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Isolation and Characterization of Prototrophic Mutants of *Escherichia coli* Unable to Support the Intracellular Growth of T7

MICHAEL CHAMBERLIN

Department of Biochemistry, University of California, Berkeley, California 94720

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Mutants of *E. coli* B/1 were isolated which grew normally but did not permit the intracellular growth of bacteriophage T7. Two classes of mutants were studied in detail (*tsnB*$^-$ and *tsnC*$^-$). These strains adsorbed T7 normally and were killed by the infection. Synthesis of T7 RNA and of early and late classes of T7 proteins occurred normally after infection. In T7-infected *tsnB*$^-$ cells, T7 DNA synthesis stopped prematurely shortly after its onset, suggesting that the *tsnB* function affects a step in the late phase of T7 DNA replication. Mutants of T7 were isolated (T7β) which could grow on *tsnB*$^-$ cells. In T7-infected *tsnC*$^-$ cells, T7 DNA synthesis was completely blocked, suggesting that the *tsnC* function affects a step in an early phase of T7 DNA replication.

The growth of a bacteriophage in an infected cell involves a series of steps in which host and phage components must interact. Since alteration of either host or phage components can block growth, the isolation and study of host range mutations has proved a profitable approach to the study of these host cell-phage interactions. Whereas some of the earliest studies of host range mutants focused on mutants blocked in the adsorption process (1), it has become clear recently that there are also steps involved in intracellular phage growth which can be blocked by mutations in either the bacterial cell or the infecting phage (7-10, 12).

The intracellular growth of phage T7 involves a variety of positive and negative control processes which may include regulation of gene expression at the level of both transcription and translation (25). The T7 genome contains information for at most 30 to 40 proteins; the genes for nearly all of these proteins have been identified and mapped genetically, and in many cases the identity and function of the protein is known as well. Thus, it is hoped that a complete understanding of the regulatory processes which occur in the growth of T7 phage is within reach. In an attempt to identify steps in the growth of T7 phage which involve interactions between host and phage components, I isolated and characterized two classes of mutants of *Escherichia coli* B which grow normally but which are unable to support the intracellular growth of T7 phage.

MATERIALS AND METHODS

The growth and handling of T7 and related phage strains was carried out as described by Studier (26). All growth curves were carried out at 30 C. Phages T7L, T3L, and amber mutants of T7 were obtained from F. W. Studier; ϕII phage and the ϕII mutant, which grow on *fsp*$^+$ strains of *Escherichia coli*, were obtained from G. G. Meynell (30) as well as *E. coli* K-12 strains, which are *fsp*$^+$ and *fsp*$^-$. The T7 mutant SS$^-$ and its host *Shigella sonnei* and T3-T7 recombinant phages were obtained from R. Hausman. *E. coli* B mutants blocked in the intracellular growth of T7 phage were isolated after nitrosoguanidine mutagenesis (2) of strain B/1. After mutagenesis, cells were grown for about four to five generations, and then samples containing about 2,500 viable cells were spread on broth plates along with from 10^6 to 10^7 T7 phage. After 14 to 20 h, large or medium-sized colonies were picked and purified by streaking to obtain single colonies. These isolates were then tested by replicating onto a plate spread with 10^9 T7 phage; strains were saved which failed to grow on such a plate and hence could presumably adsorb and be killed by T7 phage.

RESULTS

Classification of tsn$^-$ mutants of E. coli B/1. I shall designate mutants of *E. coli* B/1 which are killed by T7 phage but which do not permit normal phage growth as *tsn*$^-$. Twenty-eight *tsn*$^-$ isolates were tested further, initially by spot tests, for the ability to grow or be killed by phages T2, T4, ϕII, T3, and T7. Serial 10-fold dilutions of the phages (from about 10^9

to 10^4 phage/ml) were prepared in a honeycomb containing 25 chambers in a 5 × 5 grid pattern. A multiple-tine device was used to deliver about 10 μliters of each dilution in a single step to a plate seeded with the bacterial strain to be tested. This allowed strains to be rapidly screened for the ability to be killed by or grow the phages, and gave an estimate of the efficiency of plating (EOP). The *tsn*⁻ mutants tested were divided into three general classes as a result of these tests.

tsnA⁻ mutants (20 of 28 isolates). These strains are completely resistant to both T3 and T4 phage and hence are probably B/3,4 mutants. T7 phage killed *tsnA*⁻ strains and plated with an efficiency that varied from 0.1 to 10^{-5} for difference isolates. T7 plaques varied widely in size as reported for host range mutants of T3 phage (6).

