THE INTERACTION OF *cos* WITH CHI IS SEPARABLE FROM DNA PACKAGING IN *recA- recBC*-MEDIATED RECOMBINATION OF BACTERIOPHAGE LAMBDA

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ABSTRACT

Chi (5′-GCTGGTGG) is a recombinator in RecA- RecBC-mediated recombination in *Escherichia coli*. In bacteriophage λ vegetative recombination, Chi is fully active only when it is correctly oriented with respect to *cos*, the site that defines the ends of the packaged chromosome. Here we demonstrate that packaging from *cos* is not necessary for this cos-Chi interaction. Our evidence suggests that correctly oriented *cos* is an activator of Chi. *cos*, as an activator, is (1) dominant over cos^-, (2) active opposite an extensive heterology, (3) able to interact with Chi only when on the same (*cis*) chromosome, and (4) able to interact with Chi at distances as far as ≥ 20 kb. Thus, *cos* and Chi form a two-component recombinator system for general recombination. *cos* may serve as an asymmetric entry site for a recombination enzyme that recognizes Chi in an asymmetric way.

GENERAL genetic recombination can take place at any point between two homologous DNA sequences, but the distribution of crossovers is not uniform along the DNA molecule. Special sites ("recombinators") that influence recombination in their neighborhood were inferred by genetic analyses in fungi (LISSOUBA and RIZET 1960; LISSOUBA *et al.* 1962; MURRAY 1963; for review see STAHL 1979a,b) and later substantiated by the discovery and characterization of Chi (χ) in *Escherichia coli* (STAHL, CRASEMANN and STAHL 1975; for review see STAHL 1979b). Chi, the DNA sequence 5′GCTGGTGG (SMITH *et al.* 1981b) is a recombinator in *recA- recBC*-mediated recombination (STAHL and STAHL 1977; GILLEN and CLARK 1974). It influences recombination in P1 transduction and in conjugation (DOWER and STAHL 1981) as well as in bacteriophage λ lytic infections, where it has been studied in detail.

Figure 1a helps us to review the lytic cycle of λ. A phage particle contains a double-stranded DNA molecule with complementary single-stranded "cohesive ends." After entry into the cell, the ends are covalently closed forming a *cos* (cohesive end site) sequence. The resulting circle with a single *cos* is not a good substrate for packaging into a phage particle. Unit λ DNA bounded on each end by a separate *cos* sequence is packaged efficiently. The packaging machinery

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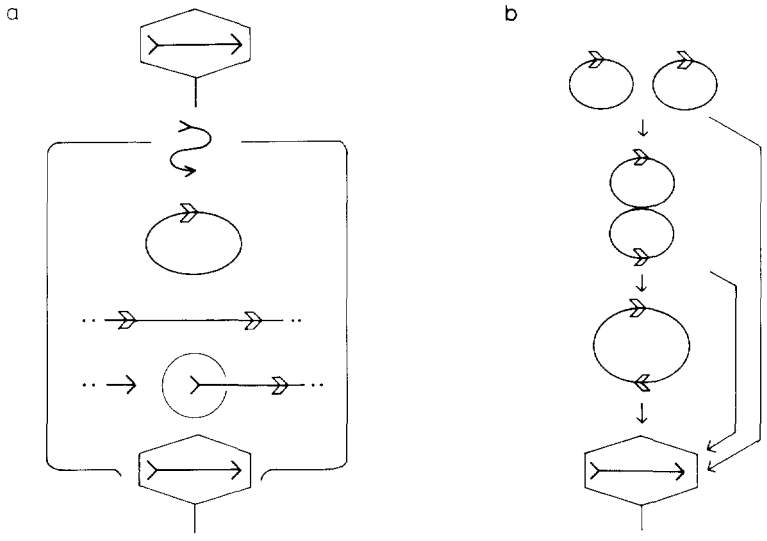

FIGURE 1.—a, λ DNA cycle in lytic infection; b, Rec-mediated recombination of λ DNA. Recombination takes place between two circular DNA molecules and produces a form from which chromosomes can be packaged, for example, a circular dimer. A figure-eight-shaped dimer, in which two circles are joined at a homologous site via a Holliday junction, is one intermediate form. The possibility that packaging could be coupled with resolution of the figure-eight, or even its formation, in Chi-stimulated recombination is indicated by the bypass arrows.

recognizes and cuts the *cos'* to regenerate the cohesive ends. This interaction is asymmetric (HOHN 1975; FEISS *et al.* 1979; FEISS and WIDNER 1982; FEISS, KOBAYASHI and WIDNER 1983), and DNA packaging proceeds in a polar way starting from the left end (EMMONS 1974; FEISS and BUBLITZ 1975; STERNBERG and WEISBERG 1975). Under standard conditions (Red$^-$ Gam$^-$) for Chi action, the only effective route to an efficiently packaged form is *recA recBC*-dependent recombination. As illustrated in Figure 1b, *recA*-dependent recombination of λ can proceed via a figure-eight ("8")-shaped intermediate form (IKEDA and KOBAYASHI 1979), in which two circles are joined at homologous sites by a Holliday junction (HOLLIDAY 1964). The resolution of the Holliday junction can convert a figure-eight into a circular dimer, which is a good substrate for packaging (IKEDA and KOBAYASHI 1979; ROSS and FREIFELDER 1976). Chi somehow enhances the overall recombination reaction, producing more packageable DNA forms and hence promoting phage particle formation (STAHL, CRASEMANN and STAHL 1975; MALONE and CHATTORAJ 1975).

Figure 2a schematizes the properties of Chi action in λ. Chi on only one of the DNA molecules is sufficient to stimulate recombination (*dominance*) (LAM *et al.* 1974). Chi enhances exchanges even when the recombining partner carries a large heterologous sequence opposite Chi (*action opposite heterology*) (STAHL and STAHL 1975). Chi can stimulate exchange as far as 10 kb away (*action at a distance*) (LAM *et al.* 1974). The stimulated crossovers are located primarily to one side of a given Chi (*bias*) (STAHL and STAHL 1975).

Study of this bias led to the discovery of *orientation-dependence*, the feature

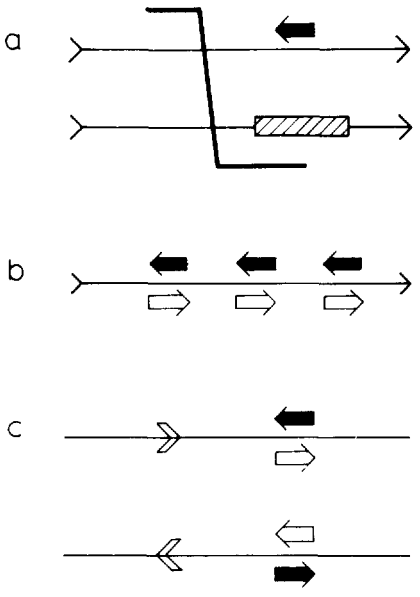

FIGURE 2.—Properties of Chi stimulation of recombination in λ lytic growth. a, Recombination is stimulated even if only one of the participating chromosome carries Chi (short thick arrow) (dominance). It is active even opposite heterology (hatched box). It can stimulate recombination at a distance. The crossovers are located primarily to the left of Chi when Chi is drawn as 5′-GCTGGTGG (leftward arrow) (leftward bias and directionality); b, Orientation-dependence. Leftward Chi is severalfold more active than rightward Chi throughout the λ chromosome; c, Chi-*cos* interaction. The orientation of *cos* defines the active orientation of Chi.

most relevant to this study. All of the Chi detected on λ have a leftward bias (STAHL and STAHL 1975; STAHL *et al.* 1980b; CHATTORAJ *et al.* 1979). By inverting DNA segments carrying Chi, only one orientation of Chi (arbitrarily called leftward orientation) was shown to give full activity irrespective of its location along the chromosome, as illustrated in Figure 2b (FAULDS *et al.* 1979; YAGIL *et al.* 1980). Consistent with this finding, all of the Chi elements detected on λ had one orientation, that is, 5′-GCTGGTGG on the *l*-strand, irrespective of their location along the chromosome (SPRAGUE, FAULDS and SMITH 1978; SMITH, SCHULTZ and CRASEMANN 1980; SMITH *et al.* 1981b,c). These results suggest that the Chi sequence has intrinsic bias (*directionality*) in its stimulation of recombination (leftward when drawn as 5′-GCTGGTGG). They also suggest that Chi has to be correctly oriented with respect to a global polarity of λ (FAULDS *et al.* 1979; STAHL *et al.* 1980a).

