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Control of Gene Function in Bacteriophage T4

I. Ribonucleic Acid and Deoxyribonucleic Acid Metabolism in T4rII-Infected Lambda-Lysogenic Hosts[1]

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Deoxyribonucleic acid (DNA) synthesis in T4rII-infected, lambda-lysogenic strains of *Escherichia coli* proceeds with one-half the rate of T4 wild-infected bacteria and stops 16 min after infection at 37 C. The rates of ribonucleic acid (RNA) synthesis, however, are the same with T4rII and T4 wild. The turnover of pulse-labeled RNA is slow in K strains (half-lives 10 to 20 min) as compared with B strains (half-lives 2.5 to 6 min). Lambda-lysogeny increases the apparent messenger (m) RNA half-lives in pulse-chase experiments. The shutoff of host RNA synthesis in T4rII infected K(λ) is incomplete. Moreover, the preferential transcription of T4 DNA ceases 13 min after infection, and transcription of host and prophage λ DNA is resumed. The T4 RNA synthesized in rII-infected K(λ) contains no late T4 mRNA. The early portion of the T4 genome, however, is transcribed completely. The T4-induced early modification of bacterial RNA polymerase does occur. Resumption of host DNA transcription at 13 min after infection is not associated with a reversal of the above polymerase modification. It is concluded that in lambda-lysogenic bacteria T4rII infections are abortive because RNA polymerase is prevented from transcribing late T4 genes.

Mutants of bacteriophage T4, defective in the rII region, are unable to grow in lambda-lysogenic host cells (2). This defect can be overcome by supplementing the growth medium with either high (5×10^{-2} M) Mg^{2+} concentrations (9) or polyamines (3, 7), but not by increasing the concentration of monovalent ions (2). On the contrary, the presence of 0.08 M Na^+ in a 0.1 M sucrose medium prevents growth of rII, whereas in the absence of Na^+ this medium promotes rII growth without requiring high Mg^{2+} concentrations (20). The functioning of the rII cistrons appears to be essential only around 10 min after infection and possibly until lysis (2). Consequently, during the first 10 min after infecting a lambda-lysogenic host, T4rII functions normally, or nearly normally, without requiring high Mg^{2+} concentrations and in the presence of 0.1 to 1.0 M monovalent ions. The functions found to be indistinguishable from r^+-infected cells during this period are the following: oxygen consumption, deoxyribonucleic acid (DNA) synthesis, overall protein synthesis (9), L-[^{14}C]alanine incorporation, deoxycytidylatemonophosphate hydroxymethylase (20), deoxycytidine triphosphatase (15, 17), DNA polymerase, α-glucosyl transferase, and β-glucosyl transferase [Josse, cited by Sekiguchi (20)]. Reduced activities are found with respect to the following: uptake of $^{28}Mg^{2+}$, ^{32}P incorporation into phospholipids, acid-soluble organic phosphates, ribonucleic acid (RNA), and DNA (in variance from Garen's observation) and phosphorylating activity (20). Between 10 min and 15 min after infection of K(λ) by T4rII (the time being dependent upon Mg^{2+} concentrations ranging from 10^{-4} to 10^{-3} M), a reduction or a halt of the following functions is observed: oxygen consumption, DNA synthesis, overall protein synthesis (2), L-[^{14}C]alanine incorporation, uptake of $^{28}Mg^{2+}$, ^{32}P incorporation, and phosphorylating activity (20). According to Rutberg and Rutberg (17), synthesis of the late T4 functions (6) and lysozyme and tail fibers does not occur. Sekiguchi (20) reports synthesis of lysozyme at about 5% normal rate. We and M. Schweiger (*personal communication*) were unable to confirm Sekiguchi's observation. Differences in cell mem-

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brane permeability between r^+- and rII-infected K(λ) were reported by Séchaud et al. (19), who observed leakage of ^{32}P-containing material from rII-infected cells and alterations in gross cellular structure by electron microscopy. In contradiction, no generalized leakage was detected by M. L. Brock (3), and no increased loss of $^{28}Mg^{2+}$ ions from rII-infected K(λ) by Sekiguchi (20).

Of the large number of observations on the physiology of T4rII-infected K(λ) cells, the majority are congruent. Of the few conflicting pieces of evidence, only the problem of permeability seems to be of importance. Nonetheless, the mechanics of prophage λ interference with growth of T4rII is not understood.

A variety of conceivable causes have been experimentally excluded. Bacteria lysogenic with phage λ i^{434}, which makes a repressor different from lambda repressor, do promote growth of T4rII (13), suggesting that no other lambda function but the immunity substance is interfering with rII growth. Heating to 45 or 48 C of K(λ) prior to rII infection removes the block against rII in some strains, but there is no complete correlation with prophage induction or temperature sensitivity of the lambda repressor in these experiments (14). Induction of prophage λ is certainly not required for removing the rII block as shown by additional experiments of Brock (3) and Tomizawa and Ogawa (21). However, Tomizawa and Ogawa (21) do report a correlation between the loss of rII exclusion by heating for bacteria lysogenic with lambda (temperature-sensitive) ts type I, but not for type II. In addition, they show that the losses of rII exclusion and of lambda immunity occur at the same rate in nonlysogenic segregants of lambda b2tsI-1-infected cells. These findings too suggest the lambda immunity substance to be instrumental both in repression of prophage λ and in exclusion of T4rII. But a locus (rex) different from cI (the structural gene for the immunity substance), which is expressed in the lysogenic state and which is responsible for rII exclusion, maps between N and cI on the lambda chromosome (12). So far, no other functions have been ascribed to this locus. The fact that rex is not part of cI is in apparent conflict with the observations of Tomizawa and Ogawa (21). Finally, the extent to which T4rII is prevented from growing in lambda-lysogenic hosts is largely determined by the bacterial strain (Mahler, Ph.D. Thesis, Brandeis Univ., Waltham, Mass., 1961).

