DNA SYNTHESIS AT THE SITE OF A RED-MEDIATED EXCHANGE IN PHAGE λ

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ABSTRACT

In phage λ, progeny particles bearing unreplicated chromosomes are recombinant by action of λ's Red system only near the right end of the chromosome. These recombinants are frequently heterozygous (heteroduplex) for markers located there. In replication-blocked crosses involving two heavy-labeled parents we find that particles in the solitary peak, containing progeny with fully conserved DNA, vary in density. Those on the heavy side of this peak are more apt to be heterozygous than are those on the light side. The data fit a model in which a double chain cut at *cos*, λ's packaging origin, is followed by partial exonucleolytic degradation of λ's *r* chain from the right end leftward. The exposed *l* chain, which thereby constitutes a 3′ overhang, invades an intact, circular homologue afrter itself suffering some degradation. Completion of the recombinant chromosome sometimes involves DNA synthesis primed by the invading chain.

CROSSES with density-labeled phage λ under conditions of limited DNA replication have revealed properties of the several recombination systems that can operate on the phage (for a review, see STAHL 1984). This paper deals with the Red pathway of λ operating in cells deprived of *E. coli* recombination pathways by a *recA* mutation. A model has been proposed for the operation of this system on phages for which DNA replication has been blocked (STAHL, KOBAYASHI and STAHL 1985; STAHL and STAHL 1985). The features of replication-blocked recombination in RecA$^-$ cells that led to the model include the following: (1) Recombination is maximal at the right end of the chromosome and declines monotonically leftward (STAHL *et al.* 1974). (2) If *cos*, the sequence that determines the ends of the phage particle chromosome, is relocated, then the high rate of recombination follows *cos* to its new location (STAHL, KOBAYASHI and STAHL 1982). (3) The particles that experienced recombination near *cos*, at least when *cos* is in its standard location, have regions of heteroduplex DNA which can exceed 20% of the length of λ (*i.e.*, 10 kb) (RUSSO 1973; STAHL *et al.* 1974). (4) The heteroduplexes may be thought of as having most of their DNA from one parent, with a minority contribution (near the right end) from the other. This minority contribution is on the chain that has its 5′ end at the right (the *r* chain) (STAHL and STAHL 1974). (5)

When one parent in a cross carries a noncuttable *cos* at the standard location, recombination occurs at the right-hand end more often than if both parents have a noncuttable *cos*; that is, a single cuttable *cos* stimulates recombination (STAHL, KOBAYASHI and STAHL 1985). (In these crosses the resulting conserved recombinant chromosomes are packaged from functional *cos* sites cloned in the middle of the λ chromosome.) (6) In $cos^+ \times cos^-$ crosses, most or all of the recombinants stimulated by the functional *cos* inherit the mutant, nonfunctional *cos*—the functional *cos* appears to be destroyed in the recombination process (STAHL, KOBAYASHI and STAHL 1985). (7) *Eco*RI cuts delivered to λ *in vivo* in $RecA^-$ cells stimulate Red-mediated recombination in their vicinity (D. S. THALER, personal communication).

The model deduced from the properties cited above and from the established *in vitro* properties of the *red* gene protein products is shown in Figure 1 and is described in the legend.

Fact (5) above implies that a double-chain cut at *cos* can provoke break-join recombination (Figure 1h). When both parents carry cuttable *cos* (Figure 1d), the same break-join event could obtain. However, in the model as presented, we have suggested that the recombination act is completed near *cos* not by break-join, but by DNA synthesis primed by the invading 3′ end (Figure 1e). Here, we present evidence for such DNA synthesis.

MATERIALS AND METHODS

The phage mutations and and bacterial strains employed are described in Tables 1 and 2, respectively. Figure 2 shows the map locations of the λ markers employed.

Phages were density-labeled with ^{13}C and ^{15}N as described in M. M. STAHL, and F. W. STAHL (1971), except that the ratio $^{13}C/C$ was 0.99.

Crosses were performed in tryptone broth supplemented with 0.05 mg/ml of each of the four deoxynucleosides and with glucose at 10 mg/ml to swamp out unincorporated isotope in the labeled λ stocks.

