COUPLING WITH PACKAGING EXPLAINS APPARENT NONRECIPROCALITY OF CHI-STIMULATED RECOMBINATION OF BACTERIOPHAGE LAMBDA BY RECA AND RECBC FUNCTIONS

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ABSTRACT

Chi (χ, 5′-GCTGGTGG) is a recombinator in RecA- and RecBC-mediated recombination in *Escherichia coli*. In vegetative recombination between two bacteriophage lambda strains, one with and the other without Chi ($a^+\chi^+b^-$ × $a^-\chi^0b^+$), the χ-containing recombinant ($a^-\chi^+b^-$) is less abundant than the non-χ-containing recombinant ($a^+\chi^0b^+$). Previously this was taken was evidence for nonreciprocality of χ-stimulated exchange. This inequality, however, is now seen to result from an event at *cos* (λ's packaging origin) that both activates Chi and initiates DNA packaging. An event at rightward *cos* leads to activation of leftward χ on the same chromosome for an exchange to its left. From the resulting circulating dimer (—*cos*-a^+-χ^0-b^+-*cos*-a^--χ^+-*b*-—), *the cos* that activated χ is more likely to be used for rightward packaging initiation than is the *cos* from the other parent. Consistent with this coupling model is "biased packaging" in λ carrying two *cos* sites per monomer genome. When their maturation is dependent on dimerization by χ-stimulated exchange, the phage particles result more often from packaging from the *cos* that activates χ than from packaging from the other *cos*. Since Chi activation and packaging can be uncoupled, we infer that some early and reversible step in packaging activates χ. A strong candidate for this step is a double-strand break at *cos* that provides an oriented entry site for a recombinase.

POLARITY in gene conversion implies special sites on DNA (recombinators) that locally enhance homologous genetic recombination (LISSOUBA *et al.* 1962; MURRAY 1963; see STAHL 1979a,b for review). Chi is a special site for the RecBC pathway of recombination in *Escherichia coli* (for reviews see STAHL 1979a,b; SMITH 1983). When Chi stimulates recombination on bacteriophage lambda, the stimulated crossovers are located primarily to the left of the Chi on the lambda genetic map (*leftward bias*) (Figure 1a) (STAHL and STAHL 1975; STAHL *et al.* 1980). By inverting DNA segments carrying Chi, FAULDS *et al.* (1979) and YAGIL *et al.* (1980) showed that only one orientation of Chi had

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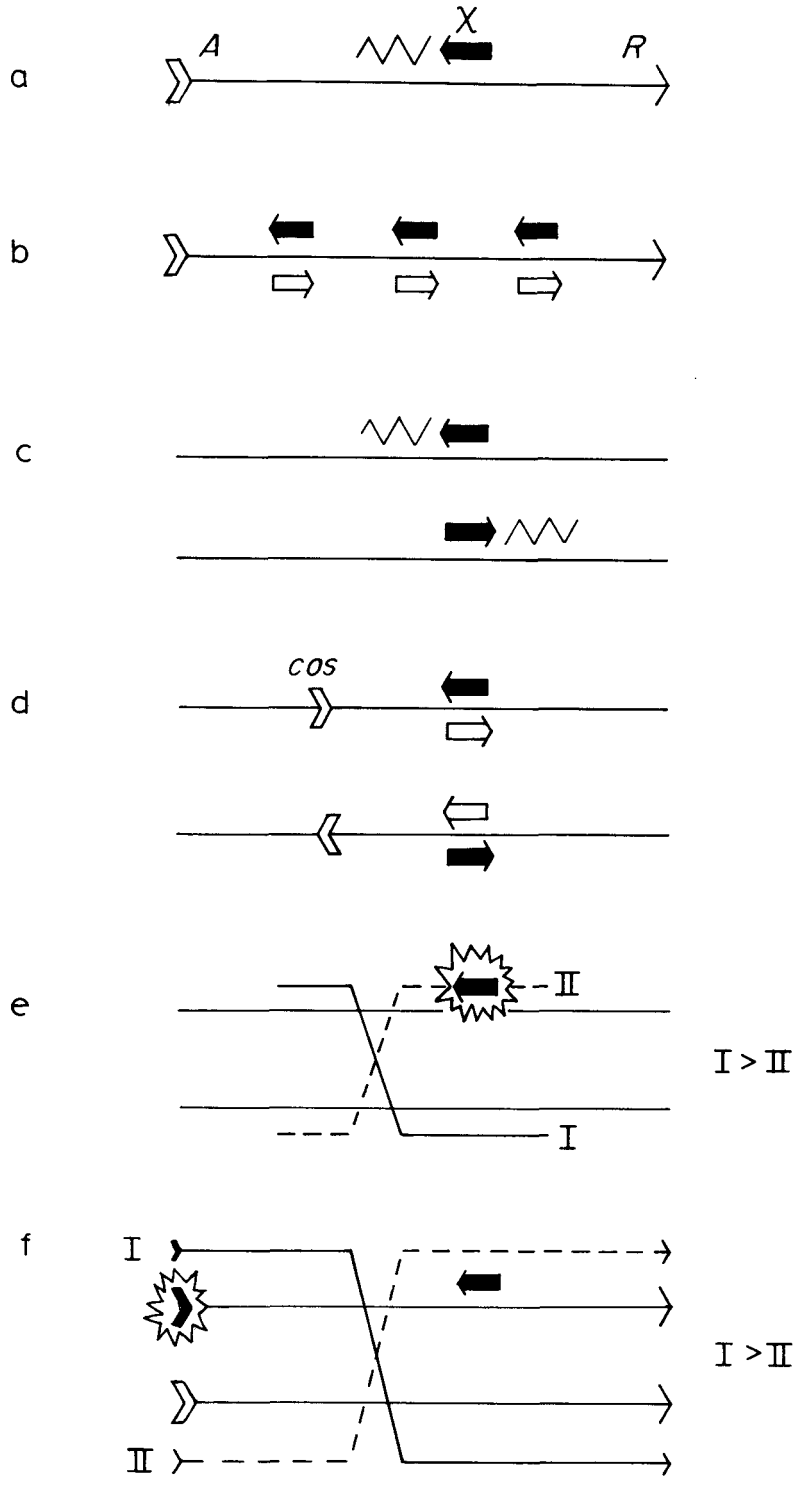

