Acid Acclimation by Helicobacter pylori

Helicobacter pylori is a Gram-negative neutralophile associated with peptic ulcers and gastric cancer. It has a unique ability to colonize the human stomach by acid acclimation. It uses the pH-gated urea channel, UreI, to enhance urea access to intrabacterial urease and a membrane-anchored periplasmic carbonic anhydrase to regulate periplasmic pH to ~6.1 in acidic media, whereas other neutralophiles cannot regulate periplasmic pH and thus only transit the stomach.

Warren and Marshall (30) first reported the key role of gastric infection by Helicobacter pylori in the development of peptic ulcer disease. Subsequently, in 1991 Parsonnet et al. (46) showed that this organism could also be a factor in the pathogenesis of gastric cancer. This hypothesis has been supported by another study (47). Indeed, persistent infection with H. pylori always produces gastritis and increases the risk of developing gastric cancer ~20-fold (54). Infection with H. pylori has also been suggested as a possible factor in the pathogenesis of other disorders, such as coronary occlusion, wherein stimulation of an immune response to the organism could also be a factor in the pathogenesis of coronary artery disease (19).

Many pathogenic neutralophiles can survive in acidic environments by maintaining cytoplasmic pH at ~5.0. However, although they can survive in an acidic environment, they do not grow. Hence, they can transit but not colonize the stomach. Examples of such organisms are Salmonella typhimurium, Vibrio cholera, and pathogenic strains of Escherichia coli and Yersinia enterocolitica (16, 30).

Although H. pylori is also a neutralophile, it is able not only to survive in but also to colonize (i.e., grow within) the human stomach (27, 30). Other Helicobacter spp. have evolved specialized processes that allow maintenance of the pH of their cytoplasm at a level that enables the organisms to survive and grow. We have termed these properties of gastric Helicobacter spp. “acid acclimation” to differentiate them from the acid-resistance (acute) or acid-tolerance (chronic) mechanisms of neutralophiles in general.

Acid Resistance/Tolerance in Neutralophiles

The energy source in aerobic bacteria is derived from synthesis of ATP driven by proton movement down an electrochemical gradient for protons (occasionally sodium). The inward-driving force for protons forms the basis of the chemiosmotic theory of bioenergetics (38). ATP synthesis depends on the flux of protons across the F$_1$F$_0$ ATP synthase energized by the pH and membrane-potential gradient. The electrogenic redox reactions are generated across the inner membrane in Gram-negative organisms or across the surface membrane in the case of Gram-positive bacteria and result in the formation of both a proton and an electrical gradient. The combination of these is termed the proton-motive force (pmf).

The pmf is calculated from the equation defining the electrochemical gradient for protons as

$$\text{pmf (mV)} = \Delta \mu_p = -RT/nF \ln[H^+] = \Delta \mu = -zF \Delta pH + \Delta \phi$$

where $\Delta \mu_p$ is the electrochemical gradient for protons, $R$ is the gas constant, $T$ is the temperature in degrees Kelvin, $F$ is the Faraday constant, $n$ is the valence of the ion, and $\Delta \phi$ is the transmembrane potential. The pmf equation predicts that there will be a reciprocal relationship between the pH gradient and the potential difference across the relevant bacterial membrane.

In the case of neutralophiles, the pmf is maintained across their cytoplasmic membrane in a range between ~160 and ~280 mV at neutral external pH. Generally, Gram-negative neutralophiles do not regulate their periplasmic pH in acidic media. Hence, for the neutralophiles that survive but do not grow in an acid environment, exposure to an acid pH causes their periplasmic pH to fall, increasing the inward proton gradient and decreasing the membrane potential or even causing development of a positive potential that could hinder proton influx. These organisms have pH-regulatory systems preventing cytoplasmic pH from falling below ~4.5 or ~5.0, a level sufficient for survival but not for replication. Not only do these neutralophiles have to tolerate a continuous acid load from their acidic environment during gastric transit, they also are exposed to several peremptory organic acids present in the intestine. Entry of the unprotonated acid is followed by acidification of...
the cytoplasm due to dissociation of the acid. The mechanisms by which individual organisms protect themselves against acid load are variable: some are common to all organisms, whereas others are unique (7).

**The F1F0 ATP synthase/ATPase**

The F1F0 ATPase may function as an outward proton transporter when the ΔpH falls below a threshold level, allowing reorientation of the γ-subunit toward F1 and away from the β-subunit toward F0 (55). This rearrangement may enable F1 to hydrolyze ATP and export protons.

