EARLY-LATE TRANSCRIPTION SWITCH: ISOLATION OF A LAMBDA DNA-RNA POLYMERASE COMPLEX ACTIVE IN THE SYNTHESIS OF LATE RNA

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Certain regions of bacteriophage DNA are transcribed at late but not at early times after infection of a bacterial cell (Hall et al., 1963, 1964). The mechanism by which these "late" regions become available to prime for RNA synthesis at late time is unknown. Escherichia coli RNA polymerase will transcribe in vitro only the "early" genes of T4 DNA (Khesin et al., 1963; Geiduschek et al., 1966) and of λ DNA (Maitra et al., 1966; Naono & Gros, 1966). Recent evidence suggests that the T4 gene 55 product allows E. coli RNA polymerase to transcribe late regions of T4 DNA in vitro (Snyder & Geiduschek, 1968) It has also been demonstrated that T4 infection alters part of the E. coli RNA polymerase (Walter et al., 1968). The present report describes the isolation of a λ DNA-RNA polymerase complex from E. coli at a late stage after infection. In vitro the complex synthesizes early and late λ mRNA in the same proportion as do the cells from which the complex was purified.

MATERIALS AND METHODS

Growth of bacteria, preparation of phage stocks and conditions for infection have been described previously (Green, 1966; Green et al., 1967). E. coli W3110(λU32) is a heat inducible lysogen carrying the prophage λc_I, U32 (Lieb, 1966; Green, 1967). Early and late in vivo ^{3}H-RNA samples were prepared by phenol extraction of W3110 cells (Green, 1966) that had been infected with λU32 (m.o.i.=3) and pulsed with ^{3}H-uracil (1 μc/ml) at 2-3 min (early) or 20-21 min (late) after infection. Lambda DNA and λdg DNA were prepared as described by Adler & Templeton (1963) and E. coli W3110 DNA as reported by Marmur

(1961). DNA-bound RNA polymerase in the gradient fractions was assayed in the absence of additional DNA (Chamberlin & Berg, 1962). Reaction mix for the assay contained 0.03M $MgCl_2$, 0.025M β-mercaptoethanol, 0.12 M tris-HCl pH 7.9, 1 mg/ml each of ATP, CTP and GTP, and 40 μc/ml ^{3}H-UTP (2c/mMole). Each incubation contained 0.2 volume of reaction mix. After 30 min. at 37°, the radioactivity in TCA precipitable material was determined by liquid scintillation spectrometry (Green et al., 1967). This value was corrected for ^{3}H-DNA, when it was present.

RESULTS AND DISCUSSION

Late λ DNA-RNA polymerase complex was isolated as described below and in the legend to Fig. 1. W3110 cells were infected with ^{32}P-λU32 (m.o.i.=3), washed once and aerated in K medium at 37°. The cells were pulsed with ^{3}H-thymidine 14-15 min after infection to label replicating DNA and immediately chilled to 0°, harvested and lysed. Fig. 1 shows the sedimentation profile of such a lysate in a sucrose gradient containing 0.2M KCl. The salt should minimize nonspecific binding of RNA polymerase to DNA (Pettijohn & Kamiya, 1967). A fast sedimenting peak of replicated DNA coincident with a peak of parental DNA is seen (fractions 5-7). Associated with the leading edge of the DNA band is a peak of DNA-bound RNA polymerase activity (approx. 70s when compared with linear λ DNA). The majority of the ^{3}H- and ^{32}P-DNA was pelleted, suggesting that much of the λ DNA is membrane bound. The slowest fraction of RNA polymerase activity (fractions 15-18) probably represents polymerase bound to DNA fragments. The free enzyme sedimented in approximately the same position, as detected by assay in the presence of added calf thymus DNA (data not presented). The late complex showed no stimulation of activity under these conditions.

DNA-DNA hybridization studies (Table 1) indicated that the replicated ^{3}H-DNA of the complex was primarily λ DNA, since 65% hybridized with unlabeled λ DNA while no hybridization to E. coli DNA could be detected. Hybridization

