MARKER EFFECTS IN THE GENETIC TRANSDUCTION OF TRYPTOPHAN MUTANTS OF *E. COLI*[1]

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ABSTRACT

Recombination frequencies have been determined in crosses involving 28 mutant strains for 20 of which the site of the alteration is known from studies of amino-acid substitutions in the protein products. Three of these mutants showed especially high frequencies of recombination when crossed to other single mutants or when crossed to a strain carrying two alterations at opposite ends of the *trpA* gene. There is no obvious molecular explanation of the high recombination of these three mutants. They include one missense mutant, one amber and one ochre. The low-frequency recombination mutants include all these same classes as well as frameshift mutants. There is nothing unique about the intragenic location of the high-recombination mutants; in each case there is at least one low-recombination mutant in the same codon.——Crosses involving mutants which were isolated in an altered wild type have shown that the behavior of a high-recombination mutant does not result from its molecular configuration alone, but from its combination with the homologous wild-type sequence from the other parent.——Several lines of evidence indicate that recombination in this system frequently involves closely-spaced double exchanges (about 40 codons apart).

MARKERS are segregating sites and are used in genetic experiments to reveal interactions between homologous chromosomes. A useful assumption is that the markers only reveal recombination and do not influence its occurrence. This assumption seems to be valid for recombination events taking place some distance (several gene lengths) from the segregating sites, but it is quite possible that events within the same gene as the segregating markers are altered in some way.

Studies of meiotic recombination in ascomycetes have suggested the occurrence of marker effects. Cases have been reported in Ascobolus (KRUSZEWSKA and GAJEWSKI 1967) and in fission yeast (GUTZ 1971) in which two mutants which are at or near the same site give very different frequencies of recombination when both are crossed to the same third mutant elsewhere in the gene. One limitation of this observation is that the basis for assuming that the first two mutant sites are close together comes from recombination—the same mysterious process that is under study.

An unambiguous test for the occurrence of marker effects can only be made with a system in which the physical positions of the markers are determined by a

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method other than recombination mapping. It is also desirable to know the base sequences in the mutant and wild-type genes in order to find out whether marker effects are based on particular molecular configurations. This paper reports the results of studies of recombination by transduction in the *trpA* gene of *E. coli*. YANOFSKY and his co-workers have amassed a large amount of information about the amino acid sequence of the protein product of this gene: the α-chain of tryptophan synthetase. Their comparison of the wild-type enzyme to those forms produced by particular mutants and revertants has revealed the positions of the genetic alterations (Table 1). In some cases they have been able to determine the base sequence for a segment of the gene and the base alterations involved in particular mutation events.

The first question posed here was: do marker effects occur? Having concluded that they do, we posed several additional questions:

1) Do marker effects result from a particular base sequence?

2) Do they result from a particular kind of mutational alteration (base substitution as opposed to addition or deletion, transition as opposed to transversion)?

3) Do marker effects depend on the mutant base sequence *per se* or on its association with a specific sequence from the other parent?

4) What aspect of the recombination process is marker-dependent? Do the high-frequency markers cause more frequent recombination events? Do they cause closer-spaced exchanges in the normal events? Or do they cause a completely different kind of recombination event?

TABLE 1

TrpA *mutants of known location*

Mutant	Codon	mRNA codon and amino acid	
		Wild type	Mutant
A38	15	AAA lys	UAA ochre
A218	22	UUU_C phe	CU^U_C leu
A33	49	GAG glu	AUG met
A88	49		UAG amber
A446	175	UAU tyr	UGU cys
A487	177	CUG leu	CGG arg
A223	183	AC^U_C thr	AU^U_C ile
A23	211	GGA gly	AGA arg
A46	211		GAA glu
$A46^{Asp}$	211		GAU asp
$A46^{Asp}MUT$	211		GAC asp
A187	213	GG^U_C gly	GU^U_C val
A58	234	GG^U_C gly	GA^U_C asp
A78	234		UG^U_C cys
A96	243	CAA gln	UAA ochre

Amino acid abbreviations: arg: arginine; asp: aspartate; cys: cysteine; gln: glutamine; glu: glutamate; gly: glycine; ile: isoleucine; leu: leucine; lys: lysine; met: methionine; phe: phenylalanine; thr: threonine; tyr: tyrosine; val: valine.

This paper seeks answers to the above questions through recombination studies with mutants known to result from specific molecular changes at specific sites in the *trpA* gene.

MATERIALS AND METHODS

Mutant strains: All the mutant strains and the molecular information about them were obtained from CHARLES YANOFSKY. The mutants of known location are described in Table 1. Some crosses involved ochre mutants of the *A* gene for which the specific location is not known (*A7*, *A13*, *A17*, *A44* and *A74*).

In the tables the mutants are designated by the isolation numbers of YANOFSKY with the prefix "*A*" omitted. Amber derivatives of ochre mutants are designated *17am*, *38am*, *54am* and *96am*. Mutant $A46^{Asp}$ is called *46A*, and $A46^{Asp}MUT$ is called *46M*.