**Na+/H+ exchange**

In prokaryotes, the Na+/H+ exchanger (NHE) is electronegative, rather than electrophoretically as found in eukaryotes. In *E. coli*, in the face of high external pH and sodium concentrations, the exchanger functions primarily to regulate intracellular Na+ concentration or to resist alkalinization from the medium. The stoichiometry of this transporter is 2H+/Na+, thus providing a driving force for Na+ extrusion and H+ uptake aided by an inward negative membrane potential. Although it is not certain that all bacterial NHEs have this stoichiometry, if the NHE transporters function like that of *E. coli*, they can only decrease cytoplasmic pH, because the exchanger is structured to import, rather than export, protons.

**Amino acid decarboxylases**

Amino acid decarboxylases appear to be the main regulatory components for cytoplasmic pH homeostasis in neutralophiles exposed to acid and are the best studied. The equation for amino acid decarboxylation, using glutamic acid as an example at pH\textsubscript{c,i} > 3.0, is

\[
\text{CH}_2\text{CH}_2\text{COOH} + \text{H}^+ \rightarrow \text{NH}_3 + \text{CO}_2
\]

Hence, a proton is consumed along with the production of CO\textsubscript{2} and γ-amino butyric acid.

The uptake of protons during decarboxylation of the amino acid is a means of elevating intracellular pH. This mechanism must be accompanied by an exchanger in the plasma membrane, exporting the product in exchange for amino acid entry. Without such an exchange, pH regulation would fail. The general scheme for these intracellular pH-regulatory amino acid decarboxylases is shown in Figure 1.

As these organisms adapt to their acidic environment, the various genes encoding the decarboxylases and the antiporters are upregulated. In *E. coli*, gadA and gadB, encoding glutamic acid decarboxylases, and gadC, which encodes the glutamate–γ-aminobutyrate antiporter (AR2), are induced by acid exposure (8). Arginine decarboxylase (adcA) and an arginine/γ-aminoguanine antiporter (AR2) also increase expression in acid (21). The internal pH generated is similar to the pH optima of these two enzymes: 4.0 for the glutamate and 5.0 for arginine decarboxylases. In *Vibrio cholerae*, gadC (lysin decarboxylase) is induced. cadC is regulated by CadC, a member of TCS transcriptional regulators that span the inner membrane with a periplasmic sensing domain and a cytoplasmic DNA-binding domain (35). ToxR appears to be required for organic acid tolerance and is linked to regulation of outer membrane proteins. Signature-tagged mutagenesis has revealed at least nine other colonization factors in this organism. Since the pH optimum of lysine decarboxylase is 5.0, this is the limit of pH regulation by this decarboxylase. Similarly in the enterohemorrhagic pathogenic *E. coli* strains, 26 genes were identified as induced when exposed to acetic acid; many are regulated by the stationary phase sigma factor, σ\text{v} (also called RpoS), including OmpC, an outer membrane protein that regulates transport across the outer membrane. The Gram-negative *Listeria monocytogenes* uses both F1F0 ATPase and similar gadA, gadB, and gadC glutamic acid decarboxylases and antiporters, respectively. Also, a two-component system encoded by *lisRK* was also identified in this organism as important for acid resistance (10).

There is also an AR1 acid-resistance system in *E. coli* that is activated by stationary-phase pH 5.5 preconditioning and involves Rpos and a cAMP receptor protein (26). This pathway is glucose repression. The genes involved in cytoplasmic pH control are not known. For these organisms to survive an acid environment, the glutamate or arginine systems are essential. For AR1 it is known that the F1F0 ATPase is essential, but whether this is because of its role as proton exporter or in synthesis of ATP is unclear.

In summary, the acid-tolerant or acid-resistant response in these neutralophiles is composed of at least two components: plasma direct decarboxylation of decarboxylases. The capacity of the outer and inner membrane system to maintain these systems is maintained, and acid adaptation. Some of these are acid-specific while others are common to all organisms, whereas others are unique, enabling adaptation to highly acidic environments.

