

Adenine Methylation of Okazaki Fragments in *Escherichia coli*

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In *Escherichia coli* *polA lig-4* bacteria, the moles percent 6-methyladenine content of 10S deoxyribonucleic acid (Okazaki fragments) is 0.96 compared with 1.4 for bulk deoxyribonucleic acid.

Deoxyribonucleic acid (DNA) from *Escherichia coli* contains 1.4 to 2.0 *N*⁶-methyladenine (MeAde) residues per 100 adenine residues (4). The MeAde moieties are the result of methylation by DNA adenine methylases that transfer the methyl group from *S*-adenosyl-*L*-methionine to specific adenine residues in polymerized DNA (5-7).

It has now been established that DNA synthesis in *E. coli* is discontinuous; small DNA pieces (Okazaki fragments) are synthesized and are subsequently joined to yield high-molecular-weight DNA (13). This poses the question as to whether adenine methylation occurs before or after ligation of the Okazaki fragments.

During a brief exposure to radioactive adenine, not enough label is incorporated into 10S DNA of normal or adenine-requiring strains to allow the determination of the moles percent MeAde. To circumvent this problem, a *polA1 lig-4* strain of *E. coli*, which accumulates 10S fragments at the nonpermissive temperature, was used.

Strain N1662 (*lig-4 polA1 rif strA thi*) (6) was cultivated in minimal salts medium (3) supplemented with 1% Difco vitamin-free, decolorized Casamino Acids, 0.2% glucose, 0.16 μ g of thiamine per ml, and 7.5×10^{-5} M [¹⁴C]adenine (specific activity, 2.5 mCi/mmol) at 30°C for three generations. At a cell density of 2×10^8 to 3×10^8 cells per ml, the culture was harvested by centrifugation, washed twice, and incubated in fresh, non-radioactive medium at 30°C for 10 min to exhaust the pool of [¹⁴C]adenine. The culture was shifted to 42°C, and 10 min later either 2×10^{-7} M [³H]thymidine (specific activity, 50 Ci/mmol) or 1×10^{-6} M [³H]adenine (specific activity, 32 Ci/mmol) was added. Two minutes later incorporation of label was stopped by the addition of an equal volume of 75% ethanol-2% phenol-21 mM sodium acetate (pH 5.3)-2 mM ethylenediaminetetraacetic acid (EDTA) (14). The cells were harvested by centrifugation, washed once, and lysed with lysozyme-EDTA-Sarkosyl (10). Por-

tions of the lysate, together with radioactively labeled bacteriophage fd DNA, were sedimented in linear 5 to 20% alkaline sucrose gradients as described previously (10).

The results of a typical experiment are shown in Fig. 1. A peak of ³H-labeled DNA that was found sedimenting at approximately 10S was arbitrarily designated as Okazaki pieces. This material is not the result of DNA degradation since no ¹⁴C label is present in this region. If pulse-labeled cells are shifted back to the permissive temperature (30°C), the label is eventually chased into high-molecular-weight DNA. Furthermore, if wild-type *E. coli* are pulse-labeled with [³H]thymidine for 15 s at 20°C, the pulse label is incorporated into DNA sedimenting at 10S.

The 10S pulse-labeled material (Fig. 1) was isolated from the alkaline gradient, dialyzed against 0.01 M tris(hydroxymethyl)amino-methane (pH 7.8)-0.001 M EDTA for 72 h, mixed with ¹⁴C-labeled native DNA, and tested for sensitivity to various nucleases (2, 9). No trichloroacetic acid-soluble material was detected after ribonuclease treatment. Nuclease S1 treatment resulted in a preferential solubilization of ³H counts, whereas incubation with pancreatic deoxyribonuclease resulted in solubilization of ¹⁴C radioactivity.

