A GENETIC ANALYSIS OF PRIMARY PRODUCTS OF BACTERIOPHAGE LAMBDA RECOMBINATION

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ABSTRACT

Primary products of bacteriophage lambda recombination that display heterozygosity as a consequence of the presence of regions of heteroduplex DNA are rare in standard λ crosses. Phage manifesting heterozygosity at a given allele are evident when recombinants, emerging from a cross, are selected for an exchange in a neighboring interval. We show that the abundance of such heterozygotes can be increased 10- to 20-fold by selection on an *E. coli* indicator that is defective in methyl-directed mismatch repair (*mut*L). Thus, the activity of the methyl-directed mismatch repair system is, at least in part, responsible for the low frequency of detectably heterozygous phage emerging from a standard cross. In a *mut*L indicator, many primary products of recombination are replicated without the intervention of mismatch repair.—The products of a six-factor phage cross have been plated on a *mut*L indicator allowing visual detection of those phage products heterozygous for one of the allelic pairs, *c*I. By genetic analysis, we show that the heteroduplex regions of these primary products of recombination are on the average about 4 kb in length and can include as much as half of the lambda genome.

GENETIC recombination can be accounted for as the consequence of a breakage and joining reaction in which homologous components of parental DNA molecules are traded. Genetic observations, as well as characterization of the primary DNA products of recombination, have demonstrated the presence of DNA with heteroduplex structure. (For review, see FOX 1978; RADDING 1982.) These heteroduplex structures are regions of the DNA duplex in which the two strands are derived from different parents. They may be present in a sequence that is flanked at both ends by DNA derived from only one of the parents (FOX and ALLEN 1964) or is flanked at the two ends by DNA heteroduplex derived from different parents (STAHL *et al.* 1974), *i.e.*, either parental or recombinant for makers outside the heteroduplex region.

Recombinants can result from two distinct processes. Those involving distant markers would be the consequence of the breakage and joining reaction. The other process, reflecting excision and repair of base-pair mismatches present in the heteroduplex region, can give rise to recombination between closely linked markers. It is this latter process that made it difficult to use genetic criteria to characterize the primary products of exchange between DNA mol-

ecules. The products of bacteriophage lambda recombination, derived from crosses carried out in *E. coli* under conditions severely restricting DNA synthesis, have been shown to harbor extensive regions of heteroduplex DNA (WHITE and FOX 1974; RUSSO 1973). Nevertheless, efforts to display the presence of heteroduplex regions formed under conditions permissive for DNA replication have generally failed (KELLENBERGER, ZICHICHI and EPSTEIN 1962). Only when selecting for recombinants has it been possible to show the occasional presence of heterozygosity at a neighboring locus (RUSSO, STAHL and STAHL 1970).

We describe here a new approach to the detection of intermediates in recombination that permits analysis of primary products of bacteriophage lambda recombination, including assessment of the extent of heteroduplex DNA present in these products. This approach was suggested by the observation that lambda DNA isolated from ordinary phage lysates is incompletely methylated at the adenine sites of GATC sequences (LACKS and GREENBERG 1977; PIKKULA *et al.* 1983). The very efficient methyl-directed mismatch repair systems of *E. coli* acts to correct base-pair mismatches in DNA that is hemimethylated at GATC sites (WAGNER and MESELSON 1976; PIKKULA *et al.* 1983; FOX and RAPOSA 1983; LU, CLARK and MODRICH 1983; WAGNER *et al.* 1984). Base-pair mismatches present in heteroduplex regions of primary products of lambda recombination would be expected to be subject to such correction. It is thus possible that heteroduplex containing heterozygous products of phage recombination are lost to detection because of the efficient correction of mismatches in the host bacteria that are used as indicators.

In order to examine this possibility, we have assessed the abundance of heterozygotes for a locus close to a genetic interval for which recombinants were selected, when wild-type bacteria or bacteria defective in methyl-directed mismatch repair, *mut*L (FOX and RAPOSA 1983), are used as indicators. We show that the abundance of heterozygotes is very substantially increased with the *mut*L indicator.

This observation encouraged us to examine the distribution of heteroduplex lengths in the primary products of lambda recombination. Phage crosses were carried out with parent's heteroallelic for six markers (point mutations), including *c*I. The progeny were plated without selection on an indicator defective in methyl-directed mismatch repair, *mut*L. Those plaques derived from phage products displaying heterozygosity for *c*I, a central marker, were identified by visual inspection as mottled plaques and were analyzed for the presence of heterozygosity for the other alleles contributed by the two parents.

