THE STIMULATION BY RIBOSOMES OF DNA TRANSCRIPTION : REQUIREMENT FOR A TRANSLATION FACTOR

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Much experimental evidence now support the original proposal by Stent (1964) that ribosomes are involved in the synthesis and release of RNA from its DNA template. In vivo experiments with extreme polar suppressible mutations (Beckwith, 1964, Contesse et al, 1966) and with ribosome depleted bacteria (Naono et al, 1966) have provided strong indication that messenger RNA was not produced if it could not form a specific complex with ribosomes. In vitro, the formation of DNA-RNA-ribosome complexes demonstrated by Byrne et al (1964) was the first proof that ribosomes could indeed become attached to the mRNA before it was released from its DNA template. Recently, Shin and Moldave (1966) clearly demonstrated that RNA synthesis by a deoxyribonucleoprotein preparation from E. coli was stimulated by the addition of ribosomes. Nevertheless, such a stimulation was not observed when purified DNA and RNA polymerase were used. This suggested that some other factor was required besides RNA polymerase and ribosomes in order to stimulate DNA transcription. In the course of studies with an in vitro system in which T_4 DNA transcription is coupled to translation (Wood and Berg, 1962) we have described the isolation from an E. coli deoxyribonucleoprotein fraction of a translation factor, specific for natural mRNA, which is required for the formation of T_4 DNA-RNA-ribosome complexes (Revel and Gros, 1966). In this communication, we present evidence that T_4 DNA transcription by purified E. coli RNA polymerase is strongly stimulated by ribosomes in the presence of this translation factor but not in its absence. Hence, a protein factor necessary for the translation of mRNA into protein appears to be involved in the control of mRNA production.

MATERIAL AND METHODS

T_4 DNA was purified according to Berns and Thomas (1965). Highly purified RNA polymerase was a gift from Dr. J.Richardson. ^{3}H-CTP was purchased

from Schwartz Biochem. and C^{14}-valine from the CEA. Electrophoretically purified DNAse was obtained from Worthington.

Translation factors were isolated as outlined in the text. Ribosomes and "S-110" free of these factors were prepared and T_4 DNA dependent valine incorporation was measured as described previously (Revel and Gros, 1966).

RNA synthesis was measured in 0.05 ml incubation mixtures containing tris-HCl pH 7.5 0.04 M, $MgCl_2$ 0.02 M, NH_4Cl 0.13 M, β-mercaptoethanol 6 mM, ATP 2 mM, GTP UTP H^3-CTP (9×10^6 cpm/μmole) 0.4 mM, phosphoenolpyruvate 4 mM, pyruvate kinase 5 μg, T_4 DNA 2 μg, RNA polymerase 1.2 μg, crystalline bovine serum albumin 15 μg, ribosomes and factors as indicated. Incubation was for 30 minutes at 34°C. The reaction was stopped with 5% TCA, 1 mg bovine serum albumin was added and the samples were filtered through Whatman glass paper (GF/B), washed with 5% TCA and with alcohol, dried and counted in a Tricarb scintillation counter.

The fate of the nascent RNA was studied by sucrose gradient analysis. Transcription was carried out under the above conditions for 30 minutes (for DNase treatment, 2 μg of DNase was then added and incubation prolonged 5 min) and the samples were diluted with 0.2 ml tris pH 7.5 0.01 M, $MgCl_2$ 0.02 M, NH_4Cl 0.06 M and layered on 5-20% sucrose gradients in the same buffer. Centrifugation was for 90 minutes at 35,000 rpm in Spinco SW 39. Fractions were collected, precipitated with TCA and counted as above.

RESULTS

1. Separation of two components of the translation factor

In our previous work, the factor used was a crude preparation rich in *E. coli* DNA and RNA polymerase and was not suitable for studies on T_4 DNA transcription. The proteins from this preparation were extracted by a polyethylene glycol-dextran phase system (Okazaki and Kornberg, 1964) and applied on a DEAE cellulose column (2.2 x 15 cm), equilibrated in 0.1 M NH_4Cl, $MgCl_2$ 2×10^{-3} M, tris pH 7.5 10^{-2} M, β mercaptoethanol 6×10^{-3}M, from which two fractions were obtained by stepwise elution : fraction "B" at 0.16 M NH_4Cl and fraction "C" at 0.28 M NH_4Cl. The effect of these fractions on T_4 DNA dependent amino-acid incorporation is shown in Table 1. Very little valine incorporation was seen in the absence of factor. Crude factor produced a 12 fold increase. Fraction B or C alone had very little activity; however, when added together to the *in vitro* system, B and C had all the activity present in the crude factor. These factors had virtually no effect on poly U dependent phenylalanine incorporation. RNA polymerase was not extracted by the phase system in our conditions and none of the purified fractions had RNA polymerase activity. In some experiments, factor "C" was further purified on Sephadex G-200.

