

Cloning and study of new DNA methyltransferase M.AluBI modifying adenine in a recognition site AGCT

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ABSTRACT

A fragment of *Arthrobacter luteus* B DNA carrying the gene of new DNA methyltransferase M.AluBI was cloned and expressed in *Escherichia coli*. The recombinant plasmid pM.AluBI-16 contains the M.AluBI gene (1515 bp in length), corresponding to a protein of 504 amino acid residues. The amino acid sequence analysis showed that M.AluBI could be an adenine-(N6)-DNA methyltransferase. A recombinant strain was grown up and the enzyme was purified by a consecutive chromatography on P-11 Phosphocellulose, Heparin-Sepharose, Sephacryl S-200 and Hydroxyapatite. M.AluBI specificity was determined by the original method based on blocking of restriction endonucleases cleavage of overlapped sites and on computer modeling. It was first shown that AluBI MTase modifies the adenine residue with formation of 5'-(m6A)GCT-3' as opposed to its prototype, M.AluI, producing 5'-AG(m5C)T-3'. A comparative sensitivity analysis of different, well known restriction endonucleases to the methylation by M.AluBI and M.AluI was done using λ and T7 phage DNA. The newly acquired data on methylation sensitivity could be useful for conducting experiments on DNA digestion with restriction endonucleases, and especially with the particular cleavage sensitivity pattern generated with the M.AluBI methyltransferase enzyme.

Keywords: gene cloning, enzyme isolation, bacterial DNA methyltransferase, enzyme specificity, restriction endonuclease, methylation sensitivity

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RESUMEN

Clonaje y estudio de una nueva metiltransferasa de ADN M.AluBI que modifica la adenina en el sitio de reconocimiento AGCT. Se clonó un fragmento de ADN de *Arthrobacter luteus* B que contiene el gen de una nueva metiltransferasa de ADN, y se expresó en *Escherichia coli*. Se obtuvo el plásmido recombinante pM.AluBI-16, que contiene el gen M.AluBI (1515 pb), codificante para una proteína de 504 aminoácidos. El análisis comparativo de la secuencia aminoacídica mostró que M.AluBI pudiera ser una metiltransferasa de ADN adenina-(N6). Se cultivó la cepa recombinante y se purificó la enzima, mediante pasos consecutivos de cromatografía en matrices de Fosfocelulosa P-11, Sefarosa-heparina, Sephacryl S-200 e Hidroxiapatita. La especificidad de M.AluBI se determinó mediante el método original de bloqueo del corte de enzimas de restricción en sitios solapados, y mediante la modelación computacional. Se demostró que la AluBI metiltransferasa modifica el residuo de adenina y genera un producto 5'-(m6A)GCT-3', a diferencia del producto 5'-AG(m5C)T-3' obtenido por la acción de su prototipo M.AluI. Posteriormente se analizó comparativamente la sensibilidad a la metilación por M.AluBI y M.AluI, de diferentes endonucleasas de restricción conocidas en el ADN de los bacteriófagos λ y T7. Los nuevos datos de sensibilidad a la metilación obtenidos pueden ser útiles en experimentos de digestión de ADN con enzimas de restricción, en particular los obtenidos con el patrón de sensibilidad generado por la metiltransferasa M.AluBI.

Palabras clave: clonaje de genes, aislamiento enzymático, metiltransferasa de ADN bacteriana, especificidad enzimática, endonucleasa de restricción, sensibilidad a la metilación

Introduction

DNA methylation is the most common form of DNA modification in prokaryotic and eukaryotic genomes. DNA methyltransferases (MTases) transfer the methyl group from S-adenosyl-L-methionine (SAM) to cytosine or adenine in double-stranded DNA. The modified bases are 5-methylcytosine (5mC or m5C), N4-methylcytosine (N4mC or m4C) or N6-methyladenine (N6mA or m6A). The MTases differ in their recognition sequence and the base to be modified within this site [1].

Most known MTase specificities are exclusively found in bacteria. These enzymes partly belong to restriction-modification (RM) systems. About 350 prototypes of RM systems and thousands of their analogs are known at present time [2]. Each RM system consists of restriction endonuclease (restrictionase) and MTase. Both cognate enzymes recognize the same site. The endonuclease cuts a recognition site thus protecting bacterial

cell from a foreign DNA invasion. MTase methylates the recognition site in host DNA and protects it from cleavage with a cognate endonuclease [3, 4]. Endonucleases of various bacteria recognizing the same site are called isoschizomers. New MTases are a perspective tool for a study of DNA-protein interactions. One of these studies is a determination of endonucleases sensitivity to a new type of methylation.

