Supplementary Appendix

A variable homopolymeric G-repeat defines small RNA-mediated posttranscriptional regulation of a chemotaxis receptor in *Helicobacter pylori*

Sandy R. Pernitzsch, Stephan M. Tirier, Dagmar Beier, and Cynthia M. Sharma

This supplement contains:

Supplementary Methods
Figures S1 to S8
Tables S1 to S8
Supplementary References

Supplementary Methods

***Helicobacter* growth conditions.** *Helicobacter* strains were grown on GC-agar (Oxoid) plates supplemented with 10% (vol/vol) donor horse serum (Biochrom AG), 1% (vol/vol) vitamin mix, 10 µg/ml vancoymcin, 5 µg/ml trimethoprim, and 1 µg/ml nystatin. For transformant selection and growth of mutant strains, 20 µg/ml kanamycin, 16 µg/ml chloramphenicol or 10 µg/ml erythromycin were added. For liquid cultures, 15 or 50 ml Brain Heart Infusion medium (BHI, Becton, Dickinson and Company) supplemented with 10% (vol/vol) FBS (Biochrom AG) and 10 µg/ml vancoymcin, 5 µg/ml trimethoprim, and 1 µg/ml nystatin were inoculated with *Helicobacter* from plate to a final $OD_{600\,nm}$ of 0.02 – 0.05 and grown under agitation at 140 rpm in 25 cm^3 or 75 cm^3 cell culture flasks (PAA). Bacteria were grown at 37 °C in a HERAcell 150i incubator (Thermo scientific) in a microaerophilic environment (10% CO_2, 5 % O_2, and 85 % N_2).

Construction of *Helicobacter* mutants. All generated mutant strains are listed in Table S3 and were cloned by homologous recombination and natural transformation of PCR-amplified constructs carrying either the *aphA*-3 kanamycin (1), the *catGC* chloramphenicol (2) or *rpsL-erm* erythromycin resistance cassette (3) flanked by ~500 bp homology regions up- and downstream of the respective genomic locus as previously described (4). Briefly, *H. pylori*, grown from frozen stocks until passage two, was streaked in small circles on a fresh plate and grown for 6-8 h at 37 °C under microaerophilic conditions. For transformation, 500 ng to 1 µg purified PCR product was added to the cells. After incubation for 14-16 h at 37 °C, cells were re-streaked on selective plates with indicated antibiotics. Genomic DNA (gDNA) of mutants was isolated using the NucleoSpin Plasmid kit (Macherey & Nagel) and mutants were checked by PCR and sequencing.

Complementation of the Δ*repG* mutant with different RepG sRNA variants. To construct RepG complementation strains, the intergenic region of HP1043 and HP1044 including the *repG* gene under control of its own promoter together with the *catGC* resistance cassette (2) was inserted into the *rdxA* locus, which is frequently used for complementation in *H. pylori* (5). A *rdxA*(500up)-*catGC*-*repG*-*rdxA*(500down) complementation construct was amplified from gDNA of a RepG complementation strain (kindly provided by F. Darfeuille and J. Reignier, University of Bordeaux, France; for sequence details see Figure S8) using oligos CSO-0017/-0018. The amplified ~2.3 kbp PCR product was transformed into the Δ*repG* mutant (strain JVS-7014, ref. (6)). The obtained strain CSS-0046 (C_{RepG}) was verified by PCR using oligos CSO-0205/-0207. Furthermore, the *XhoI/XbaI* digested ~2.3 kbp PCR fragment was cloned into plasmid pJV752.1 (7), resulting in plasmid pSP39-3, which was used for further mutant generation of RepG.

The second stem-loop mutant of RepG (SL 2, Figure 2A) was constructed by PCR amplification of pSP39-3 using oligos CSO-0080/-0081. Upon *DpnI* digestion, the PCR product

was gel-purified, self-ligated, and transformed into *E. coli* Top 10 cells. Positive clones were selected on plates with 100 µg/ml ampicillin and confirmed by colony PCR using oligos pZE-A and CSO-0205. The resulting plasmid pSP42-1 was validated by sequencing with CSO-0206. Afterwards, a PCR product amplified from pSP42-1 using oligos CSO-0017/-0018 was used for complementation of the *H. pylori ΔrepG* mutant, resulting in strain CSS-0747 (SL 2). To construct the other RepG variants, ΔCU, 3xG and 1xG*, overlap extension PCR was performed as previously described (8). First, PCR fragments were amplified from pSP39-3 using oligos CSO-0017/ -0139 and CSO-0018/-0138 for RepG ΔCU, CSO-0017/-0143 and CSO-0018/-0142 for RepG 3xG as well as CSO-0017/-0141 and CSO-0018/-0140 for RepG 1xG*. Next, the corresponding PCR-fragments were mixed in an equimolar ratio and used as templates for overlap extension PCR reactions using CSO-0017/-0018. Afterwards, gel-purified PCR reactions were transformed into the *H. pylori* Δ*repG* mutant (JVS-7014). All *H. pylori* complementation mutants were verified by PCR using oligos JVO-5069/-5257 and CSO-0207/-0205 on gDNA as template. All clones were checked by sequencing with CSO-0206.

Construction of Δ*tlpB* and *tlpB*::3xFLAG strains. The *tlpB* gene (HP0103) was deleted from strain CSS-0004 (*H. pylori* 26695 wild-type) by insertion of the *rpsL-erm* cassette, which confers dominant streptomycin susceptibility and erythromycin resistance (3). A construct containing the *rpsL-erm* cassette flanked by 500 nt up- and downstream of the *tlpB* open reading frame was generated by overlap extension PCR. PCR products corresponding to 500 nt upstream of *tlpB* (CSO-0040/-0037 on gDNA of CSS-0004), 500 nt downstream of the *tlpB* stop codon (CSO-0038/-0039 on gDNA of CSS-0004), and the *rpsL-erm* cassette (CSO-0035/-0036 on gDNA of *H. pylori* 26695 carrying a chromosomal *rpsL-erm* cassette; kindly provided from D. E. Berg, University of California, San Diego, La Jolla, CA/Washington University Medical School, St. Louis, MO; (3)) were mixed in an equimolar ratio and used as templates for overlap extension PCR with CSO-0040/-0039. The resulting gel-purified PCR product was used for natural transformation in *H. pylori* 26695. Positive erythromycin-resistant clones were checked by PCR on gDNA using CSO-0051/CSONIH-0033, resulting in strain CSS-0163 (Δ*tlpB*). For the construction of a double deletion mutant Δ*tlpB*/Δ*repG*, the Δ*repG* deletion construct (*aphA-3* flanked by 500 nt up- and downstream of *repG*) was amplified by PCR using JVO-5070 x JVO-5072 and gDNA from strain JVS-7014 (6). The purified PCR product was transformed into strain CSS-0163 (Δ*tlpB*). The double deletion strain CSS-0164 (Δ*tlpB/*Δ*repG*) was verified by PCR using JVO-5069/-5257.

To construct a *tlpB::*3xFLAG-tagged strain (CSS-0190), a plasmid (pSP57-4) containing the 3xFLAG and the *rpsL-erm* cassette flanked by 500 nt up- and downstream of the *tlpB* stop codon was cloned into *E. coli.* First, 500 nt up- and downstream of the *tlpB* stop codon were amplified from gDNA of strain CSS-0004 using CSO-0208/-0211. The resulting PCR product was *XbaI*/*XhoI* digested and introduced into likewise digested pJV752-1. Next, this plasmid was used

as template for a PCR with CSO-0245 and CSO-0210 to fuse the 3xFLAG-tag to *tlpB* and to introduce the *rpsL-erm* cassette using an *EcoRI* restriction site. In parallel, an overlap extension PCR with PCR fragments of the 3xFLAG-tag (CSO-0065/-0046 on gDNA of JVS-7033; (9)) and the *rpsL-erm* cassette (CSO-0045/-0209 on gDNA of a *H. pylori* 26695 strain carrying the *rpsL-erm* cassette) was performed and the resulting 3xFLAG::*rpsL-erm* construct was digested with *EcoRI*. Both *EcoRI*-digested PCR products (plasmid with *tlpB* up- and downstream region and 3xFLAG::*rpsL-erm*) were ligated and transformed into *E. coli*, resulting in pSP57-4. Insertion of the *rpsL-erm* cassette was verified by colony PCR (pZE-A/CSONIH-0033) and in-frame fusion of *tlpB*::3xFLAG by sequencing with CSO-0208, respectively. A PCR product amplified from pSP57-4 with CSO-0208/-0211 was transformed into *H. pylori* strains 26695 (CSS-0004) and G27 (CSS-0010). Positive erythromycin-resistant mutants were confirmed by PCR on gDNA (CSO-0050/-0046) and sequencing with CSO-0208, resulting in CSS-0190 (26695 *tlpB*::3xFLAG) and CSS-0196 (G27 *tlpB*::3xFLAG). Deletion mutants of *repG* in CSS-0190 and CSS-0196 were constructed as described above, resulting in strains CSS-0215 (26695 *tlpB*::3xFLAG/Δ*repG*) and CSS-0197 (G27 *tlpB*::3xFLAG/Δ*repG*). Strains CSS-0215 and CSS-0197 were complemented with RepG from *H. pylori* 26695 in the *rdxA*-locus by transformation of a PCR product amplified from gDNA of CSS-0046 (C_{RepG}) using CSO-0017/-0018. The resulting strains are CSS-0285 (26695 *tlpB*::3xFLAG/Δ*repG*/C_{RepG}) and CSS-0283 (G27 *tlpB*::3xFLAG/Δ*repG*/C_{RepG}).

Deletion of *repG* in diverse *Helicobacter* strains. To delete *repG* in diverse *H. pylori* strains, the sRNA deletion construct was amplified from JVS-7014 (6) using oligos JVO-5070/-5072 and transformed into *H. pylori* strains J99 (CSS-0001), B8 (CSS-0213), India7 (CSS-0099), Shi470 (CSS-0173), Lithuania75 (CSS-0101), Cuz20 (CSS-0097), and G27 (CSS-0010). Deletion of *repG* was verified by PCR using JVO-5069/-5257 on gDNA, resulting in strains CSS-0732 (J99 Δ*repG*), CSS-0733 (B8 Δ*repG*), CSS-0734 (India7 Δ*repG*), CSS-0735 (Shi470 Δ*repG*), CSS-0736 (Lithuania75 Δ*repG*), CSS-0737 (Cuz20 Δ*repG*), and CSS-0169 (G27 Δ*repG*).

Generation of compensatory base-pair exchanges in the *tlpB* 5' UTR. To delete the G-repeat (ΔG) or introduce compensatory base-pair exchanges (3xC and 1xC*) in the *tlpB* 5' UTR, a plasmid (pSP60-2) containing 500 nt up- and downstream of the *tlpB* transcriptional start site (TSS) and the *rpsL-erm* resistance cassette was constructed. First, 500 nt up- and downstream of the TSS of *tlpB* were amplified with CSO-0291/-0040 from gDNA of *H. pylori* 26695 (CSS-0004). The resulting PCR product was inserted into pJV752-1 using *XhoI*/*XbaI* restriction sites, resulting in pSP58-5. Next, the *rpsL-erm* cassette was inserted upstream of the *tlpB* promoter (P_{tlpB}) by ligation of *EcoRI*/*BamHI* digested PCR products based on pSP58-5 (CSO-0294/-0295) and an *rpsL-erm* cassette (CSO-0308/-0309 on gDNA of CSS-0163), resulting in pSP60-2. Afterwards, site-directed mutagenesis by Quick change PCR was performed with pSP60-2 as

template to delete the G-repeat (CSO-0318/-0319) or introduce triple (CSO-0316/-0317) or single G to C (CSO-0314/-0315) nucleotide exchanges, resulting in pSP64-1 (ΔG), pSP66-4 (3xC), and pSP65-4 (1xC*), respectively. All plasmids were checked by sequencing using CSO-0291. PCR fragments based on pSP60-2, pSP64-1, pSP65-4, and pSP66-4 were amplified with CSO-0291/-0040 and used for direct transformation in *H. pylori* 26695 (CSO-0004). Positive *H. pylori* clones were selected on erythromycin plates and confirmed by PCR using CSO-0051/CSONIH-0033 and sequencing of the corresponding gDNA with CSO-0291 or CSONIH-0033, which led to CSS-0384 (P_{tlpB}), CSS-0385 (*tlpB* ΔG), CSS-0386 (*tlpB* 3xC), and CSS-0387 (*tlpB* 1xC*).

Cloning of the *tlpB* promoter exchange. To exchange the *tlpB* promoter, the *cagA* promoter region was amplified with CSO-0306/-0431 from gDNA of *H. pylori* 26695 (CSS-0004) and a PCR on plasmid pSP60-2 was performed with CSO-0430/-308. Both PCR products were *BamHI* digested and ligated, resulting in pSP91-3. The plasmid was verified by colony PCR with CSO-0306/pZE-A and checked by sequencing with CSONIH-0033. PCR fragments amplified with CSO-0291/-0040 from pSP91-3 were transformed into *H. pylori* 26695. Cloness were selected on erythromycin plates and verified by PCR with CSO-0051/-0308 on gDNA and sequencing with CSONIH-0033, resulting in strain CSS-0657 (P_{cagA}). The *repG* gene was deleted in strain CSS-0657 as previously described, resulting in strain CSS-0658 (P_{cagA} /Δ*repG*). As a control, the *rpsl-erm* resistance cassette alone was inserted upstream of the *tlpB* promoter, resulting in strains CSS-0384 (P_{tlpB}) and CSS-388(P_{tlpB} /Δ*repG*) (for construction details see above).

