Mutation Research
Elsevier Publishing Company, Amsterdam
Printed in The Netherlands

RECOMBINATION IN *ESCHERICHIA COLI*

V. GENETIC ANALYSIS OF RECOMBINANTS FROM CROSSES WITH RECIPIENTS DEFICIENT IN ATP-DEPENDENT EXONUCLEASE ACTIVITY

P. G. DE HAAN, W. P. M. HOEKSTRA AND C. VERHOEF
Laboratory for Microbiology, State University of Utrecht, Catharijnesingel 59, Utrecht (The Netherlands)

(Received November 12th, 1971)

SUMMARY

A genetic analysis of recombinants from crosses with recombination-deficient recipients, lacking the ATP-dependent exonuclease activity, demonstrated differences in the inheritance pattern of donor markers compared with a Rec$^+$ recipient. In particular the donor markers proximal to the transfer origin were inherited with a decreased frequency in the recombination-deficient recombinants. A mathematical analysis of the crosses showed that the observed deviations are mainly restricted to a reduced probability of exchange events in the region near the origin of the donor fragment.

INTRODUCTION

Low[4] clearly showed that in *E. coli* K12 crosses involving *recA*$^-$ recipient strains there was hardly any progeny and that the rare progeny was partially diploid. Crosses with recipient strains, described as *recB*$^-$ or *recC*$^-$, which yielded about 0.01 to 1.0% of the progeny obtained with a Rec$^+$ recipient, resulted in haploid recombinants.

Low[4] could not demonstrate a significant difference in the linkage frequencies of unselected markers in Rec$^+$ recipients on the one hand and in *recB*$^-$ or *recC*$^-$ recipients, which lack the ATP-dependent exonuclease activity on the other. This resemblance in inheritance pattern suggested to Low[4] that in *recB*$^-$ or *recC*$^-$ strains recombination occurred in a normal way, albeit at a lower level. In this paper we show, however, that in these recombination-deficient recipients, the linkage frequency of donor markers proximal to the origin is markedly decreased.

MATERIALS AND METHODS

All bacterial strains were derivatives of *E. coli* K12. The mating type and the relevant markers are given in Table I. All recipient strains were resistant against streptomycin, whereas the donor strains were sensitive for this drug. Derivatives of

TABLE I

BACTERIAL STRAINS

Strain number	*Mating type*	*Relevant markers*					
		argB	*leu*	*proA*	*purA*	*rec*	*thr*
PC0294	F⁻	+	−	−	−	+	−
PC0295[a]	F⁻	+	−	−	−	160	−
PC0296[a]	F⁻	+	−	−	−	161	−
PC0298[a]	F⁻	+	−	−	−	163	−
PC0299[a]	F⁻	+	−	−	−	164	−
PC0300[a]	F⁻	+	−	−	−	165	−
PC0302[a]	F⁻	+	−	−	−	167	−
PC0303[a]	F⁻	+	−	−	−	168	−
PC1251[b]	F⁻	+	−	−	−	B21	−
PC1252[b]	F⁻	+	−	−	−	C22	−
AB1157	F⁻	−	−	−	−	+	−
AB2470	F⁻	−	−	−	−	B21	−
JC5489	F⁻	−	−	−	+	C22	−
PC0205	F⁻	+	−	−	−	+	−
PC0031	HfrR4	+	+	+	+	+	+
PC0008	HfrH	+	+	+	+	+	+

[a] Derived from strain 0294 after NG mutagenesis.
[b] From a *thyA*⁻ derivative of strain 0294, by transduction (see MATERIALS AND METHODS).

strain PC0294 carrying the *recB21*⁻ or *recC22*⁻ allele described by WILLETTS AND MOUNT[8] were constructed by transducing a *thyA*⁻ mutant of strain PC0294 with *P1.vir* propagated on strains AB2470 and JC5489 respectively. *thyA*⁺ transductants were selected from which UV-sensitive, recombination-deficient colonies were isolated. Conjugation experiments and recombinant analysis were performed as described by DE HAAN AND VERHOEF[3].

RESULTS

Amongst the recombination-deficient mutants isolated in our laboratory, seven independently isolated mutations were mapped between *thyA* and *argA*[6]. These mutants all lack the ATP-dependent exonuclease[5], described by BUTTIN AND WRIGHT[1]. Together with *recB21*⁻ and *recC22*⁻ derivatives of PC0294 they were crossed with the donor strain HfrR4, and *purA*⁺*strA*⁻ recombinants were selected (see Fig. 1). The transfer time was limited to 40 min so that the Rec⁺ allele was not introduced into the zygotes. The unselected marker *proA*⁺ was scored in all crosses; in crosses with the *rec*⁻ *164* mutant and the Rec⁺ parent as recipient the unselected donor markers *thr*⁺

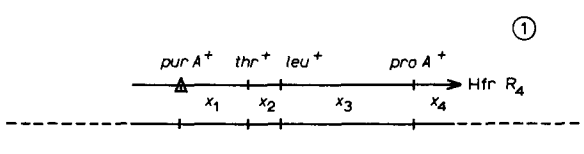

Fig. 1. Graphic presentation of a four-point cross with HfrR4 as donor. Selected donor marker *purA*⁺, unselected donor markers *thr*⁺, *leu*⁺ and *proA*⁺. Symbols x_1, x_2, x_3 and x_4 represent probabilities for crossing-over events. Δ, points to selected marker.

