

# Inactivation of a *Helicobacter pylori* DNA methyltransferase alters *dnaK* operon expression following host-cell adherence

John P. Donahue<sup>a,\*</sup>, Dawn A. Israel<sup>b</sup>, Victor J. Torres<sup>a,c</sup>, Antoaneta S. Necheva<sup>a</sup>,  
Geraldine G. Miller<sup>a,c</sup>

<sup>a</sup> Department of Medicine, Division of Infectious Diseases, A3310, MCN, Vanderbilt University School of Medicine, Nashville, TN 37232 USA

<sup>b</sup> Department of Medicine, Division of Gastroenterology, Vanderbilt University School of Medicine, Nashville, TN 37232 USA

<sup>c</sup> Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232 USA

Received 25 September 2001; received in revised form 7 January 2002; accepted 18 January 2002

First published online 18 February 2002

## Abstract

The *Helicobacter pylori* *hpyIM* gene encodes a type II DNA methyltransferase that is highly conserved among strains. To investigate the potential role of M.HpyI methyltransferase activity in controlling gene expression in *H. pylori*, we analyzed gene transcription profiles in wild-type strain J166 and an isogenic *hpyIM* mutant strain using gene arrays. This analysis showed that the expression of a majority of genes was unaffected by *hpyIM* mutation, especially in exponential phase cultures. However, in stationary phase cultures and in cells adherent to AGS gastric epithelial cells in vitro, loss of *hpyIM* function altered the expression of the stress-responsive *dnaK* operon. Complementation of the *hpyIM* mutation using a shuttle plasmid encoding a wild-type copy of the gene re-established the wild-type pattern of *dnaK* operon expression. These data suggested that *hpyIM*, encoding a DNA methyltransferase, may have a role in *H. pylori* physiology that supersedes its original function in a type II restriction–modification system. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** *dnaK* operon; DNA methyltransferase; *hpyIM*; Gene expression regulation; Chaperone; *Helicobacter pylori*

## 1. Introduction

*Helicobacter pylori* is a microaerophilic, Gram-negative bacterium that persistently colonizes the mucous layer overlying the gastric epithelium [1]. Colonization with *H. pylori* results in chronic superficial gastritis [2], which increases the risk for the development of peptic ulcer disease and distal gastric carcinoma [3–4]. However, the majority of persons colonized with *H. pylori* remain asymptomatic. Although several strain-specific factors have been identified that may be markers for the different clinical outcomes of *H. pylori* colonization [5–8], we presently have an incomplete understanding of the role these bacterial factors might have in progression to disease.

In studies designed to specifically identify virulence determinants in pathogenic versus non-pathogenic *H. pylori* strains, we identified a genetic locus that is transcription-

ally activated following adherence of an ulcer-derived *H. pylori* strain to gastric epithelial cells in vitro [8]. The locus includes a highly conserved DNA adenine methyltransferase, encoded by *hpyIM*, that modifies the sequence CATG [9] and a 5'-flanking gene, named *iceA*, that shows extensive diversity between strains [8,10] and includes two mutually exclusive gene families, designated *iceA1* and *iceA2*. In a minority of strains the *iceA1* allele encodes a functional restriction enzyme (RE) that recognizes CATG, suggesting this genetic locus originated as a type II restriction–modification (R–M) system. However, mutations in the majority of *iceA1* alleles prevent translation of a functional RE partner for the methyltransferase [10]. *iceA2* genes are highly diverse and have low homology to *iceA1* and other sequences in public databases [10], and it is not known whether *iceA2* directs the synthesis of a functional protein in vivo.

Transcription of *hpyIM* initiates at promoters located within *iceA1* or *iceA2* [11,12] and Northern blot analysis of RNA from exponential phase broth cultures demonstrates that *hpyIM* transcript levels are higher in *iceA1* strains than in *iceA2* strains (J.P. Donahue, unpublished).

\* Corresponding author. Tel.: +1 (615) 343 7869;

Fax: +1 (615) 343 6160.

E-mail address: john.donahue@memail.vanderbilt.edu (J.P. Donahue).

Because it is unlikely that *iceA1* and *iceA2* encode functional proteins in the majority of strains, we hypothesized that an association of *iceA1*-containing strains with duodenal ulcer disease [8,13], might be related to differences in DNA methylation, catalyzed by *M.HpyI*, that affect transcription of genes involved in pathogenesis.

To test the aforementioned hypothesis, we used gene arrays to compare global gene transcription in *H. pylori* wild-type strain J166 and an isogenic *hpyIM* mutant strain in exponential and stationary phase broth cultures and following adherence to AGS gastric epithelial cells in vitro. This analysis showed that in stationary phase cultures and in cells adherent to host cells in vitro, loss of *hpyIM* function significantly altered the expression of the stress-responsive *dnaK* operon [14], suggesting a role for *hpyIM* in *H. pylori* physiology that is unrelated to type II R–M systems.

