A Novel 3-Methyladenine DNA Glycosylase from *Helicobacter pylori* Defines a New Class within the Endonuclease III Family of Base Excision Repair Glycosylases*

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The cloning, purification, and characterization of MagIII, a 3-methyladenine DNA glycosylase from *Helicobacter pylori*, is presented in this paper. Sequence analysis of the genome of this pathogen failed to identify open reading frames potentially coding for proteins with a 3-methyladenine DNA glycosylase activity. The putative product of the HP602 open reading frame, reported as an endonuclease III, shares extensive amino acid sequence homology with some bacterial members of this family and has the canonic active site helix-hairpin-helix-GPD motif. Surprisingly, this predicted *H. pylori* endonuclease III encodes a 25,220-Da protein able to release 3-methyladenine, but not oxidized bases, from modified DNA. MagIII has no abasic site lyase activity and displays the substrate specificity of the 3-methyladenine-DNA glycosylase type I of *Escherichia coli* (Tag) because it is not able to recognize 7-methylguanine or hypoxanthine as substrates. The expression of the magIII open reading frame in null 3-methyladenine glycosylase *E. coli* (tag alkA) restores to this mutant partial resistance to alkylating agents. MagIII-deficient *H. pylori* cells show an alkylation-sensitive phenotype. *H. pylori* wild type cells exposed to alkylating agents present an adaptive response by inducing the expression of magIII. MagIII is thus a novel bacterial member of the endonuclease III family, which displays biochemical properties not described for any of the members of this group until now.

Living organisms have developed a variety of strategies for protecting their genetic information. DNA, the repository of this information in all cells, is constantly exposed to chemical challenges (1). A variety of reactive intracellular and environmental compounds can induce modifications in the DNA bases. If left uncorrected, these lesions may cause mutations or impair DNA replication. In the case of alkylating agents, such as the cellular methyl donor S-adenosylmethionine or genotoxic compounds such as dimethylsulfate (DMS), N-methylmethanesulfonate (MMS), or N-methyl-N-nitro-N-nitrosoguanidine (MNNG), they can react nonenzymatically with DNA to produce 7-methylguanine (7-MeG) and 3-methyladenine (3-MeA) among other alkylated bases (2). In particular, 3-MeA poses a serious problem to the cell because it blocks DNA synthesis (3–6). To eliminate this kind of modified base, most cells possess monofunctional DNA glycosylases that specifically recognize and excise the methylated bases from DNA by cleavage of the C1'-N glycosyl bond. This first step of base excision repair is followed by the repair of the resulting abasic (AP) site by excision/replacement DNA synthesis.

At least four different classes of 3-MeA glycosylases have been described, based on their sequence and substrate specificities (7). All of these are low molecular weight enzymes, and no associated AP lyase activity has been attributed to any of them. In *Escherichia coli*, the two first classes are represented. Class I is defined by the product of the tag gene that codes for a constitutive 3-MeA DNA glycosylase (8, 9). Tag is quite specific for the removal of 3-MeA, although 3-MeG would also be excised with a much lower efficiency in *vivo* (10). Class II is typified by the AlkA enzyme of *E. coli*. There is no amino acid sequence similarity between class I and II 3-MeA DNA glycosylases. AlkA has a much broader substrate specificity, being able to release not only 3-MeA but also 7-MeG, 3-MeG, 7-MeA, and O’-methylpyrimidines (11). In the last few years, it has been found that AlkA has the ability to act on a variety of modified bases other than the classical alkylation damaged. Among those, the alkA product can release oxidized bases from DNA (12), as well as deamination products of adenine such as hypoxanthine (13) or ethenopurines (14, 15). Berdal et al. (16) have shown that AlkA is even able to release normal bases from DNA. Another difference with Tag resides in the fact that alkA is induced by treatment of the cells with alkylating agents (7). This induction occurs through activation of the alkA promoter.

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The abbreviations used are: DMS, dimethylsulfate; MMS, methylmethanesulfonate; MNNG, N-methyl-N-nitro-N-nitrosoguanidine; MeG, methylguanine; MeA, methyladenine; AP, abasic; Hx, hypoxanthine; HhH, helix-hairpin-helix; EndoIII, endonuclease III; ORF, open reading frame; IPTG, isopropyl-β-D-thiogalactopyranoside; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; GST, glutathione S-transferase; PCR, polymerase chain reaction.