Density gradient centrifugation was in cesium formate at refractive index 1.3798. After 2 days' centrifugation in Beckman SW50.1 rotor at 27,000 or 30,000 rpm, fractions were collected through the bottom of the tube. [For further details, see M. M. STAHL and F. W. STAHL (1971), MCMILIN and RUSSO (1972) and STAHL *et al.* (1972a).] For the detection of *c*-hets, fractions were plated on deep tryptone agar plates (M. LIEB, personal communication). *c*-hets were scored for markers in genes *R* and *J* as follows: Each *c*-het was picked (with a sterile bit of glass tubing) into 1 ml of buffer. The buffer was then "spotted" on a top layer seeded with C600 and incubated at 42° to distinguish *Rts129* from Rts^+ and on 594 seeded with λ*Rsus5* to distinguish *Rsus5* from $Rsus^+$. The *h* marker in gene *J* was scored by spotting on C600/λ. [See ARBER *et al.* (1983) for general λ methodology.]

RESULTS

The possibility for demonstrating 3′-primed synthesis depends on degradation of the 3′ end. If there is degradation from the 3′ end, 3′-primed synthesis of varying degrees will occur, and the new DNA will be part of the packaged recombinant virion. When the phages in a genetic cross are marked near the right terminus of λ, many of the progeny particles are heteroduplex for those markers. If a parent of a given recombinant particle has suffered degradation

