Identification of a putative chromosomal replication origin from Helicobacter pylori and its interaction with the initiator protein DnaA

Anna Zawilak, Stanislaw Cebrat¹, Pawel Mackiewicz¹, Anna Król-Hulewicz, Dagmara Jakimowicz, Walter Messer², Grazyna Gosciniak³ and Jolanta Zakrzewska-Czerwinska⁴*

Ludwik Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland, ¹Institute of Microbiology, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland, ²Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 73, D-14195 Berlin-Dahlem, Germany and ³Department of Microbiology, Medical University of Wroclaw, Chanubinskiego 4, 50-368 Wroclaw, Poland

Received March 6, 2001; Revised and Accepted April 17, 2001

ABSTRACT

The key elements of the initiation of Helicobacter pylori chromosome replication, DnaA protein and putative oriC region, have been characterized. The gene arrangement in the H. pylori dnaA region differs from that found in many other eubacterial dnaA regions (rmpA-rmpH-dnaA-dnaN-recF-gyrB). Helicobacter pylori dnaA is flanked by two open reading frames with unknown function, while dnaN-gyrB and rmpA-rmpH loci are separated from the dnaA gene by 600 and 90 kb, respectively. We show that the dnaA gene encoding initiator protein DnaA is expressed in H. pylori cells. The H. pylori DnaA protein, like other DnaA proteins, can be divided into four domains. Here we demonstrate that the C-terminal domain of H. pylori DnaA protein is responsible for DNA binding. Using in silico and in vitro studies, the putative oriC region containing five DnaA boxes has been located upstream of the dnaA gene. DNase I and gel retardation analyses show that the C-terminal domain of H. pylori DnaA protein specifically binds each of five DnaA boxes.

INTRODUCTION

The events that occur at the replication origin (oriC) are central to the processes regulating DNA replication (1). In bacteria, replication of a circular chromosome starts from a replication origin oriC and proceeds bi-directionally until the replication forks reach the termination site (ter). The structure of the oriC region has been analyzed in Gram-negative and Gram-positive bacteria. The sequences of oriC regions are conserved only among closely related organisms. Sequence analysis revealed that the origins of various bacteria contain short, conserved motifs that are essential for oriC function: AT-rich regions and non-palindromic 9 bp sequences named DnaA boxes (2). Spacer regions, which vary in nucleotide composition and length, separate these conserved sequences. The initiator protein DnaA plays an important role in the initiation and regulation of chromosomal replication. It binds to the origin of replication, specifically to the DnaA boxes. Among bacteria, the initiation of replication is best understood in Escherichia coli (2,3). Within the E.coli oriC region five DnaA boxes are present. Binding of 10–20 DnaA protein molecules promotes a local unwinding within the AT-rich region of the oriC. The unwound region provides the entry site for the helicase complex (DnaB6–DnaC6), followed by other proteins required to form replication forks (2–4).

Helicobacter pylori is a Gram-negative, spiral-shaped pathogenic bacterium that was first isolated and cultured from biopsy specimens by Marshall and Warren (5). Since that time extensive studies of H. pylori biology have been carried out. This organism is a human gastric pathogen associated with peptic ulcer disease as well as chronic gastritis. Recent epidemiological studies demonstrated that H. pylori is a primary risk factor for the development of intestinal type gastric adenocarcinoma.

Recently the genome sequences of two unrelated isolates of H. pylori, 26695 and J99, have been determined. The 26695 and J99 circular chromosomes are 1 667 867 bp (6) and 1 643 831 bp (7) in size, respectively. The gene content of two sequenced H. pylori genomes suggests that the basic mechanism of chromosomal replication is similar to that of other eubacteria (6,7). However, experimental data concerning the replication of H. pylori are scarce. The genomic analysis revealed few surprising features, in particular in the initiation of replication. The typical eubacterial block of replication genes, dnaA-dnaN-recF-gyrB (2,8,9) does not exist; the dnaA gene is located ~600 kb away from the dnaN-gyrB genes (see Fig. 8), while the recF gene is missing. The dnaC gene encoding DnaC protein, which delivers the DnAB helicase to the prepriming complex, is absent. Moreover, an origin of DNA replication has not been identified and is not evident from the genome sequence. Thus, it is interesting to better understand the initiation of chromosomal replication in this extensively studied pathogen.