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by the Ada protein. Class III is typified by the mammalian methylpurine DNA glycosylases. These enzymes are biochemically quite similar to AlkA but differ markedly in their primary sequence. The recent determination of the crystal structures for the human 3-MeA glycosylase AAG (17) and the bacterial AlkA (18, 19) confirmed that class II and class III enzymes are structurally unrelated, despite the fact that they share a broad substrate specificity. Indeed, for its structure, AlkA belongs to the superfamily of DNA glycosylases, the archetype of which is the EndoIII from \textit{E. coli}, an N-glycosylase that excises oxidized pyrimidines from DNA (18). This structural correspondence is striking in view of the limited homology between the members of this family, restricted to the helix-hairpin-helix (HhH) motif present opposite a catalytic aspartic acid in the active site. A fourth class of 3-MeA glycosylases has been proposed after the identification of the MpgII enzyme from \textit{Thermotoga maritima} (20). This enzyme, as in the case of AlkA, belongs to the superfamily of the EndoIII, as defined by the HhH aspartic motif (21, 22), is able to release not only 3-MeA but also 7-MeG from N-methyl-N-nitrosourea-treated DNA; and lacks an AP endonuclease activity. Despite those similarities, the lack of sequence homology to AlkA, other than the HhH aspartic motif, has prompted the authors to define the class IV of 3-MeA DNA glycosylases (20).

In our search for new members of the EndoIII family in \textit{Helicobacter pylori}, we identified an open reading frame (ORF), HP602, that although labeled as EndoIII (23, 24), codes for an enzyme that does not recognize oxidized bases or AP sites as substrates. The lack of potential methylpurine DNA glycosylase-encoding genes in the genomic sequence of this pathogen, together with the fact that the protein coded by this ORF has the HhH aspartic motif but lacks the lysine at the active site and the canonical 4Fe-4S cluster characteristic of endonuclease III, prompted us to investigate the possibility that ORF HP602 could be the gene for a methylpurine DNA glycosylase. We present here data showing that the enzyme coded by ORF HP602 is a functional 3-MeA DNA glycosylase, MagIII, inducible at the transcriptional level by MNGG. During the preparation of this manuscript and based on its sequence, the product of this ORF was attributed to the MpgII group (20). Although it presents predicted structural similarities with members of the HhH aspartic motif superfamily also displayed by AlkA and has an overall sequence similarity to the members of the MpgII class, this 3-MeA DNA glycosylase has a more restricted substrate specificity, comparable to the one characteristic of Tag. These characteristics suggest that this enzyme \textit{from} \textit{H. pylori} defines a novel class of 3-MeA DNA glycosylases.

### EXPERIMENTAL PROCEDURES

#### Bacterial Strains, Media, and Plasmids—

| Strain                | Relevant genotype          | Source or reference |
|-----------------------|----------------------------|---------------------|
| \textit{E. coli} BL21 | FompT hsdS(rtb m_b) gal dcm | Novagen             |
| GC4801                | AB 1157, X-Tn5             | 4                   |
| GC4803                | AB 1157, X-Tn5, alkAI, tagAI | 4                   |
| \textit{H. pylori} 13/5 | Wild type                 | Laboratory collection |
| ADM1                  | Wild type                 | Laboratory collection |
| HAS141                | magIII                    | Laboratory collection |
| HAS141 magIII         | magIII                    | This work           |
| 26695                 | Wild type                 |                     |

\textit{Bacterial Strains, Media, and Plasmids}—Strains used in this study are listed in Table I. \textit{E. coli} strains were grown in Luria-Bertani (LB) broth or agar supplemented with ampicillin (200 μg/ml) or kanamycin (30 μg/ml); Minimal A salts were prepared as described by Miller (25). \textit{H. pylori} strains were grown on blood brucella agar plates at 37 °C in a 5% CO2 and 95% humidity atmosphere. Disruption mutant \textit{H. pylori} strains were cultured with 20 μg/ml kanamycin. pGEX-4T-1 and pET29a (+) were obtained from Amersham Pharmacia Biotech and Novagen, respectively.

#### Materials and Reference Compounds—

All chemicals were, unless otherwise stated, from Sigma. [32P]ATP (3000 Ci/mmol), [1H]dimethyl sulfoxide (3.8 Ci/mmol), and Nensorb-20 nucleic acid purification cartridges were from New England Nuclear. T4 polynucleotide kinase, T4 DNA ligase, and restriction enzymes were purchased from New England Biolabs.

