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The effect of various physiological conditions on host-controlled restriction in *Escherichia coli* K(P1)

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INTRODUCTION

Arber, Hattman & Dussoix (1963) have shown that the DNA of phage λ.C grown in *Escherichia coli* strain C is restricted in *E. coli* strains K and K (P1) and its DNA is broken down after injection into these strains. Recent experiments indicate that an essential factor in the restriction of non-glucosylated T4 phage DNA is a surface-located nuclease (Fukasawa, 1964; Molholt & Fraser, 1965) and there is evidence that the restriction of λ.C DNA by K(P1) may involve a surface located enzyme (Schell & Glover, 1966*a*, *b*). Shortman & Lehman (1964) have described conditions under which the levels of several nucleases are greatly reduced in *E. coli* B, K and C and the results reported in this paper indicate that the same conditions that lead to a reduction in the level of nucleases also lead to a marked reduction of the ability of K(P1) to restrict the growth of λ.C.

MATERIALS

Bacterial strains and bacteriophages: (see Schell & Glover, 1966*a*).

Media: The media used to grow K(P1) were tryptone broth (TB) (Arber & Dussoix, 1962); Yeast-extract–phosphate–glucose medium (YE); glucose-salts medium. (M9 supplemented with threonine, leucine and vitamin B1); and glucose-salts medium in which the NH_4Cl was replaced by 0·8% yeast extract (M9 + YE); (Shortman & Lehman, 1964).

RESULTS AND DISCUSSION

K(P1) cells were grown in tryptone broth, supplemented M9, M9 plus YE and YE media as standing cultures at 37°. At 4, 12 and 36 hours, samples were withdrawn and the restriction of λ.C was measured by adsorbing the phage to the cells, resuspended in λ adsorption buffer, at a multiplicity of about 0·1 and assaying the efficiency of plating (e.o.p.) of the infective centres on K(P1) indicator bacteria. It is clear from the results in Table 1 that when cells are grown to stationary phase for 36 hours in YE medium, designated 'K(P1)YE', the fraction that will permit the growth of λ.C is very much greater than for early log-phase cells sampled at 4 hours or late log phase cells at 12 hours. This effect does not occur to anything like the same extent in cells at any phase of growth in tryptone broth or M9 media. The host-controlled restriction of λ.K and λ.B by K(P1) and the restriction of λ.C and λ.B by strain K are also reduced under these conditions. It appears that some essential component of the host-controlled restriction process disappears from the cells under these conditions. This essential component may be a nuclease

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Table 1. *The restriction of λ.C by K(P1) cells in different media*

Age of culture (hours)	Growth medium			
	YE	TB	M9	M9+YE
4	10^{-7}	10^{-7}	10^{-6}	10^{-7}
12	10^{-7}	10^{-7}	10^{-6}	10^{-6}
36	10^{-1}	10^{-7}	10^{-5}	10^{-5}

K(P1) bacteria grown in the four media were harvested at 4, 12 and 36 hours, resuspended in λ adsorption buffer and challenged with λ.C at a multiplicity of 0·1. The e.o.p. of the infective centres was assayed on K(P1) indicator bacteria.

which Shortman & Lehman (1964) have shown largely disappears from *E. coli* B grown in the same manner.

When K(P1)YE cells are resuspended in fresh YE medium, growth is quickly resumed and they regain their ability to restrict λ.C after about 90 min. (Fig. 1, curve 1). This recovery of restriction can be inhibited by 50 μg. per ml. of chloramphenicol (Fig. 1, curve 2).

However, the ability of these K(P1)YE bacteria to restrict λ.C is very rapidly restored when the cells are resuspended in hypertonic media (Fig. 1, curve 3). The e.o.p. of λ.C on K(P1)YE in λ adsorption buffer is about 10^{-1} but when NaCl or any of the following salts Na, K, Li, NH_4, Mg, Mn, Ca, as chlorides, sulphates or iodides are added to the adsorption buffer the restriction immediately reappears to an extent dependent upon the concentration of the added salt. Table 2 illustrates the effect of various concentrations of NaCl on the reappearance of restriction in K(P1)YE cells. This influence on restriction

Table 2. *The effect of NaCl on the reappearance of restriction in stationary phase* K(*P*1) *bacteria grown for* 36 *hours in yeast-extract–phosphate–glucose medium*

