*PROPHAGE INDUCTION AND FILAMENT FORMATION IN A MUTANT STRAIN OF ESCHERICHIA COLI**

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In lysogenic bacteria, a specific system of repression, genetically determined by the prophage, prevents the expression of the phage genes necessary for phage replication and the production of infectious particles. With some prophage strains, treatment with ultraviolet (UV) or X radiation or with various chemical agents causes the repression system to break down and allows the production of phage. Mutant strains of bacteriophage with a thermosensitive repression system have been described.[1–3]

Recently a bacterial thermosensitive mutant has been isolated.[4] The mutant strain, when lysogenic for an inducible prophage, grows relatively normally in complete medium at 30°, but at 40° phage production is induced. This paper describes some of the properties of this mutant in which elevated temperature alters conditions within the bacterium and thereby leads to prophage induction. This strain, when cured of prophage, forms filaments due to a defect in the formation of cross-septa. The conditions for filament formation are similar to those for induction of prophage in the lysogenic strain.

Materials and Methods.—Bacteria and bacteriophage: Escherichia coli K-12, C-600, *thr, leu, thi,* lysogenic for λ, was the parent of the strains used in this study. The lysogenic and nonlysogenic strains of C-600 were used as controls for experiments with the thermoinducible strain, whose isolation is described below. AB1899 *lon,* a strain of *E. coli* which forms filaments after low doses of UV light was obtained from Dr. P. Howard-Flanders. Hfr A-235 was obtained from Dr. S. E. Luria.

Media and chemicals: Minimal medium M-63[5] was supplemented with 0.2% glucose, 0.2% Difco casamino acids (fortified with 0.01% each L-tryptophan, L-serine, and L-threonine), and 0.01% thiamin hydrochloride. Nutrient broth was prepared with either 16 gm. per liter of Difco nutrient broth powder or with 5 gm of meat extract, 10 gm of peptone, and 5 gm of NaCl per liter with the pH adjusted to 7.4 with NaOH. Hadacidin was provided by Dr. Shigeura, Merck, Sharp, and Dohme, Rahway, N.J.

Growth experiments: Cultures which had been grown overnight at room temperature were diluted into M-63 medium containing 0.2% glucose, 0.2% fortified casamino acids, 0.01% thiamin HCl, and 0.01% guanosine and cytidine. The cultures were then shaken at 26–28° until in log phase. They were quickly chilled and centrifuged and the bacteria were washed and resuspended in cold M-63 medium containing glucose, casamino acids, and thiamin. Ten-ml samples of these suspensions were added to 125-ml nephelometer flasks containing the appropriate additions. The cultures were then shaken at 40°, and the optical density of the cultures was followed at 660 mμ with a Klett colorimeter.

Sensitivity to UV irradiation: Cells were grown in nutrient broth until in log phase, then chilled and resuspended in cold M-63 minimal medium to a density

of approximately 5×10^6 cells/ml. The cells were irradiated with a General Electric G1578 15-watt lamp with mechanical shutter producing approximately 13.5 ergs/mm^2/sec at a distance of 55 cm. The cells were diluted in M-63, plated, and incubated for approximately 24–30 hours.

Results.—Isolation and characterization of a thermoinducible lysogenic strain: The mutant, C-600–T-44 (λ), to be designated as T-44 (λ), was detected during experiments designed to isolate thermosensitive mutants of the λ C_I region. C-600 (λ) was treated with N-methyl-N′-nitro-N-nitrosoguanidine, plated on nutrient agar, and incubated at 30°. Colonies were then replicated on the same medium and selected for growth at 30° but not at 40°. The mutants isolated were primarily lysogens carrying λ with mutations in the C_I region. When phage progeny from these strains were plated on C-600 and incubated at 40°, clear plaques were noted. However, one mutant, T-44 (λ), produced phage which yielded turbid plaques at 40°. In broth at 30° this lysogenic strain grew at a nearly normal rate, but the level of phage present in the medium due to spontaneous lysis was approximately ten times that found in the normal lysogenic strain. When shifted to 40° the culture lysed after 60 minutes, yielding about 100 phage per cell. The λ phage, released by thermal induction of T-44 (λ), did not have thermoinducible properties when used to lysogenize the normal C-600 strain. T-44 (λ) was cured of prophage by heteroimmune superinfection with λi^{434} and then relysogenized with UV-inducible phages λ, 424, 434, λi^{434}, or 21. At 40° each of these phages was induced in the host T-44, although none of them was thermoinducible in C-600. When T-44 was lysogenized with phage P1, which is normally poorly induced by UV light, cultures grown at 40° produced about 10 phage particles per bacterium. T-44, lysogenic for the noninducible phages 18 or 299, did not lyse when incubated at 40°. Also, no lysis was observed at 40° for T-44 lysogenic for λ ind$^-$, a mutant of λ which cannot be UV induced.[6]