\textit{Bacterial Strains, Media, and Plasmids—}

#### Sensitivity to Methylmethane Sulfonate—

Plasmids pGHP602-I and pGHP602-V were used as controls for pSHP602-I and pSHP602-V, respectively. E. coli BL21 cells were electroporated with plasmids after induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). IPTG was added to a final concentration of 1 mM, and growth was continued overnight at 18 °C with shaking. Cells were harvested by centrifugation at 4000 g, washed once with 10 mM Tris-HCl, pH 7.5, and resuspended in 10 mM Tris-HCl, pH 7.5, containing 50 mM NaCl. The expression of the S-tag-MagIII fusions in \textit{E. coli} was confirmed by S-tag Western blot according to the manufacturer’s instructions. Cells were used to inoculate 500 ml of LB medium supplemented with kanamycin and were incubated at 37 °C with shaking until the A\text{max} reached 1. IPTG was added to a final concentration of 1 mM, and growth was continued overnight at 18 °C with shaking. Cells were harvested by centrifugation at 4000 g, washed once with 10 mM Tris-HCl, pH 7.5, and resuspended in 10 mM Tris-HCl, pH 7.5, containing 50 mM NaCl. The column was eluted with 30 ml of the same buffer (0.7 ml/min), and 500-μl fractions were collected. Protein elution profile was followed with a UV monitor. Under these conditions, purified MagIII eluted at 12 min. Protein concentrations were determined by the Bradford protein assay method (26).

#### DNA Substrates—

The 34-mer oligodeoxyribonucleotides used in this study have the following sequence: 5′-GCCCTCATGCTTGCTCCAGACCTGCTTGATACCG-3′, where X represents uracil, hypoxanthine (Hx),...
or dihydrothymine residue. Except for the latter, a kind gift from Dr. J. Cadet (Commissariat à l’Energie Atomique, Grenoble, France), these oligonucleotides and their complements with each of the four bases opposite the lesion in the duplex were purchased from Oligo Express (Grenoble, France). To generate AP sites, the uracil-containing duplex was treated with 0.5 M dihydrothymine for the product of the GST fused to either 3'-GCTGGAGAGACTA-5' or 5'-TTTGGTCTTGAGAATGTTTA3'-5'. Relative quantification of mRNA levels was performed in the exponential phase of amplification as described previously (36). Briefly, PCR amplification of target template and of 16 S RNA as external control were run in parallel. DNA amplification was carried out in 1× PCR buffer supplemented with 200 µM dNTPs, 2 µM each of 5'- and 3'-specific primers, 1 unit of Taq polymerase (Roche Molecular Biochemicals) in a final volume of 50 µl. The exponential phase of amplification was determined by carrying out the reaction for 16–38 cycles using a fixed quantity of cDNA. The number of cycles was set for magIII to 26 sequential cycles at 95 °C for 30 s, 45 °C for 45 s, 72 °C for 40 s, and for 16 S RNA to 20 cycles at 95 °C for 30 s and 60 °C for 30 s. In this phase of amplification, the amounts of the amplicons are proportional to the initial amounts of templates. The CDNA was serially diluted in water from 1:5 (10 ng of RNA) to 1:15625 (3.2 pg of RNA). 20 µl of magIII and 16 S RNA PCR products amplified in parallel from the same serial dilutions were loaded on agarose gel (1% agarose, 0.05% ethidium bromide). A negative control in which cDNA was omitted was run in parallel with each experiment.

