The Effect of Heat on Host-controlled Restriction of Phage λ in *Escherichia coli* κ (P1)

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SUMMARY

The growth of phage λ .C (i.e. phage λ grown in *Escherichia coli* c) in *E. coli* κ lysogenized by phage P1 is normally restricted so that the efficiency of plating of phage λ .C on κ (P1) compared to c is about 10^{-7} . When κ (P1) bacteria are heated before infection with phage λ .C this restriction may be decreased as much as a million-fold. The time of exposure to elevated temperature (49° and above) required to achieve this increase in e.o.p. of phage λ .C decreased with increasing temperature up to temperatures which began to inhibit the capacity of bacteria to grow phage. Heated κ (P1) bacteria recovered their ability to restrict phage λ .C following the resumption of growth at 37°. Part of this recovery can be inhibited by chloramphenicol. A more dramatic recovery of restriction is observed when heated κ (P1) bacteria are resuspended in hypertonic media. Experiments are described which indicate that phage λ .C is restricted at an early step after adsorption and that, if phage escapes this restriction, it can grow in heated bacteria subsequently converted into restricting hosts by resuspension in hypertonic media.

INTRODUCTION

The host range of some bacteriophages is changed after growth in a new bacterial host. This change has been shown to be non-mutational in character and is called 'host-controlled modification' (see Luria, 1953; Arber & Dussoix, 1962). Two distinct host-controlled functions can be recognized: modification and restriction. Modification is a process which acts directly on DNA and probably takes the form of a specific alteration in certain base sequences (Arber, 1965; Klein & Sauerbier, 1965). DNA synthesized in a particular bacterial strain therefore carries a modification pattern specified by the host bacterium. Restriction is a process which can occur when foreign DNA enters a bacterium. When this foreign DNA does not carry a modification pattern which is compatible with the recipient bacterium, it is rapidly degraded to small molecular weight components (Dussoix & Arber, 1962). Mutants which are genetically unable to restrict foreign DNA have been isolated (Glover, Schell, Symonds & Stacey, 1963; Colson, Glover, Symonds & Stacey, 1965; Wood, 1965). However, certain environmental factors are known to decrease the ability

of wild-type bacteria to restrict the growth of unmodified phage particles (Luria, 1953; Lederberg, 1957; Uetake, Toyama & Hagiwara, 1964; Glover & Colson, 1965).

The experiments reported here were designed to elucidate further the mechanism of host-controlled restriction. Host specificity of a phage is indicated by a symbol representing the phage followed by a symbol representing the last host in which the phage was grown (Arber & Dussoix, 1962). The system chosen for study was the restriction of phage λ , grown in *Escherichia coli* c and designated λ .C, by *E. coli* strain κ (P1) (i.e. strain κ lysogenized by phage P1). The efficiency of plating (e.o.p.) of phage λ .C on *E. coli* κ is about 10^{-4} but, when κ is made lysogenic for phage P1, the e.o.p. of λ .C on κ (P1) decreases to about 10^{-7} . The restricted λ .C DNA is degraded in *E. coli* κ and in *E. coli* κ (P1) shortly after injection (Arber, Hattman & Dussoix, 1963). It can be asked whether the observed breakdown of the restricted λ .C DNA is the primary cause of the failure of phage λ .C to grow in κ (P1) or whether it is a secondary feature consequent upon the failure of phage λ .C to grow in *E. coli* κ (P1) for some other reason. To answer this question we have used methods which temporarily decreased restriction in κ (P1) and studied the kinetics of the loss of restriction and its subsequent recovery before and after infection with phage λ .C. The results reported here indicate that the initial phase of restriction took place very shortly after adsorption and might be due to a surface-localized nuclease.

METHODS

Bacteria. *Escherichia coli* κ -12 strain c600 (Appleyard, 1954) here designated κ , and its P1-lysogenic derivative κ (P1); *E. coli* c (Bertani & Weigle, 1953), designated c, were used.

Bacteriophage. Phage λ v, a virulent mutant of phage λ (Jacob & Wollman, 1954).

Media. Tryptone broth: 1% (w/v) Difco Bacto tryptone, 0.5% (w/v) NaCl, thiamine 10 μ g./ml.; adjusted to pH 7.1. Solid media were formed with 1.5% (w/v) Difco agar.

