Thermal induction of bacteriophage P1
by Shih-shun Lee

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
Montana State University
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Abstract:
The induction of a triply auxotrophic (thy- , ura- , met-) E_. coli lysogenic for P1 was studied under different nutritional and temperature conditions. At 37°C, as reported by others, induction occurs only under thymineless conditions. At 45&degC, induction occurs under thymineless conditions, but also under conditions of amino acid deprivation.

Although thermal induction appears to be a different process than thymineless induction, the fact that the two interact synergistically suggests that both involve repressor inactivation. Thermal induction is generally less complete than thymineless induction at 37°C. The fact that pretreatment at 37°C under condition of amino acid deprivation lowers subsequent thermal induction under conditions where thymine, uracil and methionine are all withheld suggests that cells that have just completed one round pf DNA replication and have not initiated another round are relatively insensitive to thermal induction. Finally, non-induced cells undergo little, if any, thymineless death at 45°C, although induced cells slowly lose plaque-forming ability at this temperature.

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TABLE OF CONTENTS

LIST OF FIGURES

Page

ABSTRACT

The induction of a triply auxotrophic (thy^-, ura^-, met^-) E. coli lysogenic for P1 was studied under different nutritional and temperature conditions. At $37^\circ C$, as reported by others, induction occurs only under thymineless conditions. At $45^\circ C$, induction occurs under thymineless conditions, but also under conditions of amino acid deprivation. Although thermal induction appears to be a different process than thymineless induction, the fact that the two interact synergistically suggests that both involve repressor inactivation. Thermal induction is generally less complete than thymineless induction at $37^\circ C$. The fact that pretreatment at $37^\circ C$ under condition of amino acid deprivation lowers subsequent thermal induction under conditions where thymine, uracil and methionine are all withheld suggests that cells that have just completed one round of DNA replication and have not initiated another round are relatively insensitive to thermal induction. Finally, non-induced cells undergo little, if any, thymineless death at $45^\circ C$, although induced cells slowly lose plaque-forming ability at this temperature.

INTRODUCTION

Lysogeny is the hereditary property of producing bacteriophage with-
out infection with external particles. A lysogenic bacterium possesses
and transmits to its progeny the capacity to produce phage. Lysogeny
appears to occur widely in nature. An important problem in the study
of lysogeny is the nature of the prophage-bacterium relationship.

A characteristic feature of this relationship is that the "lytic
cycle genes" are inactive for long periods of time and through many cell
generations. Since prolonged inactivation of genes is also character-
istic of cellular differentiation, any information obtained about the
nature of the prophage-bacterium relationship is of considerable general
interest.

There are two different approaches to this problem. One is to
study the process of lysogenization, i.e. to study how cells infected
by temperate phage can incorporate the phage genome without being lysed.
The other is to study the mechanism of prophage induction, i.e. to
study how the incorporated phage genome proceeds to replicate autono-
mously and lyses the host cells. In this study, the latter approach
is adopted.

The induction of lysogenic bacteria was first described by Lwoff
(1953). He defined induction as the "Action of provoking the develop-
ment of bacteriophage. Induction corresponds to a conversion of the
prophage into gonophage or to the initiation of the vegetative state".
In a lysogenic system, the replication of the prophage is coordinated
with bacterial reproduction. During the growth of a lysogenic

population, it is always observed that a few free phages are released by the bacteria. The release of phages by lysogenic bacteria into the surrounding media is called spontaneous induction. Spontaneous induction is relatively rare and only a small fraction of the bacteria releases phage particles. However, by means of proper treatments, the frequency of induction of lysogenic bacteria can be greatly increased.

A number of conditions or agents which provoke induction are known. Among these, ultraviolet light, mitomycin, thymine deprivation, and 5-bromouracil are commonly used. Ionizing radiations, nitrogen mustard, epoxides, ethyleneimines, desiccation, and nalidixic acid also can serve as inducing agents.

