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THE LOCATION OF THE RESTRICTION LOCUS FOR λ·K IN *ESCHERICHIA COLI B*

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SUMMARY

Analysis of recombinants from *E. coli* K 12 Hfr × *E. coli* B F crosses showed that one locus on the chromosome of *Escherichia coli*, controlling restriction and probably also the modification of phage λ, is located between the leading point of the Hfr H chromosome and the locus for threonine synthesis.

The restriction locus also controls the restriction of the fertility factor of K 12 and the restriction of chromosomal DNA of K 12 in *E. coli* B.

The presence of the defective prophaga X in *E. coli* B causes an additional restriction for phage λ and for the fertility factor.

Abbreviation: e.o.p., efficiency of plating.

INTRODUCTION

Escherichia coli B infected with phage λ propagated on *E. coli* K 12 shows the well-known restriction behaviour described by ARBER AND DUSSOIX[3].

Following the ideas and conventions of these authors, the DNA of $\lambda \cdot$ K (λ propagated on *E. coli* K 12) carries a host specificity improper to the new host *E. coli* B. This implies a destruction of $\lambda \cdot$ K DNA in most of the infected B cells. In only a minor fraction of the cells ($3 \cdot 10^{-4}$) is the destroying action of the host overcome and plaque formation found to occur. The progeny phages produced in these rarely yielding B cells are host modified: the parental K 12 specificity is not transferred and they acquire a host specificity for the host *E. coli* B.

One might imagine a restriction locus on the chromosome of *E. coli* causing in one way or another the destruction of DNA having improper host specificity. Supposing that restriction and modification are independently controlled, one might infer, in the same way, the presence of a modification locus determining whether or not the phage will receive a host-induced specificity.

Independent genetic control of restriction and modification in host-induced modification controlled by phage P 1 has been described by GLOVER *et al.*[9]. Recently HOLLOWAY AND ROLFE[15] described independent control in the host-induced modification of *Pseudomonas aeruginosa* phages, and they located a phage restriction locus in *P. aeruginosa*.

In this paper the location of a restriction locus in *E. coli* will be described. This locus is probably also responsible for the modification; otherwise the loci for restriction and modification are closely linked. The influence of this restriction locus on other known restriction phenomena in *E. coli*: low frequency of transfer of F′ from *E. coli* K 12 to *E. coli* B (refs. 10, 11) and poor genetic linkage in *E. coli* K 12 × *E. coli* B crosses[5], will be described and discussed.

MATERIALS AND METHODS

Bacterial strains

The *E. coli* K 12 strains employed were: C 600 *str*^{r}, indicator strain for $\lambda \cdot$ K (ref. 2); Hfr H λ^- *str*^{s} (ref. 12); Hfr R_4 *met*$^-$ *str*^{s} (ref. 21) and its derivative *ade*^-_k *arg-ura*$^-$ (ref. 24); 5832 *met*$^-$ *str*^{s} (F-*gal*$^+$) (strain F 8 of HIROTA AND SNEATH[13]); P 678 *thr*$^-$ *leu*$^-$ *thi*$^-$ *gal*$^-$ *lac*$^-$ *str*^{r} (ref. 16).

Most of the *E. coli* B strains were derivatives of the CB 80 *arg*$^-$ *leu*$^-$ *gal*$^-$ *mal*$^-$ *str*^{r} strain (ref. 22). The λ^s derivative of CB 80 is the strain CB 80–3 *arg*$^+$ *leu*$^-$ *gal*$^-$ *mal*$^+$ *str*^{r}, an *arg*$^+$ *mal*$^+$ *str*^{r} recombinant isolated from a Hfr $R_4 \times$ CB 80 cross. The efficiency of plating (e.o.p.) of $\lambda \cdot$ K on this strain is 3.10^{-4}.

The following mutants were derived from CB 80–3: *thr*$^-$ *leu*$^-$ *pro*$^-$ *ilva*$^-$ and *pro*$^-$ T_1r.

The *E. coli* B strains not derived from strain CB 80 are strain Bc 258* (ref. 4) and its *thr*$^-$ derivative.

All B strains used were F$^-$.

The symbols for markers are those as proposed by DEMEREC[8]. The restriction

* Strain Bc 258 was obtained through the courtesy of Dr. W. ARBER.

locus is abbreviated as *res*; *res K* indicates the wild-type K 12 allele, *res B* the wild-type B allele. The hypothetical modification locus is abbreviated as *mod* and, in the same way as for the restriction locus, indicated as *mod K* or *mod B*.

