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Temperature-Sensitive Modification and Restriction Phenotypes of an *Escherichia coli dnaD* Mutant

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A mutant of *Escherichia coli* temperature-sensitive for deoxyribonucleic acid synthesis, *dnaD*, was found to have temperature-sensitive modification and restriction phenotypes. In contrast to the original observation by Carl (1970), the mutant could support the growth of λ phage at 41 C. However, the λ phages thus produced were able to form plaques with normal plating efficiency only on *E. coli* C, a restriction-less strain, but not on *E. coli* K. Since the λ phages produced in the mutant at 30 C could form plaques equally well on both *E. coli* strains, it was concluded that the *dnaD* mutant has a temperature-sensitive modification phenotype. Furthermore, since the *dnaD* mutant allowed some growth of unmodified λ·C phages at 41 C but less at 30 C, the mutant is also temperature sensitive in restriction. The relationship, if any, between temperature-sensitive deoxyribonucleic acid synthesis and temperature-sensitive modification-restriction in the *dnaD* mutant is not known. Similar experiments were done with three *dnaC* mutants and one *dnaA* mutant. Two *dnaC* mutants were found to have altered restriction phenotypes at 41 C, but none of the mutants were defective in modification.

Temperature-sensitive mutants of deoxyribonucleic acid (DNA) replication have been classified into seven groups (*dnaA-G*) (18). Two of these groups (*dnaA*, *C*) are presumed to be related to the initiation of chromosome replication (9, 4). The *dnaA* mutants were shown to map in the vicinity of the *ilv* locus, at 73 min on the standard *Escherichia coli* chromosome (9). The *dnaC* mutants map at 89 min near *dra* (18). One more mutant, *dnaD*, is also cotransducible with *dra* (18), and in vivo complementation tests suggest that *dnaD* and *dnaC* may be one gene (17). The purification and study of the *dnaC* gene product also support this suggestion, although they do not eliminate other possibilities (19). The original classification of the *dnaC* and *dnaD* mutants into separate groups (4, 18) was mainly due to a physiological difference between them. Carl (4) found that the temperature-sensitive *dnaD* mutant could not support the growth of bacteriophage λ at the nonpermissive temperature, whereas a *dnaC* mutant could.

The purpose of the present work was to examine the difference in the behavior of *dnaC* and *dnaD* mentioned above. Our experiments show that both *dnaC* and *dnaD* mutants support λ phage production at 41 C and that these phages can be assayed on a restriction-less strain (3), *E. coli* C. However, the progeny phages produced in the *dnaD* mutant at 41 C plate only poorly on an *E. coli* K strain, presumably because their DNA is not properly modified and gets degraded by the K-specific restriction endonuclease (7, 13). In addition to this temperature-sensitive modification, the *dnaD* mutant also has a temperature-sensitive restriction phenotype.

Most of the available *dnaC* mutants and a *dnaA* mutant were also examined for their abilities to modify and restrict λ phage at 41 C. Two of the *dnaC* mutants were found to possess altered restriction phenotypes at 41 C.

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are listed in Table 1.

Bacteriophages. λ*vir*·K was a generous gift of B. Allet. Large phage stocks were prepared by infecting *E. coli* K (CR34) with λ*vir*·K in tryptone broth cultures. λ*vir*·C was prepared in the same way except that *E. coli* C was used at the host. λ phages prepared on *E. coli* K-12 and *E. coli* C are designated as λ·K and λ·C, respectively, according to Arber and Dussoix (2).

Determination of cell numbers. Cell concentrations were routinely determined by measurement of turbidity in a Klett photometer. Viable cell number was assayed by colony count on tryptone agar plates, incubated overnight at 30 C.

