Genet. Res., Camb. (1985), **45**, *pp.* 1–8
With 3 text-figures
Printed in Great Britain

Rec-mediated recombinational activity of two adjacent Chi elements in bacteriophage lambda

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SUMMARY

Chi is a sequence of eight nucleotide pairs which stimulate *recBC*-mediated recombination (Smith, 1983*a*, *b*). The effect of two linked Chis on *recBC*-mediated recombination was tested in bacteriophage lambda. It was noticed that the Chi element located on the right side of the phage chromosome is epistatic on the other Chi. These findings support a model proposed by Stahl *et al.* (1983) which suggests that the recombination machinery moves unidirectionaly in the phage chromosome from right to left. The results also suggest that in the presence of more than one Chi only the rightmost one stimulates recombination.

1. INTRODUCTION

The major recombination system of *Escherichia coli* is dependent on the genes *recB* and *recC* which code for the two subunits of exonuclease V (Hickson & Emmerson, 1981; Sasaki *et al.* 1982). Chi is a sequence of eight nucleotide pairs in DNA which stimulates genetic recombination mediated by the *recBC* pathway. In *E. coli* Chi sequences are scattered along the chromosome at a frequency of about 1 per 5000 base pairs. The chromosome of the wild-type bacteriophage λ does not contain any complete Chi sequence but an active Chi site can be induced by mutation in either of four sites along the phage chromosome. Chi stimulates genetic exchange in its vicinity with a leftwards polarity on the standard genetic map of λ. Chi activity is dominant and it acts also when located on heterologous DNA. The eight base pairs which form an active Chi are an asymmetric sequence and are active in one orientation only. Recombination and proliferation of a *red gam* mutant of λ in a wild-type host bacterium is dependent on the host's *recBC* system and hence it is stimulated by an active Chi site on the phage chromosome. These and other properties of Chi have been well characterized and reviewed (Stahl, 1979; Smith, 1983*a*, *b*):

The transposon Tn*5* does not contain any active Chi but such a site has been introduced into Tn*5* by mutation. When this transposable Tn*5*χ^+ element is inserted into various locations on the λ chromosome its introduced Chi element is active in one (the same) orientation only (Yagil *et al.* 1980). With the help of

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Tn*5*χ^+ we constructed a phage with two adjacent Chi elements in order to study if the two linked Chis have a different effect on recombination than does each Chi separately.

2. MATERIALS AND METHODS

(i) *Organisms*

A list of λ and of *E. coli* strains used in this study, their relevant genotype and source is given in Table 1.

Table 1. *List of strains*

Strain	Relevant genotype	Source
	(i) Bacteriophage	
105	*χ^+B121 red3 gam(am)210 CI857*	F. Stahl
EY101	*△b519 △b515 int29 red3 gam(am) 210 CI(ts)857 △nin5*	Yagil *et al.* (1980)
EY115	*gam* ::Tn*5*χ^0 insertion into EY101	Yagil *et al.* (1980)
EY116	*gam* ::Tn*5*χ^+ mutation of EY115	Yagil *et al.* (1980)
IS10	*χ^+B121* derivative of EY101	This paper
IS15	*gam* ::Tn*5*χ^0 insertion into IS10	This paper
IS16a-e	*gam* ::Tn*5*χ^+ insertions into IS10	This paper
RM251	*h int red3 gam(am)210 CI26 S(am)7*	Malone *et al.* (1980)
	(*ii*) *E. coli*	
594	*rec*$^+$ Su$^-$	Malone *et al.* (1979)
594(P2)	P2 lysogen of 594	Malone *et al.* (1979)
EY253	JC8679 ::Tn*5*χ^+	Yagil *et al.* (1980)
EY254	JC8679 ::Tn*5*χ^0	Yagil *et al.* (1980)
JC8679	*recB21 recC22 sbcA23*	Malone *et al.* (1979)
RM66	*recB21* $\lambda^r\lambda h^s$ Su$^-$	Malone *et al.* (1979)

(ii) *Media*

Tryptone plates were used for λ plating (Davis, Botstein & Roth, 1980). Indicator bacteria were grown in tryptone broth with 0·2 % maltose and 20 μg/ml thiamine. For pyrophosphate plates (Parkinson & Huskey, 1971) 2·5 mM sodium pyrophosphate was included in tryptone agar.

(iii) *Phage crosses*

These were performed as described by Stahl & Stahl (1977).

(iv) *Construction of λ strain IS10*

Strain 105 was crossed with EY101 and plated on pyrophosphate plates to select for the deletions (Parkinson & Huskey, 1971) using 594(P2) as indicator bacterium. A large plaque (χ^+B) was selected and purified. The presence of the three deletions (Fig. 1), which are essential to make room for the transposon insertion, was verified by restriction analysis.

