JOURNAL OF BACTERIOLOGY, Aug. 1987, p. 3625–3632
0021-9193/87/083625-08$02.00/0

Vol. 169, No. 8

Expression of the Cloned Coliphage T3 *S*-Adenosylmethionine Hydrolase Gene Inhibits DNA Methylation and Polyamine Biosynthesis in *Escherichia coli*

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Received 16 January 1987/Accepted 3 May 1987

We have developed a new research tool for the study of *S*-adenosylmethionine (AdoMet) metabolism by cloning the coliphage T3 AdoMet hydrolase (AdoMetase; EC 3.3.1.2) gene into the M13mp8 expression vector. The recombinant bacteriophage clones expressed an AdoMetase activity in *Escherichia coli* like that found in T3-infected cells. High levels of AdoMetase expression impaired AdoMet-mediated activities such as *dam* and *dcm* methylase-directed DNA modifications and the synthesis of spermidine from putrescine. Expression vectors containing the cloned AdoMetase gene thus provide an alternate approach to the use of chemical inhibitors or mutants defective in AdoMet biosynthesis to probe the effect of AdoMet limitation.

S-adenosylmethionine (AdoMet) has been shown to be involved in a wide variety of biochemical processes (for comprehensive reviews, see references 40 and 53). Perhaps the two most widely studied activities of AdoMet include its contribution of an aminopropyl moiety during the biosynthesis of the polyamines spermidine and spermine (49) and its donation of a methyl group to many different biological compounds (8, 36). These methylations have been shown to provide a necessary control element for processes such as chemotaxis (45), DNA replication fidelity and repair (3), and restriction-modification in *Escherichia coli* (10) and other procaryotes. A wide variety of AdoMet-mediated methylations are also found in eucaryotes. For instance, methylation of DNA in higher eucaryotes has been implicated in the control of gene expression (13, 37).

A variety of strategies have been used to study the roles of AdoMet in cellular metabolism. Analogs of methionine (e.g., cycloleucine and ethionine) inhibit AdoMet biosynthesis (23), but are also incorporated into proteins in place of methionine and/or provoke general responses to amino acid starvation (2, 35), thus obscuring conclusions based on their use. Absolutely or conditionally lethal mutants have never been isolated for AdoMet biosynthesis, and all *E. coli metK* (AdoMet synthetase) mutants isolated have demonstrated some residual AdoMet synthesis and nearly normal levels of AdoMet-mediated activities (19, 32). Finally, mutants or chemical inhibitors which prevent specific methylations (26, 52), spermidine and spermine biosynthesis (50, 54), and other well defined activities involving AdoMet often present obscure phenotypes, multifaceted effects, or reactions unrelated to AdoMet metabolism (33, 41).

There is another infrequently used strategy for assessing the metabolic consequences of AdoMet deprivation in *E. coli*. The first gene of the coliphage T3 encodes an enzyme which hydrolyzes AdoMet to homoserine and 5′-methylthioadenosine (MTA) (18) and acts as part of the defense of the phage against the host type I restriction system (47). The AdoMet hydrolase (AdoMetase, also called the AdoMet cleaving enzyme or AdoMet lyase; EC 3.3.1.2) gene can be expressed to high levels of activity in cells infected with UV light-inactivated T3. These sterile phage have been used to infect growing cells and successfully block the synthesis of cyclopropane fatty acids (11), inhibit the restriction of unprotected transformed plasmids (38), and block DNA methylations (17). Unfortunately, this technique is limited to T3-sensitive strains of *E. coli*, results may be influenced by the activities of other phage genes and/or the infection process itself, and experiments are necessarily short term owing to the presence of wild-type phage or complementing mutations leading to productive bursts. These demonstrations and the completely unmethylated state of T3 DNA (17) show that the enzyme can profoundly affect AdoMet-mediated activities in bacteria infected with T3.

The effectiveness and specificity of AdoMetase for eliminating AdoMet from the cell and its potential, once its gene is purified from other T3 genes, for producing few effects unrelated to AdoMet metabolism led us to attempt the cloning of this gene. This report documents the success of this effort and presents preliminary evidence that *E. coli* tolerates high levels of AdoMetase gene expression, despite the consequent inhibition of activities involving AdoMet.