A lambda-lysogenic bacterial cell infected by T4 represents a differentiated cell containing three chromosomes which act synergistically, antagonistically, or competitively. They force the cell to transgress through a variety of differentiated states. Observations on the process of information transfer with such cells may suggest models for differentiation. Hopefully, they will explain the failure of T4rII to perform late T4 functions in lambda-lysogenic hosts.

MATERIALS AND METHODS

Bacteria. We used the following strains of *Escherichia coli*: B; B/r(λ); K12-112, referred to as K; and K12-112(λ), referred to as K(λ).

Bacteriophages. The following phages were used: T4B, $T4Bh2^+$, T4r102(2), T4N82(6), T4N82r102, and T4LB3(1).

Growth media. Nutrient broth [8 g of Nutrient Broth (Difco), 5 g of NaCl, and 1 liter of water] and M-9A [11.5 g of $Na_2HPO_4 \cdot 7H_2O$, 1 g of NH_4Cl, 3 g of KH_2PO_4, 10 ml of 40% (w/v) glucose, 4 g of Casamino Acids, 2 ml of 0.5 M $MgSO_4$, 10 ml of 0.5% (w/v) tryptophan, and 1 liter of water] were used.

Other media. Phosphate buffer (5.4 g of $Na_2HPO_4 \cdot 7H_2O$, 3 g of KH_2PO_4, 4 g of NaCl, and 1 liter of water, *p*H 6.9); Tris-magnesium acetate (TMA) buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris) base, 0.022 M NH_4Cl, 0.01 M magnesium acetate, and 0.001 M β-mercapto-ethyl alcohol, adjusted to *p*H 7.5 by acetic acid]; and 5% and 10% (w/v) trichloroacetic acid for precipitation of nucleic acids were used. Dische's diphenylamine reaction mixture (1 g of diphenylamine, 100 ml of glacial acetic acid, and 2.75 ml of concentrated sulfuric acid) and SSC (0.15 M NaCl plus 0.015 M sodium citrate, *p*H 7.2) were also used.

Measuring host DNA degradation and phage DNA synthesis. Host DNA degradation and phage DNA synthesis were measured by the following procedures. (i) By the diphenylamine reaction of Dische (4), B/r(λ) or K(λ) is infected by T4N82r102, T4N82, T4r102, and $T4Bh2^+$ with a multiplicity of infection (MOI) of 5 to 10 in M-9A and sedimented at various times after infection; 5×10^9 complexes are resuspended in 0.5 ml of water. Of the Dische reaction mixture, 1.0 ml is added and boiled for 15 min at 95 to 96 C, and the optical density (OD_{595}) was measured against a blank treated the same way without bacteria. (ii) By acid solubility of ^3H-thymidine-labeled DNA, ^3H-thymidine is supplemented to M-9A-grown K(λ) at 0.5 μc/ml at various times before infection by T4r102. To remove unincorporated radioactive thymidine, the bacteria are sedimented, washed, sedimented again, and finally resuspended in M-9A. Before and after phage infection, 0.5-ml portions are withdrawn, transferred into 1.0 ml of 2% sodium dodecyl sulfate (SDS) at 75 C, chilled 3 min later, and precipitated by adding 2 ml of cold 10% trichloroacetic acid. The precipitable material is then filtered onto B-6 membrane filters (Schleicher & Schuell Co., Keene, N.H.) and dried and counted in a liquid scintillation counter.

Rate of RNA synthesis by pulse labeling with cytosine-5-^3H. Pulses (3 to 10 min) of cytosine-*5-^3H* (0.5

μc/ml) were given to uninfected and phage-infected bacteria (5×10^8/ml). Bacteria were then lysed by SDS, precipitated with trichloroacetic acid, and filtered as in the DNA assays (ii).

Half-life of mRNA. Half-life of mRNA was determined as follows. To cytosine-5-^3H-labeled complexes, a 10^4-fold excess of cytosine was added, and the loss of trichloroacetic acid-precipitable radioactivity was determined by using the same technique as in the assay for DNA (ii).

Isolation of mRNA. For isolation and partial purification of T4 mRNA, we used a prelayered CsCl gradient, which gave better than 60% recovery of the pulse-labeled RNA (18).

DNA-RNA hybridizations. DNA-RNA hybridizations were conducted (i) in liquid by the technique of Hall et al. (11); the total volume per hybridization was 0.3 ml of 4.2 × SSC, *p*H 7.2. Total denatured T4 DNA was 2.0 g; input radioactive mRNA was approximately 1 μg containing 1,200 counts per min of ^3H. Temperature during hybridization was 67.5 C; incubation time was 5 hr. Unhybridized RNA was digested for 25 min at 37 C by 10 μg of deoxyribonuclease-free pancreatic ribonuclease (Worthington, Freehold, N. J.).