*To whom correspondence should be addressed. Tel: +48 71 3732274; Fax: +48 71 3732587; Email: zakrzew@immuno.iitd.pan.wroc.pl
Present address:
Anna Król-Hulewicz, Institute of Biochemistry and Molecular Biology, University of Wroclaw, Tamka 2, 50-137 Wroclaw, Poland
To prepare chromosomal DNA from DNA manipulations

µ (final concentration 25

replication initiation from

H. pylori

2252

Nucleic Acids Research, 2001, Vol. 29, No. 11

were PCR amplified using primers, one of which was 5

fragments for footprinting experiments and gel retardation

kits according to the manufacturers' protocols (Qiagen). DNA

phenol–chloroform and precipitated with ethanol.

incubated at 65

°

added to final concentrations of 1% and the cell lysate was

cultivated on Columbia agar medium supplemented with horse

blood (10%) under micro-aerobic atmosphere (5% O2, 15%

strains were grown in

Escherichia coli

(J99 strain was

supE

α

1741 bp fragment)

H.pylori

E.coli oriC

DnaA protein

ORI–

KCl, 0.144% Na2HPO4, 0.024% K H2PO4, pH 7.4). Total RNA

was extracted with TRI REAGENT (Molecular Research

was added to the

region. We demonstrate that the

DnaA protein

region. Using

in silico

methods, the putative

region

H.pylori

DnaA protein

containing five DnaA boxes has been located upstream of the

dnaA
gene.

Here we characterize the key elements of chromosomal replication initiation from

H. pylori, DnaA protein and the putative oriC region. We demonstrate that the dnaA gene encoding DnaA protein is expressed in

H. pylori

and that the binding domain of the

H. pylori

DnaA protein specifically recognizes the DnaA boxes from the

E. coli oriC region. Using

in silico

and

in vitro

methods, the putative

H. pylori

oriC region

containing five DnaA boxes has been located upstream of the

dnaA
gene.

MATERIALS AND METHODS

Bacterial strains, media and culture conditions

The

E. coli

and

H. pylori

strains used and their origins are listed in Table 1. Escherichia coli DH5α served as host for all plasmid constructs and AG115 strain was utilized as host for the overproduction of the fusion protein glutathione S-transferase (GST)-DnaA(IV). Escherichia coli strains were grown in Luria–Bertani medium at 37° C for 18–24 h.

DNA manipulations

To prepare chromosomal DNA from

H. pylori,
cells were scraped from an agar plate and suspended in 200 µl of ice-cold STE buffer (150 mM NaCl, 10 mM Tris–HCl pH 8.0, 1 mM EDTA) containing lysozyme (100 µg/ml), and incubated at 37°C for 10 min. Sodium dodecyl sulfate (SDS) was then added to final concentrations of 1% and the cell lysate was incubated at 65°C for 10 min. After addition of proteinase K (final concentration 25 µg/ml), the samples were incubated at 50°C for 2 h (10). Chromosomal DNA was extracted using phenol–chloroform and precipitated with ethanol.

Purification of plasmids and DNA fragments was done using kits according to the manufacturers’ protocols (Qiagen). DNA fragments for footprinting experiments and gel retardation were PCR amplified using primers, one of which was 5’-end-labeled using [γ-32P]ATP and T4 polynucleotide kinase (11). Enzymes were supplied by Roche, Fermentas MBI and Gibco BRL. Isotopes were supplied by BRL. Isotopes were obtained from Amersham. The oligonucleotides used for PCR or for sequencing were chemically synthesized (Sigma-ARK Scientific, Darmstadt, Germany). DNA sequencing was performed using a Thermo Sequenase cycle sequencing kit (Amersham). For both strands, the nucleotide sequences were determined.

RNA isolation and RT–PCR analysis

Colonies of

H. pylori

were harvested from blood agar plate and resuspended in phosphate-buffered saline (0.8% NaCl, 0.02% KCl, 0.144% Na2HPO4, 0.024% KH2PO4, pH 7.4). Total RNA was extracted with TRI REAGENT (Molecular Research Center, Inc.). Subsequent steps of RNA isolation were carried out according to Chomczynski and Sacchi (12).