These experiments established two points: first, that the mutation responsible for the thermoinducibility of T-44 (λ) resided in the host strain and not in the prophage; and second, that all prophages which were induced by UV light when in C-600 were also induced at elevated temperatures when in T-44. Those phages which were not UV inducible were not induced in T-44 by elevated temperature.

The absence of thermal induction of λ ind$^-$ in T-44 is evidence that the alteration in T-44 at elevated temperatures affects the repressor, rather than acting by some undefined process to circumvent the function of the repressor.

Effects of nucleosides on thermal induction: Addition of purine and pyrimidine nucleosides can affect significantly the thermal induction of T-44 (λ), as shown in Figure 1. When shifted to 40°, the control culture of T-44 (λ) lysed after about 60 minutes and released a high level of phage. Guanosine and cytidine provided considerable protection from lysis at 40°. Uridine was able to substitute for cytidine, but guanosine was necessary for maximum protective effect. The free bases were relatively inactive; the deoxyribosides were less effective than the ribosides. Thymine and thymidine had no effect.

The addition of adenine promoted induction and reversed the protection afforded by guanosine and cytidine. In this instance, the free base was more effective than any of its derivatives.

The antimetabolite hadacidin (N-formyl-N-hydroxy amino acetic acid),[7] which

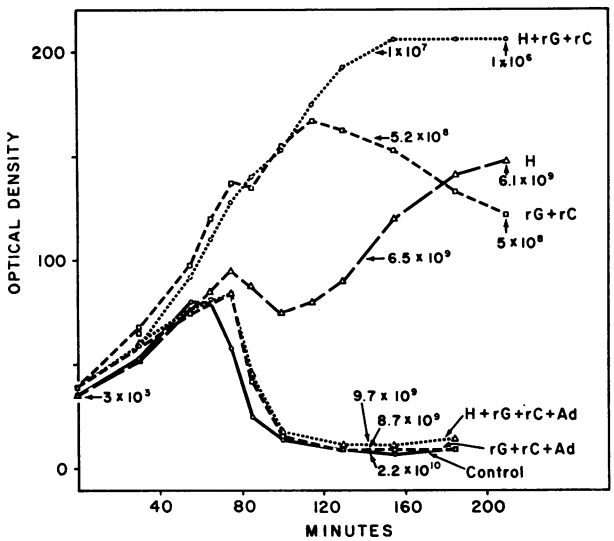

FIG. 1.—T-44 (λ) was pregrown at 30°, then shifted to 40° in the presence of: (1) control = no additions; (2) rG + rC = 100 μg/ml of guanosine and of cytidine; (3) rG + rC + Ad = 100 μg/ml of guanosine and of cytidine, 100 μg/ml of adenine; (4) H = 1 mg/ml of hadacidin; (5) H + rG + rC = 1 mg/ml of hadacidin, 100 μg/ml of guanosine and of cytidine; (6) H + rG + rC + Ad = 1 mg/ml of hadacidin, 100 μg/ml of guanosine and of cytidine, 100 μg/ml of adenine. The numbers at 140 and 210 min indicate phage titers.

inhibits the conversion of inosinic acid to adenylic acid,[8] also protected T-44 (λ) from thermal induction. When added to guanosine and cytidine, hadacidin afforded virtually complete protection. In both cases protection was reversed by the addition of adenine (Fig. 1).

Hadacidin, at the concentrations used, could not have completely blocked the synthesis of the adenine derivatives, because the strain grew at a nearly normal rate. This level of hadacidin must have been acting only to reduce the concentration of the adenine derivatives below a certain level necessary for induction.