MATERIALS AND METHODS

Reagents, bacteria, and bacteriophages. *E. coli* B/2 and phage T3 were obtained from C. K. Mathews, and *E. coli* JM103 and the M13mp8 and M13mp9 phage cloning vectors (30) were purchased from Bethesda Research Laboratories, Inc. Plasmid pKK223-3 was obtained from Pharmacia Fine Chemicals. Bacteria were usually grown in YT medium (25) and occasionally (as indicated) in M9CA medium, which contains M9 salts (31) supplemented with glycerol to 0.4% (vol/vol), Casamino Acids (Difco Laboratories) to 0.15%, and 1 μg of thiamine hydrochloride per liter. [^{14}COOH] AdoMet (60 mCi/mmol) and [1,4-^{14}C]putrescine (118 mCi/mmol) were purchased from Amersham Corp., and [$^{14}CH_3$] AdoMet (40 mCi/mmol) was obtained from Research Products International. Restriction enzymes, T4 DNA ligase, and

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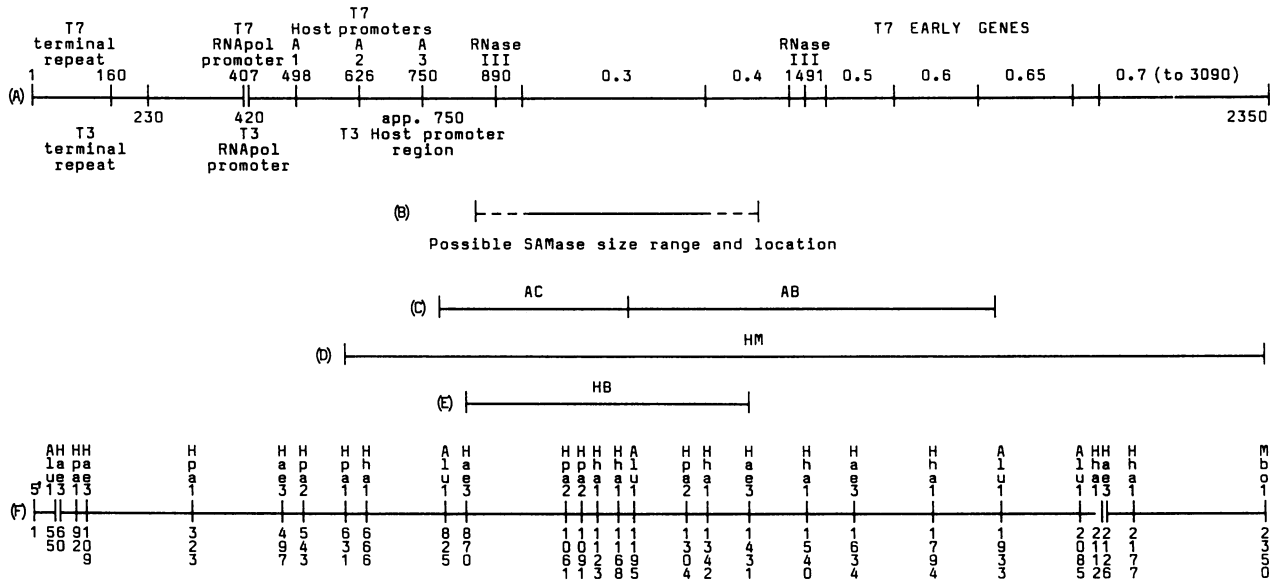

FIG. 1. Estimated location of the AdoMetase gene. This analysis was used to determine the probable location of the T3 AdoMetase gene by taking advantage of the similarity of the nucleotide sequences (12) and physical maps (6) between T3 and the closely related phage T7. (A) The T7 sequence (14) was used to superimpose a map of its earliest genes upon the T3 *Mbo*I G fragment. Below line A, the positions of comparable T3 phage elements are shown as deduced from the literature (see reference 16 concerning the terminal repeats, reference 1 concerning the T3 RNA polymerase promoter, and reference 51 concerning the *E. coli* RNA polymerase-binding studies that located these promoter sites). (B) Length of DNA necessary to encode the AdoMetase protein (reported to be as small as 11.5 kDa by Studier and Movva [47] [——] and as large as 20 kDa by Spoerel and Herrlich [43] [– – –]) and is positioned beneath its T7 homolog, the 0.3 gene. The two *Alu*I fragments (AB and AC) in line C, the *Hpa*I-to-*Mbo*I fragment (HM) in line D, and the *Hae*III fragment (HB) in line E are shown in their proper location underneath the T3/T7 physical map and are noteworthy because inaccuracies in the mapping, the uncertainty of the size of the AdoMetase, and the inexactness of the T3-T7 comparison could account for any of them encoding the entire gene (F) Restriction map determined for the *Mbo*I G fragment; this line was derived by normalizing the fragment sizes estimated by agarose gel electrophoresis (±5%) to the 2,350-base-pair length for the *Mbo*I fragment determined by Bailey et al. (4). Four *Hha*I sites known to exist between bases 1794 and 2112 and one between 2177 and 2350 were not positioned or placed on the map in line F.

all other chemical reagents were purchased from standard biochemical and molecular biology supply sources.