Our results suggest that the heteroduplex regions present in the products of recombination are variable in length and can be as long as 20 kb. Furthermore, the genetic composition of these recombination products reflects the outcome of the exchange event with respect to whether the markers flanking the heterozygous region are parental or recombinant. The use of strains defective in base-pair mismatch repair (*mut*L) as hosts for plating of the products of a cross thus provides the basis for an examination of the structure of the primary products of phage recombination. Mismatched base pairs present in

TABLE 1

Bacterial strains that were used in this work

Designation	Relevant genotype	Source
C600	*thr, leu, lac*Y, *su*II	APPLEYARD
C600 *mut*L	*thr, leu, lac*Y, *su*II Tn*10*::*mut*L218	Tet transductant of C600 by P1 growth on M182 *mut*L (this work)
AB1157	*thr, leu, his, org*E, *pro*A, *lac*Y, *gal*K, *su*II	
AB1157 *mut*L	*thr, leu, his, org*E, *pro*A, *lac*Y, *gal*K, *su*II, Tn*10*::*mut*L218	Tet^R transductant of AB1157 by P1 growth on M182*mut*L (this work)
M182	*lac*Z, *gal*U, *gal*K, *rps*L	E. SIGNER
M182*mut*L	*lac*Z, *gal*U, *gal*K, Tn*10*::*mut*L218, *rps*L	S. RAPOSA
D6432	*lac, pro, arg*Eam, *met*B, *nal*R, *rif*R, *su*II	J. MILLER
CA85(*imm*434*c*I70*am*29)		M. FOX
CA85(*imm*434*c*I7*Pam*80)		M. FOX
LE289	lysogen for *int, am*Y29, *cI*-m, *gal*T	S. GOTTESMAN

the heteroduplex regions of matured phage give rise to plaques with a mixture of the genotypes representing the composition of each of the strands of the heteroduplex.

MATERIALS AND METHODS

Bacterial strains: The strains used in this work are listed in Table 1.

Phage strains: λcI60 *Oam*29 and λ*Pam*80 come from the M. FOX collection.

The λcI60 *Oam*29 *plac*5 phage derived from λcI60 *Oam*29 successively rendered λcI60 *Oam*29 *plac*5 by a cross with λ*plac*5 and λcI60 *Oam*29 *placZoc*U108 by a cross with λ*imm*434 *plac*5 (M. LICHTEN collection).

The λ*int*4 *plac*5 *Pam*80 *Ram*5 was obtained from the cross between λ*int*4 *plac*5 and λ*imm*434 *Pam*80 *Ram*5 (M. FOX collection).

*Oam*29, *Pam*80 and *Ram*5 are all amber mutations suppressed with a reasonable efficiency by the bacterial suppressor *su*II. *lacZoc*U118 is an ochre mutation in the *Z* gene not suppressed by *su*II; *int*4 is a missense mutation.

Media: The bacteria were grown in λy medium (tryptone 10 g/liter, NaCl 5 g/liter, yeast extract 1 g/liter). λyM is λy medium supplemented with maltose at 4 g/liter.

Phage were plated on λy medium supplemented with Difco agar (11 g/liter) with 3 ml of λ top agar (6 g/liter).

λy TTC lac (or λTTC gal) plates contain, in addition, 0.5 g/liter of TTC (2,3,5 Triphenyltetrazolium Chloride) and 5 g/liter of lactose (or galactose).

Phages were usually diluted and plaques were resuspended in SMO (NaCl 5.85 g/liter; Tris-HCl 1 M, pH 7.5, 20 ml/liter) and $MgSO_4$ 10 mM.

Genetic crosses: The crosses were performed as follows. An overnight culture of bacteria in λyM medium was diluted 100-fold into the same medium and was incubated at 37°. One milliliter of the culture at about 2×10^8 cells/ml was simultaneously infected by both parental types of phage at a multiplicity of five for each. After 15 min

of adsorption at 37°, the mixture is diluted 100-fold in λy broth, incubated for 90 min at 37° and a drop of chloroform is added.

