EFFECTS OF DELETION MUTATIONS ON HIGH NEGATIVE INTERFERENCE IN T4D BACTERIOPHAGE

HILLARD BERGER AND A. JANE WARREN

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218
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HIGH Negative interference (HNI) was initially described in T4 bacteriophage by CHASE and DOERMANN (1958). It is the phenomenon in which the frequency of double recombinants in crosses of the type $r_a r_c$ by r_b is much greater than the value predicted from the frequencies of recombination in the intervals $r_a r_b$ and $r_b r_c$ and the assumption that double recombinants arise from independent events. We wish to report the results of three-factor crosses using *rII* markers in which the frequency of recombination and the level of HNI is determined with parent phage in which the central marker is either a deletion mutation or a point mutation. The major observation is that HNI, as determined from the frequency of *rII*+ double recombinants, is reduced when crossovers are required on both sides of a deletion mutation as compared to crosses requiring crossovers on both sides of a point mutation. This observation suggests that HNI is partially due to a mechanism which requires fairly precise base complementarity in the appropriate regions of the parental genomes. Further speculation concerning the relevance of these observations to some current molecular models for phage recombination will be presented in the DISCUSSION.

MATERIALS AND METHODS

Crossing procedures: Crosses were carried out at 30°C using methods and media described by CHASE and DOERMANN (1958). *Escherichia coli* B was used as host. Total progeny were determined by plating lysates on *E. coli* S/6. The *rII*+ recombinants were scored by plating lysates on *E. coli* K-12 (λh).

Phage stocks: The markers employed are in the *rII* region of T4D phage. Their location on the *rII* map is shown in Figure 1. Multiple mutants were isolated from appropriate crosses using the techniques of DOERMANN and BOEHNER (personal communication). The deletions employed are moderately short. The deletion *r61* does not cover any of the available point-mutation sites in T4D; *rdb52* covers the separable point mutant markers *r59* and *r77* (BERGER 1965); *rJ101* covers the point mutation *rb42*, and *rb17* covers the point mutation *r71*.

All of the deletions exhibit a reversion frequency of less than 10^{-9} and do not accumulate

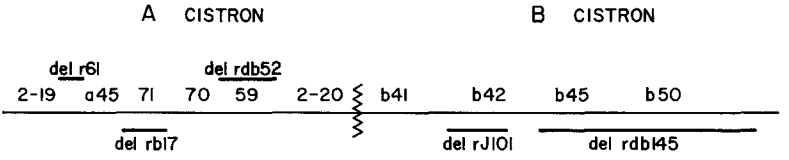

FIGURE 1.—Order of the *rII* point mutation and deletion mutation markers. Map is not drawn to scale.

heterozygotes when crossed with wild type in the presence of FUdR (BERGER 1965, and unpublished observations; DOERMANN and PARMA 1967).

RESULTS

Table 1 shows the results of crosses done to determine the HNI when either the deletion mutant *rd52* or the point mutant *r59* is used as the central marker in three-factor crosses. In all cases the level of HNI is decreased when *rdb52* is used as the central marker as compared to the crosses using *r59* as the central marker. The comparative decrease ranges from greater than 2-fold with the most distant outside markers (*r2-19* to *rb42*) to nearly 5-fold with the closest set of outside markers (*r70* to *r2-20*).

We have also performed similar although less extensive crosses using several other deletion mutations. The results of these crosses are shown in Table 2. With the deletions *rJ101*, *rB17*, and *r61* as central marker HNI is consistently reduced as compared to control crosses using the point mutants *rb42*, *r71*, and *ra45*. The reductions generally are less pronounced than observed with the deletion *rdb52*.

TABLE 1

Decreased HNI when crossovers are on both sides of the deletion mutation rdb52 *as compared to the point mutation* r59

Outside markers (r_a–r_c)	Percentage of *rII*+ recombinants between outside markers	Cross	*rdb52* (r_b) Observed* percent	*rdb52* (r_b) Expected† percent	*rdb52* (r_b) Coefficient of coincidence	*r59* (r_b) Observed* percent	*r59* (r_b) Expected† percent	*r59* (r_b) Coefficient of coincidence
r2-19–rb42	3.1	a)	.0829	.0236	3.5	.277	.0361	7.7
		b)	.0831	.0212	3.9	.246	.0395	6.2
		c)	.0838	.0208	4.0	.276	.0298	9.3
		average	....	....	3.8	...	....	7.7
r2-19–r2-20	2.0	a)	.0204	.00480	4.2	.160	.0133	12.0
		b)	.0237	.00562	4.2	.181	.0143	12.7
		average	....	....	4.2	...	....	12.3
r71–rb41	1.7	a)	.0362	.00705	5.1	.228	.0179	12.7
r70–rb42	1.5	a)	.0122	.00176	6.9	.132	.00630	21.0
		b)	.0130	.00249	5.2	.113	.00737	15.3
		average	....	....	6.0	...	....	18.4
r70–rb41	1.3	a)	.0117	.00183	6.4	.129	.00750	17.2
r71–r2-20	1.0	a)	.0125	.00126	9.9	.130	.00552	23.6
		b)	.0153	.00200	7.7	.125	.00645	19.4
		average	....	....	8.8	...	....	21.5
r70–r2-20	0.6	a)	.00576	.000700	8.2	.097	.00276	35.2
		b)	.00517	.000623	8.3	.121	.00327	37.0
		average	....	....	8.2	...	....	36.1

* The values are the percentages of *rII*+ recombinants.

