

Inducibility of Lambda Phage Development in *Escherichia coli* Mutants Thermosensitive for DNA Replication

Akira Taketo

Department of Biochemistry, School of Medicine, Kanazawa University, Kanazawa

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In a thermosensitive *dnaZ* mutant lysogenic for λ , induction of the prophage development was provoked at the restrictive temperature. In *dnaD* strain, spontaneous release of λ proceeded even at 43 °C, but distinct induction of the prophage development was not caused by a heat treatment. Similarly, shift up of growth temperature did not lead to induction of λ in lysogens carrying thermosensitive mutation in *dnaH* function or DNA polymerase I. In *dnaH* mutant, multiplication of λ -virulent phage proceeded normally at 43 °C.

Introduction

By affecting DNA replication, various physical and chemical agents exert phage-inducing effect on lysogenic bacteria. Inhibition of DNA synthesis by thymine starvation also induces phage development in thymine-auxotrophic lysogens. In addition, it has recently been shown that high-temperature treatment induces prophage development in certain *dna* mutants¹ and in a DNA ligase mutant². Inhibition of DNA replication, however, does not always trigger the phage induction. Thus, phenethyl alcohol, one of the inhibitors of DNA synthesis, did not exhibit the phage-inducing effect on λ lysogens (unpublished observation). Furthermore, λ phage is not induced at 42 °C in lysogens of *dnaA* type¹.

This communication deals with thermoinducibility of λ phage development in lysogenic *E. coli* carrying temperature-sensitive mutation in DNA polymerase I, *dnaD*, *dnaH*, or *dnaZ* functions.

Materials and Methods

Bacteria and phage strains

The following *E. coli* strains were used: PC-7 *dnaD*³, obtained from Dr. M. Bowes; HF4704S *dnaH* and HF4704 *dna*⁺⁴, from Dr. T. Komano, AX727 *dnaZ* and AX729 *dna*⁺^{5,6}, from Dr. J. R. Walker; BT4113 *polA*^{ts}, JW149^{ts} *polA*^{ts} and JW149 *pol*⁺⁷, from Dr. F. Bonhoeffer; W3110 *pol*⁺ was the parental strain of BT4113 *polA*^{ts}. PC-7-12 *dna*⁺ revertant and BT4113-3 *pol*⁺ revertant were isolated

by the author. These strains were lysogenized by wild type λ and checked for the prophage inducibility by ultraviolet irradiation. In some experiments, virulent mutant of λ (λ vir) was used. To avoid the complication due to modification and restriction, strain C was used throughout as the indicator for λ .

Media

Nutrient broth contained, per liter, Kyokuto Ehrlich Broth 10 g, Wako Polypepton 10 g and NaCl 2 g. The pH was adjusted to 7.2 with NaOH. For plating, the nutrient broth was solidified by addition of 1.4% agar (bottom layer) and 0.7% agar (top layer). Dilution fluid contained, per liter, Wako Polypepton 1 g, NaCl 3 g and MgSO₄·7 H₂O 0.1 g.

Measurement of induction

Lysogenic bacteria were grown to log-phase at 30 °C with shaking, and the density was adjusted to OD₆₆₀ = 0.12–0.13. The temperature of the culture was then shifted up to 43 °C and shaking was continued. After 2 or 3 hours, aliquot of the culture was diluted again into fresh medium and shaken successively at 30 °C.

Turbidity of the cultures was followed at 660 nm, with a Bausch & Lomb Spectronic 20 spectrophotometer. For assay of free phage, aliquots were withdrawn periodically, treated with chloroform, and plaque-forming unit was determined after suitable dilution.

Growth of λ vir

Log-phase culture of HF4704S *dnaH* was divided into 3 portions and further incubated: (A), for 120 min at 30 °C; (B), for 110 min at 30 °C and then for 10 min at 43 °C; (C) for 120 min at

Requests for reprints should be sent to Dr. Akira Taketo, Department of Biochemistry, School of Medicine, Kanazawa University, Kanazawa, Ishikawa 920, Japan.

43 °C. To each culture, λ vir was added at a multiplicity of about 0.01. After 10 min, the infected cultures were diluted 100-fold into prewarmed medium and incubation was continued at 30 °C (A) or 43 °C (B, C). At the indicated time, aliquots were removed, treated with chloroform and free phage was titrated.

