Purification of the Novel Endonuclease, Hpy188I, and Cloning of Its Restriction-Modification Genes Reveal Evidence of Its Horizontal Transfer to the Helicobacter pylori Genome*

Received for publication, December 22, 1999, and in revised form, February 25, 2000
Published, JBC Papers in Press, March 22, 2000, DOI 10.1074/jbc.M910303199

Qing Xu‡, Shawn Stickel§, Richard J. Roberts§, Martin J. Blaser‡¶, and Richard D. Morgan§‖

From the ‡Department of Microbiology and Immunology, §Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 and †New England Biolabs, Inc., Beverly, Massachusetts 01915

We have isolated a novel restriction endonuclease, Hpy188I, from Helicobacter pylori strain J188. Hpy188I recognizes the unique sequence, TCNGA, and cleaves the DNA between nucleotides N and G in its recognition sequence to generate a one-base 3′ overhang. Cloning and sequence analysis of the Hpy188I modification gene in strain J188 reveal that hpy188IM has a 1299-base pair (bp) open reading frame (ORF) encoding a 432-amino acid product. The predicted protein sequence of M.Hpy188I contains conserved motifs typical of amino-methyltransferases, and Western blotting indicates that it is an N-6 adenine methyltransferase. Downstream of hpy188IM is a 513-bp ORF encoding a 170-amino acid product, that has a 41-bp overlap with hpy188IM. The predicted protein sequence from this ORF matches the amino acid sequence obtained from purified Hpy188I, indicating that it encodes the endonuclease. The Hpy188I R-M genes are not present in either strain of H. pylori that has been completely sequenced but are found in two of 11 H. pylori strains tested. The significantly lower G + C content of the Hpy188I R-M genes implies that they have been introduced relatively recently during the evolution of the H. pylori genome.

Restriction-modification systems were first recognized in Escherichia coli more than four decades ago (1, 2) because of their role as enzymatic barriers against genomic invasion by phages. The restriction endonuclease recognizes a specific sequence in DNA, and cleaves the DNA, whereas the cognate methyltransferase modifies DNA at the same recognition sequence, preventing cleavage by the endonuclease. Based on subunit composition, co-factor requirements, DNA specificity characteristics, and reaction products, R-M systems may be classified as type I, type II, or type III (3). Type II R-M systems have been described in detail. In this study, we purified a novel restriction endonuclease Hpy188I with a new specificity (TCNGA) from H. pylori strain J188, and further cloned the genes of this R-M system. The M gene contains the conserved motifs of aminomethyltransferases, but the R-gene is unique. The system is present in some but not all H. pylori strains, and DNA analysis suggests that it was acquired by horizontal transfer.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Growth Conditions, and Reagents—The bacterial strains used in this study (Table I) are from our laboratory collection and were cultured as described (15). Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA). All columns used for protein purification were obtained from Amersham Pharmacia Biotech (Piscataway, NJ), unless otherwise indicated. Oligonucleotides used in this study (Table II) were synthesized either at New England Biolabs or at the Vanderbilt University Cancer Center DNA Core Facility using a Milligen 7500 DNA synthesizer.

DNA Techniques—Chromosomal and plasmid DNA were prepared as described (16). PCR1 and DNA sequencing were performed as described (15). Computer analyses of DNA and protein sequences were performed with the GCG programs (17, 18) and data base similarity searches were performed at the National Center for Biotechnology Information using the BLASTX algorithm (19, 20).

Purification of Hpy188I—Hpy188I cells were resuspended in ice-cold buffer A (20 mM Tris-HCl, 0.5 mM EDTA, 1 mM dithiothreitol, pH 7.5), then sonicated until ~50 mg of protein/ml of cells was released. After centrifugation, the supernatant was applied to a 20-ml heparin Hy-Per-D column (Biosepra, Marlborough, MA). The column was washed with buffer A containing 0.05 M NaCl, and eluted with a 200-ml linear gradient of 0.05–1.0 M NaCl. Fractions were assayed for endonuclease activity by incubation at 37 °C for 1 h in New England Biolabs buffer 4 (50 mM KCl, 20 mM Tris-OAc, 10 mM Mg(OAc)2, 1 mM dithiothreitol).

