THE CODING NATURE OF VALYL-TRANSFER RNA BINDING TO RIBOSOMES DURING METHIONINE STARVATION IN *E. COLI* 113-3

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SUMMARY

The results presented here indicate that the binding to normal ribosomes of val-tRNA present in *E. coli* 113-3 after methionine starvation is reduced by 1/4 while the binding of normal val-tRNA to the ribosomes present after methionine starvation is reduced by 1/3. Since rRNA synthesized during six hours methionine starvation is about 1/3 of the total rRNA present, we conclude that only ribosomes formed during exponential growth are able to bind normal val-tRNA. Two possible explanations concerning the inability of rRNA synthesized during methionine starvation to function normally in the binding of the normal val-tRNAs are suggested.

In previous work, we have demonstrated that *E. coli* 113-3 a mutant requiring vitamin B_{12} or methionine is a relaxed strain in which RNA synthesis continues during either methionine or vitamin B_{12} starvation while protein synthesis stops (1,2). Transfer-RNAs synthesized during methionine starvation are undermethylated and a new species of val-tRNA has been separated under these conditions. Similar phenomena have been observed in other strains of bacteria (3,4,5). Undermethylated tRNA and rRNA can be enzymatically methylated by S-adenosylmethionine (6-12). Undermethylated tRNAs can also be acylated as can methylated tRNAs but the degree of acylation obtainable varies (1,2,13-16). The coding properties of undermethylated tRNA are different from those of methylated tRNA and miscoding has been produced by undermethylated tRNA (17-20).

The methyl group of S-adenosylmethionine is also incorporated into

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rRNA (8-12). Ribosomal RNAs synthesized during methionine starvation are also undermethylated, but their coding properties have not been established. Work with E. coli 58-161 a methionine requiring relaxed mutant (RC^{rel}) has shown that during methionine starvation 20S and 23S ribonucleoprotein particles accumulate but little synthesis of ribosomes takes place (21). These particles contain protein derived from the cells as well as 16S and 23S RNA (22). In RC^{rel} the proportion of residual protein synthesis devoted to ribosomal protein is more than twice as great as the proportion during exponential growth (23), however, only a few species of ribosomal proteins are produced. Thus it appears that ribosomes are not completed in the absence of methionine. Similarly in HeLa cells ribosomes are not matured during methionine starvation (24), while other essential amino acid starvation allows continuing maturation but at a reduced rate (25).

It has been shown by Leder and Nirenberg (26) that an RNA codeword for valine is GUU but that the binding of val-tRNA to ribosomes is more sensitive to the addition of poly UG than of poly GUU. The purpose of this investigation was to study the poly UG directed binding of val-tRNA and rRNA produced during methionine starvation of E. coli 113-3.

MATERIALS AND METHODS

The cultivation of E. coli 113-3 and the preparation of normal and six hour methionine-starved tRNA and the charging of normal and six hours methionine-starved tRNA by valine have been described in detail (1,2). Ribosomes were prepared as described by Nishimura et al. (27), except that E. coli 113-3 was used. Ribosomal-RNAs were prepared from this strain according to the method of Hurwitz et al. (10). Ribosomal-RNA methylating enzymes were prepared from rat liver by the method of Srinivasan et al. (12). Poly UG (1:1 ratio) was purchased from Miles Laboratories.

The Assay of Methylation of rRNA. The measurement of the methylation of rRNA by S-adenosylmethionine was performed as described by Hurwitz et al. (10). The reaction mixture (0.4 ml) contained 40 μmoles of Tris-HCl buffer,

pH 8.2, 4 μmoles of 2-mercaptoethanol, 2 μmoles of $MgCl_2$, 150 μg of methylating enzymes (18), 12.5 mμmoles of ^{14}C-methyl-labeled S-adenosylmethionine (44,000 cpm/mμmole), 3 O.D. 260 units of rRNA.

Following incubation at 38° for 20 min, the reaction mixture was chilled in ice, made 2% with potassium acetate pH 4.5, and 2.5 volumes of absolute ethanol were added immediately. After standing at 0° for 30 min the precipitate was collected by centrifugation. The precipitate was washed 3 times. The amount of ^{14}C radioactivity was determined by dissolving the washed precipitate in 0.2 ml of water and counting in the Tri-Carb liquid scintillation counter.

The Assay of Binding Val-tRNA to Ribosomes. The assay procedure described by Leder and Nirenberg (26) was used. The reaction mixture (0.05 ml) contained 100 mmoles of Tris-acetate buffer pH 7.2, 20 mmoles of Magnesium acetate, 50 mmoles of potassium chloride, 1.75 O.D. 260 units of ribosomes, 8.85 μμmoles (2520 cpm, 284 cpm/μμmole) of ^{14}C-valine attached to 0.7 O.D. 260 units of normal tRNA or 0.58 O.D. 260 units of tRNA from E. coli 113-3 methionine starved for six hours. Incubations were carried out at 37° for 13 min. The binding of ^{14}C-val-tRNA to ribosomes was determined as described by Nirenberg and Leder (28).

RESULTS

The Methylation of Ribosomal-Ribonucleic Acids. One way to determine the degree of undermethylation of the RNA being studied is to investigate its capacity to accept methylation by S-adenosylmethionine in the presence of methylating enzymes compared to "normal" RNA from non-methionine starved cells. We have demonstrated that tRNA isolated in our laboratory from E.coli 113-3 after six hours methionine starvation is undermethylated (1). Since the amount of methylation of tRNA is greater than that of rRNA and is both strain and species-specific (10), we used methylating enzymes from rat liver as described by Srinivasan et al. (12) to study possible differences in the amount of methylation of rRNA obtainable in the rRNA from normal and methi-

Table I The capacity of normal and methyl deficient rRNA for methylation.