Phage growth and test for modification. Cells were grown at 30 C in tryptone broth (1%) to a

TABLE 1. *E. coli* strains used

E. coli K-12 strains	*dna* locus	Description	Reference
DG75		*thy*⁻, *leu*⁻, *str*ᴿ	4
PC-7	*D*	Derived from DG75	4
PC-2	*C*	Derived from DG75	4
BW824	*C*	Derived from DG75	20
AB1157		*thy*⁻, *arg*⁻, *his*⁻, *leu*⁻, *thr*⁻, *pro*⁻, *thi*⁻, *str*ᴿ, T6ᴿ	15
CT28-3b	*C*	Derived from AB1157	15
CR34		*thy*⁻, *thr*⁻, *leu*⁻, *thi*⁻, *lac*⁻, *tonA*, *strA*	18
E517	*A*	Derived from CR34	18
E. coli C		CGSC 3121, λ+, S-13, D prophage	3

concentration of 2×10^8 cells/ml. Six milliliters of the culture was centrifuged for 5 min at 8,000 × *g* and resuspended in 3 ml of adsorption medium (0.01 M $MgSO_4$, pH 7.0). After 5 min of preincubation at either 30 or 41 C, λ*vir*·K phage was added at a multiplicity of 3. Incubation continued for 25 min at the same temperature, and then an equal volume of prewarmed 2% tryptone broth was added. Unless indicated, the unadsorbed phage were not removed in order to avoid changing the temperature of the culture at any time after infection. The turbidity of the culture was followed with a Klett photometer. Phage titers were determined by measuring plaque-forming units on agar overlay plates. Indicator bacteria (0.2 ml), grown in tryptone broth to late logarithmic phase, were suspended in 2.5 ml of tryptone soft agar containing 0.01 M $MgSO_4$. Appropriate dilutions of phage lysates were added, and the contents of the tubes were poured onto tryptone agar plates. Incubation was overnight at 37 C.

The modification of the progeny phages was measured by using both *E. coli* C and an *E. coli* K (DG75 or AB1157) strain as indicator bacteria. The efficiency of plating is the ratio of phage titer obtained on *E. coli* K over the titer on *E. coli* C.

Test for restriction. Due to the inability of the temperature-sensitive *dna* mutants to grow at 41 C, the standard plating assay for restriction (10) could not be used at this temperature. For this reason the test for restriction was changed to measure the number of infective centers present 10 min after dilution of the infected cell suspension into tryptone broth (41 C).

Cultures were grown and infected with λ·C or λ·K, at a multiplicity of 0.01, after a preadsorption period of 15 min in 0.01 M $MgSO_4$ (pH 7.0), as described above. After adsorption (30 min), the cell suspension was diluted with an equal volume of prewarmed 2% tryptone broth. Infection was allowed to proceed for 10 min at 41 C or 20 min at 30 C; then the cells were collected by centrifugation at 5 C for 5 min at 8,000 × *g*. The supernatant was tested for unadsorbed phage, which in these experiments was 1 to 4%. The infected cells were resuspended in tryptone broth and immediately plated on *E. coli* K (DG75) at 30 C. Using these conditions, it was possible to use the restricting strain *E. coli* K (DG75) as indicator bacteria even for λ·K- or λ·C-infected *dnaD* (41 C). Plating on *E. coli* K essentially eliminated the contribution of residual unadsorbed λ·C (0.2%) to the number of infective centers.

RESULTS

Growth of λ phage in dnaD (PC-7) and dnaC (PC-2) mutants at 41 C. To study the growth of λ phage in the temperature-sensitive mutants, the cells were grown at 30 C and then shifted to 41 C in the adsorption medium shortly before infection with λ·K. Both of the mutants (PC-7 and PC-2) tested were originally investigated by Carl (4). He reported that PC-2 (*dnaC*) could support λ phage production at 41 C, whereas PC-7 (*dnaD*) could not. However, cultures of both *dnaD* and *dnaC* lysed in less than an hour after infection with λ·K at the nonpermissive temperature (Fig. 1). Titration of the lysate on a restriction-less strain (3), *E. coli* C, indicated that in each case about 40 progeny phages per bacterium were produced (Table 2).

Evidence for a temperature-sensitive modification phenotype in the dnaD mutant. As mentioned above, the *dnaD* mutant does support the growth of λ phage at 41 C and the lysate can be assayed on *E. coli* C. However, the progeny phages produced at 41 C (λ·*dnaD* [41 C]) plated poorly on an *E. coli* K strain (Table 2). This is in contrast to the results obtained with the parent strain (DG75) of *dnaD* (data not shown) or with the *dnaC* mutant, PC-2 (Table 2).

