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Genetic Analysis of Two Bacterial RNA Polymerase Mutants that Inhibit the Growth of Bacteriophage T7

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Summary. The *Escherichia coli* mutants 7009 and BR3 are defective in the growth of bacteriophage T7. We have previously shown that both of these mutant hosts produce an altered RNA polymerase which is resistant to inhibition by the T7 gene 2 protein (De Wyngaert and Hinkle 1979). In both strains, the mutation which prevents T7 growth is closely linked to *rif*A (*rpo*B). Both mutants are complemented by transformation with a multicopy plasmid carrying *rpo*B and *rpo*C but not by a plasmid carrying only *rpo*B. This indicates that the mutations reside in *rpo*C, the structural gene for the β' subunit of RNA polymerase. When a single copy of the wildtype *rpo*C allele is introduced into the mutant using the transducing phage $\lambda drif^d18$, the mutant allele is dominant over wildtype. The $\lambda drif^d18$ transductant also remains unable to support the growth of T7 in the presence of rifampin. This supports our conclusion that the mutation is in *rpo*C. We have measured the growth of T7 phage, the kinetics of phage DNA synthesis, and the structure of replicative DNA intermediates in several transductants, and compared these results with those obtained in the original mutant strains.

Introduction

The isolation and characterization of bacterial mutants which are defective in their ability to propagate bacteriophage have often been fruitful in elucidating the role of bacterial proteins in phage development. Several bacterial mutants have been isolated which grow normally but which are unable to support the intracellular growth of T7 phage (Studier 1973; Chamberlin 1974; Yamada et al. 1978). We have been studying two of these mutants, *E. coli* 7009 (*tsn*B) (Chamberlin 1974) and BR3 (Studier 1973), which appear to cause defects in late stages of T7 DNA synthesis and DNA packaging (De Wyngaert and Hinkle 1980). Our biochemical analysis of these mutants indicated that these defects probably result from a mutation in the bacterial RNA polymerase. RNA polymerase purified from either mutant was completely resistant to inhibition by the T7 gene 2 protein (De Wyngaert and Hinkle 1979). A similar result has been obtained with the *E. coli* mutant Y49 (Shanblatt and Nakada 1982). The gene 2 product, a 7 kdal protein which is required for T7 growth, binds tightly to wildtype *E. coli* RNA polymerase holenzyme and inhibits template binding (Hesselbach and Nakada 1977; De Wyngaert and Hinkle 1979).

A second T7 protein, the product of gene 0.7, has also been implicated in the "shutoff" of host RNA polymerase (Brunovskis and Summers 1972). The gene 0.7 protein is a protein kinase which phosphorylates a number of host proteins during infection, among them the bacterial RNA polymerase (Rahmsdorf et al. 1974; Zillig et al. 1975). This protein kinase is not required for growth on normal hosts but its presence slightly enhances T7 growth in the mutant hosts 7009 and BR3. For example, $T7^+$ plates with a normal efficiency on *E. coli* BR3 at 37° C although the plaques are small and are not formed at 30 °C. However, a T7 gene 0.7 mutant will not form plaques on BR3 at either 37° C or 30° C (Studier 1973).

This paper describes the genetic analysis of the *E. coli* mutants 7009 and BR3. We present evidence that in both of these strains the mutation which prevents the growth of T7 phage resides in *rpo*C, the structural gene for the β' subunit of RNA polymerase.

Materials and Methods

Media. The following media were used: T broth (1% Bacto-Tryptone, 0.5% NaCl), L Broth (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% NaCl) and M9 minimal (Miller 1972) supplemented with 0.2% glucose and for growth of *E. coli* WA802 and its derivatives 20 μg/ml L-methionine.

Bacterial and Phage Strains. The genotypes and sources of the *Escherichia coli* and phage strains used in this study are listed in Table 1. The spontaneous Rif^r mutants SRB4, SRB5, and SRB6 were selected on L broth agar plates containing 50 μg/ml rifampin. The transformation of *E. coli* 7009 and BR3 with the plasmids pGA43 and pGA45 (to construct SRB7, SRB8, SRB11 and SRB12) was carried out as described by Cohen et al. (1972) and the transformants were selected by plating on L broth agar plates containing 12.5 μg/ml choramphenicol. The transformants were also resistant to rifampin. The $\lambda drif^d18$ transductant SRB14 was constructed by procedures described by Miller (1972) using λAb as helper. The transductants were selected for resistance to rifampin and screened for temperature sensitivity. We also demonstrated that SRB14 could be cured of its transducing prophage by brief incubation at 42° C. The cured derivatives remained unable to grow $T7_{0.7}$ phage but they were no longer rifampin resistant nor temperature sensitive.

