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An Enhancing Role for DNA Synthesis in Formation of Bacteriophage Lambda Recombinants

(density label/DNA-negative mutants/Red system)

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ABSTRACT **Recombination in some intervals of the map of phage lambda is associated with more DNA synthesis than in other intervals. Blockage of DNA synthesis by high temperature in a host temperature-sensitive for DNA synthesis results in the relative reduction of recombinant frequencies in those regions having the larger amounts of recombination-associated synthesis. Reduction of DNA synthesis at normal temperatures by a combination of the bacterial mutation and a mutation in one of the phage genes required for DNA synthesis has the same consequence. Therefore, DNA synthesis enhances recombinant particle formation more in some map intervals than in others.**

DNA synthesis associated with recombination is revealed in crosses between two density-labeled parents under conditions in which DNA duplication is suppressed (1, 2). Synthesis associated with recombination in a given map interval is quantitated by comparison of the mean density of recombinants in that interval with the mean density of the total phage progeny. Recombinants in centrally located map intervals were observed to have mean densities corresponding to an inheritance of 10–25% less parental label per particle than the average inheritance in the total progeny. This finding was interpreted to imply an average amount of synthesis of from 10 to 50% of the chromosome specifically associated with the formation of particles recombinant in central intervals (2).

Two observations suggested (1) that the recombination-associated synthesis played a nonessential role in lambda recombination—(*i*) some acts of recombination appeared to be consummated without DNA synthesis, and (*ii*) the overall frequency of recombinants in crosses conducted under conditions of moderate (1, 2) or severe (3) inhibition of DNA synthesis was not less than that observed in standard lambda crosses. However, observation (*i*) overlooks the possibility that DNA synthesis is essential for recombinant formation in those instances in which recombination is associated with DNA synthesis. Observation (*ii*) fails to anticipate that recombination (i.e., the action of the recombination systems) is prerequisite for particle production among phage that have not duplicated (4). This last realization renders observation (*i*) suspect *as an observation*, since our definition of fully heavy (H/H) refers to the modal density of the heaviest *observed* particles, and these particles may contain DNA newly synthesized in association with the recombination act that rendered them maturable. The consequence of the role of recombination in maturation relevant to observation (*ii*) is the gross one that inhibition of DNA duplication imposes an absolute block on the maturation of any chromosome that has not "recombined." Thus, in crosses in which DNA synthesis is severely inhibited, *all* emerging particles (though there may not be many) have necessarily been acted upon by the recombination systems, and the frequency of recombinants among them is understandably high.

A resolution to our dilemma was suggested by certain features of density crosses conducted with slight DNA synthesis (1), as compared to others conducted with more-stringent blocks (3, 4). Fig. 1, previously published (1), shows the density distribution for total progeny and for recombinants in three intervals from a cross conducted in a host temperature-sensitive for DNA synthesis. The temperature used allowed some synthesis. Recombination in the central interval (J–*c*I) involves the most DNA synthesis, that in the right arm (*c*I to R) involves the least, and recombination in the left arm (W to J) has an intermediate amount of synthesis (see Fig. 3). Fig. 2, some of the data in which have previously appeared (4), shows the density distribution from a (heavy) × (light) cross of recombinants for markers in terminal genes *A* and *R*. This cross was conducted under conditions that would give a nearly symmetric peak of only H/H phage if all infecting particles were heavy; the infecting particles were mutant in a lambda gene required for chromosome duplication, and the host was genetically blocked for DNA synthesis as well. The recombinant density distribution can be interpreted as a graph of the per nucleotide rate of recombination along the chromosome. This rate is seen to be lowest through the middle of the chromosome, highest toward the right end, and intermediate toward the left end. This nonuniform recombination rate contrasts with the nearly uniform rate from standard crosses (5). In fact, the rates along the chromosome in the absence of DNA synthesis look about as they would if blockage of the DNA synthesis in Fig. 1 simply resulted in the disappearance of the particles containing the newly synthesized DNA, i.e., of particles lighter than the H/H mode. Such would be the result if the DNA synthesis (or some part of it) were, in fact, essential for the formation of those recombinant particles. The present paper is directly aimed at that interpretation. Our experiments show that under Red$^+$ conditions (both *rec*A plus and minus), severe inhibition of DNA synthesis results in the relative loss of recombinants in map intervals in which recombination shows large amounts of associated synthesis.

