Helicobacter pylori perceives the quorum-sensing molecule AI-2 as a chemorepellent via the chemoreceptor TlpB

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INTRODUCTION

Directed motility in response to environmental chemical cues, a process called chemotaxis, is an important trait for many bacterial pathogens. Helicobacter pylori, a Gram-negative gastric pathogen, is proposed to situate itself within the gastric mucosa in response to specific chemical cues such as pH (Schreiber et al., 2004). Dysregulation of chemotaxis compromises the ability of H. pylori to colonize the murine and gerbil stomach, and results in its abnormal distribution and modulation of inflammation in this tissue (Foynes et al., 2000; McGee et al., 2005; Terry et al., 2005; Williams et al., 2007).

The chemotactic response, which has been studied most extensively in Escherichia coli, is mediated by chemoreceptors termed methyl-accepting chemotaxis proteins (MCPs) (Armitage, 1999). The H. pylori chemosensory machinery is similar but not identical to that of E. coli. The core signal-transduction apparatus (CheW, CheA and CheY) is present in both bacteria, but H. pylori also possesses three CheV proteins composed of both CheW and response-regulator motifs (Lowenthal et al., 2009; Pittman et al., 2001). The H. pylori genome encodes four chemoreceptors, TlpA, TlpB, TlpC and TlpD (originally called HylB or HlyB), although strain G27 does not express TlpC due to a frameshift mutation in the gene. Unlike peritrichous E. coli cells, which exhibit forward swimming and tumbling behaviour, we and others have observed that H. pylori cells swim forward, reverse and stop (Lowenthal et al., 2009; Schweinitzer et al., 2008). Once the cells stop, they usually resume movement in a different direction, thus allowing them to explore new spaces, similar to the tumbling behaviour of E. coli.

Only a handful of chemotactic signals have been demonstrated for H. pylori chemoreceptors. In strain 26695 the chemoreceptor TlpA has been shown to respond to AI-2 (Schreiber et al., 2004; Williams et al., 2007).
arginine and bicarbonate (Cerda et al., 2003), and in strain SS1 the chemoreceptor TlpB has been demonstrated to respond to acid (Croxen et al., 2006). The intracellular chemoreceptor TlpD has been described as an energy sensor, although the molecular nature of the cues it perceives is not known (Schweinitzer et al., 2008). There are no known ligands for TlpC.

One environmental condition that *H. pylori* experiences is the endogenously produced quorum-sensing molecule autoinducer-2 (AI-2) (Forsyth & Cover, 2000; Joyce et al., 2000; Rader et al., 2007). Autoinducers (AIs) are bacterially produced extracellular signalling molecules that trigger quorum sensing, a form of bacterial cell–cell communication (Ng & Bassler, 2009). AI concentration increases as a function of bacterial cell density, and at critical concentration thresholds it initiates coordinated gene regulation. Several types of quorum-sensing systems have been characterized. Typically, Gram-positive and Gram-negative bacteria use oligopeptides and acylated homoserine lactones as AIs, respectively. Many Gram-positive and Gram-negative bacteria possess a quorum-sensing system that produces the furanone signal AI-2, which functions as a signal for interspecific communication. AI-2 is produced as a metabolic byproduct of the reaction carried out by LuxS, which cleaves S-ribosylhomocysteine, producing homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD). DPD undergoes rapid dehydration and cyclization, existing in equilibrium as several molecules collectively termed AI-2 (Schauer et al., 2001). In many bacteria, LuxS is a key enzyme in the production of the activated methyl donor, Sadenosylmethionine (SAM); however in *H. pylori*, the homocysteine produced by LuxS is metabolized to produce cysteine (Doherty et al., 2010).

Previous reports have shown that several *H. pylori* strains produce AI-2 in a luxS-dependent fashion, as detected through a *Vibrio harveyi* luminescence assay (Forsyth & Cover, 2000; Joyce et al., 2000; Lee et al., 2006; Rader et al., 2007; Shen et al., 2010). Mutation of the luxS gene has been associated with reduction of the flagellin flaA transcript and protein, enhanced biofilm formation, decreased motility on soft agar plates, and colonization defects in mouse and gerbil infection models (Cole et al., 2004; Lee et al., 2006; Loh et al., 2004; Osaki et al., 2006; Rader et al., 2007). We have previously reported that LuxS-produced AI-2 functions as a signal molecule that modulates transcript levels of the flagellar regulator FlhA, thereby influencing global flagellar regulation (Rader et al., 2007). Although it is clear that AI-2 signalling affects flagellar gene expression, we found that the flagella in luxS mutants are morphologically normal and thus the altered flagellar gene regulation in this strain may not explain fully its motility defect on soft agar plates. Because colonial expansion in these plates requires both motility and chemotaxis, we sought to examine how AI-2 affects the latter process. In this study we identify a novel function for AI-2 as a chemorepellent that is sensed by the chemoreceptor TlpB.

