We have characterized *Escherichia coli* DNA adenine methyltransferase, a critical regulator of bacterial virulence. Steady-state kinetics, product inhibition, and isotopic exchange studies are consistent with a kinetic mechanism in which the cofactor S-adenosylmethionine binds first, followed by sequence-specific DNA binding and catalysis. The enzyme has a fast methyl transfer step followed by slower product release steps, and we directly demonstrate the competence of the enzyme cofactor complex. Methylation of adjacent GATC sites is distributive with DNA derived from a genetic element that controls the transcription of the adjacent genes. This indicates that the first methylation event is followed by enzyme release. The affinity of the enzyme for both DNA and S-adenosylmethionine was determined. Our studies provide a basis for further structural and functional analysis of this important enzyme and for the identification of inhibitors for potential therapeutic applications.

Bacterial DNA methyltransferases generate N⁴-methylcytosine, C⁵-methylcytosine, and N⁶-methyladenosine in an *S*-adenosylmethionine-dependent reaction (1). Bacterial DNA methylation plays critical roles, including DNA repair, phage protection, gene regulation, and DNA replication, in diverse biological pathways. The majority of DNA methyltransferases form one-half of a restriction-modification system that protects the host bacteria against bacteriophage infection. Together with cognate restriction endonucleases, which generally cleave a short palindromic sequence, these restriction-modification systems provide the foundation for many recombinant DNA manipulations; the endonucleases and methyltransferases have provided many structural and mechanistic insights into the process of sequence-specific DNA recognition and modification.

Not all DNA methyltransferases have an endonuclease partner or at least one which is known. Thus, DNA adenine methyltransferase (DAM,1) methylates the adenine in GATC) in γ-proteobacteria (2, 3), and the cell cycle-regulated methyltransferase (CcrM, methylates the adenine in GANTC) in α-proteobacteria (3, 4) are involved in post-replicative mismatch repair, DNA replication timing, cell cycle regulation, and the control of gene expression. DAM and CcrM have been identified as new targets for antibiotic development (5) because some pathogenic bacteria are either avirulent or not viable when the corresponding genes are removed. DNA adenine methylation regulates the pili formation genes in *Escherichia coli* and *Salmonella*, providing one of the first and clearest examples of epigenetic gene regulation (2). This DNA-mediated gene regulation involves differentially methylated GATC sites, which represent a small minority of the ~5,000–20,000 GATC sites found in a typical bacterial genome.

*E. coli* DAM is a functional monomer of 278 amino acids (6). Our present understanding of how this enzyme functions is based largely on a small number of reports (6–10). Herman and Modrich (6) first characterized the enzyme with plasmid DNA, providing various kinetic parameters and evidence in support of a non-processive path during the methylation of multisite substrates. The apparent *Kₘ* values (AdoMet and DNA), and the apparent turnover number (19 min⁻¹) are similar to those determined for other DNA methyltransferases. The finding that the enzyme dissociates from DNA after an initial methylation cycle, at least with DNA in which the next site is 2455 bp away, has several biological implications (6). For example, the methylation state of two GATC sites in the Pap regulon in *E. coli* demonstrates that EcoRI DNA methyltransferase (M.EcoRI) is not processive with substrates in which adjacent sites are only 50 bp apart. We also identified the interesting trend that methyltransferases not part of a known restriction-modification system act processively, whereas those that are part of a restriction-modification system act distributively (11, 12). The demonstration that CcrM is a processive enzyme certainly fits into this pattern (13). Based on the results of Hermann and Modrich (6), DAM appears not to follow this trend. Subsequently, Bergerat et al. (8), provided evidence that the ability of DAM to methylate a particular GATC site depends on DNA sequences involving 2–3 bp flanking the recognition site. This qualitative analysis provided intriguing insights about how DAM might differentially methylate a subset of the thousands of GATC sites in a bacterial genome. The number and sequence context of such differentially methylated GATC sites remains poorly characterized (8). In contrast to the earlier work, Bergerat et al. (8) showed clear evidence for processive catalysis when adjacent sites were as close as 18 bp apart and in large substrates with longer inter-site distances. Recently, Urig et al. (7) also showed evidence for processive catalysis with DNA of various lengths.