Analysis of recombination products of λcI60 *Oam*29 by λ*Pam*80: The crosses were carried out in AB1157 and AB1157 *mut*L strains. The total progeny titrated permissively on C600(*su*II) and O^+P^+ recombinants selected either on the M182 or M182 *mut*L strain. The genotypes of these recombinants were further analyzed by picking 200 plaques, at random, with a fine platinum wire and streaking them out on an M182 lawn. Streaks that included both clear and turbid plaques were classified as progeny of phage heterozygous for $c(c/c^+)$.

Transfection with artificial heteroduplex DNA molecules: DNA was isolated from λcI60*Oam*29 *placZoc*U118 and λ*Pam*80*Ram*5 *int*4 *plac*5, and heteroduplexes were formed by denaturation and annealing, as described in LICHTEN and FOX (1983), except that the annealing mixture was incubated for only 1 hr at 37° and was not precipitated with ethanol.

One hundred microliters of such a heteroduplex mixture was diluted in SMO + Mg buffer to 1 μg of DNA per milliliter, heated 3 min at 65° to monomerize the DNA molecules, mixed with 200 μl of ice-cold calcium-treated competent cells C600 *mut*L (MANDEL and HIGA 1970) and successively incubated for 15 min in ice and 30 min at 37°. Infective centers were plated on λy agar plates, together with 0.2 ml of a fresh overnight culture of C600. The relative abundance of pure c^+, pure c or mixed c^+/c infective centers was determined by picking random infective centers and streaking them on a lawn of C600. In the case of mixed c/c^+ infective centers, further analysis for the presence of other alleles is described below. The genotypes of phage from each of three turbid and three clear plaques from each mixed infective center were characterized.

Analysis of cI heterozygotes formed *in vivo*: The products of a cross between phages λcI60 *Oam*29 *placZoc*U118 and λ*int*4 *Pam*80 *Ram*5 *plac*5 phages in AB1157 *mut*L were plated on C600 *mut*L in order to obtain about 100 plaques per plate. Mottled plaques containing both λc^+ and λc phage were detected by visual inspection. Each of these mottled plaques was picked and resuspended in 0.5 ml of SMO + $MgSO_4$ buffer. Aliquots of this ministock were mixed with D6432 (*su*II) and plated on λyTTC lac plates in order to obtain about 100 plaques per plate. Under these conditions lac^- phage make white plaques, and lac^+ phage make red plaques. In most of the cases we found predominantly two types of phage in each mottled plaque (λc^+ lac^+ and λc lac^-, or λc^+ lac^- and λc lac^- or λc^+ lac^+ and λc lac^+). In some cases, in addition to the two predominant genotypes c^+ lac^+ and c lac^-, we found a few phage with a different genotype. The frequency at which these were present (a few percent) makes it likely that they are products of recombination events occurring during formation of the mottled plaque. Thus, we decided to ignore these rare phage and to consider the mottled plaques as derived from a phage particle harboring the two predominant genotypes that were further characterized. For each mottled plaque, three of each clear (red or white) and turbid (red or white) plaques were characterized with respect to the other alleles.

The Int^+ phenotype recognized on the basis of complementation with c^+ *int*4 phage on the LE289 strain plated on TTC Gal (ENQUIST and WEISBERG 1976). In contrast to int^-, int^+ phage generate red papillae under these conditions.

The O and the P phenotypes were recognized by testing on CA85 (λ*imm*434 *c*17 *Oam*29) or CA85 (λ*imm*434 *c*17 *Pam*80), where the λ*Oam*29 or λ*Pam*80, respectively, fail to grow.

The *R* character was determined directly by plating on C600. After one night of incubation at 37°, the plates were inverted for 15 min on a glass Petri dish containing 1–2 ml of chloroform. The plates were then left open for 15 min at room temperature. After this treatment, spot tests with plaques of R^+ phage, but not of R^- phage, show a marked halo (WHITE and FOX 1975).

Since, as is *lac*, *R* is an external marker in our experiment, we expect the presence

of some phage recombinant for *c*I and *R* to be formed within the mottled plaque, as is the case for the *c*I-*lac* interval. The presence of these recombinants could lead us to occasional misclassification. We therefore examined more than three plaques of each clear and turbid phenotype in some cases. An aliquot of the suspended mottled plaque was plated on C600 in order to obtain about 100 plaques per plate, which were tested for the R^+ and R^- phenotype by the chloroform method. As in the case of the *lac* marker we found, for the most part, two kinds of phages in each mottled plaque (λc^+R^- and $\lambda c\ R^+$, or λc^+R^- and $\lambda c\ R^-$ or λc^+R^+ and $\lambda c\ R^+$). The occasional presence of phage with other genotypes was assumed to result from plate recombination. We considered a mottled plaque to be the product of a heterozygous phage, with one genotype resulting from each of the strands of the heteroduplex.