Cloning of translational reporter fusions to *gfpmut3*. To generate a translational reporter fusion**,** we fused the regions corresponding to the promoters, 5' UTRs, and a fraction of the N-terminal coding region of *tlpB* or *cagA* to *gfpmut3* (10) and introduced them together with the *catGC* resistance cassette (2) into the *rdxA* locus of *H. pylori* G27. First, a transcriptional *ureA*::*gfpmut3* fusion was inserted into plasmid pSP39-3 (amplified with CSO-0442/-0443) using a PCR product amplified with CSO-0440/-0441 on plasmid p463 (*ureA*::*gfpmut3* based on pTM117; kindly provided by D. S. Merrell, USU, Bethesda, MD). The PCR products were digested with *SalI*/*NotI* and ligated, resulting in pPT3-1 (*ureA*::*gfpmut3*) which served as backbone for the generation of the translational fusions of *tlpB* and *cagA* to *gfpmut3*. The *tlpB* promoter region and its 5' UTR including the first five amino acids of the *tlpB* coding region (regarding the annotated ATG) were amplified from gDNA of *H. pylori* 26695 (CSS-0004) using CSO-0581/-0126. Similarly, the *cagA* promoter, its 5' UTR and the first 28 amino acids of the *cagA* coding region were amplified with CSO-0284/-0590 from gDNA of *H. pylori* G27 (CSS-0010). The purified PCR products were digested with *ClaI/NheI* and ligated with a likewise digested PCR product, which was amplified from pPT3-1 using CSO-0146/-0683, resulting in pSP109-6 (*tlpB*-5th::*gfpmut3*) and pMA5-2 (*cagA*-28th::*gfpmut3*). These plasmids were checked by colony PCR

using CSO-0581/pZE-XbaI for pSP109-6 and CSO-0590/pZE-XbaI for pMA5-2, respectively, and in-frame fusions to *gfpmut3* were validated by sequencing with CSO-0206 and/or JVO-0155. PCR products amplified with CSO-0017/-0018 from pPT3-1, pMA5-2, and pSP109-6 were transformed into *H. pylori* G27 wild-type (CSS-0010) and/or Δ*repG* (CSS-0169) strains. Positive transformants were checked by PCR with CSO-0205 and CSO-0207 and the in-frame fusion of *tlpB* or *cagA* to *gfpmut3* was verified by sequencing with CSO-0206 and/or JVO-0155. The corresponding *H. pylori* G27 strains are CSS-0748 (*tlpB*-5th::*gfpmut3*), CSS-0751 (*tlpB*-5th::*gfpmut3*/Δ*repG*), CSS-0804 (*cagA*-28th::*gfpmut3*), and CSS-0805 (*cagA*-28th::*gfpmut3*/Δ*repG*).

Construction of markerless *tlpB*::3xFLAG* strains. Markerless *tlpB*::3xFLAG* strains were constructed as described in (3) by using a contraselectable streptomycin susceptibility determinant. For this purpose, a streptomycin resistant *H. pylori* 26695 strain (26695 Str^R, CSS-0024) was generated by introduction of two point mutations in the *rpsL* gene (K43R and K88R). A PCR product amplified from genomic DNA of *H. pylori* 26695 with JVO-5702/-5703 was used for mutagenesis. Transformants were selected on plates containing 10 µg/ml streptomycin and positive clones were checked by sequencing of gDNA using JVO-5704. For the construction of a markerless *tlpB*::3xFLAG* strain, a PCR product amplified from pSP57-4 with CSO-0208/-0211 was transformed into *H. pylori* 26695 Str^R (CSS-0024) and clones were selected on erythromycin plates. The 3xFLAG-tagging of *tlpB* was confirmed as described above. Furthermore, we constructed pSP70-1, which contains 500 nt up- and downstream of the *tlpB* stop codon including a 3xFLAG tag. To construct this plasmid, cycle PCR with CSO-0428/-0429 on pSP57-4 was performed and the resulting PCR product was digested with *DpnI* and transformed into *E. coli*. Loss of the *rpsL-erm* resistance cassette was verified by PCR using pZE-A/CSONIH-0033 and in-frame fusion of *tlpB::*3xFLAG was checked by sequencing with CSO-0208. A PCR product amplified from pSP70-1 with CSO-0208/-0211 was used for transformation and removal of the *rpsL-erm* resistance cassette from CSS-0461 (26695 Str^R *tlpB*::3xFLAG). Positive *H. pylori* mutants were selected on plates containing 10 µg/ml streptomycin and removal of the *rpsL-erm* resistance cassette was checked by plating of streptomycin resistant mutants on plates containing 10 µg/ml erythromycin. The markerless *tlpB*::3xFLAG* tagged 26695 strain (CSS-0464, 26695 Str^R *tlpB*::3xFLAG*) was checked by PCR on gDNA with CSO-0050/-0046 or CSO-0050/CSONIH-0033 and sequencing with CSO-0208. The *repG* gene was deleted in CSS-0464 as described before resulting in strain CSS-0467.

Variation of the G-repeat length in the *tlpB* 5' UTR of *H. pylori* 26695. *H. pylori* 26695 strains with varying G-repeat length of 6-16 Gs were generated by cycle-PCR on pSP64-1 with CSO-0318/-0448 to -0457 (see Tables S4 and S6). The obtained PCR products were *DpnI* digested, self-ligated and transformed into *E. coli*. The resulting plasmids pSP73-1 to pSP82-1 (Table S5),

pSP64-1 (ΔG), and pSP60-2 (represents the 26695 wild-type 5' UTR of *tlpB* with 12 Gs) were sequenced with CSONIH-0033. Afterwards, these plasmids were used as templates for PCR with CSO-0040/-0291. The PCR products with different G-repeat lengths were transformed into CSS-0464 (wild-type background) and CSO-0467 (Δ*repG*). Positive clones were checked by colony PCR on gDNA with CSO-0051/CSONIH-0033 and sequencing with CSONIH-0033/CSO-0277, resulting in strains CSS-0471 to CSS-0493 (see Table S3).

Primer extension of RepG. 10 μg DNase I treated RNA from *H. pylori* strains 26695 and G27 was used for primer extension. For the sequencing ladder, 1.5 pmol labeled oligo JVO-5126 and ~100 ng PCR product, which was amplified from gDNA of *H. pylori* strains 26695 and G27 using JVO-5126/CSO-0083, were used in sequencing reactions with the SequiTherm EXCEL™ II DNA Sequencing Kit. Primer extension was performed in 10 μl reactions. After denaturation at 80 °C, 10 μg RNA and 0.5 pmol labeled JVO-5126 were annealed by gradual cooling of the reaction to 42 °C. Reverse transcription was performed in 1x AMV reaction buffer (50 mM Tris-HCl, 75 mM potassium, 8 mM magnesium, 10 mM DTT, pH 8.3) by addition of 10 mM dNTPs and AMV Reverse Transcriptase (NEB, #M0277). After incubation for 1 h at 42 °C, the reaction was stopped by addition of 10 μl RNA loading buffer. 10 μl of the primer extension reactions and 2 μl of the ladder with 8 μl loading buffer were separated on 6% (vol/vol) PAA/7M urea sequencing gels. Afterwards, gels were dried and analyzed using a PhosphoImager (FLA-3000 Series, Fuji).

Gel-shifts, in vitro structure probing, and in-line probing assays. DNA templates that contain the T7 promoter sequence were generated by PCR using oligos and DNA templates listed in Table S7. T7 transcription was carried out using the MEGAscript® T7 kit (Ambion) and sequences of the resulting T7 transcripts are listed in Table S8. For *tlpB* mRNA leader variants (6G-16G) see also Table S6. In vitro transcribed RNAs were quality checked and 5' end labeled (γ^{32}P) as previously described (11, 12).

Gel-shift assays were performed with ~0.04 pmol 5'-end labeled RNA (4 nM final concentration) and increasing amounts of unlabeled RNA in 10 μl reactions. After denaturation (1 min at 95 °C), labeled RNAs were cooled for 5 min on ice and 1 μg yeast RNA and 10 x RNA structure buffer (Ambion) were added. Increasing concentrations of unlabeled RNA were added to final concentrations of 8 nM, 16 nM, 62.5 nM, 125 nM, 250 nM, 500 nM, and 1000 nM. For gel-shift assays with ^{32}P-labeled RepG and *tlpB* mRNA leader variants, ~0.04 pmol ^{32}P-labeled RepG was incubated with 1000 nM of unlabeled *tlpB* leader that either lacks the G-repeat (ΔG) or comprises different G-repeat lengths (6-16G). After incubation for 15 min at 37 °C, samples were immediately loaded after addition of 3 μl 5x native loading dye (0.5 x TBE, 50 % (vol/vol) glycerol, 0.2 % (wt/vol) xylene cyanol and 0.2 % (wt/vol) bromophenol blue) to a native 6 % (vol/vol) PAA gel. Gel electrophoresis was done in 0.5 x TBE buffer at 300 V. Afterwards, gels

were dried and analyzed using a PhosphoImager (FLA-3000 Series, Fuji) and AIDA software (Raytest, Germany).

For structure probing and footprinting assays, ~0.1 pmol 5' end labeled RNA was subjected in absence or presence of unlabeled target mRNA or sRNA to RNase T1 (Ambion, #AM2283), lead(II)-acetate (Fluka, #15319) or RNase III (NEB #M0245S) treatment in 10 µl reactions as previously described (7). In brief, ~0.1 pmol 5' end labeled RNA was denatured for 1 min at 95 °C and chilled on ice for 5 min. Next, 1 µg yeast competitor RNA and 10 x RNA structure buffer were added (provided together with RNase T1, Ambion). Unlabeled wild-type or mutant RepG/*tlpB* mRNA leader RNAs were added at 10- or 100-fold excess (see Figure legends). After incubation for 10 min at 37 °C, 2 µl RNase T1 (0.01 U/µl) or 2 µl freshly prepared lead(II)-acetate solution (25 mM) were added and reactions were incubated for 3 min or 90 sec, respectively. RNase III cleavage reactions were performed for 6 min at 37 °C in 1 x structure buffer containing 1 mM DTT and 1.3 U/µl enzyme. For RNase T1 ladders, ~0.2 pmol labeled RNA were denatured in 1x structure buffer for 1 min at 95 °C and afterwards incubated with 0.1 U/µl RNase T1 for 5 min. OH ladders were generated by the incubation of ~0.2 pmol labeled RNA in 1x alkaline hydrolysis buffer (Ambion) for 5 min at 95 °C. All reactions were stopped by addition of 12 µl RNA loading buffer (95% (vol/vol) formamide, 18 mM EDTA, and 0.025% (wt/vol) SDS, xylene cyanol, and bromophenol blue) on ice. Ladders and samples were denatured 3 min at 95 °C and separated on 6% (vol/vol) PAA/7M urea gels in 1 x TBE buffer. Afterwards, gels were dried and analyzed using a PhosphoImager (FLA-3000 Series, Fuji) and AIDA software (Raytest, Germany).

For in-line probing assays (13), ~0.2 pmol labeled RNA (20 nM final concentration) was incubated in absence or presence of 20 nM or 200 nM unlabeled sRNA or mRNA leader for 40 hrs at room temperature in 1x in-line probing buffer (50 mM Tris-HCl, pH 8.3 at 20 °C, 20 mM $MgCl_2$, and 100 mM KCl). For RNase T1 ladders, ~ 0.2 pmol labeled RNA was incubated in 0.25 M sodium citrate buffer (pH 5.0 at 23 °C) with 1 U/µl RNase T1 for 5 min at 55 °C. For alkaline ladders, ~0.2 pmol labeled RNA was denatured for 5 min at 95 °C in Na_2CO_3 buffer (0.5 M Na_2CO_3, pH 9.0 at 23 °C and 10 mM EDTA). All reactions were stopped by adding 10 µl colorless gel-loading solution (10 M urea, 1.5 mM EDTA, pH 8.0 at 23 °C) on ice. Cleavage products were analyzed on 8 or 10 % (vol/vol) PAA gels under denaturing conditions and visualized as described above.

Rifampicin assays and determination of *tlpB* mRNA half-life by qRT-PCR. To determine the half-life of *tlpB* mRNA in *H. pylori* 26695 wild-type, *ΔrepG,* and RepG complementation (C_{RepG}) strains*,* cells were grown to an OD_{600nm} of 1.0 and treated with rifampicin (final concentration 500 µg/ml). RNA samples were harvested at indicated time points (0, 1, 2, 4, 8, 16, and 32 min) and RNA decay was analyzed by quantitative real-time PCR (qRT) as previously described (6, 12,

14). All qRT-PCR experiments were carried out in triplicates on a CFX96 system (Biorad) using Power SYBR Green RNA-to-C_T™ 1-Step Kit (Applied Biosystems) according to the manufactures' instructions. The specific oligo sets are JVO-5267/-5268 for *tlpB* mRNA and CSO-1173/-1174 for 6S RNA, which served as internal standard.