**METHODS**

**Bacterial strains and culture conditions.** *H. pylori* strain G27 and its isogenic mutants were used in this study and are listed in Table 1. All *H. pylori* strains were maintained on blood agar plates consisting of Columbia agar (Difco) and 5 % defibrinated horse blood (Hemostat) (CHBA), or CHBA plates supplemented with 0.02 mg β-cyclodextrin ml⁻¹ (Sigma), 8 mg amphotericin B ml⁻¹ (Sigma) and 20 μg vancomycin ml⁻¹ (Sigma), and incubated at 37 °C in 10 % CO₂. Selective plates were supplemented with 10–15 μg kanamycin ml⁻¹ (Fisher), 18 μg metronidazole ml⁻¹, 10 μg chloramphenicol ml⁻¹ or 80 mM sucrose. *H. pylori* liquid medium (BB10) consisted of filtered sterilized Brucella broth (Difco) supplemented with 10 % fetal bovine serum (Gibco) and 20 μg vancomycin ml⁻¹ (Sigma). Liquid cultures were grown in 50 ml conical tubes (BD Falcon) with loosened lids shaking at 37 °C in anaerobic jars (Oxoid) with CampyGen microaerobic sachets (Oxoid) or in glass test tubes (20 × 148 mm) shaking in a 37 °C/8 % CO₂ incubator.

**Table 1.** Strains used in this study

<table>
<thead>
<tr>
<th><em>H. pylori</em> strain</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G27 (KO01)</td>
<td>Wild-type</td>
<td>Covacci et al. (1993)</td>
</tr>
<tr>
<td>luxS (BR08)</td>
<td>G27, luxS::kan sacB</td>
<td>Rader et al. (2007)</td>
</tr>
<tr>
<td>ΔluxS (BR09)</td>
<td>G27, deletion of luxS</td>
<td>Rader et al. (2007)</td>
</tr>
<tr>
<td>luxS* (BR10)</td>
<td>G27, deletion of luxS and rdx::luxS</td>
<td>Rader et al. (2007)</td>
</tr>
<tr>
<td>mG27 (KO625)</td>
<td>Mouse-passaged strain G27</td>
<td>Castillo et al. (2008)</td>
</tr>
<tr>
<td>ΔtlpA (KO1002)</td>
<td>mG27, deletion of tlpA</td>
<td>This study</td>
</tr>
<tr>
<td>tlpB (KO1003)</td>
<td>mG27, tlpB::kan-sacB</td>
<td>This study</td>
</tr>
<tr>
<td>ΔtlpB (KO1004)</td>
<td>mG27, deletion of tlpB</td>
<td>This study</td>
</tr>
<tr>
<td>tlpB* (BR31)</td>
<td>mG27, deletion of tlpB and rdx::tlpBSS1-aphA-3</td>
<td>This study</td>
</tr>
<tr>
<td>luxS tlpB (CW30)</td>
<td>mG27, tlpB::cat and luxS::kan sacB</td>
<td>This study/Williams et al. (2007)</td>
</tr>
<tr>
<td>tlpC (KO1005)</td>
<td>mG27, tlpC::aphA-3</td>
<td>This study</td>
</tr>
<tr>
<td>tlpD (KO1006)</td>
<td>mG27, tlpD::cat (made using pL30A2cat12)</td>
<td>This study/Williams et al. (2007)</td>
</tr>
<tr>
<td>cheA (KO629)</td>
<td>mG27, cheA::cat reverse (made using pKT22)</td>
<td>This study/Terry et al. (2005)</td>
</tr>
<tr>
<td>tlpB cheA (CW31)</td>
<td>G27, tlpB::kan sacB and cheA::cat reverse</td>
<td>This study</td>
</tr>
</tbody>
</table>
Construction of H. pylori mutants. All transformations were performed using natural transformation. PCR for cloning purposes was done with either Pfu Turbo (Stratagene) or Phusion (NEB), and all DNA manipulation enzymes were from New England Biolabs. The G27 luxS, ΔluxS, and ΔluxS isogenic strains (strains BR08, BR09 and BR10, respectively) were constructed as previously described (Rader et al., 2007). Throughout, we use ‘Δ’ to designate deletion mutations, in which most of a gene’s coding sequence is removed by allelic exchange, and the superscript ‘*’ to designate complemented strains in which most of a gene’s coding sequence is removed by allelic exchange, and the superscript ‘*’ to designate complemented strains.

The cheA mutant (strain KO629) was created by transforming H. pylori mG27 with pKT22 (Terry et al., 2005) and selecting for resistance on chloramphenicol CHBA.