1 The abbreviations used are: PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; ORF, open reading frame; R, restriction; M, modification; bp, base pair(s); kb, kilobase(s).

* This work was supported in part by a Dissertation Enhancement Grant from the Vanderbilt Graduate School, National Institutes of Health Grants GM56534 and DK53707, and a Vanderbilt Cancer Center Core Grant. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequences reported in this paper have been submitted to the GenBank™/EBI Data Bank with accession numbers AF202061 (the sequence of the hpy188IM-Hpy188IR locus in strain J188), AF215914 (the sequence of the corresponding region in strain A101), AF215915 (that in strain J262), AF215916 (that in strain J178), and AF215917 (that in strain 6190).

† To whom correspondence should be addressed: New England Biolabs, Inc., 32 Tozer Rd., Beverly, MA 01915. Tel.: 978-927-5054; Fax: 978-921-1350; E-mail: morgan@neb.com.

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
Published, JBC Papers in Press, March 22, 2000, DOI 10.1074/jbc.M910303199

17086 This paper is available on line at http://www.jbc.org
One unit of fractions from the heparin-TSK column was titered by serial dilution.

Amino Acid Sequencing of Hpy188I—Hpy188I cleavage sites in these DNAs. The sizes of the DNAs were digested into well defined fragments using Hpy188I. The Hpy188I endonuclease was identified by comparison with the N-terminal amino acid sequence obtained from the M gene. These clones were also assayed for the presence of restriction endonuclease activity.

Preparation of Antibodies—Hapten-protein conjugates were prepared by periodate oxidation of the methylated nucleosides as described (25). Rabbits were immunized by injecting 500 µg of the protein conjugate, in complete Freund’s adjuvant, intradermally and subcutaneously for the primary injections, and 250 µg in incomplete adjuvant via the subcutaneous route for each boost. The first test bleeds were taken 1 month after the initial injection and then at 3-week intervals.

RESULTS

Purification and Amino Acid Sequencing of Hpy188I from H. pylori Strain J188—A crude extract of H. pylori strain J188 cells (8 g) was applied to a heparin Hyper-LD column, and eluted with a linear NaCl gradient. A type II activity, designated Hpy188I, was detected in eluted fractions between 0.3 and 0.38 M NaCl, which were pooled and applied to a Mono S column. Hpy188I eluted between 0.26 and 0.3 M NaCl from this column, and Hpy188I positive fractions were then applied to a Poly-Cat A column. After elution, Hpy188I activity appeared in a broad peak between 0.3 and 0.38 M NaCl, with a trace of a second endonuclease activity. To purify Hpy188I further, positive frac-
tions were passed through a Mono Q column onto a heparin-TSK column, and the Hpy188I activity eluted between 0.38 and 0.42 M NaCl from the heparin-TSK column (Fig. 1, A and B). Hpy188I activity among these final fractions was titered on φX174 DNA. In total, more than 10,000 units of Hpy188I activity were present in fractions 39–46. Fraction 42 had the highest endonuclease activity (8 units/ml), followed by fraction 43 (4 units/ml) (Fig. 1B). SDS-PAGE of the relevant fractions (Fig. 1C) revealed that a protein band of ~21 kDa was present only in lanes of fractions 42 and 43, which had the highest enzyme activity, but not other lanes with less activity. The density of this band in the lane 42 is higher than that in the lane 43, which is consistent with the presence of higher enzyme activity in fraction 42. The size of this protein is in the range typical for type II endonucleases (3). Thus, we predicted that it was Hpy188I. N-terminal sequencing on this protein resulted in a sequence of 27 amino acids: XKRKDIILKSVDLDKDX-IDXKDFXYYK (X, not identified).

