BINDING OF TERMINATION FACTOR RHO TO RNA POLYMERASE AND DNA

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1. Introduction

Meaningful chain initiation by *E. coli* RNA polymerase requires the protein factor σ, normally found in a tight association with the enzyme [1]. On the other hand, a termination factor, named rho (ρ) [2], causes the termination of RNA synthesis at appropriate sites on the DNA template. The functional relationship between the initiation and termination events lead us to study the molecular mechanism of chain termination and particularly the possible interaction of ρ with RNA polymerase and DNA. First, the binding of ρ to T_7 DNA was demonstrated and second, a complex of ρ and RNA polymerase was isolated by polyacrylamide gel electrophoresis.

2. Material and methods

The ρ factor was purified according to Roberts [2] with slight modifications: the DEAE step was omitted and replaced by a 10–30% glycerol gradient centrifugation conducted for 12 hr at 65,000 rpm in a buffer containing 0.05 M potassium phosphate, 10^{-4} M DTT, 10^{-4} M EDTA. Under these condition, ρ was purified close to homogeneity. Active fractions were concentrated by dialysis against 60% glycerol containing 0.05 M tris pH 8, 10^{-4} M EDTA and 10^{-4} M DTT. RNA polymerase was purified and assayed as already reported [3] except that the KCl concentration of the assay was lowered to 0.05 M. Labelled and unlabelled T_7 DNA was prepared in our laboratory by J.P.Dausse. Polyacrylamide gel electrophoreses were carried out as already reported [3].

3. Results and discussion

3.1. *Interaction between ρ factor, RNA polymerase and T_7 DNA*

The ability of the ρ factor to bind to RNA polymerase and T_7 DNA was investigated using polyacrylamide gel electrophoresis. This method has already proved very useful to show the complex formation between core enzyme and σ factor and the binding of core enzyme to T_4 DNA [1, 3].

Fig. 1-A shows that purified ρ factor migrates poorly in two broad and ill-defined bands in 5% polyacrylamide gels. When DNA is added to the factor no protein bands are seen in the gel, but the material remains on the top of the gel demonstrating the binding of ρ to the nucleic acid (not shown). When the complex ρ-DNA is degraded by pancreatic DNase, a new rapidly migrating band appears in the gel (fig. 1-C). This result is very similar to the behavior of RNA polymerase under the same conditions (fig. 1-D) [see 3]. Mixing the ρ factor and RNA polymerase containing σ does not give rise to the respective protein bands (fig. 1-E). Instead one sees a new band of intermediate mobility probably representing a complex of ρ and RNA polymerase.

The last experiment shows the separation of a mixture containing ρ, RNA polymerase and T_7 DNA, after treatment with pancreatic DNase. One recognizes the different components already cited, namely, from top to bottom, (ρ-DNA), (ρ-RNA polymerase), RNA polymerase, (RNA polymerase-DNA). This shows that ρ was bound to both DNA and RNA polymerase.

Similar experiments using the core enzyme instead of complete RNA polymerase were difficult to interpret due to the similar migration properties of the ρ factor and core enzyme and no strong evidence could be found for a complex between the two proteins.