DNA isolation and analysis. T3 phage (collected from lysed cultures of *E. coli* B/2 grown in M9CA) and M13 replicative-form (RFI) DNA were purified through cesium chloride density gradients, the DNA was purified and stored, and standard procedures were used to analyze restriction digests by agarose gel electrophoresis (25). The T3 *Mbo*I G fragment was electroluted from a 0.8% agarose gel into dialysis tubing and passed through a NACS PREPAC column (Bethesda Research Laboratories) to remove contaminants from the DNA. These G-fragment preparations were then used in restriction mapping experiments (Fig. 1) and as a source for the *Alu*I and *Hae*III restriction fragments used to generate the M13 recombinant phage. All other fragments used for mapping or cloning were cut out of low-melting-point agarose gels. Gel slices containing the desired restriction fragment were melted at 68°C without further dilution, and each fragment was extracted twice with buffer-saturated phenol and twice with water-saturated ether and then precipitated by the method described by Maniatis et al. (25). Restriction subfragments were named alphabetically according to size. We used the polyacrylamide gel electrophoresis (PAGE) system of Laemmli (22), omitting the sodium dodecyl sulfate (SDS) and using gel slabs 15% in acrylamide, to track small DNA restriction fragments against known standards. The C-test to determine M13 insert orientations was performed essentially by the method of Messing (29).

Recombinant phage and plasmid production. DNA cloning experiments were performed, and DNA was analyzed by standard procedures (25, 29) and as follows. Each of the subclones in Table 1 (except M13ab9) was produced by ligating low-melting-point agarose gel-purified DNA fragments from *Alu*I or *Hae*III digests of the T3 *Mbo*I G fragment into the *Sma*I site of M13mp8. M13ab9 was produced by purifying the *Eco*RI-*Hin*dIII-liberated AB fragment (from M13ab6) and ligating it into the likewise-digested M13mp9 vector. Fragment sizes were estimated by agarose gel electrophoresis against DNA markers of known size. The HB fragment purified from M13hb1 digested with *Eco*RI and *Hin*dIII was ligated into the polylinker of pKK223-3 to produce pHBF1KK and position the AdoMetase gene behind the P_{tac} promoter.

Enzyme assays and digestions. β-Galactosidase activity was determined by the method of Miller (31), and protein concentrations were determined by the method of Lowry et al. (24). AdoMetase samples were taken from cell extracts prepared in the French pressure cell during the purification of the enzyme (43) or by freeze-thawing cells treated with lysozyme. The latter was accomplished by pelleting (in a Microfuge [Beckman Instruments, Inc.]) 1 ml of mid-to-late-log-phase cells grown at 37°C (made 0.3 mM in isopropyl-β-D-thiogalactopyranoside [IPTG] 1 h previously, as indicated in Table 2) and then microcentrifuging and washing the cells and suspending them in 0.2 ml of 50 mM Tris hydrochloride (pH 8.0)–10 mM EDTA, supplemented with 0.1 mg of lysozyme in 50 μl of the same buffer. These suspensions were digested for 5 min at room temperature, frozen (−80°C) and thawed twice, and microcentrifuged for 2 min, and the clear supernatant was tested for enzyme activity. The

AdoMetase assay takes advantage of the affinity of Affi-Gel 601 (Bio-Rad Laboratories) for *cis*-diols as are found on the ribose moiety of AdoMet, MTA, and 5'-methylthioribose (MTR) but not homoserine. AdoMetase samples were made 100 mM in Tris hydrochloride (pH 7.5), 10 mM in EDTA, 5 mM in dithiothreitol, and 0.1 mM in AdoMet (with 10,000 cpm of [^{14}COOH]AdoMet) in a total volume of 20 μl. Reactions were stopped with 100 μl of 0.2 M perchloric acid, and the debris removed by centrifugation. The supernatant was neutralized with 18 μl of 1 M KOH and then loaded onto a 2-ml (bed volume) column of Affi-Gel 601 which had been neutralized with 10 mM Tris hydrochloride (pH 7.2). ^{14}COOH-labeled homoserine was washed from the column with 6 ml of the 10 mM Tris hydrochloride, and the unreacted [^{14}COOH]AdoMet and unlabeled MTA and MTR were eluted with 6 ml of 0.1 M formic acid. Samples of the Tris hydrochloride and acid washes (3 ml each) were assayed for radioactivity by liquid scintillation counting to determine the extent of AdoMet hydrolysis. The results were corrected for nonenzymatic AdoMet hydrolysis by comparison with results for controls lacking cell extract.

Identification of AdoMetase reaction products. Radiolabeled reaction products of the T3 and M13hb1 AdoMetase digestions with the [^{14}COOH]AdoMet and [$^{14}CH_3$]AdoMet labels were separated on a thin-layer chromatography (TLC) system consisting of a cellulose F_{254} matrix and an *n*-butanol–acetone–acetic acid–water (70:70:20:40, vol/vol/vol/vol) solvent mix. The digestion products and standards were identified by UV light absorbency, ninhydrin staining, Affi-Gel 601 adherence, radio-scanning of the TLC strip, and/or comparison with known standards. Radioactive spots were scraped from the chromatogram, quantified in a liquid scintillation counter, and reported (see Table 3) as percentages of the total counts removed from each chromatogram. Extracts of uninfected B/2 and JM103 cells showed no detectable enzymatic AdoMet hydrolysis under the same conditions, and the radio scans showed activity peaks at only discrete, identifiable positions (see Table 3).