A model we call the "resolution-packaging model" (FAULDS *et al.* 1979; STAHL 1979b) can explain these and other properties of Chi action in λ lytic infections, especially "orientation-dependence." As diagrammed in Figure 3a, the model proposes that Chi helps the *recBC* enzyme to resolve the Holliday junction of a figure-eight that has been formed by *recA* protein. *recBC* enzyme enters a figure-eight at the Holliday junction and recognizes Chi. When Chi and the Holliday junction are correctly oriented, *recBC* enzyme resolves the junction.

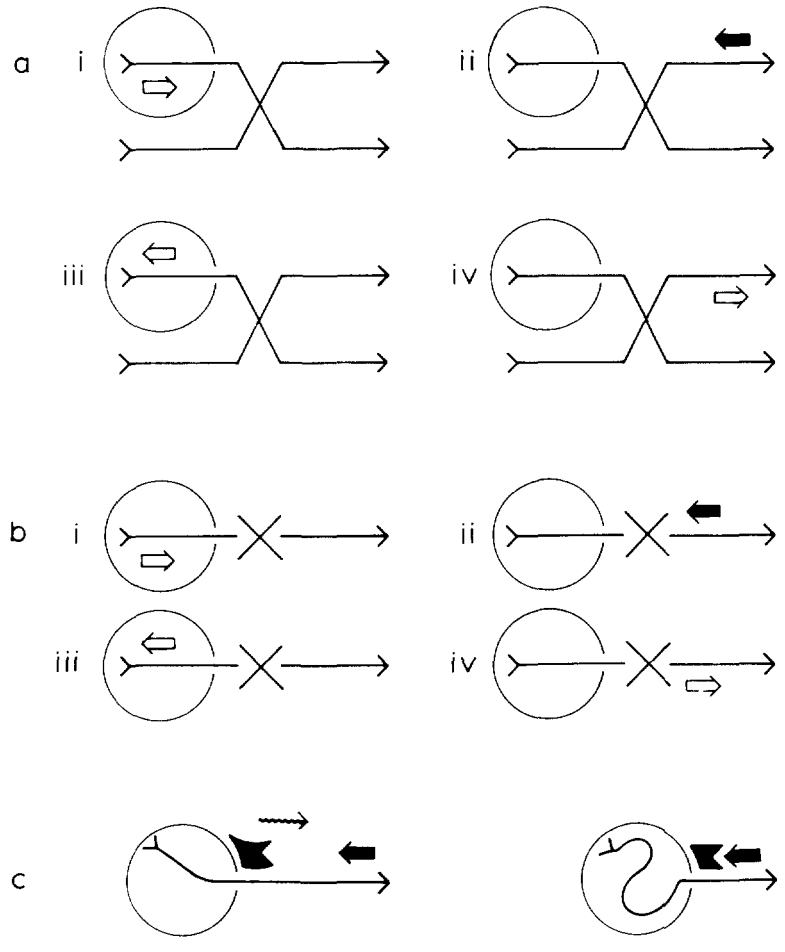

FIGURE 3.—Packaging models for Chi-*cos* interaction. a, The resolution-packaging model (modified from FAULDS et al. 1979, and from STAHL 1979b). As soon as a figure-eight intermediate (drawn here in a linear version) is formed, it is packaged from the *cos* rightward up to the junction. Chi enhances resolution of the junction. Rightward Chi to the left of the junction is masked by the head and cannot perform its action (i). Leftward Chi to the right of the junction can stimulate resolution (ii). In (iv) Chi is looking away from the junction and thus cannot help resolve it. In (iii) Chi is masked and looking in the wrong direction. Something is presumed to prevent Chi action until the packaging machinery is available. b, A packaging model at a more abstract level. As soon as a recombinogenic alteration (indicated by a cross) is produced, λ *DNA* is packaged from *cos* up to that alteration. Or, alternatively, the packaging machinery may produce that recombinogenic alteration. In either case only leftward (antiparallel to *cos*) Chi can act on that altered structure as in (a). c, One specific example of a positive packaging model. A recombination enzyme travels along with the packaging machinery in one orientation and in one direction (rightward) from the left *cos*. The enzyme interacts only with Chi antiparallel to *cos*.

To explain orientation-dependence, the model supposes that, as soon as a figure-eight is formed, it is packaged from *cos* rightward up to the Holliday junction. A rightward Chi, which would be otherwise active, is inactive since it is masked by the head and is inaccessible to the *recBC* enzyme [Figure 3a (i)]. An

unspecified mechanism is assumed to set the timing of Chi action at the stage of packaging.

A central prediction of this model is that the orientation of *cos* defines the active orientation of Chi. By constructing λ variants carrying *cos* in the two possible orientations, we have verified this prediction (KOBAYASHI *et al.* 1982). To be fully active, Chi and *cos* have to be correctly oriented relative to each other as diagrammed in Figure 2c. A second prediction of the resolution-packaging model is that packaging from *cos* is essential for the interaction of *cos* and Chi. In this work we test this prediction and find it not to be the case. Packaging from *cos* is not necessary for its interaction with Chi. In contrast to the predictions of the resolution-packaging model, our results indicate that *cos activates* Chi. Its properties lead us to think of *cos* as one part of a two-component recombinator system for generalized recombination.

MATERIALS AND METHODS

Bacterial strains: *Escherichia coli* strains used are listed in Table 1. Other strains were described previously (YAGIL *et al.* 1980).

Phage genetic elements and nomenclature: The λ mutants used are listed in Table 2 and shown in Fig. 4. λdv021 extends from about 77% λ to 83% λ (7% λ in length). The intracellular form of λdv021 in JC9937 [λdv021] was analyzed (STAHL *et al.* 1982).

Nomenclature of *cos* is slightly modified from previous usage (KOBAYASHI *et al.* 1982) (see also Table 2 and Figure 4). The ends of λ's genetic map define the site for the primary *cos*. The middle of λ's genetic map sometimes has a secondary, cloned *cos*. The cloned *cos* is a ϕ80-λ hybrid *cos* (ϕ80 right end, $-\lambda$ left end) but has *cos*λ specificity (that is, it is recognized by the λ packaging machinery). The primary *cos* is oriented rightward by definition. *cos*λ at the primary *cos* site can be intact (*cos*$^+$) or inactivated by the *cos2* mutation (*cos*$^-$). The secondary *cos* can be oriented either leftward or rightward. We list the primary *cos* genotype first and then the secondary *cos* genotype.

Nomenclature of χ is as follows (KOBAYASHI *et al.* 1982): A given DNA segment either has (χ^+) or does not have (χ^0) a Chi sequence (SMITH *et al.* 1981b). On the λ map χ^+ can be oriented either leftward or rightward. χ^+ is defined to be oriented leftward in λ when 5′-GCTGGTGG is on the l-strand. The phenotype can be Chi$^+$ or Chi$^-$. When χ^+ and *cos* have the same orientation, they are said to be parallel. Otherwise they are antiparallel.

Phage strain construction involving cos: The interaction of χ and *cos* (KOBAYASHI *et al.* 1982) was exploited during strain construction to test the orientation and/or genotype of *cos*. The method involves backcrosses to tester phage and has been outlined previously (STAHL, KOBAYASHI and STAHL 1982). The corroborating test was gel electrophoresis of restriction fragments from rapid DNA preparation or purified DNA as described later.

