ALTERATION OF RNA POLYMERASE DURING TRANSCRIPTION OF PHAGE λ DNA "IN VIVO"

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SUMMARY

λ DNA-RNA polymerase complex was isolated from λ-infected E. coli at a late stage of phage development. The polymerase was separated from the λ DNA and characterized with regard to the presence of σ-like initiation factor. The data support the hypothesis that initiation factor dissociates from RNA polymerase following the initiation of RNA synthesis.

DNA-dependent RNA polymerase (RP) from Escherichia coli has recently been shown to contain several different protein subunits (Walter et al., 1968; Burgess et al., 1969). One of these, called σ, can be dissociated from RP by passing the enzyme through a phosphocellulose column, leaving a "minimal" or "core" enzyme (PC). The "core" enzyme has considerably less activity on T4 DNA than on other templates (Burgess et al., 1969). The σ subunit, when added to PC, stimulates RNA synthesis to varying extents with different DNA templates, ranging from over 60-fold on T4 DNA to 2-fold on calf thymus DNA (Burgess et al., 1969). Three other properties of σ are relevant to the present report: 1) σ stimulates RNA synthesis at the initiation step prior to the completion of the first phosphodiester bond (Dunn and Bautz, 1969); 2) After the initiation step "in vitro", σ is released from the RP and can be utilized by another polymerase molecule to start a new RNA chain (Travers and Burgess,

1969); 3) RNA polymerase isolated from T4-infected cells is modified in at least one of its subunits (Walter et al., 1968) and is lacking σ (Bautz and Dunn, 1969).

Evidence will be presented that indicates that σ dissociates from RP after initiation of phage λ RNA synthesis "in vivo". However, unlike the case of T4 infection, the majority of RP isolated after λ development contains σ.

MATERIALS AND METHODS

Growth of bacteria, preparation of phage stocks and DNA, and conditions for infection were as described previously (Green, 1966; Green et al., 1967). The strains of E. coli used were W3110 and its lysogenic derivative, W3110($\lambda c_{I,U32}$) (Lieb, 1966).

"Late complex" containing λ DNA and RNA polymerase (RP) was isolated from W3110 at a late stage of λ development (18 min at 37°C in K medium). The method of isolation of λ complex and its characterization have recently been described (Hayward and Green, 1969 a,b). The late complex is transcribed "in vitro" predominately from the left half ("late region") of the λ genome (Chesterton and Green, 1968) and contains covalently closed circular λ DNA (Chesterton, unpublished; Hayward and Green, 1969b).

The following procedure was established for the isolation of RP from late complex. The peak fraction (3-5 ml) from a sucrose gradient purification of late complex (Chesterton and Green, 1968) was diluted (in order to reduce the sucrose concentration) with approximately 60 ml of TEDB buffer [0.01 M Tris-HCl, pH 7.9, 10^{-4} M EDTA, 10^{-4} M dithiothreitol, 0.1 mg/ml bovine serum albumin (BSA)] and concentrated by centrifugation in a Spinco SW25.2 rotor for 4 hours at 24,000 rpm at 4°C. The bottom 1.5-1.8 ml was adjusted to 5% glycerol, 0.015 M $MgCl_2$. The DNA was degraded by incubation (10 min at 15°C) with DNase I and snake venom phosphodiesterase (Worthington Biochemical Corp.) and pancreatic RNase (Sigma Chemical Co., Type 1-A) (30 μg each, weighed immediately before use) in the presence of a limiting amount of ATP, GTP, UTP and CTP (0.9 μM each). The mixture was then layered onto a 10-30% linear glycerol gradient in TEDB and

centrifuged for 12 hours at 33,000 rpm in a Spinco SW41 rotor at 2°C. Fractions (0.8 ml) were collected into an equal volume of ice cold 50% glycerol and assayed for RP activity. The enzyme sedimented as a single peak approximately half way through the gradient and was stimulated at least 20-fold by exogenous calf thymus DNA. Peak fractions were stored in 50% glycerol at -20°C.

