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Infectious DNA from Coliphage T1

II. Host-Cell Reactivation of UV-Irradiated Molecules

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Summary. Infectious DNA from phage T1 was inactivated by UV-light (2,537 Å). No effect of irradiation on the kinetics of the assay in a spheroplast system could be observed. UV-damaged molecules compete with unirradiated DNA for the infection. Infectious T1-DNA is subject to host-cell reactivation of UV-damage, the amount of which depends on the physiological conditions of the spheroplasts. Though UV-radiosensitivity of T1 particles is not influenced by the presence of the radical scavenging compound cysteamine, infectious DNA can be protected effectively by this chemical (0.01 M) against UV-damage when HCR-negative spheroplasts are used for the assay. Incorporation of 5-bromouracil radiosensitizes infectious T1-DNA in the presence and absence of HCR. This effect can be eliminated when the DNA is irradiated in the presence of cysteamine. The mechanism of radioprotection is discussed.

Introduction

Irradiation of biologically active DNA by ultraviolet light has been used widely to elucidate problems in molecular radiobiology and genetics (McLaren and Shugar, 1964; Rupert, 1964). The availability of infectious DNA from coliphage T1 (Hotz and Mauser, 1969) prompted us to investigate the radiobiological behavior of this system (Hotz, 1968a), since many data have been published on irradiated whole phage particles of this type but so far no information is available on free DNA of odd-numbered T-phage.

Host-cell reactivation (HCR) of UV-damaged infectious DNA has been reported for RF-DNA of phage ϕX-174 (Jansz *et al.*, 1963), for infectious DNA of phage virulent to *Bac. subtilis* (Reiter and Strauss, 1965; Okubo and Romig, 1965) and for DNA extracted from phage infecting *Haemophilus influenzae* (Harm and Rupert, 1963).

Sensitization by 5-bromouracil (BU) after incorporation into the DNA of UV-irradiated phage T1 is a well known effect (Sauerbier, 1961) and is believed to be mostly due to blocking of HCR. The block to the enzymic process of HCR which might be caused by base lesions (not restricted to BU-molecules) in the DNA (Hotz and Walser, 1970; Stephan *et al.*, 1970) and/or UV-damage to the deoxyribose (Hotz and Reuschl, 1967) can be abolished effectively by compounds of the cysteine-cysteamine group (Hotz, 1963; Rupp and Prusoff, 1964).

In the present report evidence is given for effective HCR of infectious DNA extracted from phage T1. The presence of 0.001 M cysteamine during the period of DNA-adsorption to HCR-positive spheroplasts results in an increase of HCR of unsubstituted T1-DNA. As in phage, HCR is blocked in T1-DNA when the

molecules have been substituted with BU. The presence of 0.01 M cysteamine during UV-irradiation increases considerably the survival rate of unsubstituted and of BU-DNA after adsorption to HCR-positive as well as to HCR-negative spheroplasts.

Materials and Methods

Strains: Origin and genetic pattern of phage T 1, of host range mutant T 1 *h* and of the bacteria used in our experiments, the preparation of infectious phage-DNA and of spheroplasts, as well as the assay of T1-DNA have already been described in the first part of this study (Hotz and Mauser, 1969). *E. coli K12* is capable of HCR, whereas strain KA16 (*E. coli K12, hcr-*), is deficient in this respect. For use in our spheroplast system mutants resistant to T1 and T1 *h* were selected from all bacterial strains.

Preparation of 5-bromouracil substituted phage: A standard method was used to grow BU-T1 phage on *E. coli B* wild-type. The amount of baseanalogue substituted was calculated from the increase in density measured by CsCl-density-gradient centrifugation and from the increase in UV-sensitivity. Our standard technique employed has already been described (Hotz and Zimmer, 1963). Usually about 60% of thymine was replaced by BU.

UV-irradiation: Phage purified by CsCl-density-gradient centrifugation or infectious DNA was diluted in adsorption-buffer (Hershey and Chase, 1952) and in NCE-buffer (0.179 M NaCl, 0.02 M Na-citrate, 10^{-3} M EDTA), respectively. The concentration of biological material usually amounted to 10^9 phage particles per ml and to about 5 μg DNA per ml, respectively. 4 ml of the suspensions were irradiated in a petri dish (10 cm ⌀) at room temperature with a 6 W-Hanau UV-lamp (mercury-vapor low-pressure, model NK 6/20) giving about 1.6 ergs/ $mm^2 \cdot sec$ at a distance of 45 cm and emitting 95% of the radiation at 2,537 Å. In this geometry the thickness of fluid corresponding to about 1 mm provides an essentially transparent layer. In the presence of 0.01 M cysteamine·HCl (Calbiochem, Los Angeles) used in our experiments the transmission of 2,537 Å wavelength for 1 mm light path was 90%. To avoid dose survival curves based on experiments with different experimental conditions the curve for only one set of data is drawn in the figures if no significant deviation was observed.