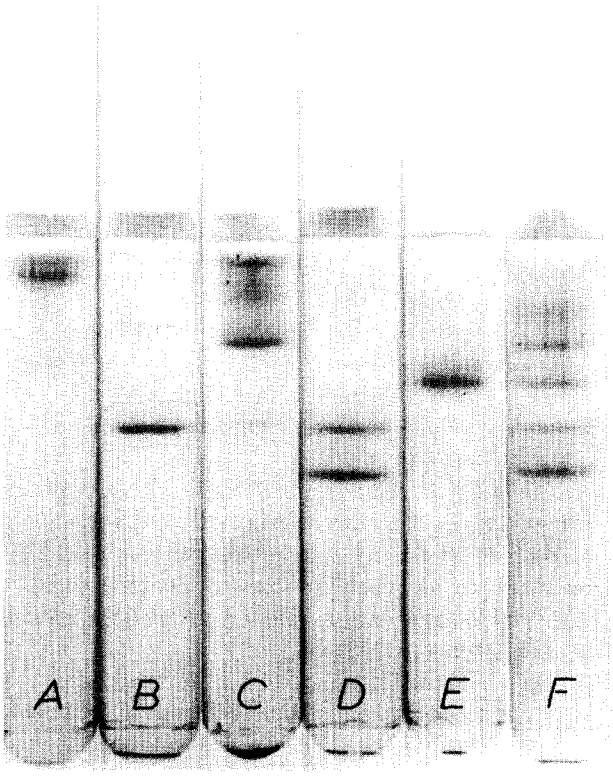

Fig. 1. Binding of ρ to RNA polymerase and T_7 DNA as seen by polyacrylamide gel electrophoresis. (A) ρ factor (6 μg); (B) RNA polymerase (12 μg); (C) ρ factor and T_7 DNA (9 μg) then pancreatic DNase; (D) RNA polymerase and T_7 DNA then pancreatic DNase (0.5 μg) [see 3]; (E) ρ factor and RNA polymerase; (F) ρ factor, RNA polymerase, T_7 DNA then pancreatic DNase.

3.2. *Study of the complex between ρ and RNA polymerase*

The question was then asked whether σ is present or not in the complex between ρ and RNA polymerase and how many subunits of ρ are involved in forming this complex. The first question was answered by cutting out the ρ-RNA polymerase complex band before, or even after staining with Coomassie blue, and rerunning the protein on a 0.1% SDS gel to dissociate the subunits of RNA polymerase. The experiment is shown in fig. 2-A together with a control gel containing purified RNA polymerase (fig. 2-B). One can see in the gel that the isolated band contains all the subunits of RNA polymerase and a sharp band, the molecular weight of which was calculated to be

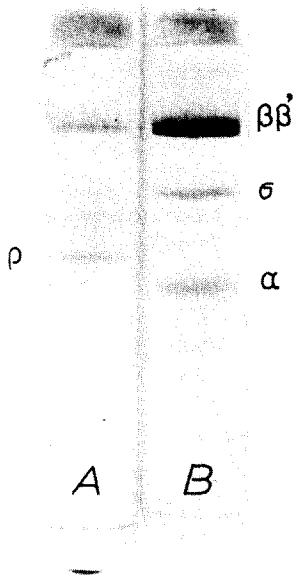

Fig. 2. Analysis of the ρ-RNA polymerase complex by polyacrylamide gel electrophoresis in 0.1% SDS. (A) Three protein bands as seen in fig. 1-E were pooled and rerun on SDS gel [1]; (B) RNA polymerase.

50,000 daltons, corresponding to the subunits of ρ. The amount of ρ present corresponds roughly to the sum of β and β' subunits based on the density of absorbed Coomassie brillant blue.

Thus a complex exists between ρ and RNA polymerase containing all the subunits of RNA polymerase (ρ-E^{σ}). To estimate the number of subunits involved in this complex, its molecular weight was estimated by gel electrophoresis according to the method of Hedrik and Smith [4]. This method was already used to estimate the molecular weight of RNA polymerase (E^{σ}), σ, and core enzyme (E) and of the complex E-DNA obtained by DNase treatment [4]. The molecular weight of the (ρ-E^{σ}) complex was found to be 750,000 ± 5% daltons which indicates that 5 (±1) subunits of ρ are present in the complex.

The ability of ρ to bind both RNA polymerase or DNA raises the question concerning the molecular mechanism of RNA chain termination. When the DNA binding property of ρ is followed at different ionic strengths with the conventional nitrocellulose membrane technique [5] it is observed that 2 μg of ρ are needed to achieve total retention of 2 μg of ^{3}H-T_7 DNA on the nitrocellulose filter. In addition

retention of T_7 DNA is only reduced 2 fold by 0.17 M KCl which totally inhibits chain termination by the factor. Complete inhibition of the DNA retention by ρ is obtained at 0.3 M KCl.

3.3. *Cyclic reuse of the termination factor*

The strikingly low amount of factor needed to saturate RNA polymerase in order to get maximum depression of total RNA synthesis [2] led us to think that, as in the case of the initiation factor σ, there might be a recycling of the termination factor. That this is the case is shown in fig. 3. Kinetics of RNA synthesis was followed with ρ factor at *limiting concentration* or without factor. As extra RNA polymerase and T_7 DNA are added every 20 min, the ratio of RNA polymerase to ρ goes from 20 (by weight) to 100. The results demonstrate that the same level of depression of total synthesis is obtained through all the kinetics showing that the ρ factor is not depleted during the first round of RNA synthesis and can be reused by additional RNA polymerase molecules.

