Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6318–6321, October 1982
Genetics

Distance from cohesive end site *cos* determines the replication requirement for recombination in phage λ

(*red* system/break and copy mechanism/density transfer)

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Contributed by Franklin W. Stahl, July 9, 1982

ABSTRACT **Previous work showed that crossing-over in the middle of the chromosome of phage λ requires more DNA replication than does crossing-over near the termini. Relocation of *cos*, the sequence that determines the λ termini, alters the requirements for replication in a given marked interval, demonstrating that distance from *cos* determines the amount of DNA replication that is required for genetic exchange. This result supports a break and copy mechanism for recombination mediated by the *red* system of phage λ.**

McMilin *et al.* (1) introduced an experiment that assesses the distribution of exchange points along nonreplicating chromosomes of phage λ. In their technique, density-labeled and ordinary phages genetically marked at terminal loci are crossed in the absence of DNA replication, achieved by a double genetic block. Under these conditions, the density of each emerging phage particle, as determined by isopycnic centrifugation, is a direct measure of the relative amounts of DNA inherited from the two infecting phage types. Among recombinants, density is a measure of the location of the genetic exchange event that generated that particular recombinant chromosome.

When this technique was applied to crosses in which the recombination systems of both λ phage and *Escherichia coli* were genetically wild type, a complex result was obtained (2). It was observed that exchanges were rare per unit of physical length in the middle of the chromosome. Most of the recombinants produced were the result of exchanges near the right end. This result was in apparent disagreement with the reported congruence (3) between the λ linkage map obtained from standard lytic cycle crosses and the physical map deduced from microscopic examination of heteroduplexes. Resolution of the disagreement lay in the previously reported (4, 5) DNA replication associated with recombination in the central region of the λ chromosome. That association had been shown in density-transfer experiments of a different sort. Both parents were density-labeled with heavy isotopes, and a single leaky block to replication was used. The block was variously *dnaB* of the host (4), *P* or *O* of the phage (6, 7), immunity (5), or *dnaG* (unpublished data). In all cases, recombinants for the central region of λ were relatively scarce in the conserved (heavy) peak but were represented better in the lighter ones. It was possible to conclude (6) that replication was not only associated with but also was necessary for a full level of recombination in the central region of the λ chromosome.

Experiments with various combinations of phage and bacterial mutants deficient in recombination-related functions revealed that only the *recBC* pathway (8) of *E. coli* operated without such differential behavior of medial and terminal recombination. The *red* pathway of phage λ and probably the *recF* pathway of *E. coli* showed the differential behavior (2), as did the *recE* pathway (9). The described relationship between replication and recombination has been interpreted in terms of a break and copy mechanism of phage λ recombination occurring in all the pathways of generalized recombination except for the *recBC* one. The models invoked have as their basic postulate the idea that a broken chain of one chromosome primes a replication fork that must move to the end of the other chromosome to complete the recombinant (10, 11). In this view, exchanges far from *cos*, the end of the chromosome, must replicate more DNA than those near the end. Subterminal exchanges may be completed by extension of the broken chain without generation of a replication fork.

The details of the models need not concern us because they are not challenged by the present paper. Instead, this paper challenges the central idea that distance from the chromosome end is, in fact, the relevant variable responsible for the differential behavior of medial and terminal regions. A concrete alternative explanation identifies distance from *ori*, the origin of phage λ replication, as the relevant variable. Based on this view (M. Fox, MIT, personal communication), replication potentiates break–join exchange. Abortive replications can stimulate exchange near *ori*, which is only 20% from the right end of phage λ, whereas full replication would be required to stimulate recombination far from *ori*.

The two views could be distinguished by comparing phages in which either *cos* or *ori* has been relocated. In this paper we describe experiments involving phages in which *cos* has been relocated. The results demonstrate that distance from *cos* is indeed a variable that influences the extent of replication required for recombination. Thus, the results stand in support of a break and copy model for λ recombination.

METHODS AND MATERIALS

Phage and Bacterial Strains. The phage λ mutations used are listed and described in Table 1. Their map positions are shown in Fig. 1. Strains of *E. coli* are listed and described in Table 2. In most cases, desired λ genotypes were isolated from UV-stimulated crosses (UV to phage only). All phages carried the phenotypically neutral deletion *nin5* in order to accommodate a supernumerary *cos* fragment. Isolation and characterization of the *cos2* and *cos3* mutations have been described (22).