There is a well known RM system AluI from *Arthrobacter luteus* with a recognition site AGCT. M.AluI modifies this site with formation AG(m5C)T [5]. Recently, we described a new RM system AluBI from *Arthrobacter luteus* B with the same substrate specificity AGCT [6]. RM-systems AluI and AluBI must differ in DNA methylation because AluBI cuts DNA modified by M.AluI [6]. This work was aimed to cloning of M.AluBI and the study of the recombinant enzyme

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properties and substrate specificity. A comparative study of sensitivity was performed of different endonucleases to DNA methylation by M.AluBI and M.AluI.

Materials and methods

Enzymes, DNA and reagents

M.AluI DNA methyltransferase and S-adenosyl-L-methionine (SAM) were from New England Biolabs Inc, USA. Restriction endonucleases, alkaline phosphatase (calf intestinal) and T4 DNA ligase as well as pUC19, phage λ (dam $^+$, dcm $^+$) and T7 DNAs, ATP, reaction buffers were obtained from Sibenzyme Ltd, Russia. Lysozyme was from Helicon, Russia. Bovine serum albumin (fraction V) (BSA) was from Americanbio, USA.

Strains and culture medium

Escherichia coli RR1 (Δ merC-mrr recA $^+$) was from New England Biolabs Inc, USA. Arthrobacter luteus B was from Sibenzyme Ltd, Russia. L-broth contains 10 g/L Tryptone (Organotechnie, France), 5 g/L Yeast extract (Organotechnie, France), 5 g/L NaCl, 0.5 g/L MgCl $_2$, 1 mg/L thiamin. For plates L-broth was supplemented with 15 g/L agar (C.T. Roeper GmbH, Germany). Ampicillin was added up to 100 μ g/mL and streptomycin up to 25 μ g/mL.

Transformation and colony screening procedures

A. luteus B was grown in flasks containing 300 mL L-broth at 30 °C, with 130 rpm for 40 h. Six micrograms of *A. luteus* B DNA, isolated as described [7], was separately digested in 200 μ L of reaction mixtures with 10-20 units of SphI (GCATG C) or BstNSI (RCATG Y) for 1 h at 37 °C. The DNA from each digest was purified by phenol extraction and precipitated with ethanol. Hybrid plasmids of pUC19 were constructed by ligation of 2.5 μ g of SphI-digested *A. luteus* B DNA and 5.0 μ g of BstNSI-digested *A. luteus* B DNA with 3.0 μ g phosphatase-treated SphI-digested pUC19 by using 2000 units of T4 DNA ligase. The reaction was allowed to proceed for 16 h at 4 °C in 20 μ L of 50 mM Tris-HCl, pH 7.8, 10 mM MgCl $_2$, 10 mM DTT, 1 mM ATP. The ligated DNA was precipitated with ethanol and dissolved in 12 μ L of water.

Electroporation was performed in a mixture of 2.5 μ g of ligation product to 50 μ L of approximately 3.0×10^9 RR1 cells, by using an EasyJet Prima electroporator according to the manufacturer's instructions (EquiBio, UK). After electroporation, the mixture was added to 1.0 mL of L-broth and incubated for 1 h at 37 °C. For calculation of transformants, 10 μ L of the culture were plated on L-agar with ampicillin. Eighty colonies grew at 37 °C after 16 h. Thus about 8000 recombinant clones were obtained. The genomic library (1.0 mL) was grown in 100 mL of L-broth with ampicillin for 16 h at 37 °C with shaking.

Total plasmid DNA of 8000 recombinant clones from 100 mL culture was isolated by using the Qiagen Plasmid Maxi Kit (Germany) according to the manufacturer's instructions. Ten micrograms of plasmid DNA preparation was digested with 50 U of AluBI for 4 h at 37 °C, precipitated with ethanol and dissolved in 15 μ L of water. Following the protocols [7], digested DNA was incubated with 3.0×10^7 RR1 cells

that had been treated with CaCl $_2$. Cells were plated on L-agar containing ampicillin and 16 clones were obtained after 16 h at 37 °C. Each clone was grown in L-broth with ampicillin and plasmid DNA was isolated by Qiagen Plasmid Miniprep Kit (Germany). DNAs from 16 clones were cut with AluBI and analyzed by electrophoresis in 1% agarose gel. The 16 DNA preparations were resistant against AluBI digestion and carried an insert about 8 kb in length. One of DNAs was cut with Acc65I, ligated with Acc65I-digested pUC19 DNA and used for RR1 retransformation. The obtained clone was named *E. coli* N16 (pM.AluBI).

DNA sequencing

The sequencing of DNA was carried out with the ABI 3130xI Genetic Analyzer device (Applied Biosystems, USA).

Production and purification of DNA methyltransferase M.AluBI from *Escherichia coli* N16 (pM.AluBI)

E. coli N16 (pM.AluBI) cells were grown till stationary phase in L-broth with ampicillin in a 20-L Vessel (New Brunswick Scientific, USA) at 30 °C with aeration for 8 h. Cells were harvested at $8000 \times g$ and stored at -20 °C.