**Determination of the Recognition Sequence and Cleavage Site of Hpy188I**—To determine the recognition sequence, Hpy188I was used to digest pUC19, pBR322, or φX174 DNAs (data not shown). The patterns of the well defined fragments from Hpy188I-digested DNA were analyzed, using the SITES program (22), which indicated that these differed from those of all known endonucleases. The sizes of digested fragments from each substrate DNA are consistent with cleavage at TCNGA symmetric sites. Mapping by digestion with additional endonucleases also predicted Hpy188I digestion to occur at TCNGA sites. Thus, we concluded that Hpy188I is a novel endonuclease with the specificity TCNGA.

To determine the cleavage site of Hpy188I within its recognition sequence, the extension products, using M13q1 and M13q2 as primers, and M13mp18 as template, were digested by Hpy188I. The digestion of the M13q1-extension product produced a band that migrated identically with the dideoxy termination product of the unspecified nucleotide in the recognition sequence, TCNGA (N is G in this location) (Fig. 2), indicating cleavage between the N and the G of the recognition sequence. Digestion of the M13q2-extension product with Hpy188I produced a band that also co-migrated with the unspecified nucleotide of the Hpy188I recognition sequence TCNGA (in this case, the N is the C on the opposite strand of DNA from the G in the M13q1 reaction) (Fig. 2). This result confirms cleavage between the N and G of the recognition sequence on this strand of DNA as well. Thus, Hpy188I cuts DNA symmetrically between N and G in its recognition site (TCNGA).
were 3–4 kb long. The Sau3AI and HindIII digestion patterns of the plasmids were similar, indicating that all inserts cloned in these plasmids were from the same genomic locus, although their sizes were slightly different.

DNA sequence analysis showed that plasmid p#16 carried an ~3.6-kb genomic DNA insert. This insert possessed two complete ORFs of 1299 and 513 bp, oriented in the same direction and overlapping by 41 nucleotides, and two incomplete ORFs of 555 and 566 bp, one at the 5’ and the other at the 3’ end of the insert (Fig. 4). The 1299-bp ORF had regions similar to the nine conserved motifs found in aminomethyltransferases (27), indicating that it is the gene for \( Hpy^{188I} \), \( hpy188IM \). The 513-bp ORF showed no similarity to any known gene in GenBank on either the DNA or the amino acid level. The two partial ORFs showed strong matches to genes identified in \( H. pylori \) strain 26695. The 555-bp partial ORF at the 5’ end matched HP#1117 (omp27) that encodes an outer membrane protein, and the 566-bp partial ORF at the 3’ end was similar to HP#1118 (deoD) that encodes a purine-nucleotide phosphorylase.

\( hpy188IM \) would encode a predicted 432-amino acid product with a molecular mass of 50.9 kDa, which is in the typical size range of DNA methyltransferases. Nine motifs identified in its product, including the (N/S/D)(Y/F) motif, are arranged in the order of motif X, and I to VIII. The longest variable region is near the C-terminal, where the target recognition domain (TRD) presumably is located (27). The 1299-bp ORF of \( hpy188IM \) uses GTG as a translation start site. There is a potential translation start site, ATG, 155 bp downstream of the GTG. Expression of \( hpy188IM \) in \( E. coli \) starting from the GTG site generated a functional methyltransferase, while expression from the downstream ATG site did not (data not shown), indicating that the GTG site is the start codon for \( hpy188IM \) translation.

By endonuclease assay, we found that all \( Hpy^{188I} \)-resistant clones demonstrated weak endonuclease activity (data not shown), suggesting that \( hpy188IR \) was also present in the inserts of these \( Hpy^{188I} \)-resistant plasmids. The 513-bp ORF encodes a 170-amino acid product with a molecular mass of 20.3 kDa, which matches the size of the purified \( Hpy^{188I} \) (Fig. 1C). Its predicted N-terminal sequence also matched the 27-amino acid sequence obtained from the purified \( Hpy^{188I} \) protein. Furthermore, expression of this ORF in \( E. coli \) generated a functional \( Hpy^{188I} \) (data not shown), indicating that it is the gene encoding \( Hpy^{188I} \).

