**Helicobacter pylori** Initiates the Stringent Response upon Nutrient and pH Downshift

Derek H. Wells† and Erin C. Gaynor*

Department of Biological Sciences, Stanford University, Stanford, California, and Department of Microbiology and Immunology, Stanford University, Stanford, California

Received 31 August 2005/Accepted 2 March 2006

* Corresponding author. Present address: Department of Microbiology and Immunology, University of British Columbia, Life Sciences Centre, 2558-2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada. Phone: (604) 822-2710. Fax: (604) 822-6041. E-mail: egaynor@interchange.ubc.ca.
† Present address: Division of Infectious Diseases, University of California San Francisco, San Francisco, California.

**Helicobacter pylori** was previously reported to lack a stringent response. In contrast, we show that after nutrient downshift, *H. pylori* produced abundant ppGpp and less total RNA. pH downshift also caused (p)ppGpp accumulation. Our observations indicate that nutrient deprivation and acid shock activate the stringent response in *H. pylori*.

The stringent response controls the ability to adapt to certain nutrient stress conditions, is associated with the onset of stationary phase in many bacteria, and directs a general decrease in metabolic activity. Hallmarks of the stringent response include rapid accumulation of guanosine tetraphosphate (ppGpp) and inhibition of stable RNA (sRNA [rRNA and tRNA]) synthesis.

Using model systems like *Escherichia coli*, it has been shown that upon amino acid starvation, the presence of uncharged tRNA molecules triggers ribosome-associated RelA to produce guanosine pentaphosphate (pppGpp), which is subsequently hydrolyzed to ppGpp (4). ppGpp is thought to bind to and alter the affinity of RNA polymerase for various promoters, such as those associated with adaptation to adverse conditions (4, 6). In *E. coli*, SpoT, a protein homologous to RelA, is responsible for ppGpp hydrolysis (4) but also has the capacity to synthesize ppGpp (25) under certain conditions (21).

All bacteria have either separate RelA and SpoT proteins, similar to *E. coli*, or dual-function enzymes (23, 24) that both synthesize ppGpp and hydrolyze ppGpp at different active sites (13). Since there is at least one relA/spoT homolog in all bacteria (16), the stringent response serves as a highly conserved means of fine-tuning metabolic activity in response to stresses.

Recent work has shown that persistence of pathogenic bacteria within specialized host niches requires the stringent response (9, 12, 17). For example, the stringent response allows for the persistence of *Mycobacterium tuberculosis* within the host (17), the activation of pathogenesis-related genes in *Legionella pneumophila* (2, 12), and the interaction of the enteric pathogen *Campylobacter jejuni* with host cells (9). Such observations strongly suggest that changes brought about by ppGpp allow these bacteria to adjust to and thrive within a specialized host environment.

Although the genes responsible for ppGpp production are broadly conserved and required for the success of several pathogenic interactions, it was previously reported that *Helicobacter pylori*, a widespread gram-negative bacterium that colonizes the stomach and causes peptic ulcer disease and gastric carcinoma (7), lacks a stringent response (18). In that study, initiation of the stringent response in *H. pylori* was tested by addition of the amino acid biosynthesis inhibitors pseudomonic acid and serine hydroxamate (SH). After addition of these compounds, the authors assayed protein synthesis and the abundance of a single rRNA transcript. They observed that although translation was inhibited, rRNA continued to accumulate. The authors concluded that *H. pylori* does not initiate the stringent response during translational pausing and that this characteristic was unique among all eubacteria (18).

Despite the fact that both sequenced *H. pylori* strains harbor *relA/spoT* homologs annotated as spoT (8), the authors further hypothesized that bacteria inhabiting "protected" niches (e.g., the epithelium of the stomach), in contrast to those found in the general environment, do not require and thereby have lost the ability to induce the stringent response (18).