RESULTS

Identification of a New Member of the Endonuclease III Glycosylase Family in H. pylori—The availability of the complete genome sequence of H. pylori allowed us to identify several putative members of the EndoIII family of DNA glycosylases involved in the repair of modified bases. Two open reading frames were annotated as homologs of the EndoIII from E. coli, HP602 and HP585 (24). To analyze whether these two ORFs coded for bona fide homologs of the EndoIII and whether they corresponded to redundant activities, the putative coding sequences from two independently isolated strains (13/5 and AD1M1) were amplified by PCR and cloned in E. coli vectors that allow their expression as fusions to the C terminus of the GST protein. For HP585 the purification of the GST fusion protein confirmed that this ORF codes for an active EndoIII-like enzyme (data not shown). Sequencing of the plasmids harboring the HP602 ORFs established that the sequences differed between the two isolates (Fig. 1). Comparison of the coded proteins and those of the two strains of which the genes are completely sequenced shows that for any pair, there are four or five amino acid differences among the 218 residues. In particular, for the two newly sequenced ORFs, a polymorphism was found, GIG versus GVG, at what has been determined to be the active site for the members of this family (22) (HhH motif, Fig. 1). As a consequence, the analysis of both clones (herein denominated 602-1 and 602-2) was carried out in parallel. HP602 also presented regions (Fig. 1) structurally homologous to the EndoIII. The three-dimensional PDB web server was searched for HP602 structurally related proteins. The strongest structural similarities were found with respect to EndoIII (E value, 2.7 × 10−7) and AlkA (E value, 2.8 × 10−2) crystals (37, 38). In the predicted secondary structure of the HP602 protein it is possible to define, with a high level of confidence (>50%) the helices αC to nM of EndoIII and AlkA, which constitute the regions at which fold and topology are shared by both enzymes (18) (Fig. 1). Moreover, identical amino acids are clustered over these conserved secondary structures. However, HP602 protein lacks the pattern of cysteines giving rise to the iron-sulfur cluster present in EndoIII and MpgII proteins. No activity was detected on DNA substrates containing dihydrothymine for the product of the GST fused to either of the HP602 ORF variants (data not shown). Furthermore, neither of these constructs was able to complement the phenotype of an E. coli strain deficient in both EndoIII and EndoVIII DNA glycosylases (data not shown). We therefore examined whether these ORFs could code for a function related to the other member of the EndoIII family, AlkA, which also shares a similar overall folding pattern with EndoIII, as well as the HhH aspartic motif (Fig. 1).

Complementation of an E. coli alkA tag Mutant—To examine
whether *H. pylori* HP602 can substitute for *E. coli* AlkA and/or Tag in the repair of damaged DNA, HP602/GIG or HP602/GVG fused to GST were expressed in *E. coli* GC4803 (4), a strain phenotypically sensitive to alkylating agents due to null mutations in tag and alkA. To confirm that the proteins were expressed, SDS-PAGE analyses of crude *E. coli* extracts were performed (data not shown). Independent transformants for each plasmid and the isogenic wild type strain GC4801 were incubated for 3 h with IPTG to allow gene expression and were subsequently challenged with 0, 5, 10, 25, or 50 mM MMS before spreading on LB plates to calculate the survival fraction. Fig. 2 shows that both HP602-I and HP602-V products are able to partially restore the wild type resistance to MMS in the alkA tag double mutant. Although it represents only the 10–15% of the DNA alkylation products, 3-MeA is considered to be the major cause for the cytotoxicity induced by alkylating agents (7). This result indicates that ORF HP602 is likely to code for a protein with a 3-MeA DNA glycosylase activity.

**Substrate Specificity of HP602 Gene Product—**To further investigate the characteristics of these DNA glycosylases, the crude extracts of *E. coli* GC4803 harboring the pGHP602-I or pGHP602-V plasmid or the vector were assayed for their ability to liberate tritiated bases from [3H]DMS-DNA or poly(dG-dC)2poly(dG-dC) (Table II). Both enzymes were able to release methylpurines from [3H]DMS-DNA but not from [3H]DMS-poly(dG-dC)poly(dG-dC). This difference in the substrate specificity suggested that MagIII could not recognize 7-MeG, the major alkylation product. We therefore named the corresponding gene *magIII* for 3-MeA DNA glycosylase.

In order to unambiguously characterize the substrate specificities, HP602-I and HP602-V were subcloned into pET29a+ (Novagen, Inc.) to yield pSHP602-I and pSHP602-V, respectively. The plasmids encode the fusion proteins S-tag-MagIII(I) and S-tag-MagIII(V), respectively. This allowed the isolation of the fusion peptide and subsequent cleavage and purification of the native protein. The SDS-PAGE protein profile, after induction with 1 mM IPTG, showed a prominent band of about 26.5 kDa that was present in cells carrying pSHP602-I and pSHP602-V but absent when pET29a+ was present in the same strain. After purification of the fusion protein, the thrombin-eluted fractions were concentrated and loaded onto a Superdex 75 column. Both MagIII proteins were purified to yield...
a single Coomassie Blue-stained band migrating in SDS-PAGE with a molecular mass of 25 kDa, which corresponds to the full-length protein coded by the ORF HP602 (Fig. 3). The capacities of both purified forms of MagIII to release specific modified bases from [3H]DMS-DNA were examined using HPLC. Fig. 4 shows that MagIII is able to release 3-MeA but not 7-MeG from alkylated DNA, which is consistent with the lack of activity on [3H]DMS-poly(dG-dC)poly(dG-dC). This was also apparent after 4 h of reaction. Under conditions where the amounts of enzyme were normalized for their capacity to excise 3-MeA with respect to AlkA, no excision of 7-MeG was detected.