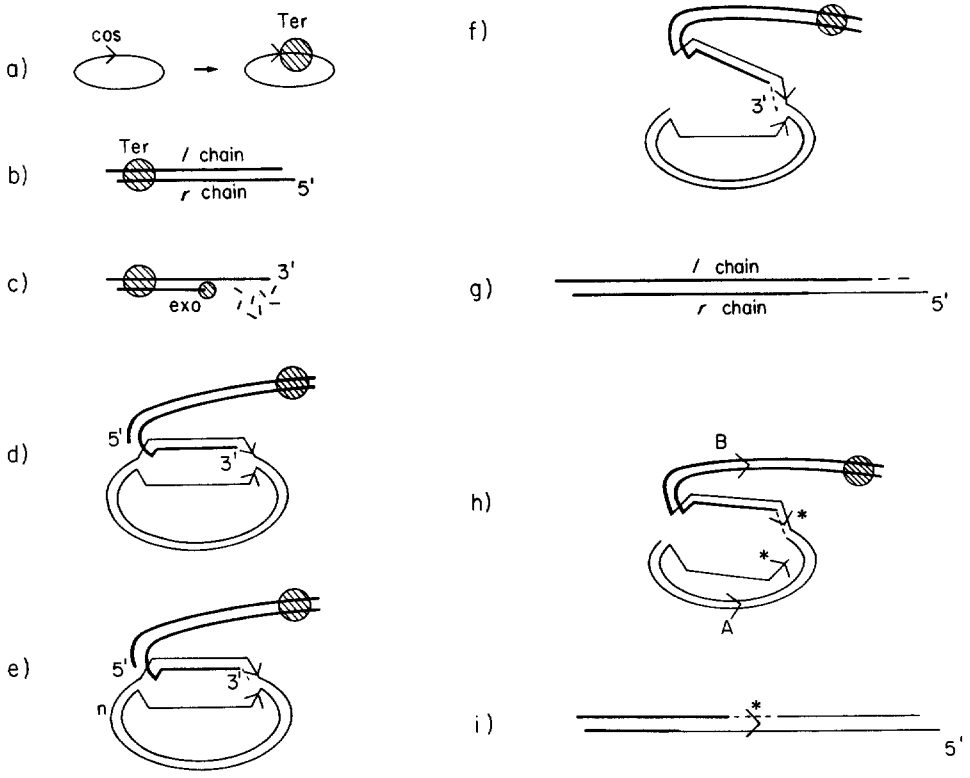


FIGURE 1.—Model for Red-mediated recombination stimulated by *cos* (from STAHL and STAHL 1985). a, Following injection, the sticky ends of λ anneal, creating *cos* and circularizing the chromosome. Following expression of λ's late genes, terminase (Ter) can bind to the right of *cos*. b, Ter cuts the *cos* site to its left, recreating sticky ends. Ter remains bound at the left end of the linear chromosome. c, The 5′-specific exonuclease of λ's Red system digests away the right-hand sticky end and continues leftward, creating the 3′ overhang. d, With the aid of the helix-destabilizing product of λ's Red *beta* gene, the 3′ end of the *l* chain invades an intact helix and establishes complementary base pairing. e, The resulting D-loop expands, perhaps via the coordinate action of the exonuclease and the *beta* protein, until a nick (n) is encountered. f, The 5′ end is ligated to the 3′ end provided by the nick. Synthesis primed by the invading 3′ end, at the right side of the D-loop, recreates a *cos*. g, The completed recombinant can then be packaged with the aid of the terminase that originally opened the invading chromosome, and which is still bound. The complete, packaged chromosome has derived most of its DNA from the invading duplex and has a single chain contribution, on the *r* chain, derived from the invaded chromosome. Depending on the degree of any degradation suffered by the invading 3′ end, there will be more or less DNA synthesis at the right end of the *l* chain. h, If the *cos* on the invaded chromosome is a noncuttable allele (*), the 3′ invading end can, perhaps after priming some DNA synthesis, join with a cut appropriately introduced into the displaced chain of the D-loop. The nick-translating nucleolytic activity of *PolI* may catalyze that cut. i, The resulting recombinant can then be packaged from the secondary *cos* sites (A and B) cloned centrally in the two parents. The resulting recombinant is a conserved Red-mediated recombinant with a medially placed point of exchange induced by the double-chain cut site, *cos*. The gene order in the recombinant is a permutation of the normal order.

TABLE 1

Phage markers

Marker	Description	Reference/source
h	Host range mutation in *J*	(?) KAISER (1962)
Jts15	Heat-sensitive mutation in *J*	BROWN and ARBER (1964)
int4	Unconditional *int* mutation	GINGERY and ECHOLS (1967)
cI26	Clear plaque mutant	MESELSON (1964)
cII68	Clear plaque mutant	IRA HERSKOWITZ
Psus80	Amber mutation in *P*; blocks DNA replication	CAMPBELL (1961)
Rsus5	Amber mutation in *R*	CAMPBELL (1961)
Rts129	Heat-sensitive mutation in *R*	CAMPBELL and DEL CAMPILLO-CAMPBELL (1963)
Rts2	Heat-sensitive mutation in *R*	BROWN and ARBER (1964)

TABLE 2

Bacterial strains

Strain	Description	Reference
FZ14	Su^- *recA56 dnaBts22*	STAHL *et al.* (1972b)
C600	$SuII^+$	APPLEYARD (1954)
C600/λ	$SuII^+$, resistant to λ h^+, sensitive to λ *h*	
594	Su^-	WEIGLE (1966)

from *cos* leftward beyond the marked site, then the resulting packaged chromosome will be homoduplex for that site. If degradation does not reach the marked site, then the particle will be heteroduplex. The heteroduplex nature of a progeny particle can be detected by a plaque assay; the amount of DNA synthesis in a progeny particle is given by its position in a cesium salt equilibrium density gradient. Our model predicts that the amount of DNA synthesis in a particle will correlate positively with homozygosis for markers near the right end of the chromosome.

Our crosses to assess this correlation have the following features: The labeled (^{13}C, ^{15}N) parents differ by two clear-plaque mutations. One parent is *cI26* and the other is *cII68* (see Figure 2). Particles that are heteroduplex over both genes will be trans heteroduplexes and will be expected to make mottled plaques, since the two *c* mutations will both complement and recombine to produce turbid areas in the plaques. Particles that are wild type on one chain but mutant on the other at one or the other locus will likewise make mottled plaques. Phage that are wild type on both chains are expected to be rare, and are so observed; they will make turbid plaques. We shall call all of these particles, which are detectably biparental in the *c* region, "*c*-hets." Our first datum is simply the frequency of *c*-hets among the progeny of a cross as a function of position in the conserved, "fully heavy" peak.