Inversion of *cos*. The relocated *cos* in our strains was originally cloned in inverse orientation into the *Eco*RI B fragment of phage λ (22). Inversion of this *cos* to the standard *cos* orientation was accomplished by inverting the entire B fragment. To that end, we digested λ carrying the cloned *cos* with *Eco*RI, ligated the fragments, and packaged the DNA *in vitro*. Proof of inversion in isolated clones was obtained from gel electro-

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Table 1. Phage λ mutations

Mutation	Description	Ref.
susP80	SuII$^+$ suppressible amber in *P*. Poorly suppressed by SuIII$^+$	12
susS7	SuIII$^+$ suppressible amber in *S*. Not suppressed by SuII$^+$	13
cI857	*ts* in *cI*	14
cos2	22-bp deletion eliminating the standard *cos*	22
cos3	≈25-bp deletion eliminating the cloned *cos*	22
nin5	Deletion of 5.4% of λ	15
imm^{434}	Immunity region of 434 replacing that of λ; net deletion of 2% of λ	16

bp, base pairs.

phoresis of *Sma* I restriction fragments (23) prepared from a "rapid DNA preparation" modified from that described by Davis *et al.* (24).

Strain Construction Involving Mutant *cos*. Kobayashi *et al.* (22) showed that the plaque size on a *rec*$^+$ host of Red$^-$Gam$^-$ phage λ carrying the χ mutation depends on the orientation of χ and *cos* with respect to each other. This property of χ and *cos* was exploited in the construction of strains used herein. When a strain carries two *cos* sequences with opposite orientation, the state (active or inactive) or those sequences can be determined by crossing to a Red$^-$Gam$^-$ λ carrying χ and one active *cos* in known orientations. The χ is inseparably linked to the deletion or substitution inactivating *red* and *gam*. The cross progeny is then plated on a phage P2 lysogen of a *rec*$^+$ strain of *E. coli* to select Red$^-$Gam$^-$ λ (25). If the tester strain by itself made large plaques on the host, implying antiparallel χ and *cos* (22), then the appearance of small plaques in the cross lysate implies that the strain being tested is active for its *cos* that is parallel to the χ. On the other hand, if the tester phage makes small plaques, then the appearance of large plaques in the lysate implies that the strain to be tested is active for its *cos* that is antiparallel to the χ.

A stock of any strain bearing two *cos* sequences, one of which is inactive, will contain some particles that are active for both. These homogenotes may influence the results of the test crosses.

Density Labeling. All phages carried the temperature-sensitive mutation *cI857*. Density-labeled stocks were made by heat induction of lysogenic K12SH28 *E. coli* grown in heavy synthetic medium (^{15}N and ^{13}C). Conditions for isopycnic centrifugation in cesium formate have been described (21). The resolution obtained in these experiments, which used glucose substituted 50% with ^{13}C and NH_4Cl substituted 99% with ^{15}N, was less than that obtained in previous experiments. Mass spectroscopic analysis of the glucose (provided by Merck, Sharp and Dohme) revealed that most of the isotope was in about half of the molecules, which were nearly fully substituted. This isotopic distribution contrasted with that of previous batches from the same manufacturer, in which 50%-substituted glucose was composed of molecules, most of which were about 50% substituted. Our reduced resolution suggests an isotopic effect that causes infected cells to use lightly substituted glucose preferentially. Despite this disappointing nonideality, stocks of the two infecting phage types were nearly equally labeled as shown by isopycnic centrifugation (Fig. 2). Furthermore, results of the experiments contained their own proof that resolution was adequate.

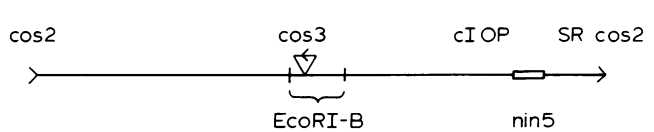

FIG. 1. Map of bacteriophage λ carrying a supernumerary *cos* site, showing features referred to and markers used.