**Acid Acclimation**

*H. pylori* is an obligate intracellular parasite of the stomach cells of other neutralophiles and esophageal cancer patients. Acid acclimation pathways in these organisms are unique, enabling acid adaptation. Some of these systems have been used to maintain cytoplasmic pH compatible with the cytoplasmic pH needed for acid adaptation, and others have been explored in secretions known as *H. pylori*.
cytoplasmic or inner membrane proteins of neutralophiles. However, the acid-acclimation pathway developed by this organism are at least two components: those affecting the cytoplasm directly, such as the ATPase and amino acid decarboxylases, and those affecting the permeability of the outer membrane, as in the case of Gram-negative neutralophiles such as ToxR. It should be emphasized here that although the cytoplasmic pH is maintained within viable limits, most bacterial processes cease to function at pH < 5.0; hence these are acid-resistant or acid-tolerant, not acid-acclimation responses. H. pylori does not express any of the amino acid decarboxylases or the antiporers, so its response to acidic media is clearly different from these other neutralophiles.

Urease

Y. enterocolitica expresses an intrabacterial urease with a pH optimum of 5.0 and a sharp pH activity curve. It is activated with the production of NH₃ and CO₂ when the cytoplasmic pH falls to 5.0 (83). At this pH, sufficient buffer is generated to allow the organism to survive with a cytoplasmic pH of 5.0 but not to grow. In contrast, the urease of H. pylori has a pH optimum between 7.5 and 8.0 and is virtually inactive at pH 5.0.

Acid Acclimation in H. pylori

H. pylori is Gram-negative neutralophile with outer and inner membranes, and it shares some of the pH-cytoplasmic, pH-regulatory systems with other neutralophiles. However, the acid-acclimation pathways developed by this organism are unique, enabling H. pylori to maintain its periplasmic pH in acidic medium at levels at which both the cytoplasmic pH and membrane potential are compatible with growth (29). The several systems used to maintain periplasmic pH form the basis for acid acclimation and growth in the gastric environment. Several such processes have been explored in some detail, but much remains to be learned.

The urease system

H. pylori expresses a neutral pH optimum urease at higher levels than any other known bacteria (39), and the generation of NH₃ and CO₂ provides both acid-neutralizing and acid-buffering capacity. The urease gene cluster of this organism consists of seven genes, an upstream promoter sequence, then ureA and ureB, the structural subunits of the enzyme, followed by a second promoter region, and then five other genes: ureC, ureD, ureG, and ureI (1, 11). The structural subunits and the last four genes show considerable homology to other urease cluster genes such as those in Klebsiella aerogenes (39). UreA and UreB form a complex that requires Ni²⁺ insertion for activity. Ni²⁺ insertion is mediated by a pair of complexes (UreE/UreG and UreF/UreH) that have been defined both biochemically in Klebsiella (41, 42) and by yeast two-hybridization methods in H. pylori (57). The UreA/UreB complex constitutes as much as 15% of the protein of H. pylori. The system encodes an organism as a whole: urease-negative mutants are unable to colonize animal models, showing that this urease is essential for gastric habitation (15). Hence urease activity is vital for gastric colonization by H. pylori, and regulation of urease occurs at several levels: biosynthesis of the structural genes, insertion of Ni²⁺ into the apoenzyme, and regulation of urease access to the urease itself.

Regulation of H. pylori content of active urease

There is evidence for upregulation of ureC and ureD expression under acidic conditions, perhaps due to activation of a two-component histidine kinase, HP0165/HP0166 (1, 48). The urease promoter is modular, contains a pH-responsive region responsible for basal transcription, and contains upstream sequences responsible for further regulation of transcription of these two subunit genes (12).
UreA and UreB are present largely as apoenzymes; therefore, regulation of Ni²⁺ concentrations inside the organism plays a vital role in acid survival. NixA is a specific Ni²⁺ transporter with eight transmembrane segments that exists in the inner membrane of *H. pylori* and functions as a monomer (20). Deletion of this gene reduces but does not prevent infection of the mouse stomach (43), but many other genes may also be implicated not only in uptake but also in storage of this essential cation. *H. pylori* also expresses nifK, which functions as a general repressor of several genes, including *ureA* and *ureB*, in the presence of high Ni²⁺ concentrations (9). 

PuA, a gene involved in flagellar expression, was identified as a gene reducing urease activity in *E. coli* expressing the *H. pylori* urease locus and nixA gene (33). HP0548, a gene that may encode a DNA helicase in the cag pathogenicity island, and HP0551, a gene that potentially encodes a lipoprotein, enhance urease expression in this foreign host (32). Their role in *H. pylori* itself has not been examined.