Detection of the Bases in the Recognition Sequence Methylated by \( Hpy^{188I} \)—DNA methyltransferases have been divided into three distinct groups, \( \alpha \), \( \beta \), and \( \gamma \) (27), based on the order of motifs and sequences in these motifs. The order of the \( M. Hpy^{188I} \) motifs (X and I to VIII) and the sequences present in its motifs are the same as or similar to those from N-6 adenine methyltransferases in the \( \gamma \) group, indicating that \( M. Hpy^{188I} \) is a member of the \( \gamma \) group. Thus we hypothesized that \( M. Hpy^{188I} \) is most likely an N-6 adenine methyltransferase. Although no N-4 cytosine methyltransferases have yet been found to belong to the \( \gamma \) group, we could not rule out the possibility just on the basis of sequence analysis.

To determine whether our hypothesis is correct, Western blotting was performed against \( M. Hpy^{188I} \)-methylated DNA. \( M. Hpy^{188I} \)-methylated DNA, p#16, was prepared in \( E. coli \) DB23 which has no endogenous N-6 or N-4 methyltransferases.\(^2\) p#16 from DB23 was resistant to digestion by \( Hpy^{188I} \), while pUC19 from DB23 was susceptible (data not shown), as expected, indicating modification of p#16 but not pUC19 DNA. pUC19 grown in a \( dam + E. coli \) strain was used as a positive control for N-6 adenine-methylated DNA, while pBamM (28, 29) from DB23 was used as a positive control for N-4 cytosine-methylated DNA. When antibodies against N-6

\( hpy188IM \) and \( hpy188IR \) were each represented by solid arrows, indicating the direction of transcription. The thin lines between the arrows represent the flanking regions.

\( \alpha \)-HindIII/\( \phi X174-HaeIII \) DNA ladder.

\( \alpha \)-HindIII/\( \phi X174-HaeIII \) DNA ladder.

\( \alpha \)-HindIII/\( \phi X174-HaeIII \) DNA ladder.
adenine-methylated DNA were used as the probe (Fig. 5A). p16 DNA gave a strong signal, like the positive control pUC19(N6-A) DNA. pBamM(N4-C) and the negative control DNA, pUC19(2) from DB23 as negative control. The total amounts of DNA spotted on membranes were indicated on the left of each panel.

FIG. 5. Determination of the M.Hpy188I-methylated residue in its recognition sequence. Anti-N-6 methyladenine antibodies (Panel A) and anti-N-4 methylcytosine antibodies (Panel B) were used for Western blotting of M.Hpy188I-methylated DNA p16. pUC19(N6-A) and pBamM were used as positive controls for N-6 methyladenine and N-4 methylcytosine, respectively, while pUC19(2) from DB23 as negative control. The total amounts of DNA spotted on membranes were indicated on the left of each panel.

FIG. 6. Comparison of the Hpy188I R-M locus from strain J188 and the corresponding loci from strains 26695 and J99. The black solid arrows labeled as deoD and omp27 represent the conserved coding regions of the two genes among the three strains. The hatched boxes represent the 96-bp conserved noncoding region just upstream of omp27. The solid lines between the black arrows and hatched boxes represent unconserved regions between J188 and 26695 (and J99). The open boxes labeled with 92 represent 92-bp direct repeats in J188 and the open boxes labeled with 49 represent 49-bp conserved segments among J188, 26695, and J99. The arrows indicate both the location and direction of each ORF. “H” indicates the locations of the Hpy188I recognition sites. The figure was not drawn to scale. The sizes of DNA fragments are indicated on the top of each schematic structure.