Our interest in DAM from *E. coli* and other bacteria is to understand how DNA adenine methylation regulates gene ex-
Functional Characterization of E. coli DAM

MATERIALS AND METHODS

SyberGold, calf thymus DNA, and ScintiVerse scintillation fluid were purchased from Molecular Probes, Roche Applied Science, and Fisher, respectively. Vistra Green, [methyl-H]AdoMet (average specific activity: 75 Ci/mmol), and [γ-32P]ATP were from Amersham Biosciences. DE81 filters were from Whatman. S-Adenosylmethionine and sinefungin were from Sigma. T4 polynucleotide kinase was from New England Biolabs.

Preparation and Purification of DAM—We purified DAM from two expression constructs. DAM, overexpressed in E. coli strain XL-2Blue (Stratagene) harboring plasmid pDAL572 (provided by Bruce A. Braaten, University of California, Santa Barbara, CA) was grown in LB media (10 g of bacto-tryptone, 5 g of bacto-yeast, and 10 g of NaCl/liter) supplemented with 50 μg/ml kanamycin and 25 μg/ml tetracycline at 37 °C. The cells were induced with 1 mM isopropyl-1-thio-b-D-galactopyranoside at 20 °C and DAM (60 nM, 150 nM) were preincubated for 3 min at 37 °C. Calf thymus DNA (0.1 mg/ml) was added, and the reaction was stopped after 2 min by heating at 80 °C. An 8-liter growth yielded 5 mg of 90% pure hDAM.

Circular Dichroism Analysis of Protein Structure—Circular dichroism spectra were obtained using purified DAM and hDAM in 50 mM sodium phosphate, pH 7.0, at room temperature. Data were collected on an Aviv 202 circular dichroism spectrophotometer using a 500-μm quartz cuvette with a 0.2-cm slit width (Starna). Data were collected between 190 and 265 nm, and spectral data were fit to curves using KaleidoGraph (Synergy Software).

Determinations of Protein Concentration—The isotope partitioning experiments, a 15-methyl-3H]AdoMet, and 0.2 mg/ml BSA) was preincubated for 3 min at 37 °C, and DAM (5 μM, [methyl-H]AdoMet, and 0.2 mg/ml BSA) were incubated at 37 °C for 10 min (18). DAM was diluted into protein dilution buffer. Samples were loaded onto pre-run 10% non-denaturing polyacrylamide gels. Gels were run at 300 V for 2 h at 4 °C. Gels were dried, exposed to image plates, and analyzed on a STORM 840 densitometer (Amersham Biosciences). Densitometry was performed using ImageQuant software (Amersham Biosciences). Densitometry of protein bands was derived from data fit to rectangular hyperbolic equations using KaleidoGraph.

Kinetic Burst and Isotope Partitioning—[methyl-H]AdoMet (30 μM) and DAM (60 nM, 150 nM) were preincubated for 3 min at 37 °C. Thymus DNA (0.1 mg/ml) was added, and the reaction was stopped after 10, 30, 70, and 90 s by placing an aliquot onto DE51 filters (14, 19). For the isotope partitioning experiments, a 15-methyl reaction volume (50 μl) contained DAM, 5 μM [methyl-H]AdoMet, and 0.2 mg/ml BSA was preincubated at 37 °C for 3 min; a 4-μl aliquot was diluted to 100 μl containing MRB, calf thymus DNA (1 μM), and [methyl-H]AdoMet (5 μM) (hot chase) or unlabeled AdoMet (cold chase). Aliquots of the chase solutions were placed onto DE51 filters and analyzed as described (14).

Pro cessivity Analysis—Processivity was determined with a 326-bp PCR fragment with GATC sites at positions 122 and 225, which
includes the Pap operon (20), using a processivity assay as described previously (11, 12). DAM (10 nM) was incubated with the 326-bp PCR-amplified fragment (1/9262M) and AdoMet (30/9262M) in MRB. Aliquots (5/9262l) were removed every minute up to 10 min and stopped with 195/9262l of Tris EDTA (10 mM Tris, pH 8.0, 1 mM EDTA) and 200/9262l of phenol-chloroform. The isolated DNA was digested with DpnII endonuclease (cuts GATC sites only if not methylated), run on an 8% PAGE, stained with Sybergold, and analyzed by densitometry.

