sequence from the University of Washington Genome Center (http://kandinsky.genome.washington.edu/Maripalidis/html/top.html), the *M. maripalidis* proS gene was cloned from genomic DNA by PCR. The complete gene was sequenced (GenBank accession number AF163997) and cloned into pCB51 to generate pCBS-Mm-proS. The *E. coli* strain QU819 [cys+; K. Bohman and L. A. Isaksson, Mol. Gen. Genet. 176, 53 (1979)] was transformed at 30°C with these plasmids and the resulting transformants tested for growth on Luria-Bertani agar supplemented with ampicillin (100 mg/liter), chloramphenicol (34 mg/liter), and Cys (0.5 mg/ml) at 30°C and 42°C.

14. V. Busiolo, M. Di Girolamo, C. Ceri, C. De Marco, Biochim. Biophys. Acta 564, 311 (1979). Thiopropionate was also found to completely inhibit Cys-tRNA synthesis by *M. jannaschii* total protein extracts, indicating that ProS is solely responsible for this activity.


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A H⁺-Gated Urea Channel: The Link Between *Helicobacter pylori* Urease and Gastric Colonization

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Acidic media trigger cytoplasmic urease activity of the unique human gastric pathogen *Helicobacter pylori*. Deletion of *ureI* prevents this activation of cytoplasmic urease that is essential for bacterial acid resistance. UreI is an inner membrane protein with six transmembrane segments as shown by in vitro transcription/translation and membrane separation. Expression of UreI in *Xenopus* oocytes results in acid-stimulated urea uptake, with a pH profile similar to activation of cytoplasmic urease. Mutation of periplasmic histidine 123 abolishes stimulation. UreI-mediated transport is urea specific, passive, nonsaturable, nonelectrogenic, and temperature independent. UreI functions as a H⁺-gated urea channel regulating cytoplasmic urease that is essential for gastric survival and colonization.

The Gram-negative pathogen *H. pylori* is unique in its ability to colonize the human stomach. *H. pylori* infection is acquired during childhood, persists lifelong if not eradicated, and is associated with chronic gastritis and an increased risk of peptic ulcer disease and gastric cancer (1). An acid-tolerant neutralophile, *H. pylori* expresses a neutral pH—optimum urease to maintain proton motive force (PMF) and to enable gastric colonization (2).

Most urease is found in the bacterial cytoplasm, although up to 10% appears on the surface, owing to cell lysis during culture (3). Surface or free urease has a pH optimum between pH 7.5 and 8.0 but is irreversibly inactivated below pH 4.0 (4, 5). The activity of cytoplasmic urease is low at neutral pH but increases 10- to 20-fold as the external pH falls between 6.5 and 5.5, and its activity remains high down to pH ~2.5 (5). Thus, cytoplasmic, not surface, urease is required for acid resistance. The unmodified urea permeability of the inner membrane is insufficient to supply enough urea to intrabacterial urease for urease activity to buffer the bacterial periplasm in the face of gastric acidity (the median diurnal acidity of the human stomach is pH 1.4). The data here show that *H. pylori* expresses a urea transport protein with unique acid-dependent properties that activates the rate of urea entry into the cytoplasm.
The permeability of urea across phospholipid bilayer membranes, $4 \times 10^{-6}$ cm s$^{-1}$(6), is insufficient to saturate intracellular urease. At neutral pH, this rate of urea entry is not able to saturate intracellular urease even with 100 mM external urea. In acidic media, the apparent Michaelis constant $K_M$ of internal urease becomes equal to that of free urease, $\sim$ 1 mM (5), with 99.9% protein permeabilizes the inner membrane, as shown by penetration of propidium iodide, without disrupting its morphology (Fig. 1). The urease of $C_{12}E_8$-treated intact organisms is fully active at neutral pH (intact bacteria, $0.25 \pm 0.1$ μmol of urea per minute per milligram of protein; bacterial homogenate, $2.76 \pm 0.27$ μmol of urea per minute per milligram of protein; bacteria with 0.01% $C_{12}E_8$, $2.65 \pm 0.10$ μmol of urea per minute per milligram of protein). Thus, an increase of urea permeability of intact $H. pylori$ accounts for the activation of cytoplasmic urease in acidic media.

The urease gene cluster consists of seven genes. $ureL$ and $ureB$ encode the urease structural subunits, and $ureE$, $F$, $G$, and $H$ encode accessory proteins necessary for Ni$^{2+}$-insertion into the apoenzyme (7). $ureL$ encodes a membrane protein with homology to putative amide transporters such as AmiS, AmiS2, and ORFP3 (8), and its absence impairs acid survival (9). UreI may be an acid-activated urea transporter crucial for acid resistance of $H. pylori$. Its function was determined in deletion mutants and by expression in Xenopus oocytes.

