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Variations in Genetic Recombination Due to Amber Mutations in T4D Bacteriophage

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Recombination experiments were performed to assess the affect of amber mutations in 12 genes of T4D bacteriophage on genetic recombination. Crosses were performed in various suppressor-containing bacterial hosts to permit the production of progeny phage. Amber mutations in genes *32*, *46*, and *47* caused decreased recombination, amber mutations in genes *30*, *41*, *42*, *43*, *56*, *61*, and *62* caused increased recombination, whereas mutations in genes *63* and *37* showed no demonstrable effect on recombination.

Genetic and biochemical investigations with T4 and λ bacteriophages have indicated that one or more bacteriophage genes participate in genetic recombination after phage infection (4, 9, 11, 12). The more than 50 genes identified in T4 bacteriophage have been classified into groups which affect early and late bacteriophage functions (5, 6). We selected amber conditional lethal mutations in 11 early function genes and 1 late function gene of T4D bacteriophage and attempted to ascertain their effect on recombination and on heterozygosis of progeny phage. Since most of the amber mutants are lethal in *Escherichia coli B* (because this strain lacks an effective amber or ochre nonsense suppressor), crosses were done in a set of isogenic strains of *E. coli* W3110, into which we introduced one of five different nonsense suppressors. Two of the suppressors, *suA* and *suC*, are ochre suppressors and result in approximately a 3 to 5% restoration of gene function to a given amber mutant of *E. coli* [*see* Garen (7) for a review]. The three other suppressors, *su1*, *su2*, and *su3*, are amber suppressors which restore from 30 to 50% gene function in various amber mutants of *E. coli*. Although the effectiveness of a nonsense suppressor gene has been determined primarily with studies of amber mutants in various genes of *E. coli*, it was generally found that the degree of permissiveness for T4 amber mutants in the various *su* bacterial strains was in agreement with the previously determined suppression levels. In general, T4 amber mutants produced low burst sizes in the *suA* and *suC* strains and nearly normal burst sizes in the *su1*, *su2*, and *su3* bacterial strains.

Bernstein (1; Cold Spring Harbor Symp. Quant. Biol., *in press*) also investigated the role of various T4 genes in recombination by studying the effects of temperature on the recombination frequencies in phage stocks containing temperature-sensitive conditional lethal mutations. In agreement with Bernstein's studies, our results indicate that limited function of genes *46* and *47* causes a marked decrease in phage recombination. In addition, our studies indicate that limited function of gene *32* results in decreased recombination frequencies. Heterozygosity for the point mutant *r48* is also decreased when the function of genes *32*, *46*, or *47* is inhibited.

MATERIALS AND METHODS

Phage stocks. The amber mutants were kindly supplied by R. Edgar. The *rII* mutants, *r70* and *r2–20*, and the *rI* mutant *r48* were obtained from A. H. Doermann. Stocks of *r2–20* and *r70*, which contained a specific amber mutation, were constructed by recombination.

Bacterial strains. The strains of *E. coli* W3110 AB24 containing, respectively, the suppressor genes *sul*, *su2*, *su3*, *suA*, and *suC* were obtained from C. Yanofsky. Cross lysates were plated on *E. coli* CR63 (permissive for both amber and *rII* mutants) to determine total progeny and on *E. coli* CR63 [(λh) restrictive for rII mutants] to determine the percentage of rII$^+$ recombinants.

Phage crosses. Crosses of $am_x r70$ by $am_x r2–20$ were carried out according to the methods of Chase and Doermann (2). For each amber gene tested, simultaneous crosses of am^+r70 by $am^+r2–20$ in each of the host strains were carried out. In each experiment, a minimum of 250 total progeny and 250 rII$^+$ recombinants were counted. Heterozygotes for the *r48* marker were determined by scoring for mottled plaques (8). Control crosses of $am^+r48 \times am^+r48^+$ were done simultaneously, and all scoring was done from coded plates to prevent bias. The gene *47* mutant, *amA456*, produces small plaques when plated on *E. coli* CR63,

