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Urea Transport in Bacteria: Acid Acclimation by Gastric *Helicobacter spp*

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Abstract. Urea transporters in bacteria are relatively rare. There are three classes, the ABC transporters such as those expressed by cyanobacteria and *Corynebacterium glutamicum*, the Yut protein expressed by *Yersinia spp* and the UreI expressed by gastric *Helicobacter spp*. This review focuses largely on the UreI proton-gated channel that is part of the acid acclimation mechanism essential for gastric colonization by the latter. UreI is a six-transmembrane polytopic integral membrane protein, N and C termini periplasmic, and is expressed in all gastric *Helicobacter spp* that have been studied but also in *Helicobacter hepaticus* and *Streptococcus salivarius*. The first two are proton-gated, the latter is pH insensitive. Site-directed mutagenesis and chimeric constructs have identified histidines and dicarboxylic amino acids in the second periplasmic loop of *H. pylori* and the first loop of *H. hepaticus* UreI and the C terminus of both as involved in a hydrogen-bonding dependence of proton gating, with the membrane domain in these but not in the UreI of *S. salivarius* responding to the periplasmic conformational changes. UreI and urease are essential for gastric colonization and urease associates with UreI during acid exposure, facilitating activation of the UreA and UreB apoenzyme complex by Ni^{2+} insertion by the UreF-UreH and UreE-UreG assembly proteins. Transcriptome analysis of acid responses of *H. pylori* also identified a cytoplasmic and periplasmic carbonic anhydrase as responding specifically to changes in periplasmic pH and these have been shown to be essential also for acid acclimation. The finding also of upregulation of the two-component histidine kinase HP0165 and its response element HP0166, illustrates the complexity of the acid acclimation processes involved in gastric colonization by this pathogen.

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Introduction

Urea is a small-molecular weight polar and relatively lipid-insoluble substance which is ubiquitous in nature. It has diverse functions. In organisms containing the enzyme urease, a nickel-dependent metalloenzyme present in bacteria, fungi and plants, urea is primarily used as a source of nitrogen necessary for growth. However, since urease metabolizes urea to CO_2 and ammonia, thus providing a ready source of base, metabolism of urea by urease can also enable microorganisms to respond to acid challenges. In mammals, it is the primary waste product of amino acid catabolism. It is osmotically active and changes in its concentration in various locations can contribute to osmoregulation. Thus, urea is a versatile substance and its role largely depends on whether it is an end-product or can be further broken down and if so, the utilization of the breakdown products varies considerably, either for anabolic processes or for buffering under acidic conditions.

Role of Urea in Mammals

In mammals, urea is the main nitrogenous waste product of protein metabolism, produced by the deamination of amino acids. It is synthesized in the liver, the process consuming one mole of carbon dioxide and two moles of ammonia by the urea cycle. Urea is normally eliminated from the body by glomerular filtration, and impaired renal function leads to its retention and that of other toxic byproducts in the blood with possible deleterious effects on organ function (uremia) (Vanholder, Glorieux & Lameire, 2005).

In individuals with normal renal function, accumulation of urea in the medullary interstitium plays an important role in the formation of a urine more concentrated than blood, a process important for the retention of free water by the body. The high levels of urea along with sodium in the medulla provides a favorable osmotic gradient for the diffusion of water from the tubular lumen when antidiuretic hormone causes water channels to be inserted into the apical membranes of the collecting duct, thereby markedly increasing water permeability and increased water retention by the kidney.

Although urea movement into cells was first thought to be due to simple diffusion through the lipid bilayer, studies in red blood cells (Goodman, 2002) and perfused inner medullary collecting ducts (Sands, Nonoguchi & Knepper, 1987) revealed its rate of movement was more rapid than could be explained by simple diffusion alone. Moreover, subsequent studies also indicated that urea transport demonstrated saturation kinetics, could be attenuated by exposure to specific inhibitors, and functioned in the presence of a favorable concentration gradient—characteristics consistent with the presence of a urea transporter (Mayrand & Levitt, 1983). Studies of the movement of glycerol and water also revealed that their transfer across membranes was facilitated by the presence of specific transporters or channels, glycophorin and aquaporin, respectively (Nielsen et al., 2002).

Two urea transport genes have been cloned from human and rodent tissue and their function as urea transporters has been confirmed after expression in the *Xenopus* oocyte, since the oocyte does not express a urea transporter (Hediger et al. 1996). The UT-A gene encodes five proteins and the UT-B gene encodes a single protein (Sands, 2003). UT-1, 3, and 4 are expressed in the inner medullary collecting ducts and UT-2 in the thin descending limb of Henle, whereas UT-5 is expressed in the testes (Sands, 2004). The first four are involved in generating the osmotic gradient involved in regulation of water excretion by the kidney.

UT-B was cloned from an erythroid line and in humans is the Kidd antigen of red blood cells (Olives et al., 1994). It is also expressed in the vasa recta of the kidney, blood vessels involved in the countercurrent process important for preservation of the highly osmotic environment of the medulla. Expression of these transporters has also been described in other tissues including the liver, heart, and brain (Shayakul & Hediger, 2004). In the liver and heart, they are postulated to allow the elimination of excess urea produced as a consequence of activity of the urea pathway (liver) or of the polyamine pathway.

UT-B in red blood cells aids in the maintenance of an appropriate internal osmolality in the red blood cells by augmenting urea entry into the cell: an increase in red blood cell urea aids in protection of the cell from disruption as it traverses the high osmolarity of the interstitium. Of interest, examination of the expression of these transporters in other species shows they also contribute to osmoregulation, for example, in fish exposed to the high osmolarity of the ocean (Mathai, 2005). More extensive discussion of urea transporters in mammals and other eukaryotes are covered in detail elsewhere in this issue.

Role of Urea in Bacteria

In bacteria, the enzyme urease, as in other species, catalyzes the hydrolysis of urea to ammonia and carbamic acid and the latter spontaneously hydrolyzes to form carbonic acid and an additional molecule of ammonia. Urea hydrolysis in most bacteria is used as a nitrogen or carbon source for anabolic processes. However, in gastric tract-dwelling bacteria and other bacteria that encounter acid during their life cycles, urea hydrolysis contributes to their ability to survive in acid. Moreover, in gastric tract-dwelling bacteria, this process confers on them the unique ability to colonize the stomach.

The reaction catalyzed by urease is:

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \text{ (urease)}$$

$$NH_2COOH + H_2O \rightarrow H_2CO_3 + NH_3 \text{ (spontaneous)}$$

The ammonia and the carbonic acid formed from these reactions are used for anabolic processes in most bacteria. However, in *Yersinia spp* and gastric *Helicobacter spp*, these moieties are for cytoplasmic buffering in the former and periplasmic and cytoplasmic buffering in the latter (Young, Amid & Miller, 1996; Marcus et al. 2005).

Despite the recognition that urea movement through mammalian membranes was not due to passive diffusion but was accomplished by urea transporters, bacteriologists generally assumed that urea moved through bacterial membranes by passive diffusion. Measurement of urea permeability through bacterial membranes was found to have a permeability coefficient $P = 10^{-7}$ to 4×10^{-6} cm/s as in other bilayers (Tien, 1974). Most urea uptake experiments in prokaryotes have shown, rather than the presence of a urea transporter, simply incorporation of label into bacteria protein and this depended on urea diffusion, not transport. However, in 1967 it was demonstrated that urea entry into the ureolytic bacterium *Proteus rettgeri* was the rate-limiting step of urea hydrolysis (Magaña-Plaza, 1967). This was shown by a dramatic increase in the pH of unbuffered medium due to ammonia production in toluene-permeabilized cells as compared to intact bacteria.

