay in the presence of the purified bifunctional enzyme GlmU was used (for details, see Materials and Methods). Strain GPM83 grown at 42°C presented negligible phosphoglucosamine mutase activity, 2% of that of the wild type (Table 2). The reduced growth rate and subsequent lysis of this strain are thus, indeed, a consequence of progressive GlmM depletion. Measurement of the phosphoglucosamine mutase activity in strain GPM83 complemented by the *ureC* gene thus allowed direct evaluation of *H. pylori* mutase activity with no significant interference from the host (*E. coli*) GlmM activity.

In strain URE831, the phosphoglucosamine mutase activity of UreC was low, only 6% of wild-type GlmM activity (Table 2). This was, however, sufficient to allow growth of the corresponding cells, despite an apparently imperfect cell wall. In strain URE832 carrying the *ureC* gene under control of the p*Trc* promoter on pMLD106, in the absence of IPTG, the *H. pylori* phosphoglucosamine mutase activity was only 12% of wild-type GlmM activity (Table 2). Interestingly, this low activity level is apparently sufficient for *E. coli* to show normal

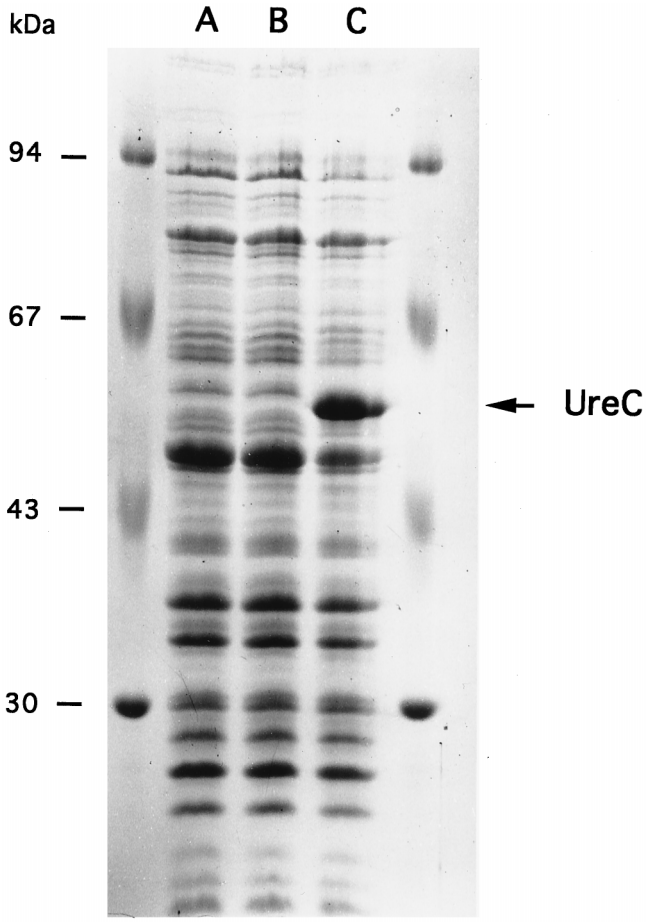

FIG. 2. Overproduction of the phosphoglucosamine mutase from *H. pylori* (UreC) in *E. coli* cells. Lanes: A, crude extract from JM83(p*Trc*99A) cells grown in the presence of 1 mM IPTG. B, crude extracts from URE832 cells grown in the absence of IPTG. C, crude extracts from URE832 cells grown in the presence of 1 mM IPTG. Molecular mass standards, indicated on the left in kilodaltons, are as follows: 94, phosphorylase *b*; 67, bovine serum albumin; 43, ovalbumin, 30, carbonic anhydrase.

morphology. Phosphoglucosamine mutase specific activity in strain URE832 grown in the presence of 1 mM IPTG was 19 times higher than wild-type GlmM activity (Table 2). This is consistent with the very strong UreC overproduction observed by SDS-PAGE analysis of crude extracts. This excess phosphoglucosamine mutase activity also seems to be deleterious for cell wall synthesis, since these cells presented abnormal shapes and an apparent septation defect.

Identification of a new family of proteins: the phosphoglucosamine mutases. Searches of the data banks identified proteins similar to GlmM in the following organisms that have been the subjects of partial or total genome sequencing projects (Fig. 3): *Haemophilus influenzae* (4), 73% identity along 445 residues (accession no. P45164); *Mycobacterium leprae*, 47% identity along 463 residues (accession no. U00020); *Clostridium perfringens* (partial sequence): 45% identity along 140 residues (accession no. X86494); *Synechocystis* sp. strain PCC6803 (5), 40% identity along 481 residues (accession no. D90908); *Methanococcus jannaschii* (1), 31% identity along 448 residues (accession no. U67553). No function has previously been attributed to these putative proteins, although relatedness to phosphoglucomutases has been suggested for some of them. Six sequences significantly similar to that of GlmM, including the *H. pylori* UreC sequence (43% identity along 445 residues), are compared to the *E. coli* GlmM sequence in Fig. 3. There are highly conserved regions, possibly those involved in the enzymatic specificity of these proteins. The presence of GlmM homologs in these organisms also suggests that they have a metabolic pathway leading to UDP-GlcNAc analogous to that described in *E. coli.*