The enzyme was purified at 4 °C by using Buffer A (10 mM Tris-HCl, pH 7.5; 0.1 mM EDTA, 7 mM β -mercaptoethanol) and Buffer B (10 mM Potassium Phosphate, pH 7.2; 0.1 mM EDTA; 7 mM β -mercaptoethanol). Harvested cells (100 g) were suspended in 500 mL of Buffer A with 0.2 M NaCl, 5 % glycerol, 0.5 % Triton® X-100 (non-ionic detergent, Sigma) and 0.1 mM PMSF (proteases inhibitor). Cell suspensions (100 mL each) were sonicated on Soniprep 150 (MSE, UK) with a nozzle 2-cm in diameter and receiving 5 maximal impulses for 1 min with cooling in ice for 1 min. Cell debris was removed by centrifugation at $20\,000 \times g$ for 1 h. The supernatant was loaded onto a Phosphocellulose P11 (Whatman, UK) of 300 mL bed volume and eluted with 3 L of 0.2 to 0.6 M NaCl gradient in Buffer A for 15 h.

Fractions were assayed for M.AluBI activity as described below. The activity-containing fractions were pooled, dialyzed against 3 L of Buffer A with 0.05 M of NaCl for 4 h and loaded onto a Heparin-Sepharose (Bio-Rad, USA) of 50 mL bed volume column. Protein was eluted with 1 L of a 0.05-0.5 M NaCl gradient in Buffer A. Fractions containing activity were pooled, loaded onto a Sephadryl S-200 (Bio-Rad, USA) of 1 L bed volume column and eluted with 1 L of 0.8 M NaCl in Buffer A. Subsequently, fractions containing activity were loaded onto a Hydroxyapatite (Bio-Rad, USA) of 50 mL bed volume column and eluted with 1 L of a 0.01-0.2 M K-phosphate gradient, pH 7.2 in Buffer B. Finally, fractions containing activity were pooled, concentrated by a dialysis against 1 L of Buffer A with 50 % glycerol, 0.05 M of NaCl and stored at -20 °C.

DNA Methyltransferase activity assay

The assay based on a protection of methylated DNA from cognate restriction endonuclease M.AluBI sample was incubated in 50 μ L with 50 μ g/mL λ DNA (dam $^+$, dcm $^+$), 1 mM SAM, 33 mM Tris-acetate, pH 7.9, 1 mM EDTA, 66 mM potassium acetate 1 mM

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DTT, 0.1 mg/mL BSA in a well of 96 Well Microplate (Medpolymer, Russia) at 37 °C for 1 h. Then, the microplate was warmed up on a bath at 65 °C for 10 min to inactivate the enzyme. To cleave not modified DNA, the mixture was supplemented with 5 µL of 1000 U/mL AluBI restriction endonuclease diluted with 10 mM Tris-HCl pH 7.6, 50 mM KCl, 0.1 mM EDTA, 200 µg/mL BSA, 1 mM DTT and 100 mM magnesium acetate. After incubation at 37 °C for 1 h, the mixture was analyzed by electrophoresis in 0.8 % agarose gel as described [7]. One unit of *M. AluBI* activity methylated 1 µg of λ DNA in 1 h blocking the activity of AluBI restriction endonuclease.

DNA methylation with *M. AluBI* and *M. AluI*

The reaction mixture was prepared in a 2 mL Eppendorf tube, containing 0.4 mg/mL λ or T7 DNA, 10 mM SAM, 33 mM Tris-acetate pH 7.9, 1 mM EDTA, 66 mM potassium acetate 1 mM DTT, 0.1 mg/mL BSA and 100 U/mL *M. AluBI* or *M. AluI* enzyme, in a total volume of 1.0 mL, and incubated at 37 °C for 20 h. Then, 0.1 mL of 10 % SDS (Sigma) and 0.2 mL of 3 M KCl were added. The mixture was warmed at 65 °C for 10 min to dissolve SDS-precipitate and cooled at 0 °C for 15 min for precipitate formation. The supernatant, pooled after centrifugation at 10 000 \times g for 3 min, was mixed with 0.6 volume of isopropanol. DNA pellet was rinsed with 80 % ethanol, dried on air and dissolved in 0.7 mL of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) to the 0.5 mg/mL concentration.

DNA cleavage with restriction endonucleases and electrophoresis in agarose gel

Native or *M. AluBI*- and *M. AluI*-methylated λ or T7 DNA were cleaved in 50 µL of the reaction mixture containing 50 µg/mL DNA, 0.1 mg/mL BSA, appropriate SE buffer, 125 U/mL restriction endonuclease in a well of 96 Well Microplate, incubated for 2 h at the temperature recommended by the manufacturer (Sibenzyme Ltd). The reaction was stopped by addition 10 µL of 0.25 M Na-EDTA, pH 8.5, 50 % sucrose and 0.5 % bromphenol blue. The Microplate was warmed up on a bath at 65 °C for 10 min and cooled on ice. The electrophoresis was carried out in 0.8 % of LE agarose (Selegenic) in TAE buffer with 0.5 µg/mL ethidium bromide (Sigma) at 5 V/cm for 2.5 h as described [7]. The fluorescence of DNA was revealed on the Herolab GmbH device.