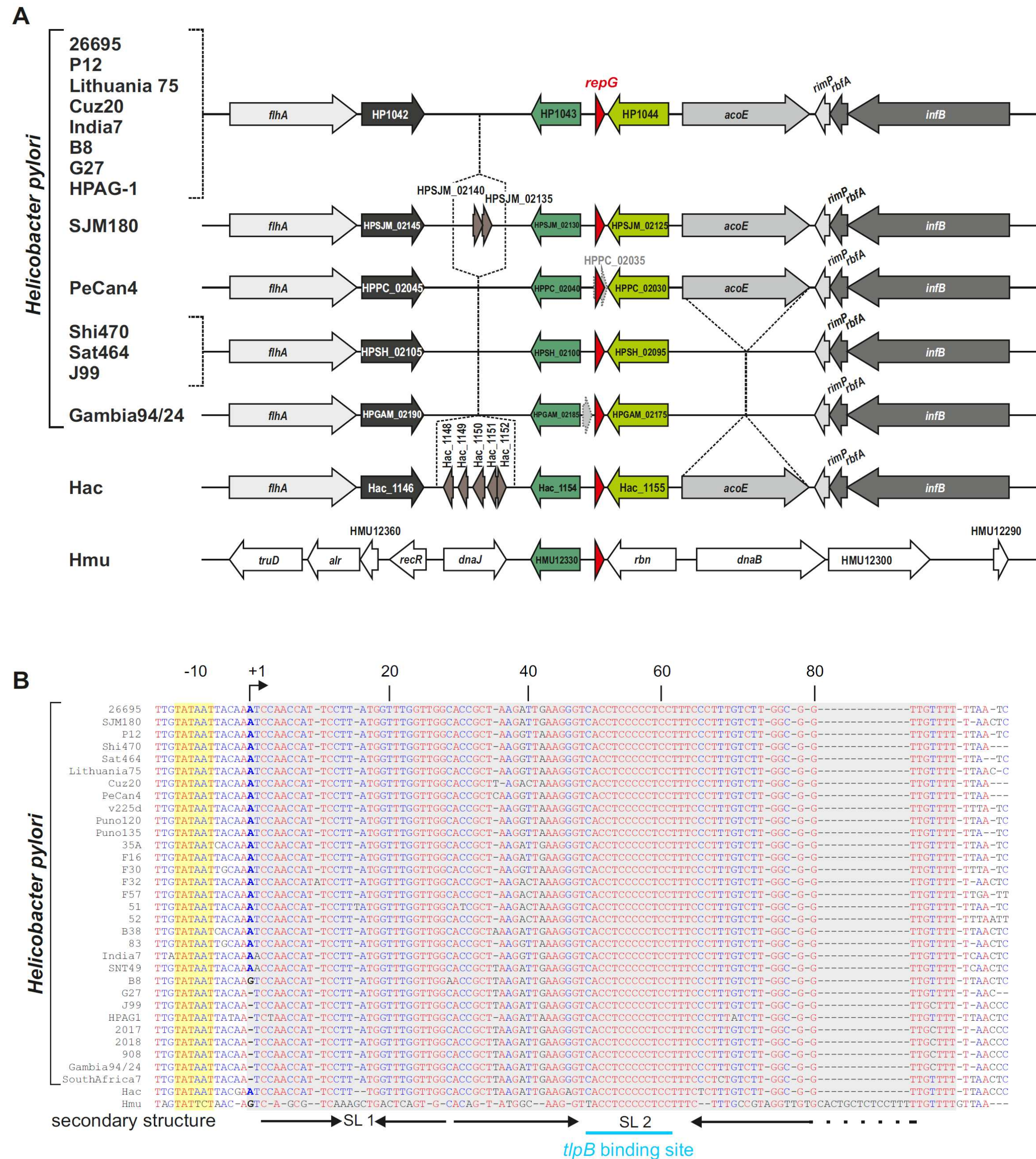
Antibodies and antisera. To detect the four chemotaxis receptors TlpA, B, C, and D, a polyclonal rabbit TlpA22-antiserum (1:2000 in 3% (wt/vol) BSA/TBS-T) that recognizes a conserved cytosolic domain (kindly provided by K. Ottemann, University of California, Santa Cruz, CA) and secondary anti-rabbit IgG (GE-Healthcare) were used. TlpB::3xFLAG was detected by a monoclonal anti-FLAG antibody (1:1000 in 3% (wt/vol) BSA/TBS-T; Sigma-Aldrich, #F1804-1MG) and secondary anti-mouse IgG (GE-Healthcare). GroEL, was visualized by monoclonal anti-GroEL antibody (1:10,000 in 3% (wt/vol) BSA/TBS-T; Sigma-Aldrich, # G6532-5ML) and anti-rabbit IgG (GE-Healthcare). CagA was visualized using an anti-cagA antibody (1:3000 in 3% (wt/vol) BSA/TBS-T; kindly provided by R. Haas, Max von Pettenkofer Institute, LMU Munich) and anti-rabbit IgG.

In vitro translation assays. Translation reactions were carried out with PureSystem (Cosmo Bio, PGM-PURE2048C) according to the manufactures' instructions. In brief, 1 pmol of in vitro transcribed mRNAs (*tlpB*-5th::*gfpmut3*, *cagA*-28th::*gfpmut3*, *tlpB*::3xFLAG, *tlpB* ΔG::3xFLAG, *tlpB* 10G::3xFLAG, *tlpB* 11G::3xFLAG, *tlpB* 13G::3xFLAG, and *tlpB* 14G::3xFLAG) were denatured in the absence or presence of 1, 10, 50, and 100 pmol of RepG or RepG mutants (ΔCU, 3xG, 1xG; see also Figure legends) for 1 min at 95 °C and chilled on ice for 5 min. The mRNA and sRNA were pre-incubated for 10 min at 37 °C before addition of PureSystem mix, and translation was performed at 37 °C for 30 min. Reactions were stopped by addition of 60 µl acetone, chilled for 15 min on ice and proteins were collected by centrifugation for 10 min at 10,000 g and 4 °C. In vitro translated TlpB or CagA was quantified by Western blot analysis using monoclonal anti-FLAG or anti-GFP and anti-mouse IgG (GE-Healthcare) antibodies. The ribosomal protein S1 served as a loading control and was detected by an S1 antibody, (1:10,000, kindly provided by M. Springer, IBPC Paris, France) and anti-rabbit secondary antibody (GE-Healthcare).

Determination of the G-repeat length in the *tlpB* leaders from sequential *H. pylori* isolates. The lengths of the G-repeat in the 5' UTR of the *tlpB* mRNA from different *H. pylori* strains that are listed in Table S1 were extracted from NCBI database (if available) or from the literature. Furthermore, *tlpB* 5' UTR sequences of sequential *H. pylori* isolates with available 454 genome sequences from the study of Kennemann *et al.* (2011) (15) were re-sequenced using Sanger sequencing. The genomic DNA of these strains was kindly provided by S. Suerbaum and C. Josenhans, Medizinische Hochschule Hannover, Germany.

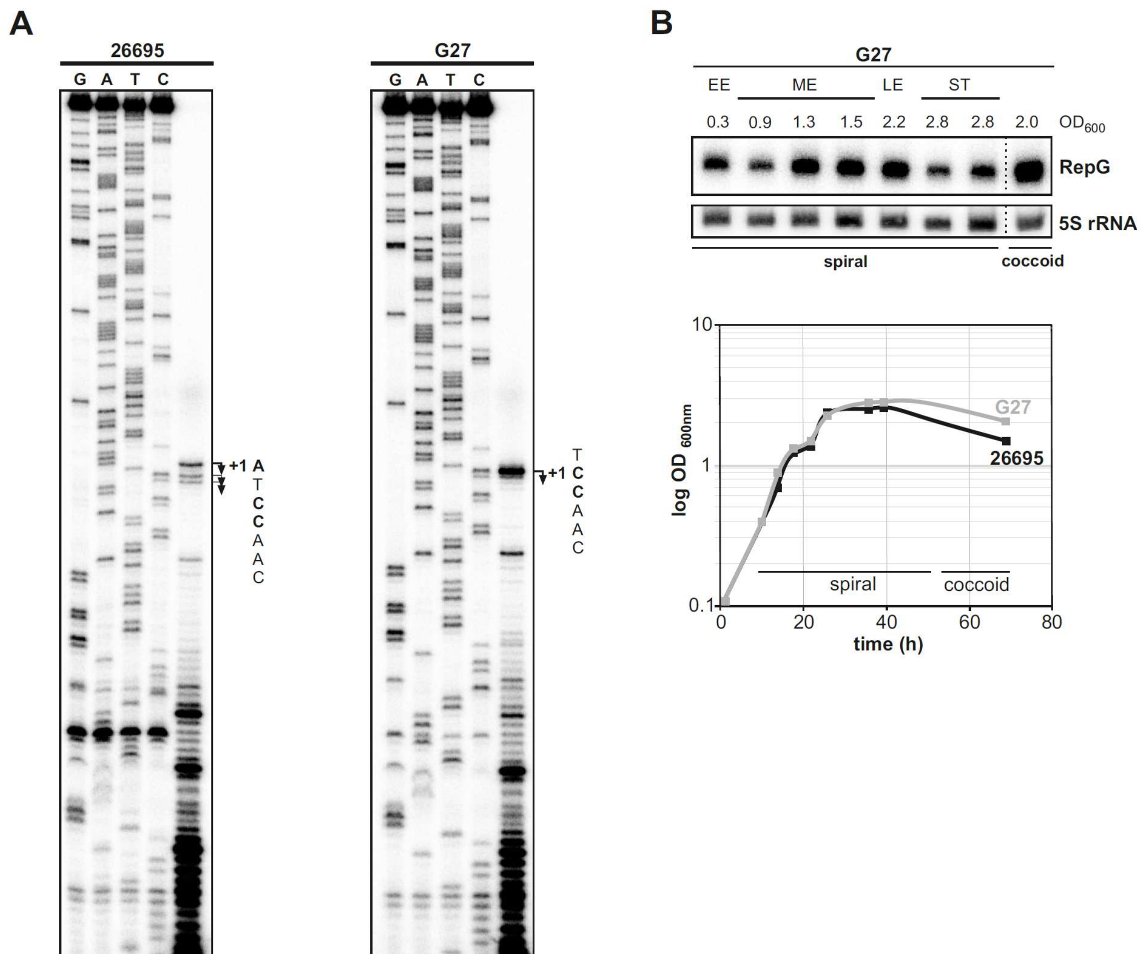
Supplementary Figures

Figure S1



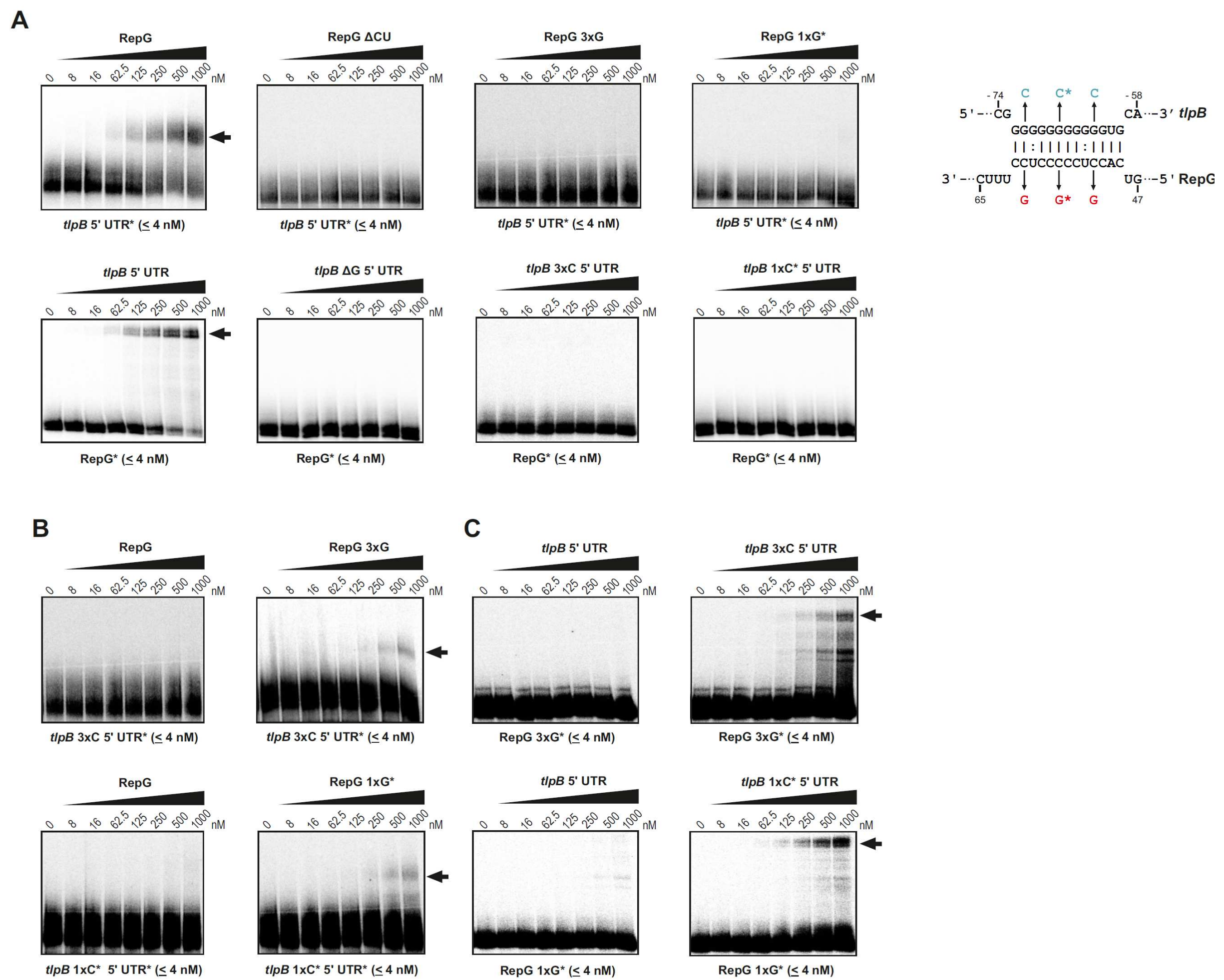
Genomic context and sequence alignment of *repG* homologs from different *H. pylori* strains, *H. acinonychis*, and *H. mustelae*. (A) The *repG* (red) gene is encoded between HP1043 (dark green) and HP1044 (light green), which encode for an orphan response regulator and a hypothetical protein, respectively. Homologs in different *H. pylori* strains as well as *H. acinonychis* (Hac) are illustrated by the same colors, whereas unrelated genes are indicated in white for *H. mustelae* (Hmu). Gene insertions or deletions are marked by dotted lines. A potential open reading frame (dotted line gray arrow) is annotated next to *repG* in *H. pylori* strains PeCan4 and Gambia94/24. **(B)** Arrows below the *repG* alignment indicate the predicted stem-loop structures SL 1 and SL 2 of RepG. The C/U-rich *tlpB* binding site in SL 2 is indicated by a blue bar.