*hyphA* and *hyphB* genes are involved in assembly of the hydrogenase complex, perhaps by assembly of Ni²⁺ into hydrogenase. It was found that deletion of these genes helps to form hydrogenase activity but sharply reduced urease activity as well, by ~200-fold. This is attributed to a role for these genes in Ni²⁺ incorporation into urease (44).

The *fur* gene plays a role in both iron regulation and acid resistance in a variety of different bacteria. The Fur gene product is a transcriptional repressor that decreases iron uptake in the presence of a high-iron environment (53). In addition to a role in iron regulation in Gram-negative bacteria, the *fur* gene also appears to play a role in the acid-resistance mechanisms of several different organisms. The most widely studied organism in terms of fur and acid resistance is *S. typhimurium*, a bacteria that must survive transit of the stomach. The *S. typhimurium fur* mutant is acid sensitive (16). *fur*, like *rpoS* and *phoP*, controls a distinct set of acid-sensitive genes (9).

Like *S. typhimurium*, the *H. pylori* *fur* mutant is acid sensitive, but its role is unrelated to iron regulation (56). The amount of iron available to the bacteria has no effect on acid sensitivity (6). Fur does not affect *H. pylori* survival to acid shock, but it does play a role in growth in acid.

**The role of urease in acid acclimation by *H. pylori***

The earliest hypothesis to explain the gastric acidity-protective effect of this neutral-pH-optimus urease was that, following lysis of individual organisms, urease was bound to the surface of neighboring bacteria and the NH₄⁺ produced generated a neutral pH environment around them (49). There are several difficulties in accepting this “altruistic lysis” hypothesis (14). Firstly, the neutral-pH optimum of the urease of *H. pylori* would prevent any activity in the acid environment presumably inhabited by the organism, which must be at least as low as pH 5.0. Secondly, the methods used to demonstrate binding of urease were such that the urease produced by lysis of the bacteria was not separated from the intact organisms, hence resulting in an artifact exaggerating the quantity of surface urease (28). Finally, the neutral-pH microenvironment.

The key experiment showing that the crucial urease compartment was cytoplasmic and not surface bound was measurement of urease activity in intact bacteria by ¹⁴CO₂ release from labeled urea at neutral pH and in progressively acidic buffered media, as shown in FIGURE 2A. At neutral pH, little urease activity was measurable in intact organisms in contrast to lysed bacteria. But as the pH in the medium was reduced from pH 6.5 to 5.5 and down to pH 2.5, ¹⁴CO₂ release sharply increased in intact bacteria. The simplest explanation for these observations was that acidic pH activated a urea transport property in intact organisms, allowing access to cytoplasmic urease. The stable activity down to pH 2.5 also shows that even down to this medium pH, cytoplasmic pH does not fall at the 5-MM urea concentration used in these experiments; otherwise urease activity would be inhibited (50). Deletion of *ureI*, whose sequence predicted an integral membrane protein, prevented the pH-dependent increase of urease activity (50). Hence it is *ureI* acting as an acid-activated urea transporter providing urea to the cytoplasmic urease that allows buffering of the periplasm of the organism to allow at least survival in acidic medium. Expression of *Urel* is essential for colonization in mice or gnotobiotic mice (40, 51).

The *ureI* gene appears to be uniquely expressed by gastric *Helicobacter* spp. (50). Topographic analysis showed that this protein is a six-transmembrane segment polytopic membrane protein, and fractionation studies localized it to the inner membrane of the organism (58). Expression of *Urel* in *Xenopus* oocytes was used to determine its urea-transport properties, as had been done for the discovery of mammalian urea transporters (62). Urel displayed characteristics of a pH-gated urea channel (nonsaturable, voltage independent), having low open probability at neutral pH and high open probability at pH 5.0 and below, with half-maximal opening at pH 5.9. It was very selective for urea, with even then the protein is armi...
To explore the amino acids responsible for proton gating of the channel, site-directed mutagenesis was carried out on the protonatable exoplasmic amino acids of the Urel of H. pylori and H. hepaticus, a simpler pH-activated urea channel with half-maximal opening at pH 6.8, one pH unit higher than that of *H. pylori*. Mutations and chimeras were also generated with the Urel of *S. salivarius*, an even simpler exoplasmic urea channel variant that lacks pH-dependent activation (60). There is strong homology in the membrane domain, significant homology in the cytoplasmic domain, and none in the periplasmic loops, but a protonatable or protinated residue is present at the COOH terminus of these channels could be mutated to a neutral amino acid without effect on the pH dependence of urea uptake.