T7 phage isolated from plaques grown on *tsnA*⁻ strains had an improved EOP on the *tsnA*⁻ strains. Fresh isolates of *tsnA*⁻ strains plated φII nearly normally; however, this ability was lost on propagation and the strains became /φII. It seems likely that the *tsnA*⁻ mutants are altered at the ϕ^R site (3) and hence are impaired in adsorption although not completely blocked. I have not studied these strains further.

tsnB⁻ mutants (3 of 28 isolates). These strains are able to grow T3 and T4 normally by spot tests, and are killed by, but do not grow, T7 and φII phage. Strain 7009 was selected for further study; it plates φII and T7 at an EOP of about 10^{-7} to 10^{-8}, and T3 at an EOP of 0.4 relative tc that of B/1. Strain 7009 grows normally at 37 C in either rich or minimal medium.

tsnC⁻ strains (5 of 28 isolates). These strains grow T4 phage normally by spot testing and are killed by, but fail to grow, T3, T7, and φII phage. Strain 7004 was selected for further study; it plates T7 with an efficiency of less than 10^{-9}. Strain 7004 grows normally at 37 C in either rich or minimal medium.

T7 mutants which grow on tsnB⁻ (7009). Plaques were observed when T7⁺ was plated on strain 7009 at an EOP of about 10^{-7} to 10^{-8} relative to B/1. These plaques contained phage which grew on 7009, although the EOP varied considerably among different phage isolates. In general, phage derived from large, clear plaques grew on 7009 with the greatest efficiency; several such phage strains were isolated and were designated as β⁻ mutants signifying their ability to grow on *tsnB*⁻ strains. T7β was inactivated by a rabbit antiserum prepared against T7⁺ with a K = 3,000 as compared to a k of 3,200 for T7⁺. T7β showed an EOP of 0.3 to 0.5 on strain 7009 relative to strain B/1. This ability to grow on 7009 was not lost after growth on *E. coli* B/1. Thus, T7β appears to be a T7 mutant able to grow on both *tsnB*⁻ and *tsnB*⁺ host strains. A comparable φII mutant was obtained which showed an EOP of 0.8 on 7009 relative to B/1.

Effects of T7 infection on tsnB⁻ and tsnC⁻ strains. (i) Adsorption and killing of the infected cell. The observation that T7⁺ was able to kill *tsnB*⁻ and *tsnC*⁻ strains in spot tests suggested that T7⁺ was able to adsorb to and kill these strains normally. However, this was tested directly in two ways. First, by direct measurement of viable cell count, in the presence of a multiplicity of infection of 20 of T7⁺, from 94 to 96% of the cells in a culture of B/1, 7004, or 7009 were killed in a 2-min period. Second, when samples containing 10^9 cells of bacterial strains B/1, 7004, or 7009 per ml were exposed to 10^8 phage per ml for 5 min and then lysed with chloroform, there was a 85 to 90% loss in viable phage in the suspension. In contrast, a strain of *E. coli* B/7 showed at most a 30% loss in viable phage. Thus, adsorption of T7⁺ phage to a *tsn*⁻ cell leads to killing of the cell and inactivation of the infecting phage.

(ii) Lysis. *E. coli* B infected with T7⁺ lyse after about 28 min at 30 C (26). Strain 7004 lysed well from 30 to 40 min after infection with T7⁻, whereas strain 7009 lysed slowly over the period from 30 to 60 min after infection.