Determination of DNA methyltransferase specificity

M. AluBI recognition sequence and its methylated basis were defined using methylation sensitivity of restriction endonucleases [2] according to Dedkov [8]. As described above T7 DNA, as native and *M. AluBI*-methylated, were cut by restriction endonucleases *AluI* (AG[^]CT), *AluBI* (AG[^]CT), *Tru9I* (T[^]TAA), *Fsp4HI* (GC[^]NGC), *BstC8I* (GCN[^]NGC) and *Bst2BI* (CACGAG(-5/-1)). Then, DNA fragments were analyzed by electrophoresis in 0.8 % agarose. Beforehand, blocking of restriction endonucleases by the methylation was modeled to reveal an overlapping of the methylation and the cleavage. Methylation of (mA)GCT was simulated as editing T7 DNA sequences by replacements AGCT with NGCN and designated

(A=>N)GC(T=>N). Then, simulated cleavage of native and edited DNA by restriction endonucleases and electrophoresis was performed with Vector NTI program. In the program, a restriction endonuclease didn't cut the recognition site if any of bases was replaced by N. Thus the methylated site was examined by cleavage with restriction endonucleases. *M. AluBI* specificity was determined by the analysis of experimental and simulated results.

Results and discussion

Selection of clones carrying the *M. AluBI* and DNA sequencing

MTase clones were selected by the resistance of recombinant DNA to the cognate restriction endonuclease according to [9]. A genomic library of *A. luteus* B DNA was obtained in pUC19/Sph I vector DNA as 8000 *E. coli* RR1 transformants. A total DNA of hybrid plasmids was digested with AluBI and used for additional RR1 transformation. Plasmid DNAs from 16 clones were cut with AluBI and analyzed by electrophoresis in 1 % agarose gel. All these plasmids were protected from AluBI restriction endonuclease and contained ~8.0-kb insertions of *A. luteus* B DNA including the *M. AluBI* gene. Then 8.0-kb insertion was cut with Acc65I and 2101 bp DNA fragment with *M. AluBI* gene was subcloned into pUC19/Acc65I vector DNA. The obtained clone was named *E. coli* N16 (pM. AluBI). Final plasmid DNA, pM. AluBI-16, was sequenced. A restriction map of this recombinant plasmid with ORF of *M. AluBI* gene is shown in Figure 1.

The Acc65I-fragment of *A. luteus* B DNA cloned in pUC19 contained 2101 base pairs with a single open reading frame (ORF) corresponding to *M. AluBI* of 504 amino acids (in single-letter): MTFTTSWRA-RIFEEAELLPTDLISDLSIGEVSVLYEYTHAIA-DADARKTNGQFFTPDDVASFMAGFADRFPR-GRWLDPCAGIGNLSWHLIASQEDPEEFLL-ERMLLSDTDELALLVARALLTAQRRHLNLF-HEIEENFVVFDFLSVSDSGTSKIGALAAIPSHD-FVIVNPPYLATKGRDSRFETAESSDLYAYFLEN-VIKTSRGFISVTPQSFTNAKKFMGLRSLLR-SYSNLEILCFDNIPGNLFRGVKGSRNSNTANS-IRAAITVALPQPGVPSITSMLRWKGTERQRLF-AEVERFKSHVPLTAEFPKVSTVFEDLYRWT-VELPRLGSLCQRTETEPLHVPSAPRYFIPALK-SPVARSSQRTLYFPNSAARDRAYLLINSSLMY-WWWVRDGGMTLSQETLLSMPPLPDFAVRD-DLVTRELSEETNKVYKLNAGVSQENVKH-SPELIADLNAHVPIHAAPLLTHLNSEFAQFEY-LGRADDAASSARSAIVANGAS.