Bacteria can be classified into three groups, dependent on the preferred pH of their environment: acidophiles that grow best in acidic pH, neutralophiles that grow best at neutral pH and alkalophiles that grow best at alkaline pH. At least three urea uptake systems have been identified in neutralophilic bacteria that contribute to their ability to survive or colonize acidic environments, particularly that of the stomach. The ABC-type urea transporter is energy-dependent and requires ATP to transport urea across the cytoplasmic membrane. The two energy-independent urea transporters, Yut and UreI, appear to be channel-like structures that allow urea to enter the cytoplasm through a urea pore powered by a favorable concentration gradient from gastric juice urea that is maintained by rapid hydrolysis of the entering urea by intrabacterial urease.

ABC-Type Urea Transporters

The ABC (ATP-binding cassette)-type urea transporter is typical of ABC transport systems, which consist of a lipid-anchored substrate binding protein, two integral membrane transport subunits and two ATP-binding proteins. This urea transport system has been extensively studied in *Corynebacterium glutamicum* a Gram-positive, non-pathogenic and fast growing soil bacterium, *Methylophilius methyltophus* and *Anabaena*, a filamentous, heterocyst-forming cyanobacterium. *C. glutamicum*, *M. methyltophus* and *Anabaena* utilize urea as a nitrogen source. When urea is abundant in the environment of *C. glutamicum*, sufficient urea enters the cytoplasm by passive diffusion where it is hydrolyzed by urease (Siewe et al., 1998).

However, in nitrogen-limiting conditions, an energy-dependent urea uptake system is synthesized. This energy-dependent system is encoded by the *urtABCDE* gene cluster (Beckers et al. 2004). *urtA* encodes the lipid-anchored urea binding protein, *urtB* and *urtC* encode integral membrane proteins comprising the two subunits of the urea permease and *urtD* and *urtE* encode for ATP-binding protein.

The urea permease subunits of *C. glutamicum*, *urtB* and *urtC* are similar to the putative urea permease subunits *fmdE* (40.8% aa identity) and *fmdF* (35.2% aa identity) identified in the *fmd* gene cluster of *M. methyltophus* and *urtB* (49.3% aa identity) and *urtC* (40.4% aa identity) in the *urt* gene cluster in *Anabaena* (Mills et al., 1998; Valladares et al., 2002). Hydrophobic and topological predictions of *urtB* of *C. glutamicum* indicate it has six membrane-spanning segments with both the N and C termini extracellular, similar to the topography determined for UreI (Weeks et al., 2000). Hydrophobic and topological analysis predicts 12 membrane segments with both the N and C termini extracellular for the protein encoded by the *urtC* gene. Thus, there is significant heterogeneity in the putative transporters in these organisms.

Inactivation of *urtC* as well as *urtA*, the putative urea-binding protein and *urtE*, an ATP-binding protein, in *Cg* by insertional mutagenesis resulted in a dramatic loss of urea uptake. Likewise, mutations of the *urtA*, *urtB* and *urtE* genes of *Anabaena* decreased urea uptake (Beckers et al., 2004). These mutational studies confirm that urea uptake in these organisms is facilitated by an ABC-type transporter.

The ABC-type urea transporter of *Cg* is synthesized under conditions of nitrogen starvation. In nitrogen-free medium, urea uptake increased greater then 3-fold. However, in the presence of 5 mM ammonia, 5 mM glutamine or 5 mM glutamate, urea transporter synthesis was abolished and urea uptake was prevented. In the absence of a nitrogen source, urea transporter synthesis was inhibited by the presence of 50 μM chloramphenicol, suggesting the transport system was regulated at the level of gene expression.

Transcription of the *Cg urtABCDE* operon is regulated by AmtR (Beckers et al., 2004). AmtR is a repressor protein with two binding sites upstream of *urtA*. In AmtR deletion mutants there was uncontrolled expression of *urtA* even in the presence of abundant nitrogen sources. Repression of the *urtABCDE* operon is released by the binding of the adenylated signal transduction protein GlnK to AmtR (Beckers et al., 2004).

Physiological regulation of the ABC-type urea transporter *fmd* operon of *Methylophilius methyltophus* appears to be similar to that of Cg (Mills et al., 1998). Quantitative western blot analysis of the urea binding protein FmdD from bacteria grown with methanol as a carbon source and several amides as a nitrogen source was performed to study the regulation of the *fmd* operon. Under urea-limiting conditions, the concentration of FmdD was greatly increased as compared to acetamide- and ammonia-limiting conditions. Indeed, high concentrations of ammonia either by direct addition to the medium or as the result of urea hydrolysis decreased expression of FmdD, indicating that the *fmd* operon of *Mm* may be under the control of repressor protein similar to AmtR of *Cg*. This type of transporter has not been described in organisms that inhabit mammals and the mechanism of urea permeation through the membrane has not been studied intensively in this group of urea permeases in bacteria.

Yut Urea Transporter

Yersiniae spp have developed a unique strategy enabling survival of brief exposure to gastric acidity using an acidic pH-optimum urease. Since urea uptake by Yut is not pH-regulated, unrestricted urea entry and subsequent hydrolysis by urease to 2 NH_3

and CO_2 would alkalize the cytoplasm to lethal levels if its urease were active at neutral pH. *Yersinia* has overcome this problem by expressing an intrabacterial urease with a sharp pH optimum at 5.5 (Young et al., 1996; de Koning-Ward & Robins-Browne , 1997). When the organism is at neutral pH its cytoplasm is ~pH 8.0, a pH at which the enzyme has little or no activity even in the presence of urea. As the bacterium encounters an acid challenge (pH < 3.0), its cytoplasmic pH falls, allowing initiation of urease activity and thence buffering of the cytoplasmic pH to ~5.0. At this cytoplasmic pH, *Yersinia* can survive in the mammalian stomach, but it cannot live there since the pH is too low to allow important biosynthetic processes.

A single-component urea transporter, Yut, from the gram negative bacterium *Yersinia peseudotuberculosis* was analyzed after expression in the *Xenopus* oocyte and by insertion into *H. pylori*, where UreI had been deleted (Sebbane et al., 2004). The Yut urea permease is a 35 kD protein with ten predicted membrane-spanning helices. Yut has no homology to other bacterial urea permeases; however, it does have some homology (21–23%) to eukaryotic urea transporters, including the human UTA-1 urea transporter found in the inner medullary collecting duct (22% identity, 38% similarity). Yut also contains the tandem N and C termini urea transport signature sequence repeats (L/V)PXXTXXF similar to those found in eukaryotic urea transporters (Sands, 1997). Measurement of urea uptake in *X. laevis* oocytes injected with Yut mRNA revealed that uptake was independent of temperature and was not saturable — characteristics of a channel-like structure. This is an interesting finding in light of the sequence similarity to the eukaryotic urea transporters which, however, are facilitated transporters requiring binding of substrate and conformational changes in the transporter for transmembrane urea movement. It was also found that Yut selectively transported urea and not thiourea but, unlike the UreI urea channel discussed below, it is pH insensitive. These properties indicate that the putative Yut urea channel exists only in the open state, allowing urea to enter into the cytoplasm down its concentration gradient. Insertion of Yut was able to compensate for the absence of UreI in *H. pylori* deletion mutants, consistent with its role as a passive urea transport protein (Sebbane et al., 2004).