The Hpy188I R-M System in H. pylori 17090

17090

The Hpy188I R-M System in H. pylori

Analysis of the hpy188IM-hpy188IR locus of strain J188 reveals 92-bp direct repeats with only 3 mismatches, which flank the R-M genes (Fig. 6). The 92-bp repeat on the right is located at the junction of the 2457-bp region and the conserved deoD ORF (Fig. 6), and 79 bp of this repeat corresponds to the 3′ end of the deoD ORF, a region conserved among all three strains. The 92-bp repeat on the left is located 171 bp downstream of the conserved omp27 ORF (Fig. 6). The sequence of the 171-bp region is completely different from those in the corresponding regions of 26695 or J99, and has no homologs elsewhere in either of the sequenced strains, suggesting that it has a different origin. The 92-bp direct repeats are not present in the corresponding intergenic region of 26695 and J99, suggesting these repeats are related to the acquisition event of the Hpy188I R-M system in strain J188.

There is a 49-bp segment located 4 bp downstream of the
right 92-bp repeat and 217 bp upstream of the hpy188IM ORF in strain J188 (Fig. 6). This segment shows strong similarity to segments of the same length in strains 26695 (with 7 mismatches) or J99 (with 11 mismatches) that lie 2 or 6 bp downstream of the deoD ORF (Fig. 6). This segment is not found elsewhere in the 2 sequenced H. pylori genomes. Thus, it is unlikely that the J188 version represents a chance similarity. This arrangement in which the Hpy188I R-M genes adjoin the 49-bp segment and deoD suggests that a module containing the R-M system may have integrated specifically into the region between the 49-bp segment and the 3’ end of deoD. The event may have resulted in the 92 bp duplication that is now seen. Considering the possibility of Hpy188I involvement in DNA mobility, we checked for the presence of its recognition site, TCNGA, in the related regions. However, the locations found (Fig. 6) do not suggest that they were directly involved in the integration event.

To further investigate the origin of this R-M system, the G + C content of these regions were calculated. The G + C content of the 2457-bp J188-specific region in strain J188 was 28.8%, while that of the Hpy188I R-M ORFs was 29.9%. The G + C content in the 365-bp intergenic region of strain 26695 is 32.3%, and a similar G + C content is observed in the J99 equivalent region. In contrast, the G + C content in the flanking regions including omp27 and deoD, was 39.5%, which matches the overall G + C content (39%) of H. pylori (13, 14, 37). The significantly lower G + C content of the 2457-bp J188-specific region strongly suggests that the hpy188IM-hpy188IR locus was introduced during the evolution of the H. pylori genome.

Study of Hpy188I Diversity Among Various H. pylori Strains—To further study the diversity of the Hpy188I R-M system, chromosomal DNAs from 10 H. pylori strains, including J188 and 26695, were examined for their modification at TCNGA sites. As expected, the DNA of J188 was resistant to Hpy188I digestion, indicating modification at TCNGA sites, whereas DNA from 26695 was digestible, corresponding to the absence of the R-M system in its genome (Fig. 7A). DNA from seven other strains was digestible, but strain J166 was resistant, suggesting that the Hpy188I R-M system is present in J166, but not the other strains. To confirm this observation, a pair of primers, QII ORF-F and QII ORF-R corresponding to the 5’ end of hpy188IM and the 3’ end of hpy188IR, respectively, were used to amplify the same set of DNAs (Fig. 7B). Only DNA from J188 and J166 gave PCR products with the predicted size of 1.8 kb, whereas no PCR products were observed for the other strains. Thus, only J188 and J166 among the strains tested have the Hpy188I R-M system.