Media for adsorption of phage λ : 0.01 M-MgSO₄ or 0.01 M-MgSO₄ in 0.01 M-tris HCl buffer at pH 7.3.

Techniques. The general phage techniques used were as described by Adams (1950). Special techniques relating to phage λ were as described by Arber (1958, 1960). The efficiency of plating (e.o.p.) of infective centres = count on *E. coli* κ (P1)/count of phage λ .C stock on *E. coli* c.

RESULTS

The effect of heat on restriction

Uetake *et al.* (1964) showed that exposure of *Salmonella butantan* to temperatures between 44° and 51° for a few minutes before infection with phage ϵ^{15} previously grown on *Salmonella anatum*, increased the e.o.p. of the phage from 2×10^{-2} to 5×10^{-1} . We have studied the effect of heat on the restricting ability of *Escherichia coli* κ (P1) by measuring the increase in the e.o.p. of phage λ .C on the heated host bacteria. *E. coli* κ (P1) organisms were grown in tryptone broth to a colony count of 10^9 bacteria/ml. The bacteria were harvested by centrifugation and resuspended to a count of 10^{10} /ml. in 0.01 M-MgSO₄ and starved for 1 hr at 37°. The bacteria were

heated by diluting the starved bacterial suspension 10-fold into pre-heated 0.01 M-MgSO₄ at the chosen temperature. After a given time at the elevated temperature the bacteria were transferred to a tube at 37° containing 1×10^8 phage particles/ml. of the challenge phage λ .C. After 15 min. adsorption at 37°, the infected cultures were diluted and the number of infective centres assayed on *E. coli* κ (P1) indicator bacteria. The kinetics of heat-inactivation of restriction by temperatures from 48°

Fig. 1

Fig. 2

Fig. 3

Fig. 1. Kinetics of the heat inactivation of phage λ .C restriction in *Escherichia coli* κ (P1). κ (P1) bacteria were grown to colony count 10^9 /ml. in tryptone broth, harvested and resuspended to colony count 10^{10} /ml. and starved in 0.01 M-MgSO₄. The suspension was heated by making a 1/5 dilution into preheated 0.01 M-MgSO₄. After heating the bacteria were returned to 37° and challenged with phage λ .C. The number of infective centres was assayed on *E. coli* κ (P1). ●—● 48°; ○—○ 49°; ▲—▲ 50°; △—△ 52°.

Fig. 2. Kinetics of the heat inactivation of phage λ .C restriction in *Escherichia coli* κ (P1). Experimental procedure as in Fig. 1. △—△ 55°; ●—● 57°; ○—○ 60°.

Fig. 3. Time of exposure to heat treatment required to obtain the maximum decrease in the restriction of phage λ .C by *Escherichia coli* κ (P1).

to 60° are illustrated in Figs. 1 and 2. The e.o.p. of phage λ .C on *E. coli* κ (P1) increased by a factor up to 10^6 when the host bacteria were heated before infection. The time of exposure to elevated temperatures required to achieve the maximum effect (the highest e.o.p. of phage λ .C) decreased with increasing temperature (Fig. 3).

The colony count of the cultures did not decrease when they were heated for up to 60 min. at temperatures up to 50°. Above 50° the viability decreased both as a function of the temperature and of the exposure time. Fig. 4 shows that after long exposure times at 57° the decrease in the number of infective centres both for

phage λ .C on *Escherichia coli* c and for unrestricted phage λ .K (P1) on *E. coli* κ (P1) followed roughly the same kinetics as the decrease in the number of viable bacteria. The capacity of *E. coli* κ (P1) to grow phage λ .C appeared to be rather more heat-sensitive than its capacity to grow phage λ .K (P1). The result could be explained if there were a heat effect on the ability of *E. coli* κ (P1) to modify phage λ .C so that a fraction of the infected κ (P1) bacteria released bursts of phage λ .C which would not plate on the κ (P1) indicator bacteria. However, when infected bacteria taken at this part of the curve were allowed to lyse in liquid media at 37°

Fig. 4. Effect of heat on the viability and the capacity of *Escherichia coli* κ (P1) and *E. coli* c to grow host-modified phage λ . Experimental procedure as in Fig. 1. ■—■ *E. coli* κ (P1) colony count; Δ — Δ infection of *E. coli* c with phage λ .C; ●—● infection of *E. coli* κ (P1) with phage λ .K (P1); ○—○ infection of *E. coli* κ (P1) with phage λ .C.

the bursts consisted entirely of modified λ .K (P1) phage particles. Alterations in the physiological condition of the bacteria before heating, and in the medium in which they were heated, showed that the heat effect was most pronounced with starved stationary-phase bacteria heated in 0.01 M-MgSO₄. Bacteria in other phases of growth heated in tryptone broth responded less well.