## 2. Materials and methods

### 2.1. Bacterial strains, plasmids and genomic DNA isolation

*H. pylori* strains J166 (wild-type) and J166M<sup>-</sup> (*hpyI-M::aphA*) have been previously described [8,15]. Plasmid pVU1041 was constructed by inserting a 2000-bp DNA fragment encoding strain J166 *iceA1-hpyIM* (HP1209-HP1208)[16] into the *Escherichia coli*–*H. pylori* shuttle vector, pHel2 [17]. Plasmid pVU1041 was methylated in vitro using J166 cell-free extract prior to natural transformation of *H. pylori* strain J166M<sup>-</sup> [15]. For preparation of genomic DNA, *H. pylori* cells were harvested from 24-h cultures grown on agar plates and genomic DNA was isolated as described [18].

### 2.2. *H. pylori* growth and adherence to human AGS cells

*H. pylori* strains were grown in Brucella broth containing 10% fetal calf serum at 37°C and 5% CO<sub>2</sub> with shaking (100 rpm). For exponential phase cultures, 25 ml of broth in a 250-ml culture flask was inoculated with a fresh overnight broth culture to a final optical density at 600 nm of 0.15–0.2, harvested after two to three doublings in density and immediately processed for RNA isolation. For stationary phase cultures, and to obtain cells used for in vitro adherence to AGS gastric epithelial cells, 75 ml of broth in a 250-ml culture flask was inoculated as described for exponential cultures and incubated for 42 h. Stationary phase cells were collected by centrifugation and immediately processed for RNA isolation or were suspended in RPMI 1640 to an estimated final concentration of  $1 \times 10^{10}$  CFU ml<sup>-1</sup>. Human AGS gastric epithelial cells were grown in RPMI 1640 containing 10% fetal calf serum. Confluent cultures of AGS cells in T75 culture flasks (approximately  $1 \times 10^7$  cells) were washed once with RPMI 1640 and infected with  $1 \times 10^{10}$  *H. pylori* cells in a total

volume of 10 ml of RPMI 1640. Infected AGS cell monolayers were incubated at 37°C (5% CO<sub>2</sub>). After 1 h, non-adherent bacteria were removed and AGS cell monolayers with adherent bacteria were washed with ice-cold PBS prior to isolation of total RNA.

### 2.3. RNA isolation

RNA was isolated from *H. pylori* exponential and stationary phase cells and from *H. pylori*-infected AGS gastric epithelial cell monolayers essentially as described [19]. Exponential and stationary phase bacterial cell pellets in 15-ml polypropylene centrifuge tubes or AGS cells with adherent *H. pylori* in T75 culture flasks were each lysed with 3 ml of lysis solution [0.5% SDS, 10 mM Na<sub>2</sub>EDTA (pH 8.0)]. Then, 3 ml of ice-cold wash solution [0.1 M sodium acetate (pH 5.2), 10 mM Na<sub>2</sub>EDTA (pH 8)] was added to the cell lysates in 15-ml polypropylene tubes. Cell lysates in culture flasks were transferred to 15-ml polypropylene tubes and flasks were rinsed with 3 ml of ice-cold wash solution which was then combined with cell lysates. Each cell lysate was then extracted with 6 ml of H<sub>2</sub>O-saturated phenol and centrifuged at 2600×g for 10 min at 4°C. The aqueous phase (5.5 ml) was removed and added to 0.66 ml 1 M Tris–HCl (pH 8) and 0.27 ml 5 M NaCl and the RNA precipitated by the addition of 12 ml 100% ethanol and incubation at –20°C for a minimum of 30 min. The RNA was collected by centrifugation, washed with 70% ethanol, air dried and dissolved in 50 µl of DEPC-treated H<sub>2</sub>O. RNA was incubated with amplification grade DNase I (0.5 U µg<sup>-1</sup> RNA; Life Technologies) as described by the manufacturer. The integrity of the RNA was assessed by agarose gel electrophoresis prior to labeling for hybridization to *H. pylori* gene arrays.

### 2.4. Gene array hybridization

<sup>33</sup>P-labeled cDNA was synthesized from 15 µg RNA (exponential and stationary phase bacteria) or 75 µg RNA (adherent bacterial and AGS cell total RNA) using random hexamer primers and Superscript II reverse transcriptase (Life Technologies). Reactions were incubated at 42°C for 2 h and then 70°C for 15 min. After this incubation, RNA was hydrolyzed by incubation in 0.3 N NaOH, 17 mM Na<sub>2</sub>EDTA, 0.3% SDS at 68°C for 30 min. Hydrolysis reactions were neutralized by adding 2 N HCl and the labeled cDNA was separated from unincorporated [ $\alpha$ -<sup>33</sup>P]dATP using a Gel-Spin SS50 column as described by the manufacturer (Worthington). *H. pylori* gene arrays (Eurogentec) were pre-hybridized in 5×SSC (pH 7.5), 5×Denhardt's solution, 0.5% SDS [19] for a minimum of 2 h at 65°C. The <sup>33</sup>P-labeled cDNA was denatured in 0.3 N NaOH at 37°C for 10 min and hybridized to the gene array at 65°C for 20 h in the same solution used for pre-hybridization. Hybridized gene arrays