We demonstrate that, in contrast to the previous conclusions (18), *H. pylori* has a pronounced stringent response, as evidenced by the production of significant amounts of ppGpp and a marked decrease in sRNA under stringent total nutrient starvation conditions. Three *H. pylori* strains were assayed for ppGpp production: the sequenced strains J99 and 26695 (1, 20) and strain G27, a strain commonly used to investigate *H. pylori* interactions with gastric epithelial cells (10, 11, 19). Strains were grown in rich medium (brucella broth plus 10% fetal bovine serum) under microaerobic conditions to an early log optical density at 600 nm of ~0.2. The bacteria were then washed and transferred to either morpholinepropanesulfonic acid (MOPS)-MGS (14) lacking mannitol (22), a defined minimal medium that contains no carbon or phosphate, or fresh rich medium. ppGpp production was assayed by an established protocol (3). In brief, immediately after transfer to new medium, samples were labeled with 100 μCi/ml 32P (Amersham) for 45 to 60 min under microaerobic conditions and then treated with an equal volume of 2 M formic acid to lyse cells and extract nucleotides. Small volumes (typically 3 μl) were spotted onto polyethyleneimine-cellulose thin-layer chromatography (TLC) plates and developed in 1.5 M KH2PO4 to visualize intracellular nucleotide pools. Intensity was deter-
inhibition of sRNA synthesis (4). To test this in H. pylori, we measured total RNA produced upon a nutrient downshift. As previously reported, H. pylori does not harbor uracil uptake machinery (18); thus, assays of RNA turnover involving [3H]uracil were monitored for 3 h under microaerobic conditions. As expected, H. pylori did not grow in the minimal medium, although numbers of CFU in both nutrient-downshifted samples remained constant with no loss of viability over the entire 3 h (data not shown). However, the total amount of RNA per CFU declined dramatically for G27 shifted to minimal medium without chloramphenicol, in contrast to G27 maintained in rich medium or to G27 shifted to minimal medium plus chloramphenicol (Fig. 2A).

To further support these findings, precise levels of spoT RNA and 16S rRNA were assayed by reverse transcription-quantitative PCR analyses. We observed a significant (>3-fold) increase in the ratio of spoT RNA to 16S rRNA in the minimal medium samples over time compared to rich medium samples (Fig. 2B), suggesting either a relative increase in spoT RNA, a relative decrease in 16S rRNA, or a combination of both during starvation conditions. Together, the observations presented in Fig. 2 are consistent with the notion that H. pylori indeed mounts a stringent response that is induced during total nutrient deprivation.

Recently, a pH downshift was shown to result in numerous gene expression changes in H. pylori (15). We therefore sought to determine whether H. pylori initiates the stringent response...
during a pH downshift. Strain G27 was grown to early log phase in rich medium at pH 7.0 and then either downshifted to pH 4.5 or in rich medium at pH 3.0 (final pH 4.5) and labeled with 32P. Nucleotides were resolved by TLC. (pppGpp) corresponding to guanosine penta- and tetraphosphate in the pH 4.5 sample is indicated by the arrow. This spot was quantified by densitometer analysis and found to be ~4.5-fold higher than in the pH 7.0 control.

FIG. 3. H. pylori produces (p)ppGpp upon pH downshift. H. pylori strain G27 was grown to early log phase in rich medium at neutral pH and then diluted 1:1 either in rich medium at neutral pH (final pH 7.0) or in rich medium at pH 3.0 (final pH 4.5) and labeled with 32P. Nucleotides were resolved by TLC. (pppGpp) corresponding to guanosine penta- and tetraphosphate in the pH 4.5 sample is indicated by the arrow. This spot was quantified by densitometer analysis and found to be ~4.5-fold higher than in the pH 7.0 control.

We thank Sharon Long and Stanley Falkow for support and encouragement throughout the course of this work, Sarah Svensson and George Spiegelman for helpful discussions, Karen Guillemin and Kyle Moury for helpful discussions and assistance with RNA experiments, and Hirofumi Hara and William Mohn for assistance with RT-qPCR experiments.

D.H.W. was supported by NIH grant GM30962 to Sharon Long. E.C.G. is supported by a Career Development Award from the Burroughs Wellcome Fund and a grant from the Canadian Institutes of Health Research.

REFERENCES