Results and Discussion

Inducibility of λ phage in DNA polymerase I mutants

Strains BT4113 $polA^{ts}(\lambda)$ and 149 t^s $polA^{ts}(\lambda)$ are conditionally lethal at 43 °C. After transfer to the high temperature, release of λ phage continued for about 3 hours and then levelled off (Fig. 1 A).

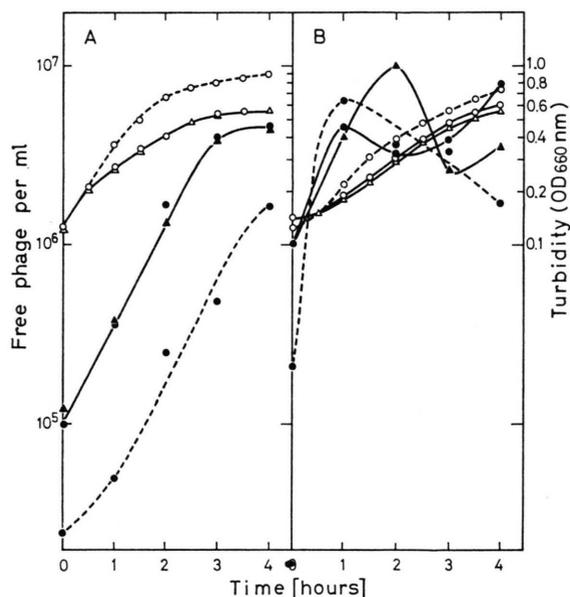


Fig. 1. Phage release in thermosensitive mutants defective in DNA polymerase I. In A, free phage yield [—●—, BT4113 $polA^{ts}(\lambda)$; —▲—, 149 t^s $polA^{ts}(\lambda)$; —●—, W3110 $pol^+(\lambda)$] and turbidity of the culture [—○—, BT4113 $polA^{ts}(\lambda)$; —△—, 149 t^s $polA^{ts}(\lambda)$; —○—, W3110 $pol^+(\lambda)$] were followed at 43 °C. In B, the lysogens grown for 3 hours at 43 °C were transferred to 30 °C at time 0, and incubation was continued at the permissive temperature. Symbols are the same as in A.

Gradual increase in phage titer indicates that DNA polymerase I is apparently not required for replication of wild-type λ . In fact, multiplication of λ vir proceeded normally in these $polA^{ts}$ strains at 43 °C (data not shown). As in parental strain W3110 $pol^+(\lambda)$, neither distinct burst of λ or mass lysis of the culture were observed in $polA^{ts}(\lambda)$ during in-

cubation at 43 °C. Phase-contrast microscopy revealed that cells of BT4113 $polA^{ts}(\lambda)$ grown at 43 °C for 3–4 hours were mostly filamentous. Induction of λ phage development was not observed when the cultures grown at 43 °C for 3 hours were transferred again to 30 °C (Fig. 1 B). In these lysogens, however, development of λ phage was efficiently induced by irradiation with ultra-violet light. As expected, heat treatments failed to induce prophage development in $polA^+$ revertants lysogenic for λ (data not shown).

Both DNA polymerase I mutant and DNA ligase mutant are defective in sealing of Okazaki pieces, are sensitive to methylmethane sulfonate and form filaments at the restrictive temperature^{2,7,8}. Despite similar phenotypes, the thermal induction of prophage development occurs in DNA ligase-defective mutant^{2,9}, but not in DNA polymerase I-less strain.

Development of λ phage in *dna* mutants

Using strain K-12 C600 as indicator for λ , Schuster *et al.*¹ have observed that PC-7 $dnaD(\lambda)$ does not induce the prophage development at 42 °C. Since the λ phages produced in the $dnaD$ mutant at the nonpermissive temperature plate only poorly