Measurements of recombination: Recombination was measured in transduction experiments using the temperate phage P1kc. Map distances within the *trpA* gene were measured by the frequency of *trp*+ progeny in crosses in which both donor and recipient carried mutations in *trpA*. The specific procedures used in the preparation of lysates and recipient cells and in performing the crosses were those of YANOFSKY and LENNOX (1959). All recipient strains were *his*−, and the frequencies of *trp*+ transductants were normalized to the frequency of the unlinked transduction to *his*+ in the same cross. All the *trp*+ recombination frequencies in the tables represent the number of *trp*+ transductants per hundred *his*+ transductants in the same cross. Absolute frequencies of *his*+ transductants were from two to four per hundred thousand recipient cells. A typical datum resulted from counts of 100–300 *trp*+ colonies and a similar number of *his*+ colonies. In many cases the same cross was repeated in two or more experiments, and these gave good agreement (see Tables 5 and 6 for examples).

Construction of trpA *double mutants:* Two stocks were constructed which each contained two ochre mutations near opposite ends of the *trpA* gene: *A38* on the left and *A96* or *A109* on the right. Double mutant *38–96* was built by transducing a *cysB A96* recipient with phage from a *cys*+ *A38* donor. *Cys*+ recombinants were selected on minimal medium plus indole. *CysB* and *trpA* are co-transduced with a frequency of about 50%, and some fraction of these are recombinant with respect to the marked sites in *trpA*. The *trpA* double mutants were recognized by virtue of the fact that ochre suppressors do not suppress *trpA* ochre double mutants (YANOFSKY and ITO 1966). The recipient parent of this cross also carried the *gal*-linked ochre suppressor su_{oc}-*A*. Thus the recipient parent was tryptophan-independent as were all progeny that carried either of the single *trp* mutants. The only tryptophan-requiring progeny were those carrying both mutations. One such strain was isolated, and its genotype was confirmed by the failure to produce *trp*+ recombinants in separate crosses to *A38* and to *A96*. The same procedure was used to isolate the double mutant *38–109*.

Selection of ochre-to-amber mutations: Spontaneous *trp*+ revertants were selected from ochre mutant strains *A17* and *A54* which carried amber suppressors. These reversions could result from any of three different genetic events: a new ochre suppressor mutation, a back-mutation at *trpA* or a change from ochre (UAA) to amber (UAG) at the mutant site in *trpA*.

The revertants which resulted from ochre suppressor mutations were screened out by a test for the ability to suppress a T4 ochre mutant (YANOFSKY and ITO 1966). Cells were streaked on nutrient agar plates and a loopful of the T4 lysate was spotted on the streak. The plates were scored after 72 hours' incubation at 37°. A clear area of lysis indicated that the bacteria carried the ochre suppressor which was required for phage growth.

P1 lysates were prepared from revertants which did not carry ochre suppressors. The lysates were used as donors in transduction with a recipient strain carrying a deletion of the tryptophan operon (this strain would grow on medium supplemented with tryptophan but not on medium supplemented with indole). Transductants were selected on minimal medium plus indole and replicated to minimal. If the donor had been a *trp*+ back mutant, all the selected transductants would grow on minimal. If the donor had been a suppressed amber mutant, transductants receiv-

TABLE 2

Frequencies of trp+ *recombinants in three-point crosses at* trpA

Single mutant parent	Donor *38–96*	Recipient *38–96*	Donor *38–109*	Recipient *38–109*
Single mutants of known location				
23	1.88 (8)	1.69	1.86 (6)	1.76
88	1.82	1.89	1.54	0.85
187		0.61 (2)		
223		0.55 (2)		
487		0.33		
58	0.26 (3)	0.25	0.42 (3)	0.12
78	0.26 (3)		0.32 (3)	
33	0.15	0	0.14	0
46	0.07 (6)	0.10	0.11 (4)	0.09
446		0.06		
46A	0.03	0.05	0	0.05
46M	0.04			
Single mutants of unknown location				
54	1.60 (2)	0.78	1.33	0.88 (2)
54am	0.02 (2)		0	0.08
44	0.09		0.08	
74	0.14		0.14	
7	0.26		0.04	
13	0.18	0.02	0.09	0.08 (2)
17	0.35 (2)	0	0.13	0.06 (2)
17am	0			
Control crosses				
38am				0
38	0.06 (6)	0.02	0.08 (5)	0 (2)
96	0.01 (5)	0	0 (4)	0
109	0 (2)		0 (2)	
96am	0		0	

Note: In those cases in which the same cross was made more than once, the results have been averaged, and the number in brackets tells how many times it was made.

ing the *trpA* gene but not the amber suppressor would grow on indole but not on minimal. The interpretation was confirmed by checking the parents and transductants of this cross for the presence of an amber suppressor by the T4 lysis test (using a T4 amber mutant).

Construction of trpE trpA *double mutant strains:* Mixtures of *cys*+ *trpA*— donor phage and *cys*— *trpE*— recipients were plated on minimal medium plus indole for *cys*+ transductants. These included wild type, *trpA* and *trpE* single mutants and the double mutant. Wild-type transductants were identified by their ability to grow on minimal medium, and the *trpE* single mutants were recognized by growth on anthranilate. The *trpA* single mutants and the double mutants both required indole for growth. The double mutants were identified by their failure to give *trp*+ transductants when mixed with a lysate from *trpE*— on minimal medium.