(ii) Hybridization on membrane filters (10): B-6 filters (Schleicher & Schuell) are loaded with 5 μg of denatured λ DNA (*p*H 13) or 100 μg of denatured K DNA (*p*H 13) in 2 × SSC by slow filtration and dried for 4 hr at room temperature and for 1 hr at 80 C. DNA was denatured by addition of KOH to give a *p*H of 13, incubated at room temperature for 13 min, chilled, and neutralized by $HClO_4$. Control filters without DNA were prepared the same way. For hybridization, a DNA-loaded filter and a control filter were placed in the same vial containing 1 to 3 μg of radioactive RDA in 1 ml of 4 × SSC incubated for 12 hr at 67.5 C, transferred to 3 × SSC, and finally to 2 × SSC containing 20 μg/ml of ribonuclease. Digestion was allowed for 1 hr at room temperature with steady shaking. Filters were then washed from both sides by filtering through 50 ml of 2 × SSC.

DNA preparations. DNA was prepared from sucrose gradient-purified T4 by three phenol extractions in 0.1 × SSC and two butyl alcohol-chloroform extractions, and from K12-112 by lysis at 67.5 C in 2 × SSC containing 2% SDS, chilling, and sedimenting the precipitated SDS, by subsequent dilution to 0.5 × SSC and several butyl alcohol-$CHCl_3$ extractions at *p*H 9.0 followed by phenol extractions. The DNA types were finally dialyzed against 0.01 × SSC.

Preparation of crude extracts for RNA polymerase assays. Crude extracts from K(λ) and T4r102-infected K(λ) were prepared by lysing the concentrated and phosphate buffer-washed cells in modified TMA (omitting the Mg^{2+}) by freezing-thawing in the presence of lysozyme. Lysates were then spun in an SW-50L rotor for 40 min at 30,000 rev/min and the supernatant fraction was tested for RNA polymerase activity.

Assay for RNA polymerase. The enzyme assay followed the one described by Zillig, Fuchs, and Millette (23), but with increased concentrations of magnesium-acetate and NH_4Cl, which allowed an almost linear rate of synthesis for 35 min at 37 C. The reaction mixture is described as follows: total volume, 0.5 ml, *p*H 7.9; DNA, 40 μg; crude extract, 0.05 ml; Tris-acetate, 0.03 M; NH_4Cl, 0.20 M; magnesium-acetate, 0.07 M; guanosine-5′-triphosphate, cytidine - 5′ - triphosphate, uridine - 5 - triphosphate, adenosine-5′-triphosphate (P. L. Biochemicals, Inc., Milwaukee, Wis.), and adenosine-^3H (generally labeled)-5′-triphosphate (New England Nuclear Corp., Boston, Mass.), specific activity 1 c/mole, all at 1 mM.

RESULTS

Effects of T4 infections on the host DNA. We first measured the amount of DNA remaining in the cell after T4LB3 infection of K(λ) at 41.5 C. There is no loss of DNA from the cell suggesting that no, or very little, degradation has taken place (Table 1). The same experiment with B/r(λ) infected by T4N82r102 shows a loss of approximately 50% of the DNA by 32 min after infection at 37 C (*see* Fig. 1, lower curve).

Since a retention of polynucleotides in K(λ) could lead to faulty results with the diphenylamine reaction, we studied the acid solubilization of ^3H-thymidine-labeled host DNA after T4LB3 infection at 41.5 C. ^3H-thymidine was supplied to the growth medium between 10 and 5 min before phage infection and subsequently washed out, and the growth medium supplemented with 20 μg/ml of cold thymidine. The counts per minute before and after infection by T4LB3 at 41.5 C are given in Table 2. To reduce the fluctuations arising from trichloroacetic acid precipitation and membrane filtration, the steps of SDS lysis, acid precipitation and membrane filtration (*see* above) were performed in duplicate. It is seen that none of the incorporated radioactivity is rendered acid-soluble after T4LB3 infection. With T4 wild- and T4r102-infected K(λ), we obtained similar results (Table 2). Possibly, with T4 wild a drop in acid precipitable counts between 0 and 6 min after infection is

TABLE 1. *OD_{595} after diphenylamine reaction of K(λ) infected by T4LB3 at 41.5 C*

Time after infection	OD_{595}
min	
0	0.081
4	0.086
8	0.085
12	0.093
16	0.093
20	0.091
24	0.094
28	0.092

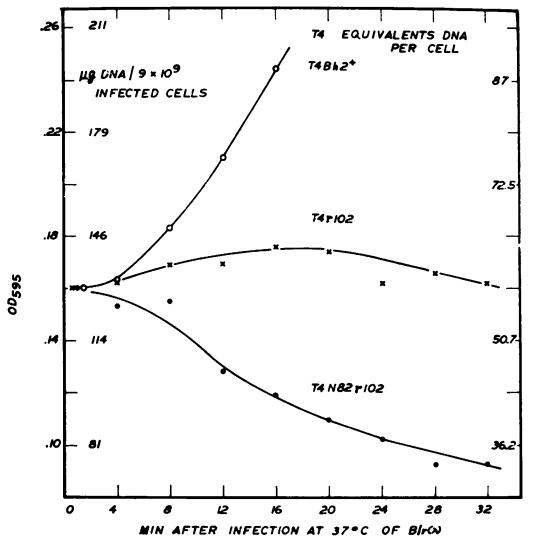

FIG. 1. *DNA synthesis at 37 C in B/r(λ) infected by T4Bh2+ (○), by T4r102 (×), and by T4N82r102 (●). The abscissa gives the time after infection, the ordinate the OD_{595} observed in the diphenylamine reaction which is converted to micrograms of DNA and T4 equivalents of DNA.*

indicated. The amount of DNA degraded did not exceed 20%.