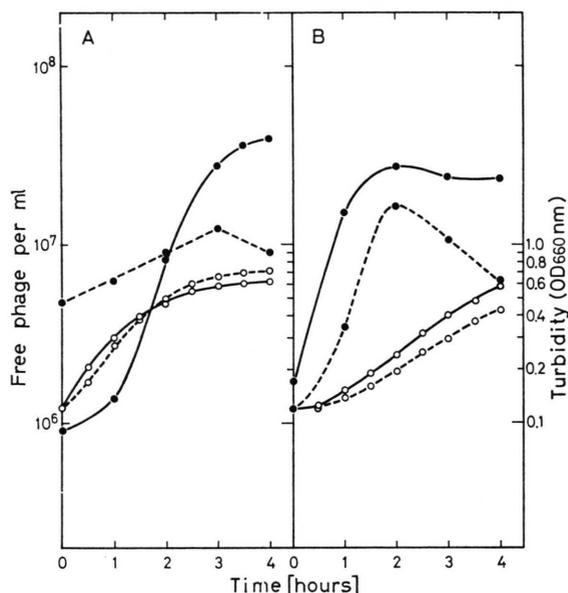


Fig. 2. Phage release in *dnaD* mutant. In A, free phage yield [—●—, PC-7 $dnaD(\lambda)$; —●—, PC-7-12 $dna^+(\lambda)$] and turbidity of the culture [—○—, PC-7 $dnaD(\lambda)$; —○—, PC-7-12 $dna^+(\lambda)$] were followed at 43 °C. In B, the bacteria grown at 43 °C for 3 hours were transferred to 30 °C at time 0, and incubated further at the permissive temperature. Symbols are the same as in A.

on *E. coli* K12^{10,11}, the prophage inducibility was reexamined using a restriction-less indicator, *E. coli* C¹². The PC-7 *dnaD* strain supported the growth of λ vir at 42–43 °C (unpublished observation). As shown in Fig. 2, yield of free λ considerably increased during and after incubation of *dnaD* (λ) at the nonpermissive temperature. However, neither distinct burst of phage nor cellular lysis were provoked by the heat treatment. On the other hand, the prophage was efficiently induced by exposing the *dnaD* lysogen to ultraviolet light or mitomycin C (data not shown).

Thermosensitive mutant HF4704S *dnaH* is defective in DNA initiation⁴. In *dnaH*(λ) strain, temperature shift to 43 °C did not provoke the induction of λ phage development (Fig. 3A). However,

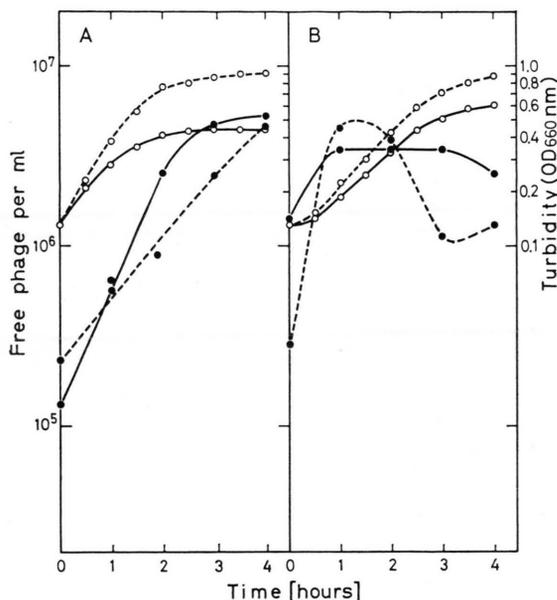


Fig. 3. Phage release in *dnaH* mutant. In A, free phage yield [—●—, HF4704S *dnaH*(λ); --●--, HF4704 *dna*⁺(λ)] and turbidity of the culture [—○—, HF4704S *dnaH*(λ); --○--, HF4704 *dna*⁺(λ)] were followed at 43 °C. In B, the bacteria grown at 43 °C for 3 hours were transferred to 30 °C at time 0, and incubated further at the permissive temperature. Symbols are the same as in A.

release of small amount of free phage continued exponentially until cessation of bacterial growth, indicating that *dnaH* function is not required for replication of λ . As shown in Table I, growth of λ vir in *dnaH* mutant was not blocked at the restrictive temperature. None of the DNA initiation mutants hitherto known are unable to support replication of λ .

Table I. Multiplication of λ vir in *dnaH* host.

Time [min]	30 °C	43 °C ^a	43 °C ^b
0	3.7×10^3	—	—
60	2.1×10^4	5.7×10^4	5.3×10^4
120	5.7×10^4	1.9×10^5	1.5×10^5
180	3.1×10^5	6.4×10^5	3.8×10^5

^a Cells grown at 30 °C were infected 10 min after shifting up to 43 °C.

^b Bacteria were infected at 43 °C after growing for 120 min at the restrictive temperature.

In contrast with *dnaC*, neither *dnaA*¹, *dnaD* nor *dnaH* induce prophage development at the restrictive temperature. At present, basis of the differences in prophage inducibility is quite unknown. Correlation with patterns of DNA degradation, residual synthesis or filamentation seems not so high.