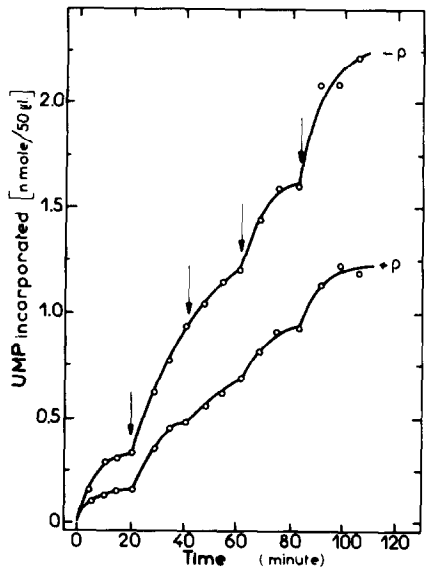

Fig. 3. Cyclic reuse of termination factor. Two incubation mixtures (1 ml) containing RNA polymerase (20 µg), T_7 DNA (12 µg), standard salts and nucleotide [3] and ^{3}H-UTP (16,000 cpm/nmole), are incubated with or without ρ (1 µg) at 37°. Aliquots of 50 µl are taken at different times, the RNA is precipitated with 5% TCA and counted. At 22 min, extra RNA polymerase (16 µg) and T_7 DNA (9.2 µg) were added; later on (arrows) RNA polymerase (8 µg) and T_7 DNA (4.6 µg) were again added.

4. Discussion

As already noted in the original report of Roberts little is known on the molecular mechanism of RNA chain termination [2]. A number of possibilities were put forward by this author implying either the binding of the factor to DNA alone or to RNA polymerase alone, or its interaction with the transcription complex when it reaches a specific stop signal. The ability of the ρ factor to form a complex with RNA polymerase was demonstrated in this work using polyacrylamide gel electrophoresis. The complex has a molecular weight of 750,000 ± 5% and includes σ, β, β' and α and 5 subunits of ρ. The ρ factor is able also to bind to T_7 DNA as can be seen on polyacrylamide gels and by conventional nitrocellulose filter technique, and to a variety of polynucleotides (J.S.Krakow, personal communication). We observed that the efficiency of ρ is independent of the amount of DNA used; moreover the factor is able to bind DNA in salt conditions which prevent RNA chain termination. These observations suggest that the DNA is not the target of ρ but rather that the factor acts through its binding to RNA polymerase. As far as we know purified RNA polymerase preparations do not contain ρ. In fact, in order to understand the low ratio of factor to polymerase needed to get maximum depression of RNA synthesis and the cyclic reuse of the factor observed, we believe that the loose association between factor and polymerase becomes tighter and effective when the transcriptional system reaches a stop signal on the DNA. Then the transcription process is stopped and the RNA chain is released together with the RNA polymerase and the factor. It should be stressed that at the low salt concentration required by the ρ factor the polymerase binds to the RNA product and is unable to reinitiate. That is why a depression of total RNA synthesis is seen in the presence of ρ. Thus the transcription in vitro system should be considered still incomplete until the reinitiation requirement is met in the presence of ρ. In conclusion one will note the striking similarities in behavior of the σ and ρ factors: both bind to RNA polymerase, dictate specific information to the transcriptional machinery and can be reused a number of times. As such they can be considered not like mere 'subunit' but as 'specifier protein' modulating the activity of core enzyme.

References

[1] R.R.Burgess, A.A.Travers, J.J.Dunn and E.K.F.Bautz, Nature 221 (1969) 43.
[2] J.W.Roberts, Nature 224 (1969) 1168.
[3] J.L.Darlix, A.Sentenac, A.Ruet and P.Fromageot, European J. Biochem. 11 (1969) 43.
[4] A.Ruet, A.Sentenac and P.Fromageot, FEBS Letters 3 (1970) 169.
[5] O.W.Jones and P.Berg, Proc. Natl. Acad. Sci. U.S. 56 (1966) 1026.