RESULTS

Three-point crosses: Two stocks were built which each carried two mutational changes near the ends of the *trpA* gene. These were used to screen for *trpA*

mutants showing high-frequency intragenic recombination. When one of the double mutants was crossed to a strain carrying a single mutation located in the middle part of the gene, prototrophic recombinants could only result from at least two exchanges within the gene. This represented the replacement of a very short segment of one parent genome with the homologous segment of the other parent. Such an event appears to be the basis of "gene conversion" in meiotic systems, the event which gives rise to non-reciprocal segregation ratios (3:1 and 1:3). Gene conversion frequencies have been reported to show dramatic evidence of marker-specific effects.

The double-mutant stocks carried ochre mutations: *A38* on the left and *A96* or *A109* on the right. One or both of the double mutants were crossed to each of twenty single mutants which were all located between the two marked sites of the double mutants (Table 2). Twelve of these single mutants are at known positions (localized to a specific codon position by protein studies).

Three of the mutants (*A23*, *A54* and *A88*) gave significant numbers of *trp*+ recombinants and will be called HR (high recombination) mutants. Most of the others gave none or were not much above the reversion frequency and will be called LR (low recombination) mutants. The crosses of mutants *A187* and *A223* gave lower frequencies of *trp*+ recombinants than the HR mutants and they will be designated IR (intermediate recombination) mutants. Some crosses were made in both directions, and in most of these cases the results of the reciprocal crosses were similar (Table 2).

Three of the five mutants which gave significant frequencies of recombinants in the crosses to the double mutants had been shown earlier to give unusually high recombination in two-point crosses. YANOFSKY *et al.* (1964) noted high frequency recombination for *A23* and *A187*, and a similar observation regarding *A88* was made by DRAPEAU, BRAMMER and YANOFSKY (1968).

Two-point crosses: Fifty-seven pairwise combinations of fifteen single mutants of known position were crossed, and *trp*+ recombinants were selected and counted. The recombination frequencies are shown in Table 3. Figure 1 presents the same data as frequencies of recombination per codon (amino acid residue) between the two segregating sites, and they are graphed against increasing distance. The same mutants which produced prototrophs in the three-point crosses (HR and IR mutants) show high frequencies in the two-point crosses, especially in those crosses in which the segregating sites are close together. Most of the crosses between LR mutants show rates of recombination per codon in the range of 0.01 to 0.02%. This rate was previously observed by YANOFSKY *et al.* (1964) to generate a recombination map length for the *trpA* gene of 4%.

Figure 1 shows that all classes of mutants (even those showing low-frequency recombination) produce somewhat elevated frequencies of recombinants per codon in the crosses in which the segregating sites are close together (less than 40 codons). This is the pattern predicted by "negative interference" (CHASE and DOERMANN 1958): the frequent occurrence of two or more exchanges in close proximity (about 40 codons apart in the present case).

TABLE 3

Frequencies of recombination in two-point crosses between trpA *mutants of known position*

L R × L R crosses				Crosses in which at least one parent is IR or HR			
Cross	Distance (residues)	Total recombination	Recombination per residue	Cross	Distance (residues)	Total recombination	Recombination per residue
				23 × 187	2	0.18	0.090
				46 × 187	2	0.085	0.042
58 × 96	9	0.28	0.031				
58 × 96am	9	0.43	0.048				
78 × 96	9	0.38	0.042				
78 × 96am	9	0.20	0.022				
				187 × 58	21	2.12	0.101
46 × 58	23	0.96	0.042	*23 × 58*	23	2.50	0.109
46A × 58	23	0.41	0.018				
				223 × 23	28	4.51	0.161
				223 × 46	28	1.09	0.039
				187 × 96	30	1.91	0.064
				223 × 187	30	1.08	0.036
46 × 96	32	0.76	0.024	*23 × 96*	32	1.93	0.060
46 × 96am	32	1.32	0.041	*23 × 96am*	32	2.32	0.069
46A × 96	32	0.87	0.027				
46A × 96am	32	0.89	0.028				
487 × 46	34	0.63	0.019	*487 × 23*	34	1.65	0.049
38am × 33	34	1.11	0.033	*38am × 88*	34	1.94	0.057
487 × 58	57	1.11	0.019				
				223 × 96	60	1.21	0.020
487 × 96	66	1.20	0.018				
33 × 487	128	1.14	0.009				
				33 × 223	134	1.34	0.010
33 × 46A	162	1.76	0.011	*88 × 46A*	162	4.31	0.027
33 × 46	162	2.47	0.015	*88 × 46*	162	9.61	0.059
33 × 487	162	2.58	0.016	*33 × 23*	162	2.70	0.017
				88 × 23	162	5.79	0.036
				38 × 223	168	3.99	0.024
33 × 58	185	1.62	0.009	*88 × 58*	185	6.06	0.033
33 × 78	185	1.15	0.006	*88 × 78*	185	5.54	0.030
33 × 96	194	2.27	0.012	*88 × 96*	194	3.85	0.020
33 × 96am	194	1.67	0.009	*88 × 96am*	194	3.26	0.017
38 × 46	196	3.61	0.018	*38 × 23*	196	3.60	0.018
38 × 46A	196	3.66	0.019				
38am × 46A	196	2.86	0.014				
38 × 58	219	3.54	0.016				
38 × 78	219	3.87	0.018				
38 × 96	228	2.74	0.012				
38 × 96am	228	2.37	0.010				
38am × 96	228	3.60	0.016				
38am × 96am	228	2.81	0.012				