A sequence similar to the putative active site of hexosephosphate mutases has been identified in the GlmM sequence (13). The following consensus motif for the phosphoglucosamine mutase active site can be deduced from the seven sequences (Fig. 3): G(IV)(VM)I(ST)AS*HNPXX(DY)NGIK. This motif includes a serine residue (S99 in UreC) the phosphorylation of which, in hexosephosphate mutases, seems to be required for catalytic activity (17). By analogy with the reaction catalyzed by the phosphoglucomutases, GlcN-1,6-diP has been proposed to be an intermediate in the catalytic process. However, it is not known whether this compound or another hexose 1,6-diP is required for the initial phosphorylation of the phosphoglucosamine mutases.

The existence of a GlmM homolog in an organism as distant from *E. coli* as the archaebacterium *M. jannaschii* suggests a very ancient and common origin for the corresponding genes, i.e., before the divergence of eubacteria and archaebacteria. Interestingly, in *M. jannaschii*, no murein-like structure has been observed and only a single proteinaceous S layer has been described (19). Possibly, the ancestral function of GlmM was not a step in the peptidoglycan synthesis pathway.

TABLE 2. Peptidoglycan content and phosphoglucosamine mutase activity measured in various *E. coli* cells

Strain (condition)	Temp (°C)[a]	Relevant genotype	Peptidoglycan content (nmol · g [dry wt] of bacteria^{-1})[b]	Sp act (U · mg of protein^{-1})[c]	Sp act amplification factor
JM83	30	Wild-type *glmM*	8,500	0.05	1
JM83	42	Wild-type *glmM*	9,000	0.04	1
GPM83	30	*glmM* Ts[d] mutant	9,000	0.11	2.2
GPM83	42	*glmM* Ts mutant	5,500	0.001	0.02
URE831	42	*glmM* Ts mutant carrying pILL594	7,000	0.003	0.06
URE832 (without IPTG)	42	*glmM* Ts mutant carrying pMLD106	9,000	0.006	0.12
URE832 (with 1 mM IPTG)	42	*glmM* Ts mutant carrying pMLD106	9,000	0.95	19

[a] Cells were grown exponentially in 2YT medium at 30°C (permissive temperature) or first at 30°C and then for 5 h at 42°C (nonpermissive temperature), the time at which the growth rate of mutant strain GPM83 began to decrease (13).
[b] The peptidoglycan content of sacculi was quantified as detailed in Materials and Methods and expressed in terms of muramic acid content.
[c] Phosphoglucosamine specific activity was measured as detailed in Materials and Methods. One unit of enzyme activity was defined as the amount which catalyzed the synthesis of 1 μmol GlcN-1-P per min.
[d] Ts, temperature sensitive.

FIG. 3. Alignment of the predicted amino acid sequences of *H. pylori* UreC (UreC-Hp) and those of ORFs found in *H. influenzae* (MRSA-Hae), *M. leprae* (UreD-Mlep), a partial sequence of *C. perfringens* (UreC-Clost), *Synechocystis* sp. strain PCC6803 (ORF-Synecho), and *M. jannaschii* (MJ1100-Metha) with the *E. coli* GlmM sequence (GlmM-Ecoli). The alignments were performed with the PILEUP program by using the Genetics Computer Group Sequence Analysis Software Package, version 7-UNIX. Stars indicate amino acids identical to those in the *E. coli* GlmM sequence. Points indicate gaps inserted to optimize alignment. The sequences are in decreasing identity percentage order; i.e., the *H. influenzae* sequence shows the highest degree of identity (73%) with the *E. coli* GlmM sequence. The position of the putative active site of these phosphoglucosamine mutases is overlined. #, putative phosphorylated serine residue.

No UreC or GlmM homologs were found in the complete genomic sequences of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*, which are known to be cell wall-less bacteria. One could propose either that these organisms possess no phosphoglucosamine mutase, possibly because this function was lost during evolution, or that the amino acid sequence of the *Mycoplasma* phosphoglucosamine mutase had considerably diverged and could not be distinguished, by data bank analysis, from those of the other hexosephosphate mutases.

In conclusion, although little data about the *H. pylori* cell wall is available, our finding of a phosphoglucosamine mutase suggests the existence of a pathway similar to that described in *E. coli* for the synthesis of cell wall peptidoglycan and lipopolysaccharide. That *ureC* is an essential gene in *H. pylori* supports this hypothesis and might open new perspectives in the search for anti-*H. pylori* drugs: this bacterial phosphoglucosamine mutase is a potential target for antimicrobial agents. Finally, the role of the product of *ureC* does not include involvement in the expression of urease activity in *C. jejuni* (8). Consequently, we favor the hypothesis that, in *C. jejuni*, the 2.2-kb-long DNA region upstream from *ureA* may be the target of a transcriptional activator essential for full expression of the *ureAB* genes in this organism. For these reasons, we decided to rename the *H. pylori ureC* gene the *glmM* gene.

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