TABLE II

LINKAGE FREQUENCIES OF UNSELECTED MARKERS IN HfrR4 × F⁻Rec⁻ CROSSES

The transfer time was 40 min. A minimum of 200 *purA*⁺*strA*⁻ recombinants from each cross were scored for unselected proximal markers.

Recipient strain	*Rec marker*	*Unselected marker*	*Linkage frequency*
PCo294	*rec*⁺	*thr*	0.64
		leu	0.62
		proA	0.55
PCo299	*rec⁻164*	*thr*	0.59
		leu	0.52
		proA	0.13
PCo295	*rec⁻160*	*proA*	0.12
PCo296	*rec⁻161*	*proA*	0.12
PCo298	*rec⁻163*	*proA*	0.14
PCo300	*rec⁻165*	*proA*	0.11
PCo302	*rec⁻167*	*proA*	0.14
PCo303	*rec⁻168*	*proA*	0.11
PC1251	*recB21*	*proA*	0.08
PC1252	*recC22*	*proA*	0.16

and *leu*⁺ were also scored (Table II). This table shows that the linkage of unselected markers is decreased in Rec⁻ recipient strains. The decrease is very pronounced for the proximal *proA* marker; 8–16% for Rec⁻ strains against 55% for the Rec⁺ parent.

To exclude the possibility that the abnormal result of the HfrR4 × F⁻Rec⁻ crosses was due to peculiar properties of one of the strains, we made a series of crosses with other donors and recipients. The crosses are visualized in Fig. 2 and the results are given in Table III. Again the linkage of unselected markers is decreased in crosses with Rec⁻ recipient strains. Thus the decrease seems to be independent of the origin and direction of transfer of the Hfr; the linkage is merely determined by the Rec allele of the recipient. Further evidence for this hypothesis was obtained from a cross between HfrR4 and a *rec⁻164*⁺ transductant from strain PCo299. In this cross an almost normal linkage frequency of 0.45 was found for the *proA*⁺ marker.

MATHEMATICAL ANALYSIS

DE HAAN AND VERHOEF[3] have presented a mathematical method for analysing isogenic and non-isogenic crosses in *E. coli*. This method, which is in agreement with a general mathematical description of recombination given by WALMSLEY[7], was based on an analysis of a four-point cross with three unselected markers (Fig. 1). DE HAAN

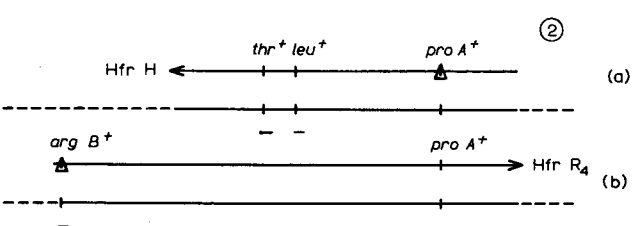

Fig. 2. (*a*) Graphic presentation of a cross with HfrH as donor. Selected donor marker *proA*⁺, unselected donor markers *thr*⁺ and *leu*⁺. (*b*) Graphic presentation of a cross with HfrR4 as donor. Selected donor marker *argB*⁺, unselected donor marker *proA*⁺. *A*, points to selected marker.

TABLE III

LINKAGE FREQUENCIES OF UNSELECTED MARKERS IN CROSSES WITH Rec⁻ RECIPIENTS

The transfer time was 30 min in the crosses with HfrH as donor and 40 min with HfrR4 as donor. A minimum of 200 recombinants from each cross were scored for unselected proximal markers.

Donor	*Recipient*	*Rec marker*	*Selected marker*	*Unselected marker*	*Linkage frequency*
HfrH	AB1157	Rec⁺	*proA*⁺	*leu*⁺	0.87
				thr⁺	0.80
HfrH	AB2470	*recB21*	*proA*⁺	*leu*⁺	0.55
				thr⁺	0.46
HfrR4	AB1157	Rec⁺	*argB*⁺	*proA*⁺	0.58
HfrR4	AB2470	*recB21*	*argB*⁺	*proA*⁺	0.20

AND VERHOEF[3] found that the frequencies of the eight recombinant classes were dependent on four parameters: the three crossing-over frequencies between the unselected markers and a parameter they called α. In isogenic crosses this parameter was equal to 0.5. The crossing-over frequency between origin and first proximal marker was not present in their formulae.

