# GROWTH OF PHAGES λ, ØX174, and M13 REQUIRES THE dnaZ (previously dnaH) GENE PRODUCT OF ESCHERICHIA COLI

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SUMMARY: A functional dnaZ (previously designated dnaH) product, known to be involved in DNA polymerization, is required for phage λ, ØX174, and M13, but not T4 or T7, growth.

The Escherichia coli genes known to be required for chromosome replication are the dna genes A, B, C(D), E, G, H, I, P, and Z (1, 2, 3, 4, 5, 6). Genes dnaA, C(D), H, I, and P are involved in initiation of replication (3, 4, 5, 6, 7, 8, 9, 10). dnaE codes for DNA polymerase III (11, 12); dnaB, G, and Z products also are involved in polymerization but in some, as yet undefined, role (2, 7, 10). The polymerization gene dnaZ, which maps near purE, previously was designated dnaH (2) but has been changed to avoid confusion with the replication initiation gene which maps near thyA and has also been designated dnaH (5, 6).

Replication of coliphages λ, M13, and ØX174 in vivo also requires functional genes products of some host dna genes. λ replication is inhibited at the nonpermissive temperature in dnaBts, Ets, and Gts mutants, but does not require dnaA, C, or P products (4, 7, 13, 14). ØX174 replication requires functional products of dnaB, C(D), and E; dnaA product is not required (15, 16, 17, 18); and the requirement for dnaG has not been tested. M13 requires the dnaA and B products but only early after infection; late after infection the A and B products are dispensable. The dna C(D), E, and G proteins are

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ts, temperature-sensitive

required continuously for M13 (19, 20). The dnaB protein functions in replication of M13 RF (21).

This paper demonstrates that a functional dnaZ product, known to be required for host DNA polymerization, is required also for production of progeny λ, M13, and ØX174, but not T4 or T7.

## MATERIALS AND METHODS

Strains: The K12 strains AX727, dnaZts, and AX729, dnaZ$^+$, were described previously (2). F'lac$^+$ derivatives of AX727 and AX729 were prepared by mating them with RV/F'lac$^+$ (obtained from M. Malamy). E. coli C was obtained from R. McKee, AB1157 from P. Howard-Flanders, λ$^+$ and λcII$_{2002}$ from D. Kaiser, M13 from D. Ray; and T4D, T7, and ØX174h8ρ$^-$ (referred to as ØX174) from C. Earhart.

Growth of λ, T4, and T7: Cultures of AX727 and AX729 were grown in YET broth containing 0.5% NaCl at 28°C, resuspended in buffer [0.01 M potassium phosphate (pH 7.0) containing 0.01 M $MgSO_4$], and incubated at 28°C for 20-25 minutes. Half of each culture was shifted to 42°C, and the cultures were incubated 5 minutes more. Phage were added at a multiplicity of infection of 0.05 to 0.15. The T4-cell mixture was supplemented with 50 μg/ml L-tryptophan. Adsorption was permitted for 5 additional minutes for T4 and T7, and for 20 minutes for λ. (In the experiments with λ, adsorption was at 28°C only.) Suspensions were then diluted 100-fold (λ) or 1000-fold (T4, T7) into prewarmed YET broth and incubated at 28°C or 42°C for 2 hours. Samples were treated with chloroform and assayed for PFU with AB1157 as indicator, using YET agar supplemented with 0.001 M $MgSO_4$, and YET top agar containing 0.5% NaCl, 0.7% agar, and .001 M $MgSO_4$.

Production of M13 Progeny: M13 growth in F'lac$^+$/AX727 and F'lac$^+$/AX729 at 28°C and at 42°C was followed after infection at a multiplicity of 4 (Ref. 20). Indicator was F'lac$^+$/AX729 at 37°C (22).

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RF, replicative form; YET, yeast extract (0.5%) and tryptone (1%); PFU, plaque-forming units.

Table 1. Production of progeny λ, T4, T7, and ØX174 in *dnaZ*$^+$ and *dnaZts* hosts at permissive and nonpermissive temperature.

| Phage | Host | PFU/input phage 28°C | PFU/input phage 42°C | PFU 42°C / PFU 28°C |
|---|---|---|---|---|
| λ$^+$ | *dnaZts* | 120 | 0.89 | .0071 |
| | *dnaZ*$^+$ | 70 | 190 | 2.7 |
| λ*cII*$_{2002}$ | *dnaZts* | 120 | 0.74 | .0064 |
| | *dnaZ*$^+$ | 64 | 140 | 2.2 |
| T4 | *dnaZts* | 84[a] | 32 | 0.38 |
| | *dnaZ*$^+$ | 87[a] | 76 | 0.87 |
| T7 | *dnaZts* | 140 | 470 | 3.4 |
| | *dnaZ*$^+$ | 200 | 670 | 3.3 |
| | | PFU/ml[b] 30°C | PFU/ml[b] 40°C | PFU 40°C / PFU 30°C |
| ØX174 DNA | *dnaZts* | $7.6\times10^6$ | $1.4\times10^4$ | 0.0018 |
| | *dnaZ*$^+$ | $2.7\times10^7$ | $2.1\times10^7$ | 0.78 |

[a]After 3 hours' incubation.

[b]PFU/ml of the ØX174 DNA-spheroplast mixture.