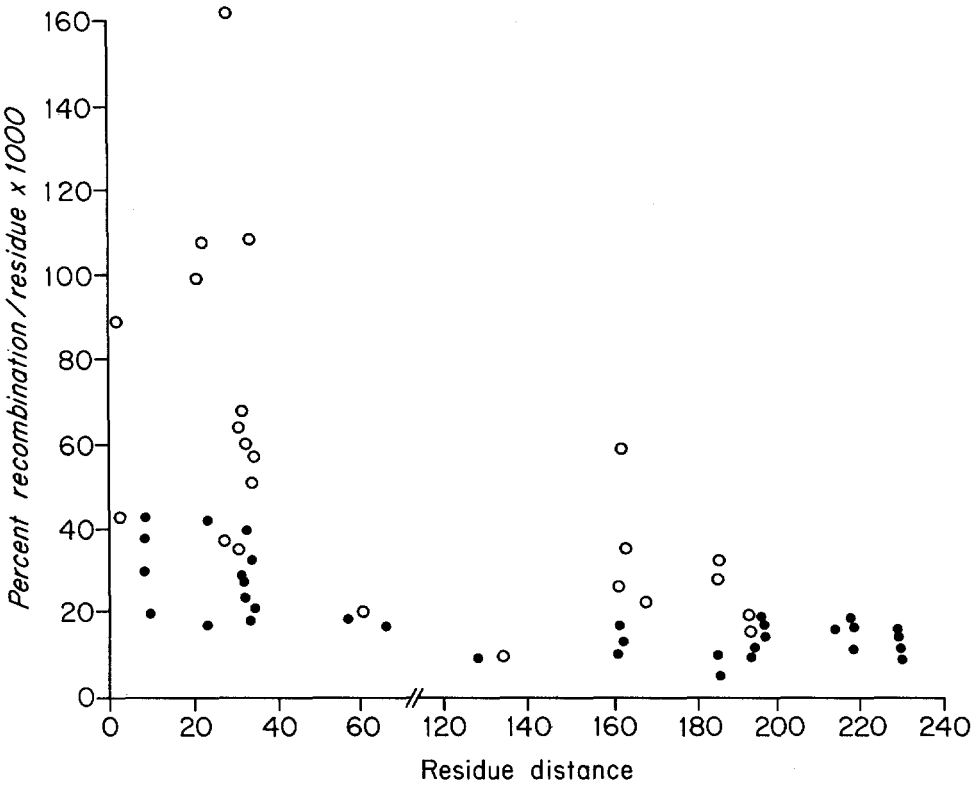

FIGURE 1.—Recombination frequency *versus* physical distance in two-point crosses between mutants of known position. Circles represent crosses in which one or both parents were HR or IR mutants. Dots represent crosses in which both parents were LR mutants.

Frameshift mutants: All the *trpA* mutations involved in the experiments described above resulted from base substitutions in the wild-type gene. Frameshift mutants result from the addition or deletion of one or two nucleotides. Three frameshift mutants in the *trpA* gene have been examined for intragenic recombination. Mutant *A9813* resulted from the deletion of an adenine nucleotide at codon 175 (BRAMMAR, BERGER and YANOFSKY 1967). Mutant *A21* resulted from either a deletion of two nucleotides at codon 215 or a single deletion at codon 216 or 217 (BERGER, BRAMMAR and YANOFSKY 1968). Mutant *A504* resulted from either a deletion of two nucleotides at codon 216 and/or codon 217 or a single deletion at codon 218 (BERGER, BRAMMAR and YANOFSKY 1968).

All three of these mutants have high frequencies of spontaneous reversion which interfere with the accurate measurement of intragenic recombination frequencies. In order to distinguish transductants from revertants, we built each of the three frameshift mutants into a double mutant with *cysB*. In the subsequent crosses we selected and counted *cys*+ *trp*+ co-transductants (MALING and YANOFSKY 1961).

It is difficult to make any *a priori* prediction about the frequency of *cys*+ *trp*+ transductants in these crosses, as it will depend on the relative positions of the two parental *trpA* mutations with respect to *cysB*. Therefore, an important control is provided by a base-substitution mutant of high recombination frequency and another of low recombination frequency located very near the site of the frameshift mutant under study. Therefore *A23* and *A46* (which are both near the sites of *A21* and *A540*) were built into double mutants with *cys* and studied in corresponding crosses to those of the frameshift mutants.