The UreI Proton-gated Urea Channel of Gastric *Helicobacter spp*

The Gram-negative pathogen *Helicobacter pylori* is unique in its ability to colonize the human stomach. *H. pylori*, a neutralophile, has adapted to living in the highly acidic gastric milieu by a process defined as acid acclimation to contrast it with the better known acid resistance or tolerance processes utilized by other neutralophiles, that allow them to maintain a viable cytoplasmic pH between 4.0 and 5.0, allowing survival but not growth (Foster, 2004; Marcus et al., 2005). Acid acclimation is the ability of this organism to buffer its periplasm to near neutrality and maintain a sufficiently high cytoplasmic pH when the organism encounters acidity, thus enabling continuation of its metabolism and gastric colonization. This mechanism appears to be unique to gastric *Helicobacter spp* and requires the presence at least of a neutral-pH optimum cytoplasmic urease, a proton-gated urea channel, and a membrane-anchored carbonic anhydrase, as discussed below.

H. pylori expresses very high levels of a urease that has a pH optimum between pH 7.5 and 8.0 (Mobley, Island & Hausinger, 1995) and is irreversibly inactivated below pH 4.0 (Scott et al., 1998). From these observations, it is the intrabacterial urease that is utilized for acid acclimation in the gastric environment since there would be no urease activity external to the organism. The urease utilization by *H. pylori* must differ from that of *Yersinia spp* to allow cytoplasmic pH to be maintained close to neutrality to allow activity of this neutral-pH optimum enzyme.

In intact *H. pylori*, urease activity is low at neutral pH but increases ten- to twenty-fold as the external pH falls between 6.5 and 5.5 and activity remains high down to pH of ~2.5 (Scott et al., 1998). At neutral pH, permeabilization of the inner membrane with 0.01% of the non-ionic detergent, $C_{12}E_8$, increased urease activity to that measured in the bacterial homogenate (intact bacteria 0.25 ± 0.1; bacterial homogenate, 2.76 ± 0.27; bacteria with 0.01% $C_{12}E_8$, 2.65 ± 0.10 μmol urea/min/mg protein) (Weeks et al., 2000). Therefore, as shown above for *Proteus rettgeri*, the cytoplasmic membrane of *H. pylori* is a barrier for adequate urea transport. Unlike *Proteus rettgeri*, the data that showed increased urease activity at pH levels < 6.0 in the intact organism was due to increased urea entry into the cytoplasm in acid.

Analysis of the *H. pylori* urease gene cluster (Labigne, Cussac & Courcoux, 1991) suggested a possible candidate for this urea transporter. The *Hp* urease gene cluster consists of seven genes, a promoter region (*pureA*) then *ureAB*, a second promoter (*pureI*), and then *IEFGH*. *ureA* and *ureB* encode the urease structural subunits, while *ureE, F, G* and *H* encode accessory proteins necessary for Ni^{2+} insertion into the apoenzyme to produce active urease. *ure*I encodes for an integral membrane protein of 195 amino acids with a molecular mass of 21.56 kDa predicted to contain six transmembrane helices with periplasmic N and C termini (*see* Fig. 2). Since urease is required for acid resistance and colonization in animal models and UreI being the only membrane protein in the urease gene cluster, it was a likely candidate for a urea transporter (Skoulibris et al.,

1998; Scott et al., 2000; Mollenhauer-Rektorschek et al., 2002). Therefore, uptake of urea was studied in UreI deletion mutants and after expression of its cRNA in *Xenopus* oocytes.

The urease activity of intact non-polar *ureI* knockout mutants of *H. pylori* failed to increase as the medium pH fell below pH 6.5, as was seen in the wild-type organism (Scott et al,. 2000). This loss of acid-stimulated urease activity was not the result of urease inactivation since $C_{12}E_8$-permeabilized *ureI* knockout mutant urease activity was identical to the WT organism. Hence, these observations already suggested that UreI was an acid-activated urea permease.

Measurement of passive urea uptake in prokaryotes, with their small volume/surface ratio, is precluded by the endogenous permeability of the inner membrane phospholipid bilayer. Therefore, *Xenopus* oocytes with a volume/surface ratio several hundred times that of bacteria were used to determine urea uptake through UreI, as had been done for mammalian urea transporters (Hediger et al., 1996, Weeks et al., 2000). UreI was expressed in *Xenopus* oocytes by injection of *ure*I cRNA. Over 30 min, urea uptake in UreI oocytes was accelerated 6- to 10-fold at pH 5.0 compared to pH 7.5, the same as in non-injected oocytes at either pH. Urea accumulation was consistent with acid-dependent UreI facilitation of urea transport into the 0.4–0.6 μl of internal oocyte water space.

UreI-dependent urea uptake in oocytes was activated with a pH profile nearly identical to the pH activation profile of cytoplasmic urease in *H. pylori*. Half-maximal activation of transport occurred at a pH of ~5.9. Uptake was highly selective for urea, with only trace accumulation of ^{14}C-thiourea or ^{14}C-mannitol (0.16 ± 0.04 and 0.14 ± 0.08 pmol/oocyte/30 min at pH 5.0). Uptake of 50 μM ^{14}C-urea at pH 5.0 was 10.87 ± 0.72 and 9.4 ± 0.3 pmoles/oocyte/30 min in the absence or presence of 100 mM unlabeled urea, respectively, showing lack of saturation even though a 2000-fold excess of cold urea was added. The addition of urea to voltage-clamped UreI-expressing oocytes resulted in no change in current. An inward current of 117 nA would be predicted, if UreI were a proton- or cation-driven urea transporter with a stoichiometry of 1:1. UreI-mediated urea uptake is therefore non-electrogenic.

Transport at pH 5.0 was temperature-independent between 15 and 30 °C. This temperature independence and the lack of saturation of uptake suggest that, after H^+ activation, urea fluxes through UreI with little interaction with the protein. These properties, temperature independence, non-saturability, electroneutrality, urea selectivity and pH dependent urea uptake, are most easily interpreted if UreI is a proton-gated urea channel where its open probability is regulated directly by the pH of its periplasmic surface.

Role of UreI in Periplasmic Buffering

Acid acclimation of this gastric pathogen depends on the maintenance of suitable levels of cytoplasmic and periplasmic pH to maintain an effective proton-motive force (*pmf*), the bioenergetic basis for growth and transport of substrates and metabolites. The *pmf* is the algebraic sum of the difference of pH (-ΔpH) and membrane potential ($+\Delta\psi$) across the inner membrane separating the cytoplasm from the periplasm.

$$pmf\ (\text{in mV}) = \Delta\bar{\mu}_H{}^+ = -RT/nF \ln [\mathrm{H}^+{}_{out}]/[H^+{}_{in}] + \Delta\psi = -61\Delta\mathrm{pH} + PD$$

Therefore, using the *pmf* equation and measuring the membrane potential of *H. pylori*, it was possible to calculate the periplasmic pH and the role of UreI in the process of periplasmic buffering. In strongly buffered medium at pH 7.4, the cytoplasmic pH was found to be 7.8–8.2 and the membrane potential ~−180 mV, resulting in a *pmf* of −220 mV (Meyer-Rosberg et al., 1996). In progressively acidic buffers in the absence of urea, the membrane potential decreased and reached 0 mV at pH 3.5, since the driving force for proton influx was now solely from the ΔpH across the inner membrane. However, the addition of 5 mM urea resulted in the maintenance of a constant membrane potential of −101 mV over a range of medium pH between 3.0 and 6.0, with a calculated periplasmic pH of 6.1. This is close to the half-maximal activation pH of UreI determined by urea uptake in oocytes expressing the UreI channel and also the pH at which the urease activity of the intact organism is half-maximal. This is also, not coincidentally, the pH maintained by a bicarbonate buffer (Scott et al., 1998; Weeks et al., 2000).