ΔtlpB (strain KO1004) was made in two steps. First, mG27 was transformed to kanamycin resistance (KmR) by the plasmid pTClB11, which bears a tlpB::kan-sacB allele. The correct nature of this mutation was verified by PCR using primers that flank the insertion site. pTClB11 was made starting with pTCB101, which has tlpB from strain SS1 cloned into pBluescript (McGee et al., 2005). pTCB101 was then subjected to iPCR using primers tlpB-30 and tlpB-40 (sequence in McGee et al., 2005). The kan-sacB construct was obtained from pKSII (Copass et al., 1997) by digesting with SmaI and Xhol. The kan-sacB and tlpB flanking pieces were ligated using T4 ligase. The correct nature of the plasmid was verified by digestion. In this construct, tlpB is transcribed in the same direction as sacB (opposite to aphA-3). This strain is called KO1003. KO1003 was then transformed with a plasmid bearing a large deletion of tlpB, pTClB113, following by selection for sucrose resistance (SucC). pTClB113 was created using iPCR with primers tlpB-30 and tlpB-40, followed by intramolecular ligation of the plasmid. The deletion removes all of tlpB except the first 27 and the last 17 amino acids, although the deletion is +1 out of frame, resulting in a 3’ end that encodes a different sequence with three additional amino acids. The tlpB region from SucC, kanamycin-sensitive (KmR) colonies was amplified using PCR with primers that flank tlpB. This PCR product was sequenced to verify that tlpB was deleted.

To create the tlpB* complemented strain (strain BR31), mG27 ΔtlpB mutant bacteria were naturally transformed with a construct encoding the rdxA locus with an insertion composed of the full-length tlpB gene, expressed from the cheY promoter, and cloned upstream of the non-polar aphA-3 cassette. Disruption of the rdxA locus confers metronidazole resistance, and the non-polar aphA-3 cassette contains two internal ribosome-binding sites flanking the kanamycin-resistance gene conferring that resistance. Transformants were first screened on kanamycin plates, further screened on metronidazole-supplemented plates, and verified by PCR amplification and sequencing of the genomic locus.

The tlpA mutant (strain KO1002) was made in two steps by first transforming mG27 to KmR with pTA12, which bears a tlpA::kan-sacB allele, to create strain KO1001, followed by transformation with a plasmid that bears a tlpA deletion (pTA14). pTA12 was made from pTA10, which has tlpA from strain SS1 cloned, with ~500 bp flanking sequence, by PCR amplification with primers TlpA6 (5’-ATTGAGCGCAAAAATAGGGG-3’) and TlpA7 (5’-TTTTCTCTGCGCAAGGCGTTGC-3’). This product was phosphorylated with T4 polynucleotide kinase, and then cloned into EcoRV-digested pBluescript K5+ (Stratagene) to create pTA10. To create pTA12, iPCR was carried out with pTA10 template plus primers TlpA12 (5’-CACCACAAATAAGTTTATATTAGGCA-3’) and TlpA13 (5’-CAAGAAATGCAAAAAGGCTCCTAAGG-3’) to create an in-frame deletion that leaves a region coding for 14 amino acids at the 5’ end, and 19 amino acids at the 3’ end of tlpA. This product iPCR was then ligated to a kan-sacB fragment that came from pKS2. pKS2 derived from pKS1, which was made via a three-piece ligation of the following pieces: (1) the aphA3 gene (also called kan) isolated from pBS-kan (Terry et al., 2005) by cutting with SmaI and Xhol, followed by dephosphorylation; (2) the sacB gene from pKSII (Copass et al., 1997) generated by cutting with BamHI, blunting with T4 polymerase followed by cutting with PstI; (3) the vector backbone from pBluescript K5+ cut with PstI and Xhol, followed by phosphorylation and gel purification of the 3 kb piece. These three pieces were ligated together to generate pKS1. pKS1 has the aphA3 promoter driving transcription of both aphA3 and the downstream sacB gene. To create pKS2, pKS1 was digested with PvuII to remove the bla gene followed by self-ligation. For cloning of the aphA3-sacB fragment from pKS2, PCR amplification using primers reverse (universal) and sacBend2 (5’-CTTCTTCCCTTTTATTCTTTGGG-3’) was carried out, to amplify aphA3-sacB without the sacB transcriptional terminator, and the resulting fragment was ligated with the PCR fragment of pTA10 to create pTA12. In pTA12, tlpA, aphA3 and sacB are all in the same transcriptional orientation. To create pTA14, the same pTA10 iPCR fragment was self-ligated. H. pylori KO1001 was then transformed with pTA14, followed by selection for sucrose resistance and screening for kanamycin sensitivity. The tlpA allele was PCR amplified from SucC KmR colonies using primers tlpA10 (5’-TCTAAGGTGGTATGAATCGGG-3’) and tlpA11 (5’-GCTGGAATCTAGAAAATGCTTATTCTCATTAC-3’), and sequenced to verify the correct deletion. This tlpA strain is also called mG27 tlpA or KO1002.