The recovery of restriction after heat treatment

Figure 5 shows the recovery of restriction after heat treatment under various conditions. In each experiment cultures of *Escherichia coli* κ (P1) were grown in tryptone broth, harvested and resuspended in phage λ -adsorption buffer and then heated at 50° for 30 min. Recovery of restriction was measured by returning the

bacteria to 37° and aerating them in the recovery medium for 4 hr; at given intervals 1 ml. samples were withdrawn and challenged with phage λ .C. After 15 min. adsorption, the number of infective centres was assayed on *E. coli* κ (P1) indicator bacteria. Curve 1 shows that restriction did not markedly 'recover' when the bacteria were maintained at 37° in phage λ -adsorption buffer after heating. Thus, heat inactivation of the restriction process was not reversed by a simple incubation at

Fig. 5. Kinetics of the recovery of restriction in *Escherichia coli* κ (P1) after heat inactivation. *E. coli* κ (P1) was grown to colony count 5×10^8 /ml. in tryptone broth, harvested and resuspended at colony count 5×10^9 /ml. in phage λ -adsorption buffer. The suspension was heated for 30 min. at 50° by making a 1/5 dilution into pre-heated phage λ -adsorption buffer. After heating the bacteria were returned to 37° and again diluted 1/5 into different recovery media to a final colony count of 2×10^8 /ml. The suspensions were aerated for 4 hr. and at intervals 1 ml. samples were removed and challenged with 1×10^7 phage λ .C particles. After adsorption for 15 min. the number of infective centres was assayed on *E. coli* on κ (P1). Recovery media: ○—○, phage λ -adsorption buffer; ●—●, tryptone broth + 0.01 M-MgSO₄ + thiamine 10 μ g./ml.; ▲—▲, tryptone broth + 0.01 M-MgSO₄ + thiamine 10 μ g./ml. + chloramphenicol 50 μ g./ml.; △—△, phage λ -adsorption buffer + M-NaCl.

37°. When heated bacteria were allowed to recover in fresh growth medium a marked recovery of restriction occurred (Fig. 5, curve 2). Colony counts made on these bacterial suspensions showed that the organisms began to divide actively only after a lag of 90 to 120 min. The initial and major part of the recovery took place

during this lag and was not dependent upon new protein synthesis, because the addition of chloramphenicol 50 $\mu\text{g./ml.}$ did not influence the recovery process during the first 90 min. (Fig. 5, curve 3). Comparison of the latter parts of curves 2 and 3 shows that, when growth was resumed, renewed synthesis of a factor involved in restriction occurred, because this portion of the recovery was inhibited by chloramphenicol.

Figure 5, curve 4 shows that when heated bacteria were allowed to recover in the presence of m-NaCl there was a dramatic and immediate response. Restriction increased immediately by a factor of 10^4 . The main factor in recovery under normal growth conditions (Fig. 5, curve 2) must therefore be the presence of 0.5 % (w/v) NaCl in the growth medium.

Table 1. *The effect of NaCl on the recovery of restriction in Escherichia coli* κ (P1) *after heat treatment*

E. coli κ (P1) was grown to colony count of $5 \times 10^8/\text{ml.}$ in tryptone broth, harvested and starved for 1 hr at 37° in 0.01 m-MgSO_4 at colony count $5 \times 10^8/\text{ml.}$ The suspension was heated for 30 min at 50° by making a 1/5 dilution into the above pre-heated media. Restriction was measured, either directly after heat treatment or after a post-heat treatment as indicated, by challenging with 5.5×10^7 phage λ .C particles and assaying the number of infective centres on *E. coli* κ (P1) indicator bacteria.