Assay for *dam* and *dcm* methylation. *E. coli* JM103 was infected with M13ac1, M13ab6, or M13hb4 and grown overnight in 3 ml of YT medium. Phage RF DNA was isolated by the method of Birnboim and Doly (7), digested with *Mbo*I, *Dpn*I, *Sau*3AI, *Eco*RII, or *Bst*NI, and electrophoresed in a 1.5% agarose gel.

Analysis of spermidine biosynthesis. Cells to be used for polyamine analyses were infected with either M13mp8 or M13hb4 and grown to 50 Klett units (optical density at 600 nm, 0.1), harvested, washed, suspended in M9CA, and incubated at 37°C for 3 h with shaking. [1,4-^{14}C]putrescine (0.5 μCi) was added, and the cultures were incubated for another 1 h. These conditions were necessary to ensure both complete phage infection of the cells and effective expression of the AdoMetase gene. The cells were then harvested, washed twice, and extracted with 5% perchloric acid for 2 h on ice, and the debris were removed by centrifugation. The supernatant was neutralized with KOH, frozen, and centrifuged again to remove the salts. These extracts and standards of spermidine, putrescine, and proline were then derivatized with dansyl chloride (15, 42). The TLC system used to separate the dansylated compounds consisted of an aluminum oxide F_{254} neutral type T matrix on an aluminum backing, developed with a chloroform–*n*-butanol (49:1, vol/vol) solvent mixture.

AdoMetase purification and analysis. *E. coli* B/2 was infected with UV-irradiated T3 (17) and harvested 30 min later, and *E. coli* JM103(pHBF1KK) was induced in the mid-log phase by addition of IPTG to 1 mM (final concentration) and then harvested after another 2 h of incubation. Portions (5 g) of the two cell pastes were subjected to a modification of the AdoMetase purification protocol of Spoerel and Herrlich (43) for the host subunit-bound B-type AdoMetase. Rather than dialyze the 25% $(NH_4)_2SO_4$ supernatant against buffer A, we ran it over a Sephadex G-200 column. The pooled AdoMetase activity (in the protein front) was then subjected to *S*-adenosylhomocysteine–agarose (Bethesda Research Laboratories) affinity chromatography. The enzyme was eluted from the column (with 2.5 mM AdoMet rather than adenosine), dialyzed against buffer A, and subjected to SDS-PAGE (22).

RESULTS

Location of the AdoMetase gene. The AdoMetase gene is the first on the T3 genome, and the AdoMet hydrolytic activity of its product can be detected within 2 min of phage adsorption to the host cell. The host RNA polymerase initially uses any of three strong promoters to transcribe a long polycistronic mRNA from which the mature AdoMetase mRNA is cleaved by RNase III (21). The location of the AdoMetase gene and other markers on the T3 DNA was approximated by using the positions of homologous genes mapped on the closely related and fully sequenced coliphage T7 genome. This estimate was supported with promoter-binding studies and physical mapping experiments performed on T3 (Fig. 1). Figure 1A shows these T7 elements and some of their T3 homologs superimposed on the 2,350-base-pair T3 *Mbo*I G fragment, the 5'-terminal *Mbo*I restriction fragment (4). A sequencing study (9) published after our use of these estimates confirmed their accuracy by locating the three *E. coli* RNA polymerase-specific promoters between bases 442 and 479, 571 and 608, and 681 and 718 and by locating the T3 RNA polymerase-specific promoter between bases 366 and 386 of the T3 genome.

Cloning strategy. We were unable to clone the fragment described by Bailey et al. (4) defined by the *Hpa*I site at base pair ca. 631 and the *Mbo*I site at base pair ca. 2350 (the HM fragment) in the T3 map (Fig. 1). To identify a more suitable fragment for cloning, we mapped the *Alu*I, *Hae*III, *Hha*I, *Hpa*I, and *Hpa*II sites on the *Mbo*I G fragment and aligned them with the approximate AdoMetase gene position derived in Fig. 1. The *Hae*III B (HB) fragment appeared most likely to encompass the AdoMetase gene, although it is conceivable that the *Alu*I B (AB) and C (AC) fragments contain the gene if the evidence used in Fig. 1 is inaccurate.

We purified the HB, AC, and AB fragments from low-melting-point agarose gels and cloned them into the *Sma*I site of the M13mp8 polylinker. M13 RFI DNA was isolated (7) from bacterial cultures inoculated with viral plaques altered in their ability to hydrolyze 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and digested with *Eco*RI and *Hin*dIII. Phages demonstrating monomeric inserts of the expected fragment lengths were selected for further study. Southern blots with a ^{35}S-labeled *Mbo*I G fragment probe verified the T3 origin of the insert (results not shown). Insert orientations relative to the lactose promoter of the M13 vector were determined by the C-test, and recombinant phage clones containing the three T3 inserts in both orientations were identified. The results of these tests characterizing the recombinant phages are shown in Table 1.

