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The breakdown of the restriction mechanism in zygotes of *Escherichia coli*

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A few cells in a culture of *E. coli* K or *E. coli* B, usually about 1 in 10^4, permit the growth of the restricted phage λ.C (Arber & Dussoix, 1962). Such cells may be genetically different from the majority of the cells in the culture or they may be temporarily in such a physiologica condition that the restriction mechanism breaks down or cannot be expressed. Mutants which are genetically unable to restrict the growth of phage normally restricted by wild-type cells have been isolated (Glover *et al.*, 1963; Colson *et al.*, 1964; Wood, 1964). But several environmental factors are known which decrease the capacity of wild-type bacteria to restrict the growth of unmodified phage particles (Luria, 1953; Lederberg, 1957; Uetake *et al.*, 1964). We shall report here observations which suggest that *E. coli* zygotes are in a special physiological condition such that their ability to restrict phage is reduced by more than a factor of 100.

These observations were made in the course of experiments in which Hfr strains of *E. coli* K were mated with *E. coli* B F^- recipients, to establish the region of the K chromosome which carries the loci controlling host-induced modification. Having found that these loci lie close to the *thr-leu* region, it was anticipated that zygotes which had received this segment of the chromosome would plate λ.K with a greatly improved efficiency. Accordingly the zygotes, blended at various times from a cross between Hfr H *thi⁻str-r* and B F^- *leu⁻str-s*, were used as plating bacteria on Difco agar containing streptomycin and challenged with λ.K. At zero time the efficiency of plating (e.o.p.) was that expected for a B strain (10^{-4}) but after 30 min. mating this had risen to 10^{-2}, at which level it remained constant for the duration of the cross (120 min.). But the same effect was observed when different Hfr strains were used as donors, e.g. Hfr Reeves-1, Hfr B9, Hfr B1, Hfr B11 and Hfr B12. These Hfr strains either do not transfer the *thr-leu* region at high frequency or transfer it at quite different times. This effect was therefore not due to the inheritance of chromosomal genes linked to *thr-leu*. This conclusion has been confirmed by the demonstration that λ phage grown on the zygotes carried the host-specificity of B in contrast to phage λ grown on recombinants known to have inherited the restriction gene from K, which invariably carried the host specificity of K (Colson *et al.*, 1964). The same effect was also observed when the zygotes from a K × B cross were challenged with λ.C. Phage λ.C is normally restricted by both the donor K and the recipient B about 10^4 fold, but is restricted only 100-fold by the zygote population. Again, phage grown in the zygotes has been shown to carry the normal host-specificity of B.

These observations suggested that there was a transient breakdown of the restriction mechanism in *E. coli* B zygotes as a result of the mating process itself, which might not be related to chromosome transfer. This prompted experiments with F′ and F^+ donors

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which transfer chromosomal genes less frequently. There is, in fact, an increase in the e.o.p. of λ.C on the zygotes from such matings although it is less dramatic and slower to appear.

In all these experiments a sample of the zygote population was used as plating bacteria. A clearer demonstration of the effect can be achieved by adsorbing phage λ to pre-formed zygotes and plating the infected bacteria on a suitable indicator for infective centres. In this experiment the zygotes were made by mating 10^9 K *Flac*$^+$ *str-s* λ-*r* donors with 10^8 B *lac*$^-$*leu*$^-$ *str-r* *F*$^-$ recipients at 37° in broth containing ·02 *M* $MgSO_4$. Samples were taken at chosen times, streptomycin was added to kill the donor and the mixture blended for 30 sec. Phage λ.K was adsorbed for 10 min. and unadsorbed phage was destroyed by antiserum. Infective centres were assayed by plating suitable dilutions on B and C indicator bacteria. The results of this experiment in Table 1 show that, 40 min. after

Table 1. *The plating of λ.K infected zygotes from a cross between* E. coli *K* Flac$^+$ str-s λ-r *and* E. coli *B* lac$^-$leu$^-$ str-r *F*$^-$ *on* E. coli *K and* E. coli *B indicator bacteria*

Minutes after mating at which zygotes were blended and challenged with λ.K	Number of infective centres per 10^4 zygotes plated	
	E. coli B	*E. coli* C
0	0	0
20	3	3
40	164	159
60	257	213
80	404	334
100	518	500
120	372	351
80*	51	56

* Sample blended at 80 min. then incubated for a further 120 min. at 37° before challenging with λ.K.

mating, restriction in the zygote population has fallen 100-fold, and since the plaque counts on B and C are similar the phage produced by the infected zygotes carries the host specificity of B.

The increase in the number of infective centres from 40 to 100 min. probably represents an increase in the number of zygotes resulting from the continuous mating of the growing populations of male and female bacteria in the mixture. By 120 min. the number of infective centres appears to decrease, and in a sample taken at 80 min. and incubated for an additional 120 min. after blending before challenging with λ.K this decrease is about 10-fold. This means that restriction has begun to recover. We have not followed this recovery further but we do know that the majority of ex-conjugants from such a mating selected after 24 hours incubation show the normal restriction properties of B.

It is possible that this decrease in restriction reflects the saturation of the restricting enzyme by the incoming unmodified DNA of the donor chromosome and this increases the probability that infecting λDNA will escape restriction. Alternatively, the mating process itself may cause a physiological change in the recipient which results in a transient break-down of the restriction mechanism.

SUMMARY

In zygotes from matings between Hfr, F′ or F^+ donors of *E. coli K* and F^- recipients of *E. coli* B the restriction of λ.K and λ.C is 100 times less than in unmated B cells. This decrease in the capacity of zygotes to restrict phage λ is transient and is not related to the transfer of genes controlling host-induced modification during the cross.

REFERENCES

ARBER, W. & DUSSOIX, D. (1962). Host specificity of DNA produced by *Escherichia coli* I. Host controlled modification of bacteriophage. *J. mol. Biol.* **5**, 18–36.

COLSON, C., GLOVER, S. W., SYMONDS, N. & STACEY, K. A. (1964). The genetic control of host-induced modification in *Escherichia coli* B, K and C 1. The genetic location of the control of host-induced modification in *Escherichia coli* B, K and C (in press).

GLOVER, S. W., SCHELL, J., SYMONDS, N. & STACEY, K. A. (1963). The control of host-induced modification by phage P1. *Genet. Res.* **4**, 138–140.

LEDERBERG, S. (1957). Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria. *Virology*, **3**, 496–513.

LURIA, S. E. (1953). Host-induced modification of viruses. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 237–244.

UETAKE, H., TOYAMA, S. & HAGIWARA, S. (1964). On the mechanism of host-induced modification. Multiplicity activation and thermolabile factor responsible for phage growth restriction. *Virology*, **22**, 203–213.

WOOD, W. (1964). *Path. Microbiol.* Lausanne (in press).