Production of ØX174 Progeny in Spheroplasts: Spheroplast suspensions of AX727 and AX729 (23) were made at 30°C, infected with ØX174 single-stranded DNA (15 molecules/spheroplast), and incubated 15 min at 30°C without aeration. The DNA-spheroplast mixture was diluted into PAM medium (24) prewarmed to 30°C or 40°C. After 2 hours, the mixtures were diluted into 0.05 M sodium borate and assayed by plating with *E. coli* C as indicator (25).

## RESULTS

The *dnaZ* gene product is essential for λ, but not for T4 or T7, growth (Table 1). When the *dnaZts* mutant was infected with λ$^+$ or λ*cII*$_{2002}$ at 28°C and then shifted to 42°C, progeny phage production was restricted to less than the number of infecting phages. However, both T4 and T7 phages replicated in the *dnaZts* mutant at the non-permissive temperature even when infection occurred at 42°C.

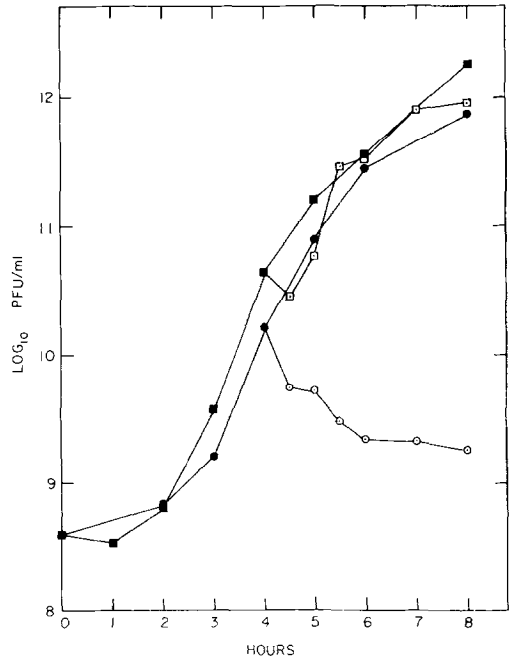


Fig. 1. Production of M13 phage at 28°C and at 42°C in dnaZ$^+$ and dnaZts hosts. ● , F'lac$^+$/dnaZts at 28°c; ○, F'lac$^+$/dnaZts at 42°C; ■, F'lac$^+$/dnaZ$^+$ at 28°C; □, F'lac$^+$/dnaZ$^+$ at 42°C. At 4 hours, half of each culture was shifted to 42°C.

Production of ØX174 phages from single-stranded DNA in spheroplasts required a functional dnaZ gene product (Table 1). Yield of progeny phages at 40°C in the temperature-sensitive dnaZts host was 0.18% of the yield at 30°C.

Similarly, M13 growth was inhibited at 42°C in an episome-containing derivative of the dnaZts mutant (Fig. 1). After infecting male strains of dnaZts and dnaZ$^+$ and incubating at 28°C until phage were being actively produced, half of each culture was shifted to 42°C. The increase in free phage concentration in the dnaZts culture stopped abruptly at 42°C, but the dnaZ$^+$ culture continued to produce phage. The decrease in titer probably is caused by adsorption of phage to cell envelopes and has been observed earlier (20). Thus the dnaZ product is needed for continued M13 production, even when the replicative forms have been given the opportunity to replicate before the temperature increase. In contrast, Primrose et al.

(19) found that M13 phage continued to be produced for at least two hours after increasing temperature in a dnaBts strain if the temperature increase was preceded by incubation at the permissive temperature long enough for free phage to be produced. The dnaB product apparently is required for the replication of the M13 RF's (21), but not for parental RF formation or for the synthesis of progeny single-stranded DNA.

## DISCUSSION

The product of the E. coli dnaZ gene is essential for cellular DNA polymerization and for growth of λ, ØX174, and M13, presumably because the dnaZ product is required for synthesis of the phage DNA also. Apparently these phages do not code for this essential material which is assumed to be a protein. However, both T4 and T7 apparently code for a product which performs a function similar to that of the dnaZ product, or replication of the DNA of these phages does not require a dnaZ-like activity.

Perhaps the dnaZ product function can be determined in an in vitro DNA synthesizing system. The in vitro complementation procedure using ØX174 single-stranded circular DNA as template (26) should provide an assay for the dnaZ product. The gene products required for ØX174 DNA replication in vivo also are required for in vitro DNA synthesis (15, 16, 17, 18, 27) in the assay described by W. Wickner et al. (26). Moreover, when single-stranded circular ØX174 DNA was used as template in an in vitro system, the addition of purified proteins of all the dna genes known to be required for ØX174 replication (i.e., dnaB, C, E, and G) still was insufficient to sustain DNA synthesis (28). The dnaZ product might complete the protein requirement, although still more factors could be required.

Recently, W. Wickner et al. (29) showed that what is presumed to be the native product of the dnaE gene (polymerase III*) and copolymerase III* are the only proteins required for in vitro DNA synthesis when the primer-template was single-stranded circular ØX174 DNA containing a small hydrogen bonded RNA polymer. Hurwitz and S. Wickner (30) reported that RNA-primed

ØX174 single-stranded circular DNA serves as template for DNA synthesis *in vitro* in the absence of *dnaB*, *C*, and *G* proteins; polymerase III and new protein Factors I and II were the only necessary proteins. Presumably Factors I and II are related to polymerase III* and copolymerase III*. The *dnaZ* product could be copolymerase III*, Factor I or II, the *E. coli* unwinding protein (31), or an additional factor.

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