JOURNAL OF BACTERIOLOGY
Vol. 88, No. 6, p. 1585-1589 December, 1964


# EFFECT OF HEAT SHOCK ON $T_4$rII MULTIPLICATION IN *ESCHERICHIA COLI*

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Received for publication 24 July 1964

ABSTRACT

KASATIYA, SHANTI-SWAROOP (University of Southern California, Los Angeles), AND MARGARET LIEB. Effect of heat shock on $T_4$rII multiplication in *Escherichia coli*. J. Bacteriol. **88**:1585-1589. 1964.—Heating certain strains of *Escherichia coli* K-12 (λ) to 45 to 48 C for 12 min before phage infection increased the fraction of bacteria that allowed multiplication of $T_4$rII mutants. The effect of heat on interference to $T_4$rII was not correlated with induction of the λ prophage, which occurred in some of the strains studied. Incubation of heated cells at 37 C before infection resulted in the recovery of interference, providing a prophage was still present. The presence of magnesium ion after infection is known to greatly decrease the interference in K-12 (λ) to $T_4$rII. When heat-shocked cells were infected with $T_4$rII, and then incubated in medium containing a suboptimal concentration of magnesium ion, more cells produced a burst of $T_4$rII progeny than with either treatment alone. It is suggested that factors produced by both the prophage and the bacterium contribute to the structure or substance responsible for interference. The variation in heat sensitivity of interference in various strains is probably due to differences in the bacterial contribution to the postulated structure.

The presence of λ prophage in *Escherichia coli* K-12 interferes with the multiplication of $T_4$ phages with mutations in the rII region. Although the lysogenic cells are killed, few lyse and release $T_4$. The probability that a K-12 (λ) cell, infected with a given rII mutant phage, will produce any phage progeny is the transmission coefficient, or TC (Benzer, 1955). Benzer reported that the TC of a given strain was different for various $T_4$rII mutants. Mahler (1961) studied several K-12 (λ) strains, and showed that the TC for a given rII mutant was determined by the bacterial strain, and not by the prophage. The level of interference was transmitted from HFr to $F^-$ as a unit, but was not closely linked to any of the markers studied. Multiplicity of infection, stage of growth, and nutritional status did not influence the TC of a K-12 (λ) strain for a given $T_4$rII (Mahler, 1961). However, Benzer (1955) reported that the TC "depends strongly upon the physiological state of the bacteria and upon temperature." Garen (1961) showed that magnesium added to the culture medium increased markedly both the TC and the number of rII obtained per phage-producing cell. For the maximal effect, the presence of magnesium was required from 10 min after $T_4$rII infection until the end of the latent period.

The nature of the block to $T_4$rII replication in K-12 (λ) cells is not known. There is considerable evidence that the "immunity substance" produced by the prophage is not responsible: (i) phage 434 hy has the immunity of λ, but does not interfere with rII replication (Kaiser and Jacob, 1957), (ii) ultraviolet induction of K-12 (λ) increases rather than decreases the TC (Mahler, 1961), and (iii) photoreactivation reverses λ induction, but does not change the TC (Mahler, 1961). In the experiments reported here, a brief heat shock increased the TC of some K-12 (λ) strains. Synergism was shown between the effects of heating and the addition of $Mg^{++}$.

## MATERIALS AND METHODS

*Bacterial strains. E. coli* M3 is a histidine-requiring derivative of K-12S. M3 (λ co) is M3 lysogenized with a clear-plaque forming λ mutant; this strain was listed by Mahler (1961) as M3 (λ). M3 (λ c1-t1) carries a heat-inducible λ mutant obtained from J. J. Weigle. There is evidence that heat induction in this strain is due to inactivation of the immunity substance produced by the prophage (Lieb, 1964). Strain B was used to assay $T_4$rII. During the course of the work reported here, single colonies from strain M3 were used to start new sensitive strains called M3a and M3b.

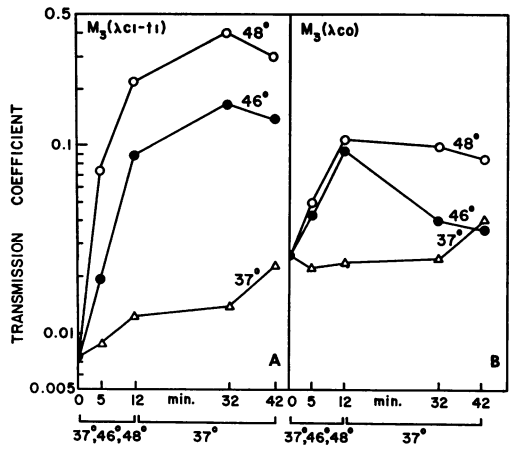


FIG. 1. *Effect of heat shock on transmission coefficient in (A) M3 (λ c1-t1) and (B) M3 (λ co).*

