Medium pH-dependent redistribution of the urease of *Helicobacter pylori*

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Helicobacter pylori is an aetiological agent of gastric disease. Although the role of urease in gastric colonization of *H. pylori* has been shown, it remains unclear as to where urease is located in this bacterial cell. The purpose of this study was to define the urease-associated apparatus in the *H. pylori* cytoplasm. *H. pylori* was incubated at both a neutral and an acidic pH in the presence or absence of urea and examined by double indirect immunoelectron microscopy. The density of gold particles for UreA was greatest in the inner portion of the wild-type *H. pylori* cytoplasm at neutral pH but was greatest in the outer portion at acidic pH. This difference was independent of the presence of urea and was not observed in the *ureI*-deletion mutant. Also, the eccentric shift of urease in acidic pH was not observed in UreI. After a 2 day incubation period at acidic pH, it was observed that the urease gold particles in *H. pylori* assembled and were associated with UreI gold particles. Urease immunoreactivity shifted from the inner to the outer portion of *H. pylori* as a result of an extracellular decrease in pH. This shift was urea-independent and UreI-dependent, suggesting an additional role of UreI in urease-dependent acid resistance. This is the first report of the intracellular transport of molecules in bacteria in response to changes in the extracellular environment.

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INTRODUCTION

Helicobacter pylori is a Gram-negative microaerobic pathogen that causes chronic gastritis, peptic ulceration and gastric cancer (Warren & Marshall, 1983; Bohnen *et al.*, 1986; Wadström, 1995). Bacterial infection occurs in young individuals and persists throughout life (Kurosawa *et al.*, 2000). The urease of *H. pylori* is essential for gastric colonization and presumably neutralizes acidity, thereby allowing bacterial survival (McGowan *et al.*, 1996). Most of the urease is in the bacterial cytoplasm and only a small amount is found on the surface of the bacterial cell (Nilius *et al.*, 1991; Bode *et al.*, 1993; Dunn & Phadnis, 1998; Marcus & Scott, 2001). Since the enzyme has an optimum pH between 7.5 and 8.0 and is irreversibly inactivated below pH 4.0 (Rektorschek *et al.*, 1998; Scott *et al.*, 1998) or pH 3.0 (Ha *et al.*, 2001), it is difficult to explain how the enzyme acts

on the surface of the bacterial cell for its protection in the stomach. Furthermore, UreI is essential for gastric colonization and survival in acidic media, and UreI does not affect surface but only intra-bacterial urease (Scott *et al.*, 2000; Weeks & Sachs, 2001; Skouloubris *et al.*, 1998; Mollenhauer-Rektorschek *et al.*, 2003). These characteristics show that it is the intracytoplasmic urease that is important for *H. pylori* colonization.

The unique gastric acid resistance of *H. pylori* may be due in part to an acid-regulated urea channel, UreI, which increases the access of urea to intrabacterial urease in acidic media (Weeks *et al.*, 2000). That is, after acidity opens a urea channel in the inner membrane of the bacterium, urea is taken up passively and hydrolysed by the enzyme in the cytoplasm. If urea and ammonia are transported across the cytoplasm in large amounts, they may cause degeneration of cytoplasmic proteins and alkalization of the bacterial cytoplasm. To overcome toxicity, the bacterium may have a protective mechanism or apparatus. It has been suggested that the urease antigen is localized near the bacterial surface (Dunn &

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Phadnis, 1998), as shown by immunoelectron microscopy of cryoultramicrosections and acrylic resin-embedded bacterial cells. Since these methods did not provide sufficient contrast of the cell wall in micrographs, it was difficult to analyse their data by any quantitative methodology. On the other hand, the urease acts in the bacterial cytoplasm (Athmann *et al.*, 2000) and localization of UreI at the inner membrane has been proven by biochemical methods (Weeks *et al.*, 2000) and by morphological methods using contrast-enhanced immunoelectron microscopy (Hong *et al.*, 2000). According to these findings, we hypothesized that a complex of urease and UreI is localized near the inner membrane and we attempted to determine the actual localization of *H. pylori* urease and UreI using contrast-enhanced immunoelectron microscopy (Hong *et al.*, 2000). UreI has been shown to interact with UreA by a variety of methods and this would be consistent with our hypothesis (Volland *et al.*, 2003; Rain *et al.*, 2001).

