Ribonucleic Acid Synthesis Under Relaxed Control*

Lewis R. Mandel** and Ernest Borek

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y.

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Continuous ribonucleic acid (RNA) synthesis in a bacterial cell depends on the presence of all requisite amino acids either from exogenous or endogenous sources. If an organism auxotrophic for an amino acid is deprived of that amino acid all RNA synthesis ceases (1). An exception to this stringent control of RNA synthesis by catalytic amounts of amino acid was reported several years ago by Borek, Ryan and Rockenbach (2). It was observed then that the methionine requiring auxotroph *E. coli* K_{12} W-6 continues to synthesize RNA without concomitant DNA and protein synthesis when cultures of this organism are incubated in the absence of methionine.

In studies on multiple amino acid auxotrophic derivatives of strain K_{12} W-6, Stent and Brenner have shown that control of RNA synthesis is relaxed not only on methionine starvation but by withdrawing other amino acids as well (3). Their studies have

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demonstrated the existence of a genetic locus, RC, (relaxed control) whichis transferable during conjugation and whose presence in the cell releases amino acid control of RNA synthesis. Consequently, multiple auxotrophic recombinants accumulate RNA when deprived of one or more of their essential amino acids. Moreover, Neidhardt has recently reported that a prototrophic derivative of K_{12} W-6, E. coli K-10, unlike other prototrophs continues to synthesize RNA when it is transferred from an amino acid enriched medium to an amino acid deficient one (4).

We have been studying the nature of the RNA which is synthesized during methionine starvation of E. coli K_{12} W-6 and have reported earlier that this RNA does not contain the methylated base nucleotides (5). It does, however, contain pseudouridylic acid, an additional minor constituent of soluble RNA*** (6,7). To determine the macromolecular character of the accumulating RNA, and thus to determine the fractions of RNA influenced by the RC locus, analyses by ultracentrifugation on sucrose gradients were undertaken (8).

Bacterial cultures were raised on a defined salts medium (9) containing methionine. When in mid-log phase, the microorganisms were suspended in methionineless medium containing $P^{32}O_4^{\equiv}$ (200 microcuries/liter). After incubation for 2 hours at 37°, the cultures were harvested and cell-free extracts were prepared by grinding the bacterial pellet with alumina in the presence of DNAase. The extract, diluted with a 10^{-2} M tris-10^{-2} M $MgCl_2$ buffer at pH 7.4, was clarified by two centri-

*** We are indebted to Dr. E. Reich of the Rockefeller Institute for this determination.

fugations at 18,000xg and was then extracted with phenol to obtain protein-free RNA.

The phenol extracted RNA was centrifuged at 25,000 rpm for 14 hours at 5° in a Spinco SW 25 swinging bucket rotor on 27 ml of a 20%-5% linear sucrose gradient. The sucrose was buffered with 10^{-2} M tris pH 7.4 and contained 10^{-4} M $MgCl_2$. At the end of the centrifugation, the bottoms of the tubes were pierced with a hypodermic needle and the contents collected in 34 equal fractions. After suitable dilution the UV absorption at 260 mμ of each fraction was taken and aliquots were plancheted for counting in a mica-end-window Geiger-Muller counter.

The nature of the accumulated RNA is indicated by the sedimentation pattern in Fig. I. In addition to the low molecular weight, non-methylated, soluble RNA (10,11), polynucleotides of ribosomal size are also synthesized during methionine depriv-

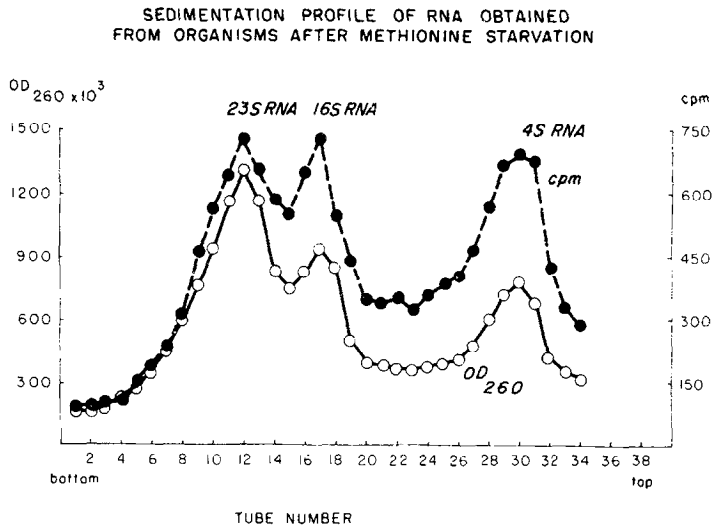

Figure I

ation of E. coli K_{12} W-6. The optical density profile of the RNA obtained from organisms in logarithmic growth phase and sedimented under identical conditions is presented in Figure II for comparison.