The results are shown in Table 4. In crosses to the double mutant *A38–109*, all three frameshift mutants behave like the base-substitution mutant *A46*. In the crosses to single mutants, *A21* and *A540* generally behave like low-recombination mutants, although the results are somewhat variable. Mutant *A9813* gives somewhat higher recombination frequencies with the single mutants at the right-hand end of the gene (*A58*, *A96*, *A109*), but *A9813* is located about 40 codons farther to the left than the other frameshift mutants or the controls.

We conclude that at least two of the three frameshift mutants show the low-frequency intragenic recombination which is characteristic of the majority of the base substitution mutants which have been examined.

BERGER, BRAMMER and YANOFSKY (1968) also studied revertants of frameshift mutants, which carried base additions or deletions at two closely-linked sites in the *A* gene. We have compared three of these revertants to wild type for their efficiency as donors in *trpA*+ transduction, and have found very similar rates. The average frequency of *trp*+ transductions in all these crosses was about 1.8 times the frequency of the unlinked *his*+ transduction, as noted previously by YANOFSKY and LENNOX (1959).

Molecular stimulus for high-frequency recombination. What are the significant molecular features which cause high-frequency recombination in crosses involving *A23* and low frequency in crosses of *A46* (a different base-substitution mutant in the same codon)? There is abundant evidence that crosses involving *A23* show high-frequency recombination whether that mutant enters the cross as donor or recipient. Evidence of this type has already been shown in the results of the three-point crosses in Table 2. Many of the two-point combinations of Table 3 were also crossed in both directions, with reciprocal crosses giving similar results. Table 5 gives results of triplicate experiments in which *A23* and *A46*

TABLE 4

Frequencies of cys+ trp+ *transductants in crosses involving frameshift mutants*

	Recipient				
Donor	his cys 23	his cys 46	his cys 21	his cys 540	his cys 9813
38–109	1.00	0.05	0.12	0.16	0.04
17	1.12	0.06	0.74	0.57	0.65
58	1.60	0.42	0.78	0.59	1.33
96	1.40	0.20	0.79	0.47	1.00
109	1.48	0.61	0.81	0.77	1.82

TABLE 5

Trp+ *transduction frequencies in reciprocal crosses*

Donor	Recipient	Frequency		
23	*his 17*	0.75	0.78	1.11
17	*his 23*	1.25	1.28	1.07
46	*his 17*	0	0.18	0
17	*his 46*	0	0.17	0.09
23	*his 96*	1.76	1.74	1.77
96	*his 23*	1.64	1.50	1.13
46	*his 96*	0.82	0.88	1.01
96	*his 46*	0.43	0.38	0.81

were crossed reciprocally with a nearby mutant on the left (*A17*) and one on the right (*A96*).

We should like to know whether it is the molecular sequence of *A23 per se* which stimulates high-frequency recombination, or the combination of this sequence with the homologous region (codon 211 and environs) of the other parent. YANOFSKY and HORN (1972) have isolated *trpA* mutants in a wild-type strain with an altered sequence in codon 211, which permits us to test this point. All the mutants in the crosses reported above were isolated in the standard wild-type *trpA* gene which has GGA (glycine) in position 211 (YANOFSKY 1963). Among *trp*+ revertants of mutant A23, YANOFSKY and HORN (1972) isolated one which had serine (codon AGU or AGC) at position 211. Starting with this 211-serine wild type, they isolated a new series of *trpA* mutants. We have crossed eight of these mutants to *A23* and *A46*, and the results are shown in Table 6. There are some fluctuations, but in most cases *A23* and *A46* give similar frequencies of recombination when crossed to the same mutant. We conclude that high recombination depends not only on *A23*, but on its combination with the homologous sequence in strains carrying 211-glycine.

We cannot be certain whether the levels of recombination shown in the crosses of Table 6 are high or low, because the exact positions are unknown for seven

TABLE 6

Trp+ *transduction in crosses to mutants in 211-serine genetic background*

Mutant with 211-serine	X 23	X 46
250	0.29, 0.59	0.10, 0.35
213	0.37, 0.39	0.67, 0.68
231a	0.54, 0.44	0.35, 0.59
245	0.60, 0.67	0.78, 0.55
215C	0.87, 0.73	0.53, 0.54
215a	1.56, 1.76	3.54, 2.85
225	2.24, 1.18	1.56, 1.13
218	2.28	2.26

of the eight mutants in the serine-211 *trpA* gene. Mutant *218* is in codon 22 (YANOFSKY and HORN 1972). Recombination in the crosses of this mutant to *A23* and *A46* occurs at typical LR rates.

Comparison of ochre mutants to amber mutants at the same site: Starting with an ochre (UAA) mutant, it is possible to obtain mutations to amber (UAG) in the same codon by selecting revertants in a strain carrying an amber suppressor. In this way we have obtained the amber mutants corresponding to ochre mutants *A54* and *A17*. Amber mutants corresponding to *A38* and *A96* were kindly supplied by C. YANOFSKY, who obtained them by the same procedure.