The *cos* genotype of each phage preparation (see Figure 9) used in $\lambda \times \lambda$dv crosses was verified by cleavage with the restriction enzyme *Sma*I as shown in Figures 5 and 6. The leftmost fragment (A) and the rightmost fragment (C) on the map are fused because the primary *cos* locus is not cleaved by *in vivo* packaging. The central fragment (B) is cleaved into two by *in vivo* packaging at the cloned secondary *cos*. The sizes of the fragments verify the orientation of the secondary *cos*.

Further testing for *cos2* in phage stocks used in $\lambda \times \lambda$dv crosses was carried out as described later in this article. The *cos2* mutation is measurably unstable as a result of homogenotization ("conversion" of *cos2* to *cos*$^+$ by interaction with the cloned *cos*). Consequently, individual *cos2* stocks must be verified for that mutation. Our *Sma*I analysis (Figures 5 and 6) showed that most phage particles in our preparation have *cos2* mutation. Procedures for genetic verification of *cos2* depend on the interaction of *cos* and Chi and, therefore, differ according to whether the cloned *cos* is leftward or rightward.

Stocks of phages with leftward cloned *cos* (MMS874 and 878) were tested by crossing them to the Chi-containing, *Sam7*, *cos2* strain MMS848 in the *recB sbcA* host JC8679 (Figure 7). The presence of large plaques on a *rec*$^+$ Su$^-$ host is a sensitive indication of particles that are not *cos2*. Our failure

TABLE 1

Bacterial strains

Strain	Properties	Source/reference
QR48	SulI$^+$ *recA*	SIGNER and WEIL 1968
KR3a	SulII$^+$ *recA*	IRA HERSKOWITZ
C600	SulI$^+$ *rec*$^+$	APPLEYARD 1954
594	Su$^-$ *rec*$^+$	WEIGLE 1966
JC8679	SulI$^+$ *recB21 sbcA20*	STAHL and STAHL 1977
JC9937	Su$^-$ *rec*$^+$	STAHL *et al.* 1982
JM1	SulII$^+$ *recB21 sbcA20*	STAHL *et al.* 1980b
JC9387	Su$^-$ *recB21 recC22 sbcB15*	STAHL and STAHL 1977

TABLE 2

λ Genetic elements employed

Genetic elements	Relevant properties	Source/reference
secondary *cos*	φ80-λ hybrid *cos*, made up of λ's leftmost and φ80's rightmost *Bgl*II fragments inserted into a *Bgl*II site at 47%.	HELIOS MURIALDO/KOBAYASHI *et al.* 1982
cos2	22-bp deletion removing nicking site of *cos*.	KOBAYASHI *et al.* 1982; FEISS, KOBAYASHI, and WIDNER 1983
χC$^+$157	Active Chi sequence within *cII* with cII$^-$ phenotype.	STAHL, CRASEMANN and STAHL 1975; SPRAGUE, FAULDS and SMITH 1978
χD$^+$123	Active Chi sequence to the left of *S*.	STAHL, CRASEMANN and STAHL 1975 SMITH *et al.* 1981a
χ$^+$76	Active Chi sequence inseparable from *b1453*.	KOBAYASHI *et al.* 1982; STAHL and STAHL 1977
b1453	Red$^-$ Gam$^-$ deletion.	HENDERSON and WEIL 1975
imm434	Immunity region of phage 434 substituted into λ	KAISER and JACOB 1957
imm21	Immunity region of phage 21 substituted into λ	CAMPBELL 1971
λ*dv021*	Circular plasmid derivative of λ (7% λ) bearing genes *O* and *P*	BERG 1974; STAHL *et al.* 1982
nin5	Deletion	FIANDT *et al.* 1971
red3	Nonconditional Red$^-$ mutation	SIGNER and WEIL 1968
gam210	Amber mutation in gene *gam* (γ)	ZISSLER, SIGNER and SCHAEFER 1971
cI857	Thermosensitive mutation in *cI*	SUSSMAN and JACOB 1962
cII2002	*cII* mutation (not Chi$^+$)	STAHL, CRASEMANN and STAHL 1975
Pam80	Amber mutation in gene *P*.	CAMPBELL 1961
Sam7	Amber mutation in *S*, suppressible by SulII$^+$ but not by SulI$^+$.	GOLDBERG and HOWE 1969

to see any large plaques, in more than 10^3 plaques examined, adequately confirms the purity of the stock.

The genetic test for *cos2* in those phage whose cloned *cos* is rightward (MMS959 and 963) is less convenient. These phage were crossed with the *cos2 Sam7* strain (MMS912) that carries a leftward cloned *cos* and a leftward Chi (χC$^+$) that confers a clear plaque phenotype (Figure 8). Selection for the *S*$^+$ allele from the phage being tested and for the *gam210* allele of MMS912 (on a *recA*$^+$ SulI$^+$ host) ensures, because of the arrangement of deletions and inversions, that essentially all clear

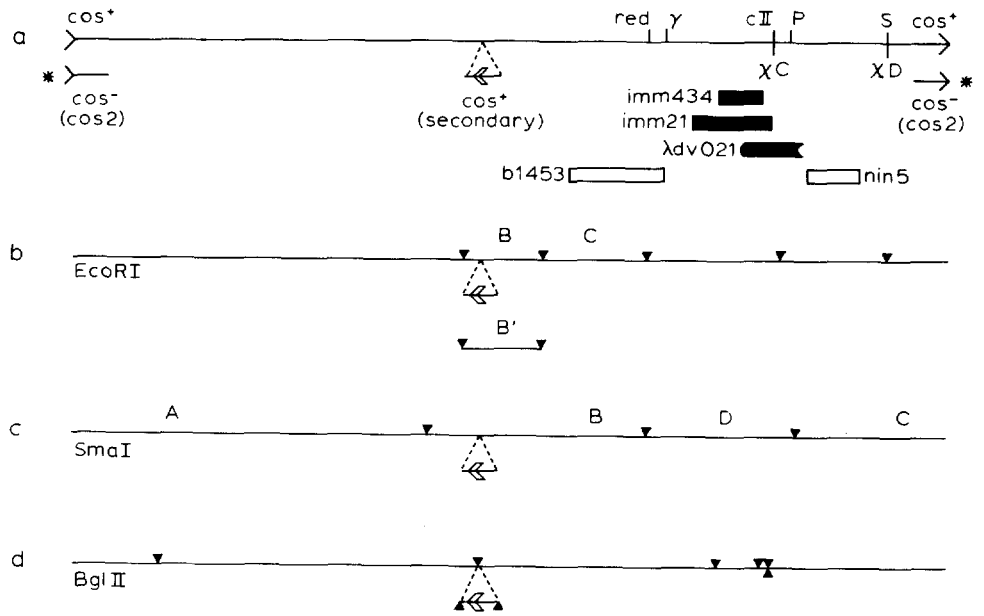

FIGURE 4.—a, λ genetic map. *red* represents two genes, *exo* and *bet*; b, λ restriction cleavage map. Deletion of *Eco*RI-C fragment makes λ Red⁻. The *Eco*RI-B fragment was inverted to invert the secondary *cos*.

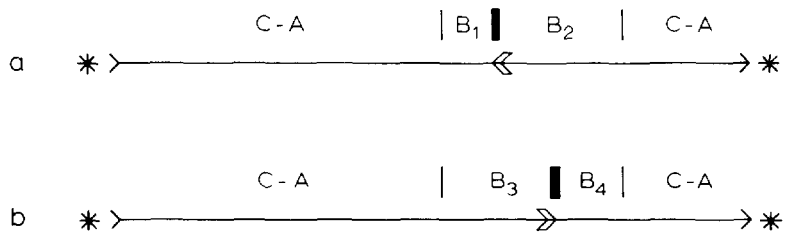

FIGURE 5.—*Sma*I cleavage map of λ *cos* mutant phage in Figure 9. * indicates *cos*⁻ mutation (*cos2*). The thick vertical lines show the cleavage at *cos* by *in vivo* packaging. The thin vertical lines show the cleavage by *Sma*I. The leftmost fragment (A) and the rightmost fragment (C) are fused because the primary, natural, *cos* is inactive and not cleaved in packaging. Instead, packaging from the cloned secondary *cos* cleaves B fragment into two pieces. The sizes of the resulting two pieces verify the orientation of the secondary *cos*. The cloned *cos*, *b1453* deletion and *imm21* substitution have made fragment sizes different from these in wild-type λ and have eliminated D fragment. a: Figure 9a; b: Figure 9b.

plaques will not only carry the leftward, clear-Chi and the leftward cloned *cos* but will also inherit their primary *cos* from the phage being tested. When these plaques are picked and streaked on a host that discriminates Chi⁺ and Chi⁻ phenotype [594(P2)], they will give tiny plaques only if they are *cos2*. We picked and tested 20 clear plaques for both strain MMS959 and 963 and found all 40 to be *cos2*.