Table 2. Strains of *E. coli*

Strain	Relevant properties	Ref.
JM1	SuIII$^+$	17
594	Su$^-$	18
K12SH28	Prototroph	19
FA22	*dnaBts* derivative of K12SH28	20
FZ14	RecA$^-$ derivative of FA22	21
C600(λ)	Selective host for λimm^{434}	

RESULTS

Plan of the Experiments. Crosses were performed in pairs. In each pair, one of the crosses utilized phages whose active *cos* is in the standard position, whereas the other cross utilized phages whose active *cos* is about in the middle of the standard λ map. The phages are derived from a strain in which a supernumerary *cos* had been cloned, in reverse orientation, within the *Eco*RI B fragment (see Fig. 1). Phages with active *cos* in the standard or the cloned position, respectively, were derived from strains in which the cloned *cos* had been mutated to inactivity (*cos3*) or the standard *cos* had been so mutated (*cos2*). In all crosses, the marked chromosomal interval is *P–S*, chosen for its proximity to the standard *cos* at the right end of the phage λ chromosome. (We used a marker in gene *S* because the first *R* marker we tried had a high reversion rate in the presence of the supernumerary *cos*. We presume that the cloned fragment contained a sequence at least remotely homologous to the vicinity of our marker, *susR5*.) In the phages with inactivated standard *cos* (*cos2* mutant phages), the *P–S* interval is about in the middle of the virion chromosome, each of which has been packaged from the cloned *cos*. Both parents in each cross were density-labeled.

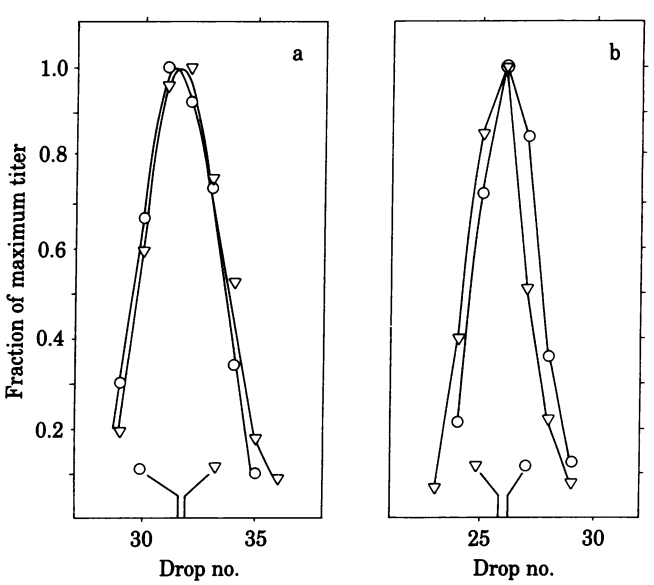

FIG. 2. Comparison of density labeling of phage λ stocks. Labeled parental phage stocks used in our experiments were centrifuged together to equilibrium in a Cs formate gradient. In each case, the density distributions are almost isomodal, and the mean densities are only slightly different. (*a*) Parent phages for Fig. 5*b*: ○, *susP80*; ▽, *susS7*. (*b*) Parent phages for Figs. 3*b* and 4*b*: ○, *susP80*; ▽, *susS7*.

The host carried a *ts* mutation in the *dnaB* gene, and infections were made at a temperature that allowed a little λ DNA replication. Lysates, collected by treating the cells with $CHCl_3$ 90 min after infection, were centrifuged in a cesium formate equilibrium gradient. Fractions were collected through a needle hole and assayed for total phage (on *E. coli* strain JM1) and for P^+S^+ recombinants (on *E. coli* strain 594). (On JM1, only the P^+ phages make large plaques, and only large plaques were enumerated).

The density distribution of the total phage provided the reference for each experiment. Under these conditions of restricted replication, many of the phage in the lysate owed their encapsidation to dimerization achieved by recombination (21). Thus, the mean density of the total phage is, in part, determined by the average amount of recombination-associated replication. The mean densities (average position in the gradient) of the P^+S^+ recombinants were determined and compared with the mean densities of the total phages. If the P^+S^+ recombinants are about as dense on the average as the total phage, we conclude that they have enjoyed about the same amount of replication per particle as has the average phage. Such behavior is characteristic of recombinants arising at the right end of standard phage λ chromosome. (Since most of the total particles are due to right-end recombination, the density of detectable right-end recombinants is likely to be similar to that of total phage.) If P^+S^+ recombinants are less dense on the average than the average of the total phage, then we conclude that the scored recombinants have enjoyed more recombination-associated DNA replication than has the total phage. Such behavior is characteristic of a marked region in the center of the standard λ virion chromosome.