Thus, we observe very little acid solubilization of host DNA between 0 and 6 min after infection, and thereafter reutilization of nucleotides for T4 DNA synthesis probably obscures any further breakdown. It is obvious that a large fraction of the host DNA remains in a macromolecular form and could possibly play some role in the T4-infected cell. Whether prophage λ stays intact remains unknown.

T4 DNA synthesis in B/r(λ). The rates of bacteriophage DNA synthesis and of mRNA synthesis could be correlated. Thus, before measuring RNA synthesis, we compared the DNA synthesis with B/r(λ) infected by T4r102, T4Bh2+, and T4N82r102, respectively (Fig. 1). In T4r102-infected cells, DNA synthesis proceeds up to 17 min after infection with one-half the rate of T4Bh2+-infected cells, and then ceases (differential plot in Fig. 2).

Rate of RNA synthesis in T4B and T4r102-infected K(λ). The rate of RNA synthesis was determined by the incorporation of cytosine-5-^3H from 2-min pulses into acid-insoluble material. Figure 3 shows similar rates of RNA synthesis with T4B- and with T4r102-infected K(λ), except for late times when there is a reduced rate of precursor uptake with T4r102. Thus, the failure of T4r102 to perform its functions in K(λ) around 10 min after infection is not caused by a failure of RNA synthesis.

Half-life of mRNA in T4-infected hosts. In a first series of experiments, the mRNA half-life was measured by pulse-chase experiments. Cytosine-5-^3H (or uracil-5-^3H) was added to T4-infected bacteria for 3 to 4 min; then, a 10^4-fold excess of nonradioactive cytosine (or uracil) was added, and the loss of trichloroacetic acid-precipitable material was observed. Table 3 gives the mRNA half-lives obtained with such experiments. It is seen that, in host *E. coli* B, the half-lives are short, both with T4B and r102 ($t_{0.5}$ = 2.5 to 6 min). T4r102-infected K displays a half-life of 11 minutes. With K(λ), no acid solubilization is detectable when infected by r102, whereas, a 22-min half-life is observed when infected by T4B.

The apparent lack of mRNA turnover with K(λ) hosts at any time after infection could be a result of poor chase, which could be caused by differences in the kinetics of the RNA precursor pools, or by an exceedingly poor uptake of chaser from the medium. The latter possibility becomes unlikely because the uptake of radioactive precursor is about the same with hosts B, B/r(λ), K, and K(λ); (see Table 2). We return to the question of the mRNA half-life later.

Nature of RNA synthesized. We saw little effect of λ-lysogeny on the rate of RNA synthesis. Therefore, we studied the homology of the RNA with T4, λ, and K12-112 host DNA by hybridization versus denatured DNA of the aforementioned species. The hybridizations with T4 DNA were performed in liquid; those using λ and K-12 DNA with membrane-immobilized DNA. Maximum hybridization efficiencies with T4 DNA were 28.4% of the input RNA, with λ DNA 7%, and with K 12 DNA 60%. The low efficiency with λ DNA is due to the small fraction of λ mRNA in the sample used for hybridization. More than 60% of this RNA preparation is K-12 mRNA, another 20% T4 mRNA.

The RNA types were extracted from T4B and T4r102-infected K(λ) at various times after infection at 10^{-3} M Mg^{2+}. The length of the preceding incubation with cytosine-5-^3H is 5 or 6 min at 37 C. In Fig. 4 are plotted the hybridization efficiencies of RNA harvested from T4B-infected K(λ) versus the time after infection. Hybridization with T4 DNA amounts to 25 ± 2.5%, with K-12 DNA 5 to 8%. Since 3.8% of the bacteria from which the RNA was harvested survived the T4 infection, we may conclude that essentially no host mRNA is synthesized in the T4-infected cells. With T4r102-infected K(λ), we see very different results (Fig. 5). Here, up

TABLE 2. *Trichloroacetic acid-precipitable counts per minute in thymidine-methyl-^3H-labeled K(λ) before and after infections by T4 phages*

Phage	Incubation temp after infection	^3H pulse[a]	Radioactivity at various times before and after infection							
			Before	2 min	4 min	6 min	8 min	10 min	15 min	20 min
	C	*min*								
T4LB3	41.5	−10 to −5	1.94×10^4[b]	2.03×10^4	2.53×10^4	2.27×10^4	1.99×10^4	2.16×10^4		
T4B	37	−40 to −20	6.33×10^4	4.97×10^4	5.71×10^4	4.99×10^4	6.27×10^4	4.90×10^4	6.03×10^4	6.14×10^4
T4r102	37	−40 to −20	6.02×10^4	5.35×10^4	6.31×10^4	6.83×10^4	5.17×10^4	6.66×10^4	6.51×10^4	6.45×10^4
T4B	37	−5 to −1	5.59×10^4	4.62×10^4	3.69×10^4	5.09×10^4	4.88×10^4	5.11×10^4	4.88×10^4	5.93×10^4
T4r102	37	−5 to −1	5.25×10^4	5.82×10^4	5.09×10^4	5.51×10^4	5.48×10^4	5.77×10^4	5.58×10^4	5.40×10^4
T4B	37	−1 to 0	4.06×10^4	3.79×10^4	3.95×10^4	3.85×10^4	3.84×10^4	3.67×10^4	4.61×10^4	3.87×10^4
T4r102	37	−1 to 0	3.69×10^4	4.66×10^4	5.19×10^4	4.89×10^4	4.61×10^4	4.23×10^4	4.66×10^4	4.44×10^4

[a] Time before infection.
[b] Expressed as counts per minute.