Figure S2



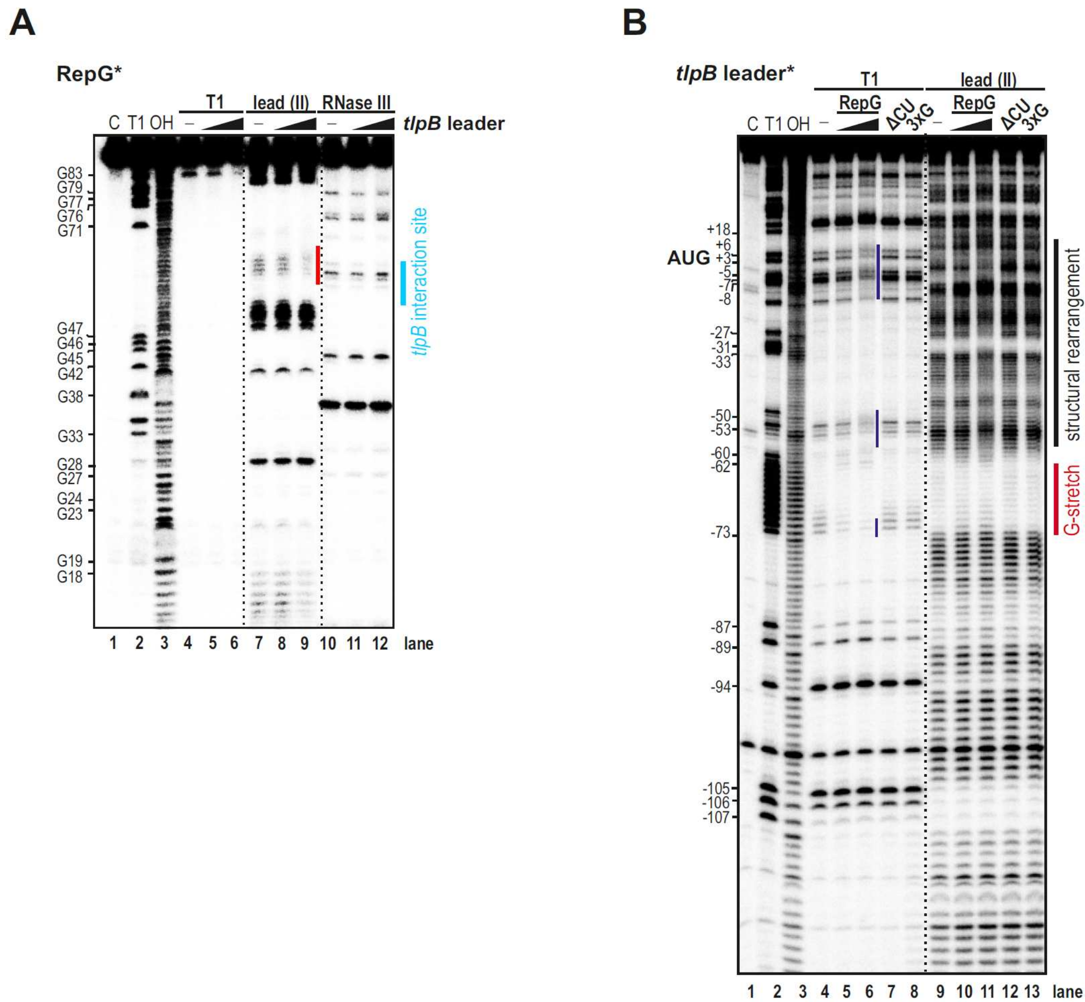
Determination of the 5' end of RepG sRNA using primer extension in *H. pylori* strains 26695 and G27 and expression analysis of RepG during growth in *H. pylori* G27. (A) Total RNA was isolated from *H. pylori* strains 26695 (*left panel*) and G27 (*right panel*) that were grown to exponential growth phase (OD_{600} of ~0.8). After DNase I treatment, 10 µg of total RNA was used in primer extension assays with ^{32}P-end labeled oligo JVO-5126. A sequencing ladder corresponding to the *repG* upstream region served as reference (lanes G, A, T, C). Primer extension revealed that the different bands in *H. pylori* strain 26695 (Figure 1B and C) correspond to RepG versions that vary at their 5'-end (87, 85, and 84-nt long). The identified transcriptional start sites of RepG (TSS, +1) are indicated by arrows and bold nucleotides. The "A" of the +1 TSS of 26695 corresponds to the TSS that was previously determined by differential RNA-seq (6). **(B)** Expression of *repG* was analyzed in *H. pylori* strain G27 by Northern blot analysis with 5 µg of total RNA and hybridization with ^{32}P-labeled CSO-0003 (RepG). 5S rRNA was used as loading control (JVO-0485). RNA samples were taken during growth at different optical densities at 600 nm (OD_{600}), which are indicated in the growth curves of *H. pylori* strains 26695 (black) and G27 (gray) from liquid culture shown in the lower panel. Both strains change their morphology from spiral to coccoid shape after approximately 60 hours of growth (EE – early exponential, ME – mid exponential, LE - late exponential, and ST – stationary growth phase).

Figure S3



Validation of a direct interaction between RepG and the *tlpB* leader using gel-shift assays. (A) (*Left upper panel)* About 0.04 pmol (4 nM final concentration) in vitro transcribed and 5' end ^{32}P-labeled *tlpB* mRNA leader was incubated without or with increasing concentrations (final concentrations of 8, 16, 62.5, 125, 250, and 1000 nM) of unlabeled RepG, RepG without the *tlpB* binding site (ΔCU), RepG 3xG, and RepG 1xG* variants (triple or single* C to G nucleotide exchange(s) in *tlpB* interaction site) for 15 min at 37 °C. RNA-RNA complex formation was investigated by direct loading of the samples on a native 6% (vol/vol) PAA gel in 0.5 x TBE running buffer. (*Left lower panel):* In a reciprocal experiment, ~0.04 pmol labeled RepG was incubated for 15 min at 37 °C with wild-type *tlpB* leader or *tlpB* leader variants that either lack the G-repeat (*tlpB* ΔG) or contain triple or single* G to C nucleotide exchange(s) (*tlpB* 3xC, *tlpB* 1xC*) in the G-repeat. (*Right panel)* RepG sRNA base-pairs with its C/U-rich terminator loop with a homopolymeric G-repeat in the *tlpB* mRNA leader. Triple and single* nucleotide exchanges in the *tlpB* binding site of RepG (C to G exchange(s)) are indicated in red and compensatory base-pair exchanges in the *tlpB* G-repeat (G to C substitution(s)) are marked in blue. **(B)** For in vitro analyses of the compensatory base-pair exchanges, labeled *tlpB* mRNA leader variants (3xC; 1xC*) were incubated with RepG wild-type (RepG) or RepG variants with corresponding nucleotide mutations (3xG; 1xG*). **(C)** V*ice versa* gel-shift assays, in which RNA-RNA complex formation between RepG 3xG or RepG 1xG* variants and the wild-type or *tlpB* mRNA leader variants with compensatory mutations (3xC; 1xC*) was examined.

Figure S4

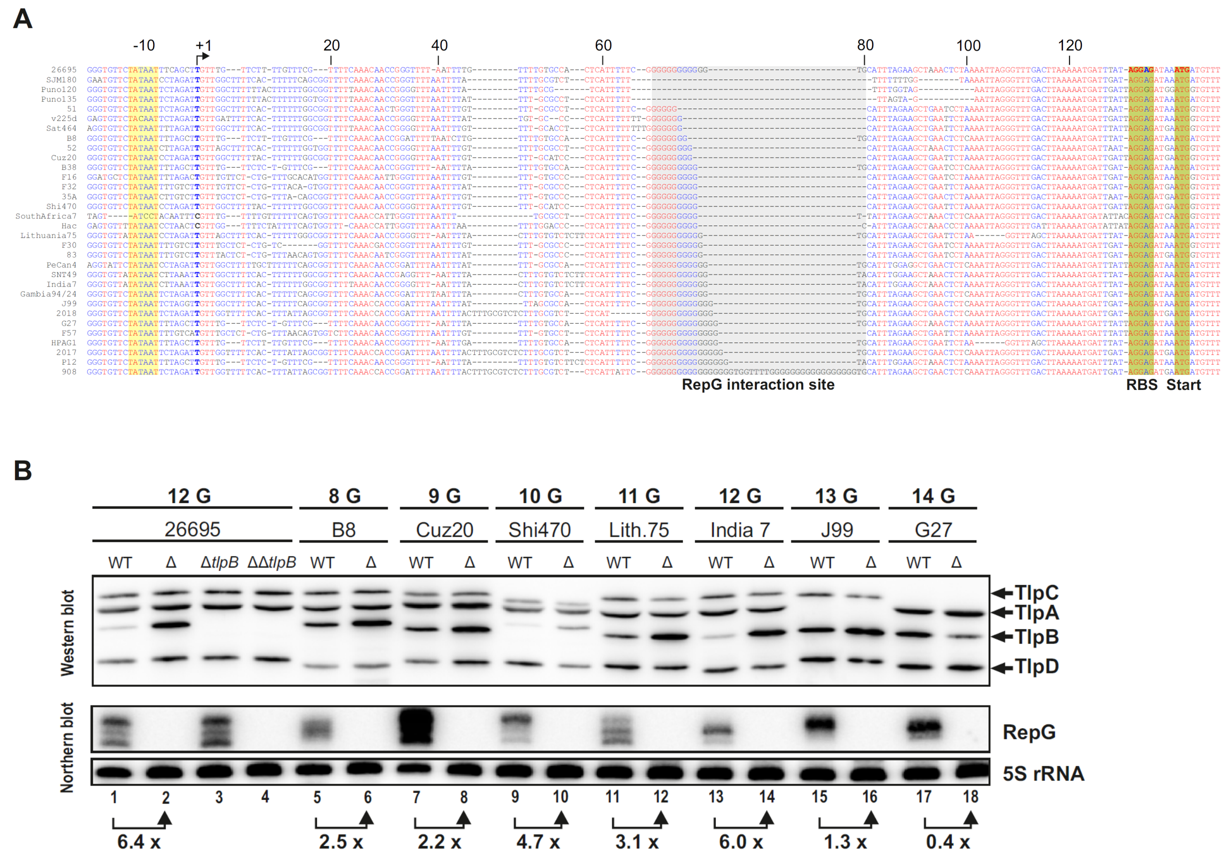


In vitro structure mapping of RepG and the *tlpB* mRNA leader and their interaction site. (A) About 0.1 pmol (10 nM final concentration) in vitro transcribed and ^{32}P-labeled RepG* was treated in the absence (lanes 4, 7, and 10) or presence of 100 nM (lanes 5, 8, and 11) or 1000 nM (lanes 6, 9, and 12) unlabeled *tlpB* mRNA leader with RNase T1, lead (II)-acetate and RNase III. Protection from lead (II)-acetate in the predicted *tlpB* interaction site (blue bar) of RepG is indicated in red. Lane C: untreated RNA; Lane T1: RNase T1 ladder of hydrolyzed and denatured RNA; Lane OH: Alkaline ladder of denatured RNA. **(B)** Structure probing of ~0.1 pmol (10 nM final concentration) radioactively labeled *tlpB* mRNA leader in the absence (lanes 4 and 9) or presence of 100 nM (lanes 5 and 10), 1000 nM of RepG (lanea 6 and 11) or 1000 nM of RepG that either lacks the *tlpB* interaction site (ΔCU; lanes 7 and 12) or harbors a triple C to G exchanges (3xG; lanes 8 and 13). Regions that are protected from RNase T1 cleavage upon addition of RepG are indicated by dark blue bars.

