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Identification of Recombinant Chromosomes and F-Merogenotes in Merodiploids of *Escherichia coli*

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Segregants from merodiploids heterozygous at two or more sites in the *lac* region were selected on the basis of containing a recombinant F-merogenote. Such recombinants frequently contained a recombinant chromosome as well. When the merodiploid was heterozygous at two sites, the frequency at which reciprocally recombinant chromosomes were present in the selected population was lower when the two marked sites were in the same gene and close together than when the sites were more widely separated. When the merodiploid was heterozygous at four sites and selection was made for an intragenic recombinational event, the recombinant chromosome was the reciprocal type about half the time and about half the time was not. Among the latter genotypes, most were nonrecombinant for the intragenic pair of markers. The data are consistent with a model in which recombination leads to the formation of two recombinant products, each containing a region of hybrid deoxyribonucleic acid.

Recombination of genetic markers carried by the bacterial chromosome and an F-merogenote frequently results in the formation of reciprocally recombinant genotypes (chromosome and F-merogenote) which are coinherited by progeny cells (5, 14). It thus appears that mitotic recombination between chromosome and F-merogenote, unlike most recombinational systems in bacteria, allows one to recover and analyze genetically more than a single product of recombination. This feature could be very useful in further delimiting the possible mechanisms of recombination: the analysis of ordered tetrads and octads in fungi has provided much information about the recombination process in these organisms. It is not clear that an analysis of recombinational products in bacteria could approach the completeness possible in fungi, but, in view of how much is known about the genetic elements of bacteria, it seems worthwhile to extend this kind of study as far as possible.

In the present work, we have asked primarily two questions about recombination in F′ strains. First, when we select for recombination between two closely linked intragenic sites, at what frequency is the reciprocal genotype coinherited, and how does this frequency compare with that found for an intergenic two-point cross? And, second, if we select for an intragenic recombinational event in a merodiploid in which sites on either side of the gene are also marked, what kinds of recombinant genotypes are coinherited?

MATERIALS AND METHODS

Bacteria. Most of the strains used in this study, together with their genotypes in the *lac* region, are listed in Table 1. *I3* is a regulator constitutive mutation. The *Z* (structural gene for β-galactosidase) mutations were first mapped by Jacob and Wollman (9). The mutations referred to in Table 1 map in the order *I3 Z2 ZS Z4 Y13*. The origin of the *Y13* (*Y* is the galactoside permease gene) mutation is unknown, and it has been given the number *13* arbitrarily. Only strain F3W13B, which requires threonine and leucine for growth, requires any growth factors besides vitamin B_1, which was provided all strains routinely. LH114 was derived from A327 by acridine treatment. Colonies of strain LH115 are Lac⁻ (phenotypic designations: Lac⁻ and Lac⁺) on lac-tet agar, because the *ZS* mutation is polar and the strain has only about 5% of the wild-type permease activity (8). A number of Lac⁺ segregants from LH115 were cured with acridine orange. The genotypes of the cured strains were tested by crosses with F3W13B, A327A, LH110, and LH101. The *lac* region of LH116 was obtained in this way. A streptomycin-resistance marker was also introduced into LH116 by selection (100 μg of streptomycin sulfate per ml). LH117 was isolated as a Lac⁺, streptomycin-resistant recombinant from a cross of LH116 and A327, which is streptomycin-sensitive. LH118 was isolated from LH117 as a Lac⁻ segregant on lac-tet agar. Data presented in Results confirm the genotype of LH119 as given in Table 1 and indicate how LH120

TABLE 1. *Bacterial strains*

Strain no.	Sex	Genotype in *lac* region						Derivation
		Chromosome			F-merogenote			
		I	Z	Y	I	Z	Y	
2320	F^-	3	2	+				(5)
A327	F′	+	S	+	+	+	+	(5)
2320A	F′	3	2	+	+	+	13	(5)
F3W13B	F′	+	+	13	+	+	13	(5)
A327A	F′	+	S	+	+	S	+	(5)
LH101	F′	3	2	+	3	2	+	(6)
LH110	F′	3	4	+	3	4	+	(6)
LH111	F′	3	2	+	+	S	+	(6)
LH104	F′	3	2	+	+	4	+	(6)
LH114	F^-	+	S	+				A327
LH115	F′	+	S	+	+	+	13	F3W13B × LH114
LH116	F^-	+	S	13				LH115, see text
LH117	F′	+	S	13	+	+	+	A327 × LH116
LH118	F′	+	S	13	+	S	13	LH117, see text
LH119	F′	3	2	+	+	S	13	LH118 × 2320
LH120	F^-	+	+	13				LH119, see text
LH121	F′	+	+	13	+	S	+	A327 × LH120