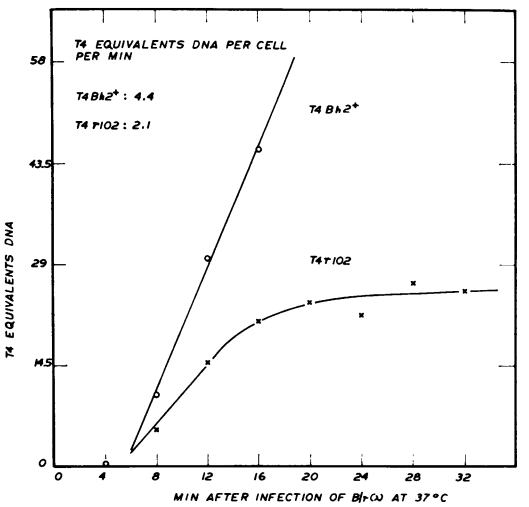

FIG. 2. *DNA synthesis at 37 C in B/r(λ) infected by T4h2+ (○) and by T4r102 (×). This is a differential plot of the data in Fig. 1.*

to 13 min after infection, T4 mRNA is preferentially synthesized (Fig. 5), but also some host mRNA is made. At around 13 min, synthesis of T4 mRNA is suddenly reduced to 20% of the previous rate, and synthesis of host RNA is resumed. Since this redirection of mRNA synthesis is brought about by prophage λ, we want to see whether λ transcription takes a lead in these events, or simply follows the host transcription. We, therefore, hybridized the same 13- to 19- and 18- to 23-min pulse-labeled RNA from T4r102-infected K(λ) with denatured λ DNA. The hybridization efficiencies obtained are 0.45% with the 13 to 19 minute RNA and 6.9% with the 18 to 23 minute material. Thus, the switch to λ transcription does not appear to precede the host DNA transcription.

Nature of T4 mRNA synthesized in T4r102-infected K(λ). One effect of λ lysogeny was to redirect the transcription from the T4 genome back to the host and prophage genomes. Since this switch occurs only after 13 min of infection and since a residual T4 transcription remains, the redirection is presumably not the primary instrument for stopping the development of T4r102. Thus, the question of whether there is a complete transcription of the T4 genome arises. We hybridized T4r102 mRNA from K(λ), pulse-labeled between 8 and 13 min, with T4 DNA in the presence of increasing amounts of "early" plus "late early" competitor RNA (Fig. 6). The complete competition indicates that no late T4 mRNA has been synthesized in the T4r102-infected K(λ). Early genes, however, are completely transcribed (upper part, Fig. 7), as the competition by 18-min T4r102 RNA from K(λ) against radioactive 7-min T4 mRNA shows. However, the competition against 18-min T4 mRNA reveals the absence of late T4 mRNA (Fig. 7, lower part).

Half-life of mRNA by hybridization studies. The mRNA half-lives with K(λ) reported above could be falsified by an insufficient chase in the pulse-chase technique. We, therefore, checked into the turnover of pulse-labeled RNA by utilizing the fact that, in T4r102-infected K(λ) host, RNA is preferentially synthesized after 13 min of infection. We infected a culture of K(λ) with r102 (MOI = 4) and divided it into two batches, one having twice the volume of the other. The large batch was pulsed with cytosine-5-^3H from 8 to 13 min and chased by a 10^4-fold excess of cold cytosine, and one-half of the volume was quickly withdrawn and poured over ice. The other half continued to be incubated until 23 min, when it was chilled. The second batch was pulse-labeled from 18 to 23 min, a 10^4-fold excess of cold cytosine was then added, and the complexes were chilled. The RNA was harvested from the three batches and hybridized with T4 and K-12 DNA. RNA isolated immediately after the pulse (13 min) should hybridize with a high efficiency with T4 DNA and with a low efficiency with K-12 DNA. RNA labeled between 8 and 13 min and isolated after a subsequent 10 min of chase by cold cytosine will efficiently hybridize with T4 DNA only if it was not turned over. RNA labeled between 18 and 23 min should hybridize poorly with T4 DNA, but efficiently with K-12 DNA. The results of the hybridizations are given in Table 4. It is seen that the 8- to 13-min labeled

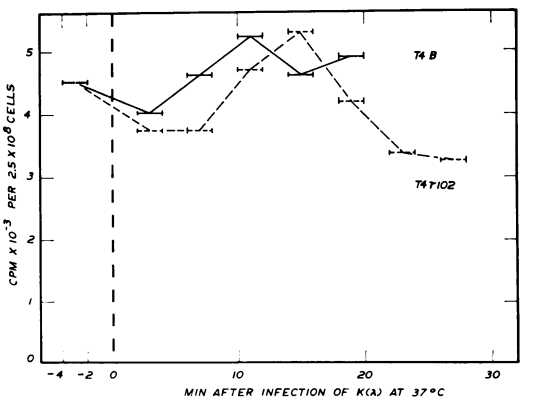

FIG. 3. *Uptake of cytosine-5-^3H by T4B (solid lines) and T4r102 (broken lines) infected K(λ). Time of infection is indicated by the vertical, broken line. The bars indicate the length of the pulses (2 min).*

TABLE 3. *Trichloroacetic acid-precipitable counts per minute in cytosine-5-^3H pulse-labeled T4-infected bacteria*[a]