**Fig. 1. Kinetic analysis of hDAM.** A, double-reciprocal plot of velocity versus DNA (20-mer) concentration (0.0075–0.2 μM). AdoMet concentrations were 10 (●), 15 (○), 30 (▼), and 100 (▼) μM. Reactions contained 2 nM hDAM. Incubations were for 20 min at 37 °C. B, double-reciprocal plot of velocity versus AdoMet concentration (5–100 μM). DNA concentrations were 0.0075 (●), 0.01 (○), 0.025 (▼), 0.05 (▼), 0.1 (■), and 0.2 ◼μM. C, product inhibition by methylated DNA versus DNA (20-mer). Reciprocal plot of velocity versus DNA concentration (0.03–0.2 μM) in the presence of saturating AdoMet (15 μM). Inhibitor concentrations were 0 (●), 0.075 (○), 0.15 (▼), and 0.3 (▼) μM. D, product inhibition by methylated 20-mer versus AdoMet. Reciprocal plot of velocity versus AdoMet concentration (5–100 μM) in the presence of saturating DNA (20-mer, 150 nM). Inhibitor concentrations were 0 (●), 0.075 (○), 0.15 (▼), and 0.3 (▼) μM. E, product inhibition by S-adenosylhomocysteine versus DNA (20-mer). Reciprocal plot of velocity versus DNA concentration (0.02–0.3 μM) in the presence of saturating AdoMet (15 μM) is shown. Inhibitor concentrations were 0 (●), 75 (○), 150 (▼), 250 (▼), and 350 ( hành) μM. F, product inhibition by S-adenosylhomocysteine versus AdoMet. Reciprocal plot of velocity versus AdoMet concentration (2–30 μM) in the presence of saturating DNA (150 nM) is shown. Inhibitor concentrations were 0 (●), 75 (○), 150 (▼), 250 (▼), and 350 ( hành) μM.

**Lineweaver-Burk Analysis**—Reactions contained hDAM (2 nM), 20-mer duplex DNA (7.5, 10, 25, 50, 100, 200 nM), [3H]AdoMet (10, 15, 30, 100 μM), and BSA (1 mg/ml) in 1× MRB (20 mM Tris-HCl, pH 8.0, 10 mM EDTA, 10 mM DTT) (14). Methylation was initiated by adding either DNA or [3H]AdoMet and incubating at 37 °C for 20 min. 1/velocity versus 1/DNA concentration and 1/velocity versus 1/AdoMet concentration double-reciprocal plots were constructed. All kinetic analyses were performed using a BASIC version of the Cleland programs (14).
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S-Adenosylhomocysteine Inhibition—Reactions with varying AdoMet and AdoHcy contained hDAM (2 nM), 20-mer duplex DNA (150 nM), AdoHcy (0, 75, 150, 250, 350 μM), [3H]AdoMet (2, 4, 8, 10, 30 μM), and BSA (1 mg/ml) in 1× MRB. Reactions with varying DNA contained 20-mer duplex DNA at 20, 50, 100, and 300 nM, and [3H]AdoMet was 15 μM. Methylation was initiated by adding [3H]AdoMet, and incubation at 37 °C was for 20 min.

Methylated DNA Inhibition—Reactions with varying methylated and unmethylated 20-mer duplex contained hDAM (2 nM), 20-mer duplex DNA (30, 50, 100, 200 nM), methylated 20-mer duplex DNA (0, 75, 150, 300 nM), [3H]AdoMet (15 μM), and BSA (1 mg/ml) in 1× MRB. For reactions where methylated 20-mer duplex and AdoMet were varied, reactions contained the same ingredients, except 20-mer duplex DNA was 60 nM, and [3H]AdoMet was 5, 7.5, 10, 15, 30, and 100 μM.

RESULTS

Expression, Purification, and Preliminary Characterization of DAM and hDAM—DAM and the His-tagged construct hDAM were both overexpressed. The two-column purification procedure for DAM generated 2–5 mg of homogeneous protein/liter of bacterial culture (data not shown); the purification procedure for hDAM provided ~1 mg of ~90% pure protein/liter of bacterial culture. The apparent $k_{cat}$ (2.5 and 0.93 min$^{-1}$), $K_m^\text{DNA}$ (3.6 and 17.4 nM), and $K_m^{\text{AdoMet}}$ (12 and 5.6 μM) for DAM and hDAM, respectively, were similar when the 20-mer DNA substrate was used.

Circular Dichroism Results—Circular dichroism spectra of DAM and hDAM were used in conjunction with CDSSTR (16) to make secondary structure predictions. Similar values were predicted for both enzymes. The predicted DAM percentages were 49% α-helix, 24% β-sheet, 5% turn, and 17% unordered, whereas the predicted hDAM percentages were 54% α-helix, 24% β-sheet, 5% turn, and 15% unordered. DNA methyltransferases utilize a broad range of secondary structures, and our calculations fall within these values.