Table 7 shows the results of experiments in which the members of an ochre-amber pair were both crossed to the same single or double-mutant parent. In three of the pairs the two members do not differ significantly in recombination behavior. In all these pairs, both members show low-frequency recombination. Ochre mutant *A54* shows high-frequency recombination. The change to the amber sequence converts it to a low-recombination mutant.

TABLE 7

Comparison of trp+ *recombinant frequencies for crosses of ochre and amber mutants at the same site*

Other parent	38oc	38am	Other parent	54oc	54am
17	2.63	2.28	*38*	2.54	0.39
88	3.84	1.94	*88*	8.60	2.26
33	0.75	1.11	*33*	1.76	0.24
46A	3.66	2.86	*13*	2.53	0.40
96	2.74	3.60	*23*	6.50	4.20
96am	2.37	2.81	*17*	6.23	3.52
13	0.66	0.48	*58*	10.57	4.12
			78	8.03	3.81
			96	9.88	3.37
			109	6.51	2.68
			38–96	1.33	0.02

Other parent	17oc	17am	Other parent	96oc	96am
38	1.55	0.50	*54*	7.99	5.42
54	2.54	4.56	*54am*	3.44	3.75
54am	1.76	2.39	*13*	1.31	1.21
23	1.11	0.56	*17*	0.69	0.46
46	0	0	*58*	0.28	0.43
33	1.03	0	*78*	0.38	0.20
88	2.99	2.65	*23*	1.93	2.32
96	0.98	0.55	*46*	0.76	1.32
38–96	0.24	0	*46A*	0.87	0.89
			88	3.85	3.26
			33	2.27	1.67
			38	2.74	2.37
			38am	3.60	2.81

NORKIN (1970) compared the recombination behavior of ochre and amber mutants in the same codon of the beta-galactosidase *z* gene of *E. coli* in a conjugation experiment. His results, like ours, showed no consistent differences between ochre and amber mutants in recombination.

Marker-specific effects and recB,C *function:* Mutation in either of the closely-linked genes *recB* and *recC* results in a drop of recombination frequency to less than 5% (WILLETTS and MOUNT 1969) and in the disappearance of a specific endonuclease (OISHI 1969). BARBOUR *et al.* (1970) have obtained revertants which have recovered normal frequencies of recombination as a result of suppressor mutations, and they have shown that the BC nuclease is still absent in these suppressed mutants. Two of these suppressed *rec* mutant strains were kindly supplied to us by A. J. CLARK along with the *rec*+ strain from which they were derived. *TrpA* mutants were introduced into these strains by transduction. They were then used as recipients in two-point *trpA* crosses in order to observe the pattern of intragenic recombination in that genetic background. The results are shown in Table 8. It is clear that the high-recombination behavior of *A23* persists in the absence of the BC nuclease. (Note that the frequencies of *trpA* recombination are higher in this genetic background, but that the difference between *A23* and *A46* is still seen, particularly in the three-point crosses.)

Segregation of an unselected marker: TrpE is linked to *trpA*, four gene lengths to the left. *TrpE* mutants grow on minimal medium supplemented with anthranilate, but *trpA* mutants do not. In crosses segregating at both loci, we can select recombinants at *trpA* on anthranilate and then score them for the unselected *trpE* marker by replicating to minimal.

In "one-point" crosses at *trpA* (recipient is *trpA*, donor is *trpA*+), about 90% of the *trpA*+ transductants carry the *trpE* marker from the donor as well (Table 9a). Among *trpA*+ recombinants from two-point crosses in which the selected site from the donor is to the left of that from the recipient, the inclusion of the donor marker from *trpE* drops to well below 90% (Table 9b). This result is another demonstration of negative interference. It means that when we select for an *exchange within trpA* (as opposed to merely selecting for inclusion of donor material from *trpA*) we are selecting a subset of transductants which frequently have another exchange nearby to the left.

TABLE 8

Marker effects in trpA *recombination in cells with a suppressed* rec *mutant*

Recipients		Donors 58	96	38–96
rec+:	*23*	2.84	4.34	3.38
	46	2.33	2.37	0.17
recB sbcA:	*23*	2.77	2.52	1.79
	46	1.31	1.06	0.18
recB sbcB:	*23*	2.28	1.70	1.75
	46	1.42	1.43	0.20

TABLE 9

Segregation of unselected marker at trpE *among* trpA+ *transductants*

a) One-point crosses at *trpA*:

Donor +		+		D *E*		+
Recipient				R		
E	*A*			+		*A*
WT × *E-33*	72/75	490/520	95%	*E* × *33*	29/360	8%
WT × *E-88*	103/124	657/735	89%	*E* × *88*	36/305	12%
WT × *E-46*	237/258		92%			
WT × *E-23*	346/375		92%			

b) Two-point crosses between LR mutants at *trpA*:

D +		+ *A2*		D *E*		+ *A2*
R				R		
E	*A1*	+		+	*A1*	+
46 × *E-33*	173/268	65%		*E-58* × *46*	138/262	53%
7 × *E-33*	132/249	53%		*E-7* × *33*	30/68	44%

c) Two-point crosses in which recipient mutant is HR:

D +		+ *A2*		D *E*		+ *A2*
R				R		
E	*A1*	+		+	*A1*	+
46 × *E-88*	393/917	43%		*E-58* × *23*	196/253	77%
7 × *E-88*	199/590	34%		*E-7* × *88*	108/131	81%
58 × *E-23*	52/191	27%				

Note: Results are recorded as fractions in which the denominator is the total number of *A*+ transductants and the numerator is the number of *E*+ *A*+ among them. At the right is the percentage of *E*+ *A*+ transductants among total *A*+ transductants.