(*v*) *Transposition of Tn5 into* gam

This was done as described before (Yagil *et al.* 1980) using strains EY254 and EY253 as donors of Tn*5*χ^0 and Tn*5*χ^+, respectively.

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(vi) *Isolation of DNA and restriction analysis*

Rapid λ DNA isolation, restriction and 1% agarose gel electrophoresis were performed as described by Davis, Botstein & Roth (1980).

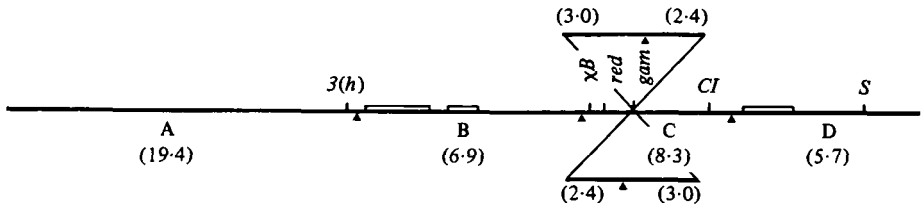

Fig. 1. A map of the λ chromosome (Daniels *et al.* 1983*a*) showing relevant loci, deletions (open bars), the sites for the restriction enzyme SmaI (triangles) and Tn*5* inserted into *gam* in two possible orientations. Letters under the map and numbers in parentheses indicate, respectively, SmaI fragments and their size in kb.

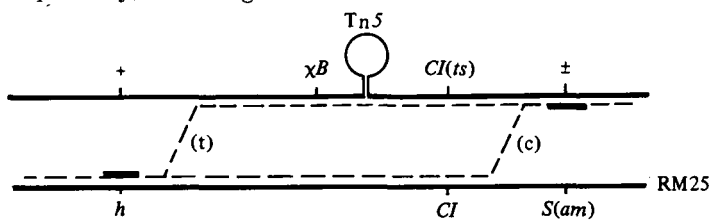

Fig. 2. Diagram of the crosses performed to detect Chi activity. Black bars indicate the selected markers, (t) shows a crossover which yields turbid plaques and (c) is a crossover which gives clear plaques.

3. RESULTS

χ^0B is one of the four loci in wild-type λ where an active Chi (χ^+B) can be induced by mutation, it is located to the left of the genes *red*, close to *gam* on the map of λ (Fig. 1; Stahl, Crasemann & Stahl, 1975). To obtain a strain with two closely located Chi elements we inserted Tn*5*χ^+ into the gene *gam* in a strain (IS10) which already carried χ^+B. The isogenic control strains which carried only one of these Chis were *IS*15 (χ^+B *gam* ::Tn*5*χ^0) and EY116 (χ^0B *gam* ::Tn*5*χ^+). The strain which carried no active Chi was EY 115 (χ^0B *gam* ::Tn*5*χ^0). To test the effect of the different combinations of Chi elements on *recBC*-mediated recombination the cross described by Malone *et al.* (1979) was utilized. Each of the above four strains was crossed with strain RM251 (Fig. 2), this strain carries the selectable host-range mutation *h*, the mutation *CI26* which yields clear plaques and a suppressible mutation in the essential gene *S*. The progeny of each cross was plated on the Su^- host RM66 selecting for recombinants which carried the gene *h* from one parent and S^+ from the other (Fig. 2). Crossovers which occurred in the *h-CI* interval yielded turbid plaques (Fig. 2(t); the plates were incubated at 30 °C). Crossovers in the *CI-S* interval resulted in clear plaques (Fig. 2*c*). The Chi elements tested were within the *h-CI* interval and hence any Chi activity would preferentially enhance recombination within this interval resulting in an increased ratio of turbid/clear plaques. Each cross was performed twice, once in the $RecBC^+$ host 594 and the other in the *recBC*$^-$ host JC8679. Chi's activity is expressed only in a $RecBC^+$ host (Stahl & Stahl, 1977). The results of the crosses in the $RecBC^+$ host (594) are given

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Table 2. *Effect of Chi elements on recombination*

Cross	Chi parent*	Host bacterium	Number plaques tested†	Ratio of turbid/clear plaques‡
I	EY115 ($\chi^0 B$ *gam*::Tn*5*χ^0)	594(RecBC$^+$)	586	0·85 (**1·00**)
II	EY116 ($\chi^0 B$ *gam*::Tn*5*χ^+)	594(RecBC$^+$)	573	1·84 (**2·16**)
III	IS15 ($\chi^+ B$ *gam*::Tn*5*χ^0)	594(RecBC$^+$)	1690	3·84 (**4·52**)
IV a–e	IS16a–e ($\chi^+ B$ *gam*::Tn*5*χ^+)	584(RecBC$^+$)	370–859	(a) 4·40 (**5·12**); (b) 4·17 (**4·91**); (c) 2·16 (**2·54**); (d) 2·30 (**2·71**); (e) 3·70 (**4·35**);
I	EY115	JC8679(*recBC*$^-$)	346	0·80
II	EY116	JC8679(*recBC*$^-$)	407	0·76
III	IS15	JC8679(*recBC*$^-$)	810	0·53
IV	IS16a–e	JC8679(*recBC*$^-$)	239–464	(a) 0·80; (b) 0·94; (c) 0·79; (d) 0·71; (e) 0·75;