To investigate the corresponding regions of the Hpy188IM-hpy188IR locus among these strains, primers QII-F and QII-R, corresponding to the 5’ and 3’ ends of the conserved deoD ORF, were used for PCR (Fig. 7C). As expected, DNA from J188 yielded a PCR product of the predicted size of ~3.0 kb. J166 DNA yielded a product of the same size, indicating that both the size and location of the Hpy188I-integrated region in J166 resembles that for J188. DNA from 26695 yielded a PCR product that matched the expected size of 1.0 kb. The other strains yielded PCR products of the same size as for 26695, except for 60190 and J178 which yielded 1.4- or 1.1-kb products, respectively. To further assess this heterogeneity, PCR fragments from strains A101, J262, 60190, and J178 were sequenced. The sequences of A101 and J262 shared >80% identity with that of 26695; and those of 60190 and J178 shared >50% identity. The presence of direct repeats (with sizes varying between 60 and 150 bp) in the J178 and 60190 sequences made their PCR products larger than those of the rest of the...
strains. These data indicate that only one genotype is present in the region between omp27 and deoD among the strains not possessing the Hpy188I R-M system. All ORFs present in the region are <125 bp, suggesting no functional genes. The 49-bp segment identified in strains J188, 26695, and J99 is also present at a similar location in the intergenic region of these 4 strains.

**DISCUSSION**

We have cloned and sequenced genes encoding the Hpy188I R-M system, a novel type II R-M system from *H. pylori* strain J188. Only two of 11 *H. pylori* strains examined possess this R-M system, and its significantly lower G + C content strongly suggests that this R-M system is a relatively recent acquisition by *H. pylori*. Comparison of the J188 hpy188IS-hpy188IR gene locus and the genomic sequence of strain 26695 indicates that the Hpy188I R-M system was introduced into a 365-bp intergenic region with a G + C content (32%) lower than average (39%) for *H. pylori*. Five regions with a significantly different G + C composition have been found in the genome of strain 26695 (13), but this 365-bp region is not located in any of these previously identified regions. The 365-bp region is also present at the same location in J99 and other strains that do not carry the Hpy188I R-M system, indicating conservation of this low G + C region. The Hpy188I R-M genes are flanked by 92-bp direct repeats, a situation that resembles the 37–40-kb cag pathogenicity island (cagPAI) in *H. pylori* (31), which also has a lower G + C content and is flanked by 31-bp direct repeats. The presence of these direct repeats further suggests that the hpy188IS-hpy188IR locus could have integrated into the *H. pylori* genome during a transposition event. A 49-bp segment, downstream of deoD, in the region of 26695 and others, is also present in the J188 region. The R-M system could have specifically integrated into the site between this small segment and deoD. However, it is unclear how this 49-bp segment was preserved while the rest of the original region was replaced by a completely different sequence.

In studies of the R-M systems of *EcoO109I*, AccI, BglII, Eco47I, and others (32–36), components of either prophages or transposons were found closely associated with their R-M genes. In the case of Hpy188I, however, no mobility genes can be identified immediately adjacent to its genes, which is also true for most of the type II R-M systems predicted in the two sequenced strains (13, 14). These data suggest that *H. pylori* uses a different mechanism for the horizontal transfer of R-M systems. One possibility is that the Hpy188I R-M system integrated into the *H. pylori* genome by homologous recombination in a region of lower G + C content. Another mechanism for horizontal transfer is exemplified by intron homing and transposition (37, 38), where endonucleases play the key role in introducing double strand breaks. While restriction enzymes have not yet been directly implicated in such events, it remains possible that they could initiate DNA insertion events. Analysis of the region between omp27 and deoD among 6 strains not possessing the Hpy188I R-M system reveals that 3 have a TCNGA site. It is conceivable that the restriction activity of Hpy188I could have facilitated the integration process of its R-M genes into the *H. pylori* genome. If this were the case, a TCNGA site could have been located in the region between the 49-bp segment and the deoD ORF, and might have provided the initial break point. Hpy188I could have cleaved this site, and subsequently, its R-M genes could have been integrated into this cleaved site. The origin of the 92-bp direct repeats of the target sequence near the 5’ end of deoD is unknown, but again is reminiscent of transposition events.