The nucleotide and amino acid sequences of *M. AluBI* were deposited in NCBI GenBank (<http://www.ncbi.nlm.nih.gov/nuccore/LN869917.1>). With the aid of Protein Blast (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) the putative conserved motifs of *M. AluBI* were detected in AdoMet_MTases superfamily with N6_MTase domain hit. This result confirms the data obtained before [6] and shows that *M. AluBI* belongs to amino-MTases and modifies the site differently than *M. AluI* (AG(m5C)T) [5]. The structure of MTases is characterized by 9 amino-acid motifs [10]. The main motifs are motif I (F-G-G) for binding SAM

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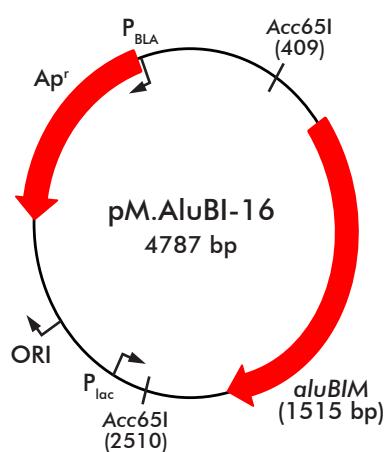


Figure 1. Restriction map of pM.AluBI-16 plasmid DNA. Acc65I, ligation site (bp); 409-2510 bp, *A. luteus* B DNA; 2510-409 bp, pUC19 DNA; ORI, replication start; P_{BLA}, β -lactamase promoter; Ap', ampicillin resistance; P_{lac}, β -galactosidase promoter; aluBIM, gene of the DNA methyltransferase M.AluBI. The map was simulated with Vector NTI program.

and motif IV (earlier – II) for catalysis: (P/F)PC in cytosine (C5) - MTases (C-MTases) and PPY in adenine (N6) - and cytosine (N4) amino MTases. Thus, as the structure of MTases which form m4C and m6A is very similar, a purification of the enzyme is necessary to determine M.AluBI specificity.

M.AluBI purification and characterization

To isolate M.AluBI enzyme preparation, *E.coli* N16 (pM.AluBI) cells were grown in L-broth with ampicillin. The enzyme was purified from cellular extract by subsequent chromatography on Phosphocellulose P11, Heparin-Sepharose, Sephadryl S-200 and Hydroxyapatite as described at Materials and methods. The traditional chromatography sequence allowed to obtain enzymatic-pure preparation of M.AluBI with activity 10 U/ μ L, which was suitable for works in molecular biology. Sufficient ionic strength and Phosphocellulose P11 were used to dissociate nucleoprotein complexes and adsorb many DNA-specific enzymes on this resin. The majority of cellular proteins wasn't adsorbed, it allowed to use rather small column already in the first chromatography.

The substrate specificity of M.AluBI was confirmed by an inability of restriction endonucleases to hydrolyze some sites and a computer simulation of this hydrolysis as described earlier [8] and indicated in Materials and methods. Figure 2 shows that the methylation of T7 DNA with M.AluBI blocks DNA hydrolysis with AluI as well as AluBI restriction endonucleases (AGCT). Moreover, M.AluBI blocked a digestion with Tru9I in the case of overlapping with an external adenine in the site TTAA. Thus M.AluBI modifies DNA producing (m6A)GCT. At the same time AluBI-methylation didn't block Fsp4HI (GC^NGC) and BstC8I (GCN^NGC) which are sensitive to m5C [11, 12]. As shown in Figure 2, Bst2BI is sensitive to the methylation with M.AluBI and doesn't cleave site CACG(m6A)G.

Thus, the results show that M.AluBI produces 5'-(m6A)GCT-3' and belongs to adenine-(N6)-DNA

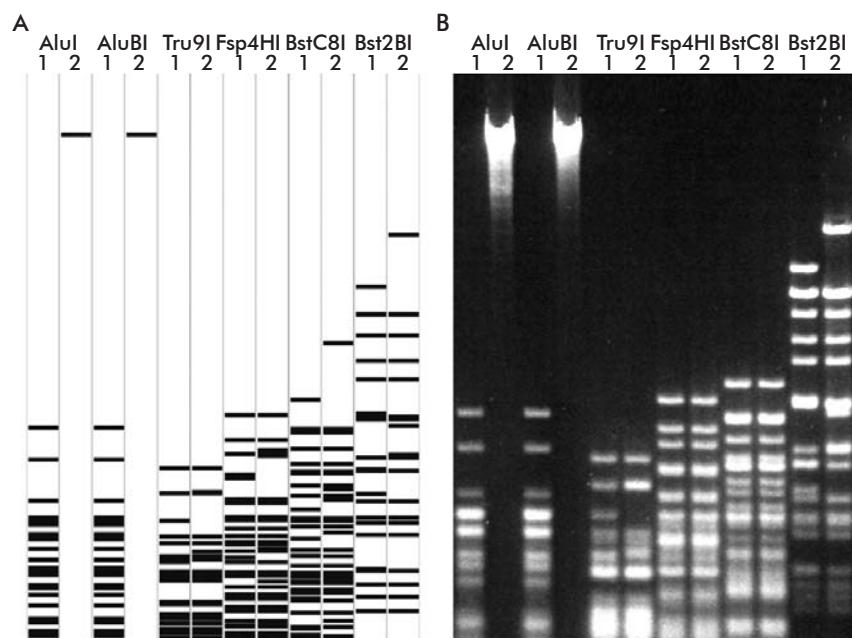


Figure 2. Determination of M.AluBI-methylation specificity. A) Computer simulation, T7 DNA edited (A=>N)GC(T=>N) with the Vector NTI program. B) Blocking of restriction endonucleases activity by DNA methylase M.AluBI. Lanes: 1, T7 DNA; 2, T7 DNA methylated with M.AluBI. DNA was digested with AluI (AG^NCT), AluBI (AG^NCT), Tru9I (T^NAA), Fsp4HI (GC^NGC), BstC8I (GCN^NGC) and Bst2BI (CACGAG(-5/-1)), followed by electrophoresis in 0.8 % agarose gels.