λ·*dnaD* (41 C) was used to infect *E. coli* K (DG75) and *E. coli* C at 37 C (Fig. 2). The phage λ·*dnaD* (41 C) grew well in *E. coli* C but not in *E. coli* K, presumably because λ·*dnaD* (41 C) was restricted in *E. coli* K but not in *E. coli* C.

At the permissive temperature, 30 C, the progeny phages produced in the *dnaD* mutant appeared to possess the normal K-specific modification of their host. These phages plated with an equal efficiency on an *E. coli* K and an *E. coli* C strain (Table 2).

These results indicate that *dnaD* is temperature sensitive in modification, and they can also explain the difference in behavior between the *dnaC* and *-D* mutants observed by Carl (4). He

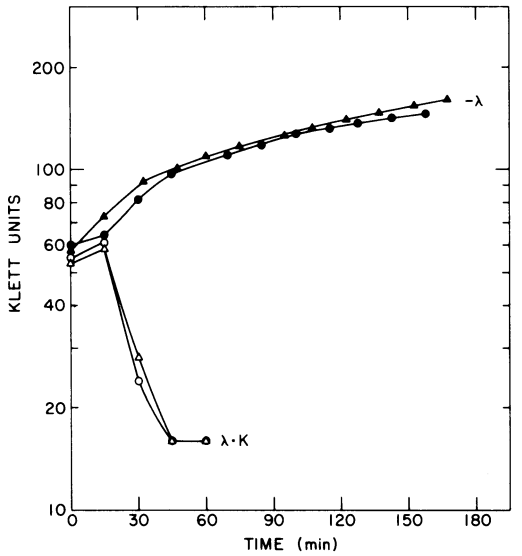

FIG. 1. *Replication of λ·K phage at 41 C in dnaD (PC-7) and dnaC (PC-2) mutants. Cells grown at 30 C were resuspended in adsorption medium and infected with λ·K phage at 41 C. The time at which the cell suspension was diluted into prewarmed (41 C) tryptone broth is designated as 0 min. Control cultures of each strain were treated in the same way except that they remained uninfected (−λ curve). The turbidity of the cultures was measured by a Klett spectrophotometer at time intervals indicated. Symbols:* ○, *dnaD (PC-7) infected with λ·K;* ●, *dnaD (PC-7) without infection;* △, *dnaC (PC-2) infected with λ·K;* ▲, *dnaC (PC-2) without infection.*

TABLE 2. *Growth and modification of λ phage in dnaD (PC-7) and dnaC (PC-2) mutants*[a]

Mutant	Temp of infection (C)	Burst size	Modification (EOP of phage progeny)
dnaD (PC-7)	41	40	10^{-2}–2×10^{-3}
dnaD (PC-7)	30	22	1.0
dnaC (PC-2)	41	38	1.0

[a] Mutant cultures were grown and infected with λ·K (multiplicity of infection, 3) at 41 or 30 C. To get a better estimate of the efficiency of plating (EOP) of the progeny produced in *dnaD* at 41 C, the unadsorbed λ·K (1%) was removed by centrifugation after the adsorption period. Burst sizes were measured on *E. coli* C and were compared with that on *E. coli* K. The EOP on *E. coli* C is taken as 1.0.

could not detect viable phages in λ-infected cultures (41 C) of the *dnaD* mutant, because λ phages produced in the culture were not able to form plaques with normal plating efficiency on a restricting *E. coli* strain.

Evidence for a temperature-sensitive restriction phenotype in the dnaD mutant. Since the restriction and modification systems of *E. coli* are closely related (5, 10, 11), we checked the possibility that both of these functions were defective in *dnaD* at the nonpermissive temperature.

The test for restriction consisted of checking the ability of unmodified λ, λ·C, to form infective centers in the mutant. By this criterion, *dnaD* was found to be defective in the restriction of λ·C at 41 C and to a smaller extent also at 30 C (Table 3). In contrast, the parent strain *E. coli* K (DG75) of *dnaD* restricted λ·C with high efficiency at either temperature.

Preincubation of a culture of *dnaD* in tryptone broth for 1 h at 41 C resulted in a large decrease in its restricting ability when it was subsequently infected with λ·C phages at either 41 or 30 C (data not shown). On the other hand, preincubation of a cell suspension for 1 h at 41 C in the adsorption medium had no such inactivating effect on the restricting activity of *dnaD* (data not shown).