Experiment	Media in which the bacteria were heated	Post-heat treatment before challenge with phage λ .C	Number of infective centres on <i>E. coli</i> κ (P1)
1	Distilled water	Addition of 0.01 m-MgSO_4	4.6×10^8
2	λ -adsorption buffer	None	5.5×10^8
3	λ -adsorption buffer + m-NaCl	None	1.5×10^1
4	λ -adsorption buffer + 2 m-glucose	None	3.3×10^3
5	Tryptone broth	Addition of 0.01 m-MgSO_4	1.3×10^8
6	Tryptone broth + 1 m-NaCl	Addition of 0.01 m-MgSO_4	1.3×10^1
7	λ -adsorption buffer	m-NaCl	5.5×10^3
8	No heat treatment	Distilled water for 3 hr	5.5×10^3
9	No heat treatment	None	1.0×10^1

The effect of hypertonic medium on the recovery of restriction after heat treatment was further investigated in a series of experiments summarized in Table 1. Comparison of expt. 2 and 3 shows that when bacteria were heated in the presence of m-NaCl there was almost no heat effect on restriction. That this was due to a protection by the hypertonic medium is shown by expt. 7 in which there was an effect of heat on restriction in spite of the fact that m-NaCl was added immediately after heating and before infection. The effect of NaCl was just as apparent when bacteria were heated in tryptone broth (expt. 5 and 6) as when heat treatment was given in distilled water or buffer (expt. 1 and 2). Experiment 4 shows that 2 m-glucose solutions which produced about the same osmotic pressure at 37° as m-NaCl also led to a rapid recovery of part of the restricting ability of heated *Escherichia coli* κ (P1). Experiment 8 on the other hand shows that when unheated bacteria were suspended in distilled water for 3 hr their ability to restrict phage λ .C was decreased.

These results suggest that the effect of m-NaCl in bringing about the rapid recovery of restriction may be due to an osmotic phenomenon. This was amply illustrated by the fact that m concentrations of the following salts, Na, K, Li, NH_4 , Mg, Mn, Ca as chlorides, sulphates or iodides when added to the adsorption buffer

produced approximately the same increase in restriction, and the addition of 2 M-glycerol to the adsorption buffer gave the same result. Furthermore, the extent to which the restriction of heated bacteria recovered when they were resuspended in hypertonic media was dependent upon the molarity of the added salt. Table 2 illustrates the effect of various concentrations of NaCl on the reappearance of restriction in heated *Escherichia coli* K (P1). It is clear that the extent to which restriction reappeared was proportional to the molarity of the medium in which the heated bacteria were resuspended. This reactivation of restriction in hypertonic media was reversible as might be expected for an osmotic phenomenon. The experiments summarized in Table 3 show that the restricting ability of heated bacteria could be made to fluctuate, depending on the osmotic pressure of the medium in which they were resuspended.

Table 2. *The effect of different concentrations of NaCl on the reappearance of restriction in Escherichia coli K (P1) after heating to 50° for 30 min*

E. coli K (P1) was grown to a colony count of 5×10^8 /ml. in tryptone broth, the bacteria harvested and resuspended in phage λ -adsorption buffer at colony count 5×10^8 /ml. The suspension was heated for 30 min. at 50° by making a 1/5 dilution into pre-heated phage λ -adsorption buffer. After heating, the bacteria were returned to 37° and the concentration of NaCl adjusted as indicated; 5.5×10^7 phage λ .C particles were adsorbed for 15 min. and the number of infective centres assayed on *E. coli* K (P1) indicator bacteria.

Concentration of NaCl (M)	Number of infective centres	Efficiency of plating corrected for adsorption (e.o.p.)
0	4.6×10^6	8.3×10^{-2}
0.05	1.6×10^6	6.3×10^{-3}
0.1	2.8×10^4	5.1×10^{-4}
0.5	2.0×10^3	3.7×10^{-5}
1.0	4.2×10^2	8.3×10^{-6}
Control a*	1.0×10^1	1.85×10^{-7}
Control b	1.0×10^7	1.0
Control c	7.5×10^6	1.0
Control d	8.1×10^6	1.0

* Control a. Unheated *E. coli* K (P1) were suspended in phage λ -adsorption buffer at colony count 1×10^8 /ml. and challenged with 5.5×10^7 phage λ .C particles.

Control b. Heated *E. coli* K (P1) in phage λ -adsorption buffer were challenged with 1.0×10^7 phage λ .K (P1) particles.

