VARIABILITY IN THE STRUCTURE OF RIBONUCLEIC ACID*

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The amount of ribonucleic acid (RNA) in a bacterial cell can be increased by environmental factors (1, 2, 3). Such variability in this component of the cell occurs uniquely upon starvation in the auxotrophic mutant E. coli K_{12}W-6. If this organism is deprived of its essential nutrient methionine, it can double its RNA content while its protein and deoxyribonucleic acid (DNA) remain constant (1).

The RNA accumulating during the methionine starvation, unlike the RNA accumulating under the influence of the drug chloramphenicol (4) is stable (5).

We have found that the organisms which possess the very high complement of RNA as a result of methionine starvation are uniquely slow in resuming protein synthesis even after the restoration of methionine (6). Such organisms were found to be unable to synthesize adaptive enzymes for as long as two hours after the restoration of methionine and they were unable to support the replication of infectious phage.

Preliminary investigations of the composition of the accumulated

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RNA revealed no change in the relative amount of the four main base components. However, a study of the minor components of the RNA revealed a profound change.

When the microorganisms are grown in the presence of methionine, the RNA synthesized contains a small percentage of methylated bases which we have isolated in the riboside form and identified as 2-methyl adenosine, 6-N-methyl adenosine, and thymine riboside. When the microorganisms are starved of methionine, there is no incorporation of methylated bases into the accumulating RNA. This alteration of RNA structure has been determined by spectrophotometric assays as well as by isotopic techniques.

The method of isolation and determination of methylated components in RNA has been described in detail by Littlefield and Dunn (7). The chromatograms we obtained were almost identical to the one illustrated by these authors; however, we did not detect 6,6-N-dimethyl adenosine.

The methionine starvations were carried out as previously described (1).

In Table I are presented the amounts of three methylated nucleosides relative to the amount of uridine in the normal and starved organisms. The decrease in the relative amounts of the methylated bases is

Table I

Proportion of Methylated Nucleosides in Parts per 100 Parts Uridine

Source of RNA	Number of Analyses	Thymine riboside	2-Methyl adenosine	6-N-Methyl adenosine
Control (non-starved)	6	.8	.3	.25
Methionine starved	4	.5	.15	less than .10

proportional to the increment of the new RNA synthesized indicating that the absolute amounts of the methylated bases remain constant.

Further support for such a conclusion was obtained by studying the fate of previously labeled pyrimidines during subsequent starvation of the bacteria. Cultures of E. coli K_{12} W-6 were grown in 4 liters of defined medium containing methionine and 100 μc of uniformly labeled DL-aspartic acid C-14. When the culture reached a population of 7×10^8 cells per ml, it was divided. Ribonucleosides were isolated from one half. The other portion was washed free of methionine and of radioactive aspartate and was incubated without methionine for 3 hours. Ribonucleosides were isolated. It is apparent from the data in Table II that the specific radioactivity of the uridine isolated from the RNA of starved organisms is approximately one half the value of the specific radioactivity at the start of the starvation, whereas that of the thymine riboside remained constant.

Table II

Specific Activities of the Pyrimidine Ribonucleosides

Compound	Source of RNA	Specific Activity in CPM per Micromole
Uridine	Control (non-starved)	1310
Uridine	Methionine starved	785
Thymine riboside	Control (non-starved)	1240
Thymine riboside	Methionine starved	1290

Adenosine and methylated adenosines were not radioactive.

Studies of the metabolism of exogenous adenine during growth and during starvation also indicated the exclusion of methylated bases from the RNA accumulating during starvation. From the data in Table III it is apparent that if adenine 8 C-14 (50 uc per liter) is added to a grow-

ing culture, the RNA yields adenosine and its methylated derivatives with essentially the same specific radioactivity. However, when the labeled adenine was administered in the same amount to a starving culture, the methylated adenosines isolated from the RNA had only a fraction of the radioactivity of the adenosine.

Table III

Specific Activities of the Purine Ribonucleosides

Compound	Specific Activity in CPM per Micromole: Adenine 8 C-14 added to growing organisms	Adenine 8 C-14 added to starving organisms
Adenosine	48,500	37,000
2-Methyl adenosine	48,000	6,000
6-N-Methyl adenosine	46,500	less than 1,000

Uridine and thymine riboside were not radioactive.

The profound structural alteration in the RNA accumulated during starvation is of particular interest in view of the uniquely retarded resumption of protein synthesis in these organisms after the restoration of methionine. It may be pertinent that the methylated bases are normally present in the "soluble" RNA (8) which is strongly implicated in protein synthesis (9). It should be of interest to study whether there is any correlation between the deposition of an RNA of altered structure and delayed protein synthesis in these organisms.

This may be a unique instance of such inhibition of protein synthesis or it may be a fortuitously exaggerated example of a more generalized biological method of suppression of protein synthesis by an RNA of altered structure.

In either case these organisms may serve as a useful model for studies of both the suppression of protein synthesis and the reversal of such suppression.

References

1. Borek, E., Ryan, A. and Rockenbach, J., J. Bacteriol., 69, 460(1955).
2. Gale, E. F., and Folkes, J. P., Biochem. J., 53, 493 (1953).
3. Takeda, Y., et al., J. Biochem. (Japan), 48, 169 (1960).
4. Neidhardt, F. C., and Gros, F., Biochim. Biophys. Acta, 25,513(1957).
5. Borek, E., and Ryan, A., J. Bacteriol., 75, 72 (1958).
6. Borek, E., Rockenbach, J., and Ryan, A., J. Bacteriol., 71,318(1955).
7. Littlefield, J. W. and Dunn, D. B., Biochem. J., 70, 642.(1958).
8. Dunn, D. B., Smith, J. D., and Spahr, P. F., J. Mol. Biol., 2, 113 (1960).
9. Hoagland, M. B., et al., J. Biol. Chem., 231, 241 (1958).