was derived from LH119. Strains not listed in Table 1 include A252 (from A. B. Pardee), an F^- which carries a deletion of the *lac* region (no recombination with F3W13B, A327A, LH101, or LH110) and A252A, which is a spectinomycin-resistant (300 μg of spectinomycin sulfate per ml in minimal agar) derivative of A252.

Media and procedures. Media were as previously described (5) except that the liquid minimal medium (used for growing cultures up for β-galactosidase assay) was supplemented with 0.25% Casamino Acids. β-Galactosidase was assayed according to the method of Pardee, Jacob, and Monod (17). Isopropylthiogalactoside was used as inducer at 5×10^{-4} M.

Various merodiploid Lac^+ cultures were treated with acridine orange by the same method as previously described (5). All strains fell into two classes: those that gave about 70% or more Lac^- colonies on lac-tet agar after acridine treatment and those that gave about 1% or less Lac^- colonies on lac-tet agar after acridine treatment.

F′ × F^- matings, which were used to check *lac* genotypes of F^- cells, were performed as follows: loopfuls (about 0.025 ml) of the F^- culture to be tested were spotted on minimal-lactose agar from overnight cultures grown in Penassay Broth. A loopful of each homozygous F′, taken from a culture growing exponentially in Penassay Broth, was placed on top of a dried spot of each F^-. The diameters of the spots were about 5 mm. After the spots dried, the plates were incubated at 37 C. Recombination between F′ and F^- resulted in completely confluent growth over the spots. Spots of parental types alone or of nonrecombining pairs gave no growth.

RESULTS

Frequency of coinheritance of reciprocal products after intragenic recombination. Tetrad analysis in fungi has shown that intergenic recombination generally gives reciprocal products but intragenic recombination often does not, even though other markers on the same chromosome segregate in the normal ratio of 2:2. This naturally leads to the following question: is the frequency of coinheritance of reciprocal products after recombination of chromosome and F-merogenote lower for intragenic recombination than for intergenic recombination? To answer this question, intragenic recombination has been studied in strains LH111 and LH104, and has been compared with strain 2320A, in which recombination is intergenic (although still intraoperonic).

The experimental procedure was precisely the same for all strains and was nearly identical to that described previously (5). Strain 2320A is Lac^- because *Z2* is an extreme polar mutation (1, 18). Single red (Lac^-) colonies on lac-tet agar were streaked on minimal-lactose agar for selection of Lac^+ recombinants. To insure that all recombinants were independently derived, only one Lac^+ segregant from each streak was picked for purification. After purification by at least two successive single-colony isolations on lac-tet agar, each Lac^+ recombinant was treated with acridine orange (*see* Materials and Methods). In about half of the cases for each strain,

acridine orange irreversibly removed *lac*+ genes from the Lac+ recombinants. We infer that in these cases recombination led to the formation of an episome with *lacZ*+ and *lacY*+ alleles and a chromosome with at least one mutation in the *Z-Y* segment. One Lac− segregant arising as a result of acridine orange curing of each of these Lac+ recombinants was purified by restreaking on lac-tet agar. None of these Lac− isolates segregated Lac+ recombinants, and all were mated with various F′ strains to check their chromosomal genotypes. Since each was derived from a recombinant carrying an episome with *Z*+ and *Y*+ genes, the reciprocally recombinant chromosome would be the double mutant type (ignoring for now the allelic state of the *I* gene). The results of these experiments are tabulated in Table 2.