Control c. Heated *E. coli* K (P1) in phage λ -adsorption buffer + M-NaCl were challenged with 1.0×10^7 phage λ .K (P1) particles.

Control d. Heated *E. coli* c in phage λ -adsorption buffer + M-NaCl were challenged with 1.0×10^7 phage λ .C particles, and the number of infective centres assayed on *E. coli* c indicator bacteria.

The timing of the restriction process

Escherichia coli K (P1) organisms in which the capacity to restrict the growth of phage λ .C had been impaired by heat treatment instantly recovered most of their restricting ability when they were resuspended after heating in a hypertonic medium. This observation was utilized in an effort to determine as accurately as possible the time after adsorption at which restriction takes place. These experiments are summarized in Table 4. When phage λ .C was adsorbed to heated *E. coli*

Table 3. *Reversibility of the reactivating effect of m-NaCl on the restriction of phage λ .C by heated Escherichia coli κ (P1)*

E. coli κ (P1) was grown to colony count 5×10^8 /ml. in tryptone broth, the organisms harvested and resuspended to colony count 5×10^9 /ml. in phage λ -adsorption buffer. The suspension was heated for 30 min. at 50° by making a 1/5 dilution into pre-heated phage λ -adsorption buffer; after heating the bacteria were returned to 37° .

Experiment 1. Heated bacteria in phage λ -adsorption buffer challenged with 1.5×10^7 phage λ .C particles.

Experiment 2. Heated bacteria in phage λ -adsorption buffer + m-NaCl challenged with 1.5×10^7 phage λ .C particles.

Experiment 3. Heated bacteria in phage λ -adsorption buffer + m-NaCl (from Expt. 2) centrifuged, washed twice in phage λ -adsorption buffer and resuspended in phage λ -adsorption buffer at colony count 1×10^9 /ml. and challenged with 1.5×10^7 phage λ .C particles.

Experiment 4. Heated bacteria from Expt. 3 finally resuspended in phage λ -adsorption buffer + m-NaCl and challenged with 1.5×10^7 phage λ .C particles.

Unheated control. Unheated bacteria in phage λ -adsorption buffer challenged with 1.5×10^7 phage λ .C particles.

In all experiments the amount of free phage after adsorption for 15 min. at 37° was measured and the number of infective centres assayed on *E. coli* κ (P1) indicator bacteria.

Experiment	Number of infective centres on <i>E. coli</i> κ (P1)	Free phage	Efficiency of plating corrected for adsorption (e.o.p.)
1	1.18×10^6	2.0×10^6	9.1×10^{-2}
2	1.17×10^2	4.5×10^6	1.1×10^{-5}
3	4.8×10^5	2.0×10^6	4.8×10^{-2}
4	1.0×10^2	4.9×10^6	1.0×10^{-5}
Unheated control	1.1×10^1	2.01×10^6	8.3×10^{-7}

Table 4. *The timing of host-controlled restriction in Escherichia coli κ (P1)*

E. coli κ (P1) was grown in tryptone broth to colony count 5×10^8 /ml., organisms harvested and resuspended in phage λ -adsorption buffer to colony count 5×10^9 /ml. The suspension was heated for 30 min. at 50° by making a 1/5 dilution into pre-heated phage λ -adsorption buffer. The bacteria were then returned to 37° and 5 m-NaCl added as indicated to a final concentration of m. The bacteria were then challenged with 1.0×10^8 phage λ .C particles.

In Expts. 1, 2 and 3, 15 min. were allowed for phage adsorption and the number of infective centres was assayed on *E. coli* κ (P1) indicator bacteria.

In Expts. 4a, 4b and 4c, phage λ antiserum was added after the adsorption period and 5 min. later the number of infective centres was assayed on *E. coli* κ (P1).

Experiment	Treatment of bacteria before challenge with phage λ .C	Treatment of bacteria after challenge with phage λ .C: NaCl added to m	Efficiency of plating corrected for adsorption (e.o.p.)
1	None	None	9.1×10^{-7}
2	Heated	None	8.3×10^{-2}
3	Heated	At time of challenge	1.3×10^{-5}
4a	Heated	2 min. after challenge	5.3×10^{-3}
4b	Heated	5 min. after challenge	2.1×10^{-2}
4c	Heated	15 min. after challenge	8.3×10^{-2}

κ (P1) at the same time as they were suspended in M-NaCl the degree of restriction was high (expt. 3), indicating that NaCl acted very rapidly. When, on the other hand, phage λ .C was allowed a short time to adsorb and to inject into heated bacteria before restriction was restored by the addition of NaCl, a much larger fraction of the infecting phage particles escaped restriction (expt. 4a, b, c). From these results we conclude that an essential step in restriction takes place very soon after adsorption, perhaps when the phage DNA is injected, and that once the phage DNA has been injected into the bacterial cytoplasm it can no longer be restricted.