Induction of auxotrophic mutations and selection of mutants

The auxotrophic mutations were induced either with nitrite by the modified Kaudewitz method[11] or by means of the mutagenic agent *N*-methyl-*N*-nitroso-*N'*-nitroguanidine as follows: 1 ml of an overnight culture was inoculated into 9 ml of pre-warmed broth and incubated for 4 h at 37° on an inclined turntable. The culture was then centrifuged and the sediment washed once with 0.1 M acetate buffer (pH 5.0). The sediment of 10 ml culture was finally suspended in 0.5 ml of acetate buffer and 0.1 ml of a *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (4 mg/ml) solution was added. After 2 h incubation at 37°, 8 ml nutrient broth was added, the suspension was centrifuged and the sediment resuspended in 10 ml nutrient broth. Thereafter the culture was incubated overnight. The survival of the *E. coli* B cells after treatment with the mutagenic agent was 20–50%.

Auxotrophic mutants were enriched with penicillin by the modified Gorini and Kaufman method[11] and selected by replica plating.

Methods of crossing

Matings between *E. coli* K 12 (Hfr or F') and *E. coli* B F^- were performed on millipore filters[19,14]. In Hfr × F^- crosses $2 \cdot 10^7$ Hfr cells and 10^9 F^- cells were brought on the filter. In the F' × F^- crosses the filter was inoculated with 10^9 F' cells and 10^9 F^- cells. The filters were incubated on pre-warmed nutrient agar. Chromosome transfer was stopped after 80 min by resuspending the cells in saline and shaking the suspension vigorously for 30 sec. In the same way episome transfer was stopped after 45 min.

Recombinants were selected in minimal medium by omitting one of the growth factors for the acceptor strain. Sexduced *gal*+ cells were selected on minimal medium with galactose as sole carbon and energy source. In both cases the donor cells were killed by the addition of streptomycin.

Mating type of sexduced cells was tested with the spot test technique as described by STOUTHAMER, DE HAAN AND BULTEN[23].

Phage techniques

Phage techniques used were in general as described in ref. 1. The e.o.p. determinations were performed using techniques and media as described by ARBER AND DUSSOIX[3]. The e.o.p. determinations were carried out in a constant-temperature room, maintained at 37°. The phage λ used was the virulent mutant, λ_{vir}. Phage variants are designated by the bacteria on which they are propagated; $\lambda \cdot$ B *res K* f.i. means that the phage is propagated on a strain of *E. coli* B into which the restriction marker of K 12 was introduced.

Transduction

Transduction experiments with bacteriophage P 1 were performed using techniques and media as described by LENNOX[18]. The transducing phage was propagated on Hfr H λ^- and concentrated by means of high speed centrifugation (4 h at 30000 × *g*).

RESULTS

Location of the restriction locus

Strain *E. coli* B is *mal*⁻ and resistant against phage λ. The loci for maltose fermentation and resistance for λ are closely linked[17]. A λ^s hybrid was isolated from a Hfr $R_4 \times$ CB 80 cross in which *mal*⁺ *str*^{r} recombinants were selected. The recombinants from this cross were tested with high titer λ suspensions for plaque formation. About 50% of the *mal*⁺ recombinants were found to be λ^s. Most of the λ^s recombinants showed the normal restriction of *E. coli* B strains, but a few recombinants showed a high efficiency of plating towards $\lambda \cdot$ K.

Analysis of the unselected markers of these recombinants showed some linkage between the introduced high efficiency of plating and the *leu*⁺ locus of Hfr R_4, suggesting that the restriction is controlled by one locus which is linked to the *leu* marker. A *leu*⁻ *arg*⁺ *gal*⁻ *str*^{r} recombinant, strain CB 80–3, with low plating efficiency towards $\lambda \cdot$ K was selected for further study.

A *pro*⁻ T_1^r *leu*⁻ derivative was isolated from CB 80–3 and crossed with Hfr H (Fig. 1). *pro*⁺ recombinants were selected and scored for transfer of the unselected markers T_1 and *leu* (cross a, Table I). The e.o.p. of these recombinants was determined with low titer $\lambda \cdot$ K suspensions. About 22% of the *pro*⁺ recombinants gave plaques,

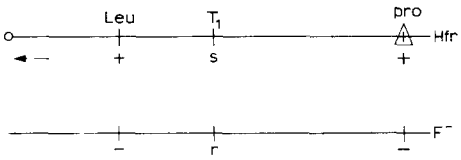

Fig. 1. Relative order of markers involved in a Hfr H $\times$ CB 80–3 *leu*⁻ T_1^r *pro*⁻ cross.
○ = origin of Hfr chromosome. The direction of transfer is indicated by →; △, indicates the selected marker.