H. pylori UreI knockout mutants failed to maintain a constant membrane potential in the presence of 5 mM urea with decreasing medium pH, in contrast to what was found in the wild-type organism (Scott et al., 1998). These data show that UreI is required for periplasmic buffering at acidic pH in the presence of urea, and therefore, for generation of an adequate *pmf* in acid. Measurement of membrane potential, however, is an indirect measure of periplasmic pH.

To determine the role of UreI in periplasmic buffering directly, the pH-sensitive fluorescent probe BCECF was used. BCECF free acid is inner membrane-impermeant, but with a molecular weight of ~600 Da it can readily enter the periplasm through the porous outer membrane of *H. pylori*. Periplasmic BCECF fluorescence in wild-type *H. pylori* incubated at a medium pH of 5.5 increased in the presence of 5 mM urea, indicating periplasmic alkalinization (Athmann et al., 2000). This increase in periplasmic fluorescence was abolished in *ureI*

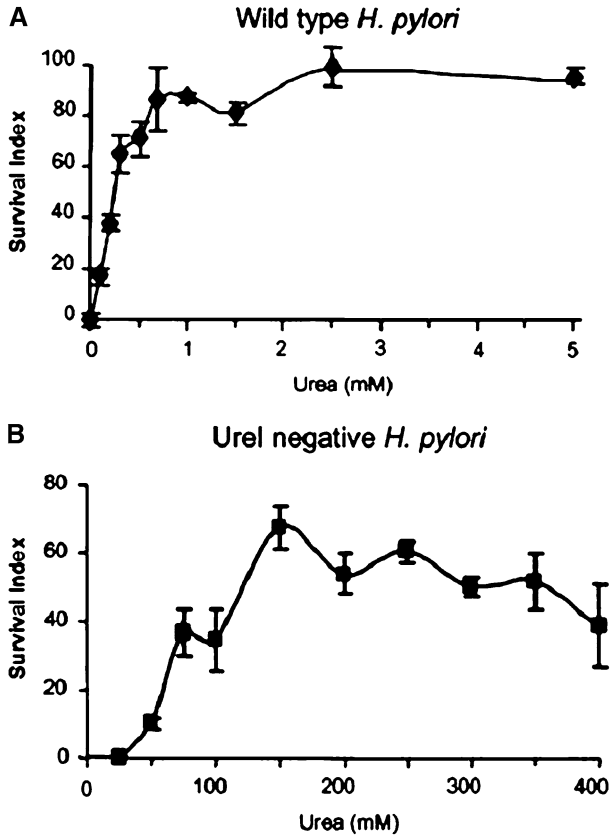

Fig. 1. The effect of deletion of UreI on acid survival in vitro at different urea concentrations. The upper graph shows that the wild type reached full survival at a urea concentration of $\sim$ 0.7 mM, whereas as shown in the lower graph, the deletion mutant showed no survival until about 100 mM and then about 60% survival at about 200 mM.

deletion mutants, showing UreI is crucial for periplasmic alkalinization.

Deletion of UreI abolished survival at pH 2.5 *in vitro* at gastric juice urea concentrations but this could be overcome by increasing urea concentration $\sim$ 300-fold, showing that UreI only catalyzed urea transport across the inner membrane (Fig. 1) (Weeks et al., 2000). Similarly, deletion of UreI prevented colonization of the mouse or gerbil stomach (Skoulibris et al., 1998; Mollenhauer-Rektorschek et al., 2002). In the case of the gerbil, inhibition of acid secretion allowed infection by the UreI deletion mutant, but infection disappeared when acid secretion was allowed to return (Mollenhauer-Rektorschek et al., 2002). UreI, like urease, is essential for gastric colonization.

Mechanism of Proton-gating of UreI

To further explore the mechanism of pH-gating of UreI, three homologues of UreI that showed different responses to changes in medium pH were investigated. Using site-directed mutagenesism, the effect on the pH dependence of urea uptake into oocytes, of the UreI of *H. pylori (Hp)* and *H. hepaticus (Hh)* (pH-gated, higher pH of activation) and that of *Streptococcus salivarius(Ss)* (pH-independent) were studied. Figure 2 illustrates the topography of the UreI membrane protein as determined by *in vitro* transcription/translation (Weeks et al., 2000, Weeks et al., 2004).

Protonation/deprotonation of specific amino-acid residues with a change in hydrogen bonding is likely the basis for activation/deactivation of urea transport by *H. pylori* and *H. hepaticus* UreI. Although there is a decrease in cytoplasmic pH of *H. pylori* as external pH falls, it is small and outside the range of protonation of any dicarboxylic amino acid in the presence of urea (Wen et al., 2003). Therefore, the residues important for UreI gating must be in the periplasmic domain. The first and second periplasmic loops of *H. pylori* each contain 7 potential protonatable residues. The periplasmically located C terminus also contains a histidine residue that is potentially involved in proton gating. The selectivity for urea as compared to thiourea and the large number of tryptophan residues in the last 3 transmembrane segments suggest that the channel is a fairly rigid structure and that only the gate for urea changes as a function of pH. The gate is presumably in the periplasmic domain and the amino acids involved were investigated by site-directed mutagenesis.

The effect of point mutations to the possible protonatable or charged residues in the periplasmic domains of *H. pylori* on urea uptake was measured using the *X. laevis* heterologus expression system (Weeks & Sachs, 2001). Alternatively, several histidine mutants were expressed in *H. pylori* and the rate of medium alkalinization determined at pH 2.2, 5.0 and 7.0 as an indirect measure of channel activity (Bury-Mone et al., 2001). Mutations that affect the pH profile of urea entry can be considered as affecting proton gating, whereas mutations that abolish urea transport may change the protein conformation and thus may not reveal a site of proton gating and interpretation of the effect of these mutations as affecting only proton gating is not possible.

There are three histidine residues in the first periplasmic loop of *Hp* UreI, H54, H70 and H71. H54 is not present in all *Helicobacter ureI* gene sequences and was not present in the N61 strain used for UreI expression in *H. pylori* (Bury-Mone et al., 2001). H54 is present in the strain used for the oocyte uptake studies and replacing it with either arginine or glycine resulted in wild-type channel activity, indicating that this histidine residue is not involved in channel gating to increase open probability (Weeks & Sachs, 2001). Single replacement of H70 and H71 with glutamine had no effect on channel gating, while replacement of both H70Q/H71Q increased urea