RT–PCR reactions were carried out using a Gibco BRL RT–PCR kit. After digestion of total RNA (3.6 µg) with DNase I, the samples were incubated at 65°C for 10 min and placed on ice. An oligonucleotide (20 pmol), PHPGR (Table 2) complementary to the 3’ region of the

dnaA
gene, was added to the
RNA samples and then a reverse transcription (RT) reaction was performed at 37°C for 90 min. An aliquot of 5 µl out of 20 µl RT solution was subjected to subsequent PCR. The amplification reaction was carried out in 50 µl using primers PHPGR and PHPGF (Table 2) for 40 cycles, with each cycle consisting of denaturation, 15 s at 96°C; annealing, 30 s at 47°C for 1–20 cycles and 66°C for 21–40 cycles; and elongation, 30 s at 72°C. Products of the RT–PCR reaction were analyzed by agarose gel electrophoresis as well as by Southern hybridization. The DNA fragments were transferred onto nylon membrane and hybridized with the digoxygenin-labeled DNA probe. Immunodetection of the hybrids was performed according to the manufacturer’s protocol (Roche).

DnaA purification

The C-terminal domain (IV) of the H. pylori DnaA protein was fused to the C-terminus of GST. Part of the H. pylori dnaA gene encoding the domain IV of the DnaA protein was amplified using the primers PHPGF and PHPGR (Table 2). The amplified fragment digested with EcoRI and BamHI was cloned into the EcoRI and BamHI sites of the pGEX-4X expression vector. The fusion protein GST-HpDnaA(IV) was overexpressed in E. coli AG115 and purified using glutathione–Sepharose 4B beads (Pharmacia) as described previously (13,14). The purified proteins were analysed by SDS–polyacrylamide gel electrophoresis (PAGE). The E. coli DnaA protein was isolated as described earlier (15).

Preparation of antisera

Antisera were obtained from rabbits by immunization with the purified GST-HpDnaA(IV) fusion protein and mixed with Freund’s complete adjuvant. Serum samples were taken 10 days after the second booster injection. Cellular particles were removed by centrifugation, and antisera were stored at −20°C.

SDS–PAGE and western blotting

SDS–PAGE was performed according to the method established by Laemmli (16). Proteins were separated by 10 or 12% SDS–PAGE and transferred to a nitrocellulose membrane. The membrane was blocked for 1 h at room temperature with TBST (10 mM Tris–HCl pH 8.0, 100 mM NaCl, 0.05% Tween-20) containing 3% bovine serum albumin (BSA), and subsequently incubated with a polyclonal anti-GST-HpDnaA(IV) antibody. Afterwards the DnaA protein was detected using a goat anti-rabbit secondary antibody conjugated with alkaline phosphatase. The membrane was stained with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium.

Electrophoretic mobility shift assay

For binding assays, DNA (50 ng) was incubated with the GST-HpDnaA(IV) or E. coli DnaA protein in the presence of a competitor FX 174 RF DNA (100 ng) at 20°C for 20 min in a binding buffer (20 mM HEPES–KOH pH 8.0, 5 mM Mg-acetate, 1 mM Na2-EDTA, 4 mM DTT, 0.2% Triton X-100, 5 mg/ml BSA and 1 mM ATP). The bound complexes were analyzed by electrophoresis on 1% agarose gels (0.25× TBE, at 4 V/cm, 4°C). Gel were stained with ethidium bromide. For radioactive shifts, 32P-labeled DNA (8 ng) was incubated with the GST-HpDnaA(IV) or E. coli DnaA in the presence of competitor poly(dI–dC) (100 ng) under conditions described above. The bound complexes were separated by electrophoresis in 8% polyacrylamide gels (0.25× TBE, at 4 V/cm, 4°C). Gels were dried and analyzed by autoradiography.

DNase I footprinting

For footprinting experiments, the promoter region of the H. pylori dnaA gene was amplified by PCR using HpS1a and HpS1b primers (Table 2). The 5′-radiolabeled DNA fragments (~10 fmol) were incubated with different amounts of the GST-HpDnaA(IV) protein in a binding buffer (20 mM HEPES pH 7.6, 5 mM Mg-acetate, 4 mM DTT, 1 mM EDTA, 3 mM ATP, 0.2% Triton X-100, 5% glycerol, 100 mM K-acetate, 5 mM Ca-acetate) at room temperature for 30 min. Then DNase I digestion was carried out according to the described procedure (17). The DNase I cleavage products were separated in 8% polyacrylamide–urea sequencing gels. Gels were dried and analyzed by autoradiography.