Our screening detected some mottled plaques containing two types of phages in which all of the markers except one were heterozygous. In those cases, in addition to the three clear and three turbid phages already analyzed, we retested ten of each type for the marker that appeared to be homozygous.

RESULTS

The methyl-directed mismatch correction system has been shown to be active in co-repairing all of the mismatches in a 2000-bp stretch of one strand or the other of a heteroduplex formed by annealing single strands of lambda DNA differing by three-point mutations (S. RAPOSA and M. S. FOX, unpublished results). It seemed likely that this kind of repair could be, in part, responsible for the rarity with which heterozygotes are observed among products of lambda recombination. To test this hypothesis we carried out phage crosses with parents differing by only point mutations, $\lambda c\ O^-$ and λc^+P^-. The products of crosses carried out in a *su*II *mut*$^+$ or a *su*II *mut*L strain were examined. The methyl-directed mismatch correction system is defective in *mut*L strain (LU, CLARK and MODRICH 1983).

If we assume that some of the O^+P^+ recombinants result from formation of a heteroduplex that includes the *c*I region, these O^+P^+ recombinants could be heterozygous for the *c*I region and, therefore, could give rise to a mixture of c^+ (turbid) and *c* (clear) progeny (as long as this heterology is not lost by replication or mismatch correction before packaging). For this kind of cross, RUSSO, STAHL and STAHL (1970) have reported that 1% of the recombinants are mixed for the *c* character. For crosses in *mut*$^+$ or *mut*L hosts, O^+P^+ recombinants were selected either on *mut*$^+$ or *mut*L indicators. The frequency of recombination observed in all these cases was between 0.1 and 0.3%.

Plaques of O^+P^+ recombinants were tested for presence of either pure c^+, pure *c* or mixed *c* and c^+ phage. The results (Table 2) show that, if the recombinants are selected on *mut*L bacteria, about 20% of the plaques are mixed for the *c* character, compared to only 1 or 2% when the selection for recombinants is carried out in the *mut*$^+$ strain. These results suggest that heteroduplexes that include the *c*I region are quite common in the products of lambda recombination and that the *mut*L-dependent mismatch correction system, acting in the indicator used to detect them, often repairs heterozygotes that are present. Surprisingly, the abundance of mixed $c^+/c\ O^+P^+$ recombinants is similar in crosses carried out in *mut*$^+$ or *mut*L strains as long as the recombinants are selected on a *mut*L indicator strain (see Table 2). These observa-

TABLE 2

λc⁺/c heterozygotes among O^+P^+ recombinants

	Phage	Cross in *mut*L (%)	Cross in *mut*⁺ (%)
Selection of O^+P^+	Mixed c^+/c	21	19
recombinants on	Pure c^+	52	53
sup⁺, *mut*L	Pure c	27	28
Selection of O^+P^+	Mixed c^+/c	2	1
recombinants on	Pure c^+	75	74
sup⁺, *mut*⁺	Pure c	23	25

The crosses between λc^+P^- and $\lambda c\ O^-$ were performed under permissive conditions in *su*II strains that were deficient (*mut*L) or proficient (*mut*⁺) in the methyl-directed mismatch correcting system. The O^+P^+ recombinants were selected in *mut*⁺ or *mut*L indicators, and the infective center composition of 200 random plaques was determined by streaking out on an *sup*⁺ *mut*⁺ lawn.

FIGURE 1.—Approximate map of the appropriate markers on the bacteriophage lambda genome.

tions suggest that many of the recombination products that are detected as O^+P^+ recombinants are made just before encapsidation and that c^+/c heterozygotes that are present are often corrected in the indicator strain.

We carried out a six-factor cross, heteroallelic for *c*, plated all of the progeny on a permissive *mut*L strain and identified by visual inspection those occasional plaques that were mottled, *i.e.*, included both phage with the c^+ and phage with the *c* genotype. Such plaques were picked, resuspended and analyzed for the presence of the other alleles. Thus, the extent of the heterozygosity could be assessed, and for those products in which both ends of a heterozygous region are evident, the parental or recombinant character of the flanking regions could be determined.