(iii) Infective centers and burst size for T7 and T7β infection of tsnB⁻ and tsnC⁻ strains. One-step growth curves for T7⁺ in strains B/1, 7004, and 7009, and for T7β in strains B/1 and 7009 were carried out (1). Less than 1% of the T7⁺ phage adsorbed to strains 7004 or 7009 gave rise to an infective center when plated subsequently on B/1. No marked increase in phage was seen during lysis of strains 7004 or 7009 after infection with T7⁺; a burst size of no more than two phage per viable infective center was estimated. Infection of *E. coli* B/1 with T7β⁺ gave a burst size of about 60 between 25 and 30 min after infection, whereas infection of 7009 with the same phage gave a burst size of 9 to 16 between 30 and 35 min. Less than 1% of the cells of strain 7004 gave rise to infective centers when infected with T7β phage. Under comparable conditions, T7⁺ infecting *E. coli* B/1 gave a burst size of 100 to 150 after 25 to 30 min.

Experiments were carried out in which *E. coli* B/1 or 7009 were infected simultaneously with both T7⁺ and T7β phage (Table 1). When both phages infected *E. coli* B/1 simultaneously, the

growth of T7$^+$ was favored to the extent expected from the differences in burst size for T7$^+$ and T7β when they grew separately on this strain. Hence, there is no apparent interaction between β and β^+ alleles. When T7$^+$ and T7β both infected 7009, there was normal growth of T7β and little or no growth of T7$^+$ phage. Thus, the β function facilitates growth only of T7β phage, which is consistent with the notion that the β function is either carried out by a site on the T7 DNA molecule or that β perhaps specifies a *cis*-acting protein component.

Infection of tsnB$^-$ and tsnC$^-$ mutants with other T7 and ϕII host range mutants and with T3-T7 recombinant phages. (i) Infection with T7 SS$^-$ mutants. Hausman has reported that although T7$^+$ will not grow on *S. sonnei* strain D2 371-48, T7 mutants can be isolated which plate normally on these strains (15). T7 SS$^-$ mutants obtained from Hausman did not plate on the *tsnB*$^-$ mutant (7009) or *tsnC*$^-$ mutant (7004).

(ii) Infection with ϕII variants that grow on fsp$^+$ strains. Williams and Meynell (30) have shown that some *E. coli* strains carry a locus (*fsp*$^+$) which restricts the growth of ϕII and other related T7-like phages. T7 phage itself is not affected; however, variants of ϕII (α) can be isolated which do grow on *fsp*$^+$ strains. (For the sake of clarity I shall call these variants α phage. In practice, the α locus probably governs adsorption; when 5×10^8 *E. coli* K-12 *fsp*$^+$ cells per ml are mixed with a multiplicity of infection of 20 of ϕIIα phage, there is less than 10% loss in viable cells in 30 min. In addition, ϕIIα plate on *fsp*$^+$ strains with the large range of plaque sizes which is characteristic of poorly adsorbing phage strains [6]. Since *fsp* does not map near the traditional ϕ^R locus [3, 30] it seems probable that this is an independent site which affects ϕII adsorption, but not that of T7 or T3.) Hence, because current strains of T7 have been propagated on *fsp*$^+$ strains, the original *fsp*$^+$-sensitive (α) character of T7 may have been lost from T7. Strains of ϕI and ϕII unable to grow on *fsp*$^+$ (ϕIα^+ and ϕIIα^+) are unable to grow on *tsnB*$^-$ or *tsnC*$^-$ strains as are their α counterparts. Occasional plaques from ϕIIα or ϕIIα^+ plated on 7009 were purified and shown to have the β character. When ϕII$\alpha^+\beta$ was replated on *fsp*$^+$ and *fsp*$^+$ strains of *E. coli* K-12, it still possessed the α^+ character. Hence, there is no relationship evident between *tsnB*$^-$ or *tsnC*$^-$ loci, and the *fsp*$^+$ locus.