MATERIALS AND METHODS

In general, techniques and strains were described (1, 3, 4). In addition, strain Q5151, a GroP strain (6) obtained from I. Herskowitz, was used to assay *sus*P phage in the presence of

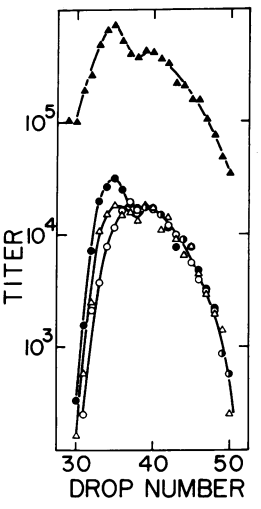

FIG. 1. Density-labeled *sus*W *ts*J and *c*I857 *sus*R were crossed in *E. coli* strain FA22 at 39°, a temperature not totally restrictive for lambda DNA synthesis. The progeny from 10^7 to 10^8 infected cells were centrifuged to equilibrium in a cesium-formate density gradient, and fractions were collected (each) into 1 ml of broth. The fractions were assayed for total progeny phage (▲), and *sus*$^+$ recombinants in the intervals W–J (△), J–*c*I (○), and *c*I–R (●). See ref. 1 for further details.

P$^+$ particles. Density-labeled phage stocks (^{13}C and ^{15}N) were prepared either by induction (heat or UV) or on plates, except in one case involving Int$^-$ phage, in which the plate method did not yield a stock of sufficiently high titer. In this case, sensitive cells were infected in liquid culture under conditions suggested to us by Enzo Russo. 5 ml of M-medium prepared with [^{13}C]glucose and $^{15}NH_4Cl$ was inoculated with 25 μl of washed K12SH28 cells from an overnight culture in the same medium (prepared with ordinary glucose and NH_4Cl). Aeration at 37° for about 4 hr gave a culture of log-phase cells

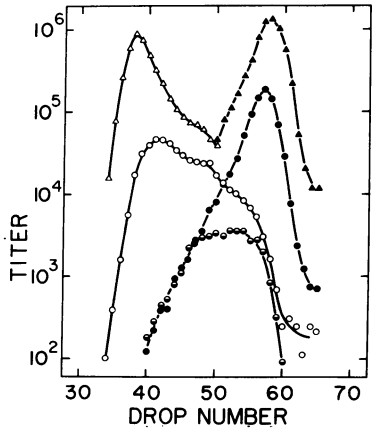

FIG. 2. Density-labeled *ts*A was crossed with light *c*I26*ts*R at 39° in FA77, a *su*$^-$ derivative of FA22. Both phages carried the mutation *sus*029, so that DNA synthesis is more severely inhibited than it is in Fig. 1. Total *c* phage (▲) and *c*$^+$ phage (△) were scored on the *su*$^+$ strain C600 at low temperature, while *ts*$^+$ recombinants were scored as *c* (●) or *c*$^+$ (○) at high temperature on the same indicator. Mottled plaques (◒) (probably representing particles heteroduplex at the *c* locus) made up an appreciable fraction of the total recombinants, as indicated. See ref. 4 for further details.