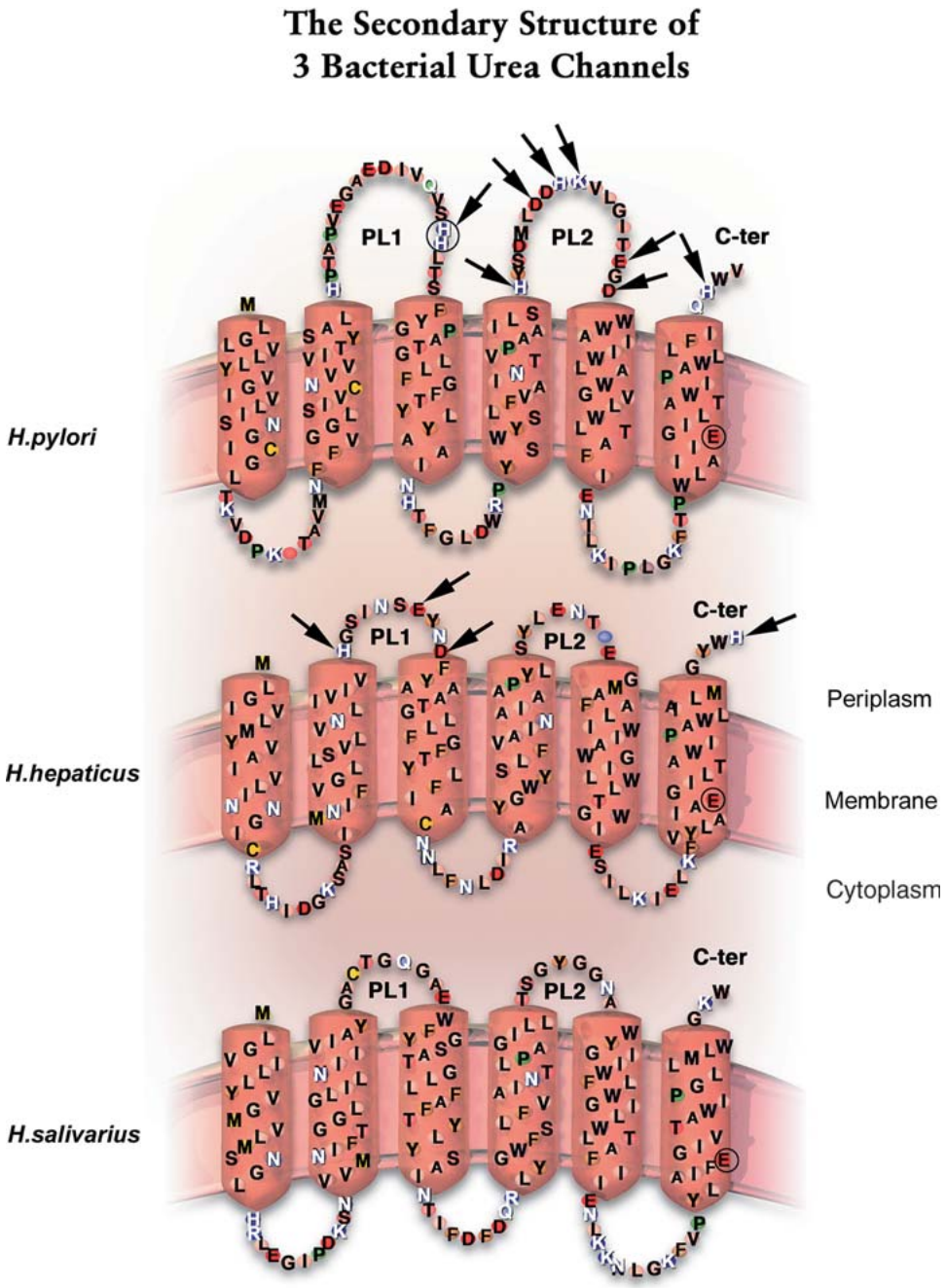

Fig. 2. The 2-D structure of the proton-gated UreIs from *H. pylori* and *H. hepaticus* and the acid- insensitive UreI of *S. salivarius*. The arrows indicate mutations that affected the pH profile of urea uptake into oocytes with maintenance of urea transport in the first pair of UreI proteins. The circle in the 6th transmembrane domain indicates the single charged intramembranal glutamate, which, when mutated to a neutral species, did not affect urea transport. Basic amino acids are in dark blue, acidic amino acids are in red, glutamine and asparagine in light blue, cysteine and methionine in yellow, hydrophobic amino acids in pale pink, tryptophan in gray and proline in green and hydroxy amino acids in pink.

uptake into oocytes at pH 7.5 by about 4-fold (5.33 ± 0.35 vs 1.40 ± 0.13) over wild type but had no effect on channel gating, suggesting these mutations have no effect on channel opening but may be involved in channel closure at neutral pH. Replacement of either of these histidines with glycine resulted in complete inactivation of the channel when expressed in oocytes. In contrast, the H71G mutation in intact *H. pylori* resulted in identical ammonia production and medium pH increase when compared to wild-type organisms (Bury-Mone et al., 2001). Mutations of the carboxylates in the first periplasmic loop, E60D, E60Q, E63Q and D64N, displayed wild-type channel activity. Thus, in the first periplasmic

loop, only the pair of histidines at positions 70 and 71 may play any role in pH regulation of *H. pylori* UreI activity (Fig. 2) and the nature of the mutation also has significant effects, perhaps due to a structural perturbation rather than an effect on acid gating. The structural perturbation may have different effects in the environment of the oocyte membrane as compared to the membrane of *H. pylori*, perhaps reconciling some of the conflicting results obtained with mutants in the two systems.

The second periplasmic loop and C-terminus contain three histidine residues (H123, H131, and H193) and 5 carboxylic amino acids (D126, D129, D130, E138, D140) and a charged lysine. Mutation of H123, H131 and H193 to arginine, asparagine, cysteine, glutamine or glycine strongly reduced or abolished urea transport at acidic pH without altering transport at neutral pH, indicating these residues are important in acid-gating of the channel. Again, using the indirect measure of channel opening, namely, ammonia production and pH elevation of the medium, the H123R and H131G mutations had properties identical to the wild-type organism, in contrast to the oocyte expression data (Bury-Mone et al., 2001). Mutations of the dicarboxylic amino acids of the second periplasmic loop indicated that these amino acids in the second periplasmic loop are also involved in channel opening. For example, the D126N, D126E and D129E transported urea at neutral pH just as in the wild-type channel, but at pH 5.0, transport was reduced by 30–50%. D130E and D130N displayed wild-type transport activity, while E138D, E138Q and D140E and D140N were inactive at acid pH, showing that E138 and D140 were involved in acid activation or urea transport (Fig. 2).

Another variant of UreI is found in *H. hepaticus* which, although acid-activated, has a higher pH at which half maximum transport is observed in oocytes, namely pH 6.8 (Weeks et al., 2004). There is strong homology in the membrane domain, significant homology in the cytoplasmic domain and none in the periplasmic loops with the sequence of UreI of *H. pylori* although these also contain protonatable residues.

All the protonatable amino acids of *H. hepaticus* could be mutated with retention of urea transport, allowing direct analysis of the role of these amino acids without the caveat of full inactivation of the channel even at neutral pH (Weeks et al., 2004). Whereas mainly mutations of the second, not the first, periplasmic loop of *H. pylori* affected the pH dependence of urea uptake, mutations mainly in the first periplasmic loop of *H. hepaticus* were those that affected the pH dependence of this UreI. These residues were H50, E56, and D59 in the first periplasmic loop. As with *Hp* mutation in the C terminus, H170, also affected pH dependence of urea uptake in oocytes (Fig. 2).

Streptococcus salivarius expresses a homologue of UreI whose expression along with the rest of the urease gene cluster is upregulated when the organism encounters an acidic environment (Chen et al., 1998; Chen & Burne, 2003). *Ss* UreI has about 82% similarity to the membrane domain of Hp UreI but none to the predicted periplasmic domains. Oocyte expression studies of *Ss* UreI also showed that it is a urea channel, i.e. highly selective for urea, temperature-independent, and non-saturable (Weeks & Sachs, 2001). Expression of *Ss* UreI in oocytes accelerated urea uptake equally at pH 5.0 and pH 7.5, indicating this urea channel was always open and did not show pH gating. Since *Ss* UreI is open independent of medium pH, it was possible to exploit this property to determine the periplasmic domains important in pH gating of *Hp* or *Hh* UreI by constructing chimeric UreI proteins by interchanging periplasmic loops between the two channels. The conserved glutamate in the 6^{th} transmembrane domain of these three channels could be mutated to a neutral amino acid without effect on the pH dependence of urea uptake.