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Table 1

Strain	Description	Source and reference
Bacteria		
B/1	*rpo*B$^+$ *rpo*C$^+$	M. Chamberlin
7009	*tsnB* (*rpo*C319)	M. Chamberlin (1974); this work
BR3	*rpo*C320	F.W. Studier (1973); this work
SRB4	Spontaneous rifampin resistant mutant of B/1	this work
SRB5	Spontaneous rifampin resistant mutant of 7009	this work
SRB6	Spontaneous rifampin resistant mutant of BR3	this work
WA802	K12 *sup*E *hsr*$^-$ *hsm*$^+$ *met*E	B. Backman; Wood (1966)
JF427	*arg*H *rif*^{r}	J. Friesen
SRB3	WA802 *arg*H *rif*^{r} by P1 607H transduction using JF427 as donor	this work
SRB7	7009/pGA43	this work
SRB8	7009/pGA45	this work
SRB9	SRB3 *arg*H$^+$ *rif*^{s} *rpo*C319 by P1 607H transduction using SRB7 as donor	this work
SRB11	BR3/pGA43	this work
SRB12	BR3/pGA45	this work
SRB13	SRB3 *arg*H$^+$ *rif*^{s} *rpo*C320 by P1 607H transduction using BR3 as donor	this work
SRB14	SRB13 (λd*rif*^{d}18) ($\lambda\Delta b$)	this work
SRB16	BR3 *rif*^{r} *rpo*C$^+$ by P1 CM *clr*100 transduction using SRB4 as donor	this work
SRB17	7009 *rif*^{r} *rpo*C$^+$ by P1 CM *clr*100 transduction using SRB4 as donor	this work
Phages		
T7	Wild type T7	F.W. Studier
$T7_{0.7}$	point mutation (JS62a) in gene 0.7	F.W. Studier (1973)
P1 607H	High frequency transducing clear plaque strain of P1	G. Pruss; Wall and Harriman (1974)
P1 CM *clr*100	P1 temperature sensitive clear plaque mutant which carries genes for choramphenicol resistance	G. Pruss; Rosner (1972)
λd*rif*^{d}18	λCI857 S7 d*rif*^{d}18	L. Lindahl; Kirschbaum and Konrad (1973)
$\lambda\Delta b$	λCI857 S7 Δb515 Δb519 (helper phage for λd*rif*^{d}18)	L. Lindahl

P1 607H was used to transduce both the *rif*^{r} and *arg*H markers from JF427 into WA802, creating strain SRB3. This strain was used as the recipient to construct SRB9 (*rpo*C319) and SRB13 (*rpo*C320). Since P1 grows poorly on 7009, SRB7 was used as the donor for *rpo*C319. SRB7 (7009/pGA43) carries a plasmid containing the *rpo*B$^+$ and *rpo*C$^+$ genes and P1 grows well on this strain.

P1 Transductions. For P1 transductions, the phage were first grown upon the donor strain according to Wall and Harriman (1974). The recipient strain was grown to mid log ($OD_{590}=0.5$ to 1.0) in L broth supplemented with 5 mM $CaCl_2$ and 10 mM $MgCl_2$ and P1 was added to give an input ratio of either 0.2 or 1. This mixture was incubated for 20 min at room temperature to allow phage absorption. The cells were centrifuged in an Eppendorf microfuge and the supernate discarded. The cells were then resuspended in the original volume of 2× Davis buffer (Sasaki and Bertani 1965) supplemented with 0.5 M Sodium citrate. To select for rifampin resistance, aliquots of the resuspended cells were spread on nutrient agar plates and incubated for 4 h at 37° C. The plates were then overlayered with 2 ml of soft agar containing 1 mg/ml of rifampin and incubation was continued overnight at 37° C. To select for Arg$^+$, the resuspended cells were plated on M-9 agar and incubated overnight at 37° C. To screen for the cotransduction of either the 7009 or BR3 phenotype, cells with the selected marker were transferred to a sterile microtiter plate containing 0.1 ml of T broth in each well. The cells were then transferred with a multitined stamper to agar plates spread with 10^4 to 10^8 T7$^+$ (to select for 7009) or $T7_{0.7}$ (to select for BR3). The plates were incubated at 37° C overnight and scored for resistance to the appropriate phage.

One Step Growth Experiments. Cells were grown in T broth to a density of 2.5×10^8 cells/ml and T7 phage were added at a multiplicity of 0.1. After 5 min the infected cells were diluted 10^{-4} to 10^{-6} in T broth and samples of the diluted cells were removed at intervals and titered. To determine the number of unabsorbed phage, a sample was removed at 5 min after infection, incubated with chloroform to kill infected cells, and titered for phage. Approximately 10% of the added phage were present as unabsorbed phage. The infective centers and the final burst sizes presented in the tables are corrected for this value. Unless otherwise indicated, all phage titers were carried out using *E. coli* B/1.