Results

1. Kinetics of the Assay of UV-irradiated Molecules

Fig. 1 shows the effect of UV-irradiation on the assay of infectious T1-DNA. UV-doses equivalent to 0.85, 1.7, and 2.2 phage lethal hits (PLH) were given to the DNA before adsorption to spheroplasts of *E. coli K12/1, 1h*. The number of hits are taken from the upper curve shown in Fig. 2. The family of curves shown in Fig. 1 reflects a decrease in phage yield proportional to the dose. The shape of the curves, however, does not deviate from the control. This result is important when dose-effect-curves have to be discussed, since an effect of irradiation on the kinetics of the assay would influence also the shape of the DNA-survival curve. It is furthermore concluded that UV-damaged DNA molecules of T1 are able to compete with unirradiated DNA for infecting the spheroplasts. The rapid decrease in the efficiency of infection at concentrations beyond 0.1 μg DNA/ml has already been observed in the assay system of unirradiated DNA (Hotz and Mauser, 1969).

2. UV-Sensitivity of Unsubstituted Molecules

Normal host-cell reactivation ability in our selected bacterial strains follows from Fig. 2 giving the UV-sensitivity of T1 wild-type phage particles and of particles of a host range mutant T1 *h* when plated on our mutant strains and on the parental strains, respectively. The slope and shape of the phage survival

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Evidence for an Early Regulatory Function in Phage P22

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Summary. A temperature sensitive mutant of P22 phage (*ts X*) was isolated and studied.

This mutant seems to have a basic regulatory function: it is defective in an early function like the typical DNA$^-$ mutant *ts 12.1*; it is unable to direct the phage DNA synthesis and does not lyse infected or induced cells.

Unlike *ts 12.1*, the mutation *ts X* seems to involve a gene product necessary for the expression of any vegetative function, since no phage protein synthesis, no alteration of host DNA synthesis, and no cell killing can be observed under non-permissive conditions.

The possible functional similarity between the N-cistron of the λ phage and the present X-cistron in P22 is discussed.

Introduction

Studies of the early genes of λ, located near the immunity region, has led to a better understanding of the regulation and growth of temperate phages. The isolation and mapping of several temperature sensitive mutants of phage P22 makes possible the study of the functions of individual genes in another temperate system whose regulation seems different from that of λ. Bezdek and Amati (1968) have reported evidence for two immunity regulator systems in phage P22 whereas immunity specificity in the lambdoid phages appear to be correlated with only one cistron (Kaiser and Jacob, 1957).

In the present paper we describe a new early temperature sensitive mutant of P22, *X*, which seems to have a basic regulatory function.

Materials and Methods

Bacterial and Phage Strains. Salmonella typhimurium strain LT2, a prototrophic revertant of the *ad*$^{-13}$ strain, has been used as a general indicator strain. *S. typhimurium*, strain 1559, restrictive for ^{P}H markers and permissive for ^{L}H alleles, has been used in some experiments (Favre *et al.*, 1968).

The following temperature sensitive (*ts*) and clear plaque mutants of phage P22 were used:

ts 6.1 and *ts 12.1* were isolated in the laboratory of M. Levine, their clear derivatives *6.1* c_1, *6.1* c_2, *12.1* c_2 were prepared in our laboratory. The temperature sensitive mutant *X* as well as the derivative *ts X* c_2 were prepared by us in Naples in 1965[1]. Lysogenic derivatives of LT2 were prepared carrying the above mentioned *ts* c^+ mutants of P22.

UV Induction. Exponentially growing cultures of lysogenic LT2 strains were used at a concentration of 2×10^8 cells/ml, centrifuged, washed once with buffered cold saline, and resuspended in buffered saline.

1 Another *X-like* mutant, *ts 25*, has been isolated in the laboratory of M. Levine (Levine pers. com.). These two mutants, *ts X* and *ts 25*, recombine but form one complementation group.