The mG27 tlpC mutant is tlpC::aphA3 (strain KO1005). This mutant was constructed by transforming mG27 with plasmid pKO150. This plasmid was made using iPCR of a plasmid containing tlpC from SS1 called pTC100 (Andermann et al., 2002) with primers tlpC31 (5’-TCTACGAAATTTTGGAGG-3’) and tlpC40 (5’-CTTAAAAGTATTGTCGAGG-3’). The product, 3.5 kb, was ligated with the aphA3 gene prepared by PCR from pBS-kan with primers Kanup (5’-GGCAGATCAGGATACCGAACG-3’) and Kandown (5’-GGCCAAAGCTTTTGTGATGATGACG-3’). The resulting product was phosphorylated with T4 kinase, and ligated to the pTC100 iPCR product to create pKO150. The tlpD mutant was made by transforming mG27 to chloramphenicol resistance using plasmid pLM02Acat2 (Williams et al., 2007), which creates tlpD::cat. Note that tlpD was previously referred to as hylB.

To generate strain CW30, with mutations in both luxS and tlpB genes, we transformed H. pylori mG27 tlpB::cat (Williams et al., 2007) with PCR DNA generated from luxS::kan sacB genomic DNA and selected for kanamycin resistance. This luxS::kan-sacB DNA was amplified with primers luxSfor (5’-AAGCGTCGGATTACGCGATG-3’) and luxSrev (5’-AAGGGCACGGCGTGAATGCTTGCA-3’).
To generate the bacterial strain CW31 with mutations in both cheA and tlpB genes, we first created a tlpB::kan-sacB mutation in wild-type G27 bacteria and then added the cheA::cat reverse mutation. To analyze the DNA for the tlpB::kan-sacB mutation, SS1 tlpB was amplified from the pK1337 plasmid using SS1TlpBF (5'-TAAG-GCGTTAGAGACGCTTTGCT-3') and SS1TlpBR (5'-AAACACG-CGGTGATACAGAACC-3') primers and ligated into the pCR 2.1-TOPO plasmid using the TOPO TA Cloning kit (Invitrogen), creating pTOPO-tpB. The kan-sacB cassette (Copass et al., 1997) was amplified from the pKSF-II plasmid using primers kansacBF (5'-CTCCATGTTTCGGCGAACCATTTGAGGTGA-3') and kansacBR (5'-GACTGAGGTTCGGCGGTTAATGACGCGGACGTCG-3'). The kan-sacB cassette was inserted into the HincII restriction site at base-pair position 735 of the tlpB gene, creating pTOPO-tpB::kansacB. The tlpB::kan-sacB sequence was amplified from pTOPO-tpB::kansacB and transformed into naturally competent G27 H. pylori. Positive tlpB::kan-sacB transformants were selected by kanamycin resistance and confirmed via PCR using primers TlpBF (5'-ACTTCAAAAGGGAGGACT-3') and TlpBR (5'-AACACGG-CGGTGATACAGAACC-3'). The tlpB::kan-sacB bacteria were transformed with PCR DNA generated from cheA::cat reverse genomic DNA, and selection for chloramphenicol resistance was applied. The correct genotype was confirmed by PCR. The cheA::cat reverse DNA was amplified with primers CheA_For1 (5'-GTGCTGAAAGGCTTAAAGAATG-3') and CheA_Rev1 (5'-GGATAATGCGCTTGTCGCG-TG-3').

Bioluminescence assay and synthetic DPD. Synthetic DPD, a kind gift from Martin Semmelhack and Bonnie Bassler (Princeton University), was prepared as described by Semmelhack et al. (2005). This DPD was synthesized with a protecting group that we removed by treatment with 15 mM H₂SO₄, followed by neutralization to pH 6.9 with potassium phosphate. As a mock DPD treatment, we neutralized 15 mM H₂SO₄ to pH 6.9 with potassium phosphate. To assay bacterial taxis away from a source of chemical repellent, we adopted the previously described barrier formation assay (Croxon et al., 2006). Wild-type mG27, ΔtlpA, ΔtlpB, ΔtlpB*, ΔtlpC, ΔtlpD and cheA isogenic strains were grown overnight, with shaking, in BB10 to an OD₆₀₀ of 1.0, followed by 2 h without shaking, as above. Samples (8 µl) of culture were spotted onto the centre of a glass slide and covered with a no. 1, 22 × 22 mm wide glass coverslip. Three sides of the coverslip were sealed with clear fingernail polish. Eight microlitres of 0.1 M HCl, 0.1 mM synthetic DPD, or mock DPD solution was placed on the open side of the coverslip. After 5–10 min incubation, the slides were examined and still images were taken using Scion Image software at ×10 through a ×40 phase filter, conditions that mimic dark-field microscopy.

