DNA-DEPENDENT RNA POLYMERASE FROM E. COLI: STUDIES ON THE ROLE OF σ IN CHAIN INITIATION

By

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Summary

The pyrophosphate exchange reaction catalyzed by E. coli RNA polymerase on phage T4 and calf thymus DNA templates was studied as a measure of synthesis of the initiating dinucleotide. The results indicate that the E. coli σ protein functions at a step prior to the completion of the first phosphodiester bond. Furthermore, incorporation of ATP into the 5' terminus of RNA chains appears to be more strongly dependent on σ than incorporation of GTP.

Introduction

Transcription of phage T4 DNA in vitro by E. coli RNA polymerase requires the function of the σ protein, which is normally associated with, but can be removed from, the basic unit of the RNA polymerase (core enzyme) (Burgess, Travers, Dunn and Bautz, 1969). The σ protein appears to be involved in initiation but not in chain elongation; it can switch DNA templates and core enzyme molecules and can thus function catalytically (Travers and Burgess, 1969). Recent studies by Krakow and Fronk (1969) have suggested that the pyrophosphate (PPi) exchange reaction by RNA polymerase from Azotobacter on a synthetic DNA template readily proceeds in the absence of RNA chain elongation if only the first phosphodiester bond is allowed to

form. We have used this exchange reaction as a tool to study initiation of transcription by E. coli RNA polymerase on T4 DNA in the presence and absence of σ, and we wish to report data suggesting that σ acts at some step prior to the completion of the first dinucleotide.

Materials and Methods

Nucleoside triphosphates were purchased from Boehringer Mannheim and purified on Dowex 1-chloride using linear LiCl gradients in 0.01 N HCl. No contaminating nucleoside triphosphates were found, and similar results were obtained using the commercial preparations or those purified by chromatography.

$Na^{32}PPi$ was purchased from New England Nuclear Corp. The assay for the pyrophosphorolytic reaction resulting in PPi exchanged into Norit adsorbable material was essentially the same as that of Krakow and Fronk except that the membrane-retained Norit was counted in a liquid scintillation counter. The presence of Norit in the toluene scintillation system resulted in approximately a 30% reduction in counting efficiency.

The isolation of DNA-dependent RNA polymerase from E. coli BA and the properties of the DC and PC forms have been previously described (Burgess, et al., 1969, and Bautz and Dunn, 1969). The enzymes used in these studies, when assayed in the presence of PPi, had activities (mμmoles UMP incorporated/10 min/mg enzyme) of: DC, 1000, and PC, 100, with T4 DNA, and 800 and 350, respectively, with CT DNA.

Results and Discussion

As shown in Table I, PPi exchange was observed under conditions of RNA synthesis, i.e., in the presence of all four nucleoside triphosphates with either T4 or calf thymus DNA as template. In the absence of σ,

Table I

Exchange of ^{32}P-inorganic pyrophosphate with nucleoside triphosphates by E. coli RNA polymerase in the presence (DC enzyme) and absence (PC enzyme) of σ[1]

Nucleoside triphosphate added	T4 DNA template				CT DNA template			
	DC	PC	PC+σ	σ	DC	PC	PC+σ	σ
ATP+UTP+CTP+GTP	2.5 (30)[2]	0.47 (3)	2.87	0.05	2.7 (30)	1.26 (13)	2.64	0.06
ATP+UTP	1.32 (0)	0.16 (0)	1.36	0.06	4.49	0.97	4.33	0.14
ATP+GTP	0.26	-			2.24	1.44		
ATP+CTP	0.74	0.04			1.62	0.46		
CTP+UTP	0.63	0.10			3.12	1.55		
GTP+CTP	0.29	0.08			1.25	0.67		
UTP+CTP	0.21	-			1.37	0.75		
ATP	0.07	-			0.63	0.18		

[1] mμmoles PPi exchanged in 10 min at 37° by 10 μg of each enzyme component (PC+σ = 20 μg) in a final volume of 0.25 ml containing: 0.05 M Tris pH 7.9, 0.01 M $MgCl_2$, 0.005 M 2-mercaptoethanol, 0.15 M KCl, 0.1 mM EDTA, 0.5 mg/ml BSA, 250 mμmoles $Na^{32}PPi$ (3000 CPM/mμmole), and 100 mμmoles of the indicated nucleoside triphosphate. T4 and CT DNA concentrations were 15 and 35 μg/0.25 ml, respectively.

[2] Values in parentheses represent total acid precipitable nucleotide incorporated (measured as UMP and corrected for base ratios of the templates).

the PPi exchange activity is reduced to a level proportional to that observed with the incorporation assay. Addition of σ fully restored the capacity to exchange PPi. In the presence of only ATP and UTP comparable levels of exchange were observed while no ^{3}H-UTP was found incorporated into acid insoluble material under these conditions. Again, the PPi exchange is strongly reduced in the absence of σ but high if σ is added back to the PC enzyme. Since Krakow and Fronk (1969) have shown that poly d(A-T) directed PPi exchange readily proceeds at the level of the first dinucleotide formed, it is probable that in the presence of T4 DNA and ATP+UTP the exchange occurs primarily at the level of the dinucleotide pppApU. Furthermore, as in the poly d(A-T) system, the PPi is exchanged predominantly with UTP as evidenced by the fact that on Dowex 1-chloride chromatography 80% of the charcoal adsorbable ^{32}P counts were recovered in the UTP peak. Thus we conclude that σ functions at some step prior to the completion of the first phosphodiester bond.

Of the pairs of nucleoside triphosphates tested with T4 DNA, A+U promote the highest PPi exchange, with A+C and G+U yielding intermediate values, whereas low levels of activity were found with the pairs A+G, G+C, and U+C, or with A alone. These data are in agreement with the findings of Maitra, Nakata, and Hurwitz (1967) that the first nucleotide incorporated is always a purine, that the second nucleotide is preferentially a pyrimidine, and that, on T4 DNA as a template, the frequency of A vs. G found at the 5' terminus is about 2:1. In addition, our data suggest that T4 RNA chains, made by complete enzyme, start most frequently with the sequence pppApU.

On calf thymus DNA as template, the PPi exchange in the presence of all four triphosphates again reflects the respective synthetic activities

of DC and PC enzymes. A comparison of the PPi exchanges observed in the presence of the different nucleotide pairs indicates that the requirement for σ is more obvious if the purine nucleotide is A rather than G. This result complements the findings of Berg, et al. (1969), and Chamberlin (personal communication) who observed that, using T7 DNA as template, the ratio of incorporation of γ-labeled ATP vs. GTP decreased as σ was removed from the enzyme.

Since σ appears to function prior to the completion of the first internucleotide bond, it could help initiation in three ways: 1) by enhancing the binding of polymerase to template; 2) by facilitating the binding of the first PuTP, and especially of ATP, to the polymerase-DNA complex (the product terminus site in the model of Krakow and Fronk, 1969); and 3) by helping the binding of the second nucleoside triphosphate to the substrate site The first possibility appears to be the least likely, since both DC and PC enzymes appear to bind equally well to T4 DNA. The second possibility is favored by di Mauro, et al. (1969), on the basis of their studies on cross resistance of PC enzyme and σ to rifampicin. A more crucial test would be to determine directly the binding of ATP to the DNA-polymerase complex in the presence and absence of σ. These studies are currently in progress.

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