GENETIC RECOMBINATION OF PHAGE S13 IN A RECOMBINATION-DEFICIENT MUTANT OF ESCHERICHIA COLI K12

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Genetic recombination in Escherichia coli can be blocked by a mutation that reduces the frequency of recombinants by more than a factor of 1000 (Clark and Margulies, 1965). The finding of this large reduction implies that the recombination mechanism affected by the mutation must be the major mechanism used by the cell. The study reported here on phage S13 recombination has two specific objectives:

1. To determine whether this same mechanism is involved in recombination of the phage genetic material;

2. To determine whether the steps involved in recombination of this phage are under the genetic control of the phage or of the host cell.

S13 was studied because of its relatively simple genetic constitution. In a comprehensive investigation (E.S. Tessman, 1965 and personal communication) only 5 complementation groups (labelled I, II, IIIa, IIIb, and IV) were found, so it is likely that the virus does not genetically specify more than about 5 protein species; and except for mutants in group II, linearity of the genetic map and additivity of the recombination frequencies were demonstrated. Because of its close relation to ØX174 (Zahler, 1958; E.S. Tessman and Shleser, 1963), S13 presumably contains only 1.7×10^6 daltons (Sinsheimer, 1959) of single-stranded DNA (Tessman, 1958 and 1959; Sinsheimer, 1959).

METHODS

A recombination-deficient mutant of E. coli K12, JC-1553, was isolated by Clark and Margulies (1965) from the non-deficient strain JC-411. Both F^- strains were kindly supplied by Dr. Clark. These K12 strains are resistant to S13 because of a failure to adsorb the phage. Therefore, the two strains were mutagenized by treatment with N-methyl-N'-nitro-N-nitrosoguanidine (Mandell and Greenberg, 1960), and colonies sensitive to S13 were isolated by replica plating. The two S13-sensitive strains used were labelled JC-411.5 and JC-1553.1; these strains will be referred to as Rec^+ and Rec^- respectively.

Because of the mutagenic treatment it was necessary to show that each of these two S13-sensitive derivatives was similar to its parent strain in the following respects. Both derivatives were still leucine-requiring (leu^-) and streptomycin-resistant, grew at 43° and also grew on a synthetic Tris-glucose medium with leucine, histidine and methionine as the only added amino acids. In crosses with a tryptophan-requiring (try^-) Hfr strain (K10), Rec^+ showed at least 1000 times more leu^+ try^+ recombinants than did Rec^-, confirming the recombination-deficient property of Rec^-. The derived strains also had the same sensitivities to ultraviolet light (UV) as the original strains; UV sensitivity is known to be related to their abilities to recombine (Clark and Margulies, 1965).

Phage recombination was compared in Rec^+ and Rec^- by crossing temperature-sensitive (t) mutants of S13 that cannot grow above 41°. Wild-type (t^+) recombinants were selected by plating progeny phage on E. coli C122 or Rec^- and incubating at 43°, a temperature at which only the wild type can form plaques. Crosses were performed by growing Rec^+ and Rec^- in tryptone broth (13 g of Bacto tryptone plus 7 g of NaCl per liter of H_2O; TB) to a concentration of 4-8 x 10^7 cells/ml and concentrating to 2 x 10^8/ml in TB plus 1 x 10^{-2} M $CaCl_2$. A pair of phage mutants was added, each mutant at a multiplicity of 5. The phage-cell mixtures were

shaken for 5 min, during which time 85-95% of the phage adsorbed. The mixtures were then diluted 100-fold into TB plus 2 x 10^{-3} M $CaCl_2$ and incubated for 40 min to allow time for the cells to burst. Adsorption and growth of the phage always took place at 37° except for one cross, t76 X t266, that was performed at 42°. The purpose of performing a cross entirely at 42° was to provide additional assurance that the cells were mixedly infected; since the t mutations are lethal at 42°, only by mixed infection could there be phage growth as a result of complementation. For all crosses, the burst sizes were between 30-50 phage/cell.

The recombination frequencies fluctuate within a factor of about 2 from day to day. In part, this may be due to the assay conditions, such as the state of the indicator, the age of the plates, and the exact temperature of the incubator. To minimize the effect of these errors, for each pair of mutants recombination was always measured in Rec^+ and Rec^- at the same time.

