METHYLATION OF RNA IN BACTERIOPHAGE T_4 INFECTED ESCHERICHIA COLI

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Methylated bases have been detected in Escherichia coli DNA and in both soluble and ribosomal RNA (Dunn and Smith, 1958; Dunn, Smith, and Spahr, 1960; Starr and Fefferman, 1964). Their presence in messenger RNA has not been established. However, none have been detected in viral RNA from TMV (Littlefield and Dunn, 1958) or from coliphage f-2 (Moore, 1966).

The methylation of nucleic acids was shown to occur at the polynucleotide level (Fleissner and Borek, 1962) by the action of methylases with S-adenosyl-methionine as donor (Gold and Hurwitz, 1964; Hurwitz, Gold, and Anders, 1964; Hurwitz, Anders, Gold, and Smith, 1965). Although the biosynthesis is understood, the function of the methylated bases is obscure.

This report deals with the in vivo methylation of RNA in bacteriophage T_4 infected E. coli and shows the incorporation of the methyl group of L-methionine into ribosylthymine of RNA. Most of the methylated RNA sediments at 4s in a sucrose gradient.

Materials and Methods

Escherichia coli, strain B, was grown on a gyrorotary shaker at 32 C in C medium containing 0.4% glucose (Roberts, Abelson, Cowie, Bolton, and Britten, 1957). An exponentially growing culture was harvested at 5×10^8 cells/ml and the cells resuspended in one-tenth the original volume of C medium minus glucose but supplemented with 20 μg/ml L-tryptophan. Wild type bacteriophage T_4 was added at a multiplicity of 5 to 10. After 3 minutes for adsorption, the culture was diluted ten-fold in C medium with 0.4% glucose and shaken gently.

At the time of dilution ($t = 0$), a sample was transferred to an excess of T_4 antiserum and assayed for surviving cells (Adams, 1959). The surviving fraction was between 10^{-4} and 10^{-3}. At appropriate time intervals, (^{3}H methyl) L-methionine (Nuclear Chicago, 4.33 c/m mole) was added at 10 μc/ml, and later, incorporation stopped by pouring the culture onto ice held at -20 C. The cells were pelleted and frozen by dipping in a bath at -40 C. Uninfected E. coli cells were treated in an analogous manner.

The SDS-phenol method (Asano, 1965) was used to purify RNA from either the frozen cell pellet or the 150 K supernatant fraction prepared by grinding the frozen cell pellet with glass beads and centrifuging the extract at 150,000 x g for 60 minutes. Except for the samples that were used in the sucrose gradient centrifugation experiment, RNA was treated with 0.50 M Tris-HCl buffer (pH 9.0) for 30 minutes at 37 C to discharge (^{3}H methyl) methionyl-sRNA and further purified by two additional ethanol precipitation steps. RNA concentrations were calculated from the absorbancy at 260 mμ (extinction of 20 cm^2/mg) and radioactivity by liquid scintillation counting of a trichloroacetic acid precipitate collected on membrane filters.

Zone centrifugation (Britten and Roberts, 1960) was performed using a 5 to 20% linear sucrose gradient in 0.01 M acetate buffer (pH 5.0) with 0.05 M NaCl. Centrifugation was at 39,000 rev./min in the SW-39 swinging bucket rotor for 5 1/2 hr. Each fraction obtained from the gradient was treated to discharge (^{3}H methyl) methionyl s-RNA, then analyzed for absorbancy at 260 mμ and trichloroacetic acid insoluble radioactivity.

Results and Discussion

Characterization of (^{3}H methyl) RNA. The specific activity of (^{3}H methyl) RNA from bacteriophage T_4 infected Escherichia coli which had been fed (^{3}H methyl) L-methionine from the fifth to the eighth minute after infection was 39 counts/min/μg. The specific activity of (^{3}H methyl) RNA from uninfected E. coli which had been treated in an analogous manner was 338 counts/min/μg. All of the radioactivity in the two RNA preparations was insoluble in 10%

cold trichloroacetic acid, but rendered trichloroacetic acid soluble by treatment with 0.3N KOH, by RNase, but not by DNase.