Replacement of the first periplasmic loop of *Hp* UreI with that from *Ss* UreI reduced urea uptake at pH 5.0 to 80% of wild-type Hp UreI activity while increasing uptake at pH 7.5 by 3- to 4-fold, results similar to the H70Q/H71Q double mutation. These mutational and the chimera data suggest that the first periplasmic loop of *Hp* is not involved in channel opening or urea transport in this variant of UreI, but may contribute to maintaining the channel in the closed state at neutral pH. A chimera between the second loop of *H. pylori* and the rest of *S. salivarius* was inactive.

However, replacement of the complete periplasmic domain of *S. salivarius* with that of *H. hepaticus* resulted in pH-independent urea uptake, hence the structure of the membrane domain of *S. salivarius* is dominant and unresponsive to the pH-dependent conformational changes in the heterologous periplasmic domain of *H. hepaticus* (Weeks et al., 2004).

Presumably the mechanism of proton gating, given the pH insensitivity of any chimeras containing the membrane domain of *S. salivarius* and the periplasmic domain of *H. hepaticus*, is a conformational change in the membrane domain of *H. pylori* or *hepaticus* induced by a change in the state of protonation of the histidines or carboxylates in the periplasmic domain that alter the conformation of the membrane domain in the acid-gated UreI members. This change then allows urea passage through the channel. A 2-D representation of the UreI homologues is shown in Fig. 2. A correct description of the mechanism of proton gating of urea transit will require a high resolution crystal structure.

The only homologous genes to *ureI* found in bacteria are the *amiS* genes of *Pseudomonas aerogenes*, *Mycobacterium smegmatis* and *Rhodococcus spp*, where there is homology in the transmembrane regions but none in the periplasmic sector (Wilson et al., 1995, Chebrou et al., 1996). The encoded proteins are postulated to catalyze amide transport but the nature of the substrate is unknown.

Other Properties of UreI

Most of the urease expressed by *H. pylori* at neutral pH is inactive. In addition to the structural genes, *ureA* and *ureB* that encode the subunits of the apoprotein, the urease gene cluster of *H. pylori* contains besides *ureI* accessory genes that are required for synthesis of catalytically active urease by nickel insertion into the apoprotein. These genes, *ureE-H*, are homologous to the urease accessory genes of *Klebsiella aerogenes*, which have been extensively studied and are required for the assembly of the nickel metallocenter within the active center of the enzyme (Brayman & Hausinger, 1996; Colpas et al., 1999; Moncrief & Hausinger, 1996, 1997; Mulrooney & Hausinger, 1990). It seems likely that the high level of expression of the urease apo-enzyme is present to facilitate acid acclimation when acidity is encountered.

Yeast two-hybrid analysis was used to determine the interactions among the accessory proteins. It was found that UreF interacted strongly with UreH, and UreG interacted strongly with UreE (Voland et al., 2003). These insertion proteins therefore function in a paired fashion. Since the yeast two-hybrid system cannot report the interaction of membrane proteins, blue native PAGE western blot analysis was first used to determine interactions of urease with UreI. It was found that, indeed, UreA and UreB were bound to the membrane protein, UreI (Voland et al., 2003).

Immuno-electron microscopy localized UreI to the inner membrane of *H. pylori* at both acidic and neutral pH, as also shown biochemically (Weeks et al., 2000; Hong et al., 2003). The location of UreI was independent of the presence of the accessory proteins involved in conversion of the apoenzyme to active urease. Urease was present predominantly throughout the cytoplasm at neutral pH, but at acidic pH urease was found at the membrane in association with UreI (Hong et al., 2003). Additionally, at neutral pH about 75% of the cytoplasmic urease was present as inactive apoenzyme, lacking the Ni^{2+} required for activity of this metalloenzyme (Scott et al., 2002). Upon exposure to acid, urease activity increased in a time-dependent manner until full activity was reached after ninety minutes. The increase in activity was abolished in the absence of Ni^{2+} and also in *H. pylori* UreI knockout mutants, but not in the presence of the protein synthesis inhibitor, chloramphenicol or urea. These results suggest the relocation at acidic medium pH of urease from a diffuse cytoplasmic localization to the inner membrane in close contact with UreI may result in nickel incorporation into the apoenzyme, thus increasing urease activity and hence, improved acid acclimation to the gastric environment. The large stoichiometric ratio between urease apoenzyme and UreI implies that after activation at the membrane surface, the active urease is removed from UreI and moves either to another membrane location or back to the cytoplasm, but now in active form.

Urease negative strains of *H. pylori* are unable to colonize the stomach, as are UreI deletion mutants (Eaton & Krakowka, 1994; Tsuda et al., 1994; Andrutis et al. 1995). Thus, as indicated previously, proteins encoded by this gene cluster are integral to the acid acclimation process in this gastric denizen.

Other Acid Acclimation Genes

Analysis of the transcriptome of *H. pylori* in progressively acidic media down to pH 4.5 in the absence and presence of urea revealed about 200 genes whose expression was increased in acid (Wen et al., 2003). These genes responded differentially to the presence of urea in the medium. One cluster of genes was no longer upregulated at a medium pH of 6.2 in the presence of urea. These findings can be interpreted as showing that the urease system is able to normalize periplasmic pH at this relatively moderate acidity and this cluster is regulated by a system responsive to periplasmic pH. At pH 5.5 and 4.5 additional genes were upregulated. These genes can be regarded as responding not only to changes in periplasmic pH but also to changes in cytoplasmic pH, which was shown to fall under these more acidic conditions and to be restored to different extents by the presence of urea, depending on medium pH.

Among these genes, there are, particularly in the cluster responding uniquely to a decrease in periplasmic pH when the medium pH is lowered to pH 6.2, genes that could be considered as potentially pH homeostatic, indicating that *H. pylori* has a rather large repertoire of genes able to contribute to acid acclimation. These putative pH homeostatic genes consisted of arginase (able to produce urea), asparaginase (able to produce NH_3), aliphatic amidase (able to generate ammonia, but the natural substrate is unknown), and both a cytoplasmic and periplasmic carbonic anhydrase that could generate bicarbonate as a buffer (Wen et al., 2003). These findings led to an investigation of the role of the carbonic anhydrases in acid acclimation.

Role of Carbonic Anhydrase in Acid Acclimation

Deletion or inhibition of the periplasmic carbonic anhydrase drastically reduced acid survival of the

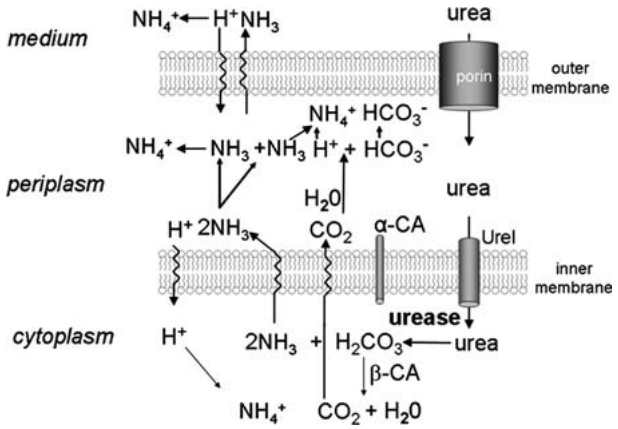

Fig. 3. A model of the role of UreI, intrabacterial urease and cytoplasmic and periplasmic carbonic anhydrase in enabling acid acclimation by *H. pylori* that is described above. In the presence of acid (pH < 6.5), UreI opens by protonation of amino acids in the second periplasmic loop. Opening of the urea channel permits urea entrance into the cytoplasm where it is hydrolyzed by active urease located largely at the membrane in association with UreI. One of the products of intrabacterial urea hydrolysis, H_2CO_3, is rapidly converted to CO_2 and H_2O by cytoplasmic carbonic anhydrase and now the $2NH_3$ and the CO_2, being gases, rapidly move out through the inner membrane into the periplasm, whereupon NH_3 becomes protonated and CO_2 is converted to bicarbonate and a proton by the membrane-anchored α-carbonic anhydrase. NH_3 consumes entering protons and the proton produced by the action of carbonic anhydrase and the HCO_3^- buffers the periplasmic pH to about 6.1.