When assayed on calf thymus DNA, at least 25% of the RP activity of the λ complex could be recovered. This figure is quite arbitrary, however, since the activity varies with different templates and can be markedly stimulated by the addition of σ factor (see Results).

Assay mixtures for RP contained 0.025 M Tris-HCl, pH 7.9 at 25°C, 0.01 M $MgCl_2$, 0.005 M 2-mercaptoethanol, 0.16 M KCl, 0.18 mM ATP, GTP and CTP, 2 μM H^3-UTP (4 C/mmole, Schwarz BioResearch, Inc.), 5 μg of DNA, and 0.1 ml of enzyme in a final volume of 0.25 ml. Due to the glycerol in the enzyme storage buffer, the final concentration of glycerol was 20%. The addition of RP to DNA was in 0.2 M KCl, thereby favoring specific binding (Pettijohn and Kamiya, 1967). Calf thymus (CT) DNA was obtained from Calbiochem. The reaction mixtures were incubated for 20 min at 37°C. RNA was precipitated by the addition of cold 10% trichloroacetic acid, the mixtures were filtered through nitrocellulose membranes, and radioactivity was measured by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Effect of λ on host RNA polymerase (RP):

A comparison of the template specificities of RP isolated from *E. coli* and from heat induced *E. coli* ($\lambda c_{I,U32}$) is presented in Table 1. Both enzyme preparations had greater activities on T4 DNA than on CT DNA and similar activity ratios (CT/T4) on the two templates. In neither case was the activity ratio significantly affected by the addition of σ factor, although a small stimulation of the "induced" RP was observed. The PC enzyme obtained from the *E. coli* RP and the "induced" RP also had similar properties. Both preparations had about one-fourth the activity on T4 DNA as on CT DNA, and were stimulated nearly 10-fold by the addition of σ factor. PC purified by only one passage through

phosphocellulose normally is stimulated 10-15-fold by σ (Bautz, personal communication).

It would thus appear that the RP isolated from cells producing λ phage contains σ or a σ-like factor. In contrast, RP isolated from T4-infected cells by the same procedure lacks the σ subunit (Bautz and Dunn, 1969).

Dissociation of initiation factor from RP "in vivo":

Preliminary observations (Chesterton and Green, unpublished) indicated that RP isolated from a λ DNA complex was modified with respect to its enzymatic activity on various templates. The results of Travers and Burgess (1969) showed that σ factor dissociates from E. coli RP after the initiation step "in vitro". We were thus led to test the possibility that σ or a σ-like factor dissociates "in vivo" after initiating RNA synthesis on a λ DNA template. Kinetic analysis showed that less than 10% of the enzymatic activity of the late complex was sensitive to Rifampicin. This indicates that most of the polymerase in the complex had initiated RNA synthesis "in vivo" (Umezawa et al., 1968; diMauro et al., 1969). The early complex has also been shown to contain over 90% of the RP in the initiated state (Hayward and Green, 1969a,b).

λ DNA-RP complex was isolated from infected E. coli at a late stage of λ development, and RP was separated from the λ DNA and purified as described in the Materials and Methods section. It should be noted that this purification method utilizes only steps that are in the procedure of Burgess et al. (1969); namely, nuclease degradation of the DNA-RP complex and glycerol gradient purification of RP. As shown by these authors, as well as the data in Table 1, this method does not cause the dissociation of σ from RP. If an initiation factor dissociates from the complex "in vivo", the resulting RP would be expected to have a relatively low activity on T4 DNA and it should be stimulated by σ factor (Burgess et al., 1969). As shown in Figure 1, both of these criteria were met. The activity was four times greater on calf thymus DNA than on T4 DNA. (This ratio varied from 3 to 10 for different preparations of enzyme.) Addition of σ (20 μl) stimulated the activity at least 11-fold on