The *cos2* strains used in the *cis-trans* experiments (MMS912, 946 and 970) all have leftward cloned *cos*. They were tested for *cos2* with crosses similar to that described for *cos2* stocks with leftward cloned *cos*.

Media and phage methodology: BBL bottom and top agars, which give larger plaques for Red⁻ Gam⁻λ than do most media, were described previously as was K-maltose (STAHL and STAHL 1971a).

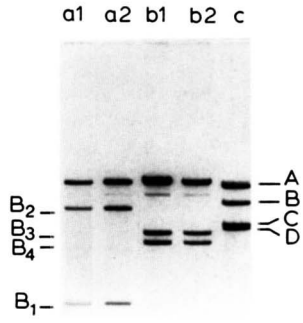

FIGURE 6.—*Sma*I cleavage of λ *cos* mutant phage in Figure 9. DNA was purified from the phage preparations used in the λ × λdv crosses (Figure 9) and in the genetic tests (Figures 7 and 8). *Sma*I digests were run through 1% agarose (see Figure 5 for explanation). The faint B bands in a1, a2, b1 and b4 resulted from cohesive end joining. Absence of C fragment indicates that few, if any, phage have *cos*$^+$ at the primary *cos* locus. a1: Figure 5a (Figure 9a). χ^+ (MMS874); a2: Figure 5a (Figure 9a). χ^0 (MMS878); b1: Figure 5b (Figure 9b). χ^+ (MMS959); b2: Figure 5b (Figure 9b). χ^0 (MMS963); c: Wild type λ (also *cI857 Sam7*).

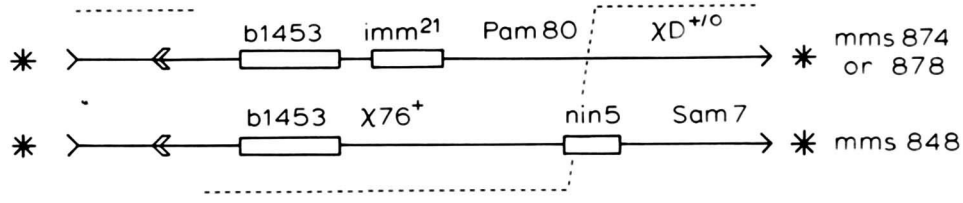

FIGURE 7.—Genetic test for *cos2* in stocks of MMS874 and 878. Phage were crossed (in strain JC8679) to MMS848, and the P^+ S^+ recombinants were selected on 594. As indicated by the dashed line, most of the recombinants inherit the *cos* allele at the standard locus from the S^+ parent. Symbolism is as defined in Figure 4. The map is not to scale.

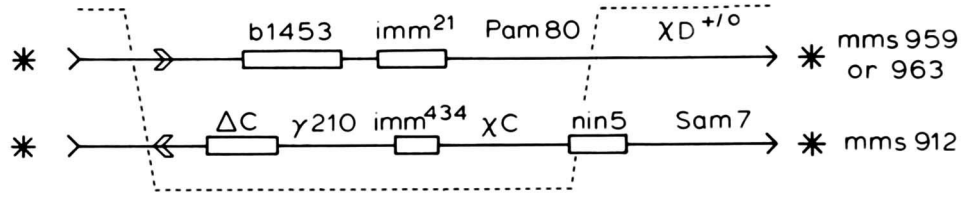

FIGURE 8.—Genetic test for *cos2* in stocks of MMS959 and 963. Phages were crossed (in strain JC8679) to MMS912, and clear (χC^+) plaques were picked off QR48, which selects against *S7* and *b1453*. All these recombinants inherit the rightward cloned *cos*, because the *Eco*RI-B fragment (between SR1 and SR2), in which the *cos* resides, is inverted in MMS959 and 963. This inversion abuts ΔC, a deletion from SR2 to SR3, which, in turn, overlaps the Red$^-$Gam$^-$ deletion *b1453*. Because of the proximity of *S* to the standard *cos* locus, most of the selected recombinants will carry the allele at that locus from the phage being tested. Each such particle that is *cos2* will make small plaques on the *rec*$^+$ Su$^-$ indicator 594 (P2). The map is not to scale; in particular, the actual distances from the left end to the cloned *cos* is about half the total λ chromosome length.

The other media and methods were standard (HERSHEY 1971). λ Tryptone SeaKem agarose plates contain λ tryptone broth and 1.2% SeaKem LE agarose (FMC Corporation) plus 0.3% glucose, 4×10^{-6} M $FeCl_3$, 7.5×10^{-5} M $CaCl_2$, 10 μg/ml of vitamin B_1, 2 mM $MgSO_4$.

DNA isolation: (1) Purified DNA. The phage particles were prepared by the confluent lysis method or by liquid infection. They were purified by high-speed centrifugation and by banding in

a cesium formate or CsCl gradient. The DNA was prepared by two cycles of phenol extraction (KOBAYASHI and IKEDA 1977). (2) Rapid DNA. The method is a modification of one by HEIDI KNICKERBOCKER and KAREN SPRAGUE. Plate lysate was prepared on fresh λ tryptone SeaKem agarose plate from 10^6 phage particles. The plate was left overnight at 4° after overlay of 5 ml of 10 mM Tris-HCl, 10 mM $MgSO_4$. The liquid was carefully removed. SeaKem plate lysate or liquid lysate freed of cell debris (0.4 ml) was mixed with 40 μl of 0.5 M EDTA (pH ~8), 20 μl of 2 M Tris acetate (pH ~7.5), and 20 μl of 10% SDS and was incubated for 15 min at 70° in an Eppendorf tube; 0.1 ml of 5 M potassium acetate was mixed, and the tube was left on ice for 30 min and then spun for 10 min. To the supernatant 0.4 ml of SSC-saturated phenol was mixed, and the tube was spun. The upper phase was removed and mixed with 40 μl of 3 M sodium acetate. Cold ethanol (1.1 ml) was mixed, and the tube was left on crushed dry ice for 10 min and then spun for 5 min. The supernatant was discarded, and the visible pellet was dissolved in 100 μl of 10 mM Tris·HCl (pH 7.5) and 2 mM EDTA and mixed with 10 μl of 3 M sodium acetate and then with 0.3 ml ethanol. The tube was left on dry ice for 5 min. The tube was spun, and the supernatant was discarded. The pellet was dried under vacuum and then suspended in 40 μl of 10 mM Tris·HCl (pH 7.5) and 2 mM EDTA and stored at −20°.

Restriction enzymes and gel electrophoresis: The enzymes *Sma*I and *Hind*III were purchased from New England Biolab, and *Eco*RI was purchased from Miles Laboratories Inc. Rapid DNA preparations were digested in the presence of 100 μg/ml of RNase (Sigma Chemical Company) that had been boiled to inactivate DNase. Restriction digests were heated at 75° for 5 min to melt annealed cohesive ends and were quickly chilled in ice water. The electrophoresis was carried out at 4°. We detected little cohesive end annealing under these conditions.