methyltransferases (EC 2.1.1.72) [1]. M.AluBI possesses a new specificity among known analogs [2] and may be used in genetic engineering and molecular biology.

Methylation sensitivity of restriction endonucleases

An essential characteristic of restriction endonucleases is methylation sensitivity of these enzymes. A number of endonucleases were tested for methylation sensitivity with λ and T7 DNAs methylated by M.AluBI or M.AluI with formation of (m6A)GCT or AG(m5C)T, respectively. M.AluBI differs from the similar enzymes such as M.AluI forming AG(m5C)T [5], or M.Mma5219II [2] and M.EsABCII [13] which form AG(m4C)T-3'. The model and experimental results are shown in Figure 3 and Figure 4 and are interpreted in the Table. In some cases, the methylation of particular sequences was simulated as described [12].

Some results shown in the Table confirm known data about methylation sensitivity of restriction endonucleases [2]. Other results were obtained for the first time (* noted cleavage %). Methylation sensitivity is one of characteristics of restriction endonucleases [2]. In particular, this property is used in studying cancer. The obtained data expand possibilities of restriction endonucleases for studying DNA methylation.

Conclusions

The gene of a new DNA methyltransferase AluBI from bacterial strain Arthrobacter luteus B was cloned in *E. coli*. A recombinant M.AluBI enzyme preparation was isolated and it was shown that this MTase modified the adenine residue in the recognition sequence AGCT producing 5'-(m6A)GCT-3'.

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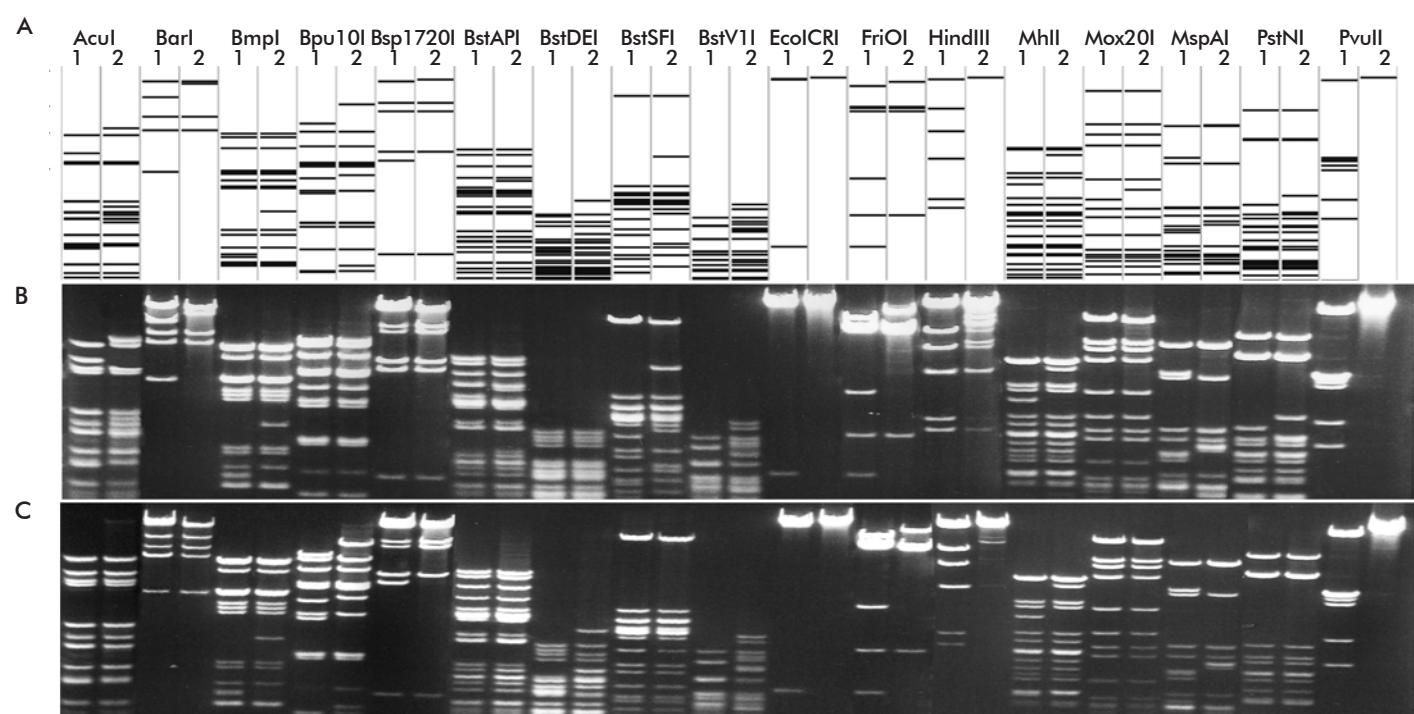