Table 1. Conditions were as described in Revel and Gros (1966). Endogenous incorporation (3 µµmoles valine) was substracted from the DNA stimulated values.

T_4 DNA dependent Valine incorporation

	µµmoles valine	per cent
No Factor added	2·6	8
+ Factor B (100µg)	3·9	12
+ Factor C (100µg)	6·2	19
+ Factor B + Factor C	32·1	98
Crude Factor (200µg)	32·8	100

2. Effect of the translation factors and ribosomes on T_4 DNA transcription

RNA synthesis by purified E. coli RNA polymerase on T_4 DNA template was measured under the ionic conditions used to study the formation of DNA-ribosome complexes (see methods). Fig. 1A shows that the amount of RNA synthesized in 30 minutes is somewhat increased when ribosomes are present

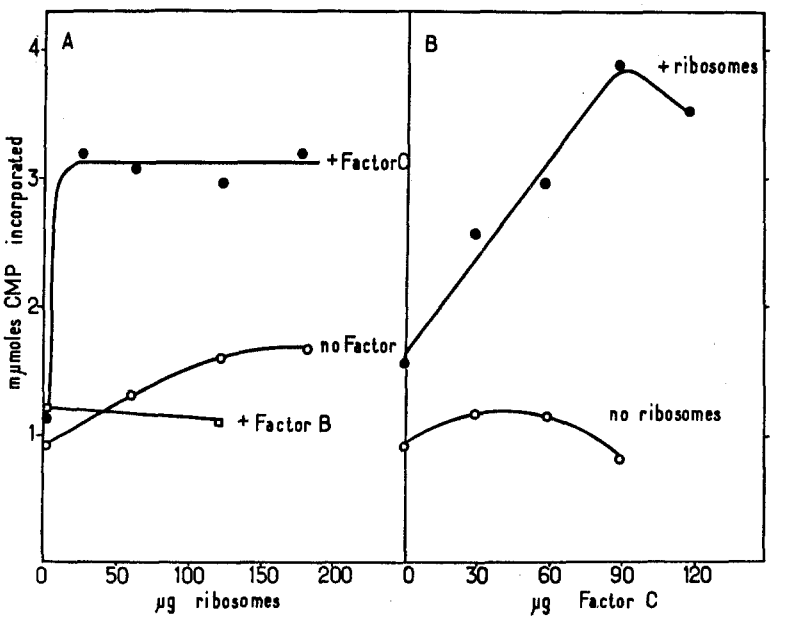

Figure 1. The effect of ribosomes and Factor C on T_4 DNA transcription
Conditions are indicated in methods. A : Effect of ribosomes in the absence of factors (open circles), with 60 µg Factor C (closed circles) or 100 µg Factor B (squares). B : Effect of Factor C in the absence of ribosomes (open circles) and in the presence of 120 µg ribosomes (close circles).

but this increase is over 4 times larger when factor C is added with the ribosomes. Addition of factor B had no effect. Fig. 1B shows that in the absence of ribosome, factor C alone had little stimulatory activity but when ribosomes were present, RNA synthesis increased linearly with the amount of factor C added.

Table 2 indicates that the synthesis of RNA stimulated by ribosomes and factor C was dependent on the presence of T_4 DNA and RNA polymerase. Addition of factor B and C together never gave more stimulation than C alone.

Table 2. The complete reaction mixture was as described in Methods with 120 μg ribosomes and 60 μg Factor C.

T_4 DNA dependent RNA synthesis

	mμmoles CMP incorp.	per cent
complete	3·0	100
omit T_4 DNA	0·12	4
omit RNA polymerase	0·30	10
omit Factor C	1·41	47
omit ribosomes	1·15	38
omit Factor C and ribosmes	0·90	30
plus Factor B	2·10	71

The kinetics of RNA synthesis with T_4 DNA are presented in Fig. 2. When RNA polymerase alone was used, RNA synthesis proceeded linearly for over 45 minutes. Addition of ribosomes increased slightly the initial rate of synthesis. Factor C alone produced a 2 fold increase in the initial rate but synthesis slowed down after 5 minutes and was virtually stopped at 20 minutes. Addition of ribosomes in the presence of factor C produced a further increase in the initial rate but now synthesis was linear for more than 30 minutes. Under these conditions, the extent of RNA synthesis was more than 100 per cent higher than in the non-stimulated system.