Two-point crosses in which the recipient carries a high-recombination mutant at *trpA* are presented in Table 9c. Here the frequencies of inclusion of the donor markers at *trpE* fall even lower. This is a further demonstration that recombination involving these mutants is like gene conversion—the inclusion of a very short segment of genetic information from one parent in a recombinant which is otherwise identical to the other parent.

DISCUSSION

Marker effects may be of two kinds. If heterozygosity *per se* had a consistent effect on recombination, this could be called a "general marker effect". If a certain segregating mutant affected recombination differently from others, this could be called a "specific marker effect".

General marker effects: One type of general marker effect has been proposed by HOLLIDAY (1964) and is called "map expansion": the close proximity of two segregating sites inhibits the effective pairing which is a necessary precondition for recombination, the degree of inhibition being some inverse function of the distance between the sites. Map expansion would generate a decreasing frequency

of recombination per distance in two-point crosses as the segregating sites approached each other. This is the reverse of the trend seen in the present study (Figure 1). Our observations could result from the opposite kind of a general marker effect: closely-spaced segregating sites *increasing* the likelihood of recombination. Alternatively, this trend might occur in the absence of general marker effects if exchanges frequently occurred in pairs separated by a distance similar to that between our closer-spaced mutants; between farther-spaced mutants there would be some double exchanges which would not yield recombinants.

Specific marker effects: The two-point and three-point crosses clearly demonstrate specific marker effects at the *trpA* locus. Strong evidence for marker-specific effects has also been observed at the *lacZ* locus of *E. coli* by NORKIN (1970). He studied intragenic recombination between markers at known sites in conjugation crosses.

What properties of the HR mutants are responsible for their recombination behavior? There are no simple molecular properties which are common to the three HR mutants and absent in the LR set. All three HR mutants resulted from base substitutions, but so did several LR mutants. The location of the HR mutants within the A gene does not appear significant. *A88* and *A54* are rather near the left end, and *A23* is rather near the right end. In each case we know at least one LR mutant in the same codon as the HR mutant. *A23* is missense, *A88* is amber, and *A54* is ochre; all three of these classes occur also among the LR mutants. *A23* resulted from a transition, and *A88* from a transversion. (The mutational origin of *A54* is not known.) The LR mutants include all classes of base-substitution mutants as well as frameshift mutants.

The base sequence in the region around HR mutant *A23* is mostly known (BERGER, BRAMMAR and YANOFSKY 1968) and that around HR mutant *A88* is partially known (DRAPEAU, BRAMMAR and YANOFSKY 1968). They have no obvious common features which could explain the specific marker effects (see Table 10).

NORKIN (1970) suggested that some marker effects might be explained by phenotypically non-mutant base substitutions which occurred at sites other than the mutant site and which affected the frequency of recombination. There is good

TABLE 10

Molecular sequences at sites of known marker effects at the trpA *locus*

leu	glu	leu		leu	gln	gly	phe	gly
$^{U}_{C}$U?	GAG	$^{U}_{C}$U?		UUG	CAG	GGA	UUU	GG$^{U}_{C}$
A88 UAG amber						*A23* AGA arg		
A33 AUG met						*A46* GAA glu		
						A46asp GAU asp		
						*A46asp*MUT GAC asp		

Base sequences represent mRNA codons for the wild-type gene and for various mutants. Underlined sequences represent HR mutants.

evidence that such second-site effects are not involved in the difference in recombination shown by *A23* and *A46*, as YANOFSKY *et al.* (1964) have pointed out. They recorded several independent occurrences of the *A23* mutation and of the *A46* mutation, and, in each case, the amino acid substitution correlated with the recombination behavior.

The nature of specific marker effects: How do the HR mutants alter the recombination process? They do not cause a significant increase in the number of basic recombination events; the frequency of *trp*+ transductants in one-point crosses is much the same for HR mutant *A23* as for LR mutants. However, if the other parent carries a nearby *trpA* mutant, the HR effect on *trp*+ recombinant frequency is maximal. Therefore, we conclude that the presence of an HR mutant increases the likelihood of nearby exchanges per recombination event. The experiment with an unselected marker (Table 9c) and the three-point crosses (Table 2) indicate that HR mutants are frequently associated with multiple exchanges spanning very short segments.

Integration of donor material into recombinants: The experiments which employed *trpE* as an unselected marker provide strong evidence for negative interference: the clustering of two or more exchanges. In the crosses in which an exchange within *trpA* was required (Table 9b), a significant fraction of the transductants had a second exchange between *trpE* and *trpA*. A similar conclusion was reached by CRAWFORD and PREISS (1972) who used P1 transduction to study intragenic recombination at *trpB* (which is adjacent to *trpA*). They had unselected markers on both sides, and this revealed that a large proportion of the intragenic (*trpB*+) recombinants had parental marker combinations, especially if the two segregating sites in *trpB* were very close together.