* The other parent was always RM251.
† The progeny was always plated on strain RM66.
‡ Boldface figures in parentheses show increase in ratio relative to cross I.

in the upper half of Table 2. The first cross (I), which included no active Chi, yielded a turbid/clear plaque ratio of 0·85. In cross II, which included an active Tn*5*χ^+ only, this ratio increased approximately twofold to 1·84. These results are consistent with the ones reported previously (Yagil *et al.* 1980). In the presence of χ^+B alone (cross III) the ratio increased by a factor of 4·52 to 3·84. Thus χ^+B is twice as active as Tn*5*χ^+. When both χ^+B *and* Tn*5*χ^+ were present two distinct kinds of results were obtained when different strains with independent *gam*::Tn*5*χ^+ insertions were tested (crosses IV a–e in Table 2). The increase in turbid/clear plaque ratio was either four- to fivefold (5·12, 4·91 and 4·35, crosses IV a, b and e, respectively) or two- to threefold (2·54, 2·71, crosses IV c and d), i.e. little if any accumulative activity of both active Chis was observed; rather, with some Tn*5*χ^+ insertions the results resembled the stronger activity of χ^+B alone (VI a, b, e) whereas other insertions resembled the weaker activity of Tn*5*χ^+ alone (VI c d). The lower half of Table 2 shows the clear/turbid plaque ratio when the same crosses were performed in a *recBC*$^-$ host. The relative homogeneity of these results indicate that those in the upper part of the table are specific to the activity of Chi.

As already pointed out, Chi is active in one orientation only. To test whether the two groups of results obtained in crosses IV a–e (Table 2) were due to different orientations of the inserted Tn*5*χ^+ we carried out a restriction analysis of the DNA molecules extracted from each of these strains. The wild-type chromosome carries three sites for the restriction enzyme SmaI (Fig. 1; Daniels *et al.* 1983*b*). Tn*5* has one site for this enzyme dividing the transposon into two fragments of 2·4 and 3·0 kilobases (kb; Jorgensen, Rothstein & Reznikoff, 1979). The insertion of Tn*5* into *gam* in one orientation is expected to yield two new fragments of 10·4 and 3·3 kb instead of the 8·3 kb fragment C (Fig. 1). In the opposite orientation two fragments of 9·8 and 3·9 kb are expected instead of C. Fig. 3 shows a gel of SmaI fragments of strains used in the crosses. Column 101 of the figure shows the fragments of the parental strain (EY101) prior to the insertion of Tn*5* with its expected four fragments. Columns I and II show the strains used in crosses I and II. These two strains are identical except for the χ^+ mutation in Tn*5* (Yagil *et al.* 1980) and in both of them the insertion of Tn*5* into *gam* created the two expected new fragments. The estimated size of these two fragments is 10·4 and 3·3 kb and hence the orientation of the active Tn*5*χ^+ fits the orientation of the lower transposon depicted in Fig. 1. Columns a–e in Fig. 3 show the SmaI fragments of the five strains constructed for crosses IV a–e, respectively. The fragments in columns c, d, whose respective crosses gave the weaker activity approximating that of Tn*5*χ^+ alone, showed the active orientation of Tn*5*χ^+ (compare with column II in Fig. 3). On the other hand, the two new fragments of 9·8 and 3·9 kb in the strains of crosses VI a, b, e (which resembled the stronger activity approximating that of χ^+B alone) fitted the inverted (inactive) orientation of Tn*5*χ^+. These results show that in the crosses where Tn*5*χ^+ was present in the active orientation its activity was epistatic on that of χ^+B. In the strains with the inactive orientation of Tn*5*χ^+ the activity of χ^+B became apparent.