Figure 3. Simulated and experimental cleavage of native and methylated λ DNA with restriction endonucleases. A) Sites of the DNA sequence edited as (A=>N)GC(T=>N) with the Vector NTI program. B) DNA methylated with M.AluBl. C) DNA methylated with M.AluI. Lanes: 1, native nucleotide sequence and DNA; 2, restricted DNA. Cleavage was performed with endonuclease activity sufficient for 5-fold excess of DNA. DNA bands in 0.8 % agarose were visualized by fluorescence with ethidium bromide.

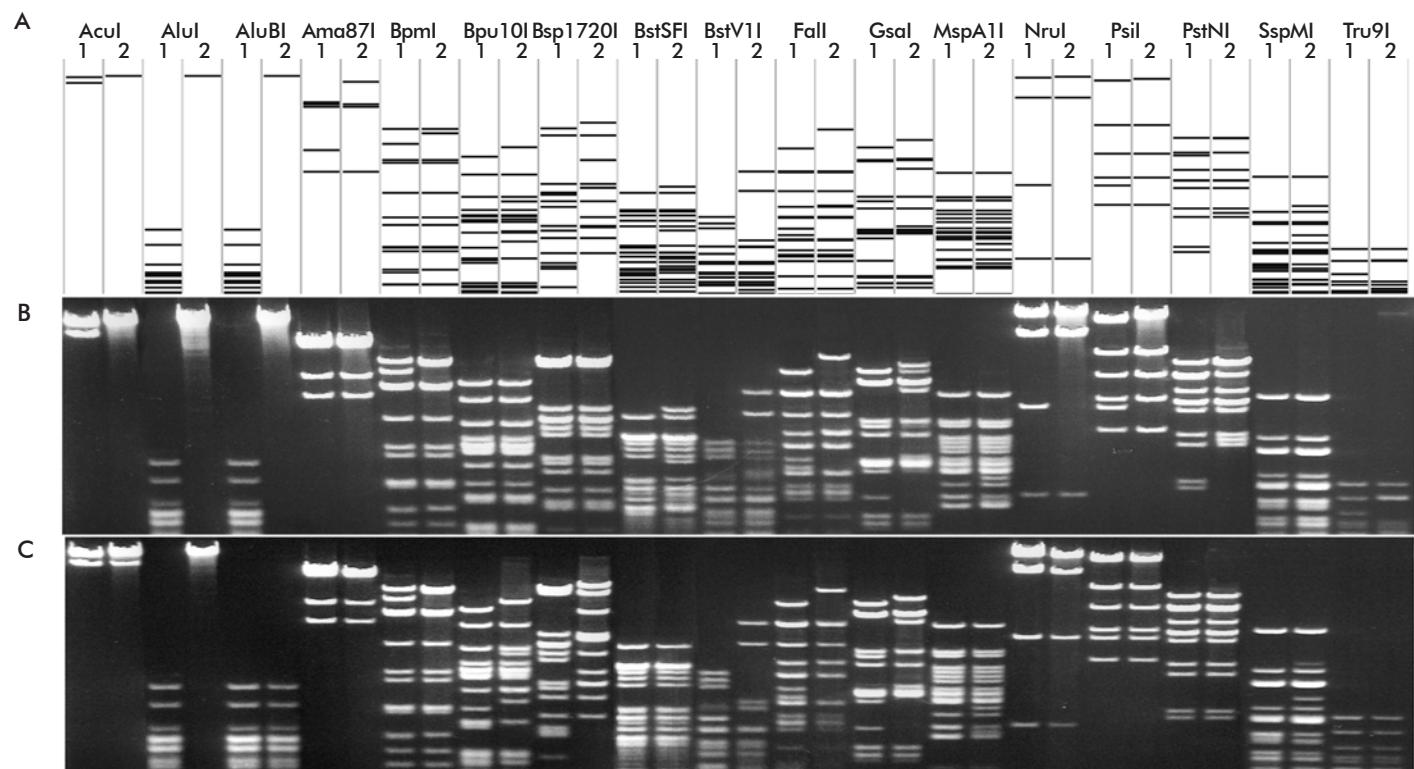


Figure 4. Simulated and experimental cleavage of native and methylated T7 DNA with restriction endonucleases. A) Sites of the DNA sequence edited as (A=>N)GC(T=>N) with the Vector NTI program. B) DNA methylated with M.AluBl. C) DNA methylated with M.AluI. Lanes: 1, native nucleotide sequence and DNA; 2, restricted DNA. Cleavage was performed with endonuclease activity sufficient for 5-fold excess of DNA. DNA bands in 0.8 % agarose were visualized by fluorescence with ethidium bromide.