Other Materials. Rifampin (Calbiochem.) was used from a stock solution (10 mg/ml) in dimethyl sulfoxide. Chloramphenicol and sucrose (ultrapure grade) were from Sigma. Tryptone and Yeast extract were from Difco and [^{3}H]-thymidine (20 ci/mmole) was from New England Nuclear Corp.

Results

The Mutations Affecting T7 Growth are Closely Linked to rif^r

The structural genes for the subunits of *E. coli* RNA polymerase have been identified and mapped on the chromosome (Bachman and Low 1980). The genes for the β and β' subunits, *rpo*B and *rpo*C, are located next to each other

Table 2. Cotransduction of mutations affecting T7 growth with rifampin resistance

Donor	Recipient	Total transductants	Number of transductants with unselected marker	Percent cotransductions
SRB4 (B/1 *rif*^{r})	BR3	999	929	93
SRB6 (BR3 *rif*^{r})	B/1	428	406	95
SRB4 (B/1 *rif*^{r})	7009	960	856	89

Transductions were carried out as described under Methods using P1 CM *clr*100. Rifampin resistance is the selected marker. The ability (or lack of ability) to grow T7 phage is the unselected marker

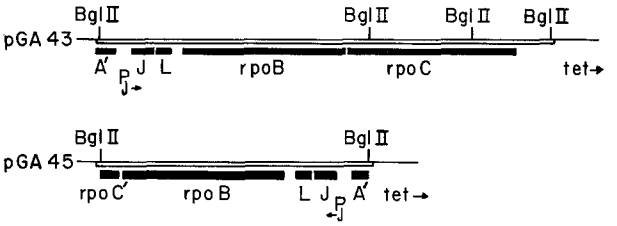

Fig. 1. Structure of plasmids pGA43 and pGA45. The structure of pGA43 has been described by An and Friesen (1980). pGA45 was constructed from pGA43 by *Bgl*II digestion followed by ligation (Friesen, personal communication). The single line indicates DNA of the cloning vehicle, pGA24, and is not drawn to scale. The double line indicates bacterial DNA. The locations of genes for ribosomal proteins and RNA polymerase subunits are indicated by the filled boxes below

at about 90 min. Resistance to the antibiotic rifampin maps in *rpo*B (Hayward and Scaife 1976), and this provided us with a convenient marker for preliminary mapping of the mutations which prevent T7 growth in *E. coli* 7009 and BR3.

We first isolated spontaneous rifampin resistant mutants of the permissive host *E. coli* B/1 and the mutants 7009 and BR3. In each case the mutation causing rifampin resistance had no effect on the ability, or the lack of ability of phage T7 to grow on the host. These *rif*^{r} strains were then used as donors for P1 transduction, and the *rif*^{r} transductants were screened for their ability to support the growth of T7 (Table 2). When the permissive host B/1 *rif*^{r} was used as donor, 93% of the BR3 *rif*^{r} transductants and 89% of the 7009 *rif*^{r} transductants had also become permissive for growth of T7. In the reciprocal transduction using BR3 *rif*^{r} as donor, 95% of the B/1 *rif*^{r} transductants were unable to support the growth of $T7_{0.7}$. The reciprocal transduction using 7009 *rif*^{r} as donor was not carried out since we were unable to obtain good P1 transducing lysates with this host. These results indicate that the mutation in both *E. coli* 7009 and BR3 which affects the growth of T7 phage is tightly linked to *rif*^{r}.

In a separate experiment we also measured the cotransduction of these markers with *arg*H, which maps at 89 min. We obtained about 30% cotransduction of either *tsn*B or the related mutation in *E. coli* BR3 with the *arg*H marker. We conclude that the mutation which renders the RNA polymerases from *E. coli* 7009 and BR3 resistant to inhibition by the T7 gene 2 protein resides in either the β or the β' subunit.

Evidence that the Mutations are in rpoC

To determine whether the mutations are in *rpo*B or in *rpo*C we made use of two recombinant plasmids, PGA43 and PGA45, which were generously provided by James Friesen. These plasmids were derived from the transducing phage $\lambda drif^d18$. They carry the transcription unit containing the promoter P_J, the ribosomal protein genes *rplJ* and *rplL* and the RNA polymerase genes *rpo*B and *rpo*C. However, PGA43 contains both the *rpo*B and *rpo*C genes while PGA45 contains a complete copy of only *rpo*B (Fig. 1). The plasmids have a dominant Rif^r mutation in *rpo*B and they also code for resistance to chloramphenicol.