Phage	Host bacteria	Uninfected bacteria	^{3}H-cytosine pulse	Minutes after chase						Half life of mRNA
				0	2	3	4	6	9	
		%								*min*
T4r102	B	<0.1	8 to 12	9.74×10^3[b]		4.44×10^3		2.12×10^3		2.6
T4r102	B	<0.1	10 to 14	5.08×10^3		3.89×10^3		2.50×10^3		5.9
T4r102	B/r(λ)	<0.1	8 to 12	9.37×10^3		6.50×10^3		4.82×10^3	3.57×10^3	6.1
T4r102	K12-112	0.1	8 to 12	6.32×10^3		5.29×10^3		5.35×10^3	4.57×10^3	11.0
T4B	K12-112(λ)	0.2	8 to 12	6.34×10^3		4.79×10^3		5.28×10^3	4.92×10^3	22.0
T4r102	K12-112(λ)	0.6	1 to 5	13.7×10^3	13.2×10^3		13.8×10^3	13.2×10^3		∞
T4r102	K12-112(λ)	0.6	9 to 13	6.24×10^3	5.94×10^3		5.80×10^3	6.15×10^3		∞
T4r102	K12-112(λ)	0.6	14 to 18	5.30×10^3	5.55×10^3		4.82×10^3	6.00×10^3		∞
T4r102	K12-112(λ)	0.6	20 to 24	3.08×10^3	3.40×10^3		3.84×10^3	3.85×10^3		∞

[a] Cytosine-5-^3H is supplied at various times after infection for 3 to 6 min and then chased by a 10^4-fold excess of nonradioactive cytosine.
[b] Values are expressed in counts per minute.

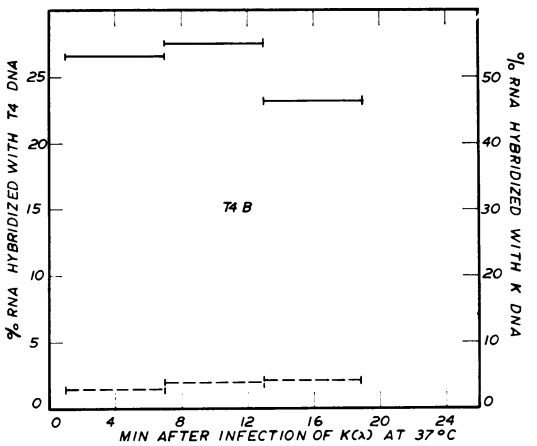

FIG. 4. *Hybridization efficiencies of cytosine-5-^{3}H-labeled RNA isolated from T4B infected K(λ). Bars indicate time and length of the pulse-labeling. Solid bars give the level of liquid hybridization with T4 DNA. The broken bars give the level with K12-112 DNA immobilized on membrane filters. Input per hybridization is 1,200 counts per min.*

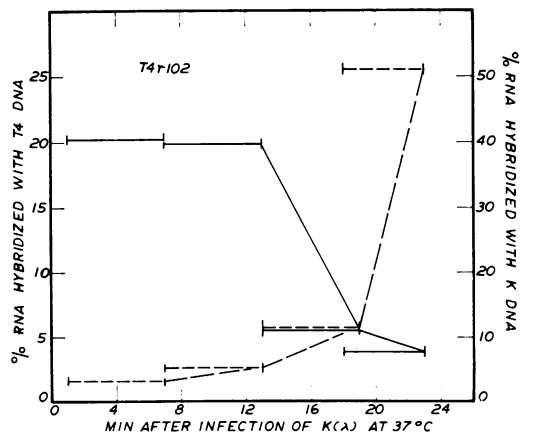

FIG. 5. *Hybridization efficiencies of cytosine-5-^{3}H-labeled RNA isolated from T4r102-infected K(λ). Bars indicate time and length of the pulse-labeling. Solid bars give level of liquid hybridization with T4 DNA. Broken bars give the levels with membrane filter-immobilized K12-112 DNA. Input per hybridization is 1,200 counts per min.*

RNA isolated 23 min after infection hybridizes with about 0.7 the efficiency of the 13-min isolate. RNA, pulse-labeled from 18 to 23 min, hybridizes with much lower efficiency. With K-12 DNA, the latter RNA shows a high degree of hybridization, whereas both the 13-min isolate and the 8- to 13-min-labeled 23-min isolate hybridize with low efficiencies.

A turnover, involving uptake of chaser cytosine, would have reduced the specific activity of the 8- to 13-min labeled RNA when isolated at 23 min after infection, which would have led to a

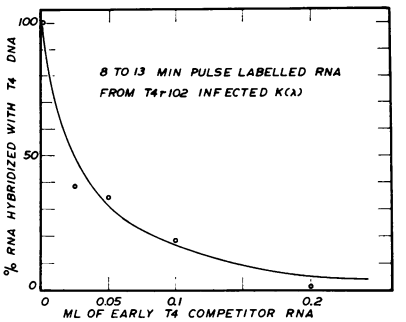

FIG. 6. *Hybridization-competition experiment with cytosine-5-^{3}H-labeled RNA from T4r102-infected K(λ) competed by T4N82 mRNA isolated 13 min after infection of E. coli B (early competitor). Concentration of competitor RNA is 40 μg/ml. Input per hybridization is 1,200 counts per min.*