Kinetic Mechanism—The kinetic mechanism was analyzed by double-reciprocal analysis using hDAM methyltransferase (14). The 1/velocity versus 1/DNA concentration plot shows intersecting lines in the second quadrant, whereas the 1/velocity versus 1/AdoMet concentration plot shows lines intersecting on the y-axis (Fig. 1, A and B). A ping-pong mechanism is unlikely because the patterns in Fig. 1, A and B are intersecting, whereas the predicted hDAM percentages were 54% for both enzymes. The predicted DAM percentages were 49% for both enzymes. The H9252/H9251 dimer for hDAM generated 2–5 mg of homogeneous protein/liter of bacterial culture. The apparent $k_{cat}$ (2.5 and 0.93 min$^{-1}$), $K_m^\text{DNA}$ (3.6 and 17.4 nM), and $K_m^{\text{AdoMet}}$ (12 and 5.6 μM) for DAM and hDAM—E. coli DAM has two tryptophans at positions 10 and 236, which provide a basis for probing ligand-induced conformational changes (6). AdoMet was added at 10 concentrations from 1 to 200 μM in MRB, and the decrease in native protein fluorescence was plotted as a function of AdoMet concentration. Triplcate determination was used to calculate the thermodynamic dissociation constant and associated standard error, 18.7 ± 1.81 (data not shown). The $K_d^{\text{AdoMet}}$ was 21.8 ± 1.81 (data not shown).
is similar to the $K_{\text{AdoMet}}^\text{DNA}$ (Table I), indicating that AdoMet is bound with comparable affinity in the binary complex and in the presence of DNA.

Determination of $K_{\text{DNA}}^m$—The affinity of the enzyme (DAM) for an unmethylated duplex (20-mer) was determined by gel mobility shift assay (Fig. 2) using $50 \mu M$ sinefungin. The dissociation constant ($K_{\text{DNA}}^m$), $119 \pm 7.3 \text{ nM}$ (Table I), was determined from the scanned gel and resultant binding isotherm. Although similar to $K_{\text{DNA}}^m$ (Table I), direct comparison is compromised by the use of sinefungin for the $K_{\text{DNA}}^m$ and AdoMet for $K_{\text{DNA}}^m$ determinations.

Burst Experiments—Burst experiments provide insight about which steps limit catalytic turnover, the amount of active enzyme, and an apparent $k_{\text{cat}}$ determination (19, 22). Under high enzyme and substrate (DNA and AdoMet) conditions, DAM manifests a burst of product formation, followed by a slower rate (Fig. 3A). Because the filter binding assay measures formation of methylated DNA, which includes product that is still associated with the enzyme, the simplest interpretation of these results is that methylation is faster than subsequent product release steps. The pre-steady-state phase (before 10 s) is followed by the steady-state phase, and the slope of the latter provides a measure of the apparent $k_{\text{cat}}$. The slope of the higher enzyme condition (squares, $150 \text{ nM}$) is correspondingly greater than the lower enzyme condition (circles, $60 \text{ nM}$). Extrapolation of both plots to zero time provides an estimate of the active enzyme, which within experimental error is >95% active. Increasing the AdoMet concentration from the $30 \mu M$ used in these experiments does not result in any changes in the product formation profiles (data not shown).

Isotope Partition—Our steady-state kinetic analysis suggests that the binary enzyme-AdoMet complex is catalytically competent. This can be directly tested through the use of an isotope-partitioning experiment (14). As in the burst experiment (Fig. 3A), preincubation of radiolabeled AdoMet and enzyme followed by the addition of DNA and more radiolabeled AdoMet in a chase results in the rapid formation of product. Adding a 25-fold excess of unlabeled AdoMet in the chase along with DNA results in a 3-fold decrease in the vertical axis intercept. A non-zero vertical intercept under these conditions requires the initial binary enzyme-AdoMet complex to be catalytically competent because a 25-fold reduction in AdoMet-specific activity results in a near zero level of detected product when the labeled and unlabeled AdoMet are premixed (data not shown). The 3-fold decrease in vertical intercept shows that upon DNA addition, the binary complex dissociates to a greater extent than proceeding to form the ternary complex and catalysis (14).

Processivity Analysis—The data in Fig. 4 show that DAM acted distributively on the DNA fragment derived from the Pap regulon, which contains two GATC sites separated by 103 bp (11, 12, 20). The gel shows the digestions (DpnII) which generated the singly methylated fragments (S1 and S2) and unmethylated fragments (U1 and U2). The percent conversion, which relates the singly (S1 and S2) and doubly (D) methylated band to the sum of all bands, is plotted. The calculated percent conversions are shown in the lower graph, and lines are shown for a hypothetical completely distributive enzyme (Fig. 4) (11, 12). Similar results were obtained when NaCl was varied up to 100 mM, and AdoMet was used at 3 mM (data not shown).