FIGURE 1.—Properties of Chi stimulation of recombination in lambda lytic growth. a, Leftward bias. Chi stimulates exchange to its left on the lambda genetic map. *A* and *R* are genetic markers. b, Orientation dependence. Chi in leftward orientation is much more active than Chi in rightward

full recombinator activity (*orientation dependence*) (Figure 1b). These asymmetries are now understood in molecular terms: (1) Chi is an asymmetric sequence, 5′-GCTGGTGG (SPRAGUE, FAULDS and SMITH 1978; SMITH *et al.* 1981) and stimulates recombination to its 5′-GC ("front") side (*bias* or *directionality*) (Figure 1c). (2) For Chi to be active, another lambda element, *cos*, has to be correctly oriented relative to Chi (*Chi-cos interaction*) (Figure 1d) (KOBAYASHI *et al.* 1982). A natural *cos* is called rightward. A rightward *cos* is an activator of leftward Chi, and a leftward *cos* is an activator of rightward Chi (KOBAYASHI *et al.* 1983).

Figure 2a illustrates the roles of *cos* in lambda lytic infections. (1) A phage particle contains a double-stranded DNA molecule with complementary single-stranded cohesive ends. After DNA injection into the cell, the ends are covalently closed forming a *cos* (cohesive end site) sequence. At the end of the life cycle, the packaging machinery recognizes and cuts *cos* to regenerate the cohesive ends. The enzyme that cleaves **cos** is called terminase. (2) A monomeric circle is not a good substrate for packaging. Unit lambda DNA bounded on each end by a separate *cos* sequence is packaged efficiently. Under standard conditions (Red^- Gam^-) for Chi action, the only effective route to such a concatemer form is *recA*- and *recBC*-dependent recombination. Chi enhances this recombination reaction, producing more packageable DNA forms and hence increasing phage yield. (3) DNA injection is probably polar, with the right end first and the left end last (KATSURA 1983). The polarity may be determined by the orientation of the cohesive ends and, therefore, by the orientation of *cos*. (4) DNA packaging proceeds in a polar way starting from the left end (EMMONS 1974; FEISS and BUBLITZ 1975; STERNBERG and WEISBERG 1975). The interaction of the packaging machinery with *cos* is asymmetric (HOHN 1975; FEISS *et al.* 1979; FEISS, KOBAYASHI and WIDNER 1983). Terminase specifically binds to the right of the *cos* that is to become the left end of a packaged chromosome (FEISS *et al.* 1979; FEISS, KOBAYASHI and WIDNER 1983).

Underlying the Chi-*cos* interaction could be the polarity of either injection or packaging. However, DNA injection is unnecessary (STAHL *et al.*, 1983a; KOBAYASHI, STAHL and STAHL 1984) for most or all of the Chi-*cos* interaction. Furthermore, to interact with Chi, *cos* does not have to become the ends of the packaged chromosome (KOBAYASHI *et al.* 1983). However, terminase and its specific interaction with *cos* is necessary for the Chi-*cos* interaction (KOBAYASHI, STAHL and STAHL 1984). Thus, we infer that a terminase-mediated transient double-stranded DNA break at *cos* activates Chi providing an entry site for a recombinase (KOBAYASHI *et al.* 1983; KOBAYASHI, STAHL and STAHL 1984). This view is supported by the activation of Chi by action of restriction

orientation. c, Intrinsic directionality. When Chi, 5′-GCTGGTGG, stimulates exchange, it does so to its 5′-GC ("front") side. d, Chi-*cos* interaction. The orientation of *cos* defines the active orientation of Chi. e, Unequal recovery of complementary recombinants interpreted as nonreciprocality of Chi-stimulated exchange. Some destructive event at χ handicaps class II recombinants. f, An alternative explanation for unequal recovery of complementary recombinants. An event at *cos* (1) activates Chi on the same chromosome for an exhange to its left and (2) often initiates rightward packaging on the resulting dimer, producing a class I recombinant phage particle.