The moles percent 6-methyladenine was determined as described previously (11), and Table 1 shows that the 10S pulse-labeled DNA contains 0.96 mol% MeAde, whereas bulk DNA contains 1.4 mol% MeAde. In other experiments, values of 0.94 and 0.98 mol% MeAde in the 10S fraction were obtained. In three similar experiments with a *lig-7* mutant of *E. coli*, the average moles percent MeAde in the 10S fraction was 0.96.

It is clear from these data that some methylation of Okazaki pieces in *E. coli* *polA1 lig-4* can occur before ligation. This should be compared to the situation in eukaryotes where newly synthesized DNA is not methylated (1). If methylation of Okazaki fragments also occurs in wild-

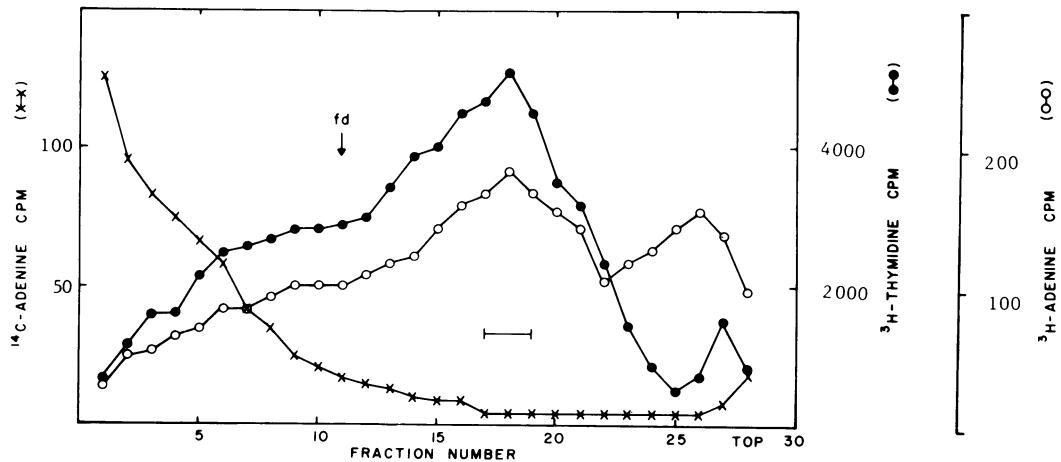


FIG. 1. Sedimentation of DNA in alkaline sucrose. Strain N1662 was grown for three generations with [^{14}C]adenine at the permissive temperature and then pulsed with either [^3H]thymidine or [^3H]adenine for 2 min at the nonpermissive temperature. The cells were harvested and lysed, and portions of the lysate, together with radioactive bacteriophage fd DNA, were sedimented in alkaline sucrose at 24,000 rpm in an SW27 rotor at 20°C for 16 h. The gradient was fractionated, and a 25- μl volume from each fraction was placed on a filter paper disk. The disks were washed three times with 5% trichloroacetic acid, once with ethanol, and once with acetone before being counted. Each wash lasted 10 min. For simplicity, the two gradients are shown superimposed. The direction of sedimentation is from right to left. The bar represents the region of 10S pulse-labeled DNA.

TABLE 1. Moles percent 6-methyladenine in DNA^a

Source of DNA	cpm		mol% ^b
	Adenine	6-Methyladenine	
Bulk DNA, N1662 grown at 30°C	711,091	10,390	1.4
Pulse-labeled DNA, 10S	12,643	120	0.96

^a Bulk DNA was isolated (12) from strain N1662 grown at 30°C with [^3H]adenine for three generations. The DNA was hydrolyzed in 1 M HCl at 100°C for 60 min, and the hydrolysate was subjected to thin-layer chromatography (9). Authentic MeAde, adenine, 2-methyladenine, and *N*⁶-dimethyladenine were included as standards. Counts per minute were calculated as gross counts per minute minus the average counts per minute in areas of the chromatogram devoid of ultraviolet-absorbing material.

^b Moles percent refers to the percentage of adenine bases that are methylated.

type *E. coli*, then the DNA adenine methylases may be part of a "replication complex" and may play a role in DNA synthesis.

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