Strains J188 and J166 have the Hpy188I R-M system integrated in the same region, suggesting that they might have arisen from the same original strain. However, their genotypes at three other loci, vacA (39), cagA (40–42), and iceA (43) (Table I) are substantially different from each other, indicating that they are not closely related. Thus, the differences at these loci may be explained by two independent Hpy188I R-M system integration events into this lower G + C region in two separate strains. If this is true, the lower G + C region may be a particularly hot site for integration. Alternatively, J188 and J166 may have arisen from the same original strain that acquired the R-M system, and has subsequently diverged at the vacA, cagA, and iceA loci.

The Hpy188I R-M genes cloned in this study were present in only two of 11 *H. pylori* strains tested. The diversity of this R-M system among various strains is consistent with studies on other type II R-M systems in *H. pylori*. These include iceA1-hpyIM, an NalIII-like R-M system (15, 43) where the R gene is allelic with a non-R gene, and a DedI isoschizomer (44) which resulted from an integration event. In addition, comparison of the genomic sequences of strains J99 and 26695 indicates that some major strain-specific components are R-M genes (14).

Finally, a study examining genomic differences between *H. pylori* strains J166 and 26695, using a PCR-based subtractive hybridization method, shows that seven of 18 DNA clones specific to J166 appear to be R-M genes (45). Although we found the Hpy188I R-M system to be present in J166, but not in 26695, this difference was not found during the previous study (45).

This study exemplifies the apparent propensity of *H. pylori* to accumulate R-M systems, presumably by integrating them into inactive positions of the genome. It is unknown, although, why this organism, for which there are no known bacteriophages, needs so many R-M systems. A feature of *H. pylori* infection is its persistent colonization in the human stomach mucosa for years or decades (10–11). Clearly, *H. pylori* is well adapted to this gastric environment and it is tempting to think that the acquisition of so many R-M systems might be related to this unique lifestyle. Restriction enzymes and their associated methyltransferases in *H. pylori* might provide a biological role that we have yet to discover.