Identification of AdoMetase activity. Samples of each clone were grown in YT medium in the presence and absence of IPTG and assayed for both β-galactosidase and AdoMetase

TABLE 1. T3 M13 subclone characterization

Subclone[a]	Parent vector	Insert origin	Cloning site	Insert size (bp)	Insert orientation[b]	Positive C-test with[c]:
M13ab6	M13mp8	T3	*Sma*I	700	+, (5′ → 3′)	M13ab9, M13hb4
M13ab9	M13mp9	M13ab6	*Eco*RI-*Hin*dIII	700	−, (3′ → 5′)	M13ab6, M13hb1
M13ac1	M13mp8	T3	*Sma*I	370	+, (5′ → 3′)	M13ac3, M13hb4
M13ac3	M13mp8	T3	*Sma*I	370	−, (3′ → 5′)	M13ac1, M13hb1
M13hb1	M13mp8	T3	*Sma*I	560	+, (5′ → 3′)	M13hb4, M13ac3
M13hb4	M13mp8	T3	*Sma*I	560	−, (3′ → 5′)	M13hb1, M13ac1

[a] All of the subclones were named for the T3 fragment they contain (e.g., M13ab6 contains the AB fragment).
[b] Orientations are expressed as the T3 strand found on the single-stranded mature M13 DNA (+ or −) and whether the RNA transcript initiated at the P_{lac} promoter of the vector would produce mRNA in the same orientation as that from the promoters of T3 (5′ → 3′) or its complement (3′ → 5′).
[c] Fragment orientations with respect to the T3 parent were established by C-testing the AB and HB clones against M13ad1 and M13ha3. These clones were derived by ligating the *Alu*I D (AD) and *Hae*III A (HA) fragments found on the 3′-terminal *Mbo*I G fragment directionally into *Sma*I-*Bam*HI-digested M13mp8.

activities. The M13ab6 and M13ac1 phage-infected bacteria have a weak inducible β-galactosidase activity (which correlates with their light-blue plaques when grown in the presence of IPTG and X-gal), while only the M13 derivatives with the HB restriction fragment inserts exhibited the AdoMetase activity (Table 2). AdoMetase gene expression directed by the M13hb1 and M13hb4 phages did not respond to IPTG or *lacI*^{q} repression as it did for β-galactosidase. Large amounts of AdoMetase were produced in either insert orientation relative to the P_{lac} promoter even though no *E. coli* promoter has been shown in this region of the T3 DNA. Furthermore, IPTG had only a modest effect on AdoMetase expression in either M13hb1 or M13hb4, despite the demonstrated effectiveness of the *lacI*^{q} repression in controlling β-galactosidase synthesis in the M13mp8-infected *E. coli* JM103 host.

Verification of AdoMetase identity. An AdoMetase has never been demonstrated in any strain of *E. coli*. The appearance of such an activity in *E. coli* only upon infection with T3 or the M13hb1 and M13hb4 recombinant phages provides strong circumstantial evidence for the T3 coliphage origin of the enzyme. Two tests were performed to compare the T3- and M13-based enzymes and their activities to further confirm our cloning of the authentic T3 AdoMetase.

Either [^{14}COOH]AdoMet or [$^{14}CH_3$]AdoMet was added to whole-cell extracts of T3- and M13hb1-infected cells, the reaction products were separated by TLC, and the position(s) of the radioactivity was located on the chromatogram. Digestion of AdoMet with either extract produced the same radio-labeled products. AdoMetase-mediated hydrolysis of methyl-labeled AdoMet yielded MTA and its metabolite MTR, while the carboxy-labeled AdoMet produced homoserine (Table 3). No other radioactive peaks appeared on scans of the chromatograms when either label was used (not shown).

AdoMetase was partially purified from extracts of *E. coli* JM103 transformed with the plasmid pHBF1KK (a construct which positions the AdoMetase gene fragment behind the IPTG-inducible P_{tac} promoter) and *E. coli* B/2 infected with UV-irradiated T3. These samples were then subjected to SDS-PAGE to visualize AdoMetase and compare the enzymes encoded by T3 and pHBF1KK (Fig. 2). The T3- and pHBF1KK-derived samples show very similar banding patterns on the gel, and the protein bands at approximately 14 and 17 kilodaltons (kDa) agree roughly with the size(s) of the AdoMetase proteins described by Studier and Movva (47) and Spoerel and Herrlich (43). Furthermore, the 45-kDa protein in the gels from both sources corresponds to the host factor that was identified by Spoerel and Herrlich (43) as part of the type B AdoMetase complex.