This suspension was irradiated using a Philips germicidal lamp (15 W, 30 sec, 77 cm distance, survival being 1% in the case of non-lysogenic bacteria). The irradiated cells were transferred into the same volume of Oxoid nutrient broth and aerated at nonpermissive temperature (39—39.5° C).

At intervals, samples were taken for measurements of optical density at 650 mμ.

Measurement of Protein Synthesis. The measurement of phage directed protein synthesis was performed following the method of Ptashne (1967) with some minor modifications. LT2 cells (1×10^9/ml), growing in M9 medium supplemented with 20 μg/ml of each aminoacid except leucine, were chilled, centrifuged, washed and transferred into a fresh medium of the same composition and then diluted to a concentration of approximately 3×10^8/ml. This suspension was irradiated on an ice bath with stirring, using a Siemens UV lamp (77 W, 4 min, 35 cm distance). The theoretical calculated survival for this dose was approximately 10^{-45}, and the protein synthesis was reduced at least 2,000 fold. These UV irradiated cells were then incubated at 37° ($t=0$). At $t=8$ minutes, the concentration of Mg^{++} was raised to 0.015 M; at 10 minutes phages were added at the multiplicities of infection (m.o.i.) indicated in the text. At 20 minutes 0.1 μc/ml, ^{14}C-leucine (New England Nuclear, specific activity: 260 μc/μM) was added. At 35 minutes the incorporation was stopped by addition of a 10% cold trichloracetic acid (TCA). The bacteria were washed by diluted TCA on membrane filters, dried and their radioactivity counted in a SELO gas flow counter.

Measurement of DNA Synthesis in the Infected Cells. The DNA synthesis was measured by the same method as described by Favre *et al.* (1968).

Results

UV induction of P22 lysogens carrying P22 wild type or *ts* mutants *X*, *6.1* or *12.1* resulted in two kinds of response (Fig. 1):

a) Lysis of the irradiated cells after about 60 minutes, characteristic of wild type and *ts 6.1* prophages.

b) No detectable lysis of the cells, characteristic for the early mutant *ts 12.1*, and *ts X*.

Infective phage particles were produced only in the case of P22 wild type prophage.

These types of response were also reflected in the pattern of DNA synthesis, when LT2 cells were infected with the respective clear derivatives of the *ts* mutants (Fig. 2).

Infection with the *ts 6.1* c_1 and *6.1* c_2 mutants resulted in the pattern of DNA synthesis typical for c_1 or c_2 mutants of P22, but no phage was produced and the cells were killed. Infection with the *ts 12.1* c_2 phage resulted in a permanent inhibition of DNA synthesis, the cells were killed and no infective particles produced.

In the case of the infection with the *ts* mutant *X* no difference in DNA synthesis was detected compared with the uninfected control with the exception of a slight depression during the first five minutes. The survival of the cells was also unaffected by the *ts X* c_2 infection and no viable phage particles were produced. The pattern of cell killing in LT2 cultures infected with the three *ts* mutants used is summarized in Table 1.

These results are in agreement with those reported in Fig. 2 since only *ts X* does not cause any significant loss of viability of the infected cells. Similar results are obtained if strain *S. t.* 1559 is used as a host despite the fact that in this strain P22 wild type infection produces bacterial killing without phage produc-

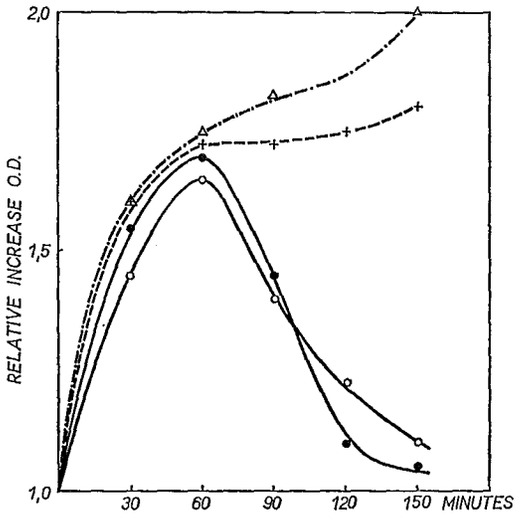

Fig. 1. Optical density measurement of LT2 cells lysogenic for various phages following UV induction and incubation at non-permissive temperature. ○—○—○ P22 *ts 6.1*, △-·-△-·-△ P22 *ts 12.1*, +--+--+ P22 *ts, X*, ●—●—● P22 wild type