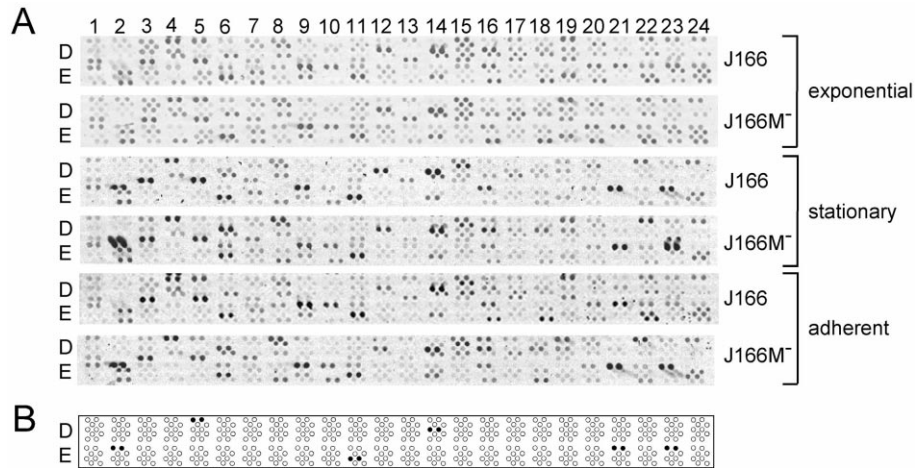


Fig. 1. Gene-array analysis of transcription in *H. pylori* wild-type strain J166 and isogenic *hpyIM* mutant strain J166M<sup>-</sup>. A: Shown are sections of autoradiographs of separate DNA arrays that were hybridized to <sup>33</sup>P-labeled cDNA probes synthesized from total RNA isolated from exponential and stationary phase cells and from cells adherent to AGS gastric epithelial cells as indicated. The section of each gene array shown comprises coordinates D1–24 and E1–24 containing a total of 216 separate genes spotted on nylon membranes in duplicate horizontally. The gene-array results shown are representative of two independent determinations. B: Diagrammatic representation of the gene arrays shown in (A) depicting the location of DNA spots (shaded) encoding the heat-shock operon genes *hrcA* (1E2), *dnaK* (1E21), *grpE* (1E23), catalase gene (*kat*, 3D14), vacuolating cytotoxin gene (*vacA*, 3E11) and the peptide release factor 1 gene (*prfA*, 1D5).

were washed with 2×SSC, 0.1% SDS at 65°C for 20 min and then with 0.2×SSC, 0.1% SDS at 65°C for 1 h. Gene arrays were autoradiographed using Kodak Bio-Max MR X-ray film.

2.5. RNA slot-blot analysis

DNase I-treated *H. pylori* RNA was applied to Hybond-N (Amersham Life Science) membranes using a slot-blot apparatus and crosslinked to the membrane by exposure to ultraviolet light as described [11]. DNA fragments used as probes were synthesized by PCR using gene-specific oligonucleotide primers and *H. pylori* J166 genomic DNA as template. DNA fragments were labeled with [ $\alpha$ -<sup>32</sup>P]dCTP by random priming using Prime-It RmT (Stratagene) as described by the manufacturer. Un-

incorporated [ $\alpha$ -<sup>32</sup>P]dCTP was removed from labeled DNA fragments using 1-ml Sephadex G-50 spin columns essentially as described [19]. RNA slot blots were pre-hybridized, hybridized to <sup>32</sup>P-labeled DNA probes, and then washed as described by us for Northern blots [11]. Blots were autoradiographed using Kodak Bio-Max MR X-ray

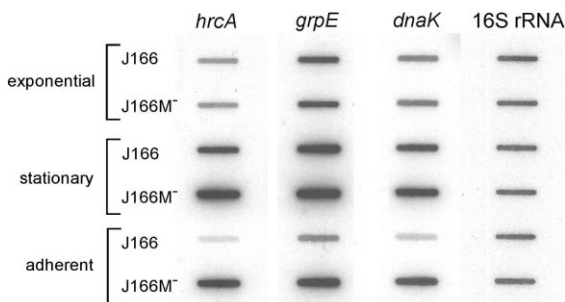


Fig. 2. RNA slot-blot analysis of steady-state *dnaK* operon transcript levels in *H. pylori* wild-type strain J166 and *hpyIM* mutant strain J166M<sup>-</sup>. The same RNA preparations used for the gene array analysis shown in Fig. 1 were applied to Hybond-N membranes and hybridized to <sup>32</sup>P-labeled DNA probes encoding *hrcA*, *grpE*, *dnaK* or 16S rRNA as indicated.