Computer analysis

The sequences were analyzed with the Wisconsin Package v9.0 Genetics Computer Group (GCG, Madison, WI). Sequence alignments were created with the Bestfit, while the PI value was predicted with the IsoElectric program modules. Secondary structure predictions were obtained from the PHD server: http://www.embl-heidelberg.de/predictprotein/predictprotein.html, which includes the algorithms developed by Rost and Sander (18). Sequences of dnaA genes were from the National Center for Biotechnology: Bacillus subtilis (P05648), Campylobacter jejuni (CAB72494), E. coli (BAA16384), H. pylori J99 (AAD06996).

DNA walks

The computational analysis was performed on the sequence of the H. pylori J99 genome downloaded from: http://www.ncbi.nlm.nih.gov. To find the point where the DNA asymmetry changes its sign, we performed DNA walks on the complete chromosome sequence. The data obtained have been presented in the form of a diagram of detrended DNA walks as described previously (19,20). In this walk, the shift of the walker in the two-dimensional space is [1,1] for guanine, [1,0] for adenine and thymine and [1,0/C] for cytosine, where G and C are total numbers of guanine and cytosine in the analyzed sequence. In such a walk, the walker starts and completes its walk at position zero on the y-axis. Coordinates on the x-axis correspond to the position on the chromosome. After a rough estimation of the region of the origin of replication, we performed detailed DNA walks in that region, analyzing differences between G and C content in the DNA sequence. Methods are also described in detail at web site http://smORFland.micrób.uni.wroc.pl.

RESULTS

Characterization of the H. pylori J99 dnaA gene and its product, initiator protein DnaA

The predicted molecular mass of the H. pylori DnaA protein is 52.7 kDa. Based on a phylogenetic tree of eubacterial DnaA proteins (not shown), H. pylori DnaA is most similar to DnaA of C. jejuni (39.9% identity). DnaA protein from distantly related E. coli still reveals 32.9% identity to H. pylori DnaA.
Expression of the dnaA gene in H. pylori cells was proved by RT–PCR (Fig. 1A) and western blotting (Fig. 1B). The antibodies against the H. pylori DnaA protein (see Materials and Methods) detected a 53 kDa H. pylori protein corresponding in size to the deduced dnaA gene product (Fig. 1B).

Based on the homology pattern, bacterial DnaA proteins have been divided into four domains, to which different functions could subsequently be assigned (2,21; Fig. 2). The DNA binding domain of E.coli DnaA has been localized in the C-terminus. In order to check whether the C-terminus of the H. pylori DnaA protein is responsible for DNA binding, its interaction with DNA was analyzed by gel retardation assay. The PCR-amplified DNA fragment of the dnaA gene encoding domain IV of H. pylori DnaA protein fused to the gst gene (Materials and Methods and Table 1) was overexpressed in E.coli AG115. The resulting fusion protein, GST-HpDnaA(IV) (38 kDa) was purified by affinity chromatography on glutathione-Sepharose 4B as described earlier (13,14). Since the H. pylori oriC region had not been known, the E.coli oriC region containing five DnaA boxes was chosen to study the GST-HpDnaA(IV) protein interaction with DNA (Fig. 3). The GST-HpDnaA(IV) protein was incubated with the E.coli oriC fragment and then the DNA–protein complexes were analyzed by a gel retardation assay (Fig. 3). The interaction of the GST-HpDnaA(IV) protein with the E.coli oriC region led to formation of discrete nucleoprotein complexes (Fig. 3). The non-specific competitor (ØX 174 RF DNA) was not bound at all by the fusion protein. Binding of the GST protein alone to the E.coli oriC fragment was not observed (data not shown).

Nucleoprotein complex formation between GST-HpDnaA(IV) protein and a single DnaA box from the E.coli oriC region occurred already at the lowest protein concentration (at a GST-HpDnaA(IV)/DnaA box molar ratio of 0.6). At higher protein concentration, sequential binding of GST-HpDnaA(IV) molecules to the five DnaA boxes caused the formation of high molecular weight complexes (Fig. 3). Thus, the data specify the C-terminal domain of the H. pylori DnaA protein as the DNA binding domain.