Several mutations of the *H. pylori* Urel inactivated urea transport, making the results of these mutations impossible to interpret in relationship to their role in transport (59). However, all of the protonatable amino acids of *H. hepaticus* could be mutated with retention of urea transport. To determine an effect, the pH at which half-maximal urea uptake was observed was taken to be the pH of the mutated transporter. Contrasting results were obtained when comparing *H. pylori* and *H. hepaticus*. Whereas mutations of the second periplasmic loop of Urel appeared to affect the pH dependence of urea uptake, mutations of the first periplasmic loop of *H. hepaticus* affected this pH dependence. However, replacement of both histidines (H13, H14) in the first periplasmic loop of *H. pylori* appeared to increase the open probability of the channel at neutral pH.

Replacement of the complete periplasmic domain of *S. salivarius* with that of *H. hepaticus* with even thiourea being impermeant (58). The protein is arranged with its NH2 and COOH terminal domains, with three periplasmic domains, two loops connecting transmembrane segments 2 and 4 to 5, and the COOH terminus also exposed to the periplasm. The pH profile of urea uptake into oocytes was virtually identical to the pH activation of urease in intact *H. pylori* (FIGURE 2B) (58).

<table>
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<tr>
<th>Medium pH</th>
<th>Urease activity</th>
<th>Urea uptake (pmol urea/oocyte)</th>
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<tr>
<td>4.5</td>
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<td>7.0</td>
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FIGURE 2. Aspects of the effect of deletion of Urel in *H. pylori*. A: effect of pH on urease activity of wild-type *Helicobacter pylori* and urel deletion mutants. There is a sharp increase in urease activity of intact wild-type *H. pylori* as the medium pH falls below pH 6. In contrast, the urease activity of the urel deletion mutant does not increase as medium pH decreases, indicating that Urel is a urea transporter. The pH optimum curve of urease from the urel gene deletion knockout strain is identical to the wild-type activity, showing an indirect effect of the Urel deletion on urease activity. B: pH dependence of urea uptake into Xenopus oocytes mirrors the pH profile of urease activity in intact *H. pylori*. C: survival of *H. pylori* wild-type and urel deletion mutants at pH 2.5. *H. pylori* increases urea permeability 300-fold, permitting acid survival at physiological urea levels in *H. pylori* compared with the urel deletion knockout strain. The wild-type organism displayed full survival at 5 mM urea and 50% survival at 0.7 mM urea, whereas the urel deletion mutants required 300 mM urea for even 50% survival. These data show the importance of Urel for acid acclimation.
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 (60). It seems likely that with acidification, protonation of the histidines or even the carboxylates results in a change of hydrogen bonding in the periplasmic domain, resulting in a conformational change in the membrane segments allowing urea entry across the channel.

**Gastric juice urea**

The urea in gastric juice is maintained at the same level as in blood, between 1 and 3 mM. There is evidence for some expression of the urea-transporter gene, *ut1*, in the rat stomach based on microarray analysis, but the cell type expressing this transporter is not known (Lambrecht N, personal communication). However, it is generally thought that urea moves into the gastric juice by free diffusion largely via a paracellular pathway. The leakiness of the paracellular pathway in the antrum, the usual site of *H. pylori* infection, is greater than that of the fundic region (52). This may be due to a combination of greater urea access to the organism and a higher pH in this absorptive region of the stomach. Microscopic observations show a larger number of bacteria are incubated at a medium pH of 5.5 in the absence of a periplasmic pH. At a medium pH of 7.4, the membrane potential was found to be about –180 mV, and as the pH of the medium was decreased, the membrane potential fell as expected from the assumption that efflux of NH3 would regulate periplasmic pH.

**Membrane potentials.** At a medium pH of 7.4, the membrane potential was found to be about –180 mV, and as the pH of the medium was decreased, the membrane potential fell as expected from the assumption that efflux of NH3 would regulate periplasmic pH.

**Consequences of ureI activation**

*H. pylori*, perhaps due to the structure of its outer membrane, is suitable for the study of its inner membrane potential and cytoplasmic pH using fluorescent dyes without the need for generation of spheroplasts, in contrast to *E. coli* (37). This proved extremely useful for analyzing the effects of urea addition at different fixed medium pH on the assumption that efflux of NH3 would regulate periplasmic pH.