TABLE 1. *Effect of amber mutation on r70 to r2–20 recombination frequency*

Gene no.	Amber mutant	Defect	Host	No. of crosses	am		No. of crosses	am		am/am$^+$ recombinants
					Avg burst size	Average per cent r^+ recombinants		Avg burst size	Average per cent r^+	
30	*amE605*	DNA- (ligase)	*su1*	1	195	0.71	1	251	0.80	0.89
			su2	1	160	0.69	1	202	0.72	0.96
			su3	1	150	0.81	1	109	0.92	0.88
			suC	1	87	1.10	1	98	0.99	1.11
			E. coli B	1	16	0.87	1	97	0.53	1.64[a]
32	*amA453*	DNA-	*su1*	4	145	0.85	3	158	0.83	1.02
			su2	4	86	0.68	3	149	0.83	0.82
			su3	4	62	0.75	3	177	0.77	0.97
			suA	2	1.1	0.22	3	206	0.62	0.35[a]
			suC	6	1.5	0.29	5	206	0.70	0.41[a]
37	*amN52*	Tail fibers	*su1*	1	191	0.86	1	172	0.61	1.41
			su2	1	167	0.66	1	200	0.55	1.20
			su3	1	206	0.71	1	202	0.58	1.22
			suA	2	6.6	0.38	2	182	0.50	0.76
			suC	2	5.0	0.40	2	233	0.48	0.84
41	*amN81*	DNA-	*su1*	1	319	0.75	1	158	0.81	0.92
			su2	1	318	0.72	1	205	0.54	1.33
			su3	1	310	0.88	1	257	0.79	1.11
			suA	1	194	1.79	1	520	0.95	1.89[a]
			suC	1	103	1.98	1	208	1.15	1.72[a]
42	*amN122*	DNA$^-$ (dCMP hydroxymethylase)	*su1*	1	245	1.20	1	190	0.53	2.26[a]
			su2	1	48	0.95	1	94	0.59	1.62[a]
			su3	1	44	1.30	1	132	0.55	2.36[a]
			suA	2	8.8	1.33	2	167	0.50	2.60[a]
			suC	2	8.0	1.50	2	220	0.47	3.20[a]
43	*amB22*	DNA- (DNA polymerase)	*su1*	1	158	0.93	1	283	0.53	1.75
			su2	1	335	0.78	1	212	0.58	1.34
			su3	1	192	0.91	1	173	0.53	1.72
			suA	1	144	1.80	1	282	0.61	2.95[a]
			suC	1	60	1.65	1	195	0.58	2.85[a]
46	*amN130*	DNA arrest	*su1*	2	228	0.68	2	213	0.65	1.0
			su2	1	211	0.61	1	193	0.75	0.81
			su3	1	180	0.53	1	166	0.82	0.65
			suA	4	92	0.23	4	198	0.55	0.42[a]
			suC	4	91	0.41	4	155	0.60	0.68[a]
			E. coli B	2	2.3	0.09	2	158	0.67	0.13[a]
47	*amA456*	DNA arrest	*su1*	2	37	0.16	2	208	0.50	0.32[a]
			suA	2	10	0.10	2	193	0.58	0.17[a]
			suC	2	11	0.19	2	101	0.67	0.28[a]
			E. coli B	2	3.0	0.08	2	158	0.57	0.14[a]
46	*amA456*	DNA arrest	*su1*	1	47	0.21	1	316	0.39	0.54[a]
47	*amN130*		*suA*	1	14	0.10	1	254	0.44	0.23[a]
			suC	1	46	0.26	1	308	0.45	0.58
			E. coli B	1	5	0.08	1	186	0.54	0.15[a]
56	*amE51*	DNA- (deoxycytidine triphosphate phosphatase)	*su1*	1	167	0.55	1	208	0.61	0.90
			suA	1	2	1.37	1	193	0.73	1.88[a]
			suC	1	1	1.49	1	101	0.72	2.06[a]