The labeled parents differed as well as the *R* locus, between *c* and the right end (see Figure 2). One parent carries a *ts* marker (*Rts129*) and the other an amber marker (*Rsus5*). Particles that are heteroduplex for both sites will be

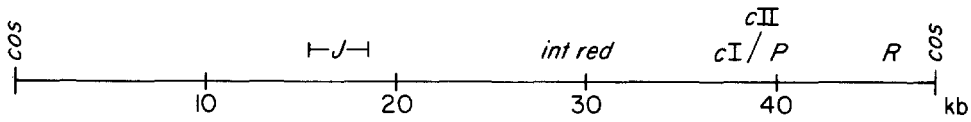


FIGURE 2.—Map of λ showing the genes to which reference is made.

trans heteroduplexes and will make plaques that contain both ts^+ and sus^+ particles; those heteroduplexes that are wild type on one chain will do likewise, as will the presumably rare particles that are wild type on both chains. We call all of these particles, which are detectably biparental at *R*, "R-hets." Our second datum is the frequency of *R*-hets among *c*-hets as a function of position in the density gradient.

If the *l* chain of λ chromosomes can be degraded from the right end to the left of *c* and can recover by synthesis back to *cos*, or part of the way back to *cos*, before completing the act by breaking and joining, the frequency of *c*-hets will vary across the conserved peak, being higher on the heavy side. On the other hand, if degradation and resynthesis cannot extend left of *c*, but can extend left of *R*, then the frequency of *c*-hets will be constant, but *R*-hets will vary across the gradient.

Both parents had an amber mutation in the *P* gene (*Psus80*), and crosses were conducted in the Su^- *dnaBts* strain FZ14 at 41.5°, so that chromosome replication was "double-blocked" (McMILIN and RUSSO 1972). The crosses were *cII68 Psus80 Rts129* × *cI26 Psus80 Rsus5*. The progeny of each cross was centrifuged to near equilibrium in cesium formate, and fractions were assayed for phage titer (Figures 3a and 4a). Each gradient gave a peak of unadsorbed phage and a single peak of progeny phage, which is lighter than the unadsorbed phage by virtue of newly synthesized protein coats. The progeny peaks appear to be slightly broader than the unadsorbed phage peaks, suggesting some DNA synthesis. In each experiment, fractions from the heavy flank and fractions from the light flank of the progeny peak were separately pooled and recentrifuged (Figures 3b and 4b). Then, fractions from the heavy side of the rebanded pool of heavy fractions were compared with fractions from the light side of the rebanded pool of light fractions. In each experiment (Tables 3 and 4) these fractions differed from each other with respect to both the frequency of *c*-hets and the frequency of *R*-hets among *c*-hets. Both frequencies were greater in the heavy fractions than in the light fractions. These data support the version of our model in which DNA synthesis, primed by the invading 3′ chain, sometimes is the route by which a *cos* is acquired by the incipient recombinant.

An alternative explanation for the DNA synthesis associated with homozygosity is that mismatches at *c* or *R* provoke mismatch correction, characterized by excision and replacement that is extensive enough to provide the density resolution that we see between hets and "nonhets." Such "long track" excision repair is methyl-dependent and does not operate on fully methylated DNA (PUKKILA *et al.* 1983; LU, CLARK and MODRICH 1983). M. FOX (MIT, personal communication) has suggested that fully conserved progeny phage are highly methylated and are, therefore, not subject to long-tract repair. Thus, mismatch correction is unlikely to be the source of the DNA synthesis seen here. As a test of the possibility, we have included a marker in λ's left arm (*h* in the *J*