Effect of AdoMetase expression on cellular metabolism. Surprisingly, high levels of AdoMetase were tolerated with little obvious effect on the bacteria. Cultures of *E. coli* JM103 infected with the M13hb1 and M13hb4 recombinants showed the same growth rates (as measured by increasing turbidity) and final viable cell number as the M13mp8-infected controls (results not shown). The only noticeable phenotypic trait conferred by the AdoMetase gene upon the cells was a tendency for them to be somewhat elongate and occasionally filamentous during early-log-phase growth. This tendency was more pronounced when AdoMetase activity was at its highest, i.e., in M13hb4- or IPTG-induced M13hb4-infected bacteria (Table 2).

To determine whether AdoMetase might be useful in inhibiting AdoMet-mediated processes, we examined the effect of the expression of the AdoMetase gene on in vivo DNA methylation and polyamine biosynthesis.

TABLE 2. T3 M13 subclone enzyme activities

Subclone	β-Galactosidase activity[a]			AdoMetase activity[b]		
	−IPTG	+IPTG	+/− ratio[c]	−IPTG	+IPTG	+/− ratio[c]
M13mp8	6.8	447	66	0	0	
M13ab6	3.8	89	23	0	0	
M13ab9	<1	<1		0	0	
M13ac1	11	85	7.7	0	0	
M13ac3	<1	<1		0	0	
M13hb1	<1	<1		8.0	12	1.5
M13hb4	<1	<1		13	8.4	0.65

[a] The β-galactosidase assay and units are those of Miller (31).
[b] The AdoMetase assay is described in Materials and Methods, and the units are expressed in picomoles of AdoMet hydrolyzed per minute per milligram of protein added to the reaction mixture.
[c] The +/− ratio was determined by dividing the activity of induced cells by that of the uninduced cultures.

TABLE 3. Identity of AdoMetase reaction products

Reaction components	R_f[a]	% Total cpm	Deduced identity
[$^{14}CH_3$]AdoMet + T3-AdoMetase	0.08	7.3	AdoMet
	0.67	36.7	MTA
	0.75	56.0	MTR
[$^{14}CH_3$]AdoMet + M13hb4 AdoMetase	0.08	3.2	AdoMet
	0.68	33.4	MTA
	0.75	63.4	MTR
[^{14}COOH]AdoMet + T3-AdoMetase	0.08	2.2	Adomet
	0.20	97.8	Homoserine
[^{14}COOH]AdoMet + M13hb4-AdoMetase	0.08	2.8	AdoMet
	0.20	97.2	Homoserine

[a] Standard R_f values: AdoMet, 0.08; MTA, 0.68; MTR, 0.75; homoserine, 0.20.

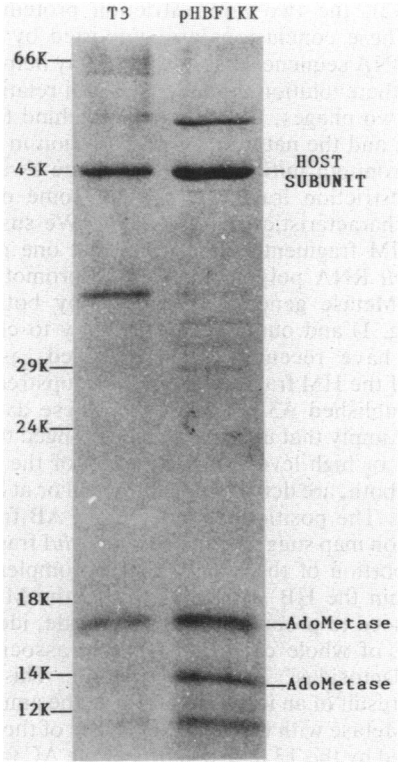

FIG. 2. Visualization of the T3 and pHBF1KK AdoMetase proteins. AdoMetase enzymes generated by T3 or the pHBF1KK plasmid were purified as described in Materials and Methods, and the *S*-adenosylhomocysteine–agarose eluates were analyzed by SDS-PAGE. Linear gradient (10 to 18%) gels were stained with Coomassie brilliant blue to reveal the similar protein patterns between the T3 (lane 1)- and pHBF1KK (lane 2)-derived samples. The numbers on the left side of the gels mark the positions (in kilodaltons) of a variety of protein standards, and labels on the right identify the AdoMetase protein and 45-kDa host subunit bands among the unidentified proteins.

E. coli K-12 strains (e.g., *E. coli* JM103) have several DNA methylases, the most prominent being the *dam* and *dcm* enzymes, which modify the internal adenosine or cytosine nucleotides in the sequences 5′-GATC-3′ and 5′-CC(A/T)GG-3′, respectively. Several type II restriction enzymes are sensitive to the methylation of these sequences and are commonly used to probe the nature and extent of their modification (48). We incubated M13ab6, M13ac1, and M13hb4 RFI DNA molecules with *Mbo*I, *Dpn*I, *Sau*3AI, *Eco*RII, or *Bst*NI to assay the effect of AdoMetase gene expression on the activities of the *dam* and *dcm* methylases. Figure 3 explains this procedure more fully and demonstrates that M13hb4 DNA is substantially unmethylated at its *dam* sites and hypomethylated at its *dcm* positions, unlike the viral DNA replicated in the absence of AdoMetase.