Western blotting. Total cell proteins were prepared from H. pylori cultured on CHBA plates for 2 days by resuspending and lysing the cells in 2× Laemmli sample buffer. Samples were separated on a 10 % SDS-PAGE gel, transferred to Immunoblot PVDF membranes (Bio-Rad) and incubated with 1:5000 dilution of anti-GST_TlpA22 (Williams et al., 2007). Rabbit polyclonal antibody recognizes the conserved CheW-interacting methyl-accepting domain of all H. pylori chemoreceptors. For visualization, the blots were incubated with the secondary antibody goat anti-rabbit-HRP (Santa Cruz Biotech) at a dilution of 1:2000, followed by incubation with luminol, p-coumaric acid and hydrogen peroxide. Luminescent blots were visualized by exposure to Biomax Light film (Kodak).

Statistical analysis. The frequency of stops was analysed statistically using either one-way ANOVA (www.physics.csbsju.edu/cgi-bin/stats/anova_pnp) when strains were tested or two-way ANOVA (http://faculty.vassar.edu/lowry/anova2u.html) when both strains and chemical treatment were tested. Post-hoc multiple comparisons to identify significance within individual hypotheses were performed using t-tests, and the P-values were adjusted using Bonferroni correction to correct for an increase in type I error. P-values of <0.001 were considered significant and used for Bonferroni correction.

RESULTS

**luxS** is required for normal swimming behaviour of *H. pylori*

When examining the swimming behaviour of wild-type and an isogenic luxS deletion mutant (referred to as ΔluxS) of *H. pylori* strain G27 (Rader et al., 2007), we observed that the ΔluxS mutant displayed a propensity for swimming in a straight line (running), as opposed to the wild-type, which exhibited more typical run-stop-run behaviour. We quantified this behaviour in bacterial cultures using video microscopy to observe swimming behaviour. We found that the ΔluxS mutant exhibited significantly fewer stops per second than the wild-type strain (Fig. 1). Complementation of the luxS mutation with a wild-type copy of the luxS gene at the rdxA locus [referred to as luxS* (Rader et al., 2007)] significantly increased the number of stops per second as compared with the ΔluxS strain (Fig. 1). It should be noted that over the course of these experiments, we observed some variation between trials in the average number of stops per second exhibited by the strains, but the responses to chemicals and differences between genotypes were reproducible across experiments. We attribute these differences to factors that
were difficult to control across all experiments, such as differences in the lots of serum used in the media.

**The LuxS product, AI-2, is a chemorepellent for *H. pylori***

To test whether the decrease in the number of stops in the ΔluxS mutant relative to the G27 wild-type and luxS* complemented isogenic strains was due to the loss of the AI-2 signal, we treated each strain with 0.1 mM synthetic DPD, corresponding to the approximate concentration of AI-2 in the cell-free supernatant of a early stationary-phase culture of wild-type *H. pylori* (Rader *et al.*, 2007). We also treated each isogenic strain with sterile water, the DPD diluent, as a negative control. Bacterial cultures were incubated with treatment for 10 min before video microscopy, and the number of stops that individual bacteria performed during 5 s was recorded. Each strain was treated with water, mock DPD, 0.1 M HCl and 0.1 mM synthetic DPD. The number of individual bacterial cells scored (n) for each condition is indicated below the column; bars indicate se. * indicates each strain that is statistically significant different from all other strains within a treatment, + indicates chemorepellent treatments (HCl, synthetic DPD) that are significantly different from controls (water, mock DPD) within a strain (P<0.001 with Bonferroni correction).

The swimming behaviours exhibited in all three isogenic strains upon addition of synthetic DPD were strikingly similar to the reported repellent response of *H. pylori* (Croxen *et al.*, 2006). Beyond this barrier, bacterial movement resembled movement proximal to the source of the acid. This increased movement proximal to the acid source ended in the formation of a visible barrier of bacterial cells toward the centre of the coverslip that accumulated presumably at a preferred concentration of the chemical treatment (Fig. 2C). Beyond this barrier, bacterial movement resembled that of the untreated controls. These observations show that *H. pylori* strain G27 responds to pH similarly to strain SS1 (Croxen *et al.*, 2006). When treated with 0.1 mM synthetic DPD, the cultures exhibited a response that was similar to that to acid: the cells showed increased frequency of stops and formed a bacterial barrier at a similar location away from the chemical source (Fig. 2D). In response to mock DPD, the percentage of motile bacteria in the population increased, but there was no obvious net directional movement, and after 5 min no bacterial barrier