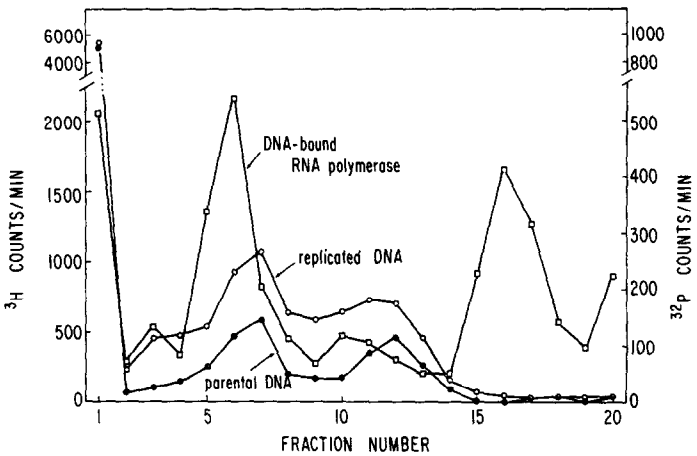

Fig. 1. Isolation of the Late Complex in a Sucrose Gradient. The direction of sedimentation is from right to left. E. coli W3110 cells (250ml, 2.7 x 10^8/ml) were infected with λU32 containing ^{32}P-DNA (Green et al., 1967) and pulsed with ^{3}H-thymidine (0.2 μc/ml culture, 17c/mMole) as described in the text. Cell lysis was achieved by the EDTA-lysozyme-Nonidet method of Hayward and Green (1968). The lysate (5 ml) was immediately layered over a 10-30% linear sucrose gradient (54 ml) containing 0.2M KCl, 0.05 mg/ml bovine serum albumin, 1mM β-mercaptoethanol, 0.001M EDTA, and 0.01M tris-HCl pH 7.9, and centrifuged at 23,000 rpm (65,000g) for 10 hr. at 5° in an SW 25.2 rotor (Beckman L-2 centrifuge). Fractions (3 ml) were collected from just above the pellet. They were assayed (0.15 ml portions) for ^{3}H-labeled (o——o) and ^{32}P-labeled (●——●) TCA precipitable material and for DNA-bound RNA polymerase activity (□——□). Fraction 1 contained the pelleted material resuspended in the residual liquid.

Table 1: DNA-DNA Hybridization Analysis of the Late Complex DNA. The procedure used was that of Hayashi (1968). Template DNA (10 μg per filter) was baked onto nitrocellulose filters at 80° and incubated with ^{3}H-DNA samples (E. coli, 16,800 cts/min; late complex, 1470 cts/min) in tris-HCl pH 7.4 containing 0.5M KCl (0.4 ml) at 65° for 18 hrs. After washing, the filters were dried and counted by liquid scintillation spectrometry.

Template DNA	H^3-DNA	Hybrid Cts/min	% of ^{3}H-DNA in hybrid
Lambda	Late complex	934	61.2
Lambda	E. coli	199	0.7
E. coli	Late complex	33	0
E. coli	E. coli	945	5.2
None	Late complex	33	-
None	E. coli	79	-

Table 2: Characterization of RNA synthesized *in vitro* by Early and Late Complexes. To avoid possible contamination of the hybrids, the complexes utilized below were isolated in the absence of any tritium labeled material. Incubation conditions (2 hr at 37°) for the synthesis of ^{3}H-RNA by the complexes were as for the RNA polymerase assay. RNA was purified by phenol extraction and concentrated by alcohol precipitation. Early and late *in vivo* ^{3}H-RNA samples (see Materials and Methods) served to indicate the relative amounts of early or late RNA which hybridized with λ and λdg DNA. For the hybridization reaction (Nygaard and Hall, 1963; Green, 1966), 20 μg of λ, λdg or *E. coli* DNA were used per tube and 15,000 cts/min early *in vivo* ^{3}H-RNA, 12,980 cts/min late *in vivo* ^{3}H-RNA, 1020 cts/min early complex *in vitro* ^{3}H-RNA, or 3160 cts/min late complex ^{3}H-RNA where indicated.

DNA	^{3}H-RNA	Hybrid Cts/min	% of Input ^{3}H-RNA in Hybrids	Ratio λ/λdg
None	Early *in vivo*	29	-	
λ	"	458	2.9	1.18
λdg	"	391	2.4	
E. coli	"	333	2.0	
None	Late *in vivo*	29	-	
λ	"	1040	7.8	3.04
λdg	"	362	2.6	
E. coli	"	297	2.1	
None	Early Complex	18	-	
λ	"	645	61.5	1.08
λdg	"	597	56.7	
E. coli	"	19	0	
None	Late Complex	42	-	
λ	"	1538	47.3	3.17
λdg	"	514	14.9	
E. coli	"	48	0	

with pulse-labeled *E. coli* ^{3}H-DNA served as a control. Sedimentation studies in neutral and alkaline sucrose gradients have shown that the complex contains closed twisted circular λ DNA (data to be presented elsewhere).

The following experiment demonstrates that the late complex synthesizes late λ RNA *in vitro* whereas an early complex, isolated in the same manner, synthesizes primarily early RNA. Early and late RNA were distinguished by comparing the amounts of each which hybridized with λ and λdg DNA. The λdg (Joyner *et al.*, 1966) is deleted for the late A-J region of the phage genome,

and thus lacks most of the late genes (Dove, 1966). Whereas early RNA made *in vivo* had the same hybridizing efficiency with λ and λdg DNA, the RNA extracted from the infected cells at late time after infection with λ had a 3-fold higher efficiency with λ DNA than with λdg DNA (Table 2). By this criterion the RNA synthesized by the late complex was similar to the late RNA made *in vivo*. This consists of RNA transcribed from both early and late regions of the λ DNA (Skalka, 1966; Taylor et al., 1967). For comparison, an early complex of λ DNA with RNA polymerase was isolated from W3110(λU32) cells infected with λU32. The infected lysogens had been heated for 25 min at 43° in the presence of chloramphenicol (100 μg/ml) in order to derepress λ RNA synthesis (Green, 1966). As seen in Table 2, the RNA synthesized *in vitro* by the early complex hybridized primarily with the early region of the λ genome. Neither the early nor the late complex synthesized detectable levels of *E. coli* RNA.

A comparison of the properties of the λ DNA-RNA polymerase complexes which are repressed or derepressed for late λ RNA synthesis should yield valuable information concerning the mechanism of the early-late switch. We are currently investigating the nature of the RNA polymerase and the DNA in these complexes. It should also be possible to determine whether some factor, perhaps coded by the λ Q gene (Dove, 1966), is required for transcription of the late genes.

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