**DISCUSSION**

Although several bacterial DNA methyltransferases are well characterized both structurally and functionally (1), few have the potential medical importance of DAM and CcrM, shown to be essential for virulence and viability in several human pathogens (2, 4). Our aim was to provide a functional description of DAM to form the basis of identifying potent inhibitors.2 We purified the recombinant enzyme from two expression vectors in an effort to obtain large quantities for both structural and inhibitor screening studies. The two forms, with (hDAM) and without (DAM) an N-terminal histidine tag, showed similar kinetic parameters (see “Results”).

Our kinetic mechanism results are most consistent with a steady-state ordered mechanism involving an initial enzyme-AdoMet complex (Figs. 1 and 3; see “Results”). A limited number of DNA methyltransferases have been characterized at this level, but this overall kinetic mechanism is similar to that of several enzymes that are important in human disease, including the family of AP endonucleases.

2 N. Mashhoon, C. Pruss, M. Carroll, P. H. Johnson, and N. O. Reich, submitted for publication.
determined for the DNA adenine methyltransferases M.EcoRI (14), M.EcoRV (23), and T4DAM (24). Other DNA adenine methyltransferases appear to have either random or alternative ordered mechanisms (25, 26). In contrast, the DNA cytosine methyltransferases M.HhaI (27, 28), M.MspI (29), and Dnmt1 (30) either have obligate DNA first mechanisms or have a strong preference for this pathway. A recent study of DAM described pre-steady-state experiments in which the preformed enzyme-AdoMet complex leads to faster rates than the preformed enzyme-DNA complex (7). The authors interpret these results as evidence for a DNA first kinetic mechanism. Although such results have many interpretations, they provide little direct evidence for any order of substrate addition. Another recent study showed that at high concentrations, DAM can bind its cognate DNA in the absence of any cofactor or cofactor analogs, which can be reconciled (31) with our proposed kinetic mechanism because the initial velocity studies describe the kinetic preferences under much lower concentrations. Further evidence for an AdoMet first kinetic preference is provided by our isotope-partitioning results (Fig. 3B) which clearly show that the enzyme-AdoMet complex is competent; this result eliminates any mechanism in which sequence-specific DNA binding first is an obligate step. Interestingly, the binary complex is shown under the conditions of the assay to partition largely back to the free enzyme and AdoMet rather than lead to the ternary complex and catalysis. As described for M.EcoRI (14), an AdoMet first mechanism requires only that the cofactor is bound prior to sequence-specific DNA binding, not to the binding of nonspecific DNA adjacent to the target site. This has implications for the mechanism of overall site location, which has not been clearly identified for DAM but was shown to involve facilitated diffusion for other DNA methyltransferases (11, 12).

Many of the structural and functional characteristics of DAM presented here are similar to those described for other DNA methyltransferases. Thus, the observed burst kinetics (Fig. 3A) show that a product release step after methyltransfer limits the catalytic turnover, as has been described for the majority of DNA methyltransferases (1). The affinity for DNA (Fig. 2) and inhibition by both AdoHcy and methylated DNA (Table I) of the enzyme are similar to those described for other DNA adenine methyltransferases. Because there is no high resolution structure for DAM, we used secondary structure predictions based on CD analysis to determine whether the structure of the protein fits that of previously characterized DNA methyltransferases and to confirm that the His-tagged enzyme is folded properly. Our secondary structure predictions for both DAM and hDAM are very similar, are within the range of observed values determined previously for DAM (32), and are from the inspection of the five related adenine methyltransferase crystal structures T4DAM, M.TaqI, M.DpnM, M.RsrI, and M.PvuII.

The proposed kinetic mechanism has implications for the design and interpretation of assays to identify DAM inhibitors. For example, despite the AdoMet first kinetic mechanism, compounds that interfere with AdoMet binding may leave the ability of the enzyme to bind DNA unperturbed. This characteristic could derive from the ability of the enzyme to bind DNA nonspecifically in the absence of its cofactor, which would not necessarily be altered if the AdoMet site were changed. Alternatively, compounds that interfere with DNA binding are likely to cause a significant reduction in the amount of enzyme-DNA complex formation. These two mechanistic outcomes are likely to result in very different cellular phenotypes even if the compounds demonstrate similar inhibition potency. In the former case, the inhibited enzyme will likely be bound nonspecifically to DNA; in the latter case, the inhibited enzyme will likely be free from DNA. Methyltransferase inhibitors that sequester the target enzyme onto the DNA are known to have significant nonspecific cellular toxicity (33).