![Graph](image-url)

**Fig. 1.** LuxS is required for normal swimming behaviour of *H. pylori*. Swimming behaviours of G27 wild-type, ΔluxS mutant and luxS* complemented isogenic strains were observed by video microscopy, and the number of stops that individual bacteria performed during 5 s was recorded. Each strain was treated with water, mock DPD, 0.1 M HCl and 0.1 mM synthetic DPD. The number of individual bacterial cells scored (n) for each condition is indicated below the column; bars indicate se. * indicates each strain that is statistically significant different from all other strains within a treatment, + indicates chemorepellent treatments (HCl, synthetic DPD) that are significantly different from controls (water, mock DPD) within a strain (P<0.001 with Bonferroni correction).
had formed and the bacteria were evenly distributed underneath the coverslip (Fig. 2B). Our data thus suggest that *H. pylori* cells respond chemotactically to synthetic DPD similarly to how they respond to HCl.

The chemotaxis receptor TlpB is required for chemotaxis away from AI-2

Bacterial tactic movement toward or away from a chemical source is generally regulated by the chemotaxis system. Key to this system are the chemoreceptors which sense environmental signals and transmit the information through a transduction cascade, eventually affecting flagellar rotation (Armitage, 1999). To test whether the response to synthetic DPD was due to detection by any of the four *H. pylori* chemoreceptors, we repeated the wet-mount assay described above, using mouse-passaged *H. pylori* mG27 isogenic strains harbouring deletions or insertional mutations in the chemoreceptor genes *tlpA* (strain ΔtlpA), *tlpB* (strain ΔtlpB), *tlpC* (strain ΔtlpC) or *tlpD* (strain ΔtlpD). The wild-type strain mG27 does not produce TlpC as detectable by Western blotting (Fig. 3A), but we included the engineered *tlpC* mutant in our analysis for completeness. Each of these strains was challenged with 0.1 mM synthetic DPD, 0.1 M HCl or mock DPD. As a negative control for chemotaxis we employed a non-chemotactic mutant with a mutation in the chemotransduction gene *cheA*. The ΔtlpA, ΔtlpC and ΔtlpD mutants all formed bacterial barriers when challenged with synthetic DPD and HCl (Fig. 2F, G, L, M, O, P). As previously reported, the ΔtlpB mutant failed to form a bacterial barrier when challenged with HCl (Fig. 2I). In addition, this mutant failed to form a bacterial barrier when challenged with synthetic DPD (Fig. 2J). As expected, the *cheA* mutant did not form a bacterial barrier in response to HCl or synthetic DPD (Fig. 2R, S). None of the five strains produced bacterial barriers in response to mock DPD (Fig. 2E, H, K, N, Q). These data implicated TlpB in the sensing of AI-2.

To further characterize TlpB as a chemoreceptor for synthetic DPD, we repeated the video taxis assay with the mG27 wild-type, ΔtlpB and ΔtlpB* isogenic strains. The average number of stops for each genotype was recorded for each strain grown in mock DPD, 0.1 M HCl or 0.1 mM synthetic DPD. As reported previously (Croxen et al., 2006), we observed that the ΔtlpB mutant displayed an increased stopping frequency as compared to its wild-type parent in medium without added chemorepellents (see below). To best represent the relative responsiveness of the different strains to HCl and DPD, we therefore show the normalized stopping frequencies for each strain in each condition relative to the average number of stops exhibited by that strain in the mock DPD medium (Fig. 3C). In accordance with the wet-mount assay, the ΔtlpB mutant exhibited significantly reduced responsiveness to DPD and HCl as compared with the wild-type strain. The complemented *tlpB* strain had restored responsiveness to DPD and the bacteria were evenly distributed underneath the coverslip (Fig. 2B). Our data thus suggest that *H. pylori* cells respond chemotactically to synthetic DPD similarly to how they respond to HCl.
and greater responsiveness to acid than the wild-type strain. We do not know the reason for this strain’s hyper-responsiveness to acid, but it could be due to altered expression of the tlpB gene under acidic conditions when expressed from a different promoter and genomic locus than those of the native gene.

As noted above, the ΔtlpB mutant displayed an increased stopping frequency as compared to its wild-type parent when grown in medium in the absence of exogenously added chemorepellents (Fig. 4A). We wondered whether this increased frequency was due to a hyper-activation of the chemotaxis signal transduction pathway, or some other effect on swimming behaviour caused by the loss of tlpB. To distinguish these possibilities, we constructed a tlpB cheA double mutant and compared the swimming behaviour of this strain to the tlpB and cheA single mutants. As expected, the cheA single mutant, which lacks a functional chemotaxis signal transduction pathway, exhibited fewer stops than the wild-type strain in BB10 (Fig 4A). The tlpB cheA double mutant was indistinguishable from the cheA single mutant, indicating that the increased stopping observed with the tlpB mutant was due to increased activation of the chemotaxis pathway, possibly as a result of increased signalling from other chemoreceptors in the absence of TlpB.