We transformed *E. coli* 7009 and BR3 with these two plasmids and determined the effect of each plasmid on the growth of T7 phage in the mutant strain. Since these plasmids are maintained in multiple copies, we would expect the products from the plasmid genes to dilute out those from the chromosome so that very little mutant RNA polymerase is formed. In fact, this is observed in transformants produced with PGA43. When this plasmid, which carries both *rpo*B and *rpo*C, is introduced into either *E. coli* 7009 or BR3, the transformants grow T7 phage with a near normal efficiency (Table 3). In contrast, when PGA45 is introduced into these cells it has very little effect on the efficiency of T7 growth.

Table 3. Growth of T7 phage in transformants of 7009 and BR3

Host	$T7^+$		$T7_{0.7}$	
	Percent input phage forming infective centers	Burst per infective center	Percent input phage forming infective centers	Burst per infective center
B/1	100	112	100	75
7009	30	2.5	<5	1
SRB7 (7009/pGA43)	100	108	60	38
SRB8 (7009/pGA45)	80	18	<5	1
BR3	80	7	<5	1
SRB11 (BR3/pGA43)	–	–	100	42
SRB12 (BR3/pGA45)	–	–	20	1

One step growth experiment were performed at 37° C as described under Methods. The fraction of input phage forming infective centers was calculated relative to the number of plaque forming units (titered on *E. coli* B/1) added to the culture

If the transformants are grown for several generations in the absence of chloramphicol, spontaneously cured derivatives can be isolated which have lost their resistances to both chloramphenicol and rifampin. These cured transformants are indistinguishable from the parental strains 7009 and BR3.

Although pGA45 has almost no effect on the efficiency of $T7_{0.7}$ growth, transformation with this plasmid does increase somewhat the efficiency of $T7^+$ growth in 7009 (Table 3). We have carried out a single burst experiment which indicates that the small increase in growth of $T7^+$ in SRB8

does not result from *rpoC*$^+$ cells in the culture, cells which might have been produced by recombination with the *rpoC* fragment carried on pGA45. There are several possible explanations for the partial complentation of 7009 by pGA45. The RNA polymerase assembly intermediate $\alpha_2\beta$ has been implicated in the regulation of expression of *rpo*B and *rpo*C (Fukuda et al. 1978) and it is possible that overproduction of the β subunit could result in a decrease in the number of RNA polymerase molecules in the cell. Since the growth of T7 in *E. coli* 7009 is apparently blocked by a failure to inhibit the bacterial RNA polymerase, such a decrease might allow limited growth of T7 in this strain. Alternatively, it is possible that the *rpo*B mutation *rif*^{d}18 carried on pGA45 enhances the growth of T7$^+$ in 7009. Finally, it is possible that while the major mutation affecting T7 growth is in *rpo*C, a second mutation in *rpo*B also affects the growth of T7$^+$ in 7009. While we have not ruled out the possibility of such a second site mutation, our experiments with the plasmids pGA43 and pGA45 indicate that the major mutation affecting the growth of T7 in 7009 and BR3 resides in *rpo*C, the structural gene for the β' subunit of RNA polymerase. The mutation in *E. coli* 7009 has been assigned the allele number *rpo*C319 and the mutation in BR3 is *rpo*C320 (B.J. Bachman, personal communication).

Properties of a λrif^d*18 Transductant of E. coli rpoC320*

The transducing phage λd*rif*^{d}18 carries the *rpo*B, C transcription unit with a dominant Rif^r mutation in *rpo*B (Kirschbaum and Konrad 1973; Kirschbaum and Scaife 1974). A transductant carrying this phage would contain two copies of *rpo*B and *rpo*C and would allow us to test whether our *rpo*C mutants were dominant or recessive to the wild type allele. Both 7009 and BR3 are *E. coli* B strains and do not contain the *lam*B (*mal*B) receptor required for absorption of phage λ. Therefore, *rpo*C319 and *rpo*C320 were transferred by P1 transduction into the *E. coli* K12 strain WA802 (see Methods). Efforts to construct a λd*rif*^{d}18 transductant of SRB9 (WA802 *rpo*C319) were not fruitful. Seventeen independent Rif^r transductants were isolated but they all proved to be stable transductants. The *rpo*C319 mutation appears to prevent the growth of phage λ. Although λvir grows well on WA802, it plates on SRB9 with an efficiency of only 10^{-10}. Similarly, when the *lam*B receptor was moved into *E. coli* 7009 by P1 transduction, the resulting strain (7009 *lam*B) would not plate λvir.

However, λvir grows well on SRB13 (WA802 rpoC320) and it was possible to construct a λ*rif*^{d}18 transductant of this strain. The results of one step growth experiments carried out at 30° C with SRB13 and the λ*rif*^{d}18 transductant SRB14 are shown in Table 4. The presence of the wild type *rpo*C gene in the transductant SRB14 does not significantly enhance the growth of either T7$^+$ or $T7_{0.7}$ phage. Although the transductant yields about twice as many infective centers, the burst of phage remains low. We conclude that *rpo*C320 is dominant to the wild type allele.