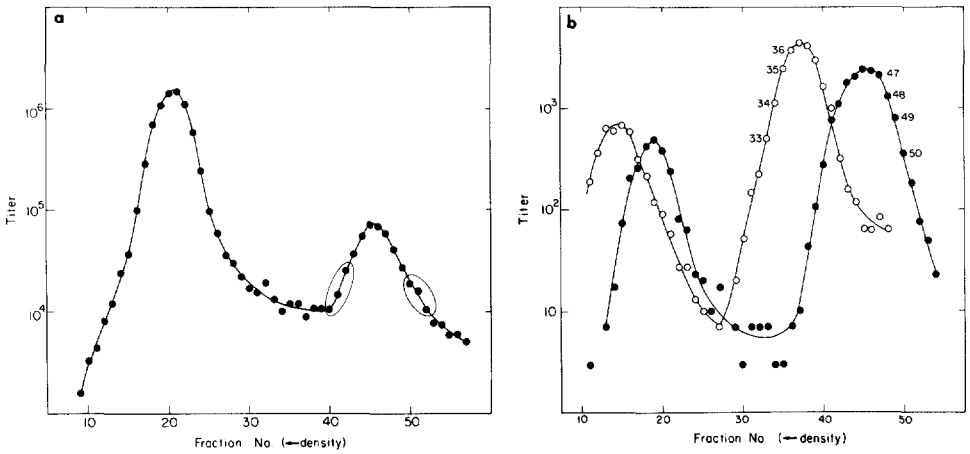
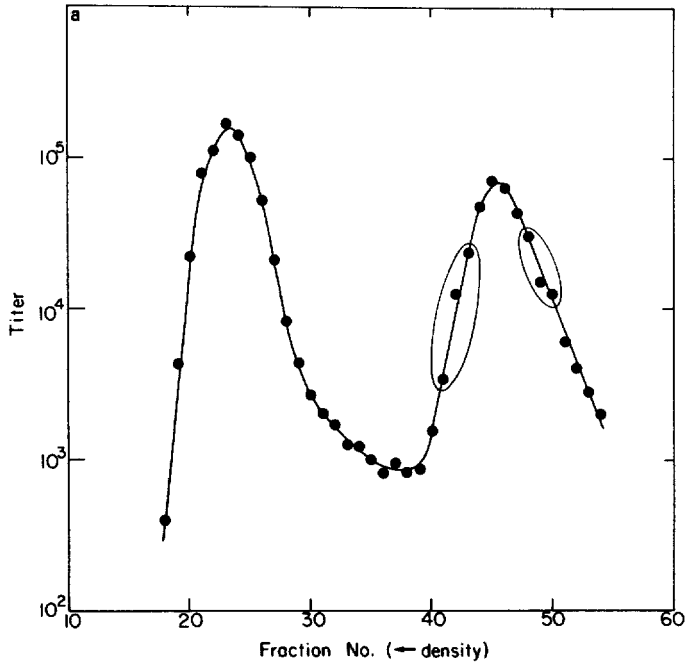


FIGURE 3.—Experiment I. a, Density-labeled phages were crossed in the *recA dnaBts* strain FZ14. Replication was blocked by high temperature (39°) and by the presence of *Psus80* in each parent. The lysate was centrifuged in cesium formate. The left-hand peak contains unabsorbed parental phages. The right-hand peak is composed of fully conserved progeny. The replication blocks have prevented the appearance of semiconserved or fully light progeny particles. b, Fractions 40–42 from (a) were pooled and recentrifuged, as were fractions 50–52, in a separate tube. Fractions 33–36 from the respun heavy fractions and fractions 47–50 of the respun light fractions were analyzed to provide the data in Table 3. Key: O, recentrifuged heavy fractions; number of fractions in this gradient was 114. ●, recentrifuged light fractions; number of fractions in this gradient was 110.

gene; see Figure 2). Our model (Figure 1g) predicts that conserved progeny that are hterozygous at *c* and homozygous at both *R* and *J* will be recombinant for *R* and *J*. The mismatch correction explanation for the DNA synthesis makes no such prediction. In Experiment III (density profile not shown) the *c126 Psus80 Rsus5* parent carried the *h* marker. In light fractions of rebanded light fractions, we scored *c*-hets for their genotype at both *J* and *R*. (In this experiment both parents were *int4*. This mutation, by blocking Int-dependent chromosome packaging, resulted in *c*-het frequencies about twice those seen in Experiments I and II.) The *J* locus was typed by spotting picked *c*-hets on C600/λ. Negative spots imply homozygosis for h^+, whereas positive spots are either *h* or h/h^+. Therefore, the phage of interest are those that are h^+, *c*-hets and homozygotes at *R*. We found that 32 of 34 of the *c*-hets that were h^+R homozygotes were *R sus,* the recombinant genotype for *J* and *R*. Thus, the DNA synthesis associated with *R*-homozygosity is comprehended by the model in Figure 1.