**In silico identification of the putative H. pylori origin of replication**

It is well documented that all eubacterial chromosomes are divided by origin (oriC) and terminus (terC) of replication (or termini in linear chromosomes) into two replichores. It has been noted that in many bacterial genomes sequenced so far, the leading strand contains more G than C. Thus, the oriC and terC regions of chromosome replication can be detected by plotting this GC skew along the genome (22). The best graphical method indicating the point where the bias in nucleotide composition of the DNA molecule changes its sign is the detrended DNA walk (19,20). This demonstrates the local deviations from the average composition (i.e. G and C content) in the analyzed consecutive DNA sequences (Fig. 4). A diagram showing the result of [G-C] DNA walks on the whole H. pylori chromosome is presented in Figure 4A. There are two evident global switch points of DNA asymmetry. To obtain more precise results, we performed a [G-C] DNA walk nucleotide by nucleotide in the region close to the putative origin of replication (Fig. 4B). Also in Figure 4B, the positions of the dnaA gene and the open reading frame (ORF) jhp1418 are indicated. According to the computational analysis the switch of DNA asymmetry is located downstream of the dnaA gene at the position 1 556 599 bp.

**In vitro identification of the replication origin from H. pylori**

In many eubacterial chromosomes including B. subtilis (23), Mycobacterium tuberculosis (24) and Streptomyces lividans (13,25), the dnaA gene is located close to the functional replication origin. The close vicinity of the global switch point of DNA asymmetry (the minimum, Fig. 4B) and the dnaA gene on the H. pylori chromosome prompted us to look for putative origin sequences (DnaA boxes). A search for DnaA box motifs...
whose sequence differs up to 2 nt from the most stringent consensus sequence for the *E.coli* DnaA box (5′-TTAT-NCACA-3′) allowed the identification of five DnaA boxes upstream of the *dnaA* gene (Fig. 5). All five DnaA boxes have the same orientation; the pairs of DnaA boxes 2, 3 and 4, 5 are closely spaced (2 bp in between) while the distance between DnaA boxes 1, 2 and 3, 4 is 33 and 13 bp, respectively (Fig. 5).

To determine whether the GST-*Hp*DnaA(IV) protein interacts with the DNA fragment containing five *H.pylori* DnaA boxes, a gel retardation assay was performed. In addition, we also evaluated whether the *E.coli* DnaA protein specifically binds these boxes. The putative *H pylori* oriC region was amplified using a pair of primers: *HpS1a* and 32P-labeled *HpS1b* (Table 2). After incubation of the labeled 187 bp DNA fragment with different amounts of the GST-*Hp*DnaA(IV) protein, the resulting nucleoprotein complexes were analyzed in an 8% native polyacrylamide gel (Fig. 6). At higher concentrations of the *E.coli* DnaA protein, five nucleoprotein complexes were observed. Binding of the GST-*Hp*DnaA(IV) protein resulted in the formation of three nucleoprotein complexes. Probably, the GST-*Hp*DnaA(IV) binds two closely spaced DnaA boxes (2, 3 and 4, 5) as a dimer due to interaction via its N-terminus (i.e. GST) (Fig. 6). The affinity of the GST-*Hp*DnaA(IV) protein to the single DnaA box 1 seems to be low since the band corresponding to the nucleoprotein complex with the lowest mobility (Fig. 6) was weak.

To confirm the location of the DnaA binding sites within the *H pylori* oriC region, DNaseI footprinting analysis was performed. The 187 bp PCR-amplified DNA fragment, labeled at one 5′-end (lower strand, see above) was incubated with various amounts of the GST-*Hp*DnaA(IV) protein and then subjected to limited DNase I cleavage (Fig. 7). The protected sites correspond to the locations of four DnaA boxes 2, 3, 4, 5 within the analyzed oriC region. At higher protein concentrations the entire DNA fragment (53 bp) containing four DnaA boxes and a short spacer (13 bp) between DnaA boxes 3 and 4 was protected. Using the upper strand (32P-labeled primer *HpS1a*), identical protection sites were determined (data not shown). However, protection of the DnaA box 1 was not observed for both strands.