Molarity of NaCl	Efficiency of plating of infective centres on K(P1)
0	$1{\cdot}1 \times 10^{-1}$
0·05	$4{\cdot}0 \times 10^{-2}$
0·1	$7{\cdot}1 \times 10^{-3}$
0·5	$5{\cdot}0 \times 10^{-4}$
1·0	$9{\cdot}7 \times 10^{-5}$
Control (*a*)	$2{\cdot}0 \times 10^{-7}$
Control (*b*)	1·0
Control (*c*)	1·0

K(P1) bacteria grown for 36 hours in yeast-extract–phosphate–glucose medium were resuspended in λ adsorption buffer containing various concentrations of NaCl. $1{\cdot}0 \times 10^8$ λ.C were adsorbed for 15 min. at 37° and the number of infective centres assayed on K(P1) indicator bacteria.

Control (*a*): λ.C adsorbed to tryptone broth grown K(P1) bacteria, resuspended in λ adsorption buffer.

Control (*b*): λ.K(P1) adsorbed to yeast-extract–phosphate–glucose grown K(P1) resuspended in λ adsorption buffer in the absence of NaCl.

Control (*c*): λ.K(P1) adsorbed to yeast-extract–phosphate–glucose K(P1) resuspended in λ adsorption buffer in the presence of 1 M NaCl.

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of the various salts tested in not a general salt effect since 2 M glucose and 2M glycerol have about the same effect as 1 M NaCl. It thus appears more likely to be due to an osmotic phenomenon. This effect of hypertonic media on the reappearance of restriction is not an artefact of the technique since hypertonic media do not influence the e.o.p. of λ.K (P1) on K(P1)YE nor of λ.C or C nor of λ.K on both K and C.

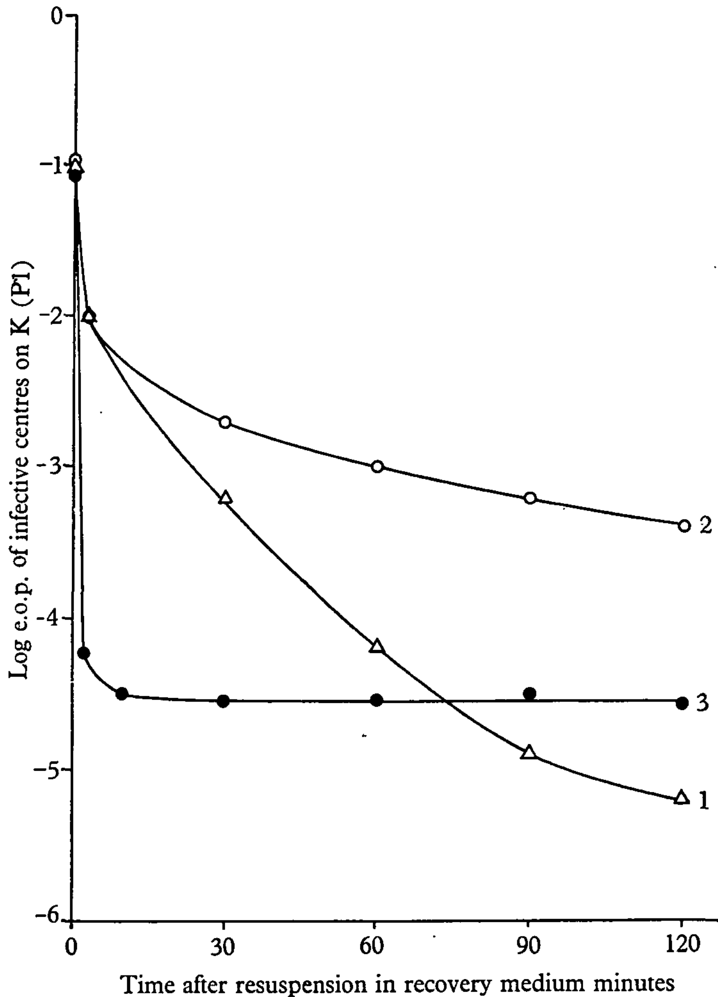

Fig. 1. Kinetics of the recovery of λ.C restriction in K(P1)YE cells. K(P1) bacteria were grown in yeast-extract–glucose–phosphate medium for 36 hours, harvested and resuspended in one-tenth of their original volume of the recovery medium indicated and then incubated at 37° with aeration. At given intervals 10-ml. samples were removed, chilled and washed with cold λ adsorption buffer. The washed cells were then resuspended at 1×10^9 cells per ml. in λ adsorption buffer and challenged with $1{\cdot}0 \times 10^8$ λ.C particles. After 15 min. adsorption at 37° the number of infective centres was assayed on K(P1) indicator bacteria.