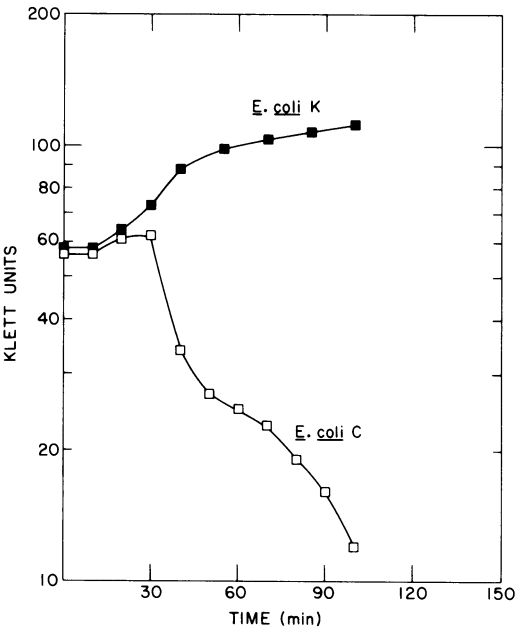

FIG. 2. *Infection of E. coli K (DG75) and E. coli C with λ·dnaD (41 C) at 37 C. The time at which the cell suspension was diluted into prewarmed (37 C) tryptone broth is designated as 0 min. Symbols:* ■, *E. coli K infected with λ·dnaD (41 C);* □, *E. coli C infected with λ·dnaD (41 C). In the case of E. coli K, there was also a drop in turbidity, but it began only 110 min after infection. Lysis was presumably due to the replication of a small fraction of the original λ·dnaD (41 C) phage stock which carried normal K-modification (see Table 2).*

Modification and restriction of λ phage by temperature-sensitive DNA initiation mutants. The data presented in this paper give evidence that the *dnaD* mutant possesses temperature-sensitive modification and restriction phenotypes. In addition, this mutant is temperature sensitive in DNA synthesis, possibly in initiation (4, 17, 19). It was of interest to us to test the possibility that some of the known DNA initiation mutants might also possess defective modification and restriction phenotypes.

Unlike *dnaD*, all of the *dnaC* and *dnaA* mutants tested produced λ phage at 41 C possessing the K-specific modification of their host. In infections with λ·K at 41 C, all of the lysates gave equal titers on *E. coli* C and *E. coli* K and are therefore designated m^+ in Table 4.

Among the *dnaC* mutants tested, CT28-3b and to a smaller extent BW824 were found to be defective in their ability to restrict λ·C at 41 C (Table 4). In contrast, *dnaC* mutant PC-2 and a *dnaA* mutant exhibited no signs of altered restriction properties. It was shown earlier (Table 3) that the parent strain (DG75) of PC-2 and of BW824 did not grow λ·C at 41 C in an identical experiment. Similar results were obtained with the parent strain (AB1157) of CT28-3b (data not shown).

TABLE 3. *Restriction of λ phage in dnaD (PC-7) mutant and in E. coli K (DG75)*[a]

Strain	Temp of infection (C)	Restriction (efficiency of forming infective centers)	
		λ·C	λ·K
dnaD (PC-7)	41	0.09	0.9
dnaD (PC-7)	30	8×10^{-3}	0.9
E. coli K (DG75)	41	7×10^{-5}	0.9
E. coli K (DG75)	30	3×10^{-5}	0.9

[a] Mutant cultures were grown and infected with λ·C or λ·K (multiplicity of infection, 0.01) at 41 or 30 C, and restriction was measured as described in Materials and Methods. Efficiency is expressed as the fraction of the adsorbed phage which forms infective centers.

DISCUSSION

Both genetic (17, 18) and biochemical (19) studies have indicated that the *dnaC* and *dnaD* gene products are either the same or very closely related. Our experiments support this hypothesis by eliminating the main reason for the original classification (4, 18) of these mutants into two groups. The apparent inability of the *dnaD* mutant to grow λ phage at the nonpermissive temperature is shown here to be due to the presence of a temperature-sensitive modification activity in this strain. In addition, the *dnaD* mutant is temperature sensitive in its ability to restrict unmodified λ phage when compared with the wild-type bacteria. The presence of a defect in both the modification and restriction systems of *dnaD* can be explained by the fact that the modification methylase and restriction nuclease are complex enzymes sharing at least two kinds of common subunits (5, 11). A change in one of the common subunits or in the assembly of the enzyme complex could lead to a simultaneous alteration of both activities.