Table 5. *The restriction of phage λ .C by Escherichia coli κ (P1) heated after adsorption*

E. coli κ (P1) was grown in tryptone broth to colony count of 5×10^8 /ml., the organisms harvested and resuspended to colony count 5×10^9 /ml. in phage λ -adsorption buffer. The suspension was heated for 1.5 min. at 57° by making a 1/5 dilution into pre-heated phage λ adsorption buffer.

Experiment 1. 5×10^9 bacteria were challenged with 5.0×10^8 phage λ .C particles; after 2 min of adsorption phage λ antiserum was added and the bacteria heated.

Experiment 2. As in Expt. 1 but 15 min. were allowed for adsorption of phage λ . In both experiments the number of infective centres after heating was assayed on *E. coli* κ (P1) indicator bacteria. To decrease error due to phage adsorption to the bacteria after heating the suspension was made to M-NaCl by adding NaCl immediately before assaying for number of infective centres.

Unheated control. 5×10^9 bacteria were challenged with 5.0×10^8 phage λ .C particles and after 2 min. adsorption, phage λ antiserum was added and after 5 min. the number of infective centres assayed on *E. coli* κ (P1) indicator bacteria.

Heated control. 5×10^9 bacteria were heated, returned to 37° and challenged with 1.0×10^8 phage λ .C particles. After 2 min. adsorption phage λ antiserum was added and after 5 min. the number of infective centres was assayed on *E. coli* κ (P1) indicator bacteria.

Expt.	Time of adsorption before heating to 57° (min.)	Number of infective centres on <i>E. coli</i> κ (P1)	Efficiency of plating corrected for adsorption (e.o.p.)
1	2	2.1×10^3	8.3×10^{-6}
2	15	2.6×10^2	1.0×10^{-6}
Unheated control	—	2.3×10^2	9.1×10^{-7}
Heated control	—	2.5×10^7	1.0×10^{-1}

If this conclusion is correct it should be impossible to 'rescue' a restricted phage by inactivating the restriction process in the host bacterium by a brief exposure to high temperature shortly after phage adsorption and injection. The following experiment was made to test this prediction. *Escherichia coli* κ (P1) organisms were grown in tryptone broth to a colony count of 5×10^8 /ml., and harvested and resuspended to 5×10^9 bacteria/ml. in phage λ -adsorption buffer. The bacteria were then infected at 37° with phage λ .C at a multiplicity of 0.1. After, respectively, 2 and 20 min. adsorption, the amount of free phage was assayed and the infected cultures heated at 57° for 1.5 min. The final number of infective centres produced by the heated bacteria was assayed on *E. coli* κ (P1) indicator bacteria. The results of this experiment are summarized in Table 5. Phage λ .C adsorbed to restricting *E. coli* κ (P1) organisms for 2 min. before heat treatment yielded 10^4 times fewer infective centres than phage λ .C adsorbed for the same time to heated bacteria. The fact that

they yielded 10 times more infective centres than phage λ .C adsorbed for the same time to unheated bacteria was probably due to phage particles which adsorbed and injected after the heat treatment had begun.

DISCUSSION

The capacity of *Escherichia coli* κ (P1) bacteria to restrict phage λ .C is heat-sensitive. The e.o.p. of phage λ .C on *E. coli* κ (P1) increased as much as a million-fold when the bacteria were heated before infection. The time of exposure to elevated temperature required to achieve the maximum increase in the e.o.p. of phage λ .C decreased with increasing temperature up to temperatures which inhibited the capacity of the bacteria to grow phage. Uetake *et al.* (1964) concluded that there was a thermolabile factor responsible for the restriction of host-modified ϵ^{15} phage in *Salmonella butantan*. However, it appears that the effect of heat on *E. coli* κ (P1) cannot simply be ascribed to the innate thermolability of the host-controlled restriction process, for two reasons. Firstly, when the bacteria are heated in a hypertonic medium, there is very little effect on restriction; secondly, when the heated bacteria are resuspended in a hypertonic medium, there is an immediate restoration of a part of their capacity to restrict. This immediate recovery of restriction is not simply due to a 're-naturation' process at 37°, because the restrictive ability of bacteria kept in phage λ -adsorption buffer after heating did not markedly increase.