Figure S5

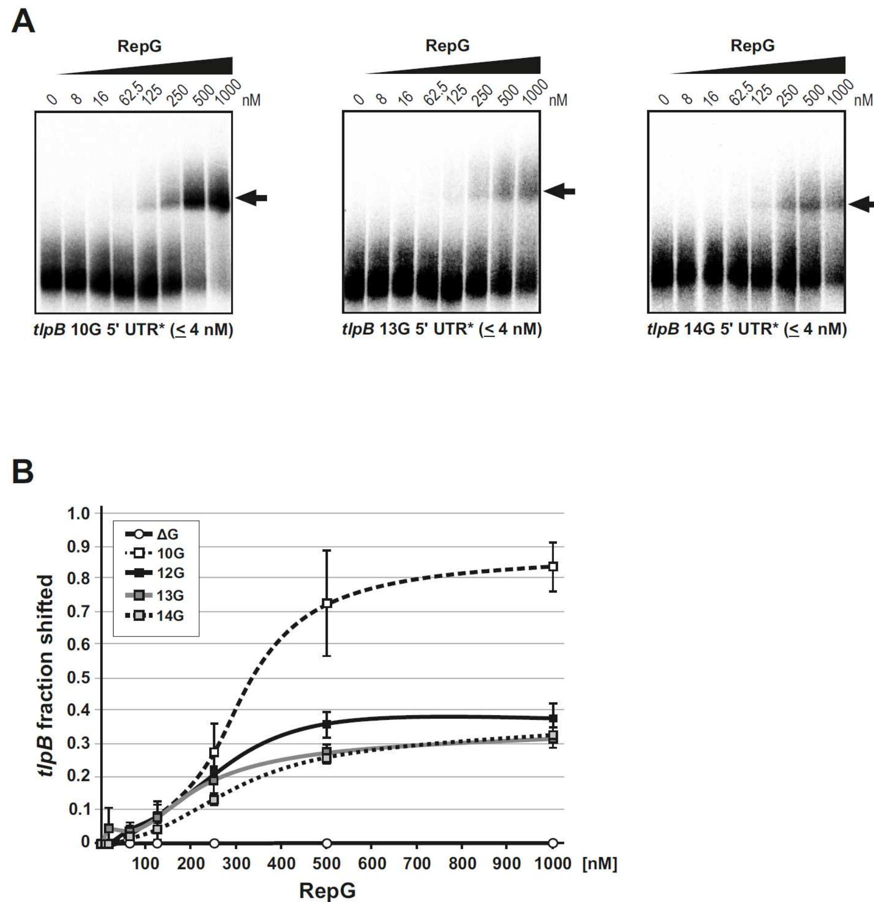
Putative secondary structures of RepG (A) and the *tlpB* mRNA leader (B) based on in vitro structure mapping, in-line probing and bioinformatics-based predictions. Arrows indicate RNase T1, lead (II)-acetate, and RNase III cleavage sites according to the cleavage patterns from structure probing assays (see Figures 3 and S4). Spontaneous cleavages of nucleotides in single-stranded RNA regions from in-line probing experiments (Figure 3) are encircled in gray. The terminator loop of RepG (SL 2) harbors the C/U-rich *tlpB* binding site (marked in blue). The homopolymeric G-repeat (red) in the *tlpB* mRNA leader was protected from RNase T1 cleavage, indicating the formation of an inter- or intramolecular structure (highlighted by a box). Secondary structures were predicted based on the structure probing data in combination with bioinformatics-based secondary structure predictions (16).

Figure S6



Strains with different lengths of the homopolymeric G-repeat in the *tlpB* leader show variations in RepG-mediated regulation. **(A)** Sequence alignment of *tlpB* leaders, promoter regions, and the first nucleotides of the open reading frames from diverse *H. pylori* strains and *H. acinonychis* (Hac)*.* The -10 promoter boxes as well as the conserved ribosome binding sites (RBS) and annotated start codons (ATG) of *tlpB* are highlighted in yellow and light green, respectively. The *tlpB* transcriptional start site (+1) is indicated in bold and the RepG interaction site according to strain 26695 is boxed in gray. **(B)** Western blot *(upper panel)* and Northern blot *(lower panel)* analyses of diverse *H. pylori* wild-type and Δ*repG* mutants grown to exponential growth phase. Protein samples corresponding to an OD_{600} of 0.01 were loaded on a 10% (vol/vol) SDS-PAA gel, blotted to PVDF membrane, and chemotaxis receptors were detected with a polyclonal rabbit anti-TlpA22 antiserum (1:2000). RepG was probed with ^{32}P-labeled CSO-0003. 5S rRNA (JVO-0485) was used as loading control.

Figure S7



Analysis of the interaction between *tlpB* leader variants with different G-repeat length and RepG using gel-shift assays. (A) About 0.04 pmol (4 nM final concentration) in vitro transcribed and ^{32}P-labeled *tlpB* mRNA leaders that comprise a 10G-, 13G- or 14G-long homopolymeric repeat were incubated without or with increasing concentrations of unlabeled RepG (final concentrations of 8, 16, 62.5, 125, 250, and 1000 nM) for 15 min at 37 °C. RNA-RNA complex formation (indicated by arrows) was investigated by direct loading of the samples on a native 6% (vol/vol) PAA gel in 0.5 x TBE running buffer. The results of a representative experiment (out of two) are shown. **(B)** Quantification of the labeled *tlpB* mRNA leader fraction that was shifted when incubated with increasing concentrations of RepG based on the gel-shift assays in panel A, Figure 3, and S3 (*tlpB* leader with 12Gs). Averages and standard deviations were calculated from at least two independent experiments.

Figure S8

RepG complementation construct: *rdxA*(500 nt up)–*catGC*–*repG*–*rdxA*(500 nt down)

CSO-0017
tctagaGATCAGCCTGCCTTTAGGGTATGTTTTTGGGAGGATTGGGAATTTTTTAAACCAGGAGCTTGTGGGAAG
AATTGTCCCCAAAGACAGCCATTTAGGGCAAATCATAGGCATTATGGTGGATAATGAGTTGCGTTATCCCAGCCA
ATTGATTGAAGCGTTTTTAGAGGGGGTTATCGTGTTTTTAATGGTAATGTGGGCTAAAAAACACACCAAAACGCA
TGGGTTGCTGATTGTGGTTTATGGTTTGGGGTATTCCTTGATGCGCTTTATTGCGGAATTTTACAGAGAGCCGGA
CAGCCAAATGGGGGTTTATTTTTTAAATTTGAGCATGGGGCAGATTTTAAGCTTATTTATGGTAATTGTTTCGTT
AGGGATTTTATTGTATGCTACAAAAAATTCTAAAAAAATAAAGGAAAATCAATGAAATTTTTGGATCAAGAAAAA
AGAAGACAATTATTAAACGAGCGCCATTCTTGCAAGATGTTTGATAGCCATTATGAGTTTTCTAGCACAGAATTA
GAAGAAATCGCTGctagagatccgccatattgtgttgaaacaccgcccggaacccga**TATAAT**ccgcccttcaac
agatccgagattttcaggagcta**AGGAAG**ctaaaATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATC
CCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTCAGCT
GGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGC
CCGCCTGATGAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCA
CCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCG
GCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTAT
TGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGA
CAACTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGAT
TCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGA
GTGGCAGGGCGGGGCGTAAtttttttaaggcagttattggtgcccttaaacgcctggttgctacgcctgaataag
tgataataagcggatgaatggcagaaattcggatcttccatacctaccagttctgcgcctgcaggtcgataaacc
gatacaattaaaggctcctttggagccttttttttggagattttcaacgtggatctgaattcgagatgcatca
agcccttgattattggttgtaaaaaatgcctttgagcgtttttatggataatttttaaaatcatttgctaaaaat
caccattttattg**TATAAT**tacaa**ATCCAACCATTCCTTATGGTTTGGTTGGCACCGCTAAGATTGAAGGGTCAC**
CTCCCCCTCCTTTCCCTTTGTCTTGGCGGTTGTTTTttaatccttgtttagttcttatttttaaccgcttgggta
ccGTATGCTCTTTAAGACCCAGCGAGTTGTTACCACACGGCCACTACATGCAAAATCTCTATCCGGAGTCTTATA
AAGTTAGAGTGATCCCCTCTTTTGCTCAAATGCTTGGCGTGAGATTCAACCACAGCATGCAAAGATTAGAAAGCT
ATATTTTAGAGCAATGCTATATCGCTGTGGGGCAAATTTGCATGGGCGTGAGCTTAATGGGATTGGATAGTTGCA
TTATTGGAGGCTTTGATCCTTTAAAGGTGGGCGAAGTTTTAGAAGAGCGTATCAATAAGCCTAAAATCGCATGCT
TGATCGCTTTGGGCAAGAGGGTGGCAGAAGCGAGTCAAAAATCAAGAAAATCAAAAGTTGATGCGATTACTTGGT
TGTGATTAAACAAAATCAAAAACTTTTTAACTATAATCAAACCTAAATTAAAGTTCAAGGAGTGGCATTTTGTTT
AAAAGAATGGTTTTAATCGCTCTTTTAGGGGTGTTTTCAAGCGTTTCATTAAGCGCTAAGctcgag
CSO-0018

rdxA 500 nt upstream: 1014605 – 1014074
catGC cassette
Intergenic region of HP1043 and HP1044 with ***repG*** under control of its native **promoter**
rdxA 500 nt down: 1013930 – 1013423

Sequence of the RepG complementation construct in the *rdxA* locus. The intergenic region of HP1043 and HP1044 (highlighted in light red) with the *repG* gene (red letters) was introduced into pSP39-3, which harbors 500 nt up- (light green) and downstream (dark green) of the *rdxA* locus of strain 26695 as well as the *catGC* resistance cassette (blue). The -10 promoter regions of *repG* and the *catGC* resistance cassette as well as its RBS are highlighted in bold. The sequences corresponding to oligos CSO-0017 and CSO-0018, which were used for amplification of the *rdxA*(500up)-*catGC*-*repG*-*rdxA*(500down) complementation construct, are underlined.

Supplementary Tables

Table S1: G-repeat lengths in the *tlpB* 5' UTRs of sequential *H. pylori* from human or after re-isolation from animals. Lines frame *H. pylori* isolates that were obtained from the same patient or that were re-isolated after mice/gerbil infection studies. The lengths of the G-repeats in the *tlpB* 5' UTRs of isolates from Narino, Colombia, from the study of Kennemann et al. (2011) (Ref. 15) were re-sequenced by Sanger sequencing.

Strain	NCBI Acc. No	Time scale	G-repeat length	G-repeat variation	Origin/Comment	Reference
908	NC_017357		17G–TGGTTTT–17G		West African duodenal ulcer disease patient in France	(17)
2017	NC_017374	10 yrs	15G	yes	Re-isolate from antrum	(18)
2018	NC_017381	10 yrs	13G	yes	Re-isolate from corpus	
NQ367	NZ_CADL00000000		15/16G°		Isolates from Narino, Colombia	(15)
NQ1671	NZ_CADM00000000	3 yrs	13G	yes		
NQ4191	NZ_CADN00000000	16 yrs	13G	yes		
NQ392	NZ_CADI00000000		13G°		Isolates from Narino, Colombia	(15)
NQ1707	NZ_CADJ00000000	3 yrs	14G	yes		
NQ4060	NZ_CADK00000000	16 yrs	15/16G°	yes		
NQ315	NZ_CADE00000000		12G		Isolates from Narino, Colombia	(15)
NQ1712	NZ_CADF00000000	3 yrs	13G	yes		
NQ352	NZ_CADG00000000		12G°		Isolates from Narino, Colombia	(15)
NQ1701	NZ_CADH00000000	3 yrs	14G°	no		
Hp141			12G-TGC#		Women with gastritis in Poitiers, France	(19)
Hp141*		150 days	10G-C#	yes	Re-isolate from female C57BL/6 inbred mice	
Hp145			10G		Women with prepyloric ulcer in Poitiers, France	(19)
Hp145*		150 days	10G	no	Re-isolate from female C57BL/6 inbred mice	
HP87			13G		Original human isolate	(20)
HP87 P7*			16/17/18G	yes	Gerbil adapted strain	
HP87 P7 *tlpD*			16/17/18G		*tlpD* mutant of gerbil adapted strain	(20)
HP87 P7 *tlpD* RI		6 weeks	16/17/18G	no	Re-isolate from gerbil antrum	

° The G-repeat length determined by Sanger sequencing differed from the genome sequence determined by 454 sequencing.
* *H. pylori* isolates that were re-isolated from C57BL/6 inbred mice or gerbils.
# Additional nucleotide variations that were identified in the flanking region of the G-repeat.