TABLE 1. *Concluded*

Gene no.	Amber mutant	Defect	Host	No. of crosses	am		No. of crosses	am		am/am$^+$ recombinants
					Avg burst size	Average per cent r^+ recombinants		Avg burst size	Average per cent r^+	
61	*amE219*	DNA delay	*su1*	1	156	1.45	1	184	0.80	1.81[a]
			su2	1	170	1.94	1	198	0.64	3.04[a]
			su3	1	180	1.90	1	440	0.40	4.75[a]
			suA	1	132	1.95	1	222	0.51	3.82[a]
			suC	1	83	3.10	1	146	0.65	4.77[a]
62	*amE1140*	DNA$^-$	*su1*	1	268	0.70	1	225	0.44	1.59
			su2	1	286	0.76	1	219	0.65	1.17
			su3	1	199	0.51	1	251	0.34	1.50
			suA	1	332	0.82	1	220	0.63	1.30
			suC	1	172	1.67	1	274	0.44	3.80[a]
63	*amM69*	DNA$^-$	*su1*	1	124	0.76	1	126	0.84	0.91
			su2	1	129	0.71	1	162	0.66	1.08
			su3	1	217	0.84	1	160	0.80	1.05
			suA	1	41	0.59	1	206	0.81	0.71
			suC	1	29	0.61	1	134	0.85	0.71

[a] Indicates ratio is significantly different from 1.0 at 95% or greater confidence level by t test.

making mottled plaque recognition extremely difficult. To overcome this difficulty, heterozygotes among the progeny of the crosses of *r48 amA456* × *r48*$^+$ *amA456* were determined by analysis of individual progeny. Individual plaques were picked, resuspended in broth, and plated at an appropriate dilution; the plates were scored for the presence of *r48* and *r48*$^+$ plaques.

RESULTS

Table 1 shows the burst size and recombination percentages obtained for the interval *r70* to *r2–20*, when stocks containing a given amber mutant were crossed in the various permissive and semipermissive hosts. Eleven genes in which amber mutants exhibit some defect in phage deoxyribonucleic acid (DNA) synthesis (early functions) and one gene involving tail fiber production (late function) were examined. Seven of the early genes, which result either in a lack of phage-specific DNA synthesis after infection of the nonpermissive host (genes *30*, *41*, *42*, *43*, *56*, and *62*) or in a delay of DNA synthesis (gene *61*; 5, 6), showed varying degrees of enhanced recombination compared to the *am*$^+$ controls. This was most evident in the lysates from crosses in the weak suppressor hosts *suA* and *suC* or in the suppressorless host *E. coli* B. In some cases (genes *42* and *61*, Table 1), the enhancement was also apparent in the strong suppressor hosts. One of the early function genes (gene *63*) and one of the late function genes (gene *37*) did not show an appreciable affect on recombination although burst sizes were decreased in the weak suppressor hosts. Most interesting are the results obtained with genes *32*, *46*, and *47*. In these cases, recombination was decreased as a result of amber mutations. Since the mutants in genes *46* and *47* produce some phage in the nonpermissive host *E. coli* B, recombination levels were also examined in this strain. As can be seen in Table 1, there was a 6- to 10-fold decrease in *rII* recombination in the presence of amber mutations in these two genes. Similar decreases were also seen in crosses with parental phage which contained two amber mutations, one in gene *46* and one in gene *47*. There is no obvious relationship between burst size and recombination. For example, low burst size is associated with unchanged recombination (gene *63*), with elevated recombination (gene *42*), and with reduced recombination (gene *47*).

Table 2 shows the results obtained from measurements of the frequency of *r48* heterozygotes in lysates from crosses done with stocks containing different amber mutations. Amber mutants which affect recombination levels similarly affect heterozygote frequencies. For example, with *amE219* (gene *61*), there was approximately a 4-fold increase in *r48* heterozygotes in crosses done with *suC* host cells (Table 2) and a corresponding 4.8-fold increase in the *r70* to *r2-20* recombination frequencies (Table 1).