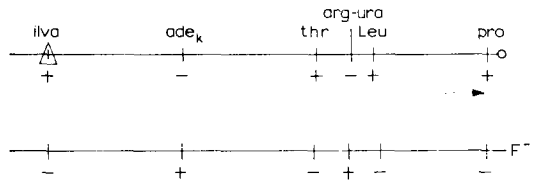

Fig. 2. Relative order of markers involved in a Hfr R_4 *ade*$_k$⁻ *arg-ura*⁻ $\times$ CB 80–3 *ilva*⁻ *thr*⁻ *leu*⁻ cross.
○ = origin of Hfr chromosome. The direction of transfer is indicated by →; △, indicates the selected marker.

indicating that they had obtained the Hfr restriction locus (*res K*). The e.o.p. of these *res K* recombinants was 0.3–0.5. The relative order of the loci is origin-*res-leu-*T_1*-pro*.

The position of the restriction locus was further analysed by crossing Hfr R_4 *arg-ura*⁻ *ade*$_k^-$ with an *ilva*⁻ *thr*⁻ *leu*⁻ derivative of CB 80–3 (Fig. 2). *Ilva*⁺ recombinants were selected and scored for transfer of the unselected markers *ade*$_k$, *thr*, *leu* and *res* (cross b, Table I). Again the e.o.p. of the *res K* recombinants was 0.3–0.5. The relative order of the loci is origin-*leu-thr-res-ade*$_k$*-ilva*. The data suggest that *thr* is nearer to

TABLE I

LOCATION OF THE λ RESTRICTION LOCUS IN *E. coli* B

Crosses were performed as described in MATERIALS AND METHODS. The order of the markers is given in Figs. 1 and 2. Recombinants prototrophic for *pro* or *ilva* were selected; 100 pro^+ recombinants and 1023 respectively 1736 $ilva^+$ recombinants were tested for the frequency of transfer of the unselected markers.

Cross	*Selected marker*	*Unselected marker*	*Frequency of transfer* (%)
a: Hfr H × CB 80-3 leu^- T_1^r pro^-	pro^+	T_1^s	68
		leu^+	35
		res K	22
b: Hfr R_4 ade_K^- *arg-ura* × CB 80-3 $ilva^-$ *thr* *leu*	$ilva^+$	ade^-_K	10.7
		res K	6.1
		thr^+	4.4
		leu^+	5.0
c: same Hfr and F⁻ as in cross b	$ilva^+$	ade^-_K	5.5
		thr^+	1.7
		leu^+	1.5

the origin than *leu*; the order origin-*leu-thr* was verified in a separate cross with the same Hfr and acceptor strain (cross c, Table I). Combining the results of cross a and b, the *res* locus is located between the origin of the Hfr H chromosome and the locus for threonine synthesis.

The *res* and *thr* markers are closely linked; about 3% of the thr^+ transductants, obtained with a P 1 suspension propagated on *E. coli* K 12 thr^+, showed a high e.o.p. towards $\lambda \cdot$ K. The co-transduction is somewhat higher than 3% as P 1 lysogenic transductants show the well-known P 1 controlled restriction towards $\lambda \cdot$ K (ref. 3). As about 70% of the transductants were lysogenic for P 1, the co-transduction of the two loci thus amounts to about 10%.

Plating efficiency

The plating efficiency of three phage variants $\lambda \cdot$ K, $\lambda \cdot$ B *res B* and $\lambda \cdot$ B *res K* on the host strains K 12, B *res B* and B *res K* was determined. Strain CB 80-3 was used as the B *res B* host; a recombinant with a high e.o.p. towards $\lambda \cdot$ K was used as B *res K* host and strain C 600 as K 12 host. The result is given in Table II. Phage $\lambda \cdot$ K plates with the usual low efficiency on strain CB 80-3 ($3 \cdot 10^{-4}$) and with an

TABLE II

PLATING EFFICIENCY OF PHAGE λ VARIANTS ON DIFFERENT HOST STRAINS

CB 80-3 was used as a B *res B* strain; the B *res K* strain is a representative K 12 × B recombinant with high e.o.p. towards $\lambda \cdot$ K.