Table S2: Location of simple sequence repeats in *H. pylori* and *C. jejuni*. Homopolymeric simple sequence repeats (SSRs) in *H. pylori* 26695 and *C. jejuni* NCTC11168 were extracted from previous studies (21-26) and classified regarding their location within promoter regions, 5' untranslated regions (5' UTR) or coding regions of annotated genes. Transcriptional start sites (TSS) from global transcriptome studies of *H. pylori* 26695 (6) and *C. jejuni* NCTC11168 (27) were used for the definition of promoters and 5' UTRs. Gene Cj0565 has two alternative promoters (primary and secondary TSS) which lead to two possible locations for the SSR.

***H. pylori* 26695**			***C. jejuni* NCTC11168**		
Promoter	**5' UTR**	**Coding sequence**	**Promoter**	**5' UTR**	**Coding sequence**
HP0009 (A14) HP0025 (T15) HP0227 (T14) HP0228 (A14) HP0349 (T15) HP0350 (A15) HP0547 (A14) HP0629 (T15) HP0651 (A7) HP0722 (T15) HP0725 (T14) HP0733 (T13) HP0896 (A14) HP0912 (T13) HP1342 (A14)	HP0103 (G12) HP0208 (A11) HP0211 (T7) HP0335 (G9) HP0585 (A8) HP0876 (T16) HP1400 (A16)	HP0058 (C15) HP0093-94 (C14) HP0143 (A7) HP0211 (A7) HP0217 (G12) HP0298 (T9) HP0379 (C13) HP0381 (G7) HP0464 (C15) HP0499 (G8) HP0580 (C8) HP0586 (A8) HP0619 (C13) HP0642 (G6) HP0651 (C13) HP0655 (G8) HP0657 (G7) HP0684-85 (C9) HP0687 (G8) HP0752 (G6) HP0753 (G7) HP0767 (G11) HP0839 (G7) HP0908 (C8) HP0919 (G9) HP1206 (A10) HP1353-54 (C15) HP1366 (A6) HP1369-70 (G10) HP1433 (C6) HP1471 (G14) HP1522 (G12)	Cj0565* (G12) Cj0628-29 (T5)	Cj0565[#] (G12) Cj0618 (G9) Cj0628-29 (G10) Cj0676 (G9) Cj1321 (G10)	Cj0031-32 (G10) Cj0045c (C10) Cj0046 (G11) Cj0170 (G9) Cj0208 (G6) Cj0279 (G6) Cj0275 (G8) Cj0348 (G6) Cj0506 (G6) Cj0617 (G9) Cj0628-29 (A5) Cj0684 (G6) Cj0685c (C9) Cj0735 (G6) Cj0959c (G5) Cj1061c (G6) Cj1130c (G6) Cj1139c (G8) Cj1144-45 (G8) Cj1184c (G6) Cj1238 (G6) Cj1295 (G9) Cj1296-97 (G9) Cj1305c (G9) Cj1306c (G9) Cj1310c (G9) Cj1318 (G11) Cj1325-26 (G10) Cj1335-36 (G9) Cj1342c (G9) Cj1370 (G6) Cj1420c (G9) Cj1421c (G9) Cj1422c (G9) Cj1426c (G10) Cj1429 (G10) Cj1437c (G9) Cj1443c (G5) Cj1643 (G6) Cj1677-78 (T7)

* considering primary TSS
[#] considering secondary TSS

Table S3: Bacterial strains

Name	Description	Strain number	*H. pylori*	Resistance
WT / 26695	Wild-type (NCBI Acc-no. NC_000915), kindly provided by T. F. Meyer (MPI-IB, Berlin, Germany); Ref. (24)	CSS-0004	26695	
Δ*repG*	*repG*::*aphA-3*	JVS-7014	26695	Kan^R
C_{RepG}	*repG*::*aphA-3, rdxA*::*repG*::*catGC*	CSS-0046	26695	Kan^R Cm^R
SL 2	*repG*::*aphA-3, rdxA*::*repG*-SL 2::*catGC*	CSS-0747	26695	Kan^R Cm^R
ΔCU	*repG*::*aphA-3, rdxA*::*repG*-ΔCU::*catGC*	CSS-0157	26695	Kan^R Cm^R
3xG	*repG*::*aphA-3, rdxA*::*repG*-3xG::*catGC*	CSS-0158	26695	Kan^R Cm^R
1xG*	*repG*::*aphA-3, rdxA*::*repG*-1xG*::*catGC*	CSS-0159	26695	Kan^R Cm^R
Δ*tlpB*	*tlpB*::*rpsL-erm*	CSO-0163	26695	Erm^R
Δ*tlpB*/Δ*repG*	*tlpB*::*rpsL-erm*, *repG*::*aphA*-3	CSO-0164	26695	Erm^R Kan^R
***tlpB*::3xFLAG**	*tlpB*-3xFLAG::*rpsL-erm*	CSO-0190	26695	Erm^R
tlpB*::3xFLAG/ Δ*repG	*tlpB*-3xFLAG::*rpsL-erm, repG*::*aphA-3*	CSS-0215	26695	Erm^R Kan^R
***tlpB*::3xFLAG/ Δ*repG*/C_{RepG}**	*tlpB*-3xFLAG::*rpsL-erm, repG*::*aphA-3, rdxA*::*repG*::*catGC*	CSS-0285	26695	Erm^R Kan^R Cm^R
P_{tlpB}	*rpsL-erm*::*tlpB* WT	CSS-0384	26695	Erm^R
P_{tlpB}/Δ*repG*	*rpsL-erm*::*tlpB* WT, *repG*::*aphA-3;*	CSS-0388	26695	Erm^R Kan^R
***tlpB* ΔG**	*rpsL-erm*::*tlpB* ΔG	CSS-0385	26695	Erm^R
***tlpB* 3xC**	*rpsL-erm* ::*tlpB* 3xC	CSS-0386	26695	Erm^R
tlpB* 1xC	*rpsL-erm*::*tlpB* 1xC*	CSS-0387	26695	Erm^R
P_{cagA}	*rpsL-erm*::P_{cagA}-*tlpB*	CSS-0657	26695	Erm^R
P_{cagA}/Δ*repG*	*rpsL-erm*::P_{cagA}-*tlpB*	CSS-0658	26695	Erm^R Kan^R
tlpB*-5th::*gfpmut3	*rdxA*::*tlpB*-5th-*gfpmut3*::*catGC*	CSS-0748	G27	Cm^R
tlpB*-5th::*gfpmut3*/Δ*repG	*repG*::*aphA-3; rdxA*::*tlpB*-5th-*gfpmut3*::*catGC*	CSS-0751	G27	Cm^R Kan^R
cagA*-28th::*gfpmut3	*rdxA*::*cagA*-28th-*gfpmut3*::*catGC*	CSS-0804	G27	Cm^R
cagA*-28th::-*gfpmut3*/ Δ*repG	*repG*::*aphA-3; rdxA*::*cagA*-28th-*gfpmut3*::*catGC*	CSS-0805	G27	Cm^R Kan^R
26695 Str^R	*rpsL-str^R*, streptomycin-resistant 26695 based on CSS-0004	CSS-0024	26695 Str^R	Str^R
26695^R *tlpB*::3xFLAG	*rpsL-str^R*, *tlpB*-3xFLAG::*rpsL-erm; tlpB*::3xFLAG tagged background strain for markerless exchange	CSS-0461	26695 Str^R	Str^R Erm^R
26695^R *tlpB*::3xFLAG*	*rpsL-str^R*, *tlpB*-3xFLAG*, markerless *tlpB*::3xFLAG tagged background strain	CSS-0464	26695 Str^R	Str^R
***tlpB*::3xFLAG*/ *tlpB* ΔG**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* ΔG	CSS-0471	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 6G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 6G	CSS-0472	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 7G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 7G	CSS-0473	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 8G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 8G	CSS-0474	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 9G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 9G	CSS-0475	26695 Str^R	Str^R Erm^R
***tlpB*-3xFLAG*/ *tlpB* 10G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 10G	CSS-0476	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 11G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 11G	CSS-0477	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* WT (12G)**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 12G	CSS-0470	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 13G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 13G	CSS-0478	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 14G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 14G	CSS-0479	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 15G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 15G	CSS-0480	26695 Str^R	Str^R Erm^R
***tlpB*::3xFLAG*/ *tlpB* 16G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *rpsL-erm*::*tlpB* 16G	CSS-0481	26695 Str^R	Str^R Erm^R
26695^R *tlpB*::3xFLAG*/ Δ*repG*	*rpsL-str^R*,*tlpB*-3xFLAG*, *repG*::*aphA-3*	CSS-0467	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* ΔG**	*rpsL-str^R*, *tlpB*-3xFLAG*, *repG*::*aphA-3, rpsL-erm*::*tlpB* ΔG	CSS-0483	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 6G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *repG*::*aphA-3, rpsL-erm*::*tlpB* 6G	CSS-0484	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 7G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *repG*::*aphA-3, rpsL-erm*::*tlpB* 7G	CSS-0485	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 8G**	*rpsL-str^R*, *tlpB*-3xFLAG*, *repG*::*aphA-3, rpsL-erm*::*tlpB* 8G	CSS-0486	26695 Str^R	Str^R Erm^R Kan^R

***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 9G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 9G	CSS-0487	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 10G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 10G	CSS-0488	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 11G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 11G	CSS-0489	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* WT (12G)**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 12G	CSS-0482	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 13G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 13G	CSS-0490	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 14G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 14G	CSS-0491	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 15G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 15G	CSS-0492	26695 Str^R	Str^R Erm^R Kan^R
***tlpB*::3xFLAG*/ Δ*repG*/*tlpB* 16G**	*rpsL-str*^{R}, *tlpB*-3xFLAG*, *repG*::*aphA-3*, *rpsL-erm*::*tlpB* 16G	CSS-0493	26695 Str^R	Str^R Erm^R Kan^R
G27	Wild-type (NCBI Acc-no. NC_011333), kindly provided by T. F. Meyer; Ref. (28)	CSS-0010	G27	
G27 Δ*repG*	*repG*::*aphA-3*	CSS-0169	G27	Kan^R
G27 Δ*tlpB*	*tlpB*::*rpsL-erm*	CSO-0167	G27	Erm^R
G27 *tlpB*::3xFLAG	*tlpB*-3xFLAG::*rpsL-erm*	CSS-0196	G27	Erm^R
G27 *tlpB*::3xFLAG/ Δ*repG*	*tlpB*-3xFLAG::*rpsL-erm, repG*::*aphA-3*	CSS-0197	G27	Erm^R Kan^R
G27 *tlpB*::3xFLAG/ Δ*repG*/C$_{RepG}$	*tlpB*-3xFLAG::*rpsL-erm, repG*::*aphA-3*, *rdxA*::*repG*::*catGC*	CSS-0283	G27	Erm^R Kan^R Cm^R
RepG complementation	*repG*::*aphA-3, rdxA*::*repG*::*catGC*; complementation of *repG* deficient mutant with RepG in *rdxA* under its native promoter; gDNA of the complementation strain was kindly provided by F. Darfeuille, University of Bordeaux, France, unpublished.		26695	Kan^R Cm^R
J99	Wild-type (NCBI Acc-no. NC_000921), kindly provided by T. F. Meyer, MPI-IB, Berlin, Germany; Ref. (23)	CSS-0001	J99	
J99 Δ*repG*	*repG*::*aphA-3*	CSS-0732	J99	Kan^R
P12	Wild-type (NCBI Acc-no. NC_011498), kindly provided by T. F. Meyer, MPI-IB, Berlin, Germany; Ref. (29)	CSS-0003	P12	
PeCan4	Wild-type (NCBI Acc-no. NC_014555), kindly provided by Douglas E. Berg, Washington University, St. Louis, MO/University of California, San Diego, La Jolla, CA	CSS-0096	PeCan4	
Cuz20	Wild-type (NCBI Acc-no. NC_017358), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA	CSS-0097	Cuz20	
Cuz20 Δ*repG*	*repG*::*aphA-3*	CSS-0737	Cuz20	Kan^R
Sat464	Wild-type (NCBI Acc-no. NC_017359), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA	CSS-0098	Sat464	
India7	Wild-type (NCBI Acc-no. NC_017372), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA	CSS-0099	India7	
India7 Δ*repG*	*repG*::*aphA-3*	CSS-0734	India7	Kan^R
Lithuania75	Wild-type (NCBI Acc-no. NC_017362), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA	CSS-0101	Lithuania75	
Lithuania Δ*repG*	*repG*::*aphA-3*	CSS-0736	Lithuania75	Kan^R
Shi470	Wild-type (NCBI Acc-no. NC_010698), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA; Ref. (30)	CSS-0173	Shi470	
Shi470 Δ*repG*	*repG*::*aphA-3*	CSS-0735	Shi470	Kan^R

SJM180	Wild-type (NCBI Acc-no. NC_014560), kindly provided by D. E. Berg, Washington University, St. Louis, MO/ University of California, San Diego, La Jolla, CA	CSS-0174	SJM180	
B8	Wild-type (NCBI Acc-no. NC_014256), kindly provided by R. Haas, Max von Pettenkofer Institute, Munich, Germany; Ref. (31)	CSS-0213	B8	
B8 Δ*repG*	*repG*::*aphA-3*	CSS-0733	B8	Kan^R
HP1334::3x-FLAG	*tlpB*::*tlpB*-3xFLAG: *aphA-3*	JVS-7033	26695	Kan^R
Hmu	Wild-type of *Helicobacter mustelae*, kindly provided by T. F. Meyer, MPI-IB, Berlin, Germany	CSS-0007		
Hac	Wild-type of *Helicobacter acinonychis* (NCBI Acc-no. NC_008229), kindly provided by T. F. Meyer, MPI-IB, Berlin, Germany; Ref. (32)	CSS-0008		
TOP 10	*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*Z Δ M15 Δ *lac*X74 *deo*R *rec*A1 *ara*D139 Δ (*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G from Invitrogen	CSO-0296	*E. coli*	

Str^R: streptomycin resistant; Kan^R: kanamycin resistant; Cm^R: chloramphenicol resistant; Erm^R: erythromycin resistant

Table S4: DNA oligodesoxynucleotides

Sequences are given in 5' → 3' direction; P~ denotes a 5' monophosphate.