† The product of the *rII*+ recombinant frequencies in region a_a–r_b and r_b–r_c obtained from 2-factor crosses done simultaneously.

TABLE 2

Effect of deletion mutants rJ101, rb17, *and* r61 *on HNI*

Outside markers (r_a–r_c)	Percentage of *rII*+ recombinants between outside markers	Central marker (r_b)	Marker Type	Average coefficient of coincidence
r70–r45	1.7	*rJ101*	del	4.7
		rb42	pt	9.2
rb41–rb50	1.2	*rJ101*	del	10.7
		rb42	pt	13.9
r2-19–r2-20	2.0	*rb17*	del	7.0
		r71	pt	13.7
r2-19–r2-20	2.0	*r61*	del	9.4
		ra45	pt	11.5
r2-19–r71	1.2	*r61*	del	15.1
		ra45	pt	18.0

DRAKE (1967) has indicated that phage which contain long deletions are prevented from undergoing some genetic interaction with normal phage. We have performed some experiments to determine if recombination in a short interval of the *rII* region is affected by the presence of outside deletions in one of the two parents in a phage cross. A phage of the type $r_a r_b r_d r_e$ where r_a and r_e are deletions was crossed with a point mutant, r_c, and the frequency of *rII*+ double recombinants was determined. The frequency of *rII*+ double recombinants was also determined from a cross of the type $r_b r_d$ by r_c (using the same point mutations but in which the deletions were not present). As seen in Table 3, the presence of nearby outside deletions does not alter the *rII*+ recombination frequency.

TABLE 3

Lack of effect of outside rII *deletion mutations on the frequency of* rII+ *double recombinants*

Cross* *with deletions* *rdb52/r2-20/rb42/rdb145* × *rb41*	Percentage *rII*+ recombinants
a	.040
b	.023
c	.026
	average = .030
without deletions *r2-20/rb42* × *rb41*	Percentage *rII*+
a	.049
b	.029
c	.022
	average = .033

*Crosses with the same letter were done simultaneously using the same host bacterial culture.

DISCUSSION

Our results indicate that HNI is decreased when the central marker in three factor crosses is a deletion mutation instead of a point mutation. This observation is most simply explained by a model in which double recombinants are formed by insertion of complementary single stranded segments of one parental genome into another (Figure 2). Presumably the insertion would be prevented or decreased when one genome contains a deletion mutation.

The decreases in HNI are most obvious with the deletion mutant *rdb52* (Table 1). In this case the decreases in HNI are from 2 to 5-fold compared to the control crosses which use a point mutation as the central marker. The greatest differences are observed when the distance between outside markers is small. The results of crosses using other deletions as central markers also show reduced HNI compared to the corresponding control crosses (Table 2). The two crosses with the deletion *rJ101* show decreases in HNI of 49% and 23%. The single cross with the deletion *rb17* shows a 49% reduction in HNI, while with the deletion *r61* two sets of crosses show decreases of only 16 to 18%. Although HNI is consistently lower when a deletion mutant is the central marker the reductions seem to be greatest with the deletion *rdb52*. We cannot adequately explain these quantitative differences among deletions, although *r61* may be a very small deletion since it has not been shown to cover any point mutation markers. It is conceivable that the number of bases deleted relative to the average size of the pairing region could affect the efficiency of segment incorporation. This suggestion does not, however, explain the observation that short deletions behave in an identical manner to longer deletions with respect to heterozygote formation in normal crosses (NOMURA and BENZER 1961) and in crosses done in the presence of FUdR (SECHAUD *et al.* 1965).

DOERMANN and PARMA (1967) also investigated the effect of deletions on HNI. From a single cross between two parents, one carrying 4 *rII* point mutations and the other carrying 4 *rII* deletion mutations, they determined the frequency of triple-mutant crossovers in which the central marker was a deletion as opposed to a point mutation. They found that the level of HNI was nearly identical when the central marker was a point mutant or a deletion mutant. However, it should be noted that DOERMANN and PARMA scored for triple-mutant recombinants while we have scored only wild-type recombinants. It is possible that deletion—wild-type genetic pairing occurs (perhaps by the formation of a loop) and that there is subsequent excision and repair of the section of DNA within the loop. Such a hypothetical process could result in the loss of wild-type double recombinants but not of triple-mutant double recombinants. It is of interest that duplex DNA containing single-stranded loops has been observed in reannealed preparations of DNA from wild type and deletion mutants of phage λ (DAVIS and DAVIDSON

FIGURE 2.—Hypothetical insertion model for the formation of double recombinants. The horizontal solid and dashed lines represent single strands of a DNA duplex.

1968). The notion that deletion—wild-type pairing occurs is also in accord with DRAKE's (1967) conclusion that moderate sized deletions can undergo genetic pairing with normal genomes.

Other possibly significant differences concern the distribution of markers in DOERMANN and PARMA's studies. In all cases in which the central marker was a point mutation the outside markers were deletions and in all cases in which the central marker was a deletion the outside markers were point mutants. Also, the distances between outside markers were generally greater than those in which we observe the most pronounced decreases in HNI.

We are currently investigating the role of repair processes and the effects of T4 phage mutations which alter genetic recombination (BERGER, WARREN and FRY 1969) on HNI.

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SUMMARY

High negative interference over short segments of the *rII* region of T4D bacteriophage is reduced when the central marker in three-factor crosses is a deletion.

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