organism even in the presence of urea, and deletion of both anhydrases prevented infection of mice (Marcus et al. 2005; Lee et al., 2003). Thus the products of urea, ammonia and CO_2 are both implicated in acid acclimation, as illustrated in the model of Fig. 3. These components interact in a coordinated manner to maintain periplasmic and cytoplasmic pH near neutrality to preserve an adequate *pmf* across the inner membrane, allowing not only survival but growth in the acidic niche occupied by *H. pylori* in the human stomach. In the presence of acid (pH < 6.5) UreI opens by protonation of amino acids in the second periplasmic loop. Opening of the urea channel permits urea entrance into the cytoplasm where it is hydrolyzed by active urease located largely at the membrane in association with UreI. One of the products of intrabacterial urea hydrolysis, H_2CO_3, is rapidly converted to CO_2 and H_2O by cytoplasmic carbonic anhydrase and now the 2 NH_3 and the CO_2, being gases, rapidly move out through the inner membrane into the periplasm whereupon NH_3 becomes protonated and CO_2 is converted to bicarbonate and a proton by the membrane-anchored α-carbonic anhydrase. Hence, NH_3 consumes entering protons and the proton produced by the action of carbonic anhydrase and the HCO_3^- buffers the periplasmic pH to about 6.1, as had been deduced from measurements of inner membrane potential under conditions of fixed medium acidity (Scott et al., 1998) between pH 3.0 and 6.2 in the presence of urea (Fig. 3).

Two-Component Histidine Kinase HP0165 0166

A second pair of genes that appeared to be of interest in this cluster was the genes encoding for the two-component histidine kinase signaling system, the inner membrane-bound sensor, HP0165, and its known response element, HP0166 (Wen et al., 2003). Various genes contain promoters that bind to HP0166, some requiring that HP0166 is phosphorylated, others not (Wen et al., 2006; Schar, Sickman & Beier, 2005). This pair also forms part of the acid acclimation response and the genes that are regulated indicate the possible complexity of the acid acclimation repertoire of genes of this gastric pathogen and potential targets for eradication, as are UreI and the carbonic anhydrases.

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References

Andrutis, K.A., Fox, J.G., Schauer, D.B., Marini, R.P., Murphy, J.C., Yan, L., Solnik, J. 1995. Inability of an isogenic urease-negative mutant stain of *Helicobacter mustelae* to colonize the ferret stomach. *Infect. Immun.* **63:**3722–3725

Athmann, C., Zeng, N., Kang, T., Marcus, E.A., Scott, D.R., Rektorschek, M., Buhmann, A., Melchers, K., Sachs, G. 2000. Local pH elevation mediated by the intrabacterial urease of *Helicobacter pylori* cocultured with gastric cells. *J. Clin. Invest.* **106:**339–347

Beckers, G., Bendt, A.K., Kramer, R., Burkovski, A. 2004. Molecular identification of the urea uptake system and transcriptional analysis of urea transporter- and urease-encoding genes in *Corynebacterium glutamicum*. *J. Bacteriol.* **186:**7645–7652

Brayman, T.G., Hausinger, R.P. 1996. Purification, characterization, and functional analysis of a truncated *Klebsiella aerogenes* UreE urease accessory protein lacking the histidine-rich carboxyl terminus. *J. Bacteriol.* **178:**5410–5416

Bury-Mone, S., Skouloubris, S., Labigne, A., De Reuse, H. 2001. The *Helicobacter pylori* UreI protein: role in adaptation to acidity and identification of residues essential for its activity and for acid activation. *Mol Microbiol.* **42:**1021–1034

Chebrou, H., Bigey, F., Arnaud, A., Galzy, P. 1996. Amide metabolism: a putative ABC transporter in *Rhodococcus sp.* R312. *Gene.* **182:**215–218

Chen, Y.Y., Weaver, C.A., Mendelsohn, D.R., Burne, R.A. 1998. Transcriptional regulation of the *Streptococcus salivarius* 57.I urease operon. *J. Bacteriol.* **180:**5769–5775

Chen, Y.Y., Burne, R.A. 2003. Identification and characterization of the nickel uptake system for urease biogenesis in *Streptococcus salivarius* 57.I. *J. Bacteriol.* **185:**6773–6779

Colpas, G.J., Brayman, T.G., Ming, L.J., Hausinger, R.P. 1999. Identification of metal-binding residues in the *Klebsiella aerogenes* urease nickel metallochaperone, UreE. *Biochemistry.* **38:**4078–4088

de Koning-Ward, T.F., Robins-Browne, R.M. 1997. A novel mechanism of urease regulation in *Yersinia enterocolitica*. *FEMS Microbiol. Lett.* **147:**221–226

Eaton, K.A., Krakowka, S. 1994. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. *Infect. Immun.* **62:**3604–3607

Flores, E., Herrero, A. 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. *Aliment. Pharmacol. Ther.* **16:**533–544

Foster, J.W. 2004. *Escherichia coli* acid resistance: tales of an amateur acidophile. *Nat. Rev. Microbiol.* **2:**898–907

Goodman, B. 2002. Transport of small molecules across cell membranes water channels and urea transporters. *Adv. Physiol. Educ.* **26:**146–157

Hediger, M.A., Smith, C.P., You, G., Lee, W.S., Kanai, Y., Shayakul, C. 1996. Structure, regulation and physiological roles of urea transporters. *Kidney Intern.* **49:**1615–1623

Hong, W., Sano, K., Morimatsu, S., Scott, D.R., Weeks, D.L., Sachs, G., Goto, T., Mohan, S., Harada, F., Nakajima, N., Nakano, T. 2003. Medium pH-dependent redistribution of the urease of *Helicobacter pylori*. *J. Med. Microbiol.* **52:**211–216

Jahns, T., Zobel, A., Kleiner, D., Kaltwasser, H. 1988. Evidence for carrier-mediated, energy-dependent uptake of urea in some bacteria. *Arch. Microbiol.* **149:**377–383

Lee, Y.-C., Takata, T., Shan, H.Y., Grandjean, B., Charney, A.N., Ando, T., Perez-Perez, G.I., Blaser, M. 2002. The carbonic anhydrase genes of *Helicobacter pylori*: characteristics and biological roles. *Gastroenterol.* **122:**A423

Labigne, A., Cussac, V., Courcoux, P. 1991. Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. *J. Bacteriol.* **173:**1920–1931

Magaña-Plaza, I., Ruiz-Herrera, J. 1967. Mechanisms of regulation of urease biosynthesis in *Proteus rettgeri*. *J. Bacteriol.* **93:**1294–1301

Marcus, E.A., Moshfegh, A.P., Sachs, G., Scott, D.R. 2005. The periplasmic alpha-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. *J. Bacteriol.* **187:**729–738

Mathai, J.C. 2005. Ammonotelic teleosts and urea transporters. *Am. J. Physiol.* **288:**F453–454

Mayrand, R.R., Levitt, D.G. 1983. Urea and ethylene Glycol facilitated transport systems in the human red cell membrane: saturation, competition, and asymmetry. *J. Gen. Physiol.* **81:**221–237