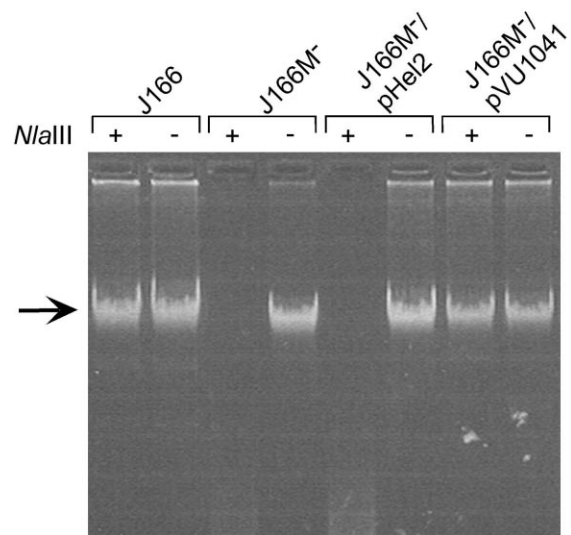


Fig. 3. Demonstration of *hpyIM* complementation in mutant strain J166M<sup>-</sup> by plasmid pVU1041 containing a wild-type copy of the *hpyIM* gene. Shown is a 0.8% TAE-agarose gel stained with ethidium bromide. The sources of genomic DNA, and the presence or absence of the restriction endonuclease *NlaIII* in reactions are indicated above the lanes. The location of undigested genomic DNA is indicated by an arrow. *M.HpyI* methyltransferase, encoded by *hpyIM*, modifies the sequence CATG rendering genomic DNA resistant to digestion by *NlaIII*.

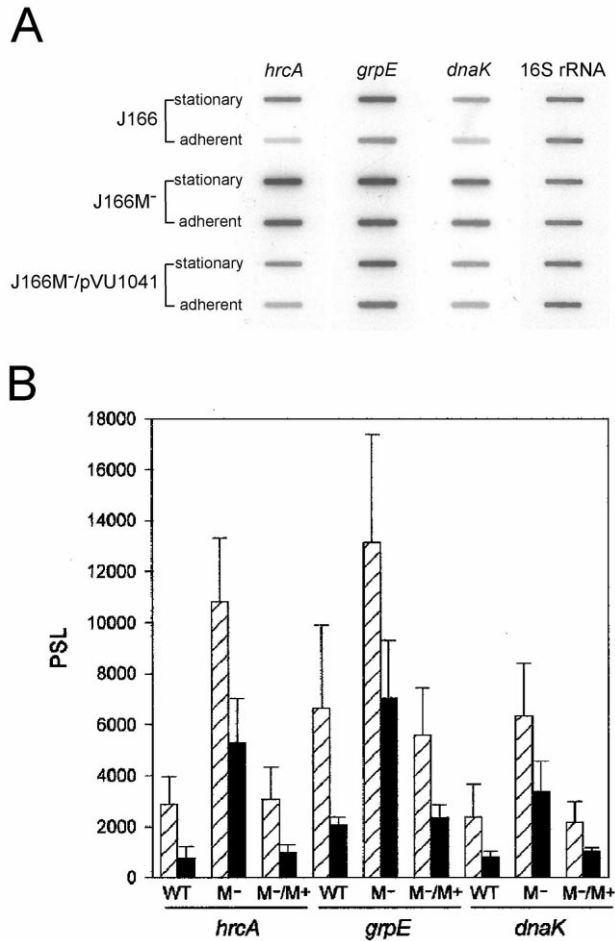


Fig. 4. Complementation of the *hpyIM* mutation re-establishes the wild-type levels of *dnaK* operon transcripts in stationary phase and adherent cells. A: RNA slot-blot analysis of transcript levels in *H. pylori* wild-type strain J166, *hpyIM* mutant strain J166M<sup>-</sup>, and J166M<sup>-</sup>/pVU1041(*hpyIM*<sup>+</sup>). RNA isolated from stationary phase cells and from cells adherent to AGS gastric epithelial cells in vitro was applied to Hybond-N membranes and hybridized to <sup>32</sup>P-labeled DNA probes encoding *hrcA*, *grpE*, *dnaK* or 16S rRNA as indicated. B: Quantitation of *dnaK* operon transcript levels by phosphorimager analysis of three independent RNA slot blots including the blot shown in (A). Photo-stimulated luminescence (PSL) values represent the mean ± S.E.M. WT, strain J166; M<sup>-</sup>, strain J166M<sup>-</sup>; and M<sup>-</sup>/M<sup>+</sup>, strain J166M<sup>-</sup>/pVU1041(*hpyIM*<sup>+</sup>). Stripped columns, RNA from stationary phase cells; solid columns, RNA from adherent cells.

film and imaged using a Fuji phosphorimaging screen (BAS-MP2040).

