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Flagellar and global gene regulation in *Helicobacter pylori* modulated by changes in DNA supercoiling

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Abstract

In *Helicobacter pylori*, a host-adapted bacterium with a small genome and few dedicated transcriptional regulators, promoter structure, and gene organization suggested a role for DNA topology in the transcriptional regulation of flagellar genes. *H. pylori* DNA supercoiling, monitored by a reporter plasmid, was relaxed by novobiocin, an inhibitor of DNA gyrase. A decrease in negative supercoiling coincided with lowered transcription of the late flagellin gene *flaA*. Targeted mutagenesis that either increased or decreased promoter spacer length in the *flaA* σ^{28} promoter lowered *flaA* transcript levels, expression of FlaA protein, and flagella formation. It also changed the promoter response to decreased superhelicity. Supercoiling of reporter plasmid DNA in *H. pylori* varied with growth phase in liquid culture. *H. pylori* σ^{28} promoters of various spacer length, as well as other supercoiling-sensitive genes, were differentially transcribed during the growth phases, consistent with supercoiling being associated with growth phase regulation. Genome-wide transcript analysis of wild-type *H. pylori* under conditions of reduced supercoiling identified flagellar, housekeeping, and virulence genes, the expression of which correlated with supercoiling change and/or growth phase. These data indicate that global supercoiling changes may help coordinate temporal (growth phase-related) regulation of flagellar biosynthesis and other cellular functions in *Helicobacter*.

Keywords: *Helicobacter*; Flagella; Type III secretion; DNA supercoiling; Transcriptional regulation; Growth cycle

Introduction

Genomic analysis reveals that the gastric pathogen *Helicobacter pylori* contains fewer regulatory factors than many other bacteria (Alm et al., 1999). Nevertheless, *H. pylori* coordinately regulates complex cellular processes. One example is the synthesis of flagella, which are essential for *H. pylori* to persist in the viscous stomach mucus where the bacterium must counter

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shedding forces, avoid gastric acidity, and orient itself in gradients essential for energy generation (Schreiber et al., 2004). As with other bacteria, flagellar biosynthesis is a hierarchical process that is subject to temporal and growth phase regulation (Spohn and Scarlato, 2001; Niehus et al., 2004; Josenhans et al., 2002a) involving sequential activation of approximately 40 genes (Niehus et al., 2004; Macnab, 2003). However, no master regulator of flagellar biosynthesis (such as the enterobacterial *flhCD* genes) has been found in *H. pylori* that could link flagellar biosynthesis with the cell cycle (Spohn and Scarlato, 1999, 2001; Niehus et al., 2004). It is unclear how formation of new flagellar filaments is initiated to create a set of new, unipolar filaments at the moment of daughter cell partition. The possibility exists that some *H. pylori* flagellar genes have evolved to respond to changes in DNA supercoiling, since in *Escherichia coli* and other bacterial species the expression of many genes is responsive to DNA topology changes, which are known to occur in response to temperature, anaerobiosis, osmolarity, pH, and cellular energy level (ATP/ADP ratio) (Drlica, 1992; Higgins et al., 1988; Hsieh et al., 1991) and entry into stationary phase (Balke and Gralla, 1987; Bang et al., 2002; Camacho-Carranza et al., 1995). A response to supercoiling could occur at many levels, ranging from the sensitivity of promoters to supercoiling effects on DNA strand separation (open complex formation) to DNA binding of accessory regulatory proteins (for reviews, see Hatfield and Benham, 2002; Travers and Muskhelishvili, 2005b). It has even been proposed that DNA twist associated with supercoiling may affect the alignment of the −10 and −35 regions of promoters and thereby influence initiation of transcription (Wang and Syvanen, 1992; Wang, 1998). Since the spacing between the −10 and −35 regions also affects the alignment of the two binding sites, spacing will influence transcription in a supercoil-dependent way.

We noticed that the major flagellin gene, *flaA*, which is expressed most strongly in the late exponential growth phase (Niehus et al., 2002, 2004; Josenhans et al., 2002a), has a σ^{28} promoter with the unusual spacing of 13 base pairs (bp). The short promoter spacer of *flaA* is conserved across 10 strains from diverse *H. pylori* populations (T. Brauer and S. Suerbaum, unpublished) and is found in other *Helicobacter* species (e.g. 11 nucleotides in *H. felis*; Josenhans et al., 1999). In contrast, other σ^{28}-dependent promoters characterized in *H. pylori* have spacer lengths of 14 or 15 bp (Niehus et al., 2004; Josenhans et al., 2002a), as is typical of *E. coli* σ^{28} promoters (Leying et al., 1992). Thus the short spacer of the *flaA* promoter is likely to be important, perhaps sensing DNA supercoiling. A second surprising observation connecting flagellar genes and supercoiling concerns the proximity of topoisomerase and flagellar genes on the *H. pylori* chromosome: *topA* is immediately adjacent to *flaB*, the second flagellin gene, with the two genes transcribed in opposite directions by overlapping promoters (Suerbaum et al., 1998). This configuration could allow transcription-dependent supercoiling from one promoter to affect expression from the other (Liu and Wang, 1987). Third, *gyrA* is in an operon containing the *flgR* gene, which encodes a response regulator that functions as a transcriptional activator for σ^{54}-dependent flagellar genes (Spohn and Scarlato, 1999; Niehus et al., 2004). These observations raise the possibility that expression of some flagellar genes respond to changes in DNA supercoiling.

In keeping with its dearth of regulatory genes, *H. pylori* appears to have a relatively simple system for maintaining supercoil homeostasis. In *E. coli*, negative DNA supercoils are introduced by DNA gyrase (Gellert et al., 1976; Levine et al., 1998), and they are relaxed by topoisomerase I (Wang, 1971; Roca, 1995) and topoisomerase IV (Kato et al., 1990; Zechiedrich et al., 2000). The net balance between supercoil-introducing and -relaxing activities is under homeostatic control (Menzel and Gellert, 1983; Snoep et al., 2002). *H. pylori* has genes encoding topoisomerase I (*topA*) and gyrase (*gyrA*, *gyrB*), but it lacks topoisomerases III and IV. Morevover, the *H. pylori topA* gene has only a single promoter (Suerbaum et al., 1998), in contrast to the four that control *E. coli topA* and apparently maintain expression throughout the growth phases (Qi et al., 1997; Tse-Dinh and Beran, 1988). Thus supercoiling may not be as tightly regulated in *H. pylori* as in *E. coli*.

The aim of the present study was to examine the hypothesis that DNA supercoiling contributes to *H. pylori* gene regulation, and in particular, to temporal regulation of flagellar gene expression. Using a series of unmarked chromosomal promoter mutations, we report that the unusual 13-bp spacing of the *flaA* promoter is optimal for *flaA* transcription under in vitro culture conditions and that the *flaA* promoter is sensitive to relaxation of DNA supercoiling induced by the gyrase inhibitor, noboviocin. Both shortening and extending the promoter spacer reduced promoter activity and reversed its response to lowered supercoiling. This finding was surprising and not readily explained. We also found that DNA supercoiling in *H. pylori* is growth phase dependent, suggesting that changes of supercoiling may link flagellar gene expression to the bacterial cell cycle. Finally, a genome-wide microarray screen revealed that a drop in supercoiling correlated with changes in expression levels for several genes of the growth phase-dependent flagellar hierarchy, as well as genes involved in stress response and virulence. These results raise the possibility that DNA superhelicity in *H. pylori* helps synchronize gene transcription.