Because AlkA can also release Hx from DNA (13), a duplex oligonucleotide harboring a unique Hx residue at a defined position was challenged with the purified MagIII and subsequently treated with 0.2 M NaOH to reveal the AP site resulting from the glycosylase activity. Fig. 5A shows that the Hx residue is not released by MagIII at detectable rates from the modified DNA. Similar experiments were carried out with oligonucleotides harboring oxidized bases as dihydrothymine or 8-oxoguanine, and no glycosylase activity was detected for these substrates harboring oxidized bases as dihydrothymine or 8-oxoguanine.

Enzymatic activities on oligonucleotide substrates. A, repair of hypoxanthine by MagIII. Lane 1, 10 μg of MagIII/GVG; lane 2, 10 μg of MagIII/GIG; lane 3, 1 μg of pure AlkA; lane 4, reaction carried out with denatured MagIII as negative control. B, AP lyase activity of MagIII. Lane 1, 5 μg of MagIII/GVG; lane 2, 5 μg of MagIII/GIG; lane 3, reaction carried out with denatured MagIII as negative control; lane 4, 2 μg of EndoIII. S and P indicate substrate and product, respectively.

Disruption of the magIII from H. pylori—As mentioned above, the examination of the complete genomic sequences of H. pylori fails to reveal ORFs potentially coding for either Tag-like or AlkA-like enzymes. To analyze whether the MagIII is indeed functional in vivo and confers resistance to alkylating agents, a disruption mutant was generated in H. pylori by insertion of a kanamycin transposon into the ORF HP602 of strain HAS141. The disruption of this gene was confirmed by sequencing of the genomic DNA. HAS141 and HAS141magIII were then treated with increasing concentrations of MMS to study the cytotoxic effect of the alkylating agent. Fig. 6 shows survival reduction of the mutant strain by concentrations of MMS that do not affect the survival of the parent strain. This result demonstrates that in H. pylori, MagIII is the main defense against the presence of 3-MeA generated by alkylating agents on DNA.

Adaptive Response of H. pylori to Alkylating Agents—The possibility of an adaptive response to the challenge by alkylating agents was explored by analyzing the levels of magIII transcripts in wild type H. pylori after treatment with sublethal doses of alkylating agents as DMS, MMS, or MNNG. As shown in Fig. 7, semiquantitative reverse transcription-PCR experiments show that exposure to 50 μg/ml MNNG induced at least a 5-fold increase of the levels of magIII transcripts. Experiments using 1:4 serial dilutions revealed that the increase of magIII mRNA induced by exposure to MNNG is between 16- and 24-fold. There was no induction of magIII mRNA after treatment of the cells with either DMS or MMS.

DISCUSSION

H. pylori is a Gram-negative bacterium that is associated with several human pathologies (39). Indeed, gastric inflammation and peptic ulcer (40, 41), as well as predisposition to gastric cancer and non-Hodgkin lymphomas of the stomach (42, 43), have been linked to infection by this pathogen. Almost half of the population of the world harbors an H. pylori infection. There is considerable evidence that the bacterial genotype is an important factor in determining the nature and severity of the disease induced by the infection (44, 45). The availability of the complete genomic sequences of two strains (23, 24) allows not only the comparison of the genomes to other organisms sequences but also a preview of the intraspecies variations in...
their coding sequences. As an example, Fig. 1 shows that in the magIII gene, there are single amino acid variations between isolates at seven positions. The genetic variability of H. pylori has been the focus of many investigations (39, 46). The study of natural isolates suggests that the genetic diversity of H. pylori exceeds the level of diversity recorded in virtually all other bacterial species studied. It has been concluded that the presence of ORFs with the capacity to code for DNA repair enzymes preclude point mutations as a possible cause of the genetic diversity. However, the inference of a gene function by sequence homology can lead to misinterpretation. This is the case especially when the homologies invoked are not to proteins for which experimental evidence of their function exists but rather to proteins for which the function has been inferred from their homology to other sequences. Moreover, in the case of H. pylori, the presence of some genes involved in particular DNA repair pathways has been identified without finding the other functions for the same pathway. In the case of the mismatch repair system, a MutS homolog has been predicted, but no ORF encoding a MutL-like protein has been identified. From the genome analysis, several enzymes from the base excision repair pathway also seem to be missing; there are no Fpg homologs (although there is a putative MutY coding sequence), and no ORF has been predicted as coding for the repair of alkylated bases by base excision repair. The description in this paper of a 3-MeA DNA glycosylase from H. pylori underscores the limitations of negative predictions about the presence of functions based on sequence comparisons.