**Membrane potential.** At a medium pH of 7.4, the membrane potential was found to be about –180 mV, and as the pH of the medium was decreased, the membrane potential fell as expected from the increase of the inward ΔpH, reaching 0 mV at a medium pH of 3.5. It is certainly possible, but as yet undetermined, that at lower pH the inner membrane potential inverts at higher medium acidity, as has been found for *E. coli* (45). The addition of urea under acidic medium pH conditions between pH 3.0 and 6.0 elevated inner membrane potential to a relatively constant value of about –101 mV, corresponding to a periplasmic pH of 6.1.

**Cytoplasmic pH.** Cytoplasmic pH was measured using BCECF-AM, a membrane-permeant fluorescent pH indicator. Cytoplasmic pH was determined over a range of medium pH of 7.4 to 4.5 in the absence and presence of urea. The BCECF fluorescence ratio increased with the addition of urea in the presence of acidic medium but did not change at neutral pH where internal pH was estimated to be ~8.0. In the absence of urea, cytoplasmic pH fell to 5.3 at a medium pH of 4.5 but was restored to pH 6.5 with urea addition.

Hence at neutral pH either in the absence or presence of urea, the pmf is about –40 mV (ΔpH = 180 mV (ΔpH) – 220 mV). At pH 4.5 in the absence of urea, the pmf is about –48 mV – 45 mV = –93 mV, but with the addition of urea at this pH the pmf rises back to –120 mV – 101 mV = 221 mV (61). Hence urease activity maintains a normal pmf at a pH at which other neutralophiles can survive but at which they allow acidification of their cytoplasm and loss of an adequate pmf for normal cell function. The key distinction between *H. pylori* (and presumably other gastric *Helicobacter* spp.) and other acid-resistant neutralophiles is the ability to maintain both cytoplasmic and periplasmic pH at a level that allows maintenance of a pmf that allows cell growth to continue in the gastric environment.

The ability of *H. pylori* to regulate its periplasmic pH is illustrated in FIGURE 3A. Here the wild-type bacteria are incubated at a medium pH of 5.5 in the presence of BCECF-free acid that can penetrate the outer but not the inner membrane, and increase of its fluorescence indicates alkalization. When urea is added to the weakly buffered medium, there is rapid periplasmic alkalization. This is not seen in ureI deletion mutants (58).

**Location of bacterial urease**

Blue native gel electrophoresis showed that UreA and UreB, soluble cytoplasmic proteins, were associated with UreI, a membrane protein (57). Further investigation using immunoelectron microscopy showed that this association was increased at an acidic medium pH of 5.5, as shown by the electron micrograph in FIGURE 3C (23). Hence urea is immediately available to the urease under acidic conditions. It is also possible that UreI is able to transport NH3 more rapidly than free diffusion across the lipid bilayer, allowing even more rapid periplasmic neutralization of entering protons.

**Permeability of the membranes of *H. pylori***

It is extremely difficult to use tracer methods to investigate passive diffusion into bacteria since the surface/volume ratio is so high. It is possible to determine uptake of tracers that are concentrated by the pmf, however, and this has been done for measurement of gradients in *H. pylori* (31). This approach provided data similar to those found with fluorescent probes as discussed above. However, little is known about the transporters in the outer membrane of this organism, but clearly after a phase of periplasmic alkalization there is alkalization of the medium with the addition of urea at acidic pH. Hence at neutral pH either in the absence or presence of NH3, the pmf is about –40 mV (ΔpH = 180 mV (ΔpH) – 220 mV). At pH 4.5 in the absence of urea, the pmf is about –48 mV – 45 mV = –93 mV, but with the addition of urea at this pH the pmf rises back to –120 mV – 101 mV = 221 mV (61). Hence urease activity maintains a normal pmf at a pH at which other neutralophiles can survive but at which they allow acidification of their cytoplasm and loss of an adequate pmf for normal cell function. The key distinction between *H. pylori* (and presumably other gastric *Helicobacter* spp.) and other acid-resistant neutralophiles is the ability to maintain both cytoplasmic and periplasmic pH at a level that allows maintenance of a pmf that allows cell growth to continue in the gastric environment.

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gas in the absence or presence of urea fell to ±50 mV (ΔpH) — 11.5 from the absence of urea to ±95 mV. At pH 7.0 the pmf was ±121 mV (86).