Cross of Red$^+$ Phage in a Rec$^+$ Host. Fig. 3 shows the density distributions of a parental phage and of P^+S^+ recombinants from a replication-diminished cross. The phages in this cross were derived by *in vivo* genetics from λ in which a second *cos* was cloned in the *Eco*RI fragment B in an orientation inverse to that in standard λ (22). When the standard *cos* was active (Fig. 3*a*), the recombinants and the total phage were essentially isodense. When the cloned *cos*, remote from the *P–S* interval, was the active one, however, the average density of the recombinants was about 1.3 drops displaced to the light side relative to that of total phage (Fig. 3*b*).

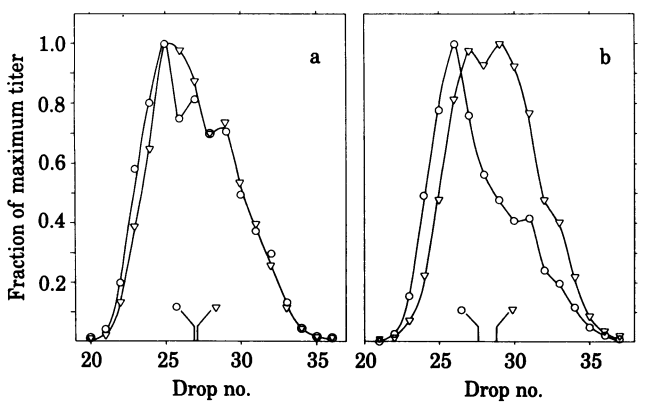

FIG. 3. Density distributions of phage and recombinants from crosses with *cos* functional in its normal position (*a*) and its translocated (cloned) position (*b*). All titers are normalized to titers of the peak fractions to facilitate comparison of the recombinant and parental phage distributions. Recombinant frequencies for the two crosses, which were carried out at 39°C on aliquots of a single culture of *E. coli* FA22, were, respectively, 13.7% (*a*) and 9.4% (*b*). ○, P^+ phage, assayed on *E. coli* strain JM1; ▽, S^+P^+ recombinants assayed on *E. coli* strain 594.

Cross of Red$^+$ Phage in a RecA$^-$ Host. Previous work (6) has shown that recombination-associated DNA replication does not require *recA* protein. Fig. 4 gives the results of crosses in a RecA$^-$ host (with diminished DNA replication) with the same labeled phages as in the previous experiment. The results are similar to those found for the Rec$^+$ host. The P^+S^+ recombinants were more dense than total phage (1.1 drops heavier) when the nearby, standard *cos* was functional but were less dense (0.7 drops lighter) than total phage when the cloned *cos* was the functional one. (The lower temperature used in this experiment allowed more DNA replication than was observed in Figs. 3 and 5).

Reinversion of the Cloned *cos*. In each of the experiments above, relocation of *cos* influenced the extent of replication associated with recombination in the *P–S* interval. In those phages, however, *cos* was not only relocated but also was inverted with respect to the orientation of standard phage λ *cos*. To rule out the possibility that orientation rather than location of *cos* was the factor determining the amount of replication associated with P^+S^+ recombinants, we reinverted the active cloned *cos* and marked the derived strain appropriately. Results of a cross involving such phages are shown in Fig. 5. The reinverted *cos* gave results like those obtained with the cloned *cos* in its original, inverted, orientation. Thus, location of *cos* with respect to the *P–S* interval is the factor that alters the amount of recombination-associated replication.

Amount of DNA Synthesis Associated with Recombination. Previously (4, 5), we estimated the average amount of new DNA per particle specifically associated with recombination in a central interval. The lower and upper limits were 10% and 50%, respectively. If the synthesis is semiconservative, these limits imply that between 20% and 100% of the chromosome is replicated in conjunction with a centrally located exchange. The density data in Fig. 5 permit an estimate of the amount of synthesis acquired by P^+S^+ recombinants as a result of the relocation of *cos*. In both parts of the figure, the distance between the heavy and light peaks is equivalent to ≈7 drops. [The density and band-shape reference phage is λimm^{434}, whose net DNA content (−2% of standard λ) is slightly less than that of the net content of our experimental phages (*nin* = −5.4% and *cos* = +4% for a net change of −1.4%); in our gradients, 1 drop was equivalent to about 1% difference in DNA content.] In Fig. 5*a* the P^+S^+ recombinants are 0.1 drop heavier than the average phage, whereas in Fig. 5*b* they are 1.4 drops lighter, for