**TlpB functions downstream of LuxS in Al-2 responses**

Our data suggest that TlpB functions as the chemoreceptor for avoidance responses to Al-2, a molecule produced by the bacteria themselves using the enzyme encoded by luxS. If this model were correct, then we would predict that cells lacking both LuxS, the enzyme that makes Al-2, and TlpB, the chemoreceptor involved in its perception, should resemble cells lacking only the receptor. We generated a luxS tlpB double mutant and compared its swimming behaviour to that of luxS and tlpB single mutants. We observed that the luxS tlpB double mutant exhibited a high stopping frequency, statistically indistinguishable from the tlpB single mutant (Fig. 4A). Importantly, whereas the luxS single mutant formed barriers to both DPD and HCl in the wet-mount assay (Fig. 4F, G), the luxS tlpB double mutant failed to form any barriers (Fig. 4I, J), similar to the tlpB single mutant.

**Fig. 3.** TlpB is required for chemotactic responses to HCl and synthetic DPD. (A) Western blot analysis of mG27 wild-type, ΔtlpB, and tlpB* isogenic strains with an antibody that recognizes a conserved region of all *H. pylori* chemoreceptors. (B) Chemotactic behaviour of the tlpB* complemented strain was assayed using the wet-mount assay in response to mock DPD, 0.1 M HCl and 0.1 mM synthetic DPD. (C) The normalized stopping frequency for mG27 wild-type, ΔtlpB and tlpB* isogenic strains treated with mock DPD, 0.1 M HCl and 0.1 mM synthetic DPD is shown, where the average number of stops for each strain in each condition is normalized to that strain’s average number of stops in mock DPD. The number of individual bacterial cells scored (n) for each condition is indicated below the column; bars indicate se. * indicates statistically significant differences in stopping frequency as compared to the mock DPD treatment of the same strain (P<0.001 with Bonferroni correction).
DISCUSSION

In this study we have shown that the quorum-sensing signal AI-2 acts as a chemorepellent for *H. pylori*, and demonstrated that the chemoreceptor TlpB is required for its perception. We previously reported that elimination of the *luxS* gene product in *H. pylori* exhibited a motility defect on soft agar (Rader *et al.*, 2007), which was complemented by exogenous AI-2, a result confirmed by others (Shen *et al.*, 2010). This assay cannot distinguish between dysfunction of the motility apparatus and dysregulation of chemotaxis. To better characterize the chemotactic response of *H. pylori* to the absence or presence of AI-2, we observed both swimming behaviour of individual bacterial cells and the response of populations of bacteria to chemical gradients. We found that *luxS*-deficient bacterial cells exhibited a decrease in stopping frequency as compared to wild-type cells. This behaviour was restored to wild-type levels by the addition of synthetic DPD. Synthetic DPD also increased the frequency of stops in the wild-type strain, and caused populations of wild-type cells to move away from a source of the chemical, suggesting that AI-2 is a chemorepellent for *H. pylori*. Our ability to modulate *H. pylori* swimming behaviour with exogenous AI-2 argues against a metabolic requirement for the *luxS* gene in chemotaxis. Additionally, although many bacteria regulate the sensitivity of their chemoreceptors via methylation, a mechanism which could be influenced by function of the SAM pathway, *H. pylori* lacks the methyl-utilizing enzymes required for this process (Szurmant & Ordal, 2004). We further demonstrated that an *H. pylori* strain deficient for the chemoreceptor TlpB failed to move away from a source of synthetic DPD, and did not display increased stopping behaviour upon addition of synthetic DPD. These behaviours were restored upon genetic complementation of the *tlpB* gene.