Temperature-sensitive mutants of lambda and P2 have been isolated, which are inducible by heat treatment (Lieb, 1966; Bertani, 1968). The mutants of lambda have been divided into two classes according to whether the lysogenic strains are not (t1) or are (t2) induced in the presence of chloramphenicol (Lieb, 1966). With respect to inducibility in chloramphenicol, the P2 mutants resemble lambda t2.

On the basis of ultraviolet light studies, Lwoff (1953) suggested that the effect of inducing agents is to increase the probability of a change of the bacterial chromosome, the change being responsible for the detachment of the prophage. The fact that many inducing agents arrest DNA synthesis suggested to Melechen and Skaar (1962) that this is critical to induction. The fact that substitution of thymine by 5-bromouracil results in P1 induction led Melechen (1964) to conclude

that it is not the absence of DNA synthesis that is responsible for thymineless induction at 37°C, but the production of abnormal DNA. The mechanism of induction suggested by Jacob and Monod (1961) is widely accepted today. Jacob and Monod suggested that induction is the result of inactivation of a repressor substance produced by a regulator gene. The effect of ultraviolet light, mitomycin, or thymine deprivation in bringing about induction is thought to be due to the accumulation of repressor-inactivating substances synthesized by the $\underline{rec}^+$ gene under these conditions (Hertman and Luria, 1967), while thermal induction of heat-sensitive mutants is thought to be the direct result of inactivation of a heat-labile repressor by high temperature (Lieb, 1966; Horiuchi and Inokuchi, 1967; Bertani, 1968).

At 37°C, induction of bacteria lysogenic for P1 occurs only under conditions which arrest or prevent normal DNA synthesis (Melechen and Skaar, 1962; Melechen, 1964). The mechanism which brings about this induction is not yet clear. In bacteria lysogenic for lambda, it has been shown (Ptashne, 1967) that the immunity repressor is protein and that only the repressor of heat-sensitive mutants can be inactivated by heat, leading to lambda induction. Neither wild-type lambda repressor nor P1 repressor has been reported to be subjected to heat inactivation.

The primary interest of this research is to study the possible mechanisms of P1 induction. In this paper, it is shown that the wild-type P1 repressor can be inactivated by heat under conditions of deprivation of a required amino acid, resulting in induction. On the

basis of the experimental results a model of P1 induction is presented.

MATERIALS AND METHODS

Bacterial strains

The bacterial strain studied in all of the experiments was MSC50-·SL4. Its genealogy is as follows. B-3 is a thymineless derivative of Escherichia coli B obtained by Dr. S. Brenner. MSC50 is a derivative of B-3 made lysogenic for P1 by Dr. P. D. Skaar. MSU4 is a derivative of MSC50 with additional requirement of uracil, obtained by Mr. D. Brinck. MSC50-SL4 is a derivative of MSU4 with the additional requirement of methionine, obtained by the author. Both MSU4 and MSC50-SL4 were isolated from among the survivors of extensive thymineless death. MSC50-SL4 is stringent; only about 4% of normal RNA synthesis occurs in the absence of methionine. The RNA synthesized is probably messenger (Forchhammer and Kjeldgaard, 1968). Hence, use of MSC50-SL4 allows one to measure induction under conditions prohibiting synthesis of (1) DNA, (2) mRNA, (3) protein and non messenger RNA, and combinations of these three.

The indicator strain used to measure induced cells was WM3S^R, a P1 sensitive derivative of E. coli B.

Media

In the following recipes, final concentrations are given as grams/liter of distilled water unless otherwise indicated. Media were sterilized before use.

Tris-glucose Minimal Medium (TG): NaCl 5.4, KCl 3.0, NH_4Cl 1.1, $CaCl_2$ 0.011, $MgCl_2$ 0.095, $FeCl_3$ 0.00162, KH_2PO_4 0.0872, Na_2SO_4 0.0227,

Tris(hydroxymethyl) aminomethane 12.1. The pH was adjusted to 7.2 with concentrated HCl and the solution was sterilized. Just before using, 9 parts were mixed with 1 part sterile glucose solution containing 20.0 grams/liter glucose.