In contrast to the large acid-induced increase in urease activity of wild-type organisms, no increase of activity in acidic medium was observed in the intact ureI mutant (10), DW504UreI$^{-}$ (Fig. 2). However, urease activity of detergent-treated ureI$^{-}$ cells ($3.0 \pm 0.25$ μmol of urea per minute per milligram of protein) or cell lysate resulted in urease activity equal to that of wild-type organisms. The normal level of urease shows that deletion of ureI did not affect the expression of the downstream urease accessory genes essential for its biosynthesis. The mutation is therefore nonpolar. Abolition of acid activation of urease in intact organisms by ureI deletion and full activation of intrabacterial urease by $C_{12}E_8$ suggest that UreI-mediated transport of urea determines the pH dependence of cytoplasmic urease.

Because activity of the $H. pylori$ cytoplasmic urease maintains an inward urea gradient, uptake does not need energy from adenosine triphosphate (ATP) or ion gradients. Measurement of passive urea uptake in prokaryotes, with their high volume-to-surface ratio, is precluded by the endogenous permeability of phospholipid bilayers. Oocytes have a volume-to-surface ratio several hundred times that of prokaryotes and express neither endogenous urea transporters nor urease activity (11). UreI was therefore expressed in Xenopus oocytes by injection of ureI complementary RNA (cRNA) (Fig. 3). Over 30 min, urea uptake in UreI oocytes was accelerated 6- to 10-fold at pH 5.0 compared with pH 7.5 and was the same as in noninjected oocytes at either pH (12) (Fig. 4, A and C). Control oocytes equilibrated to the same level as oocytes expressing UreI, but required 48 hours to reach equilibrium as compared with 1 hour for ureI-injected oocytes. No increase in internal concentration was found above the increase from equalization of the concentration gradient. Accumulation was consistent with acid-dependent UreI facilitation of urea transport into the 0.4 to 0.6 μl of internal oocyte water space (13).

UreI-dependent urea uptake was activated with a pH profile nearly identical to the pH activation profile of cytoplasmic urease in $H. pylori$ (5). Half-maximal activation of transport occurred at pH $\sim$ 6.0 (Fig. 4B). Uptake was highly selective for urea, with only trace accumulation of $^{14}$C-thiourea (Fig. 4C) or $^{14}$C-mannitol ($1.13 \pm 0.10$ and $0.41 \pm 0.14$ pmol per oocyte at pH 5.0, respectively). Uptake of 50 μM $^{14}$C-urea was $15.28 \pm 0.27$ and $13.61 \pm 0.97$ pmol per oocyte in the absence or presence of 100 mM unlabeled urea, respectively. Thus, saturation was not seen, even though a 2000-fold excess of urea was added (Fig. 4C). The addition of urea to voltage-clamped UreI-expressing oocytes resulted in no change in current. An inward current of 117 nA is predicted, if UreI were a proton- or cation-driven urea transporter with a stoichiometry of 1:1 (14). UreI-mediated urea uptake is therefore nonelectrogenic.

Transport at pH 5.0 was temperature independent between 15° and 30°C (Fig. 4D). This temperature insensitivity and the lack of saturation of uptake suggest that, after H$^+$ activation, urea fluxes through UreI with little interaction with the protein. Aquaporins, although also putative six transmembrane-segment channel-like water transport proteins, show substantial temperature dependence (15). Our data suggest that UreI functions as a specific, H$^+$-activated urea channel. A channel mechanism would allow a rate of urea uptake adequate for saturation of internal urease at physiological gastric urea concentrations (1 to 3 mM).

Western blot analysis detected the presence of UreI in purified inner but not outer membrane fractions (Fig. 3). Periodic acid–silver staining detected carbohydrate (16) in the outer but not inner membrane fraction (17), confirming the validity of the separation (18). UreI contains six hydrophobic sequences, H1 to H6. In vitro transcription/translation of various NH$_2$-terminal lengths of UreI, fused to a glycosylatable COOH-terminal tag, was used to
follow orientation of translation products in canine microsomal membranes (19). A signal anchor sequence translocates the COOH-terminus into the microsomal lumen (analogous to the bacterial periplasm), and a subsequent stop transfer sequence returns the COOH-terminus to the cytoplasmic side. Alternating signal anchor and stop transfer sequences defined (Fig. 5A) the topography of UreI.

The lack of a cytoplasmic retention signal in front of the first hydrophobic sequence, H1, and the presence of two positively charged amino acids in front of H2 imply a periplasmic location of the NH2-terminus. The COOH-terminus of the UreN1 construct, encoding Met1–Lys23, was glycosylated, signifying COOH-terminal “out” orientation (Fig. 5A). However, UreN1b, Met1–Lys27, had a COOH-terminal “in” orientation because of the additional positive charge (20). The product of UreN2, Met1–Thr56, showed strong glycosylation indicating that H1 and H2 are a membrane-inserted pair with the NH2- and COOH-termini oriented “out.” The translation product of UreN3, Met1–Arg102, lost the glycosylation of UreN2. H3 acts as a stop transfer sequence, yielding a COOH-terminus “in” orientation. The glycosylation of the product of UreN4 translation, Met1–Leu128, showed that H4 acted as a signal anchor, directing the COOH-terminus “out.” The translation product of UreN5, Met1–Lys162, showed no glycosylation, with H5 acting as a stop transfer sequence, whereas the product of UreN6, Met1–Val195, was glycosylated. H6 therefore behaved as a signal anchor. Hence, the inner membrane protein, UreI, has six transmembrane–inserted segments, with both NH2- and COOH-termini located in the periplasm (Fig. 5B).