The two parental phage used in these crosses label the right half of the lambda genome with point mutations, $plac^-\ c\ O^-$ and $plac^+\ int^-\ P^-R^-$, respectively (Figure 1). In order to assess the efficiency of mismatch repair that persists in the *mut*L strain for these markers, approximately equal amounts of DNA isolated from each of the strains were mixed, denatured, annealed and used to transform *mut*L bacteria. The mixture of the two phage DNA's used (40% of $\lambda lac^-\ c\ O^-$ and 60% $\lambda lac^+\ int^-c^+\ P^-R^-$) would be expected to give rise by random assortment to the formation of 16% of $lac^-\ c\ O^-$ homoduplex, 36% $lac^+\ int^-\ c^+\ P^-R^-$ homoduplex and 48% of heteroduplex. Analysis of infective centers obtained after transfection of *mut*L bacteria by this mixture showed that 15% of the infective centers contained only *c* phage, 39% only c^+ phage and 46% were mixed, containing both *c* and c^+ phage. Thirty-one of the infective centers with both *c* and c^+ phage were further analyzed to determine the fate of the other alleles that were present in the annealed hetero-

duplex molecules. This analysis showed 10% correction for the lac^+/lac^- mismatches, 16% for int^+/int^-, 19% for O^+/O^-, 10% for P^+/P^- and 16% for R^+/R^-. In only one case was there correction of more than one marker, giving rise to a plaque containing O^+P^- phage. These results show that there is only modest correction for any of these markers in the *mut*L strains.

These modest levels of correction convinced us that it would be possible to characterize primary products of recombination by this kind of genetic analysis. We carried out a cross between these parental phage in the permissive *mut*L host. The progeny were plated to give about 100 plaques per plate on the same bacterial strain. Phage c^+ makes turbid plaques and phage c makes clear plaques. A phage product whose DNA includes a region of heteroduplex that includes the c region would be expected to give rise to a plaque that is mottled in appearance and includes both c^+ and c phage. This allows us, by visual inspection, to identify plaques derived from phage harboring a region of heteroduplex DNA. Among a total of about 20,000 plaques examined, 112 mottled plaques were recognized. The genotypes of the phages present in these mottled plaques were analyzed, as described in MATERIALS AND METHODS. Sixty-two plaques contained phage with a complete complement of each of the two sets of parental genotypes, as well as occasional recombinants, probably produced during plaque formation. Such plaques could result from phage particles heteroduplex for the entire marked half of the genome, but cannot be distinguished from coincident plaques formed by two infective centers occurring at the same site on the plate. We assume that the latter is the most likely origin of these plaques.

The description of the genotypes present in the remaining 50 mixed plaques is displayed in Table 3. Some of the phage products are heterozygous for c only. Others include heterozygosity for various continuous portions of the genetically marked region. A few are homozygous for only one marker, *int* or *O*, and are heterozygous everywhere else. We have included these in Table 3 because they would appear to represent corrected products that were heteroduplex for the entire *lac* to *R* region.

The observed array shows that the length of heteroduplex regions is variable. Some are limited to the c region, others include increasing numbers of neighboring markers and some extend over half of the lambda genome. These lengths may be underestimates because, in some cases, terminal markers that had been heterozygous could have been subject to the residual mismatch correction that persists in the *mut*L strain.

The analysis of the 50 mottled plaques also shows a substantial number in which both ends of the heteroduplex region are evident. In 24 cases the genotypes of the termini of the heteroduplex region derive from the same parental phage, and in nine cases the flanking markers are recombinant.