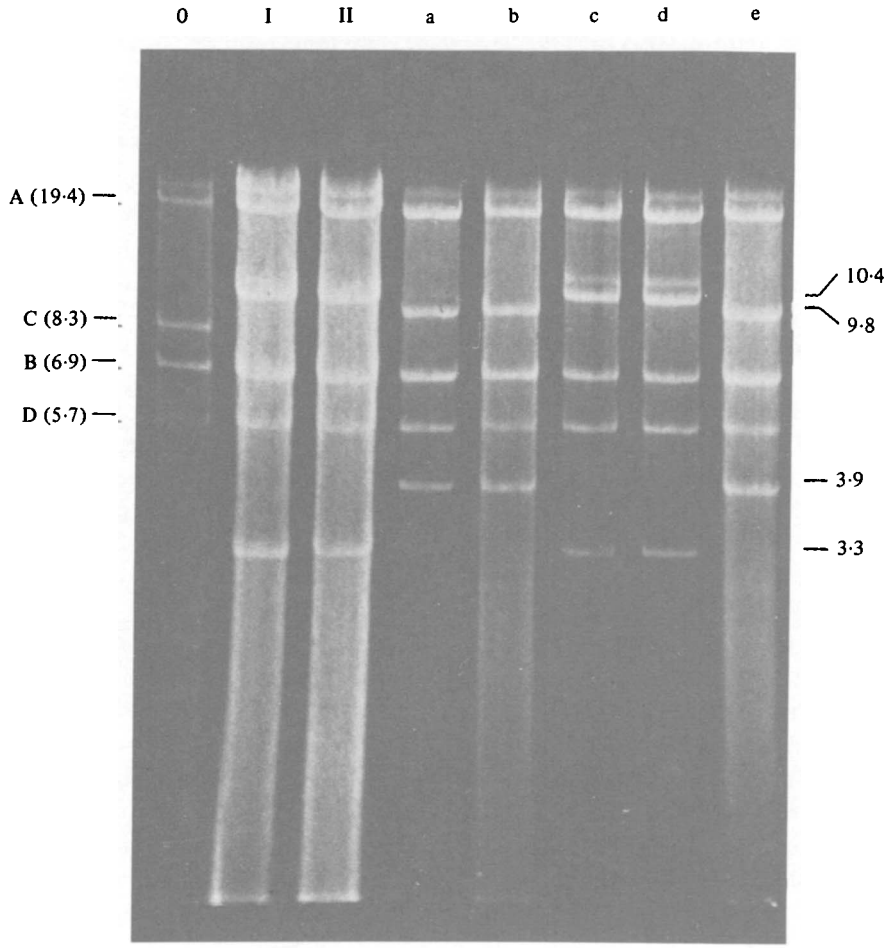

Fig. 3. A gel showing SmaI fragments of various strains. See text for details.

4. DISCUSSION

The results have shown that two active Chi elements located close to each other on the chromosome do not have an additive effect on *recBC*-mediated recombination. The activity of each Chi separately was quantitatively distinct such that Tn*5*χ^+ acted more weakly than the chromosomal χ^+B. This difference enabled us to propose that the weaker Chi element (that of Tn*5*χ^+) is epistatic on the more active χ^+B located to its left (Fig. 1).

cos is the site on the λ chromosome at which multimeric genomes of the phage are cleaved to form the encapsidated linear monomers. This staggered cleavage is performed by the enzyme terminase and results in the 'sticky' ends of the linear chromosome (Feiss & Becker, 1983). It has been shown that the orientation dependence of Chi's activity is coupled with the orientation of *cos* (Kobayashi *et al.* 1982, 1983). The dependence of Chi activity on *cos* seems to be due to the double-stranded staggered cut formed in *cos* (Stahl *et al.* 1983). It has been

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suggested that this cut enables the 'recombining machine' (which is, or includes exonuclease V, the product of *recBC*) to enter the double-stranded DNA molecule and travel in one direction along the molecule searching for a properly oriented active Chi site. Having encountered such a site the recombination event, which includes a nucleolytic activity, can take place (Kobayashi *et al.* 1983; Stahl *et al.* 1983). It has been demonstrated by electron microscopy that exonuclease V enters linear DNA molecules at an end and while traveling within the molecule it uwinds the molecule ahead and rewinds it behind (Rosamund Talender & Linn, 1979; Taylor & Smith, 1980). *In vivo*, the terminase which cuts at *cos* remains bound to the left end of the chromosome (Feiss & Becker, 1983). On the basis of these observations, Stahl *et al.* (1983) suggested that the *recBC* enzyme enters only at the free right end of the λ chromosome traveling leftwards in search of an active Chi. The data presented in this work support this model. In the chromosome with both χ^+B and Tn*5*χ^+ the 'recombining machine' enters the molecule from its right end. In search for an active Chi it reaches the location of Tn*5*χ^+ first and uses it as a substrate for the recombination event. Thus the presence of the more leftward χ^+B is inapparent. If this interpretation is correct it also implies that if more than one Chi is present on the λ chromosome only one – the rightmost – is used by the *recBC* system as a site to stimulate the recombination event.

Part of this work was performed in the hospitable laboratory of Prof. Frank and Mary Stahl. E. Y. also wishes to thank Drs Fanny and George Carroll for their hospitality.

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