We were also able to use this transductant to obtain additional evidence that the RNA polymerase mutation from *E. coli* BR3 is in *rpo*C and not *rpo*B. If the λd*rif*^{d}18 transductant SRB14 is grown in the presence of rifampin, only RNA polymerase molecules containing the Rif^r β subunit encoded by the transducing phage will be active. If our mutation was in *rpo*B, the transductant would become

Table 4. Growth of T7 phage in SRB13 (λd*rif*^{d}18)

Host	T7$^+$		$T7_{0.7}$	
	Percent input phage forming infective centers	Burst per infective center	Percent input phage forming infective centers	Burst per infective center
WA802	100	46	100	56
SRB13 (WA802 *rpo*C320)	60	7	30	3
SRB14 (SRB13 (λd*rif*^{d}18))	100	7	60	2
SRB14 + rifampin	100	9	100	3

One step growth experiments were performed at 30° C as described in Table 3. During growth with rifampin, 50 μg/ml rifampin was present in the T Broth throughout the experiment and plaque forming units were determined on agar plates containing 50 μg/ml rifampin using SRB4 (B/1 Rif^r) as indicator

permissive for T7 during growth in rifampin since all of the active RNA poymerase molecules would be sensitive to inhibition by the T7 gene 2 protein.[1] In contrast, if the mutation was in *rpo*C, about half of the rifampin resistant RNA polymerase molecules should also be resistant to inhibition by the gene 2 protein, and since resistance to the gene 2 protein is dominant over the wild type phenotype, the transductant should remain non-permissive for T7 during growth in rifampin.

The addition of rifampin to the growth medium had no significant effect on the growth of T7 in the transductant SRB14 (Table 4). This supports our conclusion that the mutation from BR3 (rpoC320) is in *rpo*C.

Growth of T7 in rpoC319 and rpoC320 Transductants

To demonstrate that the phenotypes of *E. coli* 7009 and BR3 are associated with the *rpoC* mutations, we have analyzed the defects in T7 growth in several transductants and compared these defects with these seen in the original mutant strains. Two types of P1 transductants were constructed (see Methods). First we have transduced the wild type *rpo*C allele into the original mutant strains. Second, we have transduced the mutant alleles *rpo*C319 and *rpo*C320 into a K12 strain WA802. The results of one step growth experiments carried out with these strains using either T7$^+$ or a T7 gene 0.7 mutant are shown in Table 5. The yield of either T7$^+$ or $T7_{0.7}$ in SRB9 (WA802 *rpo*C319) is very low and is essentially the same as that obtained in the original *rpo*C319 strain 7009. Neither phage will form plaques on either of these two strains. In contrast, both

1 We assume that the RNA polymerase molecules which have been inactivated by rifampin will not interfere with T7 growth, even if they are resistant to inhibition by the T7 gene 2 protein. This is a reasonable assumption since rifampin can substitute for the gene 2 protein during a T7 infection. When rifampin is added at 5 min after infection a near normal burst of phage is produced during the normally nonproductive infection with a T7 gene 2 mutant or in the mutant hosts 7009 or BR3 (Ontell and Nakada 1980; Mooney et al. 1980; Buchstein 1981)

Table 5. Growth of T7 phage in *rpo*C$^+$, *rpo*C319 and *rpo*C320 strains

Bacterial strain	$T7^+$				$T7_{0.7}$	
	Percent input phage forming infective centers		Burst per infective center		Percent input phage forming infective centers	Burst per in fective center
	30° C	37° C	30° C	37° C	37° C	37° C
B/1 (*rpo*C$^+$)	100	100	65	108	100	75
WA802 (*rpo*C$^+$)	100	100	46	93	–	–
7009 (*rpo*C319)	30	30	1	2.5	<5	1
SRB9 (WA802 *rpo*C319)	–	30	–	2.7	30	1
SRB17 (7009 *rpo*C$^+$)	–	80	–	78	70	68
BR3 (*rpo*C320)	60	80	4	7	<5	1
SRB13 (WA802 *rpo*C320)	60	70	7	7	30	3
SRB16 (BR3 *rpo*C$^+$)	100	100	76	104	100	52

One step growth experiments were performed at 30° C or 37° C as described in Table 3

phage can plate on SRB17 (7009 rpoC$^+$) and both phage produce a normal burst on this host.

$T7_{0.7}$ will not form plaques on SRB13 (WA802 rpoC320) although the yield of phage is slightly higher in SRB13 than in the original *rpo*C320 strain BR3. $T7^+$ plates with near normal efficiency at both 30° C and 37° C on SRB13 while on BR3 plaques are formed only at 37° C. However, from the one step growth experiments it is apparent that this difference in plating efficiency results from only a very small difference in burst size. In SRB13 an average burst of 7 phage per infective center is obtained with $T7^+$ at both 30° C and 37° C while in BR3 the average burst is 4 at 30° C and 7 at 37° C. In the reciprocal transductant SRB16 (BR3 *rpo*C$^+$), both $T7^+$ and $T7_{0.7}$ produce a normal burst of phage.