Cross: (1) Phage × phage crosses. Bacteria were grown in K-maltose to 1.5×10^8/ml. Equal volumes of the bacterial culture and phage mixture containing 1.0×10^9/ml each of the parental phage were mixed and aerated at room temperature for 30 min. The cultures were diluted tenfold in prewarmed K-maltose and aerated at 37° for 90 min. Chloroform and freshly prepared lyoszyme (to 20 μg/ml) were added, and tubes were left at room temperature for 10 min. (2) Phage × λdv crosses. The cells carrying λdv were grown in λ tryptone broth supplemented with maltose (0.2%) and vitamin B_1 (10 μg/ml) to 1.5×10^8/ml. The phage particles were added at a multiplicity of 7. The phage-bacteria complexes were left at 25° for 30 min and then filtered through a Millipore membrane (0.22 μm). The complexes freed of unadsorbed phage were diluted 100-fold into prewarmed broth and aerated at 37°. After 90 min, $CHCl_3$ was added, and the phage was assayed.

RESULTS AND DISCUSSION

Strategy

The resolution-packaging model as cited before is a specific example of models in which packaging from *cos* is necessary for Chi-*cos* interaction. The experiments reported here have been devised to test this class of models. Such models can be classified into two categories—positive and negative. Chi is fully active with an antiparallel *cos* and much less active with a parallel *cos*, as diagrammed in Figure 2c. An antiparallel *cos* might activate Chi (positive version), or, alternatively, a parallel *cos* might inhibit Chi that would be otherwise active (negative version). The resolution-packaging model is one example of a negative model. Figure 3b shows another, less specific, example. Packaging masks DNA from the left end up to a recombinogenic structure, which is the site of action of properly oriented Chi. Figure 3c illustrates one example of a positive version. A recombination enzyme travels together with the packaging machinery from left to right and recognizes a correctly oriented Chi.

Chi-stimulated recombination between two unit-sized λ produces a dimer in which each unit λ chromosome is bounded by a pair of *cos* sequences as

illustrated in Figure 1b. Packaging using these *cos* sequences could be coupled with the recombination process. If, however, one of the parental chromosomes carries a functional *cos* and the other does not, the *cos* without a partner cannot be used for packaging. We carried out two experiments based on this principle. The results exclude both positive and negative versions of the packaging model.

Recombination between λ and λdv

λdv is a plasmid formed by circularization of a small part of the λ genome (MATSUBARA and KAISER 1968) and may be thought of as a mutant carrying a huge deletion that removes the *cos* region (Figure 4). We crossed λdv with λ carrying only one active *cos*, in the middle of the genome, in the leftward (Figure 9a) or rightward (Figure 9b) orientation. The natural *cos* in these phage was inactivated by a 22-bp deletion, *cos2* (Table 2). A secondary, functional *cos* was placed in the two possible orientations in the middle of λ genetic map by a molecular cloning technique as described in MATERIALS AND METHODS. The presence and orientation of this *cos* was verified by analyses with a restriction enzyme as shown in Figures 5 and 6.

Reciprocal recombination between λ and λdv does not result in a product from which a chromosome can be packaged. The resulting recombinant DNA is essentially a monomeric circle in its size and in its number of *cos*. If packaging from *cos* is essential for the Chi *cos* interaction, there would be no Chi-*cos* interaction in λ × λdv recombination. Both positive and negative versions make that prediction.

In the crosses diagrammed in Figure 9, we measured the frequency of the recombinant P^+ phage in the two *cos* orientations. The results in Table 3 show that Chi stimulates λ × λdv recombination to a greater extent with an antiparallel *cos* (Figure 9b) than with a parallel *cos* (Figure 9a). In control crosses without Chi (χ^0), the orientation of *cos* did not affect recombination frequency.

The recombinant phage detected here are of two types: one is accompanied

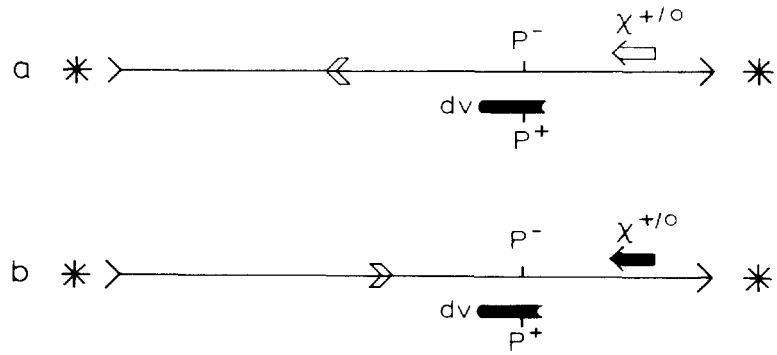

FIGURE 9.—Recombination between infecting λ and plasmid λdv. a, χ^+ and cos^+ are parallel; b, χ^+ and cos^+ are antiparallel. rec^+ cells carrying λdv plasmid were infected with λ *b1453 imm21 Pam80*. Its standard *cos* was inactivated by mutation (*cos2*), and a cloned secondary *cos* was present either in leftward orientation (a) or in rightward orientation (b). The phage is either χ^0 or χD^+. The source of the leftward secondary *cos* is described in Table 2. The entire EcoRI-B fragment (see Figure 4) was inverted in order to invert the secondary *cos*.

TABLE 3

cos-orientation-dependent Chi activity in λ × λdv cross

Cross		$\frac{\lambda P^+}{\text{total }\lambda}$ in χ^+ cross / $\frac{\lambda P^+}{\text{total }\lambda}$ in χ° cross	
cos-chi orientation	Diagram	Experiment 1	Experiment 2
Same (parallel)	Figure 9a	$\frac{6.5 \times 10^{-3}}{1.72 \times 10^{-3}} = 3.8$	$\frac{4.5 \times 10^{-3}}{1.49 \times 10^{-3}} = 3.0$
Opposite (antiparallel)	Figure 9b	$\frac{37 \times 10^{-3}}{1.62 \times 10^{-3}} = 23$	$\frac{19 \times 10^{-3}}{1.50 \times 10^{-3}} = 12.9$

rec^+ strain JC9937 carrying λ dv021 was infected with the phages shown in Figure 9. Unadsorbed phages were removed. The resulting lysate was assayed for the total phage on a $SuII^+$ strain (JC8679) and for the P^+ recombinant phage on a Su^- strain (JC9387).

by addition of λdv DNA as well as P^+ information. The other is replacement of the *Pam* allele by P^+ from the λdv unaccompanied by addition of λdv. Previous analysis demonstrated that the ratios of the two types are the same for parallel Chi-*cos* and for antiparallel Chi-*cos* (STAHL *et al.* 1982).

These results show that packaging is not necessary for the Chi-*cos* interaction. As pointed out by STAHL *et al.* (1982) they also argue that Chi stimulation of splices and of patches (STAHL 1979a) are equally dependent on *cos* orientation. Recovery of the recombinant chromosome depends on a second splice event with another λ. Further implications of these results on the role of *cos* will be discussed later.

Cross λ cos^+ × λ cos^-

The design of the second set of experiments is similar to the first one in that only one of the parents carries functional *cos*, and the other is deleted for *cos*. In this case, however, the deletion used is the 22-bp deletion *cos2*. Thus, the two parents are essentially equivalent in size.

Figure 10a illustrates the principle. To be packaged efficiently a unit λ DNA has to be bounded by a pair of *cos* sites [Figure 10a(i)]. If only one of the two *cos* is intact, the other being inactive due to the *cos2* deletion, the unit λ DNA cannot be packaged [Figure 10a,(ii)]; but if we provide another pair of *cos*, the recombinants, at least some of them, can be packaged into phage particles [Figure 10a,(iii)]. The second *cos* pair could be of the opposite orientation. Figure 10b(i) shows the recombination between two λ cos^+ produces a packageable dimer. As shown in Figure 10b(ii), recombination between λ cos^+ and λ cos^- will produce a circular dimer, but no λ can be packaged from the wild-type *cos* since it lacks a partner. If each of the parents has a second *cos*, however, DNA can be packaged from these *cos'* as shown in Figure 10b(iii). In Figure 10c the principle is again explained, this time with the λ chromosomes represented as linear.