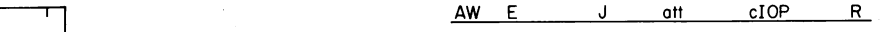

FIG. 3. Linkage map of lambda, showing the positions of genes and other landmarks referred to in this paper.

at a titer of 10^8/ml. These cells were spun down, then resuspended in 0.1 volume of the supernatant. 1.5×10^9 phage particles were added to the 5×10^8 bacteria for 10 min at room temperature. The remaining 4.5 ml of supernatant was then added, and the infected culture was incubated at 30° for about 5 hr. Lysis was completed by addition of chloroform. The somewhat heterogeneous labeling of the particles that resulted when we used this method did not appreciably distort the data (Fig. 4).

A map of lambda showing the locations of genes and sites relevant to this work is presented in Fig. 3. The following markers were used: *ts*A14, *sus*W403, *ts*E3, *ts*J15, *sus*J6, *c*I857, *c*I26, *sus*R5, *ts*R2.

RESULTS

Crosses under Red$^+$ Rec$^+$ conditions

Density-labeled *sus*J and *c*I*sus*R were crossed in the Rec$^+$ strain FA77, which is temperature-sensitive for lambda DNA synthesis. Both phages were Red$^+$, so that this experiment examines the combined behavior of the two generalized recombination systems. Because the action of Int at *att* in the J–*c*I interval could complicate the interpretation of this cross, both parents were Int$^-$. These crosses were performed on a single culture of bacteria that was split into fractions that were incubated for 10 min at 36°, 39°, 42°, and 44°. Aliquots of a mixture of the two parental phages were added to each, to give a nominal multiplicity of infection of 20, and the infected cultures were incubated for 60 min at the four different temperatures. Unadsorbed phage were eliminated by sedimentation of the infected cells, and the cultures were lysed and sterilized with egg-white lysozyme and chloroform. The lysates were then centrifuged to equilibrium in cesium formate density gradients.

The density distribution of the progeny of the 36° cross is given in Fig. 4*a*, wherein it should be noted that recombinants

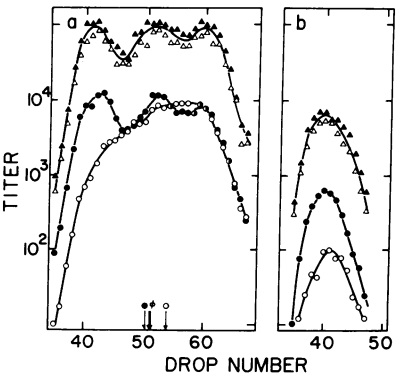

FIG. 4. Density-labeled *sus*J*int*4 and *int*4c1857*sus*R were crossed in strain FA77 at 36° (*a*) and 44° (*b*). Total progeny were scored as *c* (▲) or *c*$^+$ (△) on strain C600. *Sus*$^+$ recombinants were scored as *c* (●) or *c*$^+$ (○) on the *su*$^-$ indicator 594. Means of the recombinant and total phage (ϕ) density distributions are marked on the *abscissa* of the 36° cross. Unlike previously reported Int$^-$ crosses (2) that used *int*6, crosses using *int*4 give equally good maturation of *sus*R and *sus*$^+$ phages.

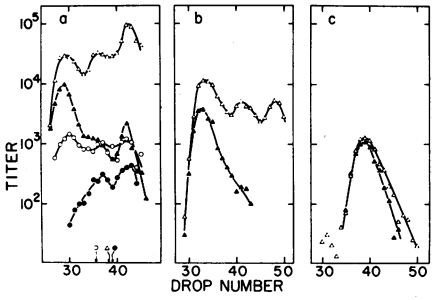

FIG. 5. Density-labeled *ts*EcI26 and *ts*J*ts*R were crossed in the *su*$^-$ *rec*A$^-$ strain FZ14 at 36° (*a*), 39° (*b*), and 42° (*c*). On the same culture at the same three temperatures, density-labeled phages carrying the mutation *sus*P, in addition to the markers above, were crossed as well. The two lysates from a given temperature were combined and centrifuged to equilibrium in cesium formate. Total progeny from the P$^+$ crosses (△) were scored on strain 594 at the permissive temperature of 30°. Recombinants from the P$^+$ cross (for Fig. 5*a* only) were scored on strain 594 as follows: Recombinants in the E–J interval (●) were taken as all plaques rising at 42°, a temperature restrictive for all the *ts* markers in the cross. Recombinants in the *c*I–R interval (○) were taken as turbid (*c*$^+$) plaques formed at 37°, a temperature restrictive for the *ts*R marker, but permissive for the others. Total progeny from the P$^-$ crosses (▲) were scored on strain Q5151.