Phage	*e.o.p. on host strain*		
	C 600	B *res B*	B *res K*
$\lambda \cdot$ K	1	$3 \cdot 10^{-4}$	$4 \cdot 10^{-1}$
$\lambda \cdot$ B *res B*	$4 \cdot 10^{-3}$	1	$5 \cdot 10^{-3}$
$\lambda \cdot$ B *res K*	1	$7 \cdot 10^{-1}$	$4 \cdot 10^{-1}$

efficiency of 0.4 on the B *res K* host. Some restriction of $\lambda \cdot$ K in the B *res K* strain is therefore still observed. Phage $\lambda \cdot$ B *res B* plates with low efficiency on the host strains C 600 and B *res K* indicating that the progeny phage is modified by the B *res B* host. Phage λ propagated on B *res K* plates with high efficiency on the strains C 600 and B *res K*. The low efficiency of plating on strain B *res B* indicates that the B *res K* cells modify the progeny phage to $\lambda \cdot$ K. Again some restriction of $\lambda \cdot$ B *res K* is observed in B *res K* cells. These results indicate that $\lambda \cdot$ B *res K* is identical with $\lambda \cdot$ K.

Attempts to separate restriction and modification

In some systems independent control of restriction and modification has been found. Supposing that in the system used in the present work restriction and modification are independently controlled by different loci, four types of recombinants from K 12 × B crosses are expected. The parental types B *res K mod K* and B *res B mod B* plate $\lambda \cdot$ K with high and low efficiency respectively; the non-parental types B *res B mod K* and B *res K mod B* are expected to give no plaques when tested with either $\lambda \cdot$ K or $\lambda \cdot$ B *res B*, as progeny phage in both strains is modified to the restricted variant.

In the crosses given in Table I, all recombinants were tested with low titer $\lambda \cdot$ K suspensions. Only B *res K mod K* cells could give plaques under this condition and the non-plaque forming cells were tentatively scored as *res B* cells. A possible independent genetic control of restriction and modification was tested by selecting 350 *thr*$^+$ recombinants from a Hfr H × B *thr*$^-$ cross. The recombinants were now tested with low and high phage concentrations. All recombinants yielded plaques; 295 recombinants with high e.o.p. and 55 recombinants with low e.o.p. were found. The experiment suggests that restriction and modification are controlled by one locus. The presence of two closely linked loci cannot, however, be excluded *a priori*.

Restriction of F-gal$^+$

The fertility factor of K 12 is restricted in *E. coli* B (ref. 10). After transfer of F-*gal*$^+$ to *gal*$^-$ acceptor strains, the episome is lost and the *gal* marker is incorporated into the chromosome with a very low frequency[11].

The influence of the restriction locus on F-*gal*$^+$ transfer was studied in crosses between 5832 (F-*gal*$^+$) and a number of B *gal*$^-$ acceptor strains. Episome tranfer was detected by selecting *gal*$^+$ colonies on minimal medium with galactose as carbon and energy source. Strain P 678 served as control in all experiments. It may be seen from the

TABLE III

FREQUENCY OF TRANSFER OF F-*gal*$^+$ FROM STRAIN 5832 TO *gal*$^-$ ACCEPTOR STRAINS

Transfer was performed as given under MATERIALS AND METHODS. The frequency of transfer of the F-*gal*$^+$ episome to P 678 under similar conditions was $4 \cdot 10^{-1}$.

Acceptor strain	*Genotype*	*Frequency of gal*$^+$ *sexduced cells per donor cell*
CB 80-3	non-cured, *res B*	10^{-6}
B *res K*	non-cured, *res K*	$3 \cdot 10^{-6}$
Bc 258	cured, *res B*	10^{-3}
Bc hybrid	cured, *res K*	10^{-1}

results given in Table III that the frequency of transfer of F-*gal*+ to CB 80–3 is very low (10^{-6}). The *gal*+ cells obtained are almost exclusively F−, indicating that CB 80–3 restricts F-*gal*+ to a very high extent. The introduction of the *res K* marker in the CB 80–3 strain increases the frequency of *gal*+ cells to $3 \cdot 10^{-6}$, but again the majority of the *gal*+ colonies are F−. Wild type *E. coli* B harbours a defective prophage X (refs. 6, 7); the curing of the acceptor strains of this defective prophage increases the frequency of transfer to 10^{-3} in Bc *res B* and to 10^{-1} in Bc *res K* cells. In both cases the majority of the *gal*+ colonies were F+.