Experiment	Activity μμmoles ^{14}C-methyl group incorporated		
	without RNA	RNA from normal cells	RNA from deficient cells
1	2.85	10.70	30.08
2	2.10	12.80	34.00

nine-starved cells. Table I indicates that rRNA prepared from methionine-starved cells accepts more methylation than does rRNA from normal cells. A small amount of methylation of normal rRNA is observed due to supermethylatio by the heterologous enzymes. Thus it appears that the substrates for the investigation of the coding properties directed by poly UG are indeed under-methylated RNAs.

The Coding Properties of the Ribonucleic Acids formed during Methionine Starvation. Since poly UG can direct the binding of phe-tRNA and val-tRNA to ribosomes (26), we selected this polynucleotide to investigate the effect

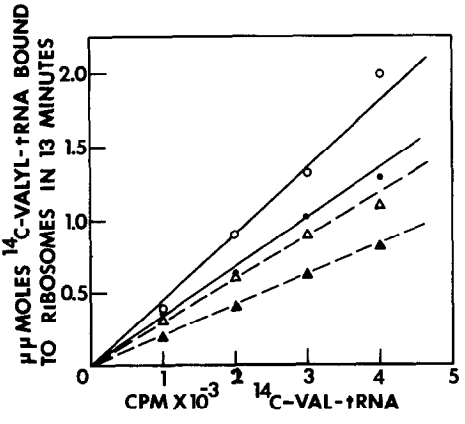

FIG. I
Effect of amount of val-tRNA on the poly UG directed binding of val-tRNA to ribosomes.
-o-o-o , normal ribosomes and normal val-tRNA
-●-●-● , normal ribosomes and methyl starved val-tRNA
-Δ-Δ-Δ , methyl starved ribosomes and normal val-tRNA
-▲-▲-▲ , methyl starved ribosomes and methyl starved val-tRNA

of methionine starvation on the binding of val-tRNA to ribosomes. In Fig. 1 are given the results of a representative experiment on the binding of ^{14}C-val-tRNA to ribosomes. Val-tRNA from normal and six hours methionine-starved cells, and ribosomes from normal and six hours methionine-starved cells were used. From Fig 1 it can be seen that, when compared with normal val-tRNA binding to normal ribosomes the binding of methionine-starved val-tRNA to normal ribosomes was reduced by 1/4, while the binding of methionine-starved ribosomes to normal val-tRNA was reduced by 1/3. The binding of methionine-starved val-tRNA to methionine-starved ribosomes was reduced by about 1/2.

The results of a second experiment in which the incubation time was varied are presented in Fig. 2. It appears quite clear from Fig. 1 and 2 that methyl deficient RNAs are not able to function normally in the poly UG direct binding of val-tRNA to ribosomes.

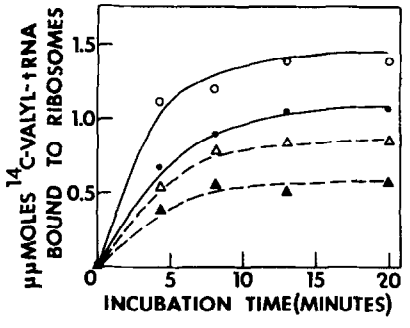

FIG. 2
Effect of incubation time on the poly UG directed binding of val-tRNA to ribosomes.
-o-o-o , normal ribosomes and normal val-tRNA
-●-●-● , normal ribosomes and methyl starved val-tRNA
-Δ-Δ-Δ , methyl starved ribosomes and normal val-tRNA
-▲-▲-▲ , methyl starved ribosomes and methyl starved val-tRNA
3000 cpm ^{14}C-val-tRNA per incubation.

DISCUSSION

Previously we have demonstrated that the amount of total RNA present is increased by about 1/3 during six hours of methionine starvation in E. coli strain 113-3 and that the new tRNA synthesized was undermethylated (1).

Since rRNA constitutes 90% of the total RNA the increase of 1/3 in RNA is primarily an increase of rRNA. In this communication, we have demonstrated that the binding of normal val-tRNA to methionine-starved ribosomes is reduced 1/3 compared to the binding of normal val-tRNA to normal ribosomes. This indicates that the rRNA synthesized during the six hours of methionine starvation is not able to function in the binding of aminoacyl-tRNA to ribosomes.

The inability of rRNA present after methionine starvation to function normally in the binding of the normal val-tRNAs may be related to the undermethylation of the new rRNA synthesized during methionine starvation.

Undermethylation per se may inhibit normal binding due to change in coding function or may inhibit binding because of inhibition of maturation due to this undermethylation (23,24).

On the other hand normal binding of normal charged tRNA may be prevented by lack of ribosome completion due to lack of available proteins because of little or no protein synthesis during methionine deprivation. This possibility appears less likely in view of the available free ribosomal proteins in the cell(22) and thus the ability to complete ribosomes (although fewer) in other essential amino acid deficiencies (25).

Explanation of lowered functional ability of both the methyl-deficient val-tRNA and the methyl-deficient ribosomal particles will require isolation, re-methylation and reassembly experiments.

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