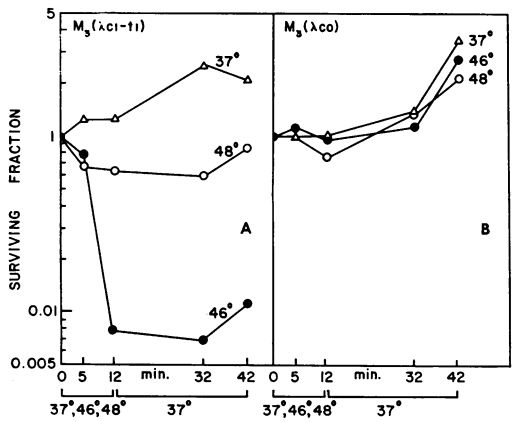


FIG. 2. *Effect of heat shock on survival of (A) M3 (λ c1-t1) and (B) M3 (λ co).*

*Phage strains.* $T_4$rII mutant N19 is a 5-bromouracil mutant obtained from E. Freese (1959). H88, a large deletion of the A cistron, and 638, a deletion of the B cistron (Benzer, 1961), were obtained from R. Edgar. Tryptophan was added to phage stocks to give 10 μg/ml.

*Media.* Tryptone (Difco) broth (TB) contained 1% Tryptone and 0.5% NaCl. Tryptone agar (TA) plates were prepared by the addition of 1.2% agar (Difco) to TB. The soft agar used for bacteriophage assay (Adams, 1950) contained 0.6% agar, 1% Tryptone, 0.8% NaCl, 0.2% sodium citrate, and 0.3% glucose. Diluting fluid contained 0.1% nutrient broth (Difco) and 0.5% NaCl.

*Experimental methods.* Bacteria for heating experiments were grown in TB at 37 C on a reciprocating shaker or a bubbler to about $2 \times 10^8$ per ml. They were chilled, centrifuged, and resuspended to 0.1 volume in cold 0.9% saline. The bacteria were diluted into TB at the appropriate temperature, and, at the desired time, 0.2-ml samples were removed for infection with $T_4$rII. The experimental culture (0.2 ml), plus 0.2 ml of $T_4$rII at $2 \times 10^9$ per ml, were incubated at 37 C for 5 min. Then, 0.2 ml of antiserum to $T_4$ was added, and, after 5 min, the cells were diluted for immediate assay or studies of phage yield. The number of bacteria infected by $T_4$ could be determined directly by assaying colony-formers both before and after exposure to $T_4$. In calculating the transmission coefficient (TC), all $T_4$-infected cells were considered to be capable of potentially producing $T_4$, even though they may have been killed by heat shock (with or without prophage induction).

## RESULTS

*Effect of heating to 46 and 48 C on interference in K-12 (λ c1-t1).* K-12 (λ c1-t1) bacteria in log phase were heated at 37, 46, or 48 C in TB for 12 min, and then transferred to 37 C. At intervals, samples were removed and infected with N19. The brief period of heating to 46 or 48 C increased the fraction of infected cells that yielded rII phage, and the TC continued to increase, even after the bacteria were restored to 37 C (Fig. 1A). Bacteria were killed at 46 C (due to induction of phage multiplication), but at 48 C, there was little killing (Fig. 2A). We presumed that at 48 C the immunity substance was destroyed, but that a reaction necessary for phage development could not proceed. When the bacteria were returned to 37 C, immunity substance regained its activity, or new immunity substance was rapidly synthesized, so that there was no induction. To determine whether the increase in TC after heating required the inactivation of immunity substance, strains lysogenic for λ, that were not heat-inducible, were tested. Heating to 46 or 48 C for 12 min increased the TC in K-12 (λ co), but there was no induction of the prophage (Fig. 1B and 2B).

*Recovery of interference after heating.* In M3 (λ c1-t1), the TC continued to rise when the heat-shocked cells were incubated at 37 C, but in M3 (λ co), incubation at 37 C resulted in a drop in TC (Fig. 1). It has been shown (Lieb,

1964) that when M3 (λ c1-t1) has been superinfected with $\lambda^+$, heating does not cause induction of the normally heat-inducible prophage. The superinfecting phage apparently produces immunity substance that is not heat-labile. In a culture of M3 (λ c1-t1) superinfected with $\lambda^+$, heating to 45 C did not result in induction (Fig. 3). However, heating did increase the TC for phage N19 (Fig. 4). When the culture was returned to 37 C, the TC decreased at the rate observed in heated M3 (λ co) cultures (Fig. 1B). Thus, the presence of prophage allowed the recovery of normal interference to $T_4$rII development.