(iii) Growth of T3-T7 recombinant strains on tsn$^-$ mutants. Genetic mapping of the β locus on T7 phage will be hampered by the lack of a host bacterial strain in which both β and β^+ phages grow equally well. Because T3 phage grows well on *tsnB*$^-$ strains, it is possible that the β region of the T7 chromosome could be localized by studying the ability of T3-T7 recombinant phages to grow on *tsnB*$^-$ strains. Such recombinant phages have been isolated by Beier and Hausmann (11). Several such recombinants grow well on strain 7009, and preliminary studies (H. Beier, personal communication) suggest that genes in the center of the

TABLE 1. *Growth of T7$^+$ and T7β in mixed infections*[a]

Mixture[b]	Time (min)	Phage conc (PFU/ml)			T7β/T7$^+$
		B/1 indicator	7009 indicator	Δ	
A	0 (input phage) mixture	2.5×10^9	1.5×10^9	1×10^9	1.5
	10 (infective) centers	8×10^8	5×10^8		
	40	4×10^{10}	1×10^{10}	3×10^{10}	0.3
B	0 (input phage) mixture	8.4×10^9	4.6×10^9	3.8×10^9	1.2
	10	4.6×10^8			
	40	5.5×10^{10}	4.4×10^{10}	1.1×10^{10}	4

[a] Five-milliliter samples of bacteria (5×10^8 ml^{-1}) growing at 30 C in a bubbler tube were infected with a mixture of T7$^+$ and T7β phage to give the final phage concentration shown at zero time. After 5 min, T7 antiserum was added to give a final concentration of $k = 1$. After 2 min, volumes were diluted 100-fold for growth. Phage or infective center assays were carried out with appropriate indicator strains at the times shown. Δ is the difference between the phage titer on B/1 and that on 7009; it is a measure of the concentration of T7$^+$ phage.

[b] Mixture A was a mixture of T7$^+$ and T7β infecting *E. coli*. Mixture B was a mixture of T7$^+$ and T7β infecting *E. Coli* 7009 (*tsnB*$^-$).

chromosome from gene 3 to gene 19 can be derived from T7 and still permit the recombinant to grow on 7009. This would tenatively place the β locus near one of the chromosomal extremities; however, much more study of the recombinants is needed before this conclusion can be made firm.

Macromolecular synthesis in T7-infected tsnB$^-$ and tsnC$^-$ cells. (i) RNA synthesis. The rate of RNA synthesis in T7-infected cells was estimated by following the uptake of [^{3}H]uracil into acid-insoluble material in short pulses. The rate of incorporation increases between 4 to 6 min after infection of *E. coli* B/1 by T7$^+$ due to initiation of synthesis of T7 RNA, then decreases markedly after 10 min (27). This same general pattern is seen in *tsnB*$^-$ and *tsnC*$^-$ mutants after infection with T7$^+$. In the normal infection, however, there is a small amount of labeling from 10 to 20 min after infection; this labeling is dependent on the synthesis of T7 DNA (M. Chamberlin, unpublished data). T7 mutants which fail to make T7 DNA did not incorporate added [^{3}H]uracil during this period (Fig. 1). Similarly, there was no incorporation of [^{3}H]uracil into T7-infected *tsnB*$^-$ or *tsnC*$^-$ cells after 10 min; this is consistent with the finding (below) that T7 DNA synthesis is defective in such infections.

(ii) Protein synthesis. The rate of protein synthesis in T7-infected cells was followed by measuring the incorporation of [^{14}C]amino acids into hot trichloroacetic acid-insoluble material in pulses. The rate of [^{14}C]amino acid incorporation in such experiments was essentially constant for the first 25 min after infection of strain B/1 by T7$^+$. Similar results were found during infection of *tsnB*$^-$ by T7$^+$ or T7β. During infection of *tsnC*$^-$ by T7$^+$, the rate of incorporation decreased about 40% during the time period from 10 to 25 min after infection.

At least three classes of T7-specific proteins have been identified by acrylamide gel electrophoresis of labeled proteins from infected cells after denaturation of the proteins with sodium dodecyl sulfate (25). To determine the effect of the *tsnB*$^-$ and *tsnC*$^-$ mutations on the amount of T7-specific proteins and on the timetable of their synthesis, ^{14}C-labeled proteins were analyzed by acrylamide gel electrophoresis using Studier's procedure.