METHODS

Bacteria and growth. The *H. pylori* strain ATCC 43504^T and the *ureI*-deletion mutant described by Weeks *et al.* (2000) were used in this study. Bacteria were cultured on Pylori agar plates (bioMérieux) in a jar containing an anaerobic gas pack (Becton Dickinson) and Campyack Microaerophilic System Envelopes (Becton Dickinson) at 37 °C for 3 days. The *ureI*-deletion mutant exhibits normal total urease activity (Athmann *et al.*, 2000). Colonies of both strains were collected, suspended and incubated in either McIlvaine buffer containing 0.1 M citric acid monohydrate and 0.2 M disodium hydrogen phosphate, pH 5 or 7, at 37 °C for 15 min or Brucella broth (supplemented with 0.1 M MES buffer and 5% horse serum, pH 5.5 or 7) at 37 °C under microaerophilic conditions for 2 days. Some of the samples were incubated in McIlvaine and MES buffer containing 1 mM urea.

pH change of urease under different pH conditions. *H. pylori* cultured on agar was collected and resuspended in Brucella broth supplemented with 5% horse serum and the turbidity was adjusted to McFarland standard 0.5. Two ml of this *H. pylori* suspension, and 1.0 ml of pH-adjusted urease assay buffer, which contained 1.3 M urea, 0.06 M NaN₃, 0.005 M phenol red and McIlvaine buffer (pH 5.0 and 7.0), were mixed in a 10 ml tube. Measurement of pH was performed in the tube at room temperature using a pH meter (Checker, HANNA).

Antibodies. The mouse monoclonal antibody against *H. pylori* urease subunit A (Austral Biologicals) and rabbit polyclonal anti-UreI antibody (Weeks *et al.*, 2000) were used as primary antibodies, and 5 nm colloidal gold-labelled anti-mouse IgG and 10 nm gold-labelled anti-rabbit IgG-labelled goat antibodies (Amersham) were used as secondary antibodies.

Fixation and staining. *H. pylori* colonies were fixed with 1% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) at 4 °C for 60 min. Fixed colonies were washed five times with 0.05 M cacodylate buffer and dehydrated in serially graded ethanol. Samples were then embedded in Lowicryl K4M resin (Electron Microscopy Science). Polymerization was allowed to occur in an ultraviolet irradiator (Dosaka EM) at -30 °C for 2 days and then at room temperature for 2 days. Ultrathin sections were cut using a Porter Blum Ultramicrotome (Sorvall MT-5000, Du Pont) and mounted on a nickel grid (300 mesh) supported by a carbon-coated collodion film.

Ultrathin sections on the grid were treated with 5% normal goat serum in 0.15 M PBS (pH 7.2) to block nonspecific reactions. Sections were then double-stained with mouse antibody against *H. pylori* UreA and

rabbit antibody against UreI (primary antibodies) and then 5 nm colloidal gold-labelled anti-mouse IgG and 10 nm gold-labelled anti-rabbit IgG-labelled goat antibodies (secondary antibodies). Sections were first reacted by adding drops of the primary antibodies at room temperature for 120 min and washed five times in PBS. Sections were then allowed to react with the secondary antibodies at room temperature for 60 min and were subsequently washed five times in PBS.

Immunostained sections were fixed with 1% glutaraldehyde in 0.05 M cacodylate buffer for 15 min and washed five times in distilled water. Finally, sections were contrast-enhanced with a mixture of alcian blue and osmium tetroxide solution and were stained with uranyl acetate aqueous solution as described previously (Hong *et al.*, 2000). All procedures for contrast enhancement were performed at room temperature.

All sections were observed under a transmission electron microscope (H-300 and H-7100, Hitachi). Photomicrographs were taken at a magnification of $\times 15\,000$ and enlarged to a magnification of $\times 30\,000$.

Morphometric analysis. The number of immunogold particles associated with *H. pylori* cells was determined using the immunoelectron photomicrographs. Areas of bacterial cells in the photomicrographs were measured using an image analyser (MCID system, Imaging Res.) and the numbers of gold particles per unit area were compared.