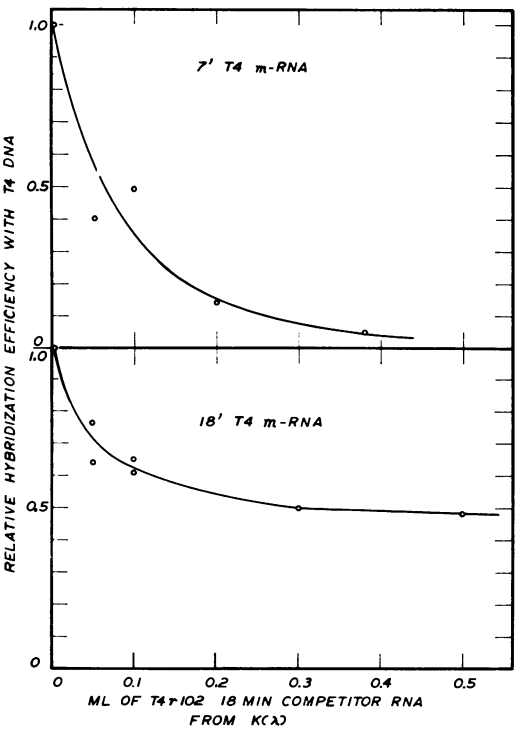

FIG. 7. *Hybridization-competition experiments with cytosine-5-^{3}H-labeled mRNA isolated at 7 min (upper part) and at 18 min (lower part) after infection of E. coli B by T4B. Competitor RNA is from T4r102-infected K(λ) isolated 18 min after infection at 37 C. Concentration of competitor RNA is 20 μg/ml. Input per hybridization is 1,200 counts per min.*

TABLE 4. *Hybridization efficiencies of cytosine-5-^{3}H-labeled RNA from T4r102-infected K(λ)*

Time[a] of ^{3}H-cytosine pulse	Time of chase	Time RNA isolated	Radioactivity[b] in isolated RNA	Per cent RNA hybridized with		
				T4 DNA (liquid)		K12-112 DNA (filter)[c]
				Expt 1	Expt 2	
8 to 13	13	13	159 × 10^3	20.4	19.6	16.8
8 to 13	13	23	164 × 10^3	13.8	13.8	10.0
18 to 23	23	23	271 × 10^3	4.1	3.5	51.0

[a] All times are minutes after infection. Uninfected bacteria, 7.5%.
[b] Expressed as total counts per minute.
[c] Averages of two experiments.

much lower hybridization efficiency with T4 DNA than observed.

In K(λ) infected by T4102, the mRNA displays a slower turnover (half-life 10 to 15 min at 37 C; Table 4) than in *E. coli* B (half-life 2.5 min at 37 C; Table 3). This conclusion is supported by the facts that the total counts per min isolated by 13 min and by 23 min are the same (see Table 4) and that the recovery in the isolation procedure is better than 60%.

Properties of RNA polymerase in T4r102-infected K(λ). *E. coli* RNA polymerase is modified immediately after the infection by T4 (22). This modification is readily demonstrated by comparing the template activities of calf thymus DNA and T4 DNA with bacterial polymerase and RNA polymerase isolated from T4-infected bacteria. With bacterial RNA polymerase, thymus DNA and T4 DNA show about an equal rate of transcription, whereas enzyme isolated from T4-infected bacteria transcribes T4 DNA at about 1/20 the rate of the bacterial enzyme (22). Thymus DNA proves to be a 10-fold better template for this RNA polymerase. Since transcription of T4r102 in K(λ) is defunct in two ways, viz., (i) late T4 genes are not transcribed and (ii) transcription switches back to host DNA after 13 min, it is conceivable that the initial polymerase modification is missing, or that we experience a reversal of the initial modification by 13 min, or that another modification takes place after 13 min. The presumed second modification would result in resumption of host transcription. Thus, it seems possible that the T4 modification of RNA polymerase is reversed, which would be demonstrable by the relative transcription of T4 and thymus DNA. We tested the RNA polymerase activities of crude extracts from K(λ) before infection and at 4 min and 22 min after infection by T4r102. The conditions of the reaction are DNA in excess, ionic strength 0.40, *p*H 7.8, and [Mg^{2+}] = 0.07 M. The transcription of thymus DNA is only slightly reduced with the 4-min crude extract and somewhat more with the 22-min extract (Fig. 8A). The transcription of T4 DNA is greatly reduced with the 4 min and the 22 min extracts (Fig. 8B). Thus, the T4-induced RNA polymerase modification (as evidenced by template specificity) does occur in the lambda-lysogenic host and is not reversed at 13 min, as we might expect from the late transcription of host and λ DNA types.

Reversal of the rII block by high Mg^{2+}. As Garen (9) had shown, the rII defect can be overcome by 3 × 10^{-2} M Mg^{2+} when it is supplied earlier than 12 min after infection at 37 C. After this time, the number of bacteria releasing rII phages drops about linearly reaching a zero value by 35 min. We attempted to dissociate the inability to produce phage from the irregular transcription of host DNA by supplementing 3 × 10^{-2} M Mg^{++} 22 min after the infection of K(λ) by T4r102. Thereafter we added cytosine-5-^{3}*H* from 24 to 29 min and isolated the RNA at 29 min. The hybridization efficiency was 3.4% with T4 DNA. From the control experiment in which no Mg^{2+} was added, we isolated RNA which hybridized with 3.25% efficiency with T4 DNA. Thus, not only is it impossible to revert the rII block by adding Mg^{2+} later than 15 min, but also it is impossible to redirect the transcription from host DNA back to T4 DNA.