Finally, the polyamine spermidine is synthesized in *E. coli* via the condensation of putrescine with the aminopropyl group of decarboxylated AdoMet; depleting the cell of AdoMet should inhibit this conversion. We added [1,4-^{14}C]putrescine to log-phase cultures of cells infected with either M13mp8 or M13hb4. Extracts of these cultures were derivatized with dansyl chloride for TLC analysis as described in Materials and Methods. Radio scans of these chromatograms showed only two radioactive peaks, which comigrated with either putrescine or spermidine (results not shown). Quantification of the radioactivity in these spots revealed the conversion of putrescine to spermidine in the M13hb4-infected cells to be 77% inhibited (relative to the M13mp8-infected controls) at 1 h after addition of the [^{14}C]putrescine.

DISCUSSION

Previously published data on the location and size of the coliphage T3 AdoMetase gene and enzyme, together with our restriction map of the 5′-proximal *Mbo*I G fragment of the phage (Fig. 1), enabled us to identify DNA fragments that might encompass the complete AdoMetase gene. M13mp8 derivatives containing the HB fragment produced a demonstrable AdoMetase activity, and comparisons between the M13- and T3-encoded enzymes further confirmed their identity.

The cloning and expression of the T3 AdoMetase gene produced a vector which should help define the effects of depleting AdoMet within cells. The enzyme can be expressed at levels sufficient to inhibit AdoMet-mediated activities (e.g., *dam* methylase function and spermidine biosynthesis) without impairing bacterial growth or M13 replication. Furthermore, the appearance of elongate and occasionally filamentous cells under conditions of enhanced AdoMetase gene expression suggests that AdoMet plays either a direct or indirect role in cell division. This correlation between AdoMetase-induced DNA hypomethylation (Fig. 3) and cell elongation or filamentation may be explained as a consequence of induction of the SOS regulon, as it is known both that *dam* methylase mutants are partially in-

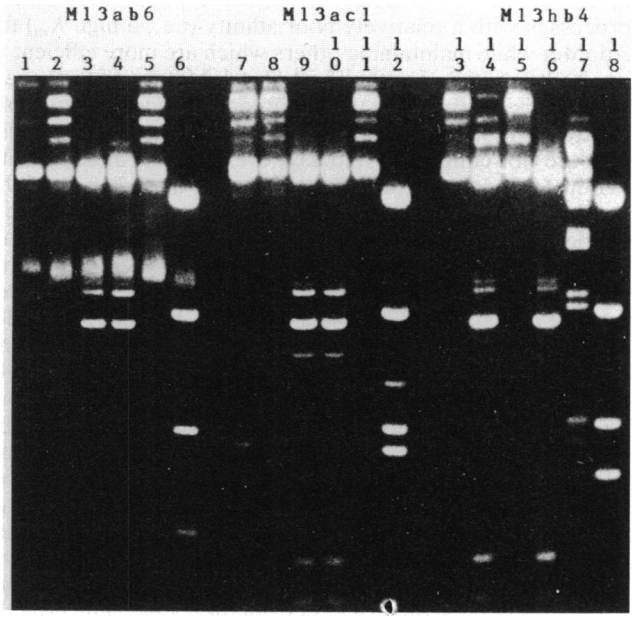

FIG. 3. Effect of AdoMetase gene expression of M13 DNA methylations. Samples were electrophoresed on an agarose gel undigested (lanes 1, 7, and 13) or digested with restriction endonucleases *Mbo*I (lanes 2, 8, and 13), *Dpn*I (lanes 3, 9, and 15), *Sau*3AI (lanes 4, 10, and 16), *Eco*RII (lanes 5, 11, and 17), or *Bst*NI (lanes 6, 12, and 18) to demonstrate the extent of restriction recognition sequence modification in vivo by *dam* or *dcm* methylases. *Mbo*I will not cut at methylated *dam* sites, *Dpn*I requires *dam* methylation, and *Sau*3AI will cut 5′-GATC-3′ sequences with or without modification. Similarly, *Eco*RII will not cut if the *dcm* site is methylated, while *Bst*NI is insensitive to modification of 5′-CC(A/T)GG-3′ sequences.

duced for the SOS response (34) and that induction of the regulon acts partly to inhibit cell division prior to the repair of DNA damage (20). AdoMetase expression vectors may be used to test other aspects of the *dam* phenotype as well, such as hypermutability (27), sensitivity to DNA-damaging agents (26), enhanced expression of genes with *dam* recognition sequences in their promoters (46), and an increased rate of transposon or insertion sequence transposition (39). Of course, *dam* methylation is only one of the many activities using AdoMet; similar studies could conceivably address such topics as RNA and protein methylation, biotin synthesis, and the specific role(s) of spermidine in the cell.

