THE SOURCE OF THE METHYL GROUP FOR THE THYMINE OF RNA*

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We have recently reported that the RNA which accumulates in E. Coli K_{12} W-6 as a result of the deprivation of methionine lacks the methylated bases which are normally present in small amounts in RNA (1). As a result of this finding we undertook a study of the origin of the methyl groups of these minor components (2).

To explore one possible source of the methyl groups, the methionine requiring auxotroph E. Coli K_{12} W-6, as well as the prototroph of this organism were grown in a defined medium (3) containing C-14 methyl labeled methionine. The methylated adenosines isolated from the RNA of both organisms were highly radioactive indicating a direct transfer of the methyl groups without extensive dilution by the one carbon fragment pool synthesized by these organisms (see lines 3,4 Table I). This observation on intact cells confirms an earlier brief note by Remy who reported the existence of an enzyme in cell free extracts which achieves a direct methylation of adenine by methionine (4). The thymine riboside which we have isolated from the radioactive RNA samples had a specific radioactivity comparable to that of the methylated adenosines (see line 6, Table I). This finding indicated that the methyl group of the thymine of RNA also stems from methionine. The synthesis of DNA thymine, on the other hand, had been shown by Friedkin and Kornberg to proceed via the methylation of deoxyuridine mono-phosphate by a one carbon fragment which does not originate from methionine (5). We also isolated the thymine from the DNA of organisms grown on C-14 methyl methionine and found that the methionine

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is but a marginal source for the methyl group of the DNA thymine (line 5, Table I). We therefore conclude that the in vivo pathways of methylation of thymine riboside and of thymine deoxyriboside are different.

Table I

The Origin of Methyl Groups for the Bases in RNA

Line	Compound	E. Coli K-12 W-6 grown in 1 L medium with 50 μc C-14 Methyl Methionine	E. Coli K-12 grown in 1 L medium with 25 μc C-14 Methyl Methionine
		Specific Activity in CPM per Micromole	
1.	Uridine	less than 100	less than 100
2.	Adenosine	200	less than 100
3.	2-Methyl adenosine	90,000	46,000
4.	6-N-Methyl adenosine	110,000	62,000
5.	Thymine from DNA	1,500	200
6.	Thymine riboside from RNA	72,000	37,000
7.	Thymine riboside from above diluted 30 fold with unlabelled thymine riboside	---	1,300
8.	Thymine degraded from thymine riboside from above	---	1,300

The organisms were grown from small inocula in synthetic medium (3). For the auxotroph the radioactive methionine was diluted with carrier yielding a specific radioactivity of 10^5 counts/min/μM L-methionine. In the case of the prototroph (second column) the methionine was not diluted and the specific radioactivity was 2 x 10^5 counts/min/μM L-methionine.

The methylated ribonucleosides were isolated from RNA by the method of Littlefield and Dunn (6). The chromatographic patterns of these minor components were identical to those reported by these authors. For the confirmation of the identity of thymine riboside, we were fortunate to have a sample of the synthetic product made available to us by Dr. George B. Brown of the Sloan Kettering Institute. The isolated thymine riboside and the authentic product had identical Rf values in the butanol-water-formic acid and in butanol-water-ammonia solvent systems which

were used for the two dimensional chromatographic separations (6). Moreover, when this synthetic unlabeled product was added to the mixture of ribonucleosides isolated from radioactive RNA, and the mixture chromatographed in two dimensions the specific radioactivity of the thymine riboside diminished in proportion to the amount of non-radioactive sample added (line 7, Table I).

That the radioactivity of thymine riboside resides in the base moiety was demonstrated by degrading the nucleoside with 8M $HClO_4$ at 100° for one hour. The free base, thymine, retained the original specific radioactivity (line 8, Table I).

We are studying the nature of the substrate which yields thymine riboside by methylation. The receptor may be some simple derivative of uracil but the possibility should not be overlooked that the receptor may be at the level of some macro-molecule containing it.

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