### 3. Results

#### 3.1. Gene array analysis of transcription in *H. pylori* wild-type and *hpyIM* mutant bacteria

To assess whether *hpyIM*, encoding a DNA adenine methyltransferase, may be involved in the control of expression of specific genes in *H. pylori*, we used gene arrays to compare global transcription in *H. pylori* wild-type

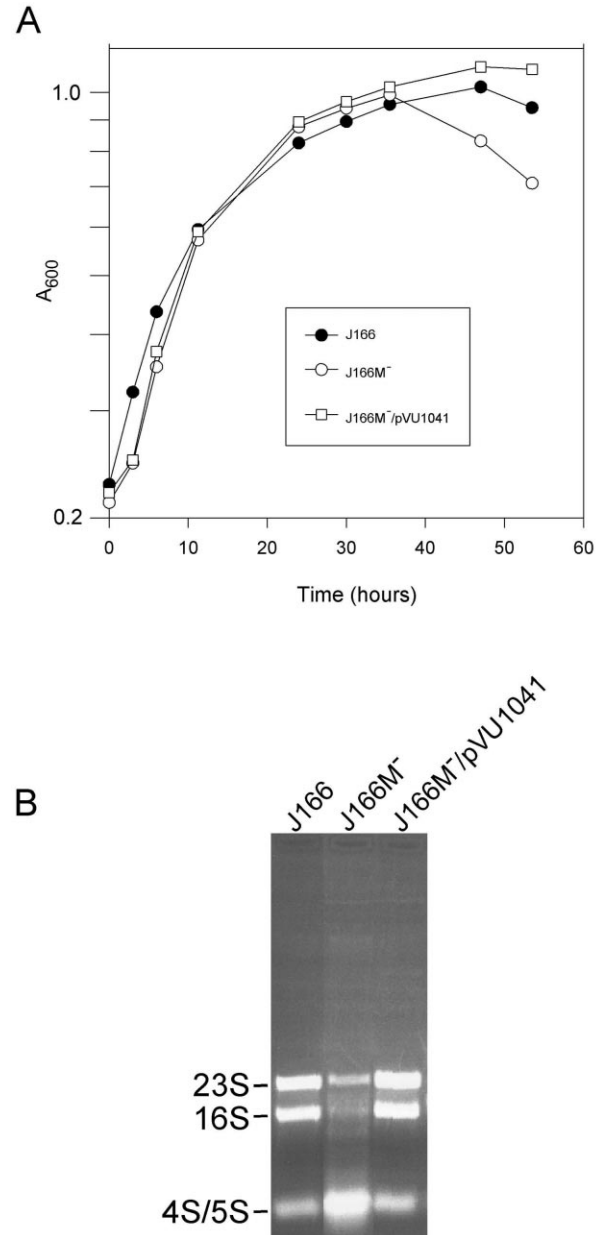


Fig. 5. Decrease in viability and RNA integrity in stationary phase cultures of the *H. pylori* *hpyIM* mutant strain. A: Shown are growth curves of strains J166, J166M<sup>-</sup>, and J166M<sup>-</sup>/pVU1041(*hpyIM*<sup>+</sup>) grown in 75 ml brucella broth plus 10% fetal calf serum as described in Section 2. Growth was monitored by measuring the change in optical density at 600 nm (A<sub>600</sub>) with time in hours. B: Shown is a 1% TAE-agarose gel stained with ethidium bromide. Total RNA (2 µg), isolated from 48-h stationary phase cultures of strains J166, J166M<sup>-</sup>, and J166M<sup>-</sup>/pVU1041(*hpyIM*<sup>+</sup>), was loaded on the gel as indicated above the lanes. The locations of 23S, 16S and 4S/5S RNA are indicated.

strain J166 and isogenic *hpyIM* mutant strain J166M<sup>-</sup> in exponential and stationary phase broth cultures and following 1 h of adherence to AGS gastric epithelial cells in vitro. Steady-state mRNA levels were analyzed using the Eurogentec *H. pylori* gene array which contains 1578 PCR products representing 97% of the ORFs from strain 26695

spotted in duplicate on a nylon membrane. This analysis showed that transcript levels of genes involved in bacterial growth and other normal cellular metabolic processes were similar in exponential phase cultures of the wild-type and *hpyIM* mutant strains (data not shown). However, analysis of the transcriptome in stationary phase broth cultures of the wild-type and *hpyIM* mutant strains revealed several potential differences. In particular, the transcript levels of the stress-responsive *dnaK* operon, comprised of *hrcA*, *grpE* and *dnaK* [14], were increased significantly in stationary phase cultures of the wild-type and *hpyIM* mutant strains, with higher levels evident in the *hpyIM* mutant (Fig. 1). Adherence of stationary phase wild-type and *hpyIM* mutant bacteria to AGS gastric epithelial cells in vitro for 1 h resulted in the rapid decrease in the levels of *dnaK* operon transcripts in the wild-type strain to pre-stationary phase levels, whereas *dnaK* operon transcript levels remained high in the *hpyIM* mutant strain. The data suggested that this was a specific effect on *dnaK* operon expression, because the transcript levels of many other genes, including catalase (*kat*, 3D14) and vacuolating cytotoxin (*vacA*, 3E11) shown in Fig. 1, remained similar in the wild-type and *hpyIM* mutant. Also, independent of the *hpyIM* mutation, we found that bacterial adherence to gastric epithelial cells resulted in an increase in the transcript levels of many genes encoding proteins predicted to be involved in bacterial growth. For example, as seen in Fig. 1, transcript levels of peptide chain release factor (*prfA*, 1D5) were clearly visible in exponential phase cells, declined to low levels in stationary phase cells, and then increased in both the wild-type and *hpyIM* mutant strains following 1 h of host-cell contact.