MagIII was originally attributed an endonuclease III-like function because of the presence of the GI/GV motif and the aspartic acid at position 150 that are the signature of the superfamily of DNA glycosylases resembling EndoIII (22, 47, 48). However, it lacks the 4Fe-4S cluster characteristic of all the EndoIII proteins described. It is shown here that MagIII is not a functional EndoIII because it does not recognize oxidized pyrimidines as substrates and does not have an AP lyase activity. We have established that MagIII is, however, a mono-functional 3-MeA glycosylase belonging to the EndoIII family of DNA glycosylases. Although the E. coli class II 3-MeA glycosylase AlkA is also a member of this superfamily, no significant primary sequence similarity is found between these proteins. Moreover, they differ significantly in their substrate specificities. While this work was in progress, Begley et al. (20) described yet another 3-MeA DNA glycosylase, MpgII from T. maritima, defining what they called class IV. They also suggested that the putative protein coded by ORF HP602 (MagIII) from H. pylori could belong to class IV of the 3-MeA DNA glycosylases by sequence similarity (28% identity). The most conserved structure is the HhH (G/P)X18-21D motif, the presence of which suggests that MagIII may use a common mechanism of base excision by nucleophilic activation. However, MagIII shows an important difference in lacking the iron-sulfur cluster, which has been implicated in DNA phosphate backbone recognition. At the sequence level, the 28% identity is not much higher than the level of identity between MpgII and MutY or EndoIII (25 or 20%, respectively). Indeed, MpgII shares a greater homology with other members of its subclass, such as the enzyme from Methanococcus jannaschii (43% identity), that lack the iron-sulfur cluster. MagIII substrate specificity is also different. Unlike MpgII and AlkA, MagIII does not excise 7-MeG. Moreover MagIII does not recognize Hx as substrate. Taking into account the alkylated lesions tested, MagIII seems to recognize almost exclusively 3-MeA, defining then a substrate specificity closer to the class I 3-MeA glycosylases such as Tag (7, 49). We therefore suggest that MagIII defines a novel class of 3-MeA glycosylases. It will be interesting to study whether the other putative MpgII described by Begley et al. (20) are MpgII-like or MagIII-like enzymes. This will shed light on the sequence features responsible for the substrate specificities.

At the physiological level, magIII, as alkA in E. coli, is transcriptionally induced by exposure of the cells with MNNG. This represents an enhanced protection against the cytotoxic effects of alkylating agents. However, no homolog for the E. coli ada gene has been reported from the sequenced genomes. The O6-methylguanine alkyl transferase deduced from the sequence of ORF HP676 lacks the signal molecule found associated to it in other bacterial species (7). Extensive data base searches using the Ada A sequence from Bacillus subtilis or the conserved regions of all the Ada-like activators (50) failed to detect H. pylori primary sequences with homology to them. The mechanisms and the genes required for this adaptive response in H. pylori remain to be found.

The construction of an H. pylori strain with a mutant allele of magIII showed that MagIII is functional in vivo and seems to be essential for the protection of the bacterial genome from the effects of alkylating agents. The sensitivity displayed by the mutants suggests that there are no efficient backup systems for MagIII in H. pylori. The presence of MagIII allows H. pylori to protect itself from the cytotoxic effects of 3-MeA adducts produced on DNA by the action of endogenous or environmental alkylating agents. However, MagIII will not excise the most abundant product generated on DNA by alkylating agents, 7-MeG. It has been suggested that this adduct, although innocuous for cell survival, could have a mutagenic effect (51, 52). This could potentially contribute to the high genetic variability found within H. pylori.

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