in the interval J–*c*I are appreciably lighter on the average than are recombinants in the interval *c*I–R. This discrepancy in density defines DNA synthesis associated with recombination in the J–*c*I interval. This synthesis could be associated with the recombination in either of two ways—the synthesis might be essential for the production of the recombinaant that manifests it or the synthesis might be merely casually related to recombination. (An example of a casual relationship would be that opportunities for recombination in the J–*c*I interval are provided by the same set of circumstances that provides for duplication of the lambda chromosome.) The two kinds of associations make different predictions with regard to the effects of blockage of the DNA synthesis. If the synthesis is essential, then blockage of synthesis will result in a reduction of J–*c*I recombinants relative to *c*I–R recombinants; i.e., blockage of synthesis will simply amount to throwing away the right-hand two thirds of Fig. 4*a*. If synthesis is but casually related to recombination in the J–*c*I interval, however, blockage of synthesis will cause recombinants in the light regions to "squeeze" into the H/H peak, and the relative frequencies of the two recombinant types will remain the same as in the total lysate of the 36° cross. The density distributions of progeny types in the 44° cross are given in Fig. 4*b*. Note that the phage emerge in a single peak, and that the recombinants look as they would if the light peaks of Fig. 4*a* were thrown away.

The relative change in recombination frequencies in the two intervals as a function of the amount of DNA synthesis is quantitated by the ratio of titers of the two types at each temperature, as measured in the unfractionated lysates. At 36° the ratio of *c*I–R recombinants to J–*c*I recombinants was 1.4; at 44° it was 8.2. At the intermediate temperatures of 39° and 42°, both the mean densities of emerging phages and the ratios of the recombinants in the two test intervals were intermediate. These ratios were 2.0 and 2.5, respectively. Thus, when Red and Rec are acting jointly, DNA synthesis appears to be essential for the formation of many of the recombinant particles. A second set of crosses, examining the behavior of the Red system in RecA$^-$ cells, is described below.

Crosses under Red$^+$ RecA$^-$ conditions

In these experiments, we compare the intervals E–J and *c*I–R. As before, crosses were performed at different temperatures on a single culture of bacteria temperature-sensitive for DNA synthesis. In this case, however, strain FZ14, a RecA$^-$ derivative of *su*$^-$ FA77, was used. At each temperature two crosses were performed. In one cross, both participating phages were wild type with respect to the DNA synthesis genes of lambda. In the other cross, both parents were defective for gene *P* function by virtue of a *sus* mutation in that gene.

Fig. 5 compares the density distributions for the total phage produced in each of the six crosses. In the P$^+$ crosses, increasing temperature progressively decreases the average amount of DNA synthesis per particle produced until, at 42°, all the phage emerge in the H/H peak. In the P$^-$ crosses, however, almost all the phage emerge in the H/H peak at each of the three temperatures. The amount of DNA synthesis that characterizes recombination in the two marked intervals can be compared in Fig. 5*a*. Recombinants in the E–J interval are less dense than overall phage, while those in the *c*I–R interval are more dense. The high density of *c*I–R recombinants compared to overall phage reflects the requirement for recombination for the appearance (presumably maturation) of unduplicated chromosomes among the progeny (4). The ratio of recombinant frequencies *c*I–R/E–J computed from the data in Fig. 5*a* is 5.1. The same ratio computed from platings of the unfractionated lysate was 4.8. For each of the three P$^+$ crosses, the *c*I–R/E–J recombinant ratio, as determined from platings of the unfractionated lysates, is given in Table 1. The corresponding ratios for the P$^-$ crosses are also given. The ratios for the P$^+$ crosses increase as temperature increases and DNA synthesis decreases. Those for the P$^-$ crosses are essentially invariant with temperature, as is the amount of DNA synthesis in those crosses. (The corresponding ratio for a standard P$^+$ cross in the ordinary RecA$^-$ strain QR48 was 1.6.) These results support the idea that for the Red system work-