Kinetics of [^{3}H]-thymidine Incorporation During T7 Infection of rpoC319 and rpoC320 Transductants

The kinetics of DNA synthesis during the nonproducitve infection of *E. coli* 7009 (*rpo*C319) by $T7^+$ phage is abnormal; synthesis decreases prematurely between 10 and 15 min after infection and then gradually increases again (Chamberlin 1974). During infection with a T7 gene 0.7 mutant, the shut off of phage DNA synthesis in *E. coli* 7009 is more complete (De Wyngaert and Hinkle 1980). When the wild type *rpo*C allele was introduced into *E. coli* 7009 to construct SRB17, the kinetics of T7 DNA synthesis was restored to a normal pattern (Fig. 2, panels A and D). The kinetics of T7 DNA synthesis is also abnormal in *E. coli* BR3 (rpoC320). When the wild type *rpo*C allele was introduced into *E. coli* BR3 to construct SRB16, there was a significant increase in the rate of DNA synthesis during T7 infection, although the kinetics of phage DNA synthesis is not completely restored to the normal pattern (Fig. 2, panels B and E).

In the reciprocal experiment we have measured the kinetics of [^{3}H]-thymidine incorporation during T7 infection of transductants of *E. coli* WA802 (Fig. 2, panels C and D). The rate of T7 DNA synthesis in both the *rpo*C319 transductant (SRB9) and the *rpo*C320 transductant (SRB13) is lower than that obtained in the parent strain WA802, although the patterns of synthesis do not exactly mimic the patterns observed during infection of the original mutants 7009 and BR3.

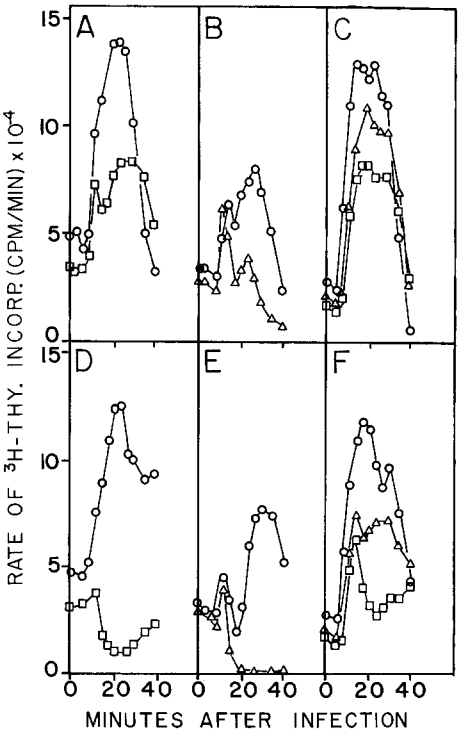

Fig. 2A–F. Kinetics of DNA synthesis in T7-infected cells. Bacteria were grown at 30° C in M9 media to a density of 2.5×10^8 cells/ml and infected with T7 phage at a multiplicity of 10. At the indicated times after infection, 0.1 ml samples were removed from the culture, incubated for 1 min at 30° C with 0.5 μCi of [^{3}H]-thymidine, and acid insoluble radioactivity was determined. Cells were **A, D**: 7009 (*rpo*C319) (□) or SRB17 (7009 *rpo*C$^+$) (○), **B, E**: BR3 (*rpo*C320) (△) or SRB16 (BR3 *rpo*C$^+$) (○), **C, F**: WA802 (*rpo*C$^+$) (○), SRB9 (WA802 *rpo*C319) (□), or SRB13 (WA802 *rpo*C320) (△). Cells were infected with **A, B, C**: $T7^+$ phage, **D,E,F**: $T7_{0.7}$ phage

Sedimentation Analysis of Pulse-Labeled DNA from T7 Infected rpoC319 and rpoC320 Transductants

We have previously shown (De Wyngaert and Hinkle 1980) that the DNA produced in T7 infected *E. coli* 7009 does not form the very large replicative intermediates seen in T7 infected wild type cells. Furthermore, the concatemers that are formed during infection of 7009 are processed into