Sucrose gradient sedimentation profiles of the two RNA preparations are given in Fig. 1. Approximately 90% of the (^{3}H methyl) RNA from phage T_4

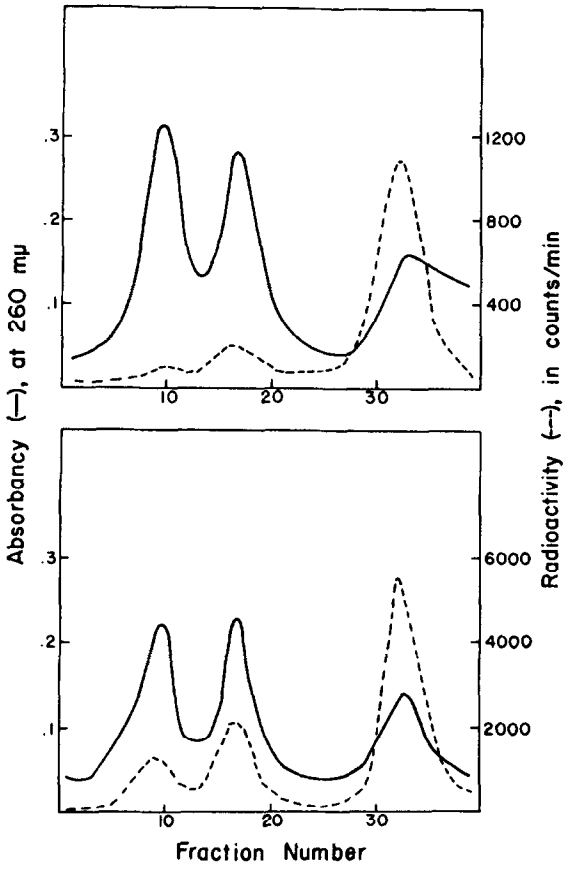

Figure 1 Sucrose gradient centrifugation profile of RNA from T_4 infected E. coli (upper frame) and from uninfected E. coli (lower frame). RNA (from left to right) sedimented at 23s, 16s, and 4s.

infected E. coli sedimented at 4s. The specific activity and relative specific activity of 4s, 16s, and 23s RNA for the two preparations are given in Table 1.

Sucrose gradient sedimentation profiles similar to that presented in Fig. 1 (upper frame) were observed for (^{3}H methyl) RNA from phage T_4 infected E. coli which had been fed (^{3}H methyl) L-methionine from the fifteenth to the

Table 1

Species of RNA	Specific Activity counts/min/μg	Relative Specific Activity
RNA from T_4 Infected *E. coli*		
4s	113	1.00
16s	10	0.09
23s	5	0.04
RNA from Uninfected *E. coli*		
4s	585	1.00
16s	163	0.28
23s	101	0.17

eighteenth minute after infection or for one minute rather than three minutes.

Identification of ribosylthymine as a product of the methylation reaction. RNA was prepared from the 150 K supernatant fraction of phage T_4 infected *E. coli* which had been fed (^{3}H methyl) L-methionine from the fourth to the tenth minute after infection, and from uninfected *E. coli*. A sample of each RNA was hydrolyzed with 70% $HClO_4$ for 60 minutes at 100 C and the resulting heterocyclic bases analyzed by paper chromatography using the isopropanol-HCl-H_2O solvent (Fig.2). The distribution of radioactivity on the chromatograms was different for the two samples. The radioactive material (R_f = 0.80) which moved faster than uracil co-chromatograms with thymine in this solvent system. No attempt was made to identify the other radioactive components present on the chromatograms.