We shall present a mathematical model based on the hypothesis that donor and repicient are completely isogenic for the segment in which the crossing-over events take place. The crosses are considered to be symmetrical and the parameter α can thus be neglected. Let x_1 be the probability of a crossing-over event in the *purA-thr* segment and x_2, x_3 and x_4 the probabilities in the *thr-leu*, *leu-proA* and *proA*-origin segments respectively (Fig. 1). The relative frequencies of the eight recombinant classes among the selected *purA*⁺*strA*⁻ recombinants can then be presented as functions of x_1, x_2, x_3, x_4 and R, when R represents the total probability of crossing-over in the segment *purA*-origin (Table IV). The parameters x_1, x_2, x_3 and x_4 can be calculated from the observed number of recombinants (a, $b \cdots\cdots h$) with the aid of the formulae presented in Table V.

Table VI presents the frequencies of the 8 recombinant classes of a cross as presented in Fig. 1, published by DE HAAN AND VERHOEF[3], and of a comparable cross between HfrR4 and a *rec⁻164⁻* recipient. From the observed numbers in the several recombinant classes, the parameters x_1, x_2, x_3 and x_4 were calculated. Using these parameters we calculated the expected number of recombinants in each class. The results (Table VI) show that both crosses give a good fit between observed and calculated frequencies. The results obtained with the Rec⁺ recipient show that the frequency of recombination in the *proA*-origin segment (x_4) is close to 0.5. This implies

TABLE IV

EXPECTED FREQUENCIES OF THE NUMBER OF RECOMBINANTS IN A FOUR POINT CROSS (Fig. 1)

Recombinant			*Expected frequency*	*Observed number*
thr	*leu*	*proA*		
+	+	+	$(1-x_1)\cdot(1-x_2)\cdot(1-x_3)\cdot x_4/\mathrm{R}$	*a*
+	+	−	$(1-x_1)\cdot(1-x_2)\cdot x_3\cdot(1-x_4)/\mathrm{R}$	*b*
+	−	−	$(1-x_1)\cdot x_2\cdot(1-x_3)\cdot(1-x_4)/\mathrm{R}$	*c*
−	−	−	$x_1\cdot(1-x_2)\cdot(1-x_3)\cdot(1-x_4)/\mathrm{R}$	*d*
−	−	+	$x_1\cdot(1-x_2)\cdot x_3\cdot x_4/\mathrm{R}$	*e*
−	+	+	$x_1\cdot x_2\cdot(1-x_3)\cdot x_4/\mathrm{R}$	*f*
+	−	+	$(1-x_1)\cdot x_2\cdot x_3\cdot x_4/\mathrm{R}$	*g*
−	+	−	$x_1\cdot x_2\cdot x_3\cdot(1-x_4)/\mathrm{R}$	*h*

TABLE V

PRESENTATION OF THE PARAMETERS x_1, x_2, x_3 AND x_4 AS FUNCTIONS OF THE OBSERVED NUMBER OF RECOMBINANTS IN THE 8 CLASSES (SEE TABLE IV)

$$\left(\frac{1-x_1}{x_1}\right)^2 = \frac{c+g}{d+e} \times \frac{a+b}{f+h}$$

$$\left(\frac{1-x_2}{x_2}\right)^2 = \frac{a+b}{f+h} \times \frac{d+e}{c+g}$$

$$\left(\frac{1-x_3}{x_3}\right)^2 = \frac{c+d}{e+g} \times \frac{a+f}{b+h}$$

$$\left(\frac{1-x_4}{x_4}\right)^2 = \frac{c+d}{e+g} \times \frac{b+h}{a+f}$$

TABLE VI

MATHEMATICAL ANALYSIS OF CROSSES INVOLVING A Rec$^+$ RECIPIENT STRAIN RESP. A *rec*$^-$ *164* MUTANT RECIPIENT STRAIN. HfrR4 WAS USED AS DONOR IN BOTH CROSSES; *purA*$^+$ *strA*$^-$ RECOMBINANTS WERE SELECTED AND SCORED FOR UNSELECTED MARKERS

Recombinant class			*Recombinants from a Rec$^+$ recipient*		*Recombinants from a rec$^-$ 164$^-$ recipient*	
thr	*leu*	*proA*	*observed*	*calculated*	*observed*	*calculated*
+	+	+	664	653	121	125
+	+	−	337	341	436	453
+	−	−	66	69	85	83
−	−	−	242	244 $\chi_3^2 = 1.55$	424	403 $\chi_2^2 = 2.09$[a]
−	−	+	125	129 $0.5 \leqslant p \leqslant 0.7$	14	15 $0.3 \leqslant p \leqslant 0.5$
−	+	+	23	26	2	4
+	−	+	38	36	5	4
−	+	−	17	14	10	10
			1512	1512	1097	1097
The calculated parameters are						
x_1				0.273		0.245
x_2				0.096		0.063
x_3				0.344		0.268
x_4				0.502		0.092