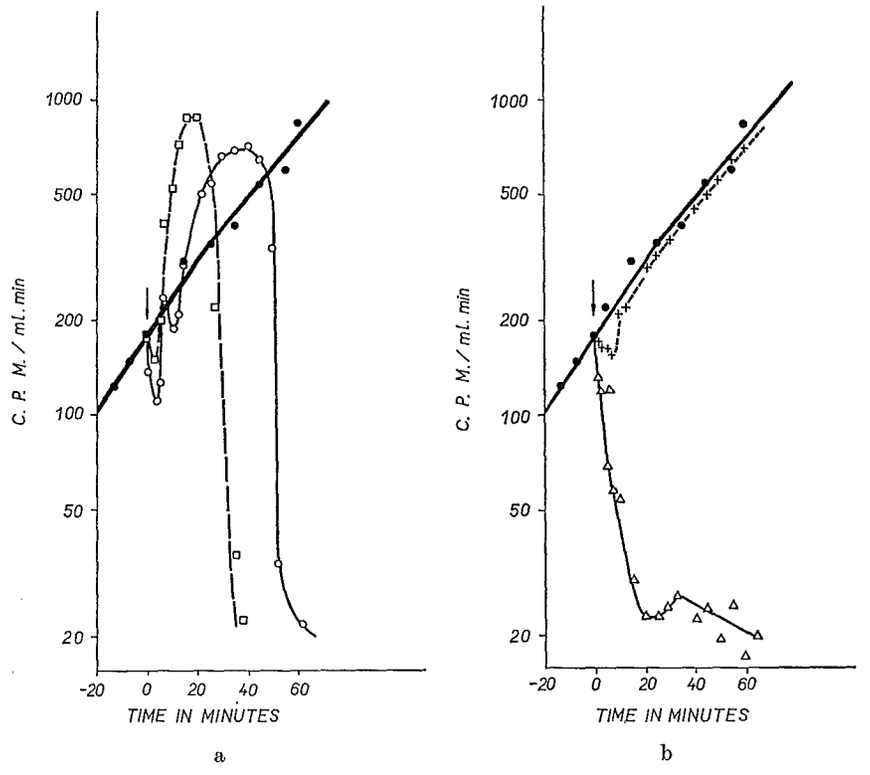

Fig. 2a and b. Rate of incorporation of thymidine ^{14}C into LT2 cells infected at m. o.i. = 20 with P22 mutants: □--□--□ *ts 6.1* c_1, ○—○—○ *ts 6.1* c_2, △—△—△ *ts 12.1* c_2, +--+--+ *ts X* c_2, and ●—●—● uninfected control

Table 1. *The killing effect on LT2 cells of P22 ts mutants under non-permissive conditions*

Mutant used (m.o.i. = 20)	Viable count in percent of uninfected control	Lysis
No phage	100	—
ts 6.1 c_1	1.3	+
ts 6.1 c_2	2.0	+
ts 12.1 c_2	1.8	—
ts X c_2	84	—

A culture of LT2 cells (at 1.5×10^8 cells/ml) growing in broth at 42° C under aeration was split into five parts and a small volume of the respective phage at m.o.i. = 20 was added. Incubation was continued under the same conditions and 10 minutes later samples were taken, diluted in prewarmed broth and plated on prewarmed EHA plates and incubated at 42° C. Lysis was observed after 80 minutes.

Table 2. *Protein synthesis in UV irradiated LT2 cells directed by various P22 ts mutants under non-permissive conditions*

Mutant used	m.o.i.	Relative protein synthesis
No phage	—	1.0
Wild type	15	2.5
c_1	15	5.9
c_2	10	6.8
ts 6.1 c_1	10	8.2
ts 12.1 c_2	6	9.4
ts X c_2	3	1.04
ts X c_2	10	1.12
ts Xc_2	30	0.55

The measurement of protein synthesis was performed by ^{14}C leucine incorporation. The relative value for UV irradiated, non-infected bacteria being 1.0, for non-irradiated, non-infected bacteria being about 2,000.

tion. This further suggests that the above mentioned gene is expressed earlier than the *H* markers (Favre *et al.*, 1968).

In both LT2 and 1559 strains, infection with *ts X* at the non-permissive temperature and shift to a permissive temperature following inactivation of unadsorbed particles lead to cell killing (and phage production with LT2 strain). This suggests that the absence of activity of the phage genome cannot be ascribed to lack of adsorption at high temperature.

