THE STIMULATION OF RNA SYNTHESIS BY RIBOSOMES IN VITRO

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It has been reported that RNA synthesized in vitro by DNA-dependent RNA polymerase and T4 or T2 phage DNA remains attached to the enzyme-template complex (Bremer and Konrad, 1964; Byrne et al, 1964); each enzyme molecule synthesizes only one RNA strand which does not dissociate spontaneously from the complex. It has been suggested (Bremer and Konrad, 1964; Byrne et al, 1964; Stent, 1964) that in vivo, an active process involving ribosomes removes the nascent RNA from the enzyme-template; this process would involve the formation of an intermediate consisting of DNA, RNA and ribosomes, and lead to the subsequent formation of polyribosomes. Thus, in vivo, ribosome attachment to nascent RNA could influence the rate and extent of RNA made by effecting the release of completed RNA molecules from the enzyme-templ complex. Byrne et al (1964) observed that when ribosomes are added to a DNA-dependent RNA polymerase (with T2 DNA)- protein synthesis coupled reaction system, an aggregate containing nascent RNA, DNA and ribosomes is formed;some free polyribosomes were also detected. These observations support the suggested role of ribosomes in RNA synthesis However, a quantitative effect of ribosomes on RNA synthesis was not observed indicating that these in vitro systems do not completely reflect the in vivo process. The present communication presents evidence that the addition of ribosomes to an RNA polymerase preparation from E. coli, containing endogenous DNA template, influences both qualitatively and quantitatively the RNA formed.

The 30,000 X g supernatant was prepared from E. coli B cells essentially as described by Matthaei and Nirenberg (1961) except that DNase was not added to the homogenate. The ribosomes were sedimented from the 30,000 X g supernatant solution at 100,000X g (max.) for 2.5 hours, washed twice with Tris-HCl (10^{-2} M), magnesium acetate (10^{-2} M) buffer, pH 7.5, and 5 times with the same buffer containing 0.5 M NH_4Cl. The 100,000 X g (2.5 hours) supernatant was centrifuged at 100,000 X g for 16 hours. The nucleoprotein pellet obtained (Stevens and Henry, 1964) was resuspended in 10^{-2} M Tris-HCl, magnesium acetate buffer, pH 7.5, and centrifuged through a linear (8 - 30%) sucrose density gradient at 90,000 X g for 26 hours. A preparation was recovered from the bottom third of the gradient which contained RNA polymerase activity and DNA (RNA polymerase-DNA complex). This preparation, approximately 25% nucleic acid, catalyzed the incorporation of C^{14}-labeled nucleoside triphosphates into RNA in the absence of exogenous DNA; all four nucleotides were required, added DNA had no effect, and incorporation was inhibited by actinomycin and DNase. RNA synthesis with some preparations was stimulated up to 200% by the addition of ribosomes. At low ribosome concentrations, below 0.3 mg per ml, the increase was proportional to the ribosomal concentration.

Incubations at 30° were carried out for 60 minutes in a total volume of 0.5 ml containing the following components: $MgCl_2$, 10 μmoles; mercaptoethanol, 5 μmoles; Tris-HCl, pH 7.5, 45 μmoles; ATP, CTP, and GTP, 0.18 μmole; C^{14}-UTP, 0.078 or 0.10 μmole (0.38 or 0.48 μCurie of C^{14}, respectively); RNA polymerase-DNA complex, 0.044 or 0.055 mg protein; and ribosomes, when present, 0.65 mg. At the end of the incubation, the reaction mixture was chilled, layered on a linear (5-20%) sucrose gradient containing 10^{-2} M Tris-HCl and magnesium acetate at pH 7.5. Centrifugations were carried out at 90,000 X g for 4 or 5 hours. Gradient fractions, 1 ml each, were analyzed for absorbance at 260 mμ and for radioactivity.