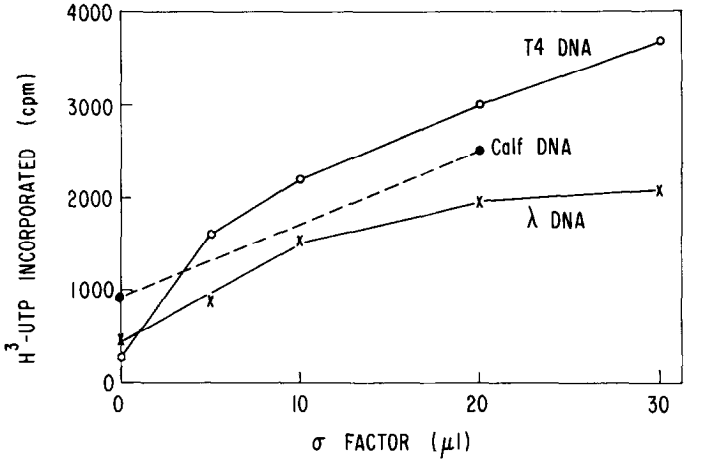

FIGURE 1: Effect of σ on RNA polymerase isolated from late λ complex. Purified RP from late complex was assayed on various templates (see Materials and Methods) and with various amounts of σ factor (27 μg/ml). Control samples with σ but no enzyme did not have significantly greater radioactivity than bottle backgrounds.

T4 DNA as compared with 2.6-fold on calf thymus DNA. The data are quite similar to those presented in Table 1 for the stimulation of PC by this preparation of σ factor.

The above results provide strong evidence that σ or a σ-like factor dissociates from RP after the initiation of RNA synthesis on a λ DNA template "in vivo". We cannot completely eliminate the possibility that initiation of RNA synthesis "in vivo" causes a greatly reduced affinity of the initiation factor for RP, such that dissociation occurs during the purification of the enzyme. This is very unlikely for the following reasons. Reconstruction experiments in which σ was added to the λ complex prior to the isolation of RP permitted the isolation of enzyme which possessed σ (Gariglio, unpublished data). More conclusive is the fact that σ stimulated RP isolated from the λ complex to the same extent as PC, indicating that the two "core" enzymes have a similar affinity for σ. However, we cannot be certain that the

TABLE 1

RNA POLYMERASE FROM E. COLI AND INDUCED E. COLI(λ)

Type of RNA Polymerase	Activity (cpm) with: CT DNA	T4 DNA	CT/T4
E. coli RP	8,917	22,172	0.42
E. coli RP + σ	10,464	21,604	0.48
Induced RP	15,834	23,503	0.67
Induced RP + σ	19,307	37,158	0.52
E. coli PC	11,248	2,822	4.0
E. coli PC + σ	16,695	23,103	0.72
Induced PC	2,747	642	4.3
Induced PC + σ	4,087	6,134	0.67

RNA polymerase was purified through two glycerol gradients, and the PC and σ species were obtained by one passage of RP through a phosphocellulose column according to the procedure of Burgess et al., (1969). σ factor (0.6 μg) was added to the enzyme at 0°C two minutes before the addition of DNA. The reaction was carried out at 37°C for 15 minutes. Calf thymus (CT) and T4 phage DNA (5 μg each) were at optimal concentrations for the polymerase reaction. All tubes were adjusted to the same final glycerol concentration as it was found that glycerol stimulates the reaction rate. Other details of the assay are described in the Materials and Methods section.

RP has not lost affinity for some other initiation factor which could have been used "in vivo" for transcription of the late region of the λ genome.

Further characterization of the RP from λ complexes is necessary in order to determine whether there is additional modification of the host enzyme or the presence of any λ-specific components. Our results lend support to the

"σ cycle" of Travers and Burgess (1969) by providing evidence that at least one step (dissociation of a σ-like factor from RP after initiation) occurs "in vivo".

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