L Agar Plus $CaCl_2$: Bactotryptone 10.0, yeast extract 5.0, NaCl 5.0, glucose 1.0, agar 10.0. The pH was adjusted to 7.0 with 1 N NaOH. After sterilization 1 ml of 2.5 M $CaCl_2$ was added.

L Broth: Same as for L agar, except omitting 10 grams agar.

Nutrient Agar: Nutrient broth 8.0, NaCl 5.0, agar 15.0.

Top-layer Agar Plus $CaCl_2$: Nutrient broth 8.0, NaCl 5.0, agar 6.5.

Saline: NaCl 8.6.

Supplements

The following were added to media, where desirable, to the indicated concentrations in grams per liter.

Thymine: 0.02.

Uracil: 0.02.

Methionine: 0.02.

Chloramphenicol: 0.05.

Methods

Preparation of culture. All experimental cultures were prepared in the following way. An overnight culture of MSC50-SL4 (ca. 15 hours in TG plus uracil, thymine, and methionine without aeration at $37^{\circ}C$) was diluted into fresh medium of the same kind to a concentration of

approximately 2 x 10^6 viable cells/ml. The culture was then aerated at 37°C for approximately three and half hours. The resulting log phase culture contained about 2 x 10^7 viable cells/ml. The cells were then washed twice by centrifugation and resuspended in TG. Then, 0.5 ml of this washed culture was diluted into 9.5 ml saline. Finally, 0.1 ml samples of the saline suspension were diluted into appropriate media to initiate the experiment. Thus, the initial population in each experiment consisted of approximately 10^5 washed, log-phase cells suspended in 10 ml of medium.

Assays of induction. In the course of growth of the experimental cultures (in different media and at different temperatures), assays of induction were made at intervals in the following way. A 0.1 ml sample was removed from the culture and mixed with 2 ml of top-layer agar plus CaCl$_2$. Then 0.1 ml of an overnight L broth culture of the indicator strain, WM3S^R, was added to the same tube. The mixture was then layered onto an L agar plus CaCl$_2$ plate. After 18 hours incubation at 37°C, the plaques appearing on the plates were counted. These were used to estimate the fraction of MSC50-SL4 cells induced at the time of plating. Skaar (personal communication) has shown that the background of induction after plating is sufficiently low and delayed that it does not significantly affect the number of visible plaques.

Assays of viable cells. During most experiments, assays of viable cells were also made at intervals. These were made by spreading 0.1 ml samples of the experimental cultures on nutrient agar, incubating the

plates and counting the resulting colonies.

   Temperature control.   During growth, the experimental cultures
were immersed in water baths maintained at either 37 or 45°C. The
fluctuation of temperature in these baths was $\pm$ 0.1°C.

RESULTS

Patterns of Induction and Survival at 45°C.

MSC50-SL4, when grown at 45°C in TG medium supplemented with thymine, uracil and methionine, grows very slowly and liberates phage at a low rate. (Approximately 5 free phage per 1000 bacteria are observed in growing cultures.)

In order to see if more extensive induction might be provoked under conditions limiting to growth, washed log phase cells were prepared as described in Materials and Methods and diluted into tubes of incompletely supplemented TG medium of the following composition.

A: TG + uracil + methionine.

B: TG + uracil.

C: TG.

D: TG + thymine.

E: TG + thymine + methionine.

F: TG + thymine + uracil.

G: TG + methionine.

The cultures were then aerated at 45°C. At intervals samples were removed to assay induced cells and viable cells. The results are shown in Figures 1 and 2. Several aspects of these results are discussed in the following sections.