The apparent altered control of *dnaK* operon transcription in the *hpyIM* mutant strain that was shown in gene-array hybridizations was confirmed by RNA slot-blot analysis. Total RNA from the same preparations used in the gene-array analysis shown in Fig. 1 was applied to Hybond-N membranes and probed with <sup>32</sup>P-labeled DNA fragments encoding *hrcA*, *grpE*, *dnaK* or 16S rRNA. RNA slot-blot results are shown in Fig. 2 and transcript levels were quantitated by phosphorimager. This analysis showed that *dnaK* operon transcript levels in exponential phase cultures of both strains were approximately equal. In stationary phase cultures *hrcA*, *grpE*, and *dnaK* transcript levels were increased in the *hpyIM* mutant strain by three-fold, 1.6-fold and two-fold, respectively, relative to the wild-type strain. Following epithelial cell adherence, *dnaK* operon transcripts declined to pre-stationary phase levels in wild-type cells while remaining high in the *hpyIM* mutant cells. This resulted in *hrcA*, *grpE* and *dnaK* transcript levels that were 36-fold, 6.5-fold, and 10.3-fold, respectively, higher in the *hpyIM* mutant strain than in the wild-type strain. For all conditions tested, 16S rRNA levels were similar, indicating that equal amounts of total bacterial RNA were present in all slots on the membrane.

### 3.2. Complementation of the *hpyIM* mutation re-establishes wild-type *dnaK* operon transcript levels

To demonstrate that mutation of *hpyIM* was specifically responsible for the observed changes in control of *dnaK* operon transcript levels, we sought to complement the *hpyIM* mutation by introducing a plasmid encoding a wild-type copy of *hpyIM* into J166M<sup>-</sup>. Plasmid pVU1041 was constructed by inserting a DNA fragment encoding *H. pylori* strain J166 *iceA1-hpyIM* into the shuttle vector pHel2 [17]. This DNA fragment contains the native transcriptional and translational regulatory signals present in wild-type strain J166 upstream of *hpyIM*. Following transformation of strain J166M<sup>-</sup>, complementation of the *hpyIM* mutation was demonstrated by the restoration of resistance of genomic DNA to digestion by the RE *NlaIII* which recognizes the sequence CATG modified by *M.HpyI* (Fig. 3). Genomic DNA from J166M<sup>-</sup> transformed with the parental shuttle vector, pHel2, remained susceptible to *NlaIII* digestion (Fig. 3).

RNA was isolated from strain J166, J166M<sup>-</sup>, and J166M<sup>-</sup>/pVU1041 stationary phase cultures and from cells adherent to AGS gastric epithelial cells in vitro and *dnaK* operon transcript levels were assessed by RNA slot-blot analysis. As shown in Fig. 4, *dnaK* operon transcripts were more strongly induced in both stationary phase and adherent cells of *hpyIM* mutant strain, J166M<sup>-</sup>, than in the wild-type strain, J166. This result was consistent with the results of gene-array and RNA slot-blot analyses shown in Figs. 1 and 2. Complementation of the *hpyIM* mutation in strain J166M<sup>-</sup>/pVU1041 resulted in the re-establishment of wild-type *dnaK* operon transcript levels in both stationary phase and adherent cells. Quantitation of transcript levels by phosphorimager analysis of the RNA slot blot shown in Fig. 4A and two other RNA slot blots from independent experiments demonstrated the reproducibility of these effects on *dnaK* operon expression (Fig. 4B). These data indicate that *M.HpyI* methyltransferase function, encoded by *hpyIM*, was responsible for the observed alterations in control of *dnaK* operon transcript levels in J166M<sup>-</sup>. The complementation observed argues against a role in this phenomenon for undetermined secondary mutations or polarity effects on gene expression as a consequence of *hpyIM* insertional mutagenesis.

