INTRACELLULAR CHARGING OF SOLUBLE RIBONUCLEIC ACID IN ESCHERICHIA COLI SUBJECTED TO ISOLEUCINE STARVATION AND CHLORAMPHENICOL TREATMENT

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To explain the inhibition of bacterial ribonucleic acid (RNA) synthesis by amino acid starvation, Stent and Brenner (1961) and Kurland and Maaloe (1962), conjecturing that amino acid starvation produces cells containing transfer RNA (S-RNA) free of amino acids, suggested that free S-RNA represses RNA synthesis, and that amino acyl S-RNA is inactive as repressor. Tissieres, et al. (1963) showed that free S-RNA inhibits the RNA polymerase in vitro, and its inhibitory effect is reduced by charging it with amino acids.

Recently, Martin, et al. (1963) showed that, in a methionine-starved Escherichia coli auxotroph incapable of making RNA, the methionine-specific S-RNA ($S\text{-}RNA_{meth}$) is fully charged, as judged by its resistance to periodate treatment. The significance of this finding for the problem of amino acid control of RNA synthesis and for the S-RNA repressor hypothesis suggested the desirability of presenting the following observations.

When the rate of RNA synthesis was controlled by means of isoleucine starvation and chloramphenicol treatment of an E. coli auxotroph, the extent of charging of the isoleucine-specific S-RNA ($S\text{-}RNA_{ileu}$) varied in a manner apparently

determined by the amino acid supply, and correlated closely with the rate of RNA synthesis.

Materials and Methods

E. coli strain JHM 544 (derived from K-12) was kindly provided by Dr. H. E. Umbarger. It lacks threonine deaminase and exhibits an absolute requirement for isoleucine.

Cells were grown exponentially in minimal medium, with added isoleucine, valine, uridine, and cytosine. They were starved 40 minutes in the same medium minus isoleucine and valine, and the experiment initiated by supplementing as desired, with isoleucine and valine (20 mcg/ml each) or chloramphenicol. After 20 minutes of incubation, 200 ml samples were quickly chilled and harvested for S-RNA extraction, and 10 ml samples were pipetted into flasks containing uridine-C^{14}. After 10 minutes of label incorporation 1 ml samples were collected by cold acid precipitation and filtration and assayed for radioactivity.

S-RNA was prepared according to von Ehrenstein and Lipmann (1961). Using this procedure on intact cells, substantial recovery of amino acid-charged S-RNA was obtained. It was established that periodate treatment of S-RNA for 30 minutes at room temperature in 0.1 M sodium succinate buffer at pH 5.6 inactivates free S-RNA with KIO_4 concentrations as low as 10^{-4} M, and has no effect on isoleucine-bound S-RNA up to at least 1.2×10^{-3} M. Thereafter, 4×10^{-4} M KIO_4 was used. After periodate treatment, 4×10^{-2} M glucose was added, and the samples incubated for 5 minutes to destroy the remaining periodate. After alcohol precipitation and washing, control and periodate-treated samples were assayed for $S\text{-}RNA_{ileu}$ according to Berg, et al. (1961). Isoleucyl RNA synthetase was prepared from E. coli B Luria according

to Bergmann, et al. (1961), through the first ammonium sulfate fractionation, except that the cells were lysed with lysozyme and freezing and thawing.

Results and Discussion

The effects of isoleucine starvation and of simultaneous chloramphenicol treatment on charging of the S-RNA$_{ileu}$ and on RNA synthesis are shown in Figure 1. During isoleucine starvation, the isoleucine on the S-RNA$_{ileu}$ is depleted. This effect is reversed by chloramphenicol. As the chloramphenicol concentration is varied, the extent of reversal is roughly proportional to the extent of stimulation of RNA synthesis. This fact suggests that both effects depend on presence of the same low concentration of isoleucine. The

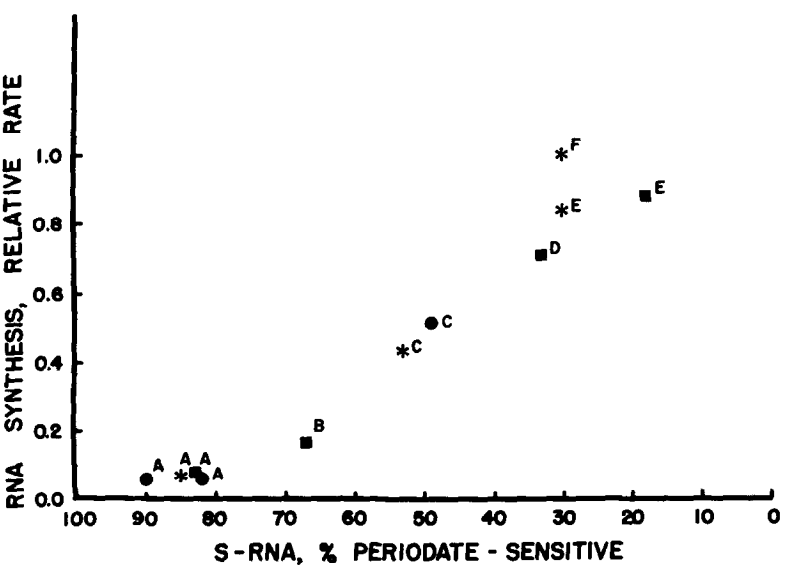

Figure 1. Effect of isoleucine starvation and treatment of isoleucine-starved cells with various concentrations of chloramphenicol, on charging of S-RNA$_{ileu}$ and on RNA synthesis, as measured by uridine-C^{14} incorporation. Points represent data from three experiments. Sample contents (mcg/ml):

	A	B	C	D	E	F
Isoleucine	0	0	0	0	0	20
Chloramphenicol	0	5	15	27	100	0

data support the conclusion of Kurland and Maaloe (1962) that the effect of chloramphenicol on RNA synthesis is a consequence of the sparing of amino acids which would otherwise be exhausted by protein synthesis.

Is the S-RNA an internal repressor of RNA synthesis? Although the correlation shown here is consistent with this hypothesis, the effect of methionine starvation reported by Martin, et al. (1963) is not. It should be mentioned that the authors report that the material charging the $S\text{-}RNA_{meth}$ in methionine-starved cells is probably not methionine. Perhaps the unknown substance prevents periodate oxidation of the $S\text{-}RNA_{meth}$ but does not prevent its function as a repressor of RNA synthesis. It would be interesting to know whether the $S\text{-}RNA_{meth}$ in this state inhibits the RNA polymerase in vitro.

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Summary

The effects of isoleucine starvation and of chloramphenicol treatment of isoleucine-starved E. coli cells on amino acid charging of the $S\text{-}RNA_{ileu}$ in vivo were examined. Isoleucine starvation produced uncharged $S\text{-}RNA_{ileu}$. The effect was reversed by treatment with chloramphenicol at the same concentrations which increased the rate of RNA synthesis.

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