Cyanide, which is known to stimulate recombination in phage T4 (Chase and Doermann, 1958; Tomizawa and Anraku, 1964), was not used, so the recombination frequencies cannot be compared with those found in previous experiments (Tessman and Tessman, 1959; E.S. Tessman and Shleser, 1963; E.S. Tessman, 1965).

RESULTS

Table 1 shows the recombination frequencies for 5 mutants crossed pairwise in 6 different ways in Rec^+ and Rec^-. Since apparently only the t^+ recombinant was observed, the frequencies are shown as twice the t^+ frequencies in order to account for the reciprocal recombinants.

In each cross the recombination frequency was significantly lower in Rec^- than in Rec^+. Not only are the recombination frequencies reduced in Rec^-, but the reduction factors suggest that there are at least two classes of crosses. For one class, involving only group I and IIIb mutants, the recombination frequencies are reduced by a factor of approximately 20 to 40

TABLE I

RECOMBINATION OF S13 MUTANTS IN *REC*$^+$ AND *REC*$^-$

Crosses[a]		Recombination frequency (in units of 10^{-5})		Frequency ratio
Mutants crossed	Complementation groups[b]	*Rec*$^+$	*Rec*$^-$	*Rec*$^+$/*Rec*$^-$
*t*39 X *t*43	I X I	2.5	0.13	19
*t*39 X *t*266	I X IIIb	15	0.61	25
*t*39 X *t*11	I X IIIb	42	1.0	42
*t*39 X *t*11	I X IIIb	92	2.2	42
*t*76 X *t*39	II X I	30	3.9	7.7
*t*76 X *t*11	II X IIIb	30	4.7	6.4
*t*76 X *t*266	II X IIIb	50	12.4	4.0
*t*76 X *t*266[a]	II X IIIb	32	8.4	3.8

[a] All crosses were performed at 37° except for the last *t*76 X *t*266 cross, which was performed at 42°.

[b] The complementation groups are functional units determined by complementation tests. The mutants were classified by E.S. Tessman (1965) with the exception of *t*11, which I classified by its failure to complement *t*266.

in *Rec*$^-$. For the other class, involving mutant *t*76, the reduction is only by a factor of approximately 4 to 8. (The variation of the *Rec*$^+$/*Rec*$^-$ ratio within each class cannot be considered significant.)

Bacterial recombination in *E. coli* K12 may involve many steps. At least one of these steps, the one blocked in *Rec*$^-$, must also be involved in the primary recombination mechanism of phage S13. Although this step is under bacterial genetic control, one cannot say what other steps are involved in phage recombination and whether these other steps are genetically controlled by the phage or by the cell.

In *Rec*$^-$, phage recombination is reduced far less than bacterial recombination is. This might be because the phage utilizes the slight residual cellular recombinational activity very effectively. But it appears much more likely that there is an important secondary mechanism

of recombination that operates only for the phage. For example, it is conceivable that one mechanism could involve breakage and rejoining, the other copy choice.

The secondary recombination mechanism is apparently relatively more effective for crosses involving the group II mutant t76 than for the mutants in groups I and IIIb. A fact that may possibly be related is that group II mutants behave anomalously in mapping experiments; S13 mutants from all the complementation groups can be consistently ordered by two-factor crosses on a linear and roughly additive map, except for mutants from group II (E.S. Tessman, 1965). A more complete set of S13 mutants must be crossed in Rec$^+$ and Rec$^-$ to determine whether the behavior of t76 is characteristic of mutants in group II.

In a cross of two rII mutants of phage T4, recombination frequencies of 8.5% and 8.0% were found in Rec$^+$ and Rec$^-$ respectively (Tessman, unpublished data). Thus phage T4 is different from both E. coli and S13 in that its primary recombination mechanism does not utilize the step blocked in Rec$^-$.

SUMMARY

The primary recombination mechanism for phage S13 is blocked in a recombination-deficient mutant of E. coli K12. When the primary mechanism is eliminated, a secondary mechanism is revealed. Crosses can be grouped into at least two classes on the basis of the relative contributions of the primary and secondary mechanisms to the recombination frequencies; the classification depends on the mutants crossed.

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