The involvement of ribosomes and factor C in DNA transcription is even clearer when the RNA produced was analysed by sucrose gradient centrifugation, as shown in Figure 3. In the absence of ribosome and factor (Fig. 3A), the newly made RNA was found in a broad peak of about 50 S which

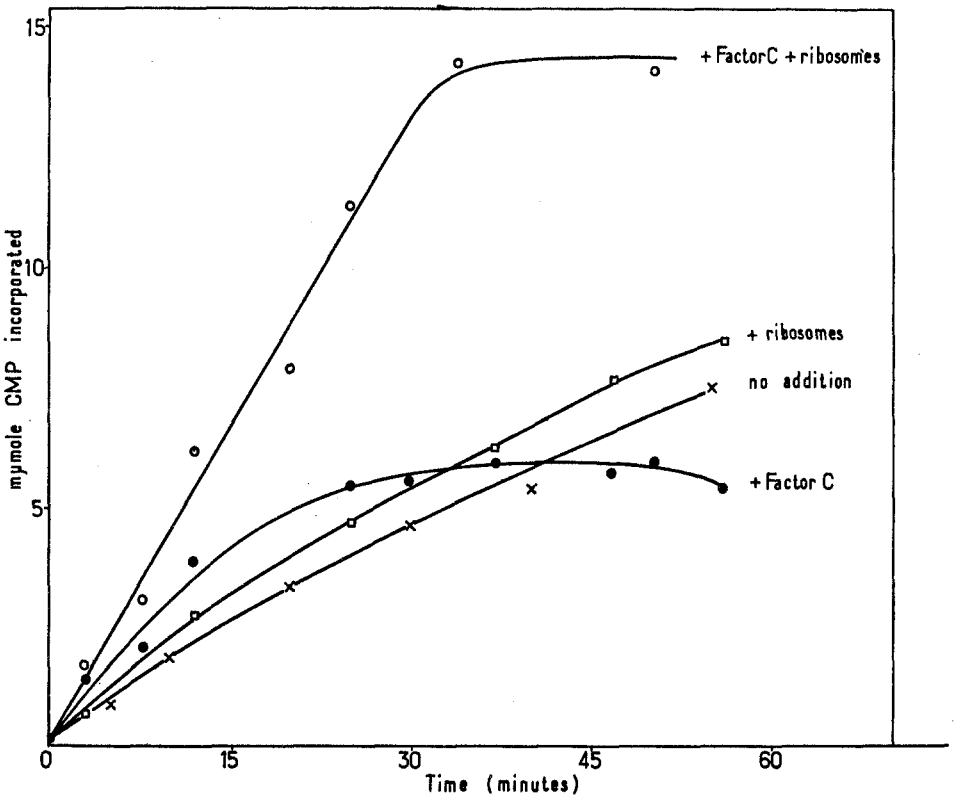

Figure 2. Kinetics of T_4 DNA transcription by E. coli RNA polymerase
A 0.125 ml incubation mixture was used with the components described in Methods. Samples were taken at various times of incubation. RNA polymerase alone (crosses), with 300 μg ribosomes (squares), 150 μg of Factor C (closed circles) or both (open circles).

corresponds to the T_4 DNA-RNA complexes described by Bremer and Konrad (1964) (accordingly, when sodium dodecylsulfate was added to this preparation little RNA heavier than 15 S was present). When RNA synthesis was carried out in the presence of ribosomes and factor C (Fig. 3B), a large portion of the newly formed RNA was found at the bottom of the centrifuge tube in heavy aggregates, sensitive to DNase, which most likely represent the DNA-RNA-ribosome complexes observed before (Revel and Gros, 1966). Some newly formed RNA was found bound to 70 S ribosomes. An appreciable amount of the radioactive RNA also sediments in the upper part of the gradient where two fractions are visible. One is sensitive to DNase and must be RNA still attached to DNA while the other appears to be RNA released from its DNA template. After DNase treatment, RNA accumulates in this latter fraction and also in a peak at about 55 S. These findings, which were confirmed by other