The following conclusions are suggested from analysis of the gel patterns. (i) There is a normal shutoff of the synthesis of bacterial

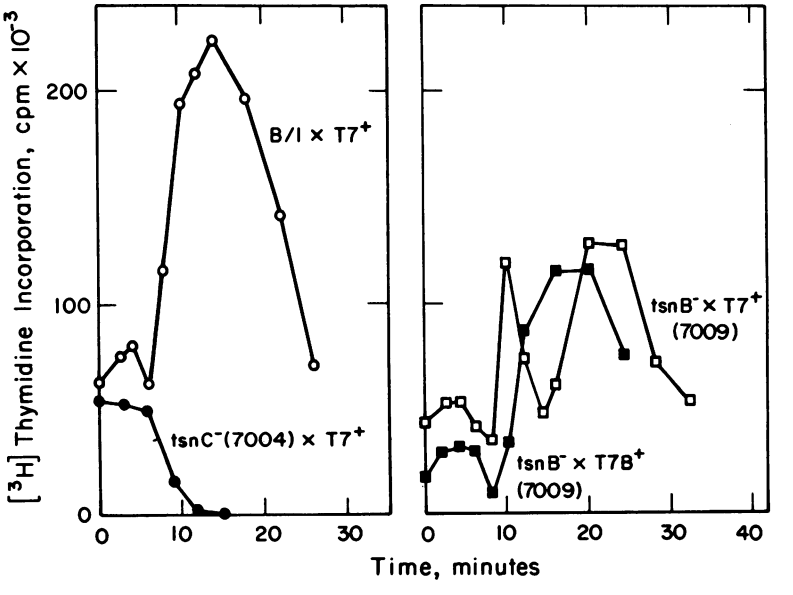

FIG. 1. *Rate of DNA synthesis in E. coli B/1 and tsn$^-$ mutants infected with T7 phage. Bacterial cultures were grown in M9 medium containing 0.4% glucose and 0.2% Casamino Acids to a density of 2.5 to 5 x 10^8 cells/ml. Cultures were chilled on ice and were diluted to 2.5 x 10^8 cells/ml. For pulse labeling, 5-ml volumes were incubated at 30 C with vigorous bubbling for 3 min and were then infected with a multiplicity of infection of 20 T7 phage. Bubbling was continued throughout infection. Samples (0.2 ml) were withdrawn at intervals and labeled with 5 μliters of [^{3}H]thymidine (100 μCi/ml, 20 mCi/μmol). After 60 s at 30 C, 2.5 ml of ice-cold 2.5% perchloric acid was added to each sample, and the total amount of acid-insoluble H was measured by filtration through a Whatman GF/c filter.*

proteins in T7$^+$ infection of both *tsnB*$^-$ and *tsnC*$^-$ strains. (ii) Class I proteins (25) including T7 RNA polymerase and DNA ligase are synthesized. Synthesis is initiated at normal times and is turned off normally. (iii) Class II proteins and class III proteins are synthesized in essentially normal amounts. Synthesis is initiated at normal times and synthesis of class II proteins appears to turn off normally.

Thus, within the limits of resolution of the analytical gel system, the synthesis and regulation of T7 protein synthesis is normal in *tsnB*$^-$ and *tsnC*$^-$ cells infected with T7$^+$. We cannot rule out the possibility that one or a small number of class II or III proteins are not made, or are made in substantially reduced amounts in *tsnB*$^-$ or *tsnC*$^-$ strains infected with T7$^+$ phage.

(iii) DNA synthesis. Synthesis of DNA in T7-infected cells was followed by measuring uptake of [^{3}H]thymidine into cold acid-insoluble material. In strain B/1 infected with T7$^+$, synthesis decreased between the time of infection and 5 to 6 min, due to the turn off of host DNA synthesis (Fig. 1); the rate then increased several-fold beginning at about 7 to 8 min due to synthesis of T7 DNA. In *tsnB*$^-$ cells, the onset of T7 DNA synthesis was delayed slightly, increased about twofold, and then rapidly decreased between 10 and 15 min. This decrease was followed by a slow, gradual increase in synthesis between 20 and 30 min. In *tsnB*$^-$ cells infected with T7β, the onset of T7 DNA synthesis was delayed slightly and then increased in a manner parallel to that seen in T7$^+$ infection of *E. coli* B/1.

Infection of the *tsnC*$^-$ mutant with T7$^+$ lead to a normal cessation of host DNA synthesis; no further incorporation of [^{3}H]thymidine was observed.