While these studies were in progress, Dagley and co-workers (12) observed in studies with the analytical ultracentrifuge the appearance of anomalous peaks of ribonucleoprotein arising from the RNA accumulated upon methionine starvation of E. coli K_{12} W-6. However, since the accumulated RNA was not preferentially labeled with isotopes and since protein-free RNA was not prepared a complete spectrum of the accumulated RNA could not be obtained.

Although sucrose gradient sedimentations could identify major RNA fractions, we were unable to determine, by this technique, whether an RNA fraction functional in protein synthesis, i.e. "messenger RNA" (13) is also synthesized. Since the hydrodynamic properties of this polynucleotide are unknown we tested for its accumulation during methionine starvation by an assay for its function in a cell-free protein synthesizing system (14). Equivalent amounts of phenol extracted RNA prepared from normal E. coli K_{12} W-6 and from methionine starved organisms were added to a constant amount of a defined incubation mixture. The ability of added phenol purified RNA to act as a functional "messenger" was expressed in terms of the amount of radioactive leucine incorporated into the hot trichloroacetic acid insoluble product obtained in the reaction. The data presented in Figure III show that the RNA accumulated

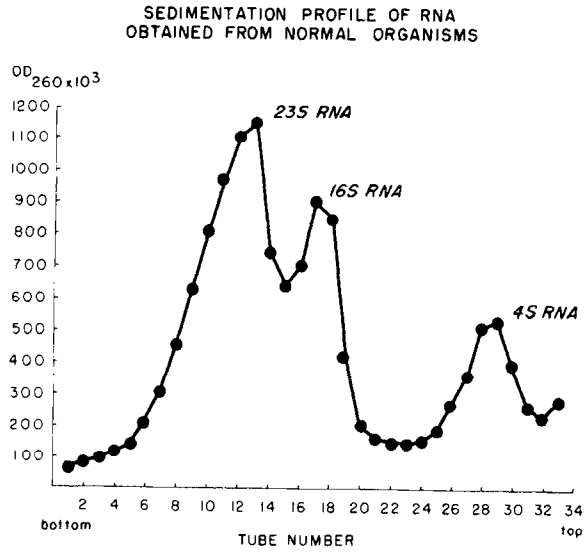

Figure II

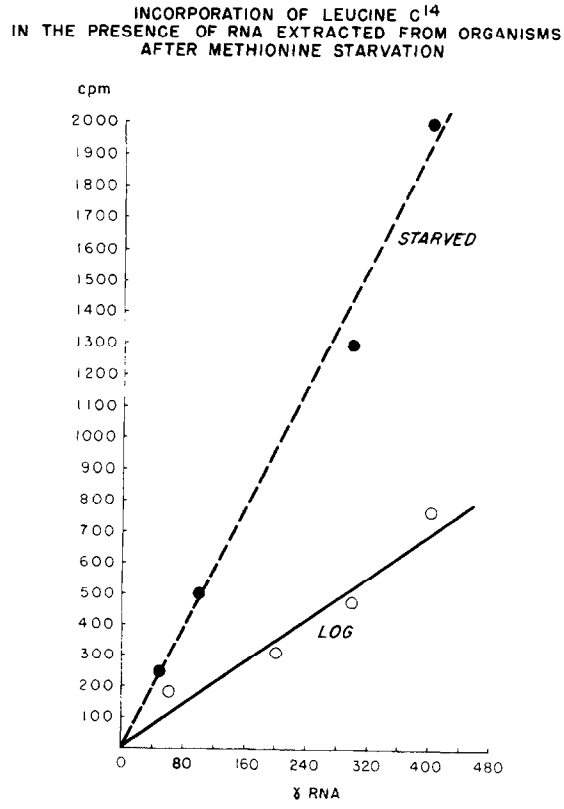

Figure III

during starvation yields a three-fold stimulation over equivalent amounts of normal RNA. Thus it appears that a fraction of RNA accumulates which is functional in stimulating amino acid incorporation into protein.****

Therefore all recognized fractions of RNA - ribosomal, transfer and the "messenger" fraction - accumulate under amino acid starvation. This finding at first sight would indicate that the control of all RNA synthesis, or rather the relaxation thereof, is invested in a single genetic locus. This is unexpected, since evidence for a divergent origin of soluble and ribosomal RNA is accumulating. For example, it has been shown in our laboratory that the synthesis of soluble and ribosomal RNA have markedly different sensitivity to ultraviolet irradiation, implying thereby that the synthetic mechanisms for these RNA fractions offer different targets for the irradiation (15).

Alternative explanations are, however, possible. If in these organisms the relaxed control of only one species of RNA is involved, and if the synthesis of all RNA fractions is interdependent, the accumulation of the whole spectrum of RNAs might result from the continued synthesis of only one of them.

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