Phage directed protein synthesis in the infected host was followed using heavily UV irradiated bacteria incapable of their own protein synthesis. The

results of these experiments (Table 2) show that of all the mutants tested, only the *ts X* c_2 did not appreciably increase the protein synthesis in the irradiated infected cells.

Discussion

On the basis of the analysis of data on UV induction of lysogenic strains (Fig. 1) and on the rate of DNA synthesis after infection (Fig. 2) it is possible to divide the three mutants studied into two classes: a) defective in late function (*ts 6.1*) which behaves as P22 wild type since despite the lack of active phage production it is able to direct phage DNA replication as well as phage protein synthesis with cell lysis; and b) defective in early function (*ts 12.1*, *ts X*) which are unable to direct phage DNA synthesis and which do not produce cell lysis. In this category the mutant *ts 12.1* behaves as typical DNA-less mutants, and has already been characterized by Botstein and Levine (1958). A third mutation *ts X* involves a gene product which seems to be necessary for any phage expression, since no alteration of DNA synthesis (Fig. 2), cell survival (Table 1) or protein synthesis in UV irradiated LT2 cells, can be observed after infection with such phage under non-permissive conditions. This characteristic clearly differentiates the mutant *X* from the typical early mutant *ts 12.1* where the defectiveness in one early function still produces a large number of other effects upon the infected cells (killing after induction or infection and stimulation of protein synthesis in UV killed cells).

The mutant *ts X* seems therefore to have a special position among the early mutants, preventing the expression of all vegetative phage functions. This conclusion is supported by the results of experiments on phage directed protein synthesis in heavily irradiated host cells. The protein synthesis in such cells, as measured by the incorporation of ^{14}C leucine, increased 5 to 20 times after infection with any of the mutants tested, except in the case of the *ts X* where the increase was very low, if any. In fact, on the basis of Ptashne's experiments (1967) with *N-sus* mutant of λ phage, a 10% increase would correspond to the synthesis of the immunity repressor only.

The *N* function of λ is necessary for the direct and/or indirect stimulation of λ vegetative functions (Dove, 1968). Gene *X* seems to be also necessary for the expression of vegetative functions in P22, as shown by the inability of *ts X* mutant to kill the host cells, to affect the DNA synthesis and to direct the protein synthesis in non-permissive conditions, thus suggesting a possible similarity between P22 gene *X* function and *N* function of λ. The main difference is in the locations of *N* and *X* with respect to the "clear" region of phages λ and P22: *X* is not linked to the P22-*c* region but is located in the region where the second immunity regulator system of P22 is operating (Bezdek and Amati, 1968), i.e., closer to m_3 marker (Levine, pers. com.) and to *ts 6.1* gene.

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Cell Division of the *Escherichia coli lon*⁻ Mutant

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Summary. Escherichia coli lon⁻ cells were subjected to treatments which produced a decrease in the DNA/mass ratio of the cell. Thymine starvation, a shift-up from minimal medium to rich medium, and exposure to BUdR each caused greater inhibition of cell division in *lon*⁻ cells than in *lon*⁺ cells. DNA metabolism was found to be the same in both *lon*⁺ and *lon*⁻ cells during these treatments. The results are consistent with the hypothesis that the *lon*⁻ defect leads to inhibition of cell division under conditions which produce a decreased DNA/mass ratio.

Introduction

The *Escherichia coli lon*⁻ mutants are conditional cell division mutants (Howard-Flanders, Simson and Theriot, 1964). After low doses of ultraviolet (UV)- or X-irradiation, septum formation is inhibited specifically (Adler and Hardigree, 1965; Howard-Flanders, Simson and Theriot, 1964; Walker and Pardee, 1967), and long multinucleate filaments result (Adler and Hardigree, 1965). Failure of filaments to form colonies results in increased UV-sensitivity of *lon*⁻ strains (Adler and Hardigree, 1964). UV exerts its effect on *lon*⁻ strains by its action on DNA (Kantor and Deering, 1967; Walker and Pardee, 1968). Specific, temporary inhibition of DNA synthesis by nalidixic acid (Kantor and Deering, 1968; Walker and Pardee, 1968) or by hydroxyurea (Kantor and Deering, 1968) causes *lon*⁻ mutants to form filamentous cells. Thus, some treatments which affect DNA metabolism uncouple indefinitely septum formation and DNA replication in the *lon*⁻ mutant.