**Acknowledgment**—We are grateful to Dr. Jack Benner for help with amino acid sequencing of Hpy188I.

**REFERENCES**

1. Luria, S. E., and Human, M. L. (1952) *J. Bacteriol.*, 64, 557–569
2. Berti, T. A., and Weigle, J. J. (1953) *J. Bacteriol.*, 65, 113–121
3. Wilson, G. G., and Murray, N. E. (1991) *Annu. Rev. Genet.* 25, 585–627
4. Kim, Y. C., Grable, J. C., Love, R., Greene, P. J., and Rosenberg, J. M. (1990) *Science* 249, 1307–1309
5. Athanasiadis, A., Vlassi, M., Kotsifaki, D., Tucker, P. A., Wilson, K. S., and Kokkinidis, M. (1994) *Struct. Biol.*, 1, 469–475
6. Winkler, F. K. (1992) *Curr. Opin. Struct. Biol.*, 2, 93–99
7. Cheng, X., Kumar, K., Posfai, J., Pfleghar, J. W., and Roberts, R. J. (1993) *Cell* 74, 299–307
8. Newman, M., Strzelecka, T., Forster, L. F., Schildkraut, I., and Aggarwal, A. K. (1995) *Science* 269, 656–663
9. Cheng, X., Balendarian, K., Schildkraut, I., and Anderson, J. E. (1994) *EMBO J.* 13, 3927–3935
10. Berg, D. E., and Logan, R. P. H. (1997) *Bioessays* 19, 86–90
11. Goodwin, C. S., Armstrong, J. A., Chalvers, T., Peters, M., Collins, M. D., Syl, L., McConnell, W., and Harper, W. (1989) *Int. J. Syst. Bacteriol.* 39, 397–405
12. Blaser, M. J., and Parsonnet, J. (1994) *J. Clin. Invest.* 94, 4–8
13. Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quakenbush, J., Zhou, L., Karkkisen, E. F., Petersen, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Giedke, A., McKinney, K., Fitzgerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T., Peterson, J. D., Kelley, J., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Wallte, Y., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C., and Venter, J. C. (1997) *Science* 280, 539–547
14. Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Guild, B. C., DeLange, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, G., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F., and Trust, T. J. (1999) *Nature* 397, 176–180
15. Xu, Q., Peek, R. M., Miller, G. G., and Blaser, M. J. (1997) *J. Bacteriol.* 179, 6807–6815
16. Schleif, R. F., and Wensink, P. C. (1981) *Practical Methods in Molecular Biology*, Springer-Verlag, New York
17. Staden, R. (1982) *Nucleic Acids Res.* 10, 4731–4751
18. Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res.* 12, 387–395
19. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403–410
20. Gish, W., and States, D. J. (1993) *Nat. Genet.* 3, 266–272
21. Hattman, S., Keisler, T., and Gottehrer, A. (1978) *J. Mol. Biol.* 124, 701–711
22. Brooks, J. E., Benner, J. S., Heiter, D. F., Silber, K. R., Snyter, L. A., Jager-Quinton, T., Moran, L. S., Slatko, B. E., Wilson, G. G., and Nwankwo, D. (1991) *Nucleic Acids Res.* 19, 841–852
30. Deleted in proof
31. Censini, S., Lange, C., Xiang, Z., Crabtree, J., Ghiara, P., Borodovsky, M., Rappuoli, R., and Covacci, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 14648–14653
32. Anton, B. P., Heiter, D. F., Benner, J. S., Hess, E., J., Greenough, L., Moran, L. S., Slatko, B. E., and Brooks, J. E. (1997) *Gene* (Amst.) 187, 19–27
33. Stankevicius, K., Pavlovics, P., Lubys, A., Menkevicius, S., and Janulaitis, A. (1995) *Gene* (Amst.) 157, 49–53
34. Vaisvila, R., Vilkaitis, G., and Janulaitis, A. (1995) *Gene* (Amst.) 157, 81–84
35. Brassard, S., Paquet, H., and Riym, P. H. (1992) *Gene* (Amst.) 103, 69–72
36. Kita, K., Tsuda, J., Kato, T., Okamoto, K., Yanase, H., and Tanaka, M. (1999) *J. Bacteriol.* 181, 6822–6827
37. Belfort, M., and Roberts, R. J. (1997) *Nucleic Acids Res.* 25, 3379–3388
38. Waite-Rees, P. A., Keating, C. J., Moran, L. S., Slatko, B. E., Hornstra, L. J., and Benner, J. S. (1991) *J. Bacteriol.* 173, 5207–5219
39. Waite-Rees, P. A., Keating, C. J., Moran, L. S., Slatko, B. E., Hornstra, L. J., and Benner, J. S. (1991) *J. Bacteriol.* 173, 5207–5219
40. Censini, S., Lange, C., Xiang, Z., Crabtree, J., Ghiara, P., Borodovsky, M., Rappuoli, R., and Covacci, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 14648–14653
41. Tummuru, M. R. R., Cover, T. L., and Blaser, M. J. (1993) *Infect. Immun.* 61, 1799–1809
42. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
43. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
44. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
45. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
46. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
47. Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A., and Berg, D. E. (1998) *Mol. Microbiol.* 28, 57–67
Purification of the Novel Endonuclease, *Hpy188I*, and Cloning of Its Restriction-Modification Genes Reveal Evidence of Its Horizontal Transfer to the *Helicobacter pylori* Genome

Qing Xu, Shawn Stickel, Richard J. Roberts, Martin J. Blaser and Richard D. Morgan

*J. Biol. Chem.* 2000, 275:17086-17093.
doi: 10.1074/jbc.M910303199 originally published online March 23, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M910303199

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 13 of which can be accessed free at
http://www.jbc.org/content/275/22/17086.full.html#ref-list-1