The *dnaZ* function is involved in elongation (polymerization) of DNA chain⁵. Recently, Truitt and Walker⁶ have shown that a functional product of the *dnaZ* gene is required for λ replication. As

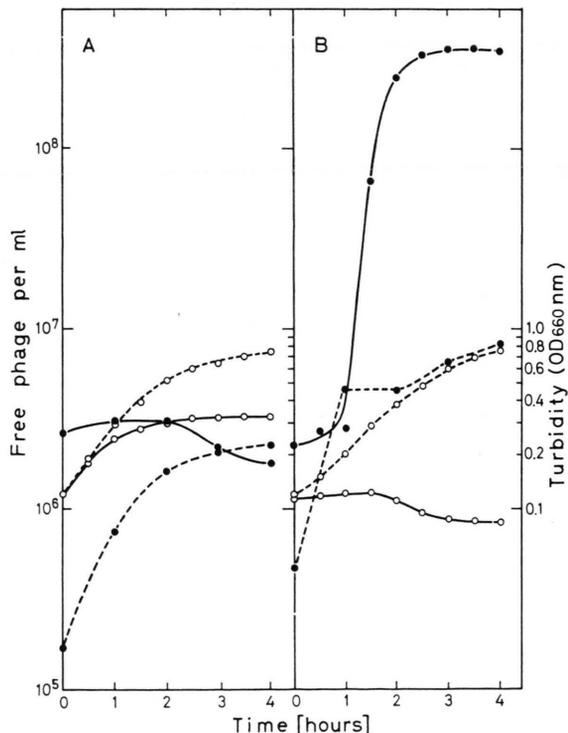


Fig. 4. Phage release in *dnaZ* mutant. In A, free phage yield [—●—, AX727 *dnaZ*(λ); --●--, AX729 *dna*⁺(λ)] and turbidity of the culture [—○—, AX727 *dnaZ*(λ); --○--, AX729 *dna*⁺(λ)] were followed at 43 °C. In B, the bacteria grown at 43 °C for two hours were transferred to 30 °C at time 0, and incubated further at the permissive temperature. Symbols are the same as in A.

seen in Fig. 4A, increase in free phage titer really stopped upon transfer of the *dnaZ*(λ) to 43 °C. Turbidity of the culture continued to increase for about two hours, whereas colony formers were markedly lost at the restrictive temperature. In addition, number of infective center (determined at 30 °C) considerably increased during growth at 43 °C (data not shown). When the *dnaZ*(λ) cells grown for 2 hours at the restrictive temperature was transferred again to 30 °C, a burst of phage occurred with concomitant decrease in turbidity (Fig. 4B). It is thus clear that in *dnaZ*(λ) strain blocking of DNA synthesis at the restrictive tem-

perature effectively induces prophage development. Upon temperature shift, the prophage induction was not observed in an isogenic *dna*⁺ strain AX729(λ).

From results of Schuster *et al.*¹ and present data, it may be generalized that elongation-type *dna* mutants cannot support λ replication at the restrictive temperature and that all mutants of this type induce λ prophage development upon a heat treatment.

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¹ H. Schuster, D. Beyersmann, M. Mikolajczyk, and M. Schlicht, *J. Virol.* **11**, 879–885 [1973].

² M.M. Gottesman, M. L. Hicks, and M. Gellert, *J. Mol. Biol.* **77**, 531–547 [1973].

³ P. Carl, *Molec. Gen. Genet.* **109**, 107–122 [1970].

⁴ H. Sakai, S. Hashimoto, and T. Komano, *J. Bacteriol.* **119**, 811–820 [1974].

⁵ C. C. Filip, J. S. Allen, R. A. Gustafson, R. G. Allen, and J. R. Walker, *J. Bacteriol.* **199**, 443–449 [1974].

⁶ C. L. Truitt and J. R. Walker, *Biochem. Biophys. Res. Commun.* **61**, 1036–1042 [1974].

⁷ B. M. Olivera and F. Bonhoeffer, *Nature* **250**, 513–514 [1974].

⁸ E. B. Konrad, P. Modrich, and I. R. Lehman, *J. Mol. Biol.* **77**, 519–529 [1973].

⁹ T. Nagata and T. Horiuchi, *J. Mol. Biol.* **87**, 369–373 [1974].

¹⁰ A. V. Paul and M. Inouye, *J. Bacteriol.* **119**, 907–912 [1974].

¹¹ J. A. Wechsler, *J. Bacteriol.* **121**, 594–599 [1975].

¹² G. Bertani and J. J. Weigle, *J. Bacteriol.* **65**, 113–121 [1953].