That the reciprocally recombinant chromosomes listed in Table 2 are true double mutants and do not simply contain deletions in the *lac* region is indicated by the following information. The one *Z2 Z4* chromosome (from LH104) and all 18 of the *Z2 Y13* chromosomes (from 2320A) were *ZS*+, as shown by mating with A327A. All of the *Z2 ZS* chromosomes (from LH111) were *Z4*+, as shown by mating with LH110. The *Z4* mutation maps very close to *ZS* but on the *Y* side, not the *Z2* side. In three cases, *Z2 ZS* chromosomes were checked further by mating with Ca 7049, an Hfr from E. Signer which contains a *Z* mutation (*U131*) located between *Z2* and *ZS* (16); *U131*+ was present in each of the three strains checked.

The difference between LH111 and LH104 in frequency of coinheritance of reciprocal products is not statistically significant, but both of them gave a lower frequency than 2320A. A χ^2 test shows that the probability is less than 0.001 that the lower frequencies observed in the intragenic crosses (LH111 and LH104) compared with 2320A may be attributed to random sampling. It is concluded that recombinational events occurring within the *Z2-ZS-Z4* segment lead to a lower frequency (by a factor of roughly 3) of coinheritance of reciprocal genotypes than do recombinational events occurring within the *Z2-Y13* segment. It should be pointed out that this difference in frequency of coinheritance of reciprocal genotypes may simply be due to the relative lengths of these two segments rather than the fact that recombination is intragenic in one segment and intergenic in the other.

The above results are not easily reconciled with the idea that the two members of a pair of coinherited reciprocal genotypes are reciprocally

TABLE 2. *Coinheritance of reciprocal genotypes in two-point crosses*

Parental merodiploid	No. of Lac+ recombinants rendered 70 to 100% Lac− by acridine treatment	No. of independent Lac− isolates with reciprocally recombinant chromosome
2320A (*Z2*/F *Y13*)	44	18
LH111 (*Z2*/F *ZS*)	58	9
LH104 (*Z2*/F *Z4*)	23	1

related down to the level of single nucleotide pairs. One can argue that the reciprocal products of a recombinational event may segregate to different daughter cells (5, 14), but it seems doubtful that such segregational events would be more frequent in intragenic crosses than in intergenic crosses. It seems more likely that many products of recombination which are reciprocally related genotypically would be found not to be reciprocally related at the nucleotide level if marked in some way all along the region between the genetically marked sites. The latter interpretation leads to the prediction that, if we analyze the products of recombination in a strain marked genetically at more than two sites, we will often recover recombinant products which are reciprocally related with respect to outer pairs of markers but which are not reciprocally related with respect to inner pairs of markers. The results given in the next section confirm this prediction.

Identification of coinherited chromosome and F-merogenote after intragenic recombination in a merodiploid heterozygous at four sites. Clearly, more information about the nature of the products of recombination can be obtained if we use a merodiploid which is marked at more than two sites. Therefore, we have constructed strain LH119, which is heterozygous at the *I3*, *Z2*, *ZS*, and *Y13* sites, as shown in Fig. 1 and 2. It is to be remembered that both the *Z2* and *ZS* mutations are strongly polar, so that merodiploid strains carrying either *ZS* and *Y13* in the *trans* configuration (LH115 and LH121) or *Z2* and *Y13* in the *trans* configuration (2320A) are Lac− on lac-tet agar. Furthermore, when *Z2*, *ZS* and *Y*+ are located *cis*, the *Y*+ gene remains inactive, as shown by permease assays performed on *Z2 ZS* strains according to the method of Beckwith (1). Therefore, the Lac+ segregants which arise by recombination in LH119 must carry the *Z2*+, *ZS*+, and *Y13*+ alleles *cis* on either episome or chromosome; and, as before, when they are on the episome they may be removed by treatment with acridine orange. Therefore, the Lac+ re-