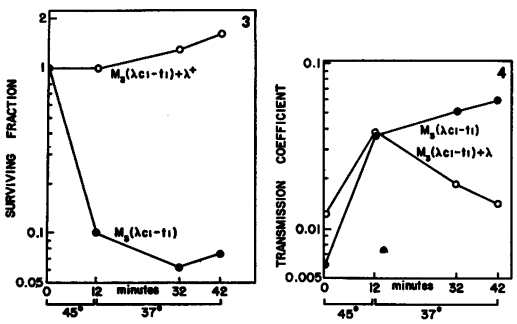


FIG. 3. *Effect of superinfection with* $\lambda^+$ *on induction of M3* (λ *c1-t1*).

FIG. 4. *Effect of superinfection with* $\lambda^+$ *on recovery of interference after heat shock. In both Fig. 3 and 4, M3* (λ *c1-t1*), *grown to* $2 \times 10^8$*/ml, was infected with* $\lambda^+$ *to give a multiplicity of about 1.5. After 10 min at 37 C for adsorption of* $\lambda^+$, *the cells were centrifuged to remove free phage, and resuspended in 30 ml of TB. They were then grown for several generations, and challenged with N19 as usual. The control, not superinfected with* $\lambda^+$, *was treated in an identical manner.*

*Optimal conditions for reduction of interference by heating.* Heating M3 (λ co) at 46 C for more than 12 min did not further increase the TC. In M3 (λ c1-t1), the TC increased at 46 C up to 24 min. However, the increase was no greater than that observed when cultures were transferred to 37 C after 12 min at 46 C (Fig. 1A). The effect of longer periods at 48 C was not studied, because of cell death and decrease in burst size. Early log-phase cultures were more sensitive to the effects of heating than were late log-phase cultures. When M3 (λ c1-t1) was first infected with N19, and then incubated at 48 C, the number of bacteria yielding $T_4$ decreased.

*Comparison of the effect of heat shock and* $Mg^{++}$. Garen (1961) reported that incubation of K-12 (λ), infected with $T_4$rII, in broth containing 0.05 to 0.1 M $MgCl_2$ (and no NaCl) allowed 50% of the cells to produce a normal burst of phage. The TC and burst size in the absence of magnesium were not given, but both appeared to be low. In a single-burst experiment, Mahler (1961) found that the addition of 0.05 M $MgCl_2$ to broth containing 0.125 M NaCl increased the TC of N19 in K-12 (λ) by a factor of six, and the burst size increased from 7.8 to 164. Four single-burst experiments were performed

TABLE 1. *Effect of heat shock and* $Mg^{++}$ *on TC and burst size*

| Treatment* | Expt 1 | | Expt 2 | | Expt 3 | | Expt 4 | |
|---|---|---|---|---|---|---|---|---|
| | TC | Burst size | TC | Burst size | TC | Burst size | TC | Burst size |
| 37 C | 0.0029 | 111 | 0.012 | 32 | 0.0050 | 90 | 0.015 | 79 |
| 48 C | 0.28 | 60 | 0.049 | 5.4 | 0.017 | 23 | 0.047† | 70 |
| 37 C + $Mg^{++}$ | ..... | ..... | 0.034 | 126 | 0.050 | 91 | 0.18 | 105 |
| 48 C + $Mg^{++}$ | ..... | ..... | 0.093 | 40 | 0.010 | 96 | 0.21† | 176 |

* Bacteria were subjected to heat shock as described in Materials and Methods. After infection with N19, the bacteria were diluted in TB, or TB plus 0.08 M $MgCl_2$, so as to have about one phage yielder per 0.5-ml sample. The relative dilutions were as follows: 37 C, 1×; 48 C, 5×; Mg + 37 C, 10×; Mg + 48 C, 20×. The total number of individual samples tested varied from 40 to 240 in the experiments reported. The samples were incubated at 37 C for the following times: experiment 1, 70 min; experiment 2, 90 min; experiment 3, 100 min; experiment 4, 120 min. At the end of the incubation time, 2 ml of soft agar seeded with B were added to each sample tube, and the entire contents were plated. The transmission coefficient is the total number of phage-yielding cells divided by the total number of infected cells in a set of samples.

† Treated at 45 C.

in which the cells were heated, infected with N19, and then incubated in TB (0.125 M NaCl) with or without added Mg (Table 1). Heating to 48 C increased the TC, but reduced the burst size. When cells were first heated, and then incubated in $Mg^{++}$, there was a greater increase in TC than with $Mg^{++}$ alone. Similar results were obtained in experiments not involving single bursts, in which the phage yield per infected cell was determined 60 min after infection.

*Effect of heat shock on TC of other $T_4$rII mutants.* The TC for two $T_4$rII deletions was increased after the host bacteria had been heated briefly (Table 2). Table 2 indicates the range of TC observed with N19 in various experiments.