**TlpB and AI-2 perception**

Most reported responses to AIs involve regulation of transcription. Recently, however, *E. coli* was shown to respond to AI-2 as a chemoattractant through a mechanism that involves both the AI-2 periplasmic binding protein LsrB and the chemoreceptor Tsr (Bansal *et al.*, 2008; Hegde *et al.*, 2011). Here we show that *H. pylori* also perceives AI-2 as a chemical cue in response to which it directs its movement, in this case by moving away from the signal. We demonstrate that the *H. pylori* chemoreceptor TlpB is required for perception of AI-2 and confirm its role in negative pH taxis. Bacterial chemoreceptors can often sense more than one ligand and can elicit both positive and negative behavioural responses. For example, the *E. coli* chemoreceptor Tsr not only senses AI-2, but also mediates taxis toward the attractants serine and related amino acids, and taxis away from weak acids, indole and leucine, and transduces oxygen and redox signals (Boyd & Simon, 1982; Rebbapragada *et al.*, 1997). We do not yet know whether TlpB senses AI-2 directly or via other binding proteins. TlpB shares no sequence similarity with the previously identified AI-2 binding proteins, *V. harveyi* LuxP (Chen *et al.*, 2002), *S. typhimurium* LsrB (Miller *et al.*, 2004) and *Aggregatibacter actinomycetemcomitans* RsbB and LsrB (Shao *et al.*, 2007), and the *H. pylori* genome lacks homologues of any of these genes. We believe that the molecular mechanism of chemotaxis from AI-2 is distinct
from AI-2-mediated regulation of flhA transcription in H. pylori, because the latter does not require tlpB (B. A. R. and K. G., unpublished results). In addition, neither tlpB nor several other chemotaxis genes appear to be regulated transcriptionally by AI-2 because their transcript levels are the same in wild-type and ΔluxS mutant strains (Rader et al., 2007; B. A. R. and K. G., unpublished results).

**AI-2-regulated motility is likely to be important in the gastric environment**

For *H. pylori*, motility is essential for colonization of piglet, gerbil and mouse stomachs, where bacterial interactions with the mucosa promote gastric pathology (Eaton et al., 1992; McGee et al., 2002; O’Toole et al., 2000; Ottemann & Lowenthal, 2002). It is well established that non-chemotactic mutants (Che−) of *H. pylori* do not infect mice to the full wild-type level (Foynes et al., 2000; Terry et al., 2005). These mutants, lacking cheA, cheW or cheY, all engage almost exclusively in straight runs without stops or changes in direction. We found that the luxS mutant exhibits a similar swimming behaviour to the Che− mutants in broth, suggesting that LuxS is responsible for production of a significant proportion of chemorepellent signals present in *H. pylori* batch culture. We have confirmed previous reports that a luxS mutant is defective in colonization of the rodent stomach; however in these experiments it was not possible to determine whether the colonization defect was due to reduced AI-2 concentrations in the mouse stomach or metabolic deficiencies in the bacteria (Lee et al., 2006; Osaki et al., 2006), (B. A. R., K. G. and K. M. O., unpublished results). Whereas the ΔtlpB mutant colonizes wild-type mice and gerbils to normal levels (McCree et al., 2005; Williams et al., 2007), it is impaired in its ability to colonize the stomachs of IL-12-deficient mice (Croxen et al., 2006). There are likely to be additional chemical cues to which *H. pylori* responds within a mouse stomach (Schreiber et al., 2004). Indeed, non-chemotactic mutants are less closely associated with mouse gastric epithelia and induce a diminished inflammatory response as compared to wild-type *H. pylori*, suggesting that chemical cues from the epithelium are important for directing *H. pylori* localization within the stomach (Williams et al., 2007).

The fact that gastrointestinal pathogens have chemotactic responses to AI-2 raises the question of the role of this response in host colonization. In the case of enteropathogenic *E. coli*, the pathogen perceives AI-2 as an attractant, possibly using it as a cue to direct itself toward the bacteria-dense colon. We hypothesize that *H. pylori* perceives AI-2 both as a repellent and as a cue to regulate flagellar gene expression as a means to coordinate its distribution and motility within the stomach. We imagine that at some threshold level of AI-2, the bacteria respond to this molecule as a chemorepellent and move away from the bulk bacterial population, thereby avoiding niche competition and promoting dispersal throughout the stomach.

Consistent with this model, luxS mutants in several *H. pylori* strains have been reported to form biofilms more readily than their wild-type counterparts (Cole et al., 2004). With sustained AI-2 levels, indicative of high bacterial populations, the elevated AI-2 may alter FlhA levels to downregulate motility. Such a response might allow bacteria to avoid wasting energy on swimming and instead focus on adherence. Several bacterial species perform reciprocal regulation of motility and adherence (Holden & Gally, 2004), although this type of response has not been shown for *H. pylori*. Furthermore, our understanding of the gastric environment has changed in part due to 16S rRNA gene enumeration studies, which identified over 120 phylotypes of bacteria in the human stomach, many of which potentially produce AI-2 (Bik et al., 2006; Federle & Bassler, 2003). It is possible that *H. pylori* will move away from AI-2 produced by coincident bacteria, providing a further means of avoiding niche competition. As disease outcome is correlated with localization of *H. pylori* populations within the stomach (Blaser & Atherton, 2004), AI-2 may be an important environmental factor in the progression of disease in *H. pylori* infections. Further understanding of those factors that regulate *H. pylori* motility and chemotaxis within the gastric environment will undoubtedly enhance our ability to predict disease outcome and design therapies that can control *H. pylori*-caused chronic inflammation and subsequent progression to gastric cancer.

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