Figure 1 shows the results of an experiment in which RNA polymerase-DNA

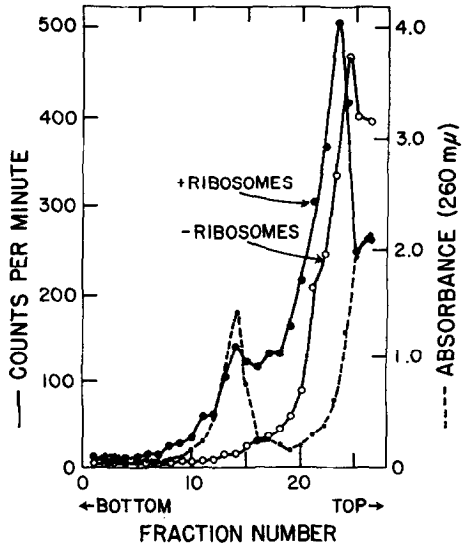

Figure 1: Sucrose gradient centrifugation of incubations of RNA polymerase-DNA complex in the presence (closed circles) and absence (open circles) of ribosomes. Incubations, as described above, contained 0.044 mg protein of RNA polymerase-DNA preparation and 0.1 μmole of C^{14}-UTP. Centrifugation time was 4 hours.

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complex was incubated and centrifuged in the presence and absence of ribosomes. Approximately 3,500 cpm of C^{14} were incorporated into RNA when ribosomes were present, as compared to 2,300 cpm in their absence. The RNA synthesized in the control incubation, without ribosomes (open circles), was recovered in the upper part of the gradient with a peak at fraction 24. Most of the DNA and the RNA polymerase activity is found in this area. When ribosomes were present in the incubation, the RNA synthesized (closed circles), sedimented slightly faster than that from control incubations, and a considerable portion was associated with the gradient region corresponding to the ribosomes.

When incubations were carried out in the absence of ribosomes, and ribosomes were added before gradient centrifugation, ribosome-RNA aggregates were formed as

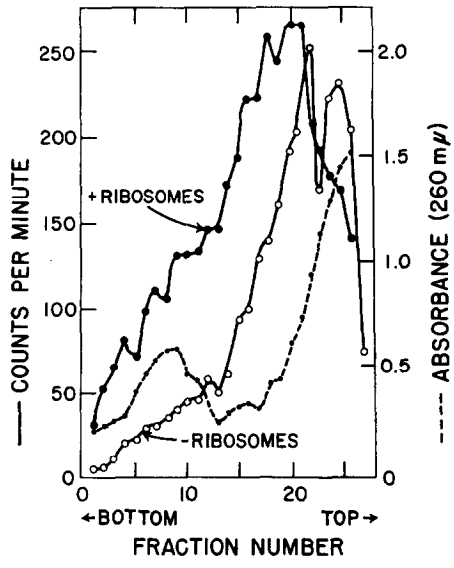

Figure 2: Sucrose gradient centrifugation of incubations of RNA polymerase-DNA complex in the presence (closed circles) and absence (open circles) of ribosomes. Incubations, as described above, contained 0.055 mg protein of the RNA polymerase-DNA preparation and 0.078 μmoles of C^{14}-UTP. Ribosomes were added to the control incubations, without ribosomes, before centrifugation. Time of centrifugation, 5 hours.

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shown in Figure 2 (open circles). However, the extent of polyribosome formation, as well as the total amount of RNA synthesized, were increased considerably when ribosomes were present throughout the incubation period (closed circles). It is interesting to note that most of the RNA synthesized in the incubation with ribosomes, including that recovered between the ribosomes and the top of the gradient, sediments with higher S values than that from control incubations. Very little of the ribosome-induced RNA is associated with the peak corresponding to that in the non-ribosome control. The nature of this material, possibly high molecular weight nascent RNA attached to RNA polymerase-DNA complex or to ribosomal sub-units, remains to be determined.

The in vitro results presented here indicate that ribosomes stimulate the synthesis

of RNA and the concomitant formation of ribosome-RNA aggregates. They are consistent with the following suggestions (Bremer and Konrad, 1964; Byrne et al, 1964; Stent, 1964): ribosomes are attached to nascent RNA before it is completed and released from the enzyme-template complex; this attachment is essential for the release of RNA, in the form of polyribosomes; the transient aggregate consisting of ribosomes, nascent RNA, RNA polymerase and DNA synthesizes RNA more extensively than in the absence of ribosome These results may reflect the ribosome-controlled process proposed for genetic transcription within the cell.

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