In order to quantify the distribution of gold particles, the densities of the particles per square micrometer in three portions of a bacterial cell were determined. The bacterial cell was divided into three portions: the outer portion is the area extending from a depth of one-sixth of the diameter to the surface of the cell; the medium portion is the area between the outer and inner portions extending from one- to two-sixths of the diameter; and the inner portion is the central area of the cell extending beyond two-sixths of the diameter to the centre of the cell. The principle of dividing portions of *H. pylori* is explained in Fig. 1. As shown, the outer portion that is between the surface and the depth of one-sixth of the shorter diameter (approx. 85 nm) is equal to the sum of the cell wall (approx. 30 nm) width, length of the antibody (approx. 25 nm) and x (approx. 30 nm).

RESULTS AND DISCUSSION

In order to measure urease activity in whole bacterial cells cultured under different pH conditions, the pH of urease assay buffer was changed. In Fig. 2, the pH change of urease assay buffer mixed with bacterial suspension is shown. Results reveal that the level of pH change at pH 5 is greater than that at pH 7; this difference was not observed in urease assay buffer mixed with the suspension of the *ureI*-deletion mutant. That is, the difference in pH change between the wild-type and *ureI*-deletion mutant at pH 5 was not the same as that observed at pH 7. In contrast, a pH change of the assay buffer was not observed in the urea-free control. The pH change of the assay buffer can therefore be taken as an indirect measure of urease activity. Our results indicate that the urease activity of wild-type *H. pylori* increases rapidly as the pH of the medium decreases, but this does not happen in the *ureI*-deletion mutant, and these results are similar to Scott *et al.* (2000).

To clarify whether the number of gold particles associated with UreA and the distribution of urease are influenced by environmental factors, the localization and the number of gold particles associated with urease were determined in cells incubated for 15 min at different pHs. The overall densities

Fig. 1. Diagram showing the procedure and direction of sectioning of *H. pylori*. (a) A lateral view of *H. pylori* in a three-dimensional diagrammatic representation. The blade of the microtome is shown cutting a longitudinal section (LS) of the bacterium. (b) A lateral view of the LS obtained after sectioning. (c) A frontal view of the LS. (d) The dorsal view of the LS, as observed through the microscope and seen in all photomicrographs presented in this paper. Dotted lines represent a frame of reference for the reader so as to give a clear picture of the area in discussion. 'a' and 'b' are two points on the same outer membrane of the LS of *H. pylori* but are separated from each other by a distance 'x' due to the curvature of the bacterial membrane. The distance 'x' is due to the limitation of two-dimensional electron microscopy. x, Maximum distance observed between any two points 'ab' owing to the curvature of the cell membrane obstructing the passage of electron wave/field of vision. S, Source of light/field of vision.

Fig. 2. Urease activity of *H. pylori* at different pHs. Wild-type (*UreI*⁺) *H. pylori* incubated at pH 7 (○) and pH 5 (●); *ureI*-deletion mutant (*UreI*⁻) incubated at pH 7 (□) and pH 5 (■); wild-type (*UreI*⁺) *H. pylori* incubated at pH 7 (◇) and pH 5 (◆) without urea. The pH change of urease assay mixture containing *H. pylori* in different pHs is shown. Urease activity of *H. pylori* at pH 5 was greater than that at pH 7 and this difference was not observed in the *ureI*-deletion mutant. The pH change of assay buffer was not observed in the urea-free control. Each assay was performed three times.

of gold particles in the bacterial cells incubated at neutral pH in the presence and absence of urea were $105.1 \pm 14.8 \mu\text{m}^{-2}$ and $117.4 \pm 35.7 \mu\text{m}^{-2}$, respectively. These values were not found to be significantly different by Student's *t*-test ($P < 0.01$). At acidic pH, the overall densities in the cell in the presence and absence of urea were $151.2 \pm 36.4 \mu\text{m}^{-2}$ and $161.1 \pm 56.8 \mu\text{m}^{-2}$, respectively. These values at the same pH were found to be statistically the same by Student's *t*-test ($P < 0.01$). The distribution of gold particles seemed to be uniform in bacterial cells incubated in the presence of urea at neutral pH in wild-type (Fig. 3a) but eccentric in those incubated at acidic pH (Fig. 3b).