We cannot answer the question of whether there is any relationship between temperature-sensitive DNA synthesis and temperature-sensitive modification-restriction in the *dnaD* mutant. Even if a single mutation is responsible for these phenotypes, their relationship might be only indirect. For example, the defect in DNA synthesis might alter the physiological condition of the cells in such a way as to decrease

TABLE 4. *Summary of modification and restriction phenotypes of temperature-sensitive DNA initiation mutants at 41 C*[a]

Strain	Mutation	Modification (EOP of phage progeny)[b]	Restriction (efficiency of forming infective centers)		Phenotype symbol
PC-7	*dnaD*	$0.01\text{–}2 \times 10^{-3}$	0.09	0.9	$r^{\pm}\ m^{\pm}$
PC-2	*dnaC*	1.0	10^{-4}	0.8	$r^{+}\ m^{+}$
BW824	*dnaC*	1.0	6×10^{-3}	1.0	$r^{\pm}\ m^{+}$
CT28-3b	*dnaC*	0.9	0.7	1.0	$r^{-}\ m^{+}$
E517	*dnaA*	0.7	2×10^{-4}	1.0	$r^{+}\ m^{+}$

[a] The modification of the progeny phages, produced in the mutants at 41 C, was measured as described in the legend to Table 2. The intermediate modification phenotype is designated as $m^{\pm}$. Restriction phenotypes of the mutants were measured at 41 C as described in the legend to Table 3. The symbol $r^{\pm}$ indicates intermediate phenotypes. The *dnaD* (PC-7) mutant is included here in the group of initiation mutants because of evidence that the *dnaC* and *dnaD* mutations might be in one gene (17, 19).

[b] EOP, Efficiency of plating.

their restricting ability. Other physiological perturbations of the host cells are known to result in a lower efficiency of restriction (3, 6, 12, 14). This idea is supported by the observation that not only *dnaD* but also two of the *dnaC* mutants are defective both in restriction and in DNA synthesis. However, with this hypothesis one cannot easily explain why the *dnaD* mutant has an $m^{\pm}$ phenotype in addition to the $r^{\pm}$ phenotype. The only physiological condition that is known to affect both restriction efficiency and the modification of the progeny phages is methionine starvation (1, 6).

An alternative explanation of the experimental data is that *dnaD* possesses two independent mutations, one in DNA synthesis and the other in modification-restriction. Nitrosoguanidine, which was used for the isolation of this mutant (4), is known to cause mutations at nearby genes (8). The *dnaD* locus and the *hs* locus for modification-restriction map very close to each other: the *dnaD* locus is cotransducible with *dra* (18), which maps at 89 min in the linkage map of *E. coli* (16), and the *hs* locus is also at 89 min (16). At least by the criterion of molecular weight, the *dnaC*, and presumably also *dnaD*, gene product is different from the modification-restriction enzyme. Recently Wickner et al. (19) have estimated the molecular weight of the purified *dnaC* protein to be about 25,000. This figure is much less than those reported for any of the three kinds of subunits of the *E. coli* B restriction nuclease (55,000, 60,000, and 135,000) (5, 11). It should be also noted that a direct relationship between DNA initiation and the modification-restriction process is unlikely in view of what is known about large numbers of previously isolated host specificity mutants. These mutants, although defective in modification-restriction (10, 21), are not affected in their growth and presumably are also unaffected in DNA synthesis.

The possibility, however, still remains that there is only one mutation in the *dnaD* mutant affecting directly both DNA initiation and the modification-restriction process. This agrees well with the fact that the *dnaD* locus maps very close to the host specificity region (*hs*) of the *E. coli* chromosome, and that two of the *dnaC* mutants tested are also less efficient in restriction than the wild-type strain (Table 4). According to this hypothesis, the *dnaD* gene product might be required not only in the initiation of DNA replication but also for a constituent of one or more of the subunits of the restriction nuclease. Depending on the position of the mutation in this gene, temperature sensitivity is caused in all or some of the three phenotypes: initiation, modification, and restriction.

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