F1F0-ATPase has three, rather than two, transmembrane segments, implying perhaps a slower inward diffusion of urea at acidic pH, indicating relatively high diffusion of NH₃ (2). The inner membrane appears to have some specialized properties that resist rapid cytoplasmic acidification. For example, the iso-electric point of the proteins is relatively high compared with E. coli, and even the c subunit of F₆F₅-ATPase has three, rather than two, transmembrane segments, implying perhaps a slower inward proton transport.

Other genes of H. pyloriregulated in acidic medium

Although the above data clarified the role of UreI in the acid-acclimation processes of H. pylori, it would be extremely unlikely that this protein, along with urease, would be the only means of combating gastric acidity by the organism. Hence gene regulation due to acid exposure was studied by using microarray analysis at pH 7.4, 6.2, 5.5, and 4.5 in the absence or presence of urea (61). About 187 genes were upregulated by exposure to acidic pH down to 4.5, and 100 were downregulated. Other laboratories have also used microarray data, some in agreement with the data from this laboratory and others not, but thus far, the data presented here are unique in investigating the effect of urea on gene expression in different pH media.

Changes in gene expression that occur at a medium pH of 6.2 in the absence, but not in the presence, of urea are likely responses to periplasmic pH since the addition of urea reverses the gene regulation, indicating that a normal periplasmic pH and membrane potential have been restored. Under these conditions, there are several genes that might be considered pH homeostatic genes able to produce NH₃, such as asparaginase, aspartate ammonia lyase, and the periplasmic u-carbonic anhydrase (HPI186) able to produce HCO₃⁻ as a periplasmic buffer. There was also upregulation in this cluster of three hydrogenase expression/forming genes, hypA, hypB, and hypC, the first two considered to have a role in NH₃ uptake, essential for adequate urease enzyme activity, although these are actually hydrogenase-related genes (5, 44).

Carbonic anhydrase. Since urease produces 2NH₃ + CO₂ from urea, this carbonic anhydrase was the first of these pH homeostatic genes that we investigated in some detail. Since an inhibitor, acetazol...
UreI restored membrane potential in acidic media, as the mutants, the addition of urea no longer a 3-log decrease in survival in the knockout organism, after regeneration of the membrane was deleted, many of the properties of the wild type. However, UreI was still functional in that there was the normal increase of bacterial urease activity with pH 4.0 and above, deletion of carbonic anhydrase had little effect on survival; hence the pH of the habitat of H. pylori must be <4.0. H. pylori the pH of the habitat of H. pylori in the mouse stomach was down to at least pHout 3.0 based on measurement of intrabacterial urease activity enable acid acclimation by H. pylori, the NH3 to neutralize entering protons, and the HCO3 to buffer the periplasm. At pH 4.0 and above, deletion of carbonic anhydrase had little effect on survival; hence the pH of the habitat of H. pylori in the mouse stomach must be <4.0.

**HISTIDINE KINASE AND OTHER GENES. H. pylori expresses a two-component system consisting of the histidine kinase sensor element (HP0165) and the response regulator HP0166 (4). It was found that there was upregulation of HP0165 at all external pH values (61) and that there was even reten-

dition of upregulation of this gene with the addition of urea at pH 6.2. There are several histidines in the exoplasmic domain of this sensor, which would change protonation at pH ~6.2. Since the response regulator for HP0165 is HP0166, we decided to examine some of the 32 genes regulated by HP0165 and disrupti-

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**References**

lamine at pH 7.4 in vivo data from mice in the 1970s (17). These studies show that the pKa is significantly higher than the cytoplasmic environment might suggest, which would indicate that intrabacterial urease is in a more alkaline environment. In fact, the pH within Helicobacter pylori is reported to be neutral to slightly alkaline, and there is evidence that urease is active at these pH levels (18).

A recent study by Leung et al. (19) investigated the role of urease in Helicobacter pylori colonization and pathogenesis. They found that urease activity is essential for colonization and survival in the gastric environment, and that urease expression is regulated by the ureA gene, which encodes for an enzyme involved in urea metabolism. This finding is consistent with previous studies showing that urease expression is upregulated in response to low pH conditions. These results support the idea that urease plays a crucial role in Helicobacter pylori colonization and pathogenesis.

However, the exact mechanism by which urease contributes to colonization and pathogenesis is still unclear. It is likely that urease mediates colonization through a combination of mechanisms, including acidneutralization, ammonia production, and/or other unknown mechanisms. Further studies are needed to elucidate the specific roles of urease in Helicobacter pylori colonization and pathogenesis.

References