The effects of specific thymine deprivation. Under conditions of specific thymine deprivation (A medium) at 45°C a large fraction of the population is induced, as is true at 37°C. A comparison of the kinetics of induction at 45°C (Figure 1) with the kinetics of induction at 37°C
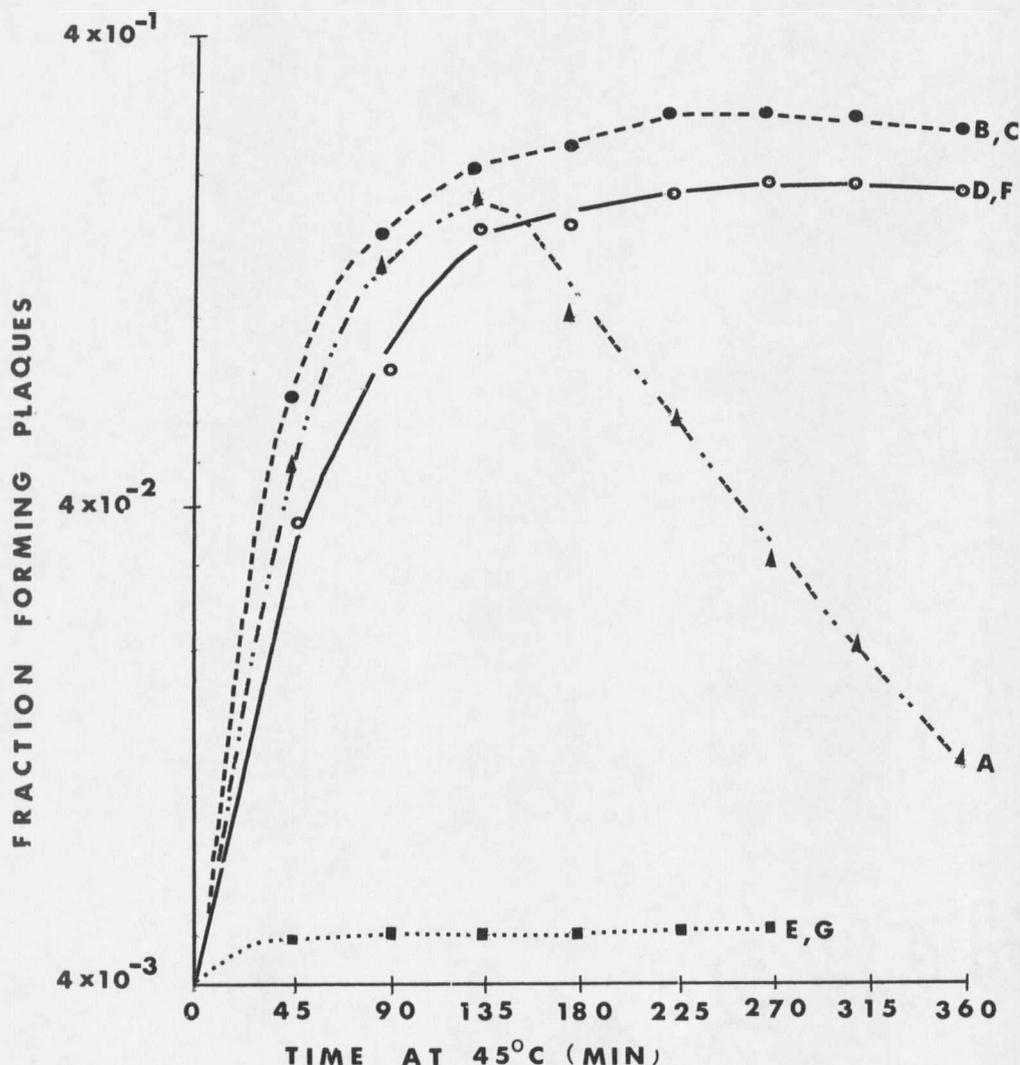
Figure 1. Induction of P1 at 45°C in different media. About 10^5 washed, log-phase cells of MSC50-SL4 were suspended in tubes containing 10 ml of incompletely supplemented TG medium. The media employed were as follows: A: TG + uracil + methionine; B: TG + uracil; C: TG; D: TG + thymine; E: TG + thymine + methionine; F: TG + thymine + uracil; G: TG + methionine. The