In addition, we evaluated in detail the interaction of the GST-*Hp*DnaA(IV) protein with individual DnaA boxes from the putative *H pylori* oriC, using a gel retardation assay. Double-stranded, 32P-labeled oligonucleotides containing a single DnaA box 1, 2 (box 3 is identical to box 2), 4 and 5 (Table 2) were incubated with various amounts of the GST-*Hp*DnaA(IV) protein and then nucleoprotein complexes were separated in an 8% native polyacrylamide gel (Fig. 7). For each analyzed oligonucleotide a single nucleoprotein complex was already visible at a low protein concentration (at a GST-*Hp*DnaA(IV)/DnaA box ratio of 1:1 or 1:2, respectively). However, binding of GST-*Hp*DnaA(IV) protein to the DnaA box 1 resulted in a faint nucleoprotein complex suggesting that this complex is quite unstable. This is in agreement with the observation from the DNase I footprinting analysis where DnaA box 1 was not protected.
DISCUSSION

Until recently, nothing was known about the initiation of chromosome replication in *H. pylori*, one of the most extensively studied pathogens. Despite the fact that the complete genome sequence of two different strains of *H. pylori*, 26695 (6) and J99 (7), has been determined, a typical eubacterial origin of replication was not identified. Here we characterize the key elements of the initiation of *H. pylori* chromosome replication: DnaA protein and the putative oriC region. In addition, we analyzed the interaction between these key elements using gel retardation and DNase I footprinting.

Comparison of numerous eubacterial genetic maps around the chromosome replication initiator gene, dnaA, showed conservation of the gene cluster rnpA-rmpH-dnaA-dnaN-recF-gyrB (2,8,9). However, the gene arrangement in the *H. pylori* dnaA region differs from that found in other eubacterial dnaA regions. Helicobacter pylori dnaA is flanked by two ORFs with unknown function (Fig. 8). The expression of the dnaA gene in *H. pylori* cells was confirmed by RT–PCR and western blotting analysis using antibodies against *H. pylori* DnaA protein (Fig. 1).

Amino acid sequence comparison of DnaA proteins and secondary structure prediction (data not shown) have shown that the DnaA protein of *H. pylori*, like other DnaA proteins, can be divided into four domains (Fig. 2). Mutational analysis of several DnaA proteins (26–30) has shown that the C-terminus of the DnaA protein (domain IV) is necessary and sufficient for specific DNA binding. The DNA binding motif of bacterial DnaA proteins consists presumably of two α-helices (A and B) with a basic loop between them, and a third long α-helix (C1-2) (21,30,31). The predicted secondary structure of the *H. pylori* DnaA protein reveals the presence of three potential α-helices...
carried out as described in Materials and Methods using $^{32}$P-labeled 187 bp
fragment (amplified by HpS1a and $^{32}$P-labeled HpS1b primers, Table 2) and varying concentrations of protein. The DNA–protein complexes were separated in an 8% polyacrylamide gel. The bottom part shows the structure of the putative H. pylori oriC region.

Figure 6. Interaction of the GST-HpDnaA(IV) protein (left) and the E.coli DnaA protein (right) with the putative H. pylori oriC region. Gel retardation assay was carried out as described in Materials and Methods using $^{32}$P-labeled 187 bp H. pylori oriC fragment (amplified by HpS1a and $^{32}$P-labeled HpS1b primers, Table 2) and varying concentrations of protein. The DNA–protein complexes were separated in an 8% polyacrylamide gel. The bottom part shows the structure of the putative H. pylori oriC region.
place within DnaA boxes or in a region adjacent to the DnaA boxes cluster.

Altogether, our results suggest that we have identified the putative *H. pylori* oriC region that is located upstream of the *dnaA* gene. The putative oriC region contains five DnaA boxes. DNase I and gel retardation assays clearly demonstrated that the C-terminal domain of the *H. pylori* initiator DnaA protein specifically binds each of the five DnaA boxes.

ACKNOWLEDGEMENTS

We thank Jurek Majka for help in isolation of the GST-*HpDnaA(IV)* protein. We are grateful to Tomasz Cierpicki for helping in secondary structure prediction of *H. pylori* DnaA. A.Z. and D.J gratefully acknowledge financial support received from the Committee of Scientific Studies (KBN grant 6P04A 025 18) and from the Foundation for Polish Science, respectively. S.C. and P.M were supported by KBN grant 6P04A 033 18. This work was in part supported by grant 436 POL 113/82/0 of the Deutsche Forschungsgemeinschaft.

REFERENCES