Although the presence of urea in the buffer did not influence the densities of gold particles in any portion of the bacterial cells, a definite change in the distribution of gold particles was observed in cells incubated at different pHs. At neutral pH, the density was highest in the inner portion and lowest in the outer portion (Student's *t*-test paired analysis, $P < 0.05$). In contrast, at acidic pH, the density was highest in the outer portion and lowest in the inner portion (Student's *t*-test paired analysis, $P < 0.05$) (Table 1).

To determine whether the eccentric distribution of urease at acidic pH is *UreI* dependent, the *ureI*-deletion mutant (Weeks *et al.*, 2000) was also observed under the same experimental conditions (Table 1). The densities at each portion were similar to those of wild-type cells incubated at neutral pH (Fig. 3c, d); i.e. the eccentric shift of the distribution of urease molecules did not occur in the *ureI*-deletion mutant at acidic pH (Student's *t*-test paired analysis, $P < 0.05$).

In order to determine whether the shift of urease distribution was due to nonspecific effects of the acidic condition, the localization of *UreI* was determined by similar methods (Fig. 3a, b). The difference in the density of gold particles for *UreI*

Fig. 3. Electron photomicrographs of *H. pylori* incubated for 15 min and then double immunostained with anti-UreA and anti-UreI antibodies. Urease gold particles (the smaller gold particles) are distributed uniformly in the bacterial cell incubated at neutral pH in the wild-type strain (a). The urease gold particles are distributed more in the outer portion than in the inner portion of the bacterial cell incubated at acidic pH in the wild-type strain (b). UreI gold particles (the bigger gold particles) in the bacterial cell incubated at neutral pH (a) are distributed in a similar manner to those at acidic pH (b). Urease gold particles are distributed uniformly in the *ureI*-deletion mutant cell incubated at neutral pH in (c). Gold particles are distributed uniformly in the *ureI*-deletion mutant cell incubated at acidic pH (d). The UreI-deletion mutant was not labelled with the anti-UreI antibody-associated gold particles (c, d). Original magnification, $\times 15\,000$.

between neutral pH (Fig. 3a) and acidic pH (Fig. 3b) was similar to that reported previously (Hong *et al.*, 2000) and was not found to be statistically significant by Student's *t*-test ($P < 0.01$). The densities of the gold particles in the outer portion of wild-type cells incubated at neutral and acidic pH were similar to those reported previously (Hong *et al.*, 2000).

To determine whether the shift occurs in proliferating bacteria, the bacteria incubated for 2 days at reduced pH were observed in the experiments (Table 2). The urease immunogold particles were distributed in a similar manner to those incubated for 15 min at neutral pH (Fig. 4a) and acidic pH (Fig. 4b). It was also observed by immunoelectron microscopy that the urease gold particles in *H. pylori* incubated for 2 days at acidic pH were gathered and associated with UreI gold particles (Fig. 4c).

Recent reports have shown that it is cytoplasmic rather than surface-associated urease that is required for the acid resistance of *H. pylori* (Rektorschek *et al.*, 1998; Scott *et al.*, 1998; Weeks & Sachs, 2001). It is unclear where cytoplasmic urease neutralizes pH in a bacterial cell under acidic conditions. Urea enters the bacterial cytoplasm through the proton-gated urea channel, UreI, after exposure of the cell to a medium of pH 6.0 or less (Marcus & Scott, 2001; Rektorschek *et al.*, 1998; Scott *et al.*, 1998; Athmann *et al.*, 2000). The urease of *H. pylori* catalyses the degradation of urea to ammonia to protect the bacterium from harmful effects of acid. However, if urea in large amounts is transported into the cytoplasm and ammonia is generated equally throughout the cytoplasm, there could be lethal protein degeneration due to alkalization of the cytoplasm. The bacterium does not survive at $pH_{in} > 8.5$. (Meyer-Rosberg *et al.*, 1996).

Fig. 4. Electron photomicrographs of *H. pylori* incubated for 2 days and then double immunostained with anti-UreA and anti-UreI antibodies. Immunogold particles are distributed in a similar manner to those in the bacterial cell incubated for 15 min at neutral pH (a) and acidic pH (b). (c) Representative of (b) at a higher magnification showing that the urease gold particles in *H. pylori* after 2 days of incubation assembled and were associated with UreI gold particles. Original magnification, $\times 15\,000$.