TABLE 1. *Effects of blocks to DNA synthesis on relative recombinant titer in two map intervals*

Temperature of cross	Corresponding Figure	*c*I–R/E–J recombinant titer	
		P$^+$ cross	P$^-$ cross
36°	5*a*	4.8	19
39°	5*b*	6.6	20
42°	5*c*	16	24

Density-labeled lambda types *ts*E *c*I26 and *ts*J *ts*R were crossed in the *su*$^-$RecA$^-$ strain FZ14 at each of three temperatures (column *1*). The density distributions of the progeny were examined in order to assess the extents of DNA synthesis (column *2*). At each temperature the ratio of recombinant titer in the *c*I–R interval to that in the E–J interval was determined for two crosses —in one cross both parents were wild type for lambda P function (column *3*), in the other case both were *sus* mutants (column *4*). Recombinant titers for the interval E–J were taken as the titer of the lysate on *E. coli* strain C600 at 42°, a temperature restrictive for each of the *ts* mutants in the cross. Recombinants in the *c*I–R interval were enumerated as turbid (*c*$^+$) plaques on C600 at 37°, a temperature permissive for *ts*E and *ts*J, but restrictive for *ts*R.

ing alone (i.e., in RecA$^-$ conditions), the DNA synthesis associated with recombination in some intervals is essential for the formation of the recombinant particles that manifest it.

DISCUSSION

We will deal briefly with two questions: (*i*) What qualifications must we put upon our conclusion that recombination-associated DNA synthesis plays an essential role in the formation of the recombinant particles that manifest it? (*ii*) What role does the observed extensive DNA synthesis play in the formation of the recombinant particle?

(*i*) We have used two methods for imposing a strong inhibition on DNA synthesis—high temperature (42–44°) in a host temperature-sensitive for lambda DNA synthesis, and elimination by mutation of the P-gene function of the phage. The two methods essentially eliminate DNA synthesis and lead to the same final result—recombination is relatively depressed in map intervals displaying large amounts of recombination-associated synthesis. If we could be certain that the two methods of blocking DNA synthesis were working by different mechanisms, we would consider our conclusion solid. Unfortunately, the known interaction between the P product and the *DNA-B* gene product manifested by the Gro-P mutants of *Escherichia coli* (6) makes the supposition suspect. It remains possible, therefore, that destruction of a part of the DNA-synthesizing apparatus involving the P/*DNA-B* gene products interferes with recombination by some mechanism other than that of interfering with DNA synthesis *per se*. This possibility is slightly weakened by the results displayed in Fig. 2, in which a block in gene *O* of lambda was used to prevent DNA synthesis.

(*ii*) The experiments described do not discriminate among various possibilities for the association between extensive DNA synthesis and recombination. The schemes we have thought most about embody one or another of the following four ideas: (*i*) Recombination (in some regions of the map) occurs best at replicating forks or on newly replicated DNA. (*ii*) Recombination (in some regions) occurs in a way that does not result in a directly maturable chromosome; subsequent duplication of the recombinant is required for maturation. (*iii*) Recombination is a source of replication forks (7); in some map regions replication from that fork is the primary route to formation of a complete, maturable recombinant molecule. (*iv*) Replication changes the topology of the chromosome and, as a consequence, the regions of the chromosome accessible to the recombination systems.

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