Figure 11a shows the cross. The primary *cos*, which is antiparallel with the Chi, is either intact or inactive due to the *cos2* mutation. Figure 11b shows the

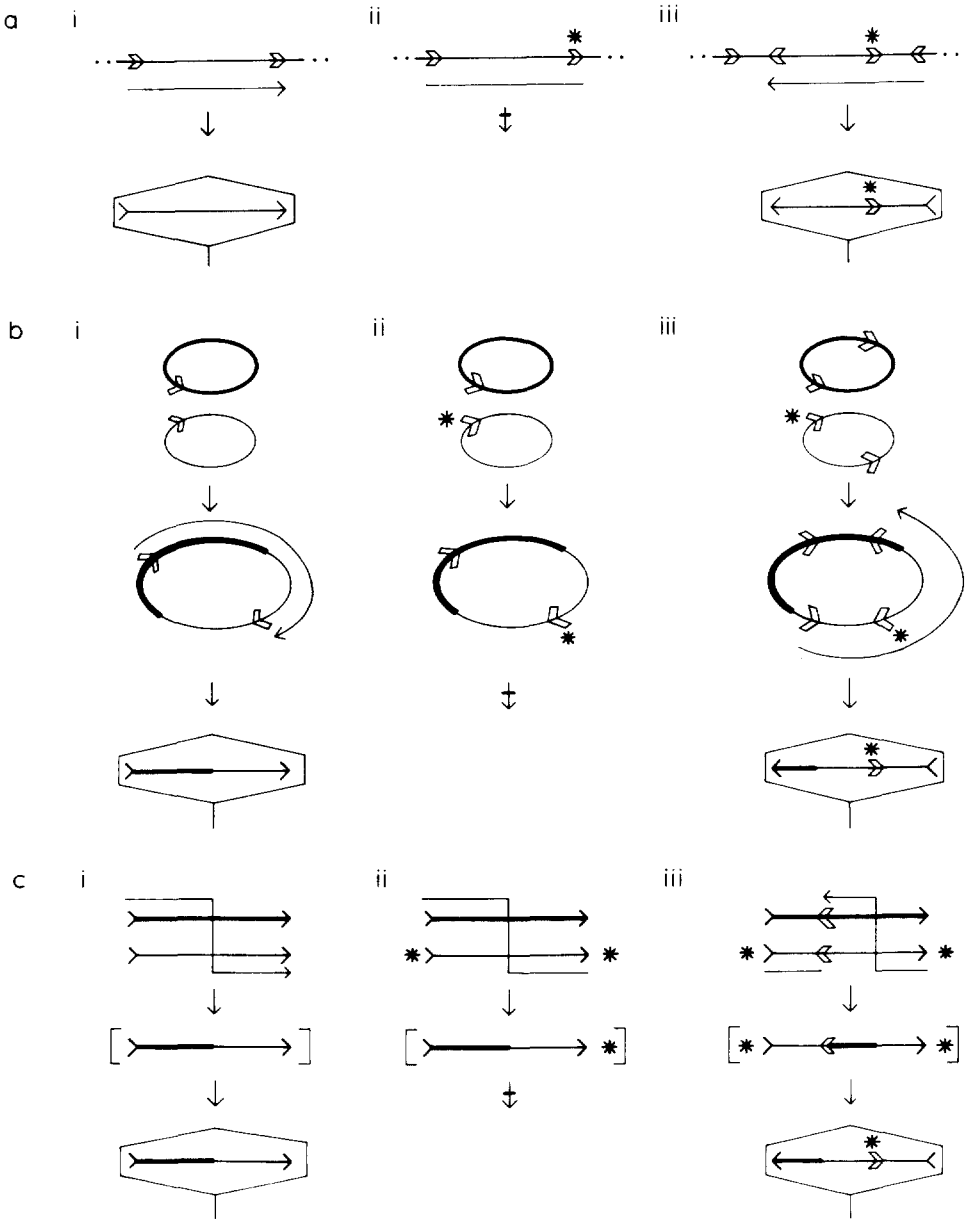

FIGURE 10.—Design of unpaired *cos* experiments. a, Principle. (i) Unit λ DNA has to be bounded by a pair of *cos* sequences in order to be packaged into a phage particle. (ii) When one of the pair is inactive due to a deletion mutation (*), the unit λ can not be packaged. (iii) However, if we provide another pair of *cos*, λ DNA can be packaged. The orientation of this second *cos* pair can be opposite to that of the first *cos* pair. b, Recombination between λ-circular version. (i) A crossover between two circles produces a concatemer form in which unit λ DNA is bounded by a pair of *cos*. The unit DNA is cut and packaged. (The crossover could be nonreciprocal, and the recombination might be coupled with packaging. But our discussion will not lose generality even if we use a circular dimer diagram). (ii) When one circle carries a mutated *cos*, the recombination does not produce a packageable structure. (iii) When both circles carry a second *cos*, the recombinant genome can be packaged by cutting of this second pair of *cos*. c, Recombination between λ-linear version. λ chromosome is represented as a line. (i)-(iii) correspond to b (i)-(iii), respectively.

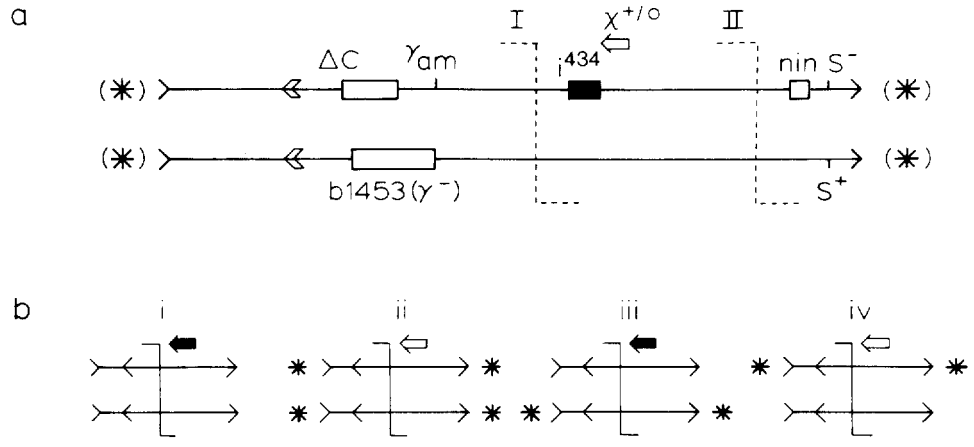

FIGURE 11.—Unpaired *cos* experiments. a, I is the interval to be stimulated by the leftward action of χC^+, and II is the reference interval. The ratio I/II is the measure of Chi activity. The primary *cos* is either active or inactivated by the *cos2* mutation. The upper phage carries either χC^+157 or $\chi^0 cII2002$. χC^+ is oriented leftward. Both phages carry secondary cos^+ in leftward orientation. ΔC; deletion of *Eco*RI-C fragment. γam; *γam210*. i434; *imm434*. nin; *nin5* deletion. S^-; *Sam7*. The recombinants (*gam210 imm*λ S^+) in the interval I (between the right end of *b1453* and the left end of *imm434*) were detected on QR48 (λ *imm434 Sam7*), and the recombinants (*gam210 imm434* S^+) in the interval II (between the right end of *imm434* and *Sam7*) were detected on QR48 (λ *red3 Sam7*). Order, but not size or position, of the markers is accurate. b, Crosses with various *cos* combinations: (i), *cos* that is antiparallel to Chi is intact both in the parent carrying Chi and in the parent not carrying Chi. (ii), the antiparallel *cos* is inactivated by mutation in both of the parents. (iii), the antiparallel *cos* is intact only in the parent carrying Chi (iv), the antiparallel *cos* is intact only in the parent not carrying Chi.

various *cos* combinations tested. I is the interval in which recombination is to be stimulated by the leftward action of Chi. II is the reference interval. Chi activity (recombination enhancement) is measured as ratio I/II.

In the positive control [Figure 11b(i)], both parents carry intact antiparallel *cos*, and Chi activity is high as shown in Table 4. The positive packaging model explains this phenomenon by saying that packaging from the antiparallel *cos* activates Chi. The negative packaging model explains it by saying that, although packaging from parallel *cos* normally inhibits Chi action, the alternative packaging from antiparallel *cos* bypasses this inhibition. In the negative control [Figure 11b(ii)], Chi activity is low. Positive packaing models explain this as the effect of the absence of packaging from antiparallel *cos*. Negative packaging models explain this as a negative influence of packaging from the parallel *cos*.