culture were then aerated at 45°C. At intervals 0.1 ml samples were removed to assay induced cells. The fraction was calculated by dividing the number of plaques at a given time by the number of colonies at zero time. Each point is the average of three independent experiments (one plating per experiment).
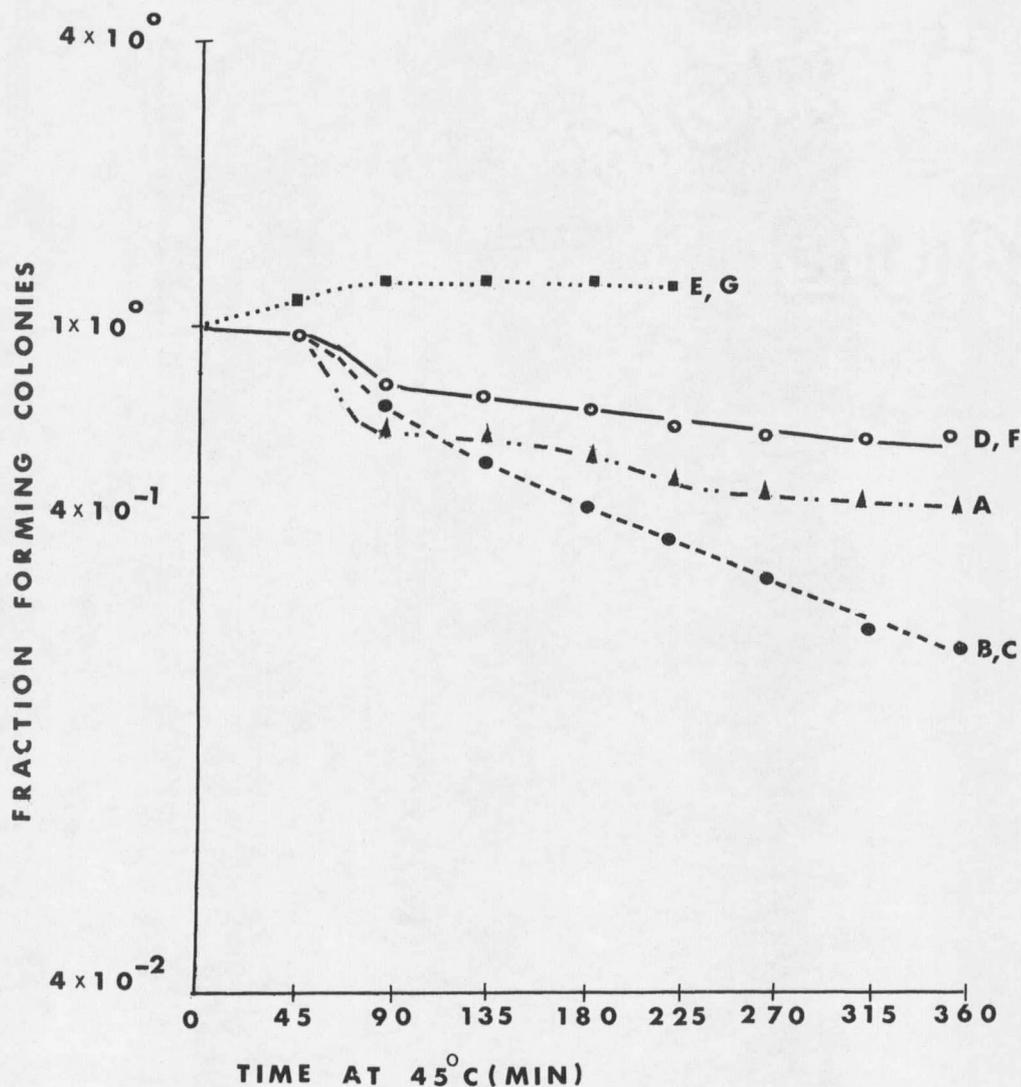
Figure 2.   Survival of MSC50-SL4 at 45°C in different media. Data are
from the same experiments illustrated in Figure 1. At intervals 0.1 ml
samples were removed and assayed for viable cells. Each point is the
average of three independent experiments (one plating per experiment).
Otherwise, procedures and symbols are the same as in Figure 1.