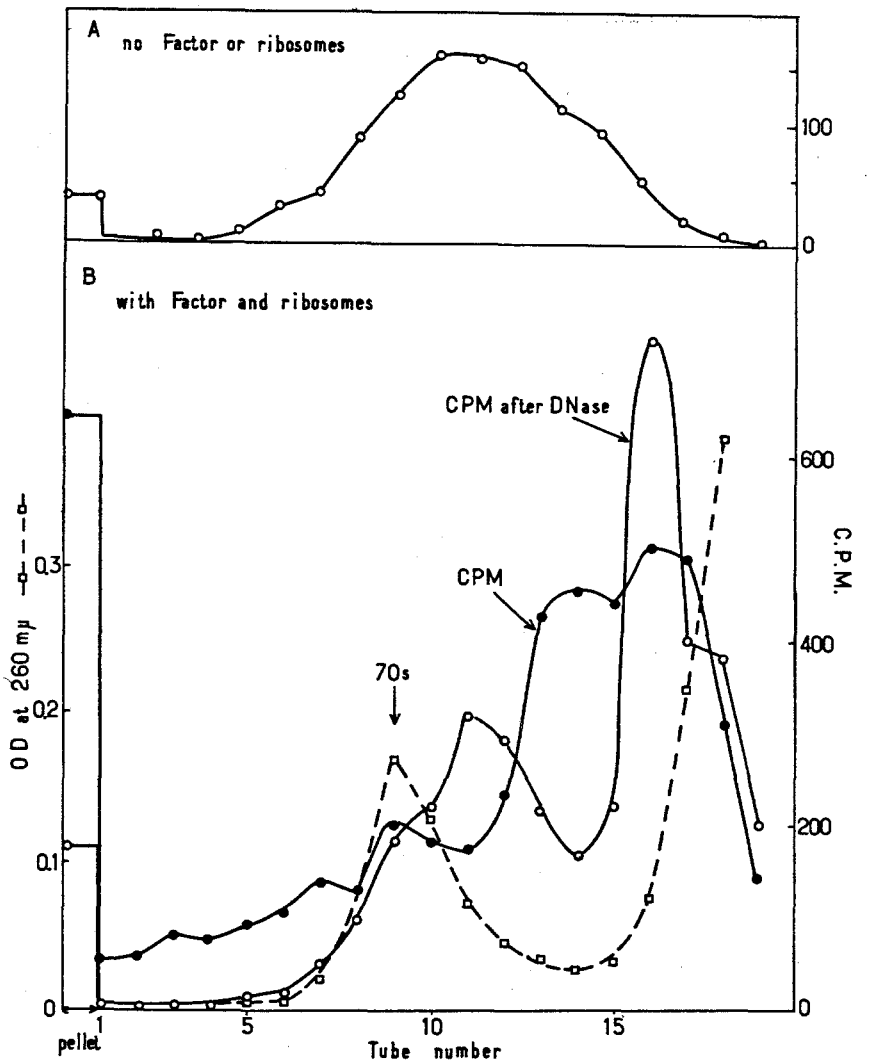

Figure 3. Sucrose gradient analysis of the incubation mixture after RNA synthesis. A : H^3-RNA formed in 30 minutes on T_4 DNA with RNA polymerase alone (open circles). B : H^3 RNA formed in 30 minutes in the presence of 60 μg ribosomes and 75 μg Factor C (closed circles). In another tube (open circles), at the end of incubation, 2 μg of DNase were added and incubation prolonged 5 minutes. All three samples were analysed as described in Methods.

experiments with labeled T_4 DNA, suggest that stimulation of DNA transcription is accompanied both by the formation of large DNA-RNA-ribosome aggregates and by the release of newly formed RNA from its template. These phenomena were observed only when both ribosome and factor C were present.

DISCUSSION

Translation of natural mRNA requires two protein factors which are

not necessary for the translation of synthetic polynucleotides lacking the initiation codon (Stanley et al, 1966, Brawerman and Eisenstadt, 1966, Revel and Gros, 1966). In our experiments, ribosomes free of these factors were prepared by centrifugation at 80,000 x g of non DNase-treated E. coli extracts, and both factors were isolated from a deoxynucleoprotein fraction sedimented at 100,000 x g. The mode of action of these two factors has not yet been established. Their simultaneous addition is required for natural mRNA dependent protein synthesis. An unfractionated mixture of these factors was found to stimulate specifically the binding of met-s-RNA_F to ribosomes (at 15 mM Mg^{++}) in the presence of T_4 mRNA (unpublished results). Stimulation of met-s-RNA_F binding to ribosomes by their factors has also been recently reported by Salas et al (1967) and by Clark and Dahlberg (1967). It is likely that the two factors we are studying, although they are prepared in a different way, are similar to those obtained by treating ribosomal preparation with high concentration of salt.

The experiments presented here show that DNA dependent RNA synthesis is strongly stimulated by ribosomes when one of the two initiation factors, factor C, is present. The other factor is not required for this effect. This is the first indication that the factors have independent functions. Either ribosome or factor C alone produce very little effects. When both ribosome and factor C are present, heavy aggregates containing DNA and newly made RNA are formed probably through the attachment of ribosomes to the nascent mRNA chains. Some RNA is released from its template in contrast to what is seen in the non stimulated reaction (Bremer and Konrad, 1964). The exact mechanism by which RNA synthesis is stimulated remains nevertheless obscure. The fact that the initiation factor promotes the translation of free mRNA (e.g. viral RNA) suggests that the effect on transcription is secondary to an effect on mRNA-ribosome interaction. In conclusion, the existence of this factor which is found in association with cell DNA provides an additional mechanism by which the cell can coordinate the rates of protein and RNA syntheses.

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