Figure 11b(iii) shows the experiment of interest and corresponds to Figure 10 (iii). The Chi-carrying parent has an intact antiparallel *cos*, but the χ^0 parent does not. The recombinant DNA cannot be packaged from the antiparallel *cos*. The positive packaging model and the negative packaging model both predict low Chi activity for the same reason as in the negative control [Figure 11b(ii)]. However, in this pair, Chi activity as high as in the positive control pair was observed (Table 4). We picked up ten of the recombinant phage plaques produced by the increased Chi activity and backcrossed the phages as diagrammed in Figure 12. These backcrosses showed that all ten of the recombi-

TABLE 4

Chi activity in crosses with unpaired cos

Cross	$\frac{\text{I/II in } \chi^+ \text{ pair}}{\text{I/II in } \chi^\circ \text{ pair}}$			
	Experiment 1	Experiment 2	Experiment 3	Average
Fig. 11b(i)	$\frac{2.2}{0.21} = 10.2$	$\frac{2.6}{0.16} = 16.5$	$\frac{3.7}{0.21} = 17.7$	14.8
Fig. 11b(ii)	$\frac{0.86}{0.23} = 3.7$	$\frac{0.87}{0.32} = 2.7$	$\frac{1.4}{0.28} = 5.0$	3.8
Fig. 11b(iii)	$\frac{1.8}{0.19} = 9.5$	$\frac{2.0}{0.28} = 7.2$	$\frac{3.4}{0.13} = 25.6$	14.1
Fig. 11b(iv)	$\frac{0.63}{0.27} = 2.4$	$\frac{0.67}{0.24} = 2.9$	$\frac{1.2}{0.36} = 3.4$	2.9

nants still carry the *cos2* mutation. In each cross we detected no large plaques among approximately 1000 plaques. This control excludes the possibility that the recombinant phage had been packaged from the primary, antiparallel *cos*. Thus, a *cos* that is present in only one of the parents, and therefore cannot be used for packaging, is sufficient to activate an antiparallel Chi (positive models) or sufficient to bypass an inhibitory action of *cos* on a parallel Chi (negative models).

In the cross Figure 11b(iv) the genotype of antiparallel *cos* is reversed in the two parents. Chi and antiparallel *cos* are *in trans*, that is, they are on different chromosomes. Here Chi activity was low. Thus, full Chi activity requires that Chi and antiparallel *cos* be *cis*, that is, on the same chromosome. Implications will be discussed later. Reconstruction experiments showed that the data were not influenced by recombination on the selective indicator plate.

The two sets of experiments provide evidence against the packaging model. In order to interact with Chi, a *cos* does not have to be used for packaging. The results exclude both positive and negative versions of the packaging model. Packaging from antiparallel *cos* is not necessary to activate Chi; packaging from parallel *cos* does not inhibit Chi.

At what stages of the λ life cycle does the cos-*Chi interaction occur?*

Then what does account for the dependence of Chi activity on *cos* orientation? Three types of models that draw upon features of the λ life cycle can be envisaged to explain this *cos*-Chi interaction; (1) *attempted packaging models*, (2) *preclosure models*, (3) *silent cos models*. Each of them can be classified into positive versions and negative versions depending on whether an antiparallel cos^+ activates Chi or parallel cos^+ inhibits Chi.

Attempted packaging models: These models assume that a reaction at *cos* that precedes packaging is responsible for the *cos*-Chi interaction even when that *cos* is not ultimately used for packaging. In our experiments, packaging from *cos* was blocked simply by removing its partner *cos*. The active *cos* might participate in early steps of a packaging act that then aborts. The negative versions of attempted packaging models are unlikely because Chi is active in a

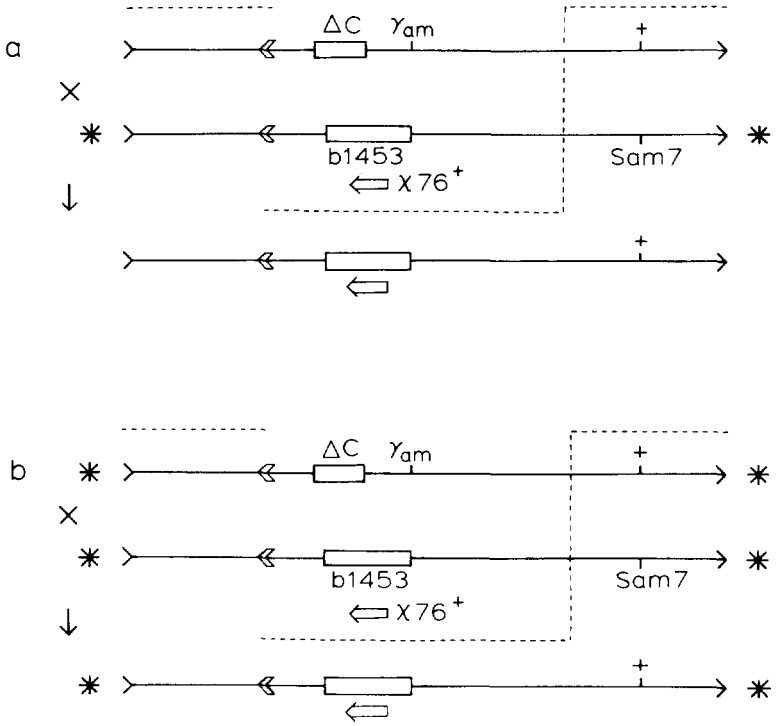

FIGURE 12.—A backcross to show that the recombinant phage produced by the interaction of Chi and an antiparallel unpaired *cos* (interval I in cross iii of Figure 10b) still carries *cos2* mutation in the primary *cos*. The upper phage is the one to be tested. The lower phage is the tester. The tester phage carries a leftward Chi, χ^+76, that is inseparable from *b1453* (*red*$^-$ *gam*$^-$) deletion. The tester shows Chi$^-$ phenotype because it lacks an antiparallel *cos*. When χ^+ is combined with rightward *cos*, it shows Chi$^+$ phenotype. *Sam7* is closely linked to the primary *cos*. *b1453* S$^+$ recombinants were selected on C600(P2), on which Chi$^+$ gives large plaques. a, If the upper phage carries *cos*$^+$, this test cross will produce many λ *cos*$^+$ (rightward) χ^+ (leftward), which makes large plaques (Chi$^+$ phenotype); b, If the upper phage carries *cos2*, no large plaque-forming phage will be produced.

chromosome carrying both an antiparallel and a parallel *cos* as shown earlier (KOBAYASHI *et al.* 1982) and as confirmed in cross (i) of Table 4. The positive versions state that the interaction of packaging proteins at *cos* (with or without a partner *cos*) activates an antiparallel Chi. A deletion, *cos2*, abolishes this interaction as shown earlier (KOBAYASHI *et al.* 1982) and as confirmed here in the two sets of experiments (Tables 3 and 4). *cos2* removes the nicking site of *cos* but leaves the terminase-binding site intact (FEISS, KOBAYASHI and WIDNER 1983). Therefore, terminase binding is not sufficient to activate Chi. That the nicking site is required suggests that cutting is one of the relevant processes. If cutting of *cos* is involved in the Chi-*cos* interaction, *cos* must be resealable to explain our results in Table 4. *In vivo*, cutting is coupled with packaging; no cutting has been detected in the absence of packaging. But, *in vitro*, terminase can cut *cos* without packaging (WANG and KAISER 1973). BECKER and GOLD (1978) showed that this cleavage reaction is not restricted by the form of the DNA outside *cos*. For example, the single *cos* on a monomeric circle can be

cleaved *in vitro* as well as *cos* on a concatemer (BECKER and GOLD 1978). They discussed the possibility that *in vivo* terminase introduces nicks at *cos* in the absence of packaging. The nicks might be quickly sealed unless the subsequent packaging makes the process irreversible (BECKER and GOLD 1978). The interaction of the packaging machinery with *cos* is asymmetric (HOHN 1975; FEISS *et al.* 1979). More specifically, the terminase-binding site is to the right of the nicking site (FEISS and WIDNER 1982; FEISS, KOBAYASHI and WIDNER 1983). This asymmetry may underlie dependence on *cos* orientation. Partial encapsidation like that originally proposed in the resolution-packaging model is not excluded as a basis of positive models.