Table 1. Distribution of urease immunoreactivity in *H. pylori* after a 15 min incubation

Double indirect immunolabelling was performed using mouse monoclonal anti-urease anti-serum, rabbit polyclonal anti-UreI antiserum and 5 and 10 nm gold particle-labelled goat anti-mouse IgG and anti-rabbit IgG.

Bacteria	pH	Urea	Number of gold particles (μm^{-2})*		
			Outer	Medium	Inner
Wild-type	7	+	90.3 $\pm$ 45.8	120.9 $\pm$ 39.7	131.6 $\pm$ 63.3
	7	–	111.0 $\pm$ 64.6	111.9 $\pm$ 37.1	121.9 $\pm$ 27.0
	5	+	205.0 $\pm$ 75.0	88.3 $\pm$ 36.3	70.7 $\pm$ 43.8
	5	–	213.9 $\pm$ 51.3	111.2 $\pm$ 85.8	47.1 $\pm$ 66.1
<i>ureI</i> -deletion mutant	7	–	132.0 $\pm$ 28.7	156.2 $\pm$ 55.2	192.4 $\pm$ 88.7
	5	–	114.6 $\pm$ 44.6	184.5 $\pm$ 86.1	177.3 $\pm$ 90.6

*The areas of visible bacterial cells were measured using photomicrographs taken at a magnification of $\times 30\,000$ using an optical image analyser. The number of gold particles (μm^{-2}) was counted in each portion of 10 bacterial cells. Outer, area extending from a depth of one-sixth of the diameter to the surface of the cell; medium, area between the outer and inner portions extending from one- to two-sixths of the diameter; inner, central area of the cell extending beyond two-sixths of the diameter to the centre of the cells.

Table 2. Distribution of urease immunoreactivity in cells of *H. pylori* after 2 days of incubation

Double indirect immunolabelling was performed using mouse monoclonal anti-urease anti-serum, rabbit polyclonal anti-UreI antiserum and 5 and 10 nm gold particle-labelled goat anti-mouse IgG and anti-rabbit IgG.

Bacteria	pH	Urea	Number of gold particles (μm^{-2})*		
			Outer	Medium	Inner
Wild-type	7	+	52.3 $\pm$ 26.3	99.9 $\pm$ 28.1	173.7 $\pm$ 48.8
	7	–	34.6 $\pm$ 8.6	59.6 $\pm$ 22.6	150.1 $\pm$ 60.8
	5	+	57.6 $\pm$ 19.1	57.0 $\pm$ 12.7	33.3 $\pm$ 19.6
	5	–	72.5 $\pm$ 21.6	69.9 $\pm$ 19.2	40.8 $\pm$ 20.0

*The areas of visible bacterial cells were measured using photomicrographs taken at a magnification of $\times 30\,000$ using an optical image analyser. The number of gold particles (μm^{-2}) was counted in each portion of 10 bacterial cells. Outer, area extending from a depth of one-sixth of the diameter to the surface of the cell; medium, area between the outer and inner portions extending from one- to two-sixths of the diameter; inner, central area of the cell extending beyond two-sixths of the diameter to the centre of the cells.

The catalytic expression of cytoplasmic urease is regulated by a mechanism that is closely associated with extracellular pH. Thus, we hypothesized that cytoplasmic urease molecules shift toward the inner membrane and assemble with UreI molecules at acidic pH. Assembly of urease and UreI has been demonstrated biochemically (Volland *et al.*, 2003). To establish this hypothesis in intact bacteria using quantitative contrast-enhanced immunoelectron microscopy (Hong

et al., 2000), we examined whether urease is redistributed to the bacterial inner membrane at low pH. The redistribution of urease was observed with a change in medium pH and was associated with the presence of UreI molecules. This was not observed in the *ureI*-deletion mutant and was independent of the presence of urea.

UreI is required for an acidic pH-induced increase in

cytoplasmic urease activity in *H. pylori* (Scott *et al.*, 2000). The data presented here show that when UreI is activated by an acidic medium pH, urease moves closer to the source of urea and presumably ammonia production occurs at or near the inner membrane. This eliminates the necessity of generalized cytoplasmic alkalization due to intra-bacterial urease.