*Comparison of related lysogenic strains.* Heat shock increased the TC of several K-12 (λ) strains for phage N19 (Table 3). However, the TC did not increase in strains M3a (λ c1-t59) and M3a (λ c1-t2), which are closely related to M3 (λ c1-t1).

TABLE 2. *Effect of heat shock on transmission coefficients of $T_4$rII mutants in strain M3 (λ c1-t1)**

| $T_4$rII mutant | 37 C | 45 C | 48 C | 50 C |
|---|---|---|---|---|
| H88 | .002 | .012 | .18 | — |
| 638 | .004 | .014 | — | .15 |
| N19 | .002–.011 | .014–.09 | .043–.39 | — |

* A log-phase culture of M3 (λ c1-t1) was held for 12 min at the temperatures indicated, and then infected with phage at a multiplicity of five.

## DISCUSSION

When bacteria of certain strains of K-12 (λ) were heated to 45 to 48 C, and then infected with $T_4$rII phages, the fraction of bacteria yielding $T_4$ progeny increased. Heat increased the transmission coefficient in M3 (λ c1-t1), which carries a heat-inducible prophage, as well as in M3 (λ co), in which the prophage is not heat-inducible. This indicates that it is not heat induction of the prophage present in the cell which causes the increase in TC. Heat shock also increased the TC when heat induction was prevented by the presence of a superinfecting phage. Heating to 48 C increased the TC of M3 (λ

TABLE 3. *Effect of heat shock on TC for $T_4$rII mutant N19 in various K-12 (λ) strains**

| Strain | Origin | Transmission coefficient | | |
|---|---|---|---|---|
| | | 37 C | 45 C | 48 C |
| M3 (λ co) | See Materials and Methods | 0.019–0.06 | 0.08–0.15 | 0.11–0 36 |
| M3b (λ)b | Lysogenic colonies picked up from plaques produced by phage $\lambda^+$ on M3b | 0.022–0.027 | 0.022 | 0.064 |
| M3b (λ)d2 | | 0.004–0.005 | 0.004 | 0.002–0.008 |
| M3a (λ c1-t59) | M3a lysogenized with λ c1-t59, which is apparently identical to c1-t1 | 0.01 | — | 0.032 |
| M3a (λ c1-t2) | M3a lysogenized with λ c1-t2, a heat-inducible λ mutant not identical to λ c1-t1 | 0.003–0.004 | 0.0039 | 0.0032–0.005 |
| M3b (λ co)c | Lysogenic colonies picked up from the plaques produced by supernatant of M3 (λ co) on M3b | 0.049 | 0.052 | |
| M3b (λ co)B | | 0.0065 | 0.013 | |
| K-14 | K-12 58-161 $F^-$ (λ) from Bertani | 0.01 | — | 0.11 |
| K-39 | C600 (λ) from Bertani | 0.225 | — | 0.80 |

* Log-phase cultures of the bacteria were held for 12 min at the temperatures indicated, and were then infected with N19.

c1-t1) without inducing a large fraction of the cells. In addition, strains M3a (λ c1-t59) and M3a (λ c1-t2) undergo heat-induction with no increase in TC.

Although the increase in TC cannot be correlated with induction, the recovery of interference, when heated cells are subsequently cultured at 37 C, apparently requires the presence of prophage. This suggests that a product of the prophage is involved in the maintenance of interference.

In the experiments comparing the effect on TC of heat shock before infection with the effect of $Mg^{++}$ added after infection, we used a mixture of NaCl and $MgCl_2$, which was not optimal for the reduction of interference. Under these conditions, there was an additive effect of heat and $Mg^{++}$ on the TC.

Heating to 46 or 48 C increased the TC for $T_4$rII N19 of two strains distantly related to M3, but had little effect on the TC of M3a or M3b carrying various prophages. We suggest that during isolation of these two substrains from M3, we inadvertently chose variants in which the bacterial structure (or substance) responsible for interference was not significantly altered by the standard heat shock. Garen (1961) suggested that the presence of the prophage may affect the bacterial membrane in such a way as to alter its permeability to certain ions, such as magnesium. The results reported here are compatible with the notion that the prophage contributes to the formation of a bacterial structure that (indirectly) blocks the replication of some bacteriophages. In some bacterial strains, a brief period at 46 to 48 C alters the structure, so as to reduce the block. If the bacterium and prophage survive the heat shock, the structure gradually regains its normal ability to block phage production.

## ACKNOWLEDGMENTS

This study was aided by Public Health Service research grant AI-05367 from the National Institutes of Health, and by Public Health Service Research Career Program Award 5-K3-GM-1546 to the junior author.

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