DISCUSSION

We choose to look upon the T4rII-infected lambda-lysogenic host as a cell with three chromosomes, T4, host, and λ, each of which is capable of controlling the synthetic apparatus. The slow degradation of the host DNA, possibly including the prophage λ (Fig. 1, Tables 1 and 2), justifies this view. Moreover, T4 DNA replication does occur in rII-infected lambda-lysogenic hosts (2, 16, Fig. 1 and 2), thus fulfilling one of the requirements for late T4 mRNA synthesis. Since it appears that continuous T4 DNA replication is not required for continuing the synthesis of

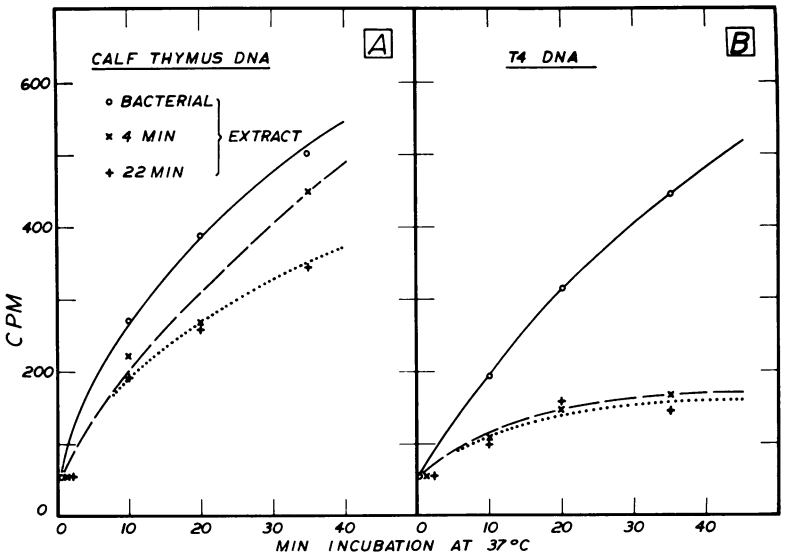

FIG. 8. *RNA synthesis by crude extracts of K(λ) and T4r102-infected K(λ) at 4 min and at 22 min after infection at 37 C. (A) With calf thymus DNA as template; (B) with T4 DNA as template.*

late T4 mRNA (Sauerbier and Bräutigam, *unpublished data*), the avenues for affecting late T4 mRNA synthesis probably are the RNA polymerase modifications and the ionic conditions.

There is little difference in the overall RNA synthesis in T4 wild and T4r102-infected K(λ) except for late times, when the precursor incorporation tapers off with T4r102 (Fig. 3). The mRNA has an unusually long half-life in K(λ), or exhibits no turnover at all, when viewed by pulse-chase experiments (Table 3). Hybridization experiments verify the slow turnover of T4r102 mRNA in K(λ) (Table 4).

When we investigated which of the DNA types is transcribed in K(λ) after T4-wild infection, we observed the regular turn off of host transcription (Fig. 4). When the infecting phage was T4r102, however, we observed the following sequence of events. (i) Immediately after infection the transcription of the host genome is greatly reduced, but not turned off. (ii) At later times (around 10 min at 37 C), host RNA synthesis is resumed, whereas (iii) T4 RNA synthesis is gradually reduced to ⅓ of the previous level, but not turned off (Fig. 5). This shift of transcription is mediated by prophage λ. We therefore wonder whether there is any sign of preceding λ activity which would lead to this result. The hybridizations with λ DNA show no unusually high transcription of λ DNA before the onset of host transcription. It thus appears that it is not a partial induction of prophage λ which leads to interference with T4r102 transcription.

The RNA synthesized from T4r102 DNA is exclusively early as far as our hybridization-competition experiments can tell (Fig. 6 and 7). The transcription of the early genes, however, seems to be complete (Fig. 7, top).

The modification of bacterial RNA polymerase by T4, as evidenced by the relative transcription of thymus and T4 DNA (22), does take place in T4r102-infected K(λ) (Fig. 8). Furthermore, the switch from T4 transcription to host and λ DNA transcription, occurring 13 min after infection, is not associated with a reversal of the polymerase modification (Fig. 8). Once the shift in transcription has occurred, it cannot be reversed by high Mg^{2+} concentrations. From Garen's data on Mg^{2+} supplementation, we infer that the shift can be prevented if Mg^{2+} is added before 13 min after infection.

We thus presume that, at 10 to 13 min after infection, a high ratio of divalent versus monovalent ions is required for the RNA polymerase (or the combined factors which determine late gene transcription) to transgress from the initial T4 modification to a second modification which enables transcription of late T4 genes. If the second modification fails, as in T4rII-infected K(λ), the preference for T4 transcription is lost and all templates are transcribed with similar efficiencies per unit length. The approximate relative amounts per cell of T4, λ, and host DNA are 1 (=20 T4 equivalents of DNA per cell; see Fig. 2 and 3), 1/50, and 3, respectively. The percentages of RNA synthesized in the same order are about 20, 10, and 70.

We conclude that in K(λ), T4rII fails to create

favorable conditions which allow the late modification of the factors involved in late T4 mRNA synthesis. This could directly be an ionic effect on the RNA polymerase, an ionic effect on the initiation factor-polymerase complex (1), an effect on the interaction of polymerase with the rex locus product (12) of lambda, an effect on the ribosomal- or transfer RNA-mediated control of RNA synthesis, or a combination of the preceding with the T4-directed synthesis of late RNA polymerase modifiers.

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