The results indicate that non-cured B *res B* and B *res K* cells restrict the episome to such an extent that incorporation of the episomal *gal*+ marker is more frequent than the successful transfer of the episome. In the absence of the defective prophage X the frequency of successful transfer is much higher than the frequency of marker incorporation.

Restriction of chromosomal DNA

In K 12 × B crosses a low number of recombinants and a decreased linkage between markers is always observed[5]. It seems that the chromosomal DNA of K 12 is to some extent also restricted in B acceptor strains. The influence of the *res* locus on chromosomal DNA restriction was studied in crosses in which a B *res B* or a B *res K* strain was used as acceptor strain.

The two acceptor strains *leu*− *gal*− *res B* and *leu*− *gal*− *res K* were crossed with Hfr H λ^-. The selected marker *gal*+ was located outside the hybrid region of the acceptor strain. The number of *gal*+ recombinants with a B *res K* acceptor strain was always 3 to 4 times larger than the number of *gal*+ recombinants obtained in crosses with B *res B* acceptor strains. This result indicates that the incorporation of a K 12 marker is better in B *res K* cells than in B *res B* cells.

The genetic linkage is also better in B *res K* cells than in B *res B* cells: 50% of the *gal*+ recombinants were *leu*+ in the cross with B *res K*, against 3% with the B *res B* strain.

DISCUSSION

The results of our experiments show that one locus is responsible for the restriction of $\lambda \cdot$ K DNA in *E. coli* B and that this locus is located between the leading point of the Hfr H chromosome and the locus for threonine synthesis. During the preparation of this paper an abstract of BOYER[5] appeared in which identical results were summarized. Our results are in agreement with those of GLOVER AND SCHELL (personal communication).

The introduction of the *res K* locus into *E. coli* B increases the plating efficiency of $\lambda \cdot$ K and changes the original modifying ability of the cell. Our results suggest that restriction and modification are controlled by a single locus or by two very closely linked loci.

Wild-type *E. coli* B harbours the defective prophage X (refs. 6, 7), the presence of which causes a small but significant restriction of $\lambda \cdot$ K. Non-cured B *res K* cells plate $\lambda \cdot$ K with an efficiency of $\pm$ 0.5 whereas the e.o.p. of $\lambda \cdot$ K in cured B *res K* cells was 1. The same phenomenon was observed when phage propagated on cured B *res B* cells was plated on non-cured *res B* cells (ARBER, personal communication).

This small but significant restriction is not accompanied by a modification of the progeny phage (Table II).

The *res* locus and the defective prophage X are also responsible for the restriction of the K 12 F-factor in *E. coli* B. The influence of the defective prophage X is more pronounced than the influence of the *res* locus. When the defective prophage X is present, the frequency of *gal*+ recombinants after F-*gal*+ transfer to *res B* cells is very low (10^{-6} per donor cell) and the majority of the *gal*+ cells have lost the F factor. The introduction of the *res K* marker increases the frequency of *gal*+ recombinants threefold but again the majority of the *gal*+ cells are F^-. The increase of *gal*+ recombinants is thus due to an increase in recombination between chromosomal DNA of episome and chromosome of acceptor cell.

The curing of the acceptor cell considerably increases the frequency of transfer; cured *res B* cells accept the F-*gal*+ factor with a frequency of 10^{-3} per donor cell and the introduction of the *res K* marker increases the frequency of successful transfer to 10^{-1} per donor cell. The *gal*+ recombinants are now predominantly F^+.

The introduction of the *res K* locus in *E. coli* B increases the number of recombinants and the genetic linkage in K 12 × B crosses. The influence of the restriction locus on the genetic linkage in K 12 × B crosses is in agreement with the results of BOYER[5] and confirms the conclusion of PITTARD[20] that phage restriction and poor genetic linkage are consequences of the same phenomenon. PITTARD suggested that low genetic linkage in restrictive crosses whould be a consequence of breakdown of the donor chromosome in the zygotes before integration. We have indications that breakdown of donor chromosome also occurs in non-restricting hosts (VERHOEF AND DE HAAN, unpublished).

A possible influence of the defective prophage X on restriction in K 12 × B crosses has not been studied.

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