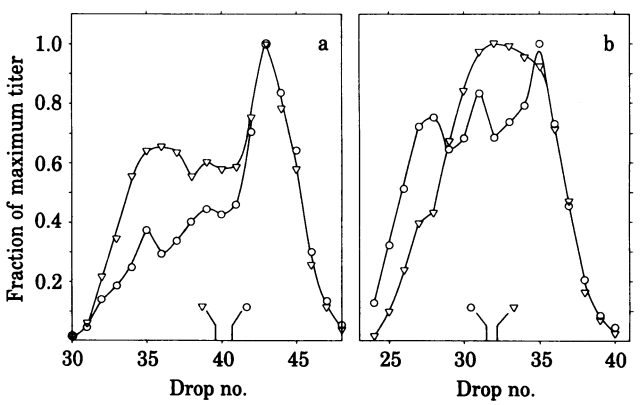

FIG. 4. Density distributions of phage λ and recombinants from crosses in Rec$^-$ *E. coli* with *cos* functional in its normal position (*a*) and its translocated (cloned) position (*b*). Key as in Fig. 3. Recombinant frequencies for the two crosses, which were carried out at 39°C on aliquots of a single culture of *E. coli* FZ14, were, respectively, 6.6% (*a*) and 2.8% (*b*).

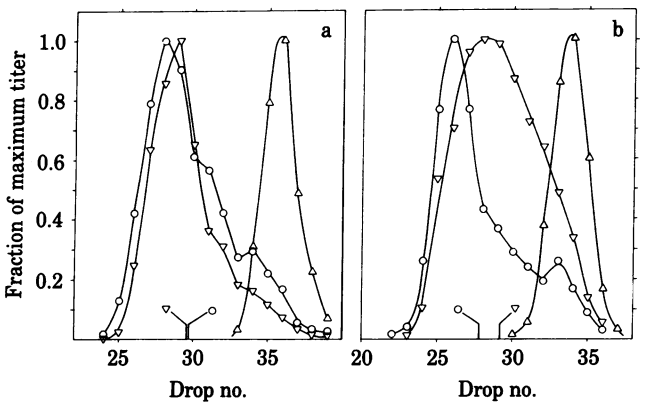

FIG. 5. Density distributions of phage λ and recombinants from crosses in Rec⁻ hosts with *cos* functional in its normal position (*a*) and its translocated position (*b*). In *b*, the translocated *cos* is oriented in the same way as the standard *cos*. In *a*, the phages are the same as in Figs. 3*a* and 4*a*. Recombinant frequencies for crosses in *a* and *b*, which were carried out at 41°C on aliquots of a single culture of *E. coli* FZ14, were, 6.9% (*a*) and 0.9% (*b*), respectively. The rightmost peak is a density reference marking the approximate position expected for fully light experimental phages. ○, P^+ phage assayed on *E. coli* strain JM1; ▽, P^+S^+ recombinants assayed on *E. coli* 594; △, λimm^{434} assayed on *E. coli* C600(λ).

a net change of 1.5 drops. Therefore, the minimal value for new DNA associated with the relocation of *cos* is 1.5/7, or 21%. For a semiconservative replication, 21% implies a fork progressing along 42% of the molecule on the average.

DISCUSSION

Our results demonstrate that the reported relationships between map position and relative dependency of recombination on replication (2, 4–7, 9, 10, 31) are due to distance from *cos*, rather than from *ori* or some other feature of the phage λ chromosome. By so doing, they support break-copy models of the sort previously presented.

Other workers (26–28) have concluded that recombination in phage T4 is predominantly by break and copy (see also ref. 29). Their proposal is similar to ones proposed for the *red* system of λ (10, 11, 30, 31). The core of such proposals is that a 3′ end of a chopped or nicked duplex, present in a recombinational intermediate, primes the formation of a replication fork. In phage λ, the recombinant molecule is completed when replication has restored a unit-length duplex flanked by *cos*.

This work was supported by National Science Foundation Grant PCM-8109799 and by National Institutes of Health Grant GM 20373.

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