Which recombination pathway generates the recombinants? Our crosses are conducted with Red^+ phage in *recA* bacteria. Since the bacterial recombi-

FIGURE 4.—Experiment II, performed as described in Figure 3. a, As in Figure 3(a). b, Fractions 41–43 from (a) were pooled and recentrifuged, as were fractions 48–50. Fractions collected from these recentrifugations were assayed only in the regions of the progeny peaks. Fractions 46 and 47 from the respun heavy fractions and fractions 56 and 57 from the respun light fractions were analyzed to provide the data in Table 4. Key: O, recentrifuged heavy fractions; ●, recentrifuged light fractions.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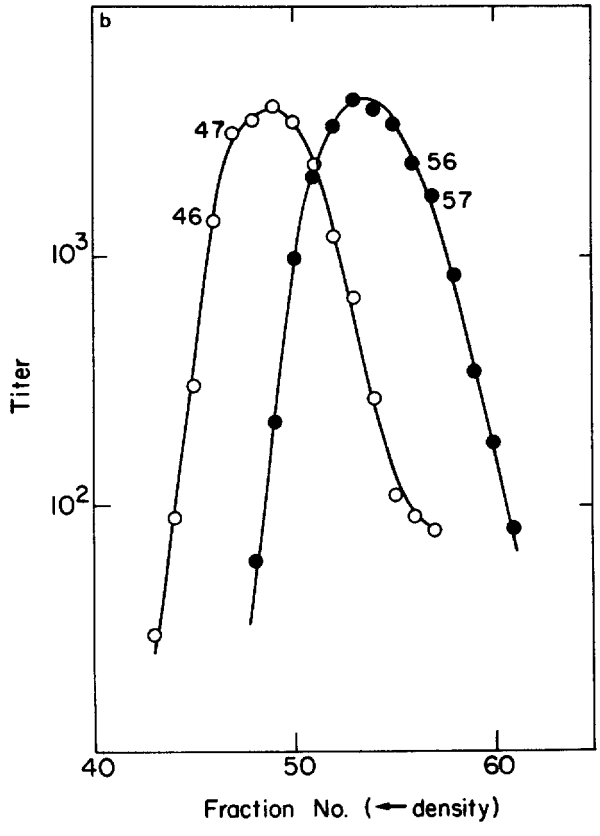
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10^3
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50
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Fraction No. (← density)

TABLE 3

Heterozygote frequencies in density fractions: experiment I

Heavy progeny				Light progeny			
	Fractions 40–42 (Figure 3a)				Fractions 50–52 (Figure 3a)		
Fraction (Figure 3b)	Plaques	*c*-hets	*R*-hets among *c*-hets	Fraction (Figure 3b)	Plaques	*c*-hets	*R*-hets among *c*-hets
33	4.0×10^2	4	2	50	2.3×10^2	3	1
34	8.5×10^2	18 (2)	14	49	5.1×10^2	8 (3)	3
35	2.2×10^3	70 (10)	57	48	9.3×10^2	20	3
36	2.2×10^3	94 (7)	71	47	1.1×10^3	16 (1)	5
Σ	5.7×10^3	186 (19)	144	Σ	2.8×10^3	47 (4)	12
	c-hets = 3.4%				*c*-hets = 1.8%		
	R-hets among *c*-hets = 77%				*R*-hets among *c*-hets = 26%		

In the columns denoting *c*-hets, the number picked is followed in parentheses by the number not picked. *R*-hets among *c*-hets denotes the number of *R*-hets found among the picked *c*-hets. In each row, analyses of the heavy and light fractions were paired. Plates were poured and plated, and plaques were picked and spotted in parallel. This procedure prevented variability in the detection or genotyping of *c*-hets from influencing the primary conclusions of this paper.

TABLE 4

Heterozygote frequencies in density fractions: experiment II

Heavy progeny				Light progeny			
	Fractions 41–43 (Figure 4a)				Fractions 48–50 (Figure 4a)		
Fraction (Figure 4b)	Plaques	*c*-hets	*R*-hets among *c*-hets	Fraction (Figure 4b)	Plaques	*c*-hets	*R*-hets among *c*-hets
46	8.9×10^2	49 (3)	37	57	1.4×10^3	24 (10)	8
47	2.2×10^3	95 (12)	75	56	2.1×10^3	56 (7)	24
Σ	3.1×10^3	144 (15)	112	Σ	3.5×10^3	80 (17)	32
	c-hets = 5.1%				*c*-hets = 2.8%		
	R-hets among *c*-hets = 78%				*R*-hets among *c*-hets = 40%		

See Table 3 footnotes.

nation pathways are RecA$^+$-dependent, it is likely that the recombinants observed in our crosses are Red-mediated. To verify this likelihood, we assessed the requirement for Red under the cross conditions we employed.