Name	Sequence 5' → 3'	Description
CSO-0003	GAAAGGAGGGGGAGGT	Northern blot probe for RepG
CSO-0017	GTTTTTTCTAGAGATCAGCCTGCCTTTAGG	RepG cloning
CSO-0018	GTTTTTCTCGAGCTTAGCGCTTAATGAAACGC	RepG cloning
CSO-0035	GGTGTTCTATAATTTCAGCTTGTATGCTTTATAACTATGGATTAA ACACTTTT	Cloning of *tlpB*
CSO-0036	CTTTTTTAATAAACTCCCCCTGATTACTTATTAAATAATTTATAG CTATTGAAAAGAG	Cloning of *tlpB*
CSO-0037	TTTAATCCATAGTTATAAAGCATACAAGCTGAAATTATAGAACAC CC	Cloning of *tlpB*
CSO-0038	GCTATAAATTATTTAATAAGTAATCAGGGGGAGTTTATTAAAAAA G	Cloning of *tlpB*
CSO-0039	GTTTTTCTCGAGTCTCAAAATCCGCTGAAATCT	Cloning of *tlpB*
CSO-0040	GTTTTTTCTAGATCAGTTGCAACCAGGAGATT	Cloning of *tlpB*
CSO-0045	AAGATGACGACGATAAATAGTAAATGCTTTATAACTATGGATTA AACACTTTT	Cloning of *tlpB*::3xFLAG
CSO-0046	TTTAATCCATAGTTATAAAGCATTTACTATTTATCGTCGTCATCT TTGT	Cloning of *tlpB*::3xFLAG
CSO-0050	GCATGAAAGATTCCTCAACCAC	Verification of *tlpB*::3xFLAG
CSO-0051	TGTCACTTATATTTACAAGTTCGCT	Verification of *tlpB* deletion
CSO-0053	ACTGCCAGGTTCGGAATGG	Northern blot probe for *Helicobacter mustelae* 5S rRNA
CSO-0065	P~GACTACAAAGACCATGACGGT	Cloning of *tlpB*::3xFLAG
CSO-0080	ACCGCTAAGATTGAAGGGTC	RepG SL 2 cloning
CSO-0081	P~TTGTAATTATACAATAAAATGGTGATTTTTAG	RepG SL 2 cloning
CSO-0083	GTTTTTATGCATCAAGCCCTTGATTATTGGTTG	Primer extension
CSO-0126	GTTTTTTGCTAGCTGAAGAAAACATCATTTATCTCCT	GFP fusion cloning
CSO-0138	AAGATTGAAGGGTACGCCCTTTGTCTTGGCGG	RepG ΔCU cloning
CSO-0139	CAAGACAAAGGGCGTACCCTTCAATCTTAGCGGTG	RepG ΔCU cloning
CSO-0140	AGGGTCACCTCCGCCTCCTTTCCCTTTGTCTT	RepG 1xG* cloning
CSO-0141	AGGGAAAGGAGGCGGAGGTGACCCTTCAATCT	RepG 1xG* cloning
CSO-0142	TTGAAGGGTCACGTCCGCCTGCTTTCCCTTTGTCTTGGC	RepG 3xG cloning
CSO-0143	ACAAAGGGAAAGCAGGCGGACGTGACCCTTCAATCTTAGCG	RepG 3xG cloning
CSO-0146	GTTTTTATCGATGTATGCTCTTTAAGACCCAGC	GFP fusion cloning
CSO-0205	AATTACAACAGTACTGCGATGAGT	RepG cloning
CSO-0206	AATCTCACGCCAAGCATTT	RepG cloning
CSO-0207	AGTTCTGATTTCATGCCCTT	RepG cloning, verification
CSO-0208	GTTTTTCTCGAGGCGCGCACACTGAAGA	Cloning of *tlpB*::3xFLAG
CSO-0209	GTTTTTGAATTCTTACTTATTAAATAATTTATAGCTATTGAAAAGA G	Cloning of *tlpB*::3xFLAG
CSO-0210	GTTTTTGAATTCAGGGGGAGTTTATTAAAAAAG	Cloning of *tlpB*::3xFLAG
CSO-0211	GTTTTTTCTAGATCTCAAAATCCGCTGAAATCT	Cloning of *tlpB*::3xFLAG
CSO-0245	P~AGTTTTAAACAAATTCACTTGTTTGTC	Cloning of *tlpB*::3xFLAG
CSO-0277	TTTATCCACCACCACCATATAAA	Cloning of *tlpB*
CSO-0278	GTTTTTTTTAATACGACTCACTATAGGTTCAAAGACATGAATTGA TTACTC	In vitro transcription
CSO-0284	GTTTTTTGCTAGCAGCCACTTGAAGATTATTGATAAAT	Cloning of *tlpB*
CSO-0291	GTTTTTCTCGAGTTAGGCATTTTATAATAAGTGTAGCCT	Cloning of *tlpB*
CSO-0294	GTTTTTGGATCCTTTATTATTTTATCTTTAAGCCTAACTTAA	Cloning of *tlpB*
CSO-0295	GTTTTTGAATTCTAAAAAATTTTATTTAACTTCACTCTCTT	Cloning of *tlpB*
CSO-0306	GTTTTTGGATCCGATCGGGCTTTTTTCAATATT	Cloning of *tlpB*
CSO-0308	GTTTTTGGATCCTGCTTTATAACTATGGATTAAACACTT	Cloning of *tlpB*
CSO-0309	GTTTTTGAATTCTTACTTATTAAATAATTTATAGCTATTGAAAAGA	Cloning of *tlpB*
CSO-0314	CTCATTTTTCGGGGGGCGGGGGTGCATTTAGAAGCTAAACTCT AAAATTAGGG	Cloning of *tlpB*; compensatory base-pair exchange
CSO-0315	TTCTAAATGCACCCCCGCCCCCCGAAAAATGAGTGGCACAAA	Cloning of *tlpB*; compensatory base-pair exchange
CSO-0316	CTCATTTTTCGGCGGGCGGGCGTGCATTTAGAAGCTAAACTCT AAAATTAGGG	Cloning of *tlpB*; compensatory base-pair exchange
CSO-0317	TTCTAAATGCACGCCCGCCCGCCGAAAAATGAGTGGCACAAA	Cloning of *tlpB*; compensatory base-pair exchange
CSO-0318	P~TGCATTTAGAAGCTAAACTCTAAAATTAG	Cloning of *tlpB*
CSO-0319	GAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0428	P~TTACTATTTATCGTCGTCATCTTT	Cloning of *tlpB*::3xFLAG

CSO-0429	TCAGGGGGAGTTTATTAAAAAAG	Cloning of *tlpB*::3xFLAG
CSO-0430	P~TTGTTTGTTCTTTTGTTTCGTTTT	Cloning of *tlpB*
CSO-0431	ATTCTTATTATACAACAATATCAAGCATT	Cloning of *tlpB*
CSO-0440	GTTTTTTGTCGACGTTGGCGTATAACATAGTATCGA	GFP fusion cloning
CSO-0441	GTTTTTTGCGGCCGCGGGAGTTAACTGCAGGTCTG	GFP fusion cloning
CSO-0442	GTTTTTGTCGACGTATGCTCTTTAAGACCCAGC	GFP fusion cloning
CSO-0443	GTTTTTGCGGCCGCCTCGAATTCAGATCCACGTT	GFP fusion cloning
CSO-0448	CCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0449	CCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0450	CCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0451	CCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0452	CCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0453	CCCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0454	CCCCCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0455	CCCCCCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0456	CCCCCCCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0457	CCCCCCCCCCCCCCCCGAAAAATGAGTGGCACAAAAC	Cloning of *tlpB*
CSO-0581	GTTTTTATCGATTTATTATTTTATCTTTAAGCCTAACTTAA	GFP fusion cloning
CSO-0590	GTTTTTATCGATGATCGGGCTTTTTTCAATAT	GFP fusion cloning
CSO-0683	GTTTTTTGCTAGCAGTAAAGGAGAAGAACTTTTCACTGGA	GFP fusion cloning
CSO-1173	GGTAGTGGTTTTTGTGTGATGG	Quantitative RT-PCR (6S RNA)
CSO-1174	CCAGATGACCGCTACTTTTACA	Quantitative RT-PCR (6S RNA)
CSONIH-0033	TCAAAGCCACTAGTAAGTCTTACTT	Verification oligo for insertion of *rpsL-erm* cassette
JVO-0155	CCGTATGTAGCATCACCTTC	Cloning of GFP fusion (33)
JVO-0485	TCGGAATGGTTAACTGGGTAGTTCCT	Northern blot probe for *Helicobacter pylori* 5S rRNA
JVO-2134	AAACCATAAGGAATGGTTGGAT	Northern blot probe for RepG
JVO-5069	CTTCACGCCCCTTGTAAATA	Verification of *repG* deletion mutant
JVO-5070	GATAAGGTTTAGCGATGTAATCGT	RepG cloning
JVO-5072	CGTTTCTTGACACGCTTAATT	RepG cloning
JVO-5125	GTTTTTTTTAATACGACTCACTATAGGATCCAACCATTCCTTATG GTT	In vitro transcription
JVO-5126	AAAACAACCGCCAAGACA	In vitro transcription
JVO-5127	GTTTTTTTTAATACGACTCACTATAGGTGTTTGTTCTTTTGTTTC GTT	In vitro transcription
JVO-5143	TTACTATTTATCGTCGTCATCTTT	In vitro transcription
JVO-5267	ACGGGGTGGTATTGTTTGAT	Quantitative RT-PCR (*tlpB*)
JVO-5268	AAGTGTAGCCTCCCCCTTTT	Quantitative RT-PCR (*tlpB*)
JVO-5953	CCCTAAACCTAAAAGAGCGG	In vitro transcription
JVO-5257	TATAGGTTTTCATTTTCTCCCAC	Verification of *repG* deletion mutant
JVO-5702	GTATTTCACACCGGGTAAATCC**C**TAACC**C**TACCCCCACG	Antisense oligo for amplification of *rpsL* fragment containing the Lys43Arg mutation (AAA129AGA) Str^R allele in primer rpsL1 and the Lys88Arg mutation (AAG263AGG) *str*^{R} allele in rpsL2 in 26695, P12 and G27 (3)
JVO-5703	CTAGGGTTTATACGACTACCCCTA**G**AAAGCCTAACTCG	Sense primer for amplification of *rpsL* fragment containing the Lys43Arg mutation (AAA129AGA) Str^R allele in primer rpsL1 and the Lys88Arg mutation (AAG263AGG) *str*^{R} allele in rpsL2 in 26695, P12 and G27 (3)
JVO-5704	AGAAGCCAGTATCGCTATGA	Sense oligo for verification of *rpsL* (HP1197) mutation in 26695, P12 and G27
pZE-A	GTGCCACCTGACGTCTAAGA	Colony PCR and sequencing of pZE12-*luc* derived plasmids
pZE-XbaI	TCGTTTTATTTGATGCCTCTAGA	Colony PCR and sequencing of pZE derived plasmids