### 3.3. The *hpyIM* mutant exhibits decreased survival in stationary phase compared to the wild-type

Under the growth conditions we used for the analysis of *dnaK* operon transcript levels in stationary phase and adherent *H. pylori*, the *hpyIM* mutant strain consistently lost viability sooner in stationary phase than the wild-type strain. This is illustrated in Fig. 5A which shows typical growth curves for strains J166, J166M<sup>-</sup> and the complemented *hpyIM* mutant J166M<sup>-</sup>/pVU1041. The growth rates of the *hpyIM* mutant, wild-type and complemented

*hpyIM* mutant strains were similar in the exponential phase of growth, and all cultures entered stationary phase after approximately 24 h of incubation. After an additional 24 h of incubation, the density of the *hpyIM* mutant culture declined relative to cultures of the wild-type and complemented *hpyIM* mutant. This decline in the culture density of the *hpyIM* mutant corresponded to an approximately three-fold reduction in the number of CFU ml<sup>-1</sup>, as determined by viable cell plate counts. Consistent with a loss in viability, RNA isolated from 48-h cultures of the *hpyIM* mutant strain appeared degraded, whereas RNA from the wild-type and complemented *hpyIM* mutant appeared intact (Fig. 5B). It should be noted that for the studies reported in this communication, RNA was isolated from cultures of the *hpyIM* mutant strain prior to the decline in viability and consequent RNA degradation.

#### 4. Discussion

DNA methylation in prokaryotes occurs primarily within the context of R–M systems. However, the Dam methyltransferase of enteric bacteria has been shown to exert a regulatory role in a number of important cellular processes, including the timing of DNA replication, methyl-directed DNA mismatch repair, and the regulation of virulence gene expression [20,21]. In this communication we showed that inactivation of the *H. pylori hpyIM* gene, encoding a type II DNA methyltransferase, altered the control of *dnaK* operon transcript levels in stationary phase cultures and following host-cell contact in vitro. These data suggest that DNA methylation, mediated by *M.HpyI* methyltransferase, may have an important role in *H. pylori* physiology that is independent of R–M systems.

It is presently unknown how the loss of *M.HpyI* methyltransferase activity caused the alteration in *dnaK* operon expression that we observed in these studies. Gene-array experiments showed that *hpyIM* transcript levels decreased in stationary phase cultures and then increased following host-cell contact. This pattern of expression is consistent with a repressive effect of *M.HpyI* activity on *dnaK* operon transcription. However, a direct effect of DNA methylation on *dnaK* operon transcription, similar to the regulation of transcriptional initiation of the *pap* operon of *E. coli* by Dam methyltransferase [22], is unlikely for two reasons. First, the *dnaK* operon promoter region does not contain the sequence CATG that is recognized by the *M.HpyI* methyltransferase [16,23]. Second, *dnaK* operon transcript levels are not induced in exponential phase cultures of the *hpyIM* mutant strain where *M.HpyI* methyltransferase activity is completely absent. Other possible mechanisms to account for our observations include alterations in *dnaK* operon mRNA stability and/or an abnormal increase in the formation of products that induce *dnaK* operon transcription, e.g., unfolded or partially folded proteins. The decreased viability of the *hpyIM* mu-

tant strain in stationary phase suggests that *M.HpyI* methyltransferase activity may function in survival mechanisms that normally operate in stationary phase, and the loss of this activity may result in intracellular conditions that lead to substantial increases in expression of the stress-responsive *dnaK* operon. Further studies are necessary to determine the mechanism(s) involved in the induction of *dnaK* operon expression as a consequence of *hpyIM* inactivation and to elucidate the potential role of DNA methylation and *M.HpyI* methyltransferase function in *H. pylori* physiology.

#### Acknowledgements

The authors thank T. Cover and R. Peek for generously providing the gene arrays used in these experiments, and J. Graham for helpful discussions. This work was supported by National Institutes of Health Grant R01-AI37659.

#### References

- [1] Marshall, B.J. and Warren, J.R. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1, 1311–1315.
- [2] Dooley, C.P., Fitzgibbons, P.L., Cohen, H., Appleman, M.D., Pérez-Pérez, G.I. and Blaser, M.J. (1989) Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *N. Engl. J. Med.* 321, 1562–1566.
- [3] Peterson, W.L. (1991) *Helicobacter pylori* and peptic ulcer disease. *N. Engl. J. Med.* 324, 1043–1048.
- [4] Nomura, A., Stemmermann, G.N., Chyou, P.-H., Kato, I., Pérez-Pérez, G.I. and Blaser, M.J. (1991) *Helicobacter pylori* infection and gastric carcinoma in a population of Japanese-Americans in Hawaii. *N. Engl. J. Med.* 325, 1132–1136.
- [5] Goossens, H., Glupczynski, Y., Burette, A., Lambert, J.-P., Vlaes, L. and Butzler, J.-P. (1992) Role of the vacuolating toxin from *Helicobacter pylori* in the pathogenesis of duodenal and gastric ulcer. *Med. Microbiol. Lett.* 1, 153–159.
- [6] Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papini, E., Xiang, Z., Figura, N. and Rappuoli, R. (1993) Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc. Natl. Acad. Sci. USA* 90, 5791–5795.
- [7] Censini, S., Lange, C., Xiang, Z., Crabtree, J.E., Ghiara, P., Borodovsky, M., Rappuoli, R. and Covacci, A. (1996) *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc. Natl. Acad. Sci. USA* 93, 14648–14653.
- [8] Peek Jr., R.M., Thompson, S.A., Donahue, J.P., Tham, K.T., Atherton, J.C., Blaser, M.J. and Miller, G.G. (1998) Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *iceA*, that is associated with clinical outcome. *Proc. Assoc. Am. Phys.* 110, 531–544.
- [9] Xu, Q., Peek, R.M., Miller, G.G. and Blaser, M.J. (1997) The *Helicobacter pylori* genome is modified at CATG by the product of *hpyIM*. *J. Bacteriol.* 179, 6807–6815.
- [10] Figueiredo, C., Quint, W.G., Sanna, R., Sablon, E., Donahue, J.P., Xu, Q., Miller, G.G., Peek, R.M., Blaser, M.J. and van Doorn, L.-J. (2000) Genetic organization and heterogeneity of the *iceA* locus of *Helicobacter pylori*. *Gene* 246, 59–68.