[a] For the χ^2 test the frequencies of classes 6 and 7 from the *rec*$^-$*164* cross were taken together.

that the probability for an odd number of crossing-over events in this region is equal to the probability for an even number of events. In the segment proximal to the origin there will thus occur a high number of crossing-over events. DE HAAN AND VERHOEF[3] pointed out that the segment proximal to the origin has something special. Based on the fact that in the Rec$^+$ cross x_4 appeared to be equal to $1-x_4$ this cross can be described by three parameters (x_1, x_2 and x_3). For the description of the Rec$^-$ cross, however, all four parameters are necessary. Moreover the calculated probabilities are lower than those calculated from the Rec$^+$ cross. The most striking deviation from the Rec$^+$ cross is the very reduced probability for crossing-over events in the origin-proximal segment (0.092 compared with 0.502).

DISCUSSION

The lack of ATP-dependent exonuclease activity in *E. coli* K12 recipient strains has two effects on recombination. Firstly it decreases the number of recombinants, and secondly it causes a decrease in the linkage of unselected markers. The decrease of un-

selected markers is very pronounced for proximal markers. The decrease in linkage frequency of the *proA*$^+$ marker was observed in nine independently isolated Rec$^-$ mutants all lacking the ATP-dependent exonuclease. The observed linkage frequencies fall within the range 0.08–0.16. The differences between these linkage frequencies in the various recipients are not statistically significant.

The mathematical analysis applied to the crosses with a Rec$^+$ or a *rec*$^-$*164*$^-$ recipient, shows that there is a very pronounced decrease in the frequency of recombination in the region proximal to the donor origin. In a Rec$^+$ recipient the proximal segment as well as the distal end of the donor fragment are regions with a high probability for crossing-overs[3]. The recombination frequency at the distal end of the donor fragment is also decreased in recipients that lack the ATP-dependent exonuclease activity (VERHOEF, unpublished results). The decreased recombination frequency at both ends of the donor fragment will in itself lower the number of recombinants in recipients lacking the ATP-dependent exonuclease. In a *rec*$^-$*164*$^-$ recipient a maximal decrease in the number of *purA*$^+$*strA*$^-$ recombinants (cross, Fig. 1) to about 4% of the yield obtained in a Rec$^+$ recipient is expected on the basis of the reduced recombination frequency (0.1–0.5) at the ends of the donor fragment. However, we observed a decrease to 0.1% for the yield of *purA*$^+$*strA*$^-$ recombinants in the *rec164*$^-$ recipient compared with a Rec$^+$ recipient. This suggests that, in recipients lacking the ATP-dependent nuclease, recombination occurs by an alternative, less active, pathway (in accordance with the hypothesis of CLARK[2]). The mathematical analysis shows that in this pathway the crossing-over events occur mainly in the middle of the transferred segment.

ACKNOWLEDGEMENT

The authors are very grateful to Misses H. S. FELIX, E. TERPSTRA-BRINK and E. M. ZUIDWEG for excellent technical assistance.

REFERENCES

1 BUTTIN, G., AND M. R. WRIGHT, DNA breakdown in *E. coli*, *Cold Spring Harbor Symp. Quant. Biol.*, 33 (1968) 259–269.
2 CLARK, A. J., *Xth International Congress for Microbiology*, in the press.
3 DE HAAN, P. G., AND C. VERHOEF, Genetic recombination in *Escherichia coli*, II. Calculation of incorporation frequency and relative map distance by recombinant analysis, *Mutation Res.*, 3 (1966) 111–117.
4 LOW, B., Formation of merodiploids in matings with a class of Rec$^-$ recipient strains of *Escherichia coli* K12, *Proc. Natl. Acad. Sci. (U.S.)*, 60 (1968) 160–167.
5 STORM, P. K., Recombination deficient mutants of *Escherichia coli* K12, *Thesis*, Utrecht (The Netherlands), 1971.
6 STORM, P. K., W. P. M. HOEKSTRA, P. G. DE HAAN AND C. VERHOEF, Genetic recombination in *Escherichia coli*, IV. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12, *Mutation Res.*, 13 (1971) 9–17.
7 WALMSLEY, R. H., The general theory of mapping functions for random genetic recombination, *Biophys. J.*, 9 (1969) 421–431.
8 WILLETTS, N. S., AND D. W. MOUNT, Genetic analysis of recombination deficient mutants of *E. coli* K12 carrying Rec mutations cotransducible with *thyA*, *J. Bacteriol.*, 100 (1969) 923–934.