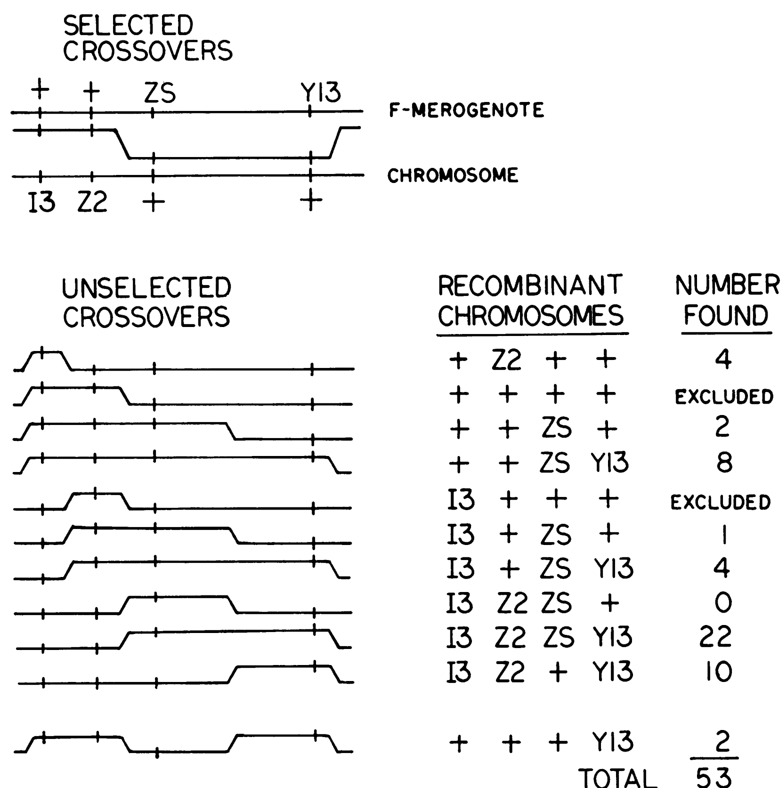

FIG. 1. *Chromosomal genotypes identified in recombinants carrying an $I3^+$ $Z2^+$ ZS^+ $Y13^+$ episome. The map distances as diagrammed are not to scale. Z2 and ZS both map near the end of the Z gene nearer I (16). The simplest crossover patterns consistent with the results have been drawn. The number of crossovers in each case has been made even, as would be necessitated by Campbell's proposal (3) that the episome, like the chromosome, is circular. All double crossover possibilities are enumerated in this figure.*

combinants which were efficiently rendered Lac$^-$ by treatment with acridine orange were inferred to have an episome carrying the $Z2^+$, ZS^+, and $Y13^+$ sites, so that the selected recombinational events can be diagrammed as in Fig. 1 and 2 (no selection was made for the allelic state of the episomal *I* gene), with the additional constraint that recombinants carrying $Z2^+$, ZS^+, and $Y13^+$ sites on the chromosome as well as episome were excluded by the selection method, because such recombinant types would not be rendered Lac$^-$ by removal of the episome.

The recombinants carrying Z^+ Y^+ episomes were also screened for ability to donate Z^+ Y^+ genes by mating them with A252A and selecting for Lac$^+$, spectinomycin-resistant recombinants. All of the strains tested were Z^+ Y^+ donors, but nearly 20% of those tested gave about 100-fold less recombination than is characteristic of F-*lac*$^+$ donors. The reason for this marked reduction in donor ability is unknown, and the strains showing it will be excluded from further consideration (although their inclusion would make little difference in the results).

The chromosomes of the remaining strains were analyzed genetically by the procedure described in the preceding section. One Lac$^-$ segregant arising as a result of the acridine curing of each selected Lac$^+$ recombinant was purified. None of these Lac$^-$ isolates segregated Lac$^+$ recombinants, and all were mated with F3W13B, A327A, LH101, and LH110. The matings with LH110 (*Z4*/F-*Z4*) were in every case positive and showed that none of the chromosomes investigated carried deletions of the *lac* region. The chromosomal *I* alleles were checked in β-galactosidase-negative strains as follows. If the strain to be checked carried *Z2*, it was mated with LH110; if it carried only *ZS*, it was mated with LH101 or LH110 (results were the same for each). Inocula from patches of Lac$^+$ recombinant growth were grown up overnight in minimal liquid and then grown in minimal liquid with and without inducer for β-galactosidase assays.