A somewhat surprising result is our demonstration that DAM shows no evidence for processive catalysis in our assay on the DNA segment derived from the regulatory region of the Pap element. The intensity of D and S1+S2 were divided by the sum of D+S1+S2+U1+U2 to determine the percent conversion of doubly and singly methylated species for a given time of reaction. D, doubly methylated fragment; S1, singly methylated (GATC I); S2, singly methylated (GATC II); U1, fragment generated from single cleavage (GATC II); U2, fragment generated from single cleavage (GATC I).
operon, which contains two GATC sites separated by 103 bp (Fig. 4) (20). Unlike the DNA substrates used in the prior studies of DAM processivity, this DNA element contains GATC sites with a methylation known to directly influence the expression of an adjacent operon (2). Although prior investigations of DAM processivity have provided conflicting interpretations, the demonstration that the enzyme can act processively (7) is compelling because many factors could interfere with this type of function under in vitro conditions. The prior work by Herman and Modrich (6) on DNA, in which two GATC sites are separated by 2455 bp, showed that the sites are methylated independently. Although not directly addressing the processivity, the work by Bergerat et al. (8) using largely plasmid substrates provided indirect evidence that DAM is processive. The most definitive study on DAM processivity was provided in the work by Bergerat et al. (8) using short synthetic DNA, PCR fragments, and λ DNA. These authors showed that DAM is processive, with an average of 55 GATC sites being methylated for one enzyme binding event with large λ DNA. Trivial reconciliations of these results with our demonstrated lack of processivity can be excluded. Our experimental results did not change with variations in salt concentrations or variations in AdoMet concentrations, and a similar insensitivity to these parameters was described by Urig et al. (7). The lack of observed DAM processivity is not likely to be because of rapid dissociation of the protein off the proximal DNA ends, as Urig et al. (7) demonstrated processive character on short substrates as well.

We suggest that the different results might be understood in the context of sequences flanking the GATC recognition sites. Evidence for this was provided in the study on plasmid DNA methylation, in which a distinct ranking of preferred DAM sites was revealed (8). The 22 GATC sites within the pBR322 plasmid have diverse flanking sequences. A small subset is shown to be particularly disfavored in that no or little methylation is observed at these sites with a methylation known to directly influence the expression of the operon (2, 6, 7). For example, although the exact number of the 19,000 GATC sites in the E. coli genome that are unmethylated or partially methylated is unknown, various studies implicate 10–50 such sites (7, 34, 35). The remaining sites are faithfully methylated during the normal cell division processes, demanding an efficient and nearly complete methylation process. As proposed by Urig et al. (7), a highly processive DAM would ensure that this large number of sites is efficiently methylated. However, other DNA methyltransferases with comparable site frequencies in the E. coli genome are either not processive, or certainly not as processive as DAM with the substrates used by Urig et al. (7). We propose that a processive DAM on the majority of sites, as described by Urig et al. (7), is compatible with our results when one considers the flanking sequence context, the different biological roles of the majority of genomic GATC sites, and the GATC sites known to be involved in gene regulation, such as those probed in our study within the Pap regulon (2, 20).

DAM has many biological functions, several of which require the enzyme to methylate the majority of genomic GATC sites (2, 6, 7). For example, although the exact number of the 19,000 GATC sites in the E. coli genome that are unmethylated or partially methylated is unknown, various studies implicate 10–50 such sites (7, 34, 35). The remaining sites are faithfully methylated during the normal cell division processes, demanding an efficient and nearly complete methylation process. As proposed by Urig et al. (7), a highly processive DAM would ensure that this large number of sites is efficiently methylated. However, other DNA methyltransferases with comparable site frequencies in the E. coli genome are either not processive, or certainly not as processive as DAM with the substrates used by Urig et al. (7). We propose that a processive DAM on the majority of sites, as described by Urig et al. (7), is compatible with our results when one considers the flanking sequence context, the different biological roles of the majority of genomic GATC sites, and the GATC sites known to be involved in gene regulation, such as those probed in our study within the Pap regulon (2, 20).

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Functional Characterization of *Escherichia coli* DNA Adenine Methyltransferase, a Novel Target for Antibiotics

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