The apparent pK of UreI activation implies protonation of one or more periplasmic histidines for activation of urea transport. Histidine 123, located at the boundary of H4, was mutated to arginine or glycine (21). Expression of UreI was unaffected by this mutation, but acid activation of urea uptake disappeared (1.83 ± 0.20 pmol per oocyte at pH 5.0 versus 1.75 ± 0.34 pmol per oocyte at pH 7.5). The protonated state of this histidine is important for acid activation of transport.

Acid survival of prokaryotes depends on the maintenance of suitable levels of cytoplasmic and periplasmic pH to maintain their PMF. Helicobacter pylori survives between pH 4.0 and 8.5 in the absence of urea and grows between pH 6.0 and 8.0 (2). A neutral

Fig. 4. Uptake experiments in ureI-injected and control Xenopus oocytes (n = 5 to 7). (A) Equilibration of 50 μM 14C-urea at pH 5.5 and 7.5. (B) Uptake of 50 μM 14C-urea or uptake of 50 μM 14C-thiourea at pH 5.0 and 7.5 in the presence of excess unlabeled urea. (C) Effect of temperature on uptake of 14C-urea at pH 5.0 between 15° and 30°C (24). Error bars indicate ± SEM.

Fig. 5. Topography of UreI. (A) SDS–polyacrylamide gel electrophoresis analysis of products resulting from in vitro transcription/translation of successive UreIN-ter/H1–ATPase β-subunit fusion constructs containing one to six of the hydrophobic sequences of UreI, with (+) or without (−) microsomes. Glycosylation (arrow) is detected by a 12.5-kD shift in the translation product. (B) Two-dimensional model of UreI from in vitro translation results (25). Arrow, histidine 123.
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14. A two-electrode voltage clamp technique [B. Mack-
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in the absence of gastric acidity, as occurs
during digestion. The combination of a high
level of a neutral pH– optimum urease and an
acid-regulated urea channel explains why H.
pylori is unique in its ability to inhabit the
human stomach. Effective inhibition of Urel
would provide a means of eradicating the
organism in the normal, acid-secreting stomach.

References and Notes
13. A poly[A] cassette (a region of repeated adenosine) was cloned into pcDNA3.1–Invitrogen, Carlsbad, CA. The ure gene sequence was inserted upstream of the poly[A] cassette and downstream of the pcDNA3.1 T7 promoter. cDNA was prepared using the messageMACHINE in vitro transcription system (Ambion, Austin, TX). Fifty nanoliters of cDNA (1 μg/μl) was injected and oocytes were maintained at 18°C in Barth’s solution for 3 days before use in uptake experiments.
15. A two-electrode voltage clamp technique [B. Mackenzie, D. F. F. Luo, E. M. Wright, J. Membr. Biol. 162, 101 (1999)] was used to study the electrical behavior of Xenopus oocytes expressing Urel. The resting membrane potential of these oocytes was not different from that of control oocytes (45 ± 2 versus 44 ± 3; n = 5). Addition of 5 mM urea at pH 5.5 had no effect on membrane potentials ranging from −150 to +50 mV. Following the electrophysiological assay, ure expression was confirmed in the same oocytes by uptake of 5 mM [3H]-urea at pH 5.0 for a value of 1.09 ± 0.03 nmole urea per oocyte per 15 min.

Vesicular stomatitis is an economically important arboviral disease of livestock. Viremia is absent in infected mammalian hosts, and the mechanism by which insects become infected with the causative agents, vesicular stomatitis viruses, remains unknown. Because infected and noninfected insects potentially feed on the same host in nature, infected and noninfected black flies were allowed to feed on the same host. Viremia was not detected in the host after infection by a black fly bite, but because noninfected black flies acquired the virus while co-feeding on the same host with infected black flies, it is concluded that a viremic host is not necessary for an insect to be infected with the virus. Thus co-feeding is a mechanism of infection for an insect-transmitted virus.

Vesicular stomatitis is an arthropod-borne viral disease that primarily affects cattle, swine, and horses; it causes vesicular lesions on the mouth, coronary bands, and teats. Many species of wildlife and humans are also at risk. The causative agents of vesicular stomatitis viruses (VSVs), are a group of antigenically related but distinct viruses of the genus Vesiculovirus, family Rhabdoviridae (1).

Despite intensive study, aspects of the epizootiology of VSVs, including modes of transmission and endemic maintenance, remain largely unknown and highly controversial. The World Health Organization (WHO) definition of an arbovirus (2) implies that only vertebrate species that develop detectable viremia after infection are significant in the epidemiology of these viruses and stipulates that vector infection