However, at least part of the restrictive factor is permanently lost by heated bacteria since they only recovered their full capacity to restrict after growth. This part of the recovery process can be inhibited by chloramphenicol which indicates that protein synthesis is required, presumably the synthesis of an enzyme responsible for restriction. The opposing effects of heat treatment and hypertonic media indicate that the heat effect is in part due to an osmotic phenomenon which results in the partial release of some of the restrictive factor and to the inactivation of the remainder. A hypertonic medium provides protection against both these effects.

That restriction of phage λ .C takes place very quickly after adsorption has been demonstrated by two different kinds of experiment. In the first, phage is adsorbed to bacteria in which restriction has been decreased by heat treatment and then restored by the addition of NaCl very shortly after adsorption. Under these conditions a significant fraction of the phage escaped restriction, indicating that once the phage DNA had been injected into the bacterium it was no longer susceptible to the restriction process. In the second, phage was adsorbed to normal restricting bacteria and then very shortly after adsorption the restriction was decreased by heat treatment. Under these conditions no significant rescue of the phage was observed.

These results can be explained by the following working hypothesis. An essential part of the host-controlled restriction process involves a nuclease located on the surface of the host bacterium, perhaps in the periplasm between cell wall and cytoplasmic membrane. This nuclease can act on DNA which lacks a compatible modification pattern when it passes through the periplasm. Support for this hypothesis has recently been obtained from two sets of experiments. Stationary phase bacteria of *Escherichia coli* strains B, κ and c grown to completion in certain media

lose most of their endonuclease I (Shortman & Lehman, 1964). Under the same cultural conditions *E. coli* κ (P1) organisms lost a large part of their capacity to restrict phage λ .C (Schell & Glover, 1966*a*). Furthermore, this nuclease has been shown to have a periplasmic location (Hilmoe, quoted by Neu & Heppel, 1964; Cordonnier & Bernardi (1965)). Conditions which result in the release of a number of surface-localized enzymes (Neu & Heppel, 1965) also lead to a decrease in the ability of *E. coli* κ (P1) to restrict phage λ .C (Schell & Glover, 1966*b*).

The opposing effects of heat and hypertonic media on the restriction of phage λ .C by *Escherichia coli* κ (P1) can be explained in the following way. Heating leads to the partial release of some of the restrictive factor and to the inactivation of the rest. When these heated bacteria are resuspended in hypertonic media plasmolysis occurs and the cytoplasmic membrane separates from the cell wall (see Mitchell, 1961). This separation of cell wall from cytoplasmic membrane increases the volume of the periplasm and could result in an extension of the period during which the phage DNA is accessible to the restricting enzyme and thus leads to a higher degree of restriction. This explanation is supported by the fact that the activation of restriction in hypertonic media is a reversible phenomenon. Phage which infects heated bacteria in a hypertonic medium may be restricted, while phage which infects heated bacteria in normal media is not restricted. The effect is specific for phages normally susceptible to host-controlled restriction and there is no comparable effect of heat or hypertonic media on the ability of phage λ .K (P1) to grow in *E. coli* κ (P1).

The protection obtained in hypertonic media against the effects of heat may also be due to plasmolysis of the cells which prevents the release and inactivation of the restricting enzyme. The host-controlled restriction process in *Escherichia coli* κ (P1) has two components, one under the control of the bacterial genome and one under the control of the prophage P1. Both these restrictions are sensitive to heat and both can be partially restored in hypertonic media (Schell & Glover, unpublished results). Therefore, these two restriction processes, though different in specificity, behave similarly to heat treatment and may have a common component.

Fukasawa (1964) suggested that a surface localized DNase is responsible for the restriction of non-glucosylated coliphage T4 DNA by *Escherichia coli* B. This suggestion is supported by the recent results of Molholt & Fraser (1965), who showed that non-glucosylated T-even coliphages were no longer restricted by spheroplasts made from normally restrictive hosts.

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