Table. Methylation sensitivity of some restriction endonucleases^a

λ phage DNA			T7 phage DNA			λ and T7 phage DNA		
Restrictase	Methylated site (5'-3'/3'-5')	Methylase cleavage (%) M.AluBI M.AluI	Restrictase	Methylated site (5'-3'/3'-5')	Methylase cleavage (%) M.AluBI M.AluI	Restrictase	Methylated site (5'-3'/3'-5')	Methylase cleavage (%) M.AluBI M.AluI
Acul	CTGAAG GACTT(m5C)	100	Acul	(m5C)TGAAG GACTTC	100*	Bpml	(m5C)TGGAG GACCTC	0*
Acul	CTGA(m6A)G GACTTC	0*	Acul	CTGAAG G(m6A)CTTC	0*	Bpml	CTGGAG G(m6A)CCTC	0*
Barl	GAAGNNNNNNNTAC CTCNNNNNNN(m6A)TG	0*	AluI	AG(m5C)T T(m5C)GA	0	Bpu10I	CCTNAG(m5C) GGANT(m5C)G	0*
BstAPI	GC(m6A)NNNNNTGC CGTNNNNNACG	0	AluI	(m6A)GCT TCG(m6A)	0	Bpu10I	CCTN(m6A)GC GGANTCG	100
BstDEI	(m5C)TNAG GANTC	0*	AluBI	AG(m5C)T T(m5C)GA	100	Bsp1720I	GCTNAG(m5C) CGANT(m5C)G	0*
BstDEI	CTN(m6A)G GANTC	100*	AluBI	(m6A)GCT TCG(m6A)	0	Bsp1720I	GCTN(m6A)GC CGANTCG	100*
FriOI	GRG(m5C)YC CY(m5C)GRG	0*	Ama87I	(m5C)YCGRG GRGCGC	100*	BstSFI	(m5C)TRYAG GAYRTC	100*
FriOI	G(m6A)GCTC CTCG(m6A)G	0*	Ama87I	CYCG(m6A)G GRGCGC	100*	BstSFI	CTRY(m6A)G GAYRTC	0*
HindIII	AAG(m5C)TT TT(m5C)GAA	5	Fall	AAGNNNNN(m5C)TT TTCNNNNNGAA	0*	BstV1I	GCAG(m5C) CGT(m5C)G	0*
HindIII	A(m6A)GCTT TTCG(m6A)A	20	Fall	A(m6A)GNNNNNCTT TTCNNNNNGAA	0*	BstV1I	GC(m6A)GC CGTCG	20*
MhII	GDG(m5C)HC CH(m5C)GDG	0	Gsal	CCCAG(m5C) GGGT(m5C)G	0*	EcoICRI	GAG(m5C)TC CT(m5C)GAG	0*
MhII	G(m6A)GCTC CTCG(m6A)G	0*	Gsal	CCC(m6A)GC GGGTGCG	10*	MspA1I	CMG(m5C)KG GK(m5C)GMC	0*
Msp20I	TGGCC(m6A) ACCGGT	100*	NruI	TCGCG(m6A) AGCGCT	0	MspA1I	C(m6A)GCTG GTCG(m6A)C	0
Pvull	CAG(m5C)TG GT(m5C)GAC	0	Psil	TTATA(m6A) AATATT	0	PstNI	CAGNNN(m5C)TG GTCNNNGAC	100*
Pvull	C(m6A)GCTG GTCG(m6A)C	0	SspMI	(m5C)TAG GATC	20*	PstNI	C(m6A)GNNNCTG GTCNNNGAC	0*
			SspMI	CT(m6A)G GATC	100*			
			Tru9I	TTA(m6A) AATT	0*			

^aThe sensitivity of the endonucleases (restrictases) was tested by cleavage of λ and T7 DNA methylated by M.AluBI ((m6A)GCT) or M.AluI (AG(m5C)T). Single letter code: R = A or G, Y = T or C, M = A or C, K = G or T, W = A or T, S = G or C, D = A or G or T (not C), H = A or C or T (not G), B = C or G or T (not A), V = A or C or G (not T), N = A or C or G or T.

* Data were obtained for the first time.

M.AluBI differs from the similar enzymes such as *M.AluI* forming AG(m5C)T or *M.Mma5219II* and *M.EsaBC1I* [13] which form AG(m4C)T-3'. The new data on sensitivity of different restriction endonucleases to *M.AluBI*- and *M.AluI*-methylation have been obtained.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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