Another sample of RNA from phage T_4 infected *E. coli* was hydrolyzed with 0.3N KOH for 18 hr at 37 C. After neutralization with Dowex-50 (H^+), the sample was fractionated by paper electrophoresis at pH 3.5. Three broad

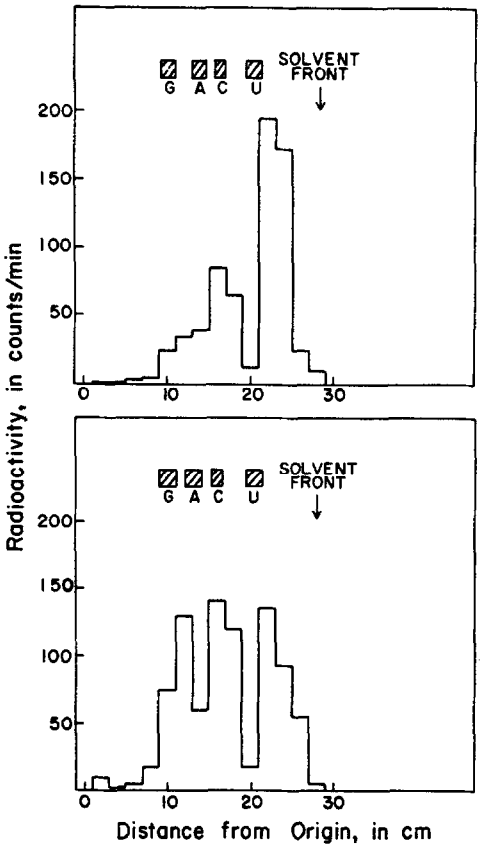

Figure 2 Paper chromatogram of perchloric acid hydrolyzed RNA from T_4 infected E. coli (upper frame) and from uninfected E. coli (lower frame). Guanine (G), Adenine (A), Cytosine (C), and Uracil (U).

radioactive regions were found on the electrophoretogram - one in the cytidylic and adenylic acid region, one in the guanylic acid region, and one between guanylic and uridylic acid. Uridylic acid and the material between guanylic and uridylic acid were eluted from the paper and dephosphorylated with E. coli alkaline phosphatase (50 μg/ml, pH 9.0, 37 C, 3 hr.). Following addition of carrier uridine, ribosylthymine, and thymidine, the material was analyzed by paper chromatography. The results are presented in Fig.3. In both the n-butyl alcohol-H_2O-NH_4OH solvent (upper frame) and the isopropanol-H_2O-NH_4OH solvent (lower frame), the radioactive material co-chromatogramed with ribosylthymine. None co-chromatogramed with either uridine or thymidine.

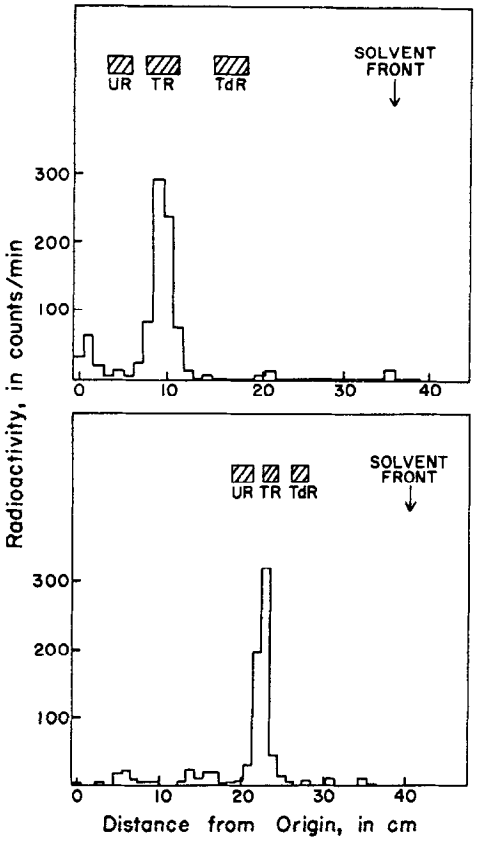

Figure 3 Paper chromatograms resolving Uridine (UR), Ribosylthymine (TR), and Thymidine (TdR). n-butyl alcohol-H_2O-NH_4OH solvent (upper frame); isopropanol-H_2O-NH_4OH solvent (lower frame).

Methylation of RNA in phage T_4 infected E. coli could occur on T_4 specific RNA, i.e., RNA synthesized from the T_4 genome in the infected cell, then methylated. Alternatively, methylation could occur on E. coli specific RNA, i.e., RNA synthesized from the E. coli genome in the uninfected cell, then modified by methylation in the infected cells. Experiments designed to decide this issue are now in progress.

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