Induction in T-44 (λ) was found to be related more to conditions of growth than to any specific temperature of growth. Partial induction could be observed at temperatures below 37°, and virtually complete induction could be produced by the addition of adenine at 30°. T-44 may be a strain in which the repressor-destroying system is delicately poised and can be easily upset by changing the growth conditions.

Properties of the cured strain: T-44 (λ) was cured of its prophage by superinfecting with λi^{434} and selecting survivors nonlysogenic for λ and λi^{434}. To show that this operation had not affected the strain other than to remove the prophage, the nonlysogenic strain (T-44) was relysogenized with wild-type λ and found to be thermoinducible.

When a culture of the cured strain was shifted to 40°, the initial rate of growth was approximately equal to that of the wild-type (see Fig. 2). However, upon continued incubation, the T-44 culture stopped growing at an optical density much less than that for the wild-type. The addition of hadacidin, guanosine, and cytidine allowed normal growth of the cells.

When the cultures of T-44 were examined microscopically after prolonged incubation at 40°, they were found to be composed primarily of nonseptate filaments up to 100 μ in length. Cells grown in cultures containing hadacidin, guanosine, and cytidine were normal in length; the addition of adenine reversed the protective effect of these three compounds. Thus, agents which were able to affect prophage

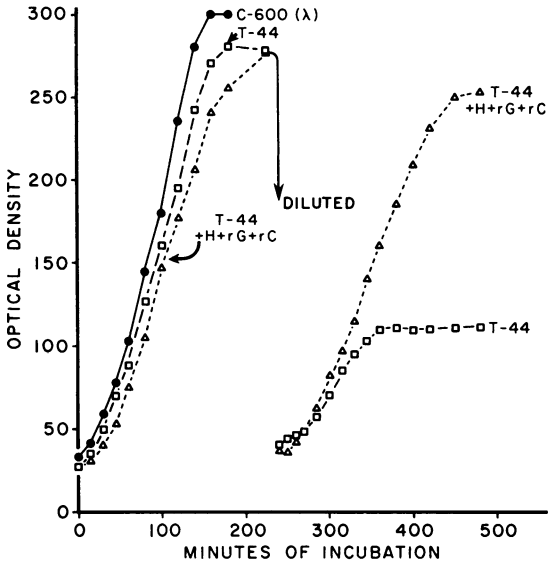

FIG. 2.—C-600 (λ) and T-44 were pregrown at 30°, then shifted to 40°. T-44 was incubated at 40° either in the presence or absence of hadacidin (1 mg/ml), guanosine (100 μg/ml), and cytidine (100 μg/ml). At 230 min the T-44 culture was diluted into fresh medium, the T-44 + H + rG + rC culture was diluted into fresh medium containing hadacidin, guanosine, and cytidine. Incubation was continued at 40°.

induction in T-44 (λ) had a parallel effect upon filament formation in the nonlysogenic strain.

DNA metabolism in T-44: Various agents that induce prophage, such as UV, X-ray, or radiomimetic chemicals, also have been shown to affect DNA. Alteration of DNA has been suspected as a cause of the induction of prophage. In the mutant T-44 the conditions for prophage induction correspond to those for filament formation. Therefore, alterations in DNA structure and metabolism were sought under conditions where cell growth continued but cell division was inhibited in the nonlysogenic strain (where the results would not be complicated by phage DNA synthesis). No abnormalities have been detected with the following techniques: (1) DNA synthesis, as measured by either diphenylamine-reacting material or incorporation of H^3-thymidine into acid-insoluble material, was the same in both T-44 and C-600 in the first 60 minutes after transfer to 40°, in the presence of either guanosine plus cytidine or of adenine; (2) examination of filaments by electron microscopy[9] and also light-microscopy using nuclear staining showed nuclear material distributed throughout the filament; (3) the amount of DNA prelabeled with H^3-thymidine at 30° remained constant during an 80-minute incubation at 40° in both T-44 and C-600; (4) there was no release of ultraviolet-absorbing material or H^3-thymidine from DNA in excess of that observed with C-600 when T-44 was incubated at 40° for 80 minutes; (5) no evidence for an increase of single-strand breaks in the DNA of T-44 was observed under conditions leading to filament formation. Cells, prelabeled with H^3-thymidine, were shifted to 40° with hadacidin, guanosine, and cytidine, or with adenine, and incubated for 40 minutes prior to lysis and centrifugation in an alkaline sucrose gradient.[10] The DNA profiles of these cells and of control cultures which were not incubated at 40° were all similar. After 90 minutes at 30,000 rpm (SW-39 rotor) the DNA peak in each case had sedimented approximately 60 per cent of the distance to the bottom of the tube. Thus no alteration in the DNA structure or metabolism was observed with these techniques.