Under double-block conditions (*i.e.*, both parents *Psus80*, host FZ14), we crossed λ*Jts15 cI26* × *Rts2*. In one cross, both parents were *red*$^+$; in a parallel cross, both parents were *red3*. In the *red*$^+$ cross, 28 of 87 J^+R^+ plaques counted were either c^+ homoduplexes or c/c^+ heteroduplexes. These recombinants are due to exchange in the *c*I-*R* interval. Most of the J^+cR^+ recombinants are presumably due to Int acting in the *J*-*c*I interval. In the Red$^-$ cross, all of the 114 J^+R^+ plaques counted were *c*. Thus, most or all of the recombination at the right end in our experiments is, in fact, due to Red.

DISCUSSION

We have observed DNA synthesis associated with homozygosity in nonreplicated, Red-mediated recombinants. We have interpreted that synthesis in terms of a model (Figure 1) in which the synthesis occurs on one chain from a point left of the homozygous site toward the right end of the recombinant chromosome. Within this framework, our observation that *c*-hets are less frequent among lighter phage suggests that this synthesis can extend from *c* to the right end of the chromosome (Figure 2). Since the synthesis is presumed to be confined to one chain, 10% or more of the DNA can become light. In Figure 3b are shown the rebanded fractions of Experiment I. When plating the fractions from this gradient, we also plated the region corresponding to the unabsorbed phage. These unadsorbed phage peaks can be used as density references. Along with the knowledge of the total number of fractions in the gradient, they allow a rough estimate of the amount of DNA synthesis that distinguishes the heavy and the light fractions. Based on many (unpublished) observations, λ progeny containing fully conserved chromosomes band halfway between the density-labeled unadsorbed phage and the fully light progeny (see also MESELSON and WEIGLE 1961). If we take the heavier progeny peak to correspond to no DNA synthesis, then the position of the lighter peak corresponds to 16% new DNA synthesis. This estimate of the amount of synthesis is compatible with the expectations of the model.

Previous work has shown that Red-mediated recombination is dependent on replication. Recombinants in the interval from λ's left end to *c* are almost totally missing from the peak of fully conserved phage, but are appropriately represented in a light peak (STAHL *et al.* 1972b). This phenomenon has been subject to two interpretations, which are not mutually exclusive. One explanation (STAHL, KOBAYASHI and STAHL 1985) supposes that, among nonreplicating chromosomes, *cos* provides the only substrate on which the Red system can act. Replicating chromosomes, on the other hand, provide suitable substrates for Red activity more or less everywhere along the chromosome. Since *cos* is presumed to act by being a double-chain cut site, we may hypothesize that replication provides other duplex ends. The tips of the tails of rolling circle replication forms are candidates for these initiation structures (SKALKA 1977). The other explanation (F. W. STAHL and M. M. STAHL 1971) supposes

not that replication is required to initiate recombination but that replication is necessary to complete some intermediate; that is, that Red is a break-copy system in which forked structures initiated by recombination enzymes evolve into replication forks [see MOSIG, SHAW and GARCIA (1984) and SKALKA (1977)]. Two advances in our understanding of Red make the latter view less useful for λ than it once was: (1) Red *can* catalyze break-join recombination (STAHL, KOBAYASHI and STAHL 1985); (2) synthesis primed by a 3′ invading end appears able to extend from left of *c* to *cox* without any apparent involvement of *dnaB* or *P* product, both of which may be essential components of a replication fork (for a review, see FURTH and WICKNER 1983). If polymerase alone can provide the necessary DNA synthesis to "repair" a segment of that length, then there may be no obstacle to its synthesizing the full length of λ. If that is so, the lack of recombination in the middle and left arm of λ in replication-blocked crosses is due to a lack of initiation structures, such as those generated by *cos*, rather than to a shortage of routes for completing the event.

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