A major portion of the nucleotides utilized for synthesis of T7 DNA are derived from the DNA of the host cell (21). Mutants of T7 which lack the T7 specific endonuclease (gene 3) or exonuclease (gene 6) fail to degrade bacterial DNA (23) and are also unable to synthesize phage DNA (26). To test whether *tsnB*$^-$ or *tsnC*$^-$ mutants might be altered in such a way as to block T7-induced breakdown of the bacterial DNA, breakdown of prelabeled bacterial DNA was followed according to the procedure of Hausmann and Gomez (13). Control infections to follow host DNA breakdown in strain B/1 and *tsnB*$^-$ after infection were carried out with a T7 amber mutant (gene 5); little breakdown could be detected during infection of B/1 with T7$^+$ due to rapid reincorporation of host DNA nucleotides into phage DNA (14). Infection of the *tsnB*$^-$ mutant with an amber mutant in gene 5, T7, or of *tsnC*$^-$ with T7$^+$, gave extensive DNA breakdown comparable to that seen during infection of the normal host (Fig. 2).

Fate of T7 DNA from the infecting phage in tsn$^-$ hosts. The observation that there is essentially normal synthesis of T7 proteins in *tsn*$^-$ hosts after infection made it unlikely that the lesion in T7 growth in these hosts was due to breakdown of phage DNA due to restriction by the host specificity system of the cell as has been shown to happen with other phages (20). However, the possibility remained that such breakdown might occur only perhaps at 10 min after infection, when sufficient nRNA had been formed to allow normal protein synthesis, but at a time before T7 DNA replication. To test this, T7 phage labeled in their DNA with [^{3}H]thymidine were prepared. Cells were infected and centrifuged to remove unadsorbed phage, and the acid-insoluble ^{3}H label in phage DNA was followed after infection (Table 2). No loss in acid-insoluble [^{3}H]DNA occurred during infection of *tsnB*$^-$ or *tsnC*$^-$ by ^{3}H-labeled T7$^+$ phage. In subsequent experiments, it has been shown

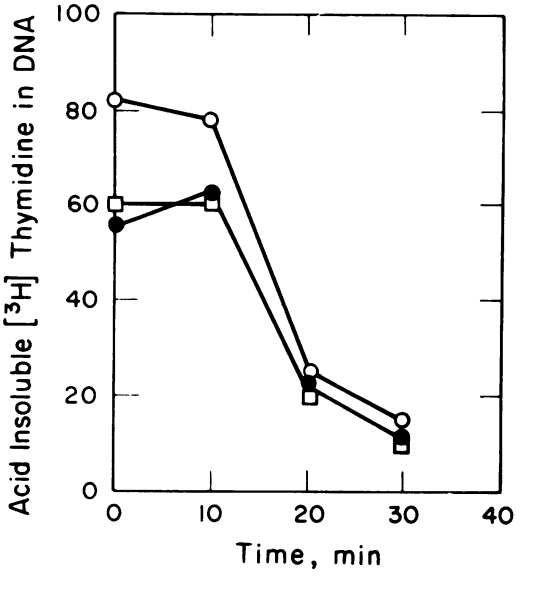

FIG. 2. *Breakdown of bacterial DNA in E. coli B/1 and tsn$^-$ mutants after T7 infection. Bacterial strains growing in M9 medium (5 ml; 5 × 10^7 cells/ml) were labeled for 4 min with 2 μCi of [^{3}H]thymidine. To each culture was added 50 μg of labeled thymidine per ml, and after 2 min the cultures were infected with a multiplicity of infection of 20 of T7$^+$ or T7 am 28 (gene V$^-$) as indicated. Samples (0.5 ml) were taken at indicated times after infection and assayed for cold acid-insoluble radioactivity. Symbols: ○ E. coli B/1 × T7 am 28; □ tsnB$^-$ × T7 am 28; ● tsnC$^-$ × T7$^+$.*

TABLE 2. *Lack of degradation of T7⁺ DNA during infection of tsn⁻ hosts*[a]