Amber *B22* (gene *43*) is the only case in which the recombinational effect, in this case increased recombination, was not parelleled by a similar increase in heterozygote frequency. Although the heterozygote frequency is higher in the presence

TABLE 2. *Effect of amber mutations on r48 heterozygote frequency*

Gene no.	Mutant	Defect	Host	am		am+		am/am+
				No. mottled	Per cent mottled	No. mottled	Per cent mottled	
30	*amE605*	DNA⁻	*su1*	41	0.47	34	0.45	1.0
			suC	46	0.60	78	0.83	0.72
32	*amA453*	DNA⁻	*su1*	114	0.87	91	0.83	1.0
			suA	43	0.30	68	0.91	0.33
			suC	55	0.47	77	0.88	0.53
43	*amB22*	DNA⁻	*su2*	46	0.90	42	0.85	1.1
			suA	45	1.08	45	1.01	1.1
			suC	175	1.33	100	0.97	1.4
46	*amN130*	DNA arrest	*suC*	42	0.68	78	0.83	0.77
			E. coli B	29	0.58	45	1.14	0.51
47	*amA456*	DNA arrest	*E. coli* B	5	0.74	6	1.95	0.38
61	*amE219*	DNA delay	*su1*	57	1.2	34	0.45	2.7
			su2	179	1.96	110	0.62	3.1
			suC	185	3.28	78	0.83	4.0

of *amB22*, the difference is slight compared to the effect on recombination.

DISCUSSION

Tomizawa and Anraku (12) showed that the gene *32* function is required for the formation of "joint" DNA molecules. These "joint" molecules have been proposed to be intermediates in the formation of T4 phage recombinants. Our findings of decreased genetic recombination under experimental conditions in which gene *32* function is limited lend support to this suggestion. Of equal interest is the observation that limiting the function of genes *46* or *47* also reduces recombination. Studies by Wiberg (14) and Warren and Bose (13) indicate that these genes are necessary for the breakdown of host DNA after T4 infection. Failure of these genes to function also results in arrest in the synthesis of phage-specific DNA. Our experiments may indicate that the gene *46* and *47* product(s) are directly involved in the recombination process. Alternatively, the effect on recombination may be an indirect one related perhaps to the presence of undegraded host DNA. Additional biochemical and genetic experiments are underway (Shah and Berger, *unpublished data*) to determine more directly the role of these genes in recombination.

The specific enzymatic defects have been identified in three of the six amber mutants which caused increased recombination. Gene *42* is the structural gene for deoxycytidine monophosphate (dCMP) hydroxymethylase (3), gene *43* is responsible for DNA polymerase (14), and gene *56* specifies a phage deoxycytidine triphosphate phosphatase (14). The product of genes *41*, *61*, and *62* has not been identified. It has been shown that phage DNA synthesis does not occur after infection with mutants in genes *41*, *42*, and *43* and *56* and *62* (5, 6). Gene *61* causes a delay in the synthesis of phage-specific DNA after infection of the nonpermissive host (5). The reason for the increased recombination frequencies which result from the presence of amber mutations in genes *30*, *41*, *42*, *43*, *56*, *61*, and *62* is obscure. Bernstein (1) has suggested that the effect of lack of DNA polymerase (gene *43*) is to leave gaps in the deoxyribose-phosphate backbone of DNA, thus increasing the probability of breakage and rejoining with homologous chromosomes. Bernstein also suggested that structural errors in DNA and the consequent alteration in DNA glycosylation resulting from mutation in gene *42* (dCMP hydroxymethylase) are responsible for the increased recombination with this gene. Since many of the mutants which cause increased recombination are defective in the synthesis of phage DNA, it is possible that the observed effect is due to inhibition of DNA synthesis per se rather than to modifications of the DNA structure. Sechaud et al. (10) showed that heterozygote and recombination frequencies are increased in crosses carried out under conditions of 5-fluorodeoxyuridine inhibition of DNA synthesis. If inhibition of

DNA synthesis per se is generally responsible for the observed recombination increases, it is possible that the decreased recombination associated with genes *32*, *46*, and *47* may be partially masked, since these genes also inhibit or modify DNA synthesis.

It can be assumed that the marker effect on recombination in the *rII* region alters recombination throughout the phage genome; hence, the effect on recombination described herein should be considered with respect to mapping experiments and studies designed to correlate the genetic and physical maps of the T4 genome. Some of the genes examined, such as *42*, *43*, and *61*, show effects on recombination even under conditions of maximum suppression (*su1* host).

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