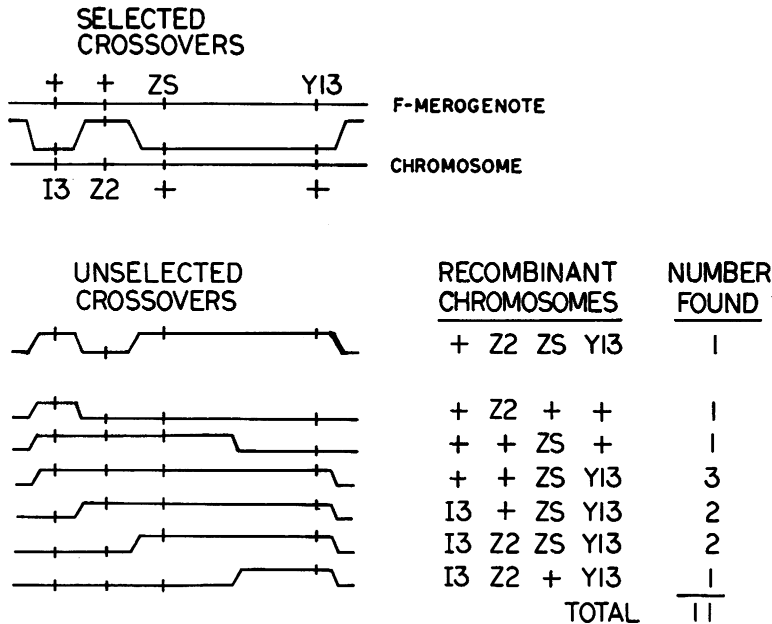

FIG. 2. *Chromosomal genotypes identified in recombinants carrying an I3 Z2+ ZS+ Y13+ episome. Only those recombinant genotypes actually found are listed.*

LH110 and LH101 are homozygous for the constitutive mutation, *I3*. The presence of a dominant wild-type gene in the recombinant population was therefore easily detected.

The allelic form of the *I* gene on the Z^+ Y^+ episome was also determined. When the chromosome carried the constitutive mutation, the episomal allele was checked simply by β-galactosidase assay on the original Lac+ segregant in minimal liquid in the presence and absence of inducer. When the chromosome was I^+, the F-Z^+Y^+ element was introduced by mating into A252A, which has a deletion of the *lac* region. Lac+, spectinomycin-resistant recombinants were purified and β-galactosidase assays were performed in the presence and absence of inducer. Each of these tests was performed in triplicate (including the isolation of independent Lac+, spectinomycin-resistant recombinants), and the results were reproducible and unambiguous. Since some of the Z^+ Y^+ episomes proved to be constitutive by this test, it is concluded that the deletion carried by A252A extends at least into the *I* gene, if not through it, and that the test is therefore valid.

The results of these identifications of genotypes are presented in Fig. 1 and 2. The data have been classified according to whether the recombinant F-merogenote carried the $I3^+$ or the *I3* site (Fig. 1 and 2, respectively). Fifty-nine per cent of the chromosomes in the selected population were found to be identical to the chromosome of the parental strain, LH119. The allelic state of the episomal *I* gene was not identified in merodiploids containing such chromosomes; therefore, these chromosomes have not been listed in Fig. 1 and 2.

As controls, 122 colonies of LH119 (Lac−, but capable of segregating Lac+ recombinants) were picked and cured of episome by acridine orange treatment. The chromosomes of the cured strains were checked by mating with F3W13B, A327A, LH101, and LH110 (the *I* gene was not checked). All but three were *Z2* ZS^+ $Y13^+$ (chromosomal genotype of LH119). The remaining three were $Z2^+$ *ZS Y13* (episomal genotype of LH119). The colonies picked for these controls were on the same set of plates as the colonies that were picked for further isolation of independent Lac+ recombinants. It is concluded that very few of the cells that initiated these colonies were recombinant in the *Z2-Y13* segment.

The results show that aside from the parental types the most common chromosomal genotype was the complementary or reciprocal type. Many noncomplementary recombinant types were also formed, and most of these were parental (chromosomal or episomal) with respect to the intragenic sites (*Z2* and *ZS*).

DISCUSSION

Our data argue against two extreme models of recombination. The first of these we term "strictly nonreciprocal." According to this model, a recombinational event leads to the formation and faithful replication of only a single recombinant product. Clearly, the high frequency at which a recombinant chromosome is found to be coinherited by cells selected on the basis of carrying a recombinant F-merogenote does not support the strictly nonreciprocal model. Such a model could be saved only if the occurrence of a nonreciprocal event very strongly increased the probability of a second nonreciprocal event occuring at the same time, such that two recombinant products would be produced. If this were the case, however, the data in Fig. 1 show that the two separate, nonreciprocal events must also be strongly correlated with respect to crossover position. In this four-point cross, by far the most frequent recombinant chromosomal genotype was the reciprocal type, but, if the recombinant chromosomes were formed by events unrelated to the selected recombinational event, then the reciprocal genotype would be expected to be infrequent compared with most other types because of the closeness of the *Z2* and *ZS* sites. The strictly nonreciprocal model loses most of its usefulness when one requires that each nonreciprocal event tends to occur at the same time and nearly in the same place as a second nonreciprocal event, such that reciprocal products are frequently produced.