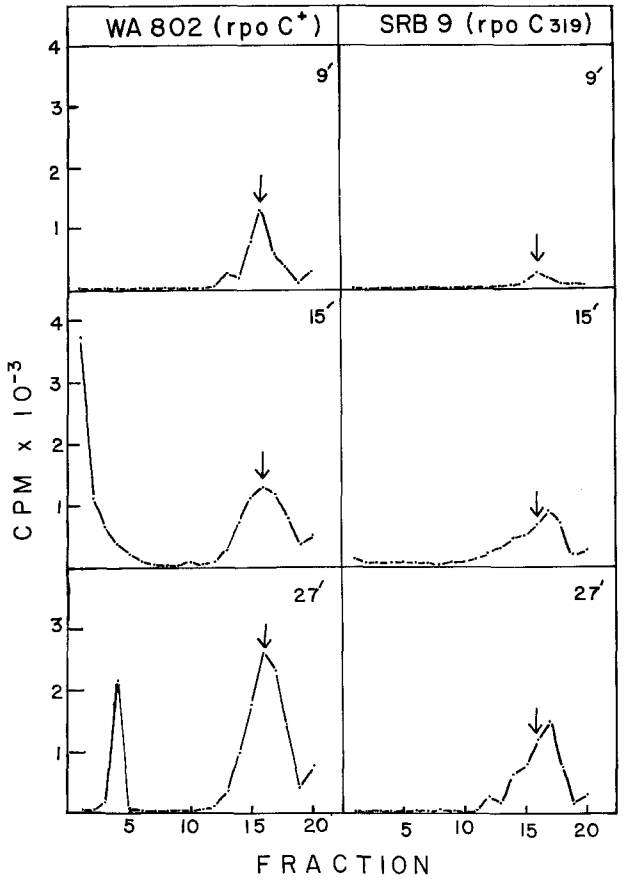

Fig. 3. Sedimentation analysis of pulse-labeled DNA from $T7_{0.7}$-infected *rpo*C$^+$ or *rpo*C319 cells. *E. coli* WA802 or SRB9 were grown and infected with $T7_{0.7}$ phage as described in the legend to Fig. 2. At the indicated times after infection, 2.5 ml portions were removed from the culture and incubated for 15 s with 1.25 μCi of [^{3}H]-thymidine. The DNA was isolated by the procedure of Paetkau et al. (1977) and analyzed by neutral sucrose gradient sedimentation as described previously (De Wyngaert and Hinkle 1980). The vertical arrows indicate the position expected for unit-length T7 DNA as determined by sedimentation of T7 [^{3}H]-DNA on a parallel gradient. The direction of sedimentation is from right to left

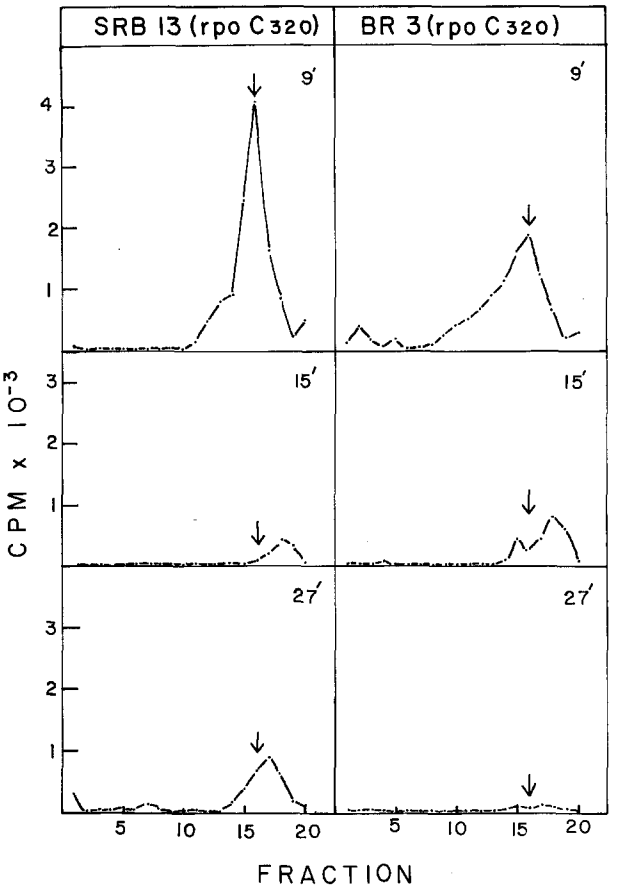

Fig. 4. Sedimentation analysis of pulse-labeled DNA from $T7_{0.7}$-infected *rpo*C320 cells. The conditions of the experiment, performed with *E. coli* SRB13 or BR3, are identical to those of Fig. 3

molecules that are smaller than unit length. These defects were most severe during infections with a T7 gene 0.7 mutant.