The extensive increase in cell size of the nonlysogenic strain at 40° indicated that there was no obvious impairment of protein or RNA synthesis. There appeared to be no alteration in induced enzyme synthesis. β-Galactosidase was not spontaneously induced in T-44 (λ) at 40°, but could be induced when thiomethyl galactoside was added.

Filament formation in the absence of prophage induction: Filament formation and prophage induction appear to be closely associated in T-44. In this strain it is not clear whether they are merely associated or whether some event in the process of filament formation causes prophage induction. If the two processes are causally related, then this relationship should be apparent with other stimuli and in other bacterial strains.

The AB1899 *lon* strain forms filaments after treatment with low doses of UV light. These filaments appear to be normal in most of their synthetic capacities, except for their inability to form cross-septa. Thus, they appear to be analogous to the filaments formed by T-44 at elevated temperatures. We have observed, however, that when AB1899 was made lysogenic for λ, filaments could be formed either spontaneously or after low doses of UV without induction of the prophage. The prophage of AB1899 (λ) can be induced by levels of UV comparable to those necessary for induction of the C-600 (λ) strain, approximately 200 ergs/mm^2, a dose 100 times that necessary for the inhibition of cell division in AB1899.

Filament formation without prophage induction was also observed under other conditions. Penicillin G (50 units/ml) in C-600 (λ), T-44 (λ), or AB1899 (λ), or crystal violet (1–5 μg/ml) in T-44 (λ) or AB1899 (λ) caused the formation of filaments, but no prophage induction resulted. (Guanosine and cytidine, or adenine, did not have any effect on filaments caused by penicillin or crystal violet in these strains.) These experiments indicate that inhibition of cell division is not *per se* the stimulus for prophage induction. Finally, a series of other thermosensitive mutants have been isolated, which at high temperature synthesize DNA but do not make cross septa so that long filaments are formed; when such strains are made lysogenic for λ$^+$, the prophage is not induced at high temperature[10a]

Effects of pantoyl lactone: The agent pantoyl lactone is effective in preventing and reversing filament formation in *E. coli* K-12 AB1899[11] and in *E. coli* B.[12] When tested in T-44 and T-44 (λ), it not only prevented filament formation, but also partially prevented prophage induction in the lysogenic strain. Moreover, its activity could be enhanced by the addition of guanosine and cytidine, and could be reversed by adenine. These purine and pyrimidine derivatives, however, were not effective in altering filament formation in AB1899. Thus, pantoyl lactone prevents filament formation in strains which have this capacity, including T-44, and also inhibits induction of prophage in T-44 (λ).

Sensitivity to UV irradiation: Survival curves for the various strains under varying doses of UV light are shown in Figure 3. Because of the sensitivity of T-44 to various growth conditions, it was necessary to grow and plate this strain on nutrient medium containing guanosine and cytidine at 25° in order to assure 100 per cent survival of unirradiated T-44. Under these conditions (Fig. 3*B*) T-44 seemed to be somewhat less sensitive than the wild-type C-600 or AB1899. Guanosine plus cytidine did not alter the UV sensitivity of the nonlysogenic strains, C-600 and AB1899. Neither guanosine plus cytidine, nor adenine had any effect in C-600 (λ) on either UV killing or phage production.

It should be noted that on nutrient agar with guanosine and cytidine at 25° AB1899 did not show the sensitivity or the diphasic survival which had been observed on yeast extract-tryptone medium at 37°.[13] Preliminary experiments have shown that this different sensitivity of AB1899 was due to differences between the nutrient broth and yeast extract-tryptone media.