Preclosure (injection) models: These models (KOBAYASHI *et al.* 1982; SMITH *et al.* 1981c) assume that the asymmetry of the two ends of λ DNA upon entry into the cell (for review, see KATSURA 1983) underlies *cos*-Chi interaction. The negative versions, where the cohesive ends inactivate parallel Chi, are difficult to imagine. The positive versions seem more likely. Here the presence of a *cos* as cohesive ends at the time of DNA entry somehow results in activation of antiparallel Chi. However, since Chi shows apparently full activity when it is in λ that is induced from either the prophage or the plasmid state (STAHL *et al.* 1983), preclosure activation cannot be the sole basis of the *cos*-Chi interaction.

Silent cos models: The models suppose that neither the interaction of *cos* with packaging proteins nor the presence of *cos* as DNA ends upon entry is relevant in Chi-*cos* interaction. Negative versions were excluded because Chi on a λ chromosome carrying both an antiparallel *cos* and a parallel *cos* is active as shown earlier (KOBAYASHI *et al.* 1982) and in Table 4 (i). However, positive versions are possible.

cos *as an activator of Chi*

For the reasons stated we favor positive models in which a single *cos* sequence activates an antiparallel Chi. The modes of activation are further limited by the following properties of *cos* revealed in the present study.

1. *cos* is active when present in only one of the two parents in a cross (*Dominance*) (Tables 3 and 4). The activity is as high as when cos^+ is present in both the parents (compare Table 4, lines 1 and 3).

2. *cos* action is not impeded by a large heterology opposite *cos* nor by heterology separating *cos* and Chi (*action opposite and across heterology*) (Tables 3 and 4). In the λ × λdv crosses, *cos* is opposite a huge deletion (Figure 9). In the λ × λ cross, the *nin5* deletion and Δ*Eco*RI-C *b1453* heterology stand between Chi and *cos*.

3. *cos* can interact with Chi on the same chromosome (*cis*) but not on a different chromosome (*trans*) (Table 4).

4. *cos* can interact with Chi *at a distance* (LAM et al., 1974; STAHL, CRASEMANN and STAHL 1975; KOBAYASHI *et al.* 1982). The distance can be as much as 20 kb. Consequently, *cos* and the exchanges can be far apart.

5. *cos* has to be antiparallel to Chi (*orientation-dependence*).

Apparently, stimulation by *cos* involves the transmission of a signal along DNA (properties 1–4). The signal must have the orientation information (5).

Presumably, some intracellular element, which we arbitrarily call a recombination machine, interacts with *cos*, and the machine changes state so that it now can interact with a Chi that is antiparallel with that *cos*. The asymmetric machine-*cos* interaction depends either on packaging-related reactions (attempted packaging models), the presence of *cos* as ends at DNA entry (preclosure models), or neither (silent *cos* models). We can not exclude the reverse of this temporal sequence; the machine-Chi interaction first, then the machine-*cos* interaction.

At a more concrete level, we speculate that the machine interacts with *cos* in an asymmetric way and travels along the DNA molecule (Figure 13). It will then recognize a Chi of antiparallel orientation. The orientation of *cos* is "memorized" either because the traveling machine takes one fixed orientation dictated by the *cos* orientation, because it chooses only one of the two strands or because it travels in only one direction.

The two enzymes implicated in Chi action, *recA* protein and *recBC* enzyme (STAHL and STAHL 1977; GILLEN and CLARK 1974), are candidates for (parts of) a machine having the properties listed. The *recBC* enzyme travels through duplex DNA (ROSAMOND, TELANDER and LINN 1979; TAYLOR and SMITH 1980). Several properties of *recA* protein are likewise suggestive; these include preferential binding to single-stranded DNA (MCENTEE, WEINSTOCK and LEHMAN 1981), *recA* protein-*recA* protein interaction (OGAWA *et al.* 1979) that leads to filament formation (MCENTEE, WEINSTOCK and LEHMAN 1981), and DNA unwinding (CUNNINGHAM *et al.* 1979). Of course, spreading by *recA* protein of an homologous strand exchange initiated at *cos* cannot explain the *cos* action opposite a heterology (property 2). Some other DNA enzymes show properties similar to those of the traveling recombination machine. For example, type I restriction enzymes have been proposed to translocate DNA (HORIUCHI and ZINDER 1972; YUAN, HAMILTON and BURCKHARDT 1980).

A related question is which step of recombination is stimulated by Chi-*cos* action. The requirement of *cos* and Chi on the same chromosome suggests that the step occurs before two molecules becomes indistinguishable. *cos* stimulates two types of Chi-mediated recombination, splicing and patching, to the same extent (STAHL *et al.* 1982). Presumably, *cos* stimulates Chi action before these two pathways diverge.

Other viruses

Chi is also active in P1-mediated transduction in *Escherichia coli* (DOWER and STAHL 1981). When Chi is on the donor DNA, the end of the transducing DNA fragment might play a role analogous to that of *cos*.

In bacteriophage T7, polarity of packaging causes asymmetry of recombination in a cell-free system (ROBERTS, SHELDON and SADOWSKI 1978). These authors postulated attachment of packaging protein(s) to one end of the phage chromosome to explain this asymmetry. In bacteriophage T4, endonuclease VII, the gene 49 product, catalyzes resolution of a Holliday structure (MIZUUCHI *et al.* 1982). Absence of this enzyme results in the accumulation of huge branched DNA that cannot be packaged (KEMPER and BROWN 1976). An interaction of this

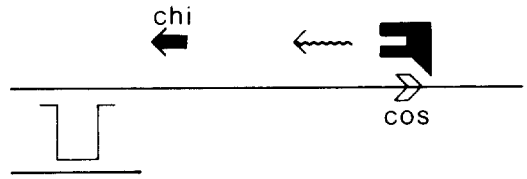

FIGURE 13.—Plausible role of *cos* in Chi stimulation of recombination. A machine (box in black) interacts with *cos* asymmetrically. It travels (wavy arrow) along λ DNA taking a fixed orientation (or on only one particular strand or in only one direction). The choice of orientation (or strand or direction) is dictated by *cos* orientation. The machine will interact with χ^+ (short arrows) antiparallel to *cos* orientation.

enzyme with the packaging machinery is not known but is plausible. In a mutant of bacteriophage P4 the head is larger than that of wild type and contains a dimer of the P4 genome. Packaging might promote recombination between monomeric circles; on the other hand, it might simply select for dimeric recombination products (SHORE *et al.* 1978).

cos can be regarded as part of a two-element recombinator system

The properties listed for *cos* are rather like those of other recombination stimulators that we call "recombinators." One example is Chi itself as described in the introduction. Another is *cog*, a well-characterized "recombinator" in Neurospora (ANGEL, AUSTIN and CATCHESIDE 1970; CATCHESIDE and ANGEL 1974; CATCHESIDE 1974). Similarities between Chi and *cog* were pointed out earlier (STAHL 1979a,b). Some of the genetic elements that are called recombinators because of their *cis* action might turn out to be a second, *cos*-like element of a dual *cis*-acting system such as this Chi-*cos* system. In fact, *cog* was proposed to be a recognition site for a recombinase (ANGEL, AUSTIN and CATCHESIDE 1970) as *cos* is proposed to be an entry site for a recombinase in the present work.

Recently, we demonstrated that Chi in an inactive state can be activated by *in vivo* action of a restriction endonuclease at a distant site (STAHL *et al.* 1983). This result lends indirect, but strong, support to the model in which *cos* acts as an (oriented) entry site for a recombination machine.

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