Quite similar evidence against the strictly nonreciprocal model has been presented by Meselson (14), who found a very strong tendency for reciprocally recombinant genotypes to occur in the same cell descended from a partial diploid carrying two λ prophages, one on the chromosome and one on an F′. Meselson employed three genetic markers well separated on the λ genome.

A second model of recombination, which we call "strictly reciprocal," is one in which recombination leads to the formation and faithful replication of two recombinant products which are reciprocally related down to the level of single nucleotide pairs. Against this model is the relatively frequent coinheritance of two recombinant genotypes which are not reciprocally related (Fig. 1 and 2). If these genotypes were produced by strictly reciprocal recombination, then they would have had to have been produced by separate events involving more than two participating *lac* regions. The occurrence of one such event in a cell would have to increase markedly the probability of a second event occurring, since recombination in the *Z2-Y13* segment was found to be extremely infrequent in unselected, control populations.

Our data most easily fit a model of recombination which is neither strictly nonreciprocal nor strictly reciprocal but which involves the breaking and rejoining of parental deoxyribonucleic acid (DNA) molecules to produce two recombinant molecules in which the joining of the parental fragments occurs at different positions along the two complementary DNA strands. Models involving such hybrid or heteroduplex regions of DNA, with some variation in their detailed aspects, have been proposed by a number of workers (7, 11, 13, 15, 19, 20). Semiconservative replication of the primary recombinant products could then generate four distinguishable (at the nucleotide level) recombinant types, which would segregate among daughter cells in the first few divisions after the recombinational event; any particular merodiploid progeny cell would contain at most two of the distinguishable recombinant types, which could be reciprocally related but would not necessarily be so. And since a cell generally contains more than a single chromosome, a recombinant episome could also segregate with a chromosome which had not participated in any recombination. [Chromosome and episome replicas apparently do not segregate independently (4), but there is no evidence to suggest that a chromosome and an episome which are destined to segregate to different daughter cells cannot recombine.] The length of the *Z* gene can be estimated at about 3,500 nucleotide pairs (2). A length of the order of 10^3 nucleotide pairs for the hybrid region (13) could easily account for the various pairs of recombinant genotypes which were found to be coinherited. Since the hybrid DNA model for recombination generally requires that the mutations involved be point mutations, it should perhaps be noted that the *Z2*, *ZS* and *Y13* mutations carried by strain LH119 revert spontaneously (*I3* has not been tested) and are thus believed to be point mutations.

An additional feature is usually proposed in the hybrid DNA model: localized single strand excision and repair in the hybrid region. The best genetic evidence in favor of such a process is gene conversion in fungi. We have as yet no comparable evidence in bacteria, and our present data provide no genetic evidence for or against a DNA repair model, unless one accepts a master strand model for DNA duplication (10, 12). If a single master strand of DNA specifies the code for both newly forming strands of DNA, one would expect a recombinational event to generate two rather than four ultimate recombinant genotypes, and without repair synthesis in the

hybrid region the two recombinant genotypes would be strictly reciprocally related. On the assumption that the master strand model of DNA duplication is incorrect (i.e., that each DNA strand is used as a complementary template during DNA synthesis), evidence for gene conversion would require the recovery and identification of all the ultimate genetic products of a recombinational event, not just those that are coinherited by one of the recombinant progeny cells. Without DNA repair in the hybrid region, one would expect the ultimate formation of two pairs of recombinant products, each pair consisting of two reciprocally related genotypes. Single-strand repair in the hybrid region would give a different result. If repair were complete throughout the hybrid region, one would expect to obtain two recombinant genotypes, which would not necessarily be reciprocally related. Less extensive repair could lead to the formation of four recombinant genotypes, all different and no two reciprocally related.

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