Time (min)	Acid-insoluble ^{3}H counts/min in strain:		
	B/1	*tsnB*⁻ (7009)	*tsnC*⁻ (7004)
0	2,091	1,186	1,492
10	2,917	2,566	3,062
20	2,817	2,058	3,187
30	3,399	2,497	3,171

[a] Bacterial cultures (5 ml; 2.5 × 10^8/ml) growing exponentially in M9 medium containing 50 μg of thymidine per ml were infected with a multiplicity of infection of 1.2 of [^{3}H]thymidine-labeled T7⁺ phage (2 × 10^5 counts/min). After 5 min the culture was centrifuged for 2 min at 5,000 rpm in the Sorvall centrifuge and resuspended in fresh unlabeled medium. Volumes (0.8 ml) were taken and assayed for radioactivity insoluble in ice-cold acid.

by sedimentation analysis that intact T7⁺ chromosomes are not fragmented after infection of either 7004 or 7009 (S. Gornicki, personal communication).

DISCUSSION

I isolated a number of bacterial mutants derived from *E. coli* B/1 which appear to grow normally but which are blocked in the intracellular growth of phage T7. Hausmann has carried out an analogous study using a large number of naturally occurring *E. coli* strains (12). Many of these strains are blocked in intracellular development of T3 and T7 phages, and the defects appear to occur at a variety of points in the phage growth cycle. This sort of study promises to reveal interesting information about the different kinds of synergistic interactions between host and viral macromolecules that appear to be an essential part of the program of viral development.

All of the *E. coli* B/1 mutants I isolated which are blocked in intracellular growth of T7 fall into two classes with distinct phenotypic characteristics; these have been designated *tsnB*⁻ and *tsnC*⁻. The properties of the two classes do not appear to be characteristic of distinct mutations at the same locus; however, the possibility must be considered open until genetic mapping establishes otherwise. Mutants of the first class (*tsnB*⁻) are able to support the growth of T3 phage but not of T7 or its close relative φII (17). Mutants of T7 and φII are obtained (T7β, φIIβ) which can grow on *tsnB*⁻ strains as well as on the parental *E. coli* B/1 strain. The T7β mutants do not enhance the growth of T7⁺ (β⁺) in a mixed infection of *tsnB*⁻ strain 7009. Wild-type T7 and T7β grow independently in *E. coli* B/1, although T7β gives a much smaller burst size. This suggests that the β function is carried out by a site on the T7 chromosome, or, alternatively, that the β locus specifies a *cis*-acting protein. The position of the β locus on the T7 chromosome has not yet been determined.

When T7⁺ infects a *tsnB*⁻ strain, the phage-induced termination of host cell functions proceeds normally and the cell is killed. The overall processes or transcription and protein synthesis in the phage-infected cell are also normal, and each of the known classes of phage proteins appears. However, the phage-directed synthesis of DNA is initiated slightly later than in a normal infection and is terminated precipitously several minutes later. This suggests that *tsnB* governs the synthesis of a bacterial component which is essential for a step involved at an intermediate point in T7 DNA replication. Alternatively, *tsnB* might govern the synthesis of a bacterial component which, in its mutant form, is able to block some intermediate step in T7 DNA replication.

E. coli B/1 mutants of the second class (*tsnC*⁻) fail to support intracellular growth of T7 phage or of any of the related female specific phages including φI, φII, and T3. No phage mutants have yet been obtained which can grow on *tsnC*⁻ strains, although these have only been sought extensively beginning with T7⁺ phage. When T7⁺ infects a *tsnC*⁻ strain, termination of host cell processes and the global pattern of RNA and protein synthesis follows as in a normal infection. As in the case of *tsnB*⁻-infected cells, all known classes of phage proteins are synthesized and regulated normally. However, in infected *tsnC*⁻ cells, there is no detectible synthesis of phage DNA. Thus, the *tsnC* gene product appears to be a host protein which affects an early step in T7 DNA replication.

The gene products specified by *tsnB* and *C* might act directly on a step or steps in T7 DNA replication or indirectly, for example, by affecting the stability of T7 DNA or the supply of DNA nucleotide precursors. Indirect action is made less likely by the finding that T7⁺ DNA from the infecting phage is not broken down in *tsnB*⁻ or *C*⁻ hosts and that host DNA breakdown, which provides precursors for T7 DNA, occurs normally in such an infection.

