SPECIES SPECIFICITY OF RIBOSOMAL RNA METHYLASES*

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Transfer RNA and ribosomal RNA contain a number of methylated components in addition to the four major bases of their primary structure. (Dunn, 1959; Dunn et al., 1960; Dunn, 1961; Dunn et al., 1963; Hall, 1963) It has recently been demonstrated (Fleissner and Borek, 1962, 1963) and corroborated in many laboratories (Gold et al., 1963; Svensson et al., 1963; Starr, 1963) that the methylated bases of transfer RNA are acquired by transmethylation at the polynucleotide level of a preformed unmethylated polymer. The in vitro demonstration was facilitated by the availability of methyl deficient transfer RNA which accumulates during methionine starvation of E. coli $K_{12}W6$. However, the RNA formed in this relaxed mutant during methionine starvation also contains ribosomal RNA (Mandel and Borek, 1963) and hence should serve as an ideal substrate in a search for enzymes which methylate ribosomal RNA. In this communication we

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wish to report the existence of enzymes in a variety of organisms which methylate ribosomal RNA obtained from methionine deprived E. coli $K_{12}W6$. Comb (1964) and Gold and Hurwitz (1964) have recently reported the in vitro methylation of ribosomal RNA.

Experimental: The ribosomal RNA from liver and Euglena gracilis were isolated by the procedures of Lipshitz-Wiesner and Chargaff (1963) and Brawerman et al. (1962) respectively. The preparation of methyl-deficient transfer RNA from starved E. coli $K_{12}W6$ has previously been described (Fleissner and Borek, 1963). The ribosomal RNA from starved cells of E. Coli $K_{12}W6$ from exponentially grown cells was obtained by repeated fractionation of the total RNA with 10 percent NaCl. The preparation of cell-free enzyme extracts from the various tissues and organisms was outlined in an earlier publication (Srinivasan and Borek, 1964).

The incubation mixtures contained 100 μmoles of Tris buffer pH 8.2, 10 μmoles each of reduced glutathione and $MgCl_2$, 0.5 ml of enzyme extract, 0.2 μc of S-adenosylmethionine-methyl-C^{14} (38.2 mc/mmole) and 1 mg of the indicated substrates in a total volume of 1 ml. The reaction mixtures were incubated at 37° for 45 minutes. The processing of the reaction mixtures for the determination of the incorporation of methyl groups followed methods described earlier (Srinivasan and Borek, 1963).

Results and discussion: The results of the various experiments performed are outlined in the Table. The capacity of the various enzyme extracts to transmethylate methyl deficient transfer RNA is also presented for comparison. All the enzyme

Incorporation of $C^{14}H_3$ from $C^{14}H_3$-S-Adenosylmethionine into Various Substrates by Heterologous Enzymes

Source of Enzyme Extract	SUBSTRATE				
	t-RNA E. coli K_{12} W-6 Starved	R-RNA E. coli K_{12} W-6 Starved	R-RNA E. coli K_{12} W-6 Log	R-RNA Liver	R-RNA Euglena gracilis
	c.p.m.	c.p.m.	c.p.m.	c.p.m.	c.p.m.
E. coli K_{12}	9,200	5,400	0	0	0
S. typhimurium	5,100	1,990	100	470	0
Ps. fluorescens	15,000	12,150	1,950	80	0
B. cereus	7,000	1,660	740	140	0
Euglena gracilis	1,690	770	610	190	0
Liver	3,760	2,100	1,110	20	0
Spinach	720	520	280	90	0
Yeast	5,470	3,180	530	0	0

t-RNA = transfer RNA; R-RNA = Ribosomal RNA.

extracts studied are able to incorporate methyl groups into ribosomal RNA. The ribosomal RNA formed during methionine starvation of E. coli $K_{12}W6$ served as the best substrate examined so far. On the other hand, the ribosomal RNA prepared from E. coli $K_{12}W6$ grown in logarithmic phase was unable to accept methyl groups from E. coli enzymes indicating that the substrate is fully methylated with respect to the homologous enzyme system. However, extracts derived from heterologous sources are capable of introducing supernumerary methyl groups into the "log" ribosomal RNA of E. coli and to a small extent into liver ribosomal RNA. This phenomenon is similar to that observed with heterologous methylation of t-RNA's and DNA's found in a variety of systems. (Srinivasan and Borek, 1963; Gold et al., 1963; Svensson et al., 1963)

Svensson et al., (1963) and Starr (1963) have reported the incorporation of methyl groups from methionine-methyl-C^{14} into the ribosomal RNA of logarithmically growing cells of E. coli and also into the ribosomal RNA of starved cells during the recovery phase. However, the first group of investigators failed to achieve in vitro methylation of the ribosomal RNA. This could be attributed to the nature of the substrate employed by them, i.e., ribosomal RNA derived from cells starved for only 60 minutes.

In the present studies we have used ribosomal RNA isolated from E. coli $K_{12}W6$ cells which were deprived of their essential nutrilite methionine for three hours. During the first hour of starvation there is some DNA synthesis (20 percent) and normal RNA synthesis. Such residual normal activity is due to the

availability of methionine from the intracellular amino acid pool. It is therefore reasonable to assume that during the first hour of starvation much of the ribosomal RNA synthesized is still of the methylated variety. Furthermore, the levels of the methylated components in ribosomal RNA are considerably lower than those found in transfer RNA. In view of these factors it is not unexpected that the ribosomal RNA formed during the first hour of depletion of methionine would be a poor substrate for *in vitro* methylations.

The function of the methylated bases in nucleic acids is still obscure. However, mounting evidence indicates that the methylation of both RNA and DNA is species specific.

Since the 16S and 23S ribosomal RNA's are determined by different regions of the genome (Spiegelman and Hayashi, 1963) it would be of interest to examine whether different ribosomal RNA methylases exist for these two species of RNA (16S and 23S), or whether such enzymes could function with either substrate but achieve different levels or patterns of methylations.

The existence of unmethylated ribosomal RNA provides a new parameter for studies of the participation of ribosomal RNA in protein synthesis. An examination of the ribosomes from starved cells as participants in protein synthesis may shed light on this problem.

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