DISCUSSION

By plating the products of a bacteriophage lambda cross on an indicator defective in methyl-directed mismatch repair, we have been able to demonstrate a substantial abundance of phage progeny harboring regions of hetero-

TABLE 3

Description of genotypes present in the mixed plaques

	Genotype of heterozygote						No.	Heteroduplex length (kb)	Flanking markers
	lac	*int*	*c*	*O*	*P*	*R*			
	2	2	1/2	2	2	2	3	<11	*P*
	1	1	1/2	1	1	1	2	<11	*P*
	1	1	1/2	2	2	2	3	<11	*R*
	2	2	1/2	1	1	1	1	<11	*R*
	1	1	1/2	1/2	1	1	2	1–12	*P*
	1	1	1/2	1/2	1/2	1	6	2–18	*P*
	1	1	1/2	2	1/2	1	1	2–18	*P*
	2	2	1/2	1/2	1/2	2	2	2–18	*P*
	2	2	1/2	1/2	1/2	1	4	2–18	*R*
	1	1/2	1/2	1	1	1	2	10–17	*P*
	2	1/2	1/2	1	1	1	1	10–17	*R*
	2	1/2	1/2	1/2	2	2	1	11–18	*P*
	2	1/2	1/2	1/2	1/2	2	2	12–24	*P*
	1	1/2	1/2	1/2	1/2	1	2	12–24	*P*
	1	1/2	1/2	2	1/2	1	1	12–24	*P*
	2	2	1/2	1/2	1/2	1/2	3	>11	
	2	1/2	1/2	1/2	1/2	1/2	1	>21	
	1	1/2	1/2	1/2	1/2	1/2	4	>21	
	1	2	1/2	1/2	1/2	1/2	1	>21	
	1/2	1/2	1/2	1/2	2	2	1	>17	
	1/2	1/2	1/2	1/2	1/2	1	3	>18	
	1/2	2	1/2	1/2	1/2	1/2	2	>24	
	1/2	1/2	1/2	2	1/2	1/2	2	>24	
	1/2	1/2	1/2	1/2	1/2	1/2	62		
Parent 1	*lac*	*int*4	*c*I60	*Oam*29	P^+	R^+			
2	lac^+	int^+	c^+	O^+	*Pam*80	*Ram*5			

duplex DNA. The heterozygosity displayed by such products is, for the most part, lost when progeny phage are plated on an indicator in which the methyl-directed mismatch repair system is functional.

The progeny of a cross between λ*c* *Oam*29 and λ*Pam* 80 are plated on a sup^+ indicator, selecting for O^+P^+ recombinants. Individual O^+P^+ plaques were tested for the genotype at *c*. When the selective indicator was proficient for methyl-directed mismatch repair, 1 or 2% of the products were heterozygous for *c*. When the selective indicator was defective in mismatch repair, *mut*L, 20% of the products displayed heterozygosity for *c*. Thus, a substantial fraction of the events that give rise to O^+P^+ recombinants are followed promptly by encapsidation. The heterozygosity present in these products is lost when they are plated on an indicator that is proficient in methyl-directed mismatch repair.

It seems likely that the phage from which these c/c^+ plaques arise are O^+/P^+ on both strands and heteroduplex at *c*, although it remains possible that they are O^+P^+ only on the transcribed strand, mutant on the other and heteroduplex at *c*. Marker rescue of the *c* allele on the mutant strand during the first cycle of growth could give rise to a plaque mixed for *c*. Such a mechanism

would not be likely to be efficient in the recovery of both *c* alleles and, thus, could lead to an underestimate of the abundance of c/c^+ heterozygotes.

The abundance of *c* heterozygotes among the recombinant products provides the basis for examining the structure of the primary products of recombination by assessing the genetic composition of individual phage emerging from a multifactor cross.

We first show that transfection of *mut*L bacteria with annealed DNA, heteroduplex for the two parental phage, gives rise to infective centers in which most of the markers present in each of the strands are represented in the plaque made by an infective center. The frequency with which individual markers appear homozygous in such products is between 10 and 20%, and infective centers in which two of the markers are homozygous are rarely observed.

Among the products of the six-factor cross we detect, by visual inspection, mottled plaques that contain both λc^+ and λc^- phage. From reconstruction experiments (data not shown) it is clear that such visual inspection probably permits detection of less than half of the plaques that, by picking and streaking, could be shown to include both λc and λc^+ phage. We estimate that perhaps 1% of the progeny could be heterozygous for *c*.

Plaques that are mixed for *c* and c^+ would include those in which the location of two parental infective centers coincided. For more than half of the plaques analyzed (62 of 112), we are not able to distinguish between heterozygosity for all markers and such coincident plaque formation.

Because of this uncertainty, we ignore these infective centers that could be coincident and focus attention on those products that display homozygosity for at least one of the markers distinguishing the two parents (see Table 3).