Meyer-Rosberg, K., Scott, D.R., Rex, D., Melchers, K., Sachs, G. 1996. The effect of environmental pH on the proton motive force of *Helicobacter pylori*. *Gastroenterology*. **111:**886–900

Mills, J., Wyborn, N.R., Greenwood, J.A., Williams, S.G., Jones, C.W. 1998. Characterisation of a binding-protein-dependent, active transport system for short-chain amides and urea in the methylotrophic bacterium *Methylophilus methylotrophus*. *Eur. J. Biochem.* **251:**45–53

Mobley, H.L., Island, M.D., Hausinger, R.P. 1995. Molecular biology of microbial ureases. *Microbiol. Rev.* **59:**451–480

Mollenhauer-Rektorschek, M., Hanauer, G., Sachs, G., Melchers, K. 2002. Expression of UreI is required for intragastric transit and colonization of gerbil gastric mucosa by *Helicobacter pylori*. *Res. Microbiol.* **153:**659–666

Moncrief, M.B., Hausinger, R.P. 1997. Characterization of UreG, identification of a UreD-UreF-UreG complex, and evidence suggesting that a nucleotide-binding site in UreG is required for in vivo metallocenter assembly of *Klebsiella aerogenes* urease. *J. Bacteriol.* **179:**4081–4086

Moncrief, M.B., Hausinger, R.P. 1996. Purification and activation properties of UreD-UreF-urease apoprotein complexes. *J. Bacteriol* **178:**5417–5421

Mulrooney, S.B., Hausinger, R.P. 1990. Sequence of the *Klebsiella aerogenes* urease genes and evidence for accessory proteins facilitating nickel incorporation. *J. Bacteriol.* **172:**5837–5843

Nielsen, S., Froklaer, J., Marples, D., Kwon, T.H., Agre, P., Knepper, M.A. 2002. Aquaporins in the kidney: from molecules to medicine. *Physiol. Rev.* **82:**205–244

Olives, B., Neau, P., Bailly, P., Hediger, M.A., Rousselet, G., Cartron, J.P., Ripoche, P. 1994. Cloning and functional expression of a urea transporter from human bone marrow cells. *J. Biol. Chem.* **269:**31649–31652

Rektorschek, M., Weeks, D., Sachs, G., Melchers, K 1998. Influence of pH on metabolism and urease activity of *Helicobacter pylori*. *Gastroenterology*. **115:**628–641

Sands, J.M. 1999. Urea transport: It's not just "freely diffusible" anymore. *TIPS* **14:**46–47

Sands, J.M. 2003. Mammalian urea transporters. *Annu. Rev. Physiol.* **65:**543–566

Sands, J.M. 2004. Renal Urea transporters. *Curr. Opinion. Nephrol. Hypert.* **13:**525–532

Sands, J.M., Nonoguchi, H., Knepper, M.A. 1987. Vasopressin effects on urea and H_2O transport in inner medullary collecting duct subsegments. *Am. J. Physiol.* **253:**F823–F832

Schar, J., Sickmann, A., Beier, D. 2005. Phosphorylation-independent activity of atypical response regulators of *Helicobacter pylori*. *J. Bacteriol.* **187:**3100–3109

Scott, D.R., Marcus, E.A., Weeks, D.L., Sachs, G. 2002. Mechanisms of acid resistance due to the urease system of *Helicobacter pylori*. *Gastroenterology*. **123:**187–195

Scott, D.R., Marcus, E.A., Weeks, D.L., Lee, A., Melchers, K., Sachs, G. 2000. Expression of the *Helicobacter pylori* ureI gene is required for acidic pH activation of cytoplasmic urease. *Infect. Immun.* **68:**470–477

Scott, D.R., Weeks, D., Hong, C., Postius, S., Melchers, K., Sachs, G. 1998. The role of internal urease in acid resistance of *Helicobacter pylori*. *Gastroenterology*. **114:**58–70

Sebbane, F., Bury-Mone, S., Cailliau, K., Browaeys-Poly, E., De Reuse, H., Simonet, M. 2002. The *Yersinia pseudotuberculosis* Yut protein, a new type of urea transporter homologous to eukaryotic channels and functionally interchangeable in vitro with the *Helicobacter pylori* UreI protein. *Mol. Microbiol.* **45:**1165–1174

Shayakul, C., Hediger, M.A. 2004. The SLC14 gene family of urea transporters. *Pluegers Arch.* **447:**603–609

Siewe, R.M., Weil, B., Burkovski, A., Eggeling, L., Kramer, R., Jahns, T. 1998. Urea uptake and urease activity in *Corynebacterium glutamicum*. *Arch. Microbiol.* **169:**411–416

Skouloubris, S., Thiberge, J.M., Labigne, A., De Reuse, H. 1998. The *Helicobacter pylori* UreI protein is not involved in urease activity but is essential for bacterial survival in vivo. *Infect. Immun.* **66:**4517–4521

Tien, H.T. 1974. Bilayer lipid membranes. Dekker, New York,

Tsuda, M., Karita, M., Morshed, M.G., Okita, K., Nakazawa, T. 1994. A urease-negative mutant of *Helicobacter pylori* constructed by allelic exchange mutagenesis lacks the ability to colonize the nude mouse stomach. *Infect. Immun.* **62:**3586–3589

Valladares, A., Montesinos, M.L., Herrero, A., Flores, E. 2002. An ABC-type, high-affinity urea permease identified in cyanobacteria. *Mol. Microbiol.* **43:**703–715

Vanholder, R., Glorieux, G., Lameire, G. 2005. New Insights in Uremic Toxicity. *Contribut. Nephrol.* **149:**315–324

Voland, P., Weeks, D.L., Marcus, E.A., Prinz, C., Sachs, G., Scott, D. 2003. Interactions among the seven *Helicobacter pylori* proteins encoded by the urease gene cluster. *Am. J. Physiol.* **284:**G96–G106

Weeks, D.L. 2001. Sites of pH regulation of the urea channel of *Helicobacter pylori*. *Mol. Microbiol.* **40:**1249–1259

Weeks, D.L., Eskandari, S., Scott, D.R., Sachs, G. 2000. A H^+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. *Science* **287:**482–485

Weeks, D.L., Gushansky, G., Scott, D.R., Sachs, G. 2004. Mechanism of proton gating of a urea channel. *J. Biol. Chem.* **279:**9944–9950

Wen, Y., Marcus, E.A., Matrubutham, U., Gleeson, M.A., Scott, D.R., Sachs, G. 2003. Acid-adaptive genes of *Helicobacter pylori*. *Infect. Immun.* **71:**5921–5939

Wen, Y., Feng, J., Scott, D.R., Marcus, E.A., Sachs, G. 2006. Involvement of the HP0165-HP0166 two-component system in expression of some acidic-pH-upregulated genes of *Helicobacter pylori*. *J. Bacteriol.* **188:**1750–1761

Wilson, S.A., Williams, R.J., Pearl, L.H., Drew, R.E. 1995. Identification of two new genes in the *Pseudomonas aeruginosa* amidase operon, encoding an ATPase (AmiB) and a putative integral membrane protein (AmiS). *J. Biol. Chem.* **270:**18818–18824

Williams, C.L., Preston, T., Hossack, M., Slater, C., McColl, K.E. 1996. *Helicobacter pylori* utilises urea for amino acid synthesis. *FEMS Immunol. Med. Microbiol.* **13:**87–94

Young, G.M., Amid, D., Miller, V.L. 1996. A bifunctional urease enhances survival of pathogenic *Yersinia enterocolitica* and *Morganella morganii* at low pH. *J. Bacteriol.* **78:**6487–6495