- [11] Donahue, J.P., Peek Jr., R.M., van Doorn, L.-J., Thompson, S.A., Xu, Q., Blaser, M.J. and Miller, G.G. (2000) Analysis of *iceA1* transcription in *Helicobacter pylori*. *Helicobacter* 5, 1–12.
- [12] Xu, Q. and Blaser, M.J. (2001) Promoters of the CATG-specific methyltransferase gene *hpyIM* differ between *iceA1* and *iceA2* *Helicobacter pylori* strains. *J. Bacteriol.* 183, 3875–3884.
- [13] van Doorn, L.-J., Figueiredo, C., Sanna, R., Plaisier, A., Schneeberger, P., de Boer, W. and Quint, W. (1998) Clinical relevance of the *cagA*, *vacA* and *iceA* status of *Helicobacter pylori*. *Gastroenterology* 115, 58–66.
- [14] Homuth, G., Domm, S., Kleiner, D. and Schumann, W. (2000) Transcriptional analysis of major heat shock genes of *Helicobacter pylori*. *J. Bacteriol.* 182, 4257–4263.
- [15] Donahue, J.P., Israel, D.A., Peek Jr., R.M., Blaser, M.J. and Miller, G.G. (2000) Overcoming the barrier to plasmid transformation of *Helicobacter pylori*. *Mol. Microbiol.* 37, 1066–1074.
- [16] Tomb, J.F., White, O., Kerlavage, A.R., Clayton, R.A., Sutton, G.G., Fleischmann, R.D., Ketchum, K.A., Klenk, H.P., Gill, S., Dougherty, B.A., Nelson, K., Quackenbush, J., Zhou, L.X., Kirkness, E.F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H.G., Glodek, A., McKenney, K., Fitzegerald, L.M., Lee, N., Adams, M.D., Hickey, E.K., Berg, D.E., Gocayne, J.D., Utterback, T.R., Peterson, J.D., Kelley, J.M., Cotton, M.D., Weidman, J.M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W.S., Borodovsky, M., Karp, P.D., Smith, H.O., Fraser, C.M. and Venter, J.C. (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 388, 539–547.
- [17] Heuermann, D. and Haas, R. (1998) A stable shuttle vector system for efficient genetic complementation of *Helicobacter pylori* strains by transformation and conjugation. *Mol. Gen. Genet.* 257, 519–528.
- [18] Wilson, K. (1994) Preparation of genomic DNA from bacteria. In: *Current Protocols in Molecular Biology*. (Ausubel, F.M., Brent R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G. and Struhl, K., Eds.), pp. 2.4.1–2.4.5. John Wiley and Sons, New York.
- [19] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- [20] Marinus, M.G. (1996) Methylation of DNA. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (Neidhardt, F., Curtiss III, R., Ingraham, J.L., Lin, E.C.C., Low, K.B., Magasanik, B., Reznikoff, W.S., Riley, M. Schaechter, M., Umberger, H.E., Eds.), pp. 782–791. American Society for Microbiology, Washington, DC.
- [21] Heithoff, D., Sinsheimer, R.L., Low, D.A. and Mahan, M.J. (1999) An essential role for DNA adenine methylation in bacterial virulence. *Science* 284, 967–970.
- [22] Braaten, B.A., Nou, X., Kaltenback, L.S. and Low, D.A. (1994) Methylation patterns in pap regulatory DNA control pyelonephritis-associated pili variation in *E. coli*. *Cell* 76, 577–588.
- [23] Alm, R.A., Ling, L.S.L., Moir, D.T., King, B.L., Brown, E.D., Doig, P.C., Smith, D.R., Noonan, B., Guild, B.C., deJonge, B.L., Carmel, G., Tummino, P.J., Caruso, A., UriaNickelsen, M., Mills, D.M., Ives, C., Gibson, R., Merberg, D., Mills, S.D., Jiang, Q., Taylor, D.E., Vovis, G.F. and Trost, T.J. (1999) Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* 397, 176–180.