Among these 50 infective centers, some are mixed only for *c*, and the remainder are mixed for *c* and one or more of the adjacent markers. The heteroduplex regions manifesting this heterozygosity appear continuous and extend over variable lengths of the marked portion of the genome. Some of these regions begin within an identifiable interval and extend beyond the marked region, but for a substantial fraction, the two ends of the region of heterozygosity are evident. The frequency with which an end occurs within a particular interval roughly reflects the nucleotide distance defining that interval, although termini occurring between *c* and *O* and between *O* and *P* appear to be overrepresented. A rough estimate, assuming that, given a heteroduplex region including *c*, the probability of finding a neighboring marker within the heteroduplex falls exponentially with the number of nucleotides separating that marker from *c*, suggests that the average length of the heteroduplex region would be about 8 kb. Because we are selecting c/c^+ heterozygotes, this estimate would reflect on overestimate of heteroduplex lengths that is similar to the overestimate characteristic of the determination of the weight average molecular weight. If the lengths were random, their average for all heteroduplexes would be about 4 kb (TANFORD 1961).

On the basis of their investigation of recombination frequencies and of mismatch repair of artificially constructed heteroduplex molecules of bacterio-

phage lambda DNA in *mut*$^+$ bacteria, WILDENBERG and MESELSON (1975) have inferred a length of heteroduplex formed per exchange of less than or approximately 3000 nucleotides for Rec-mediated recombination. The length distribution of the heteroduplex products that we have examined under conditions in which both the phage Red and the bacterial Rec functions are intact is in reasonable agreement with their calculated estimate.

Those infective centers, in which both ends of the heteroduplex region are evident, can be classified with respect to whether the two flanking regions that are homoduplex derive from one or both of the parents participating in the exchange, *i.e.*, parental or recombinant for outside markers. Although the sample is small, it would appear that most, 24 of 33 of the products, are parental for the outside markers. Here again, we confirm an earlier report by WILDENBERG and MESELSON (1975) that recombinants for a closely linked pair of markers are more often parental than recombinant for flanking markers.

The HOLLIDAY (1964) model, involving symmetric strand exchange in the formation of recombinants, might be expected to give rise, on resolution of the branch migrating intermediate, to an equal abundance of parental and recombinant structures flanking the region of heteroduplex. On the other hand, the resolution of asymmetric exchange structures suggested by HOTCHKISS (1974) and by MESELSON and RADDING (1975) might be expected to give rise to products with parental regions flanking the region of heteroduplex. LITCHEN and FOX (1984) have suggested a substantial role for asymmetric strand exchange in recombination, and the observations reported here suggest that many of the recombinants occurring late in the lytic cycle of lambda growth may be products of this kind of mechanism.

It must be acknowledged that this analysis largely ignores the modest residual mismatch correction that remains evident in *mut*L strains. Correction events at *R* and *lac* would lead us to underestimate the length of heteroduplex regions and could lead us to misclassify some of the parental and recombinant products.

We return to the question of coincident plaques. A calculation by KELLENBERGER, ZICHICHI and EPSTEIN (1962) estimates that with 100 plaques per plate the probability of superposition would be 10^{-3}, which suggests that we should have detected about 20 coincident plaques. It is possible that the heteroduplex regions are sometimes very long, covering all the markers (more than 20 kb) that we can detect. Indeed, in four cases we have found plaques that contain both clear and turbid phages and that are heterozygous for all the parental markers except one. In two cases, plaques were products of phage that were homozygous only for the *O* marker, and in two cases were homozygous only for the *int* marker. One way to account for these products is to suggest that they result from phage with a heteroduplex region that included all the markers but in which residual mismatch correction had acted at the *O* or *int* sites. Since we have shown in this work that correction for this *O* or *int* marker in *mut*L is <20%, there could be substantially more of these phage with heteroduplex regions including all of the markers present. Our decision to exclude consideration of plaques heterozygous for all markers contributes to the extent

to which we underestimate the average heteroduplex length. Whatever the real frequency of very long structures, our results show the presence of a broad distribution of lengths.

This work describes a novel way of characterizing the primary products of recombination under conditions which should not disturb the general process. It could certainly be used more generally, *e.g.*, to examine the roles of different functions involved in *E. coli* recombination (RecA, RecBC, RecF, Red, etc.) (CLARK 1973; SMITH 1983), as well as to examine the roles of structural determinants such as *chi* sites (STAHL 1979).

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