In the protein–protein interaction map of *H. pylori* urease, the urease operon that encodes UreI may be associated with the transmission of proton motive force to outer membrane receptors (Rain *et al.*, 2001). When UreI is present, acidic medium pH activation of cytoplasmic urease is observed and urease activity in intact bacteria at neutral pH depends on the presence of UreI, indicating that urease activity is membrane limited (Scott *et al.*, 2000). In this study, we confirmed that the *ureI*-deletion mutant does not induce neutralization of an acidic medium. It was also observed that the urease gold particles in *H. pylori* were associated with UreI gold particles in immunoelectron microscopy. Urease activity was associated with the presence of UreI, and urease and UreI may assemble a supramolecular complex in an acidic medium.

This is the first report that cytoplasmic molecules in bacteria are trafficked to the membrane with a change in environmental conditions outside the cell. We propose that *H. pylori* possesses a proton-regulated urease redistribution mechanism, which may be a new target for anti-*H. pylori* therapy.

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REFERENCES

- 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.
- Bode, G., Malfertheiner, P., Lehnhardt, G., Nilius, M. & Ditschuneit, H. (1993). Ultrastructural localization of urease of *Helicobacter pylori*. *Med Microbiol Immunol (Berl)* **182**, 233–242.
- Bohnen, J. M., Krajden, S., Anderson, J. G., Kempston, J. D., Fuksa, M., Karmali, M. A., Osborne, A. & Babida, C. (1986). *Campylobacter pyloridis* is associated with acid-peptic disease in Toronto. *Can J Surg* **29**, 442–444.
- Dunn, B. E. & Phadnis, S. H. (1998). Structure, function and localization of *Helicobacter pylori* urease. *Yale J Biol Med* **71**, 63–73.
- Ha, N. C., Oh, S. T., Sung, J. Y., Cha, K. A., Lee, M. H. & Oh, B. H. (2001). Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nat Struct Biol* **8**, 505–509.
- Hong, W., Morimatsu, S., Goto, T. & 8 other authors (2000). Contrast-enhanced immunoelectron microscopy for *Helicobacter pylori*. *J Microbiol Methods* **42**, 121–127.
- Kurosawa, M., Kikuchi, S., Inaba, Y., Ishibashi, T. & Kobayashi, F. (2000). *Helicobacter pylori* infection among Japanese children. *J Gastroenterol Hepatol* **15**, 1382–1385.
- Marcus, E. A. & Scott, D. R. (2001). Cell lysis is responsible for the appearance of extracellular urease in *Helicobacter pylori*. *Helicobacter* **6**, 93–99.
- McGowan, C. C., Cover, T. L. & Blaser, M. J. (1996). *Helicobacter pylori* and gastric acid: biological and therapeutic implications. *Gastroenterology* **110**, 926–938.
- 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.
- Mollenhauer-Rektorschek, M., Hanauer, G., Sachs, G. & Melchers, K. (2003). Expression of the acid-activated UreI urea channel is required for both infection and colonization by *Helicobacter pylori* in gerbil stomach. *Res Microbiol* (in press).
- Nilius, M., Bode, G., Lehnhardt, G. & Malfertheiner, P. (1991). *In vitro* inhibition of *Helicobacter pylori* urease: biochemical and ultrastructural analysis. *Eur J Clin Invest* **21**, 551–557.
- Rain, J. C., Selig, L., De Reuse, H. & 10 other authors (2001). The protein–protein interaction map of *Helicobacter pylori*. *Nature* **409**, 211–215.
- Rektorschek, M., Weeks, D., Sachs, G. & Melchers, K. (1998). Influence of pH on metabolism and urease activity of *Helicobacter pylori*. *Gastroenterology* **115**, 628–641.
- 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.
- 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.
- Voland, P., Marcus, E. A., Weeks, D. L., Prinz, C., Sachs, G. & Scott, D. R. (2003). Interactions of the seven *Helicobacter pylori* proteins encoded by the urease gene cluster. *Am J Physiol* (in press).
- Wadström, T. (1995). An update on *Helicobacter pylori*. *Curr Opin Gastroenterol* **11**, 69–75.
- Warren, J. R. & Marshall, B. (1983). Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **i**, 1273–1275.
- Weeks, D. L. & Sachs, G. (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.