We originally conceived of using the cloned AdoMetase gene as an alternative to using methionine analogs or *metK* mutants to study the effects of AdoMet deprivation in cells. For this purpose, the enzyme ideally should be under some well defined, easily manipulated, and tightly regulated expression control system. Although the M13hb4 phage expresses the enzyme at high levels, its insert orientation suggests promotion from a viral or uncharacterized promoter. On the other hand, while the M13hb1 transcript probably originates (at least in part) from the M13 P_{lac} promoter, its expression is not under firm repressor control (Table 2). We do not know whether this lack of control is due to an unknown promoter on the insert fragment or to some direct or indirect effect of the DNA hypomethylation on the binding of the P_{lac} repressor, but efforts are being made to bring AdoMetase gene expression under tight(er) control to improve its effectiveness as a research tool.

Several experiments may become possible with AdoMetase vectors that more completely regulate the expression of the gene. For instance, it might be possible to inhibit processes with a relatively poor affinity (i.e., a high K_m) for AdoMet while maintaining others which are more efficient in its use by modulating the level of AdoMetase gene expression (Fig. 2; also note the difference in *dcm* versus *dam* methylation on the M13hb4 DNA). AdoMet-mediated activities are not completely blocked even in cells infected with M13hb4, as demonstrated by the partial methylation of the *dcm* sites (Fig. 3) and continued production of small amounts of spermidine. Further (and possibly complete) inhibition of these activities also await the construction of more appropriate vectors. Such vectors may help to determine whether conditions exist by which AdoMet can be completely depleted from the cell and or by which AdoMetase gene expression can be made either conditionally or absolutely lethal. However, until suitable expression vectors are constructed, we can continue to use the M13hb1 and M13hb4 phages to study the response of cells to AdoMet starvation by performing experiments similar to those reported above.

The cloned AdoMetase gene also may help us to better understand the molecular biology of early gene expression and regulation in T3 and the genetic relationship of T3 to the similar, well characterized phage T7. The biochemical, genetic, and DNA sequence data from the T7 0.3 gene product protein, the AdoMetase homolog, show it to be a 13-kDa acidic protein that binds to and inactivates the host type I restriction enzyme (5, 14). The T3 AdoMetase also binds to the type I restriction enzyme but differs in its size, antigenic and chromatographic properties, and active AdoMet hydrolytic activity (44). Spoerel and Herrlich (43) reported the copurification of two T3 AdoMetase proteins differing in molecular weight by roughly 3 kDa, while the T7 gene encodes and produces a single peptide. These observations lead us to suspect that unlike the highly similar T3 and T7 phage RNA polymerases (28) and other regions of the phage genomes (12), the two anti-restriction proteins are quite different. These conclusions are supported by preliminary data from DNA sequencing studies and may help us to better appreciate the evolutionary and structural relationships between the two phages, the mechanism behind their genetic divergence, and the nature of viral evolution in general.

Results from our initial attempts to clone a variety of the early T3 restriction fragments prompt some observations about the characteristics of this region. We suspected that the large HM fragment contains at least one of the three early *E. coli* RNA polymerase-specific promoters and the entire AdoMetase gene, as suggested by both the early reports (Fig. 1) and our repeated inability to clone it. Our suspicions have recently been confirmed, as the *Hpa*I boundary of the HM fragment is 66 bases upstream from the complete published A3 promoter (9). These data therefore presumably imply that uncontrolled, prolonged transcription of the gene or high levels of production of the AdoMetase activity, or both, are detrimental to the cell or at least to M13 metabolism. The position of the AC and AB fragments on the restriction map suggests that the two *Alu*I fragments both contain a portion of the gene which is completely encompassed within the HB segment. Indeed, the M13ac1 clone can be induced to produce a 29-kDa peptide, identifiable by SDS-PAGE of whole cells (not shown), associated with a weak β-galactosidase activity (Table 2). This peptide is probably a result of an in-frame fusion of the amino terminus of the AdoMetase with the β-galactosidase of the M13 vector (as suggested by the T3 map position of the AC fragment, the length of the putative fusion peptide, and the enzyme activity).

Finally, we would like to emphasize the potential for the use of this gene as a research tool in organisms other than *E. coli*. It could be adapted to complement studies involving the use of potentially nonspecific chemical inhibitors of processes involving AdoMet or make unnecessary the often difficult isolation and characterization of mutants in AdoMet biosynthesis or metabolism. If the AdoMetase gene were cloned on proper expression vectors, the roles of AdoMet-related entities (e.g., 5′-methylcytosine residues on DNA and the polyamines spermidine and spermine) in processes such as chemotaxis, cell division, differentiation, transformation, and gene expression could be addressed in such diverse systems as *Bacillus subtilis*, *Saccharomyces cerevisiae*, and cultured mammalian cells.

ACKNOWLEDGMENTS

This work was supported in part by American Cancer Society grant CH-342. J.A.H. was a predoctoral trainee under Public Health Service grant GM07774-06 from the National Institutes of Health.

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