To test whether these defects were also associated with the *rpo*C319 and *rpo*C320 transductants, we have carried out the experiments shown in Figs. 3 and 4. At intervals after infection, cells were pulse-labeled (15 s) with ^{3}H-thymidine and the intracellular DNA was extracted by the procedure of Paetkau et al. (1977) and analyzed by sedimentation in neutral sucrose gradients. In *rpo*C$^+$ cells (WA802), much of the DNA pulse labeled at 15 and 27 min after infection was in a fast sedimenting form which moves to the CsCl shelf at the bottom of the tube (Fig. 3). In contrast, during infection of the *rpo*C319 transductant SRB9 none of this fast sedimenting DNA was observed and much of the labeled DNA was shorter than unit length (Fig. 3). Similar defects were observed when the original *rpo*C320 strain (BR3) or the *rpo*C320 transductant SRB13 was used in the experiment (Fig. 4). A similar result was obtained when these experiments were carried out using T7$^+$ infected cells (data not shown).

In conclusion, the defects in bacteriophage T7 DNA synthesis during growth in the three *rpo*C mutant strains SRB9, BR3 and SRB13 closely resemble the defects we had previously observed in the original *rpo*C319 strain *E. coli* 7009 (tsnB).

Discussion

RNA polymerase purified from the *E. coli* mutants 7009 and BR3 is resistant to inhibition by the T7 gene 2 protein (De Wyngaert and Hinkle 1979). We have determined that in both of these strains the mutation which prevents T7 growth is in *rpo*C, the structural gene for the *β′* subunit of the bacterial RNA polymerase. The *β′* subunit is the most basic subunit of RNA polymerase and it probably plays a direct role in DNA binding. Even in the absence of the other subunits, *β′* binds to DNA and also to an inhibitor of DNA binding by RNA polymerase, the polyanion heparin (Zillig et al. 1971). The T7 gene 2 protein, like heparin, is a competitive inhibitor of template binding by the RNA polymerase (Hesselback and Nakada 1977; De Wyngaert and Hinkle 1979). Since the mutations which prevent the binding of T7 gene 2 protein to RNA polymerase map in *rpo*C, the gene 2 protein may also bind to the *β′* subunit of RNA polymerase.

Our observation that the *rpo*C320 allele is dominant over wildtype supports the idea that the mutant RNA polymerase, which is resistant to inhibition by the T7 gene 2 protein, acts in a negative manner to interfere with normal phage development. Although we cannot rule out the possibility that the gene 2 protein also plays some positive role in phage development, the fact that rifampin can substitute for the gene 2 protein (Ontell and Nakada 1980; Mooney et al. 1980; Buchstein 1981) makes such a role unlikely.

Why is inactivation of the bacterial RNA polymerase required for T7 growth? The shutoff of synthesis of host and T7 class I proteins is essentially normal during infection with a T7 gene 2 mutant (Studier 1972), suggesting that the gene 2 protein is not required for this regulatory mechanism. Perhaps transcription by the bacterial RNA polymerase directly interferes with T7 DNA synthesis and packaging and the gene 2 protein is required for complete inhibition of the RNA polymerase. Consistent with this possibility, the gene 2 protein (or rifampin) is required for T7 DNA packaging in vitro (Le Clerc and Richardson 1979; Buchstein 1981). The transcription complex formed with the bacterial RNA polymerase may block the packaging reaction. The transcription complex formed with T7 RNA polymerase is less stable (Chamberlin and Ring 1973) and this could explain why the T7 enzyme does not interfere with DNA packaging.

The gene 2 protein is not required for T7 DNA synthesis in a crude in vitro system (Hinkle and Richardson 1974). Our measurement of the kinetics of ^{3}H-thymidine incorporation during T7 infection of *rpo*C319 and *rpo*C320 transductants indicates that while the rate of T7 DNA synthesis is reduced during infection of the *rpo*C mutants, especially using a T7 gene 0.7 mutant, other factors in the host may also affect the pattern of DNA synthesis. In all cases, however, no large replicative DNA intermediates were detected in the T7 infected *rpo*C319 or *rpo*C320 cells, and the pulse labeled DNA appeared to be somewhat shorter than unit length. We do not yet understand what factors other than the *rpo*C mutations might influence the kinetics of ^{3}H-thymidine incorporation in the T7 infected cells, and it is of course possible that these kinetics do not accurately represent the kinetics of T7 DNA synthesis. However, in some cases there is a complete cessation of ^{3}H-thymidine incorporation at about 15 min after infection of the mutant host. This strongly suggests a block in T7 DNA synthesis. It is not clear why a block in DNA packaging should so dramatically reduce the rate of DNA synthesis. Instead, transcription by the bacterial RNA polymerase may directly interfere with DNA replication.

The T7 RNA polymerase plays a direct role in the initiation of T7 DNA replication (Fischer and Hinkle 1980; Wever et al. 1980; Romano et al. 1981) and two promoters for the T7 enzyme are located at the primary origin (Tamanoi et al. 1980). This region of the genome is also transcribed by the bacterial RNA polymerase. Perhaps transcription by the bacterial enzyme interferes with initiation at this origin.

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