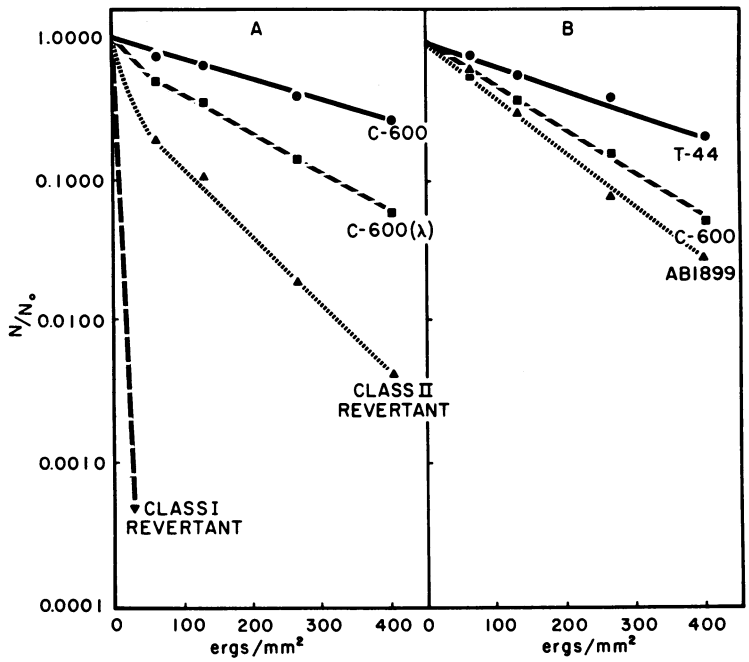

FIG. 3.—(*A*) Cells were grown in nutrient broth and plated on nutrient agar at 30°. (*B*) Cells were grown in nutrient broth containing 0.01% guanosine and cytidine, and plated on nutrient agar + rG + rC at 25°.

Isolation and characterization of revertants: T-44 (λ) will not grow when incubated on nutrient agar plates at 40°. Revertants able to grow at 40° are produced at a rather high rate (approximately 10^{-6}/cell/generation). Seventy-eight independent revertants of T-44 (λ) have been isolated. These grow normally at 40° with no tendency to lyse or form filaments. All of them are still *thr*, *leu*, immune to superinfection by λ, but sensitive to λ vir. No nonlysogenic revertants have been found.

The revertants have been divided into two general classes on the basis of their spontaneous release of free phage in stationary cultures. Class I included seventeen revertants which produced very few free phage by spontaneous induction (approximately 10^2 phage per ml in an overnight broth culture). Fifty-two of the revertants were in class II and produced approximately as many free phage as a C-600 (λ) culture (10^7 phage per ml). Nine of the revertants produced an intermediate amount of phage and have not been assigned to either class.

In all tests so far the class II revertants have behaved as though they were due to back mutations to wild-type, with the exception that they are somewhat more sensitive to UV irradiation than C-600 or T-44, as shown in Figure 3*A*. The class I revertants, however, seemed to be due to a mutation at a different site which counteracted the thermoinducibility, but at the same time endowed the bacterium with several new properties. Preliminary experiments using Hfr A-235 to transfer the threonine and leucine markers indicated that the class I revertants formed recombinants at less than 1 per cent of the rate of C-600 (λ). Also they were extremely sensitive to UV light, as shown in Figure 3*A*. They could, however, host-cell reactivate UV-treated phage, either λ or T3, to the same extent as the wild-type. In general, the class I revertants seem to behave in a manner similar

to the recombination deficient mutants previously described[14-16] in that they are rec$^-$, UV sensitive, able to host-cell reactivate, and poorly inducible.

Because of the high frequency of occurrence of the class I revertants (approximately 20% of all the spontaneous mutants found), they are presumed to be due to a single-step mutation from T-44 (λ). The frequent occurrence of this class suggests that isolation of "thermoresistant" revertants of T-44 (λ) could be a useful procedure for selecting this type of recombination-deficient mutant, and poses the interesting problem of why this phenotype should appear so frequently.