Witkin (1967) postulated that the fate of UV-irradiated *E. coli* B (which probably is *lon*⁻, see Discussion) depends on the relation between the rate of protein synthesis and the rate of DNA repair. The prediction was made that survival would be increased by any post-irradiation treatments which reduce the amount of protein synthesized before completion of DNA repair; survival would be decreased by treatments which caused increased protein synthesis. Walker and Pardee (1967, 1968) postulated that *lon*⁻ mutants are susceptible to induction of filamentous growth by "indirect effects" of the various inhibitors of DNA metabolism. Kantor and Deering (1968) predicted that the "indirect effect" which leads to filamentous growth might be a depression of the DNA to RNA and protein ratio.

These hypotheses, which imply that *lon*⁻ cells fail properly to couple cell division with DNA synthesis after alterations of the relative amounts of macromolecular constituents, can be tested without the use of inhibitors. If *E. coli*, cured of defective prophages, is starved for thymine, DNA synthesis is inhibited

but mass increases. Upon readdition of thymine, DNA synthesis begins at an accelerated rate and re-establishes the proper DNA/mass ratio, at which time cell division begins at an accelerated rate (Donachie, Hobbs, and Masters, 1968). A second way to decrease the DNA/mass ratio is to shift a culture from a poor to a rich medium (a shift-up) (Kjeldgaard, 1961).

Temporary thymine starvation and a shift-up from a poor to a rich medium inhibited cell division of the *lon*$^-$ mutant. Thus, temporary periods during which the rate of DNA synthesis is low compared to the rate of cell mass increase cause inhibition of cell division of *lon*$^-$ mutants. The conversion of *lon*$^-$ cells to filamentous growth, and subsequent inactivation of colony-forming ability by 5-bromodeoxyuridine (BUdR) (Walker and Pardee, 1968), can be explained now on the basis of specific inhibition of DNA synthesis by BUdR.

Materials and Methods

Strains. E. coli K12 strains used are listed in the Table. The low-thymine requiring AX116 and AX117 were phenotypically similar to the *deo*B$^-$ mutants of *E. coli* B (Lomax and Greenberg, 1968). The λ wild-type strain (Kaiser, 1957) was used.

Media and Culture Conditions. The yeast extract-tryptone (YET) (Howard-Flanders, Simson and Theriot, 1964) media, with only 0.5% NaCl in the broth and supplemented with 100 or 5 μg/ml thymine, were used. The minimal medium base of Howard-Flanders, Simson and Theriot (1964) was supplemented as necessary with separately autoclaved glucose or glycerol (10 mg/ml), thiamine-HCl (5 μg/ml), L-amino acids (50 μg/ml), Casamino acids (5 mg/ml), thymine (100 for *thy*A$^-$ strains or 5 μg/ml for *thy*A$^-$ *thy*R$^-$ strains), BUdR (250 μg/ml), adenosine (50 μg/ml) and uridine (50 μg/ml).

Table. *Characteristics of strains*

Strain	Characteristics	Source
2e01c	F$^-$ *thi*$^-$, *thr*$^-$, *leu*$^-$	M. Malamy
AX14	spontaneous *lon*$^-$ derivative of 2e01c	
AX104[a]	*thy*A$^-$ derivation of 2e01c	
AX105[a,b]	*thy*A$^-$ derivation of AX14	
AX116	*thy*R$^-$ derivative of AX104	
AX117[b]	*thy*R$^-$ derivative of AX105	

[a] The *thy*A mutations are isogenic.

[b] The *thy*R$^-$ mutation did not alter the relative susceptibility of the *lon*$^-$ strain to thymine starvation.

All cultures were grown at 37 C with shaking.

Preparatory to thymine starvation, strains were grown in glucose-minimal medium to about 2×10^8 cells/ml. The bacteria were resuspended in glucose-minimal medium lacking thymine by the procedure of Freifelder (1969).

Other medium changes were made by dilution into warmed media, except as noted in Results.

Determination of Cell Numbers. Total cell counts were made after dilution of cells in 0.9% NaCl—0.05% formaldehyde with a Model B Coulter Counter fitted with a 30 μ aperture. Viable cell counts were made by dilution plating.

DNA Analysis. DNA was measured colorimetrically by the diphenylamine reaction (Giles and Myers, 1965).