Table S5: Plasmids

Name	Description/Generation	Origin / marker	Reference
pJV752.1	Cloning vector, pZE12-*luc* with modified p15A origin	p15A/ Amp^R	(7)
pTM117	Cloning vector carrying a *H. pylori* origin of replication, *aphA-3* resistance cassette and a multiple cloning site upstream of the promoterless *gfpmut3* gene	oriV/ pHP666/ Kan^R	(10)
p463	GFP-expression vector carring the promoter of *ureA* fused to the promoterless *gfpmut3* gene	oriV/ pHP666/ Kan^R	Kindly provided by D. S. Merrell, USU, Bethesda, MD
pPT3-1	Plasmid for introduction of transcriptional fusion of the *ureA* promoter to *gfpmut3* into the *rdxA* locus	p15A/ Amp^R	This study
pMA5-2	Plasmid for introduction of translational fusion of the *cagA*-5' UTR including the 28th amino acid to *gfpmut3* into the *rdxA* locus	p15A/ Amp^R	This study
pSP39-3	Plasmid for complementation of *repG* deletion with RepG in *H. pylori* strain 26695	p15A/ Amp^R	This study
pSP42-1	Plasmid for complementation of *repG* deletion mutant with RepG SL 2 (30-87 nt) in *H. pylori* strain 26695	p15A/ Amp^R	This study
pSP57-4	Plasmid for FLAG-tagging of HP0103 (*tlpB*) based on pJV752.1 500 nt up- and downstream of *tlpB* stop codon, 3xFLAG, *rpsL-erm* cassette	p15A/ Amp^R	This study
pSP58-5	Intermediary plasmid for construction of pSP60	p15A/ Amp^R	This study
pSP60-2	Backbone plasmid for deletion or nucleotide exchange in G-repeat in the 5' UTR of *tlpB*	p15A/ Amp^R	This study
pSP64-1	Plasmid for deletion of G-repeat in the *tlpB* 5' UTR; based on pSP60-2	p15A/ Amp^R	This study
pSP65-4	Backbone plasmid for compensatory base-pair exchange in the G-repeat in the *tlpB* 5' UTR; based on pSP60, 1xC*	p15A/ Amp^R	This study
pSP66-4	Backbone plasmid for compensatory base-pair exchange in the G-repeat in the *tlpB* 5' UTR; based on pSP60, 3xC	p15A/ Amp^R	This study
pSP70-1	Backbone plasmid for *tlpB*::3xFLAG based on pSP57, for markerless exchange	p15A/ Amp^R	This study
pSP73-1	Plasmid for variation of G-repeat length, 6G	p15A/ Amp^R	This study
pSP74-1	Plasmid for variation of G-repeat length, 7G	p15A/ Amp^R	This study
pSP75-1	Plasmid for variation of G-repeat length, 8G	p15A/ Amp^R	This study
pSP76-1	Plasmid for variation of G-repeat length, 9G	p15A/ Amp^R	This study
pSP77-1	Plasmid for variation of G-repeat length, 10G	p15A/ Amp^R	This study
pSP78-1	Plasmid for variation of G-repeat length, 11G	p15A/ Amp^R	This study
pSP79-4	Plasmid for variation of G-repeat length, 13G	p15A/ Amp^R	This study
pSP80-1	Plasmid for variation of G-repeat length, 14G	p15A/ Amp^R	This study
pSP81-5	Plasmid for variation of G-repeat length, 15G	p15A/ Amp^R	This study
pSP82-1	Plasmid for variation of G-repeat length, 16G	p15A/ Amp^R	This study
pSP91-3	Plasmid for *tlpB* promoter exchange (*cagA* instead of *tlpB* promoter)	p15A/ Amp^R	This study
pSP109-6	Plasmid for introduction of translational fusion of the *tlpB*-5' UTR including the 5th amino acid to *gfpmut3* into the *rdxA* locus	p15A/ Amp^R	This study

Table S6: Sequences of *tlpB* leader mutants of *H. pylori* strain 26695. The length variable homopolymeric G-repeat in the 5' UTR of *tlpB* mRNA is shown in red and the RBS as well as start codon (ATG) are marked in light green. The gDNAs of *tlpB* leader mutants were used for the generation of DNA templates for T7 in vitro transcription assays (see also Table S7).

Name	Sequence 5' → 3'	Strain number
tlpB WT	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAA AATGATTTATAGGAGATAAATG	CSS-0470/ CSS-0482
tlpB ΔG	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGATTTATAG GAGATAAATG	CSS-0471/ CSS-0483
tlpB 6G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGAT TTATAGGAGATAAATG	CSS-0472/ CSS-0484
tlpB 7G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATGA TTTATAGGAGATAAATG	CSS-0473/ CSS-0485
tlpB 8G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAATG ATTTATAGGAGATAAATG	CSS-0474/ CSS-0486
tlpB 9G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAAT GATTTATAGGAGATAAATG	CSS-0475/ CSS-0487
tlpB 10G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAAA TGATTTATAGGAGATAAATG	CSS-0476/ CSS-0488
tlpB 11G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAAAA ATGATTTATAGGAGATAAATG	CSS-0477/ CSS-0489
tlpB 13G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTAA AAATGATTTATAGGAGATAAATG	CSS-0478/ CSS-0490
tlpB 14G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTTA AAAATGATTTATAGGAGATAAATG	CSS-0479/ CSS-0491
tlpB 15G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACTT AAAAATGATTTATAGGAGATAAATG	CSS-0480/ CSS-0492
tlpB 16G	**T**GTTTGTTCTTTTGTTTCGTTTTCAAACAACCGGGTTTTAATTTTGTTTTGTGCCACTCA TTTTTCGGGGGGGGGGGGGGGGTGCATTTAGAAGCTAAACTCTAAAATTAGGGTTTGACT TAAAAATGATTTATAGGAGATAAATG	CSS-0481/ CSS-0493

Table S7: Details of RNAs used for in vitro work.

RepG / *tlpB* mRNA leader	DNA template (plasmid or gDNA of *H. pylori* strain number)	Oligonucleotides	For mRNAs: 5' part to ATG [nt]	For mRNAs: 3' part from ATG [nt]	Size of the T7-transcripts [nt]
RepG	CSS-0004	JVO-5125/JVO-5126	-	-	87
RepG ΔCU	CSS-0157	JVO-5125/JVO-5126	-	-	58
RepG 3xG	CSS-0158	JVO-5125/JVO-5126	-	-	87
RepG 1xG	CSS-0159	JVO-5125/JVO-5126	-	-	87
***tlpB* leader**	CSS-0004	JVO-5127/JVO-5953	-139	+78	217
***tlpB* ΔG leader**	CSS-0385	JVO-5127/JVO-5953	-139	+78	205
***tlpB* 3xC leader**	CSS-0386	JVO-5127/JVO-5953	-139	+78	217
***tlpB* 1xC* leader**	CSS-0385	JVO-5127/JVO-5953	-139	+78	217
***tlpB* 6G leader**	CSS-0472	JVO-5127/JVO-5953	-139	+78	211
***tlpB* 7G leader**	CSS-0473	JVO-5127/JVO-5953	-139	+78	212
***tlpB* 8G leader**	CSS-0474	JVO-5127/JVO-5953	-139	+78	213
***tlpB* 9G leader**	CSS-0475	JVO-5127/JVO-5953	-139	+78	214
***tlpB* 10G leader**	CSS-0476	JVO-5127/JVO-5953	-139	+78	215
***tlpB* 11G leader**	CSS-0477	JVO-5127/JVO-5953	-139	+78	216
***tlpB* 13G leader**	CSS-0478	JVO-5127/JVO-5953	-139	+78	218
***tlpB* 14G leader**	CSS-0479	JVO-5127/JVO-5953	-139	+78	219
***tlpB* 15G leader**	CSS-0480	JVO-5127/JVO-5953	-139	+78	220
***tlpB* 16G leader**	CSS-0481	JVO-5127/JVO-5953	-139	+78	221
***tlpB-5th::gfpmut3* mRNA**	pSP109-6	JVO-5127/CSO-0441	-139	+775	917
***cagA-28th::gfpmut3* mRNA**	pMA5-2	CSO-0278/CSO-0441	-105	+844	949
***tlpB*::3xFLAG mRNA**	CSS-0464	JVO-5127/JVO-5143	-139	+1768	1907
***tlpB* ΔG::3xFLAG mRNA**	CSS-0471	JVO-5127/JVO-5143	-139	+1768	1895
***tlpB* 10G::3xFLAG mRNA**	CSS-0476	JVO-5127/JVO-5143	-139	+1768	1905
***tlpB* 11G::3xFLAG mRNA**	CSS-0477	JVO-5127/JVO-5143	-139	+1768	1906
***tlpB* 13G::3xFLAG mRNA**	CSS-0478	JVO-5127/JVO-5143	-139	+1768	1908
***tlpB* 14G::3xFLAG mRNA**	CSS-0479	JVO-5127/JVO-5143	-139	+1768	1909

Table S8: Sequences of T7 transcripts. The C/U-rich *tlpB* binding site of RepG and the G-repeat in the 5' UTR of *tlpB* mRNA are underlined. Mutations in the sRNA/mRNA interaction sites are indicated in different colors. The RBS and start codon (ATG) of *tlpB* mRNA are marked in light green and the ORF is shown in gray letters. The *gfpmut3* sequence is highlighted in green.

Name	Sequence 5' → 3'
RepG WT	**A**UCCAACCAUUCCUUAUGGUUUGGUUGGCACCGCUAAGAUUGAAGGGUCACCUCCCCCUCCUUUCCCUUUGUCUUGGCGGUUGUUUU
RepG SL 2	**A**CCGCUAAGAUUGAAGGGUCACCUCCCCCUCCUUUCCCUUUGUCUUGGCGGUUGUUUU
RepG ΔCU	**A**UCCAACCAUUCCUUAUGGUUUGGUUGGCACCGCUAAGAUUGAAGGGUACGCCCUUUGUCUUGGCGGUUGUUUU
RepG 3xG	**A**UCCAACCAUUCCUUAUGGUUUGGUUGGCACCGCUAAGAUUGAAGGGUCACGUCCGCCUGCUUUCCCUUUGUCUUGGCGGUUGUUUU
RepG 1xG*	**A**UCCAACCAUUCCUUAUGGUUUGGUUGGCACCGCUAAGAUUGAAGGGUCACCUCCGCCUCCUUUCCCUUUGUCUUGGCGGUUGUUUU
tlpB WT leader	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUCGGGGGGGGGGGUGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAAUGUUUGCUUCGUUGGGGACUCGUAUCAUGCUGGUCGUGUUAGCCGCUCUUUUAGGUUUAGGG
tlpB ΔG leader	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUCUGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAAUGUUUGCUUCGUUGGGGACUCGUAUCAUGCUGGUCGUGUUAGCCGCUCUUUUAGGUUUAGGG
tlpB 6G-16G leader	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUC-(G)$_{6-16}$-UGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAAUGUUUGCUUCGUUGGGGACUCGUAUCAUGCUGGUCGUGUUAGCCGCUCUUUUAGGUUUAGGG
tlpB 3xC leader	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUCGGCGGGCGGGCGUGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAAUGUUUGCUUCGUUGGGGACUCGUAUCAUGCUGGUCGUGUUAGCCGCUCUUUUAGGUUUAGGG
tlpB 1xC* leader	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUCGGGGGGCGGGGGUGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAAUGUUUGCUUCGUUGGGGACUCGUAUCAUGCUGGUCGUGUUAGCCGCUCUUUUAGGUUUAGGG
tlpB-5th::gfpmut3 mRNA	**U**GUUUGUUCUUUUGUUUCGUUUUCAAACAACCGGGUUUUAAUUUUGUUUUGUGCCACUCAUUUUUCGGGGGGGGGGGUGCAUUUAGAAGCUAAACUCUAAAAUUAGGGUUUGACUUAAAAAUGAUUUAUAGGAGAUAAAUGAUGUUUUCUUCAGCUAGCAGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGUCCUUCUUGAGUUUGUAACAGCUGCUGGGAUUACACAUGGCAUGGAUGAACUAUACAAAUAAAUGUCCAGACCUGCAGUUAACUCCCGCGGCCGCAAAAAAC
cagA-28th::gfpmut3 mRNA	**G**TTCAAAGACATGAATTGATTACTCAAGTGTGTAGCAGTTTTTAGCAGTCTTTGATACCAACAAGATACCGATAGGTATGAAACTAGGTATAGTAAGGAGAAACAATGACTAACGAAACCATTAACCAACAACCACAAACTGAAGCGGCTTTTAACCCGCAGCAATTTATCAATAATCTTCAAGTGGCTGCUAGCAGUAAAGGAGAAGAACUUUUCACUGGAGUUGUCCCAAUUCUUGUUGAAUUAGAUGGUGAUGUUAAUGGGCACAAAUUUUCUGUCAGUGGAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAAUUUAUUUGCACUACUGGAAAACUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCGGUUAUGGUGUUCAAUGCUUUGCGAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCCGAAGGUUAUGUACAGGAAAGAACUAUAUUUUUCAAAGAUGACGGGAACUACAAGACACGUGCUGAAGUCAAGUUUGAAGGUGAUACCCUUGUUAAUAGAAUCGAGUUAAAAGGUAUUGAUUUUAAAGAAGAUGGAAACAUUCUUGGACACAAAUUGGAAUACAACUAUAACUCACACAAUGUAUACAUCAUGGCAGACAAACAAAAGAAUGGAAUCAAAGUUAACUUCAAAAUUAGACACAACAUUGAAGAUGGAAGCGUUCAACUAGCAGACCAUUAUCAACAAAAUACUCCAAUUGGCGAUGGCCCUGUCCUUUUACCAGACAACCAUUACCUGUCCACACAAUCUGCCCUUUCGAAAGAUCCCAACGAAAAGAGAGACCACAUGGUCCUUCUUGAGUUUGUAACAGCUGCUGGGAUUACACAUGGCAUGGAUGAACUAUACAAAUAAAUGUCCAGACCUGCAGUUAACUCCCGCGGCCGCAAAAAAC

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