(as given in Melechen and Skaar, 1962) reveals two differences. First, induction is slower at 45°C; this could be a consequence of slower metabolism at that temperature. Second, although a secondary decline in plaque-forming units is observed at both temperatures, the decline is much more rapid at 37°C. Again this might be due to a difference in the rate of metabolism at the two temperatures. On the other hand, another possible factor is suggested by the "A" curve in Figure 2. Melechen and Skaar reported that, at 37°C, the number of viable cells drops sharply and extensively (about a thousand fold between 90 and 240 minutes). At 45°C (Figure 2), only a minor drop in the number of viable cells is observed even after 360 minutes. Typical "thymineless death", therefore, does not occur at 45°C. In addition to suggesting a second reason for the slower decline of plaque-forming units at 45°C, these experiments provide another example of the "uncoupling" of induction and thymineless death.

The effects of amino acid deprivation. It is clear from Figure 1 that extensive induction occurs at 45°C under conditions other than specific thymine deprivation. This is in marked contrast to the situation at 37°C. The author has verified the implication of other investigations (e.g. Melechen, 1964) that induction at 37°C occurs only when thymine alone is withheld.

The common feature of the other inducing conditions at 45°C is the absence of methionine. Wherever methionine is withheld (B, C, D, F), extensive induction occurs regardless of the presence or absence of

thymine and uracil. With the exception of specifically thymineless conditions (A), wherever methionine is provided (E, G), extensive induction does not occur.

Essentially similar results have been obtained with other triply auxotroph strains, requireing (in addition to thymine and uracil) either cysteine, or glutamate, or arginine (Lee, unpublished). Induction provoked by the deprivation of an amino acid has not been described before.

The results suggest that the maintenance of the Pl prophage state involves a heat-labile protein repressor. Clearly, if this is so, the repressor is less labile than that of the heat-sensitive mutants of lambda (Lieb, 1966), for induction of these mutants is much faster and does not require concomitant amino acid starvation.

As with induction in A medium, induction in amino acid free media is not accompanied by extensive thymineless death (see Figure 2).

The Additivity of Thermal and Thymineless Induction.

As was established in the preceding section, thermal induction occurs only under conditions of amino acid deprivation. On the other hand thymineless induction at 37° C will not occur under conditions of amino acid deprivation. Although two mechanisms of induction are suggested, both might be by way of repressor inactivation (the former by way of direct inactivation, the latter by the generation of a protein repressor-inhibitor). If both types of induction are by way of repressor

inactivation, then a synergistic interaction of the two provoking

conditions should be observed. To test for this, the following experi-

ment was performed.

A log phase culture of MSC50-SL4 was washed and resuspended in A

medium (minimal medium plus uracil and methionine). The culture was then

divided into two parts and chloramphenicol (CAP) added to one to a final

concentration of 50 μg/ml. Both were then aerated at 37°C for 45

minutes. Then each culture was washed, diluted into C medium (minimal,

no additions) and aerated at 45°C. A second dilution of the first

culture into C medium was aerated at 37°C. At intervals after 45 minutes

the three cultures were assayed for induction. In the population sub-

jected first to A medium at 37°C, then C medium at 45°C $((A)_1C_2)$, there

is the opportunity for <u>sequential</u> thymineless, then thermal, induction.

In the population subjected first to A medium at 37°C, then C medium at

37°C $((A)_1C_1)$, only 45 minutes of thymineless induction is observed. In

the population subjected first to A medium plus chloramphenicol at 37°C,

then C medium at 45°C $((A_{CAP})_1C_2)$, only thermal induction after 45

minutes is involved. Figure 3 shows the results.

As can be seen from the results given in Figure 3, the two treat-

ments applied sequentially are synergistic. That is, if one adds the

induction provoked by thymineless conditions for 45 minutes $((A)_1C_1)$ to

that provoked by thermally inducing conditions $((A_{CAP})_1C_2)$ for any period

of time after 45 minutes, the sum is <u>less</u> than the induction provoked

when thymineless and thermal conditions are imposed sequentially $((A)_1C_2)$.