Discussion.—Witkin[17] has drawn an analogy between the events leading to the inhibition of cell division in *E. coli* B after UV irradiation and the UV induction of prophage in *E. coli* K 12 (λ). The properties of T-44 (λ) suggest an even more direct relationship between these two phenomena. In T-44, conditions which promote filament formation in the cured strain, such as elevated temperature or addition of adenine, also promote prophage induction in the lysogenic strain. Similarly, conditions such as the addition of hadacidin, guanosine plus cytidine, or pantoyl lactone, which prevent filament formation, also prevent prophage induction. These observations would imply that, at least in the T-44 strain, inhibition of cell division and destruction of the λ repressor are directly related. This does not mean, however, that stopping cell division causes prophage induction, because it is possible to stop cell division in AB1899 (λ) either spontaneously or after low doses of UV light without inducing the prophage. Moreover, filaments can be formed in both T-44 (λ) and AB1899 (λ) with either penicillin or crystal violet, and in other mutant strains by elevated temperature[10a] without induction.

The observations with T-44 imply that the λ repressor and some components necessary for cell division are subject to a common factor or common sequence of events. At least in T-44 this common sequence appears to be sensitive to the levels of the purine and pyrimidine derivatives in the small molecule pool.

One possible mechanism for the control of prophage induction and of cell division would involve an interaction between a low-molecular-weight compound, possibly an adenine derivative, and a protein (or proteins). This protein, because of its enzymatic activity or its binding properties, would then affect either directly or indirectly the prophage repression and cell division systems. The mutation in T-44 could alter either the level of the low-molecular-weight component in the pool or the affinity of the protein(s) for the low-molecular-weight component. We are currently studying the levels of the various nucleotides in the small molecule pool in order to differentiate between these two possibilites.

Inducing agents such as UV, mitomycin C, etc., are known to alter the structure or metabolism of the host DNA. If an effect on DNA is a necessary condition for induction, then examination of the DNA of T-44 (λ) under inducing conditions should reveal changes. Since the conditions for induction of T-44 (λ) are identical to those for inhibition of cell division in nonlysogenic T-44, it would be expected that DNA alterations would be apparent under conditions resulting in filament formation in T-44. Our inability to detect any alterations in either DNA structure or metabolism during incubation of T-44 at 40° suggests either that more sensitive techniques are required, or that in T-44 changes in the small molecule pool occur without the need for DNA damage.

Because prophage induction and cell division do not appear to be linked in

AB1899 as they are in T-44, and because AB1899 is not sensitive to either adenine, guanosine, or cytidine, it seems likely that the T-44 mutation acts at a site different from that of AB1899. The effect of the AB1899 mutation is only upon the cell division system, whereas the T-44 mutation has an effect on both cell division and the prophage repressor.

In some lysogenic, recombination-deficient strains, the rec$^-$ mutation prevents inactivation of the prophage repressor by UV irradiation and other agents.[15, 16] Since this rec$^-$ lesion also prevents thermal induction of λ in T-44 (λ), it appears probable that the rec$^-$ lesion is suppressing the same sequence of reactions for UV induction and for thermal induction in T-44 (λ). The fact that the class I revertants do not form filaments indicates that the rec$^-$ mutation interferes with some step or steps common to both cell division and prophage induction. Thus the lysogenic and nonlysogenic strain of T-44 as well as the class I revertants relate the phenomena of prophage induction, filament formation, recombination, and UV sensitivity. The nature of this relationship remains to be clarified.

Summary.—T-44 (λ), a mutant of *E. coli* K-12 (λ) carries a mutation in the host cell which results in the induction of λ (or other inducible prophages) when grown in nutrient broth at 40°, although growth is normal at room temperature. In a defined medium this thermal induction can be prevented by hadacidin, guanosine and cytidine, or pantoyl lactone. Adenine promotes induction and reverses this protection. If the strain is cured of its prophage it forms filaments at elevated temperatures. Conditions which promote prophage induction in the lysogenic strain promote filament formation in the cured strain.

Spontaneous revertants of T-44 (λ) no longer sensitive to incubation at 40° are found to be mainly of two types. One of these is similar to the wild-type except for its sensitivity to UV irradiation, while the other has a compensating mutation which prevents prophage induction, and at the same time inhibits filament formation, recombination, and repair of UV damage.

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