If *tsnB* or *C* participate directly in T7 DNA replication, how can one explain the loss of this function in *tsn*⁻ cells which themselves grow normally? The *tsnB* or *C* proteins might, of course, be unnecessary for normal growth of *E. coli*. It is more attractive to assume that a protein which plays an intimate role in T7 DNA

replication carries out an analogous role in DNA replication in the uninfected cell. This would be possible if the *tsn* proteins were essential for *E. coli* growth, yet possessed a dual specificity. For example *tsnC* protein might function by a specific interaction with another *E. coli* protein to form a complex essential for some step in *E. coli* DNA replication. In infected cells, *tsnC* protein would form a similar complex with an analogous (but not identical) T7 protein needed for T7 DNA replication. *tsnC*$^-$ mutants would then be those in which *tsnC* protein had lost the ability to effectively interact with the T7, but not the *E. coli* protein. A similar kind of model can be constructed if a *tsn* protein interacts with a specific site on DNA, as may be the case for *tsnB* protein. If the site for functional interaction of *tsnB* protein on *E. coli* DNA and T7 DNA are not identical, mutational alteration of *tsnB* protein could lead to selective loss of the initiation with T7 DNA. The T7β mutants would then be mutants in which the DNA site had been altered to restore the fit with *tsnB* protein.

What steps in T7 DNA replication might involve *tsnB* or *C* proteins? Within 7 min after infection of the host cell, synthesis of cellular DNA has ceased, and there is a rapid onset of phage DNA synthesis (16, 26). Initial replication of the T7 genome is carried out on a linear DNA molecule (5, 31). This replication requires T7 gene 4 protein (26), T7 DNA polymerase (gene 5 protein), and possibly T7 RNA polymerase (19) and an unwinding protein (22). Subsequent replication proceeds predominantly in large replicative intermediates which contain concatemeric DNA forms (18, 24). Formation of long concatemers may be an essential step in initiation of this phase of replication of T7 DNA (28). In addition, the final mature sized viral DNA is probably cut from concatemeric intermediates (24). Phage mutants which are deficient in genes 8, 9, 10, 18, or 19 fail to carry out formation of mature-sized viral DNA from the concatemeric intermediates but do synthesize normal amounts of phage DNA (16, 26). There are no previously reported host functions required directly for T7 DNA replication. In particular, none of the known *E. coli* DNA mutants appear to be deficient in T7 DNA synthesis under nonpermissive conditions (19).

The *tsnC* protein affects an early step in replication of T7 DNA. Initiation of replication of *E. coli* DNA and that of several simpler phages, such as M13 and Φx174, involves enzyme-DNA complexes containing several *E. coli* proteins (29). This kind of interaction is potentially sensitive to a mutation of the *tsn* sort. In fact, recent biochemical studies indicate that the *tsnC* protein plays an essential role in initiation of T7 DNA replication by complexing with and activating the T7 DNA polymerase (gene 5 protein) (P. Modrick, D. Hinkle, and C. C. Richardson, personal communication). Hence, *tsnC*$^-$ mutants either contain an altered *tsnC* protein which cannot form this complex or lack *tsnC* protein entirely.

The *tsnB* protein appears to affect a later stage of replication and may involve a site on T7 DNA. The unique steps in this phase of replication are those involving concatemer formation. Host enzyme systems are probably involved in this process (28), but it is not clear which host enzymes might be site specific. One clear difference between T3 phage, which grows in *tsnB*$^-$ hosts, and T7 phage, which does not, lies in their short, terminally redundant DNA sequences (4). These sequences are probably essential for concatemer formation in replication (24, 28). However, the T7β locus does not lie simply in the terminal redundancy region; several T3-T7 hybrid phages which have T3 terminal redundancy regions (11) fail to grow on *tsnB*$^-$ hosts. Direct biochemical studies on the role of *tsnB* and *C* proteins in in vitro T7 DNA replication should prove profitable in specifying their exact roles in T7 DNA replication.

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