Curve 1. △——△: K(P1)YE cells in yeast-extract–glucose–phosphate medium.

Curve 2. ○——○: K(P1)YE cells in yeast-extract–glucose–phosphate medium + 50 μg. per ml. of chloramphenicol.

Curve 3. ●——●: K(P1)YE cells in λ adsorption buffer + 1 M NaCl.

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The rapid reappearance of restriction in K(P1)YE cells when they are resuspended in 1 M NaCl was used to obtain an estimate of the time after adsorption at which restriction occurs. If restriction takes place late in the growth cycle of the phage then infected K(P1)YE cells placed in 1 M NaCl shortly after adsorption of the phage will be able to restrict phage growth. If, on the other hand, restriction can only take place at an early stage then phage which penetrates a cell while it is still phenotypically non-restricting will grow even when restriction is restored in 1 M NaCl. When 2 min. are allowed for phage adsorption to K(P1)YE cells before the addition of 1 M NaCl 10^4 times more phage particles are able to grow than when NaCl is added before phage adsorption. When phage and NaCl are added simultaneously phage growth is as severely restricted as when NaCl is added prior to phage infection. Thus the action of NaCl is very rapid and only phage which penetrates the cell before NaCl is added can escape restriction.

Recent experiments have also shown that hypertonic medium immediately restores restriction to cells in which it has been severely impaired by heat treatment and also that phage which adsorbs to restricting hosts cannot be rescued from the restriction process even when this is lowered by heat treatment very shortly after the infection (Schell & Glover, 1966*a*). We conclude that restriction takes place very shortly after adsorption and that once phage DNA has been successfully injected into the cytoplasm of phenotypically non-restricting cells it is no longer susceptible to restriction. It seems likely that the phage DNA is restricted while passing through the periplasm. This explanation finds support from experiments which have shown that a factor essential for restriction is located in the cell-surface (Schell & Glover, 1966*b*).

SUMMARY

Growth of K(P1) bacteria under conditions which lead to a reduction in the level of nucleases also leads to a reduction of their ability to restrict the growth of λ.C. Experiments designed to estimate the time after adsorption at which restriction takes place indicate that phage DNA is probably restricted by a nuclease while passing through the periplasm.

REFERENCES

Arber, W. & Dussoix, D. (1962). Host specificity of DNA produced by *Escherichia coli*. I. Host-controlled modification of bacteriophage. *J. molec. Biol.* **5** 18–36.

Arber, W., Hattman, S. & Dussoix, D. (1963). On the host-controlled modification of bacteriophage λ. *Virology*, **21**, 30–35.

Fukasawa, T. (1964). The course of infection with abnormal bacteriophage T4 containing non-glucosylated DNA on *Escherichia coli* strains. *J. molec. Biol.* **9**, 525–536.

Molholt, B. & Fraser, D. (1965). Reversal of the restriction for host-modified T2 and T4 DNA upon conversion of non-permissive *Escherichia coli* to spheroplasts. *Biochem. biophys. Res. Commun.* **19**, 571–575.

Schell, J. & Glover, S. W. (1966*a*). The effect of heat on host-controlled restriction in *Escherichia coli* K (P1). *J. gen. Microbiol.* (in press).

Schell, J. & Glover, S. W. (1966*b*). On the localisation of a factor responsible for host-controlled restriction in *Escherichia coli* K(P1). *Genet. Res.* **7**, 277–279.

Shortman, K. & Lehman, I. R. (1964). The deoxyribonucleases of *Escherichia coli* VI. Changes in enzyme levels in response to alterations in physiological state. *J. biol. Chem.* **239**, 2964–2974.

https://doi.org/10.1017/S001667230000968X Published online by Cambridge University Press