The clusters of exchanges might occur anywhere along the donor segment which has been transferred into the recipient cell, and any cluster might include either an odd or even number of exchanges. However, the *effective* exchanges would be singles or odd-number clusters, and they would appear at both ends of an integrated segment of donor material. Even-numbered clusters would insert short segments of genetic information from one parent within a longer segment from the other parent; if the successive exchanges within a cluster were only about 0.1 gene length apart, the genetic results of even-number clusters would be negligible (unless they were much more frequent than odd-number events.)

The results of the experiments on *trpA* transduction with the unselected marker at *trpE* lead us to propose the following model. Odd-number exchanges occur at random sites along a donor chromosome fragment paired with the homologous region of a recipient chromosome. The average distance between two odd-number exchange events in the same cell is about forty gene lengths. Between or around the odd-number events there may be some even-number exchange events. The genetic consequences of the even-number events are expected to be minimal, but they may become important in regard to the HR mutants. It is possible that these mutants achieve their frequent recombination by inducing even-number events around themselves.

The estimate of 40 gene lengths for an integrated donor piece was derived from the frequency of cotransduction of *trpE* and *trpA* (Table 9a): ten percent of those transductants carrying *trpA*+ from the donor are terminated within the four-gene span to *trpE*. A similar figure (about 45 gene lengths) could be derived from the absolute frequencies of *trpA*+ transductants in one-point and two-point crosses: the average frequency in one-point crosses (wild-type donor) is 180% (of *his*+ transductants) while the frequency of recombination between two mutants at opposite ends of *trpA* is four percent; thus 4/180 of the integrated donor segments terminate within one gene length of the selected site.

DOERMANN and PARMA (1967) studied T4 crosses involving a partial phage genome and a helper phage and observed a high frequency of exchanges (negative interference) near the ends of the partial phage. It is possible that a similar phenomenon occurs in the transduction system: odd-number exchanges occurring at the ends of the donor fragment. However, this cannot be established without independent evidence on the genetic content of the donor fragment. Odd-number events occur, by definition, at the ends of the *integrated* donor segment, regardless of whether the donor segment brought in by the phage extended beyond these points. Physical studies (IKEDA and TOMIZAWA 1965) have shown that the transducing phage carries a piece of bacterial DNA with a molecular weight of 6×10^7. This would be 90,000 base pairs, which corresponds to about 70 genes of the size of those in the *trp* region (YANOFSKY *et al.* 1971).

Integration of two separate donor segments: DEMEREC and DEMEREC (1956) assumed that a double exchange was required to integrate a single donor segment (in P22 transduction in Salmonella) and a quadruple was required to integrate two separate segments. They assumed that double exchanges would be significantly more frequent than quadruples, and they used this assumption in the analysis of three-point crosses to determine the order of the genes for tryptophan synthesis. The same assumptions might apply here, with the qualification that what they termed double exchanges correspond to what we have called two separate odd-number exchange events.

In the crosses reported here in which three segregating sites are in the same gene (Table 2), there is little evidence that the double exchange type is more likely than the quadruple. (In this situation the markers are so closely spaced that the separate exchanges of a cluster may each produce an effective exchange.) The production of a prototroph should require a quadruple exchange when the donor is the single mutant, while a double exchange should suffice when the donor is the double mutant. However, most of the pairs of reciprocal crosses show very similar results. Among the six pairs of crosses involving the HR mutants, three show almost identical frequencies for reciprocal crosses; the other three show a twofold excess of the double exchange type over the quadruple exchange type. This result is ambiguous, but the absence of any pronounced excess of the double exchange type may mean that intragenic recombinants are produced from an intermediate structure in which donor and recipient genetic information have parity. This structure might be a "hybrid DNA" molecule (complementary

strands which came from separate molecules or chromosomes), as envisaged by WHITEHOUSE (1963) and by HOLLIDAY (1964).

Mutational analysis of the recombination process: The experiment involving the suppressed *recB* mutant strains (Table 8) gave a negative result: it failed to show any involvement of the *recB* gene product in the occurrence of marker effects. However, we believe this is a promising approach to the problem of the mechanism of recombination. It may be possible to dissect this complex process into its simpler components by identifying the specific roles of the genes which are involved.

Recombination may be considered as a two-step process: the formation or preparation of a structure capable of producing recombinants, and the resolution of recombinants from this structure. Numerous ideas and models have been proposed and a common feature of several of them has been a recombinogenic structure (the product of step one) involving a region of hybrid DNA.

Regarding step one, the mutation approach in phage T4 has already led to considerable insights into the sequence of events in terms of the physical structures of DNA intermediates and the function of specific enzymes (TOMIZAWA 1967; BROKER and LEHMAN 1971). Step two, however, remains largely hypothetical, although the study of transfection products from artificial heteroduplex molecules of DNA (SPATZ and TRAUTNER 1970) offers a promising approach.

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