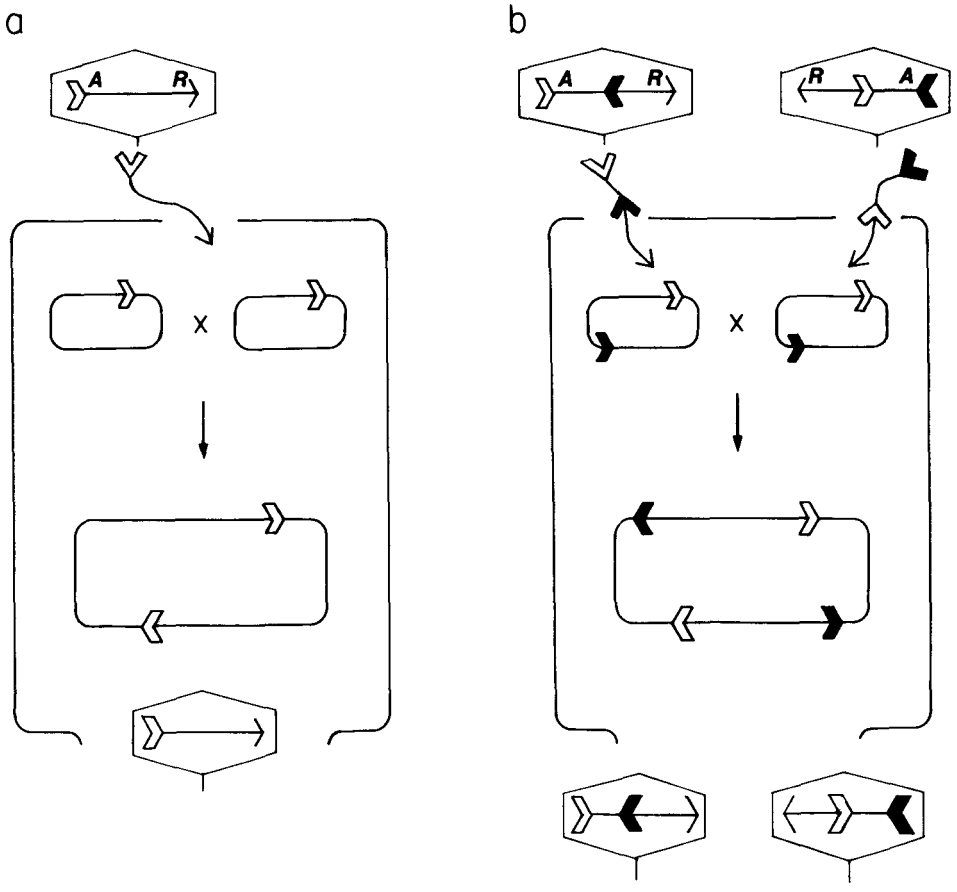

FIGURE 2.—a, Lytic DNA cycle of lambda in Red⁻ Gam⁻ background. b, DNA cycle of lambda carrying two *cos* of the opposite orientations.

endonuclease *Eco*RI at a distance (STAHL *et al.* 1983b; STAHL, KOBAYASHI and STAHL 1983).

Another asymmetric aspect of Chi action in lambda is illustrated in Figure 1e. When Chi is on only one of the recombining DNA chromosomes, the leftward action of Chi increases two complementary recombinant types, I and II. The class I is more abundant than class II (LAM *et al.* 1974; STAHL *et al.* 1980) when DNA replication is limited. This inequality was taken as evidence for nonreciprocality in Chi-stimulated exchange. For example, gapping at Chi would handicap the χ-containing class II recombinants. Such nonreciprocality would be in harmony with the destructive event *in cis* seen with known eukaryotic recombinators (reviewed by MARKHAM and WHITEHOUSE 1982). In this work, however, we demonstrate that this inequality is an aspect of the Chi-*cos* interaction rather than a property of Chi-stimulated exchange itself. The accompanying paper (STAHL, LIEB and STAHL 1984) documents symmetry in Chi action, revealed by another protocol.

TABLE 1

Bacterial strains

Strain	Properties	Reference/source
594	*rec*$^+$ Su$^-$	WEIGLE (1966)
JC8679	*recB21 sbcA20* SuII$^+$	STAHL and STAHL (1977)
QR48	*recA*$^-$ Su$^+$	SIGNER and WEIL (1968)
C600	*rec*$^+$ SuII$^+$	APPLEYARD (1954)
RA8058	*nusA1 trp*::Tn*10*	IRA HERSKOWITZ
FA77	*dnaBts* Su$^-$	MCMILIN and RUSSO (1972)
FS1189	QR48 (λ *spi6 imm*^{434} *tsR2*)	This work
FS690	C600 (P2, λ *red3 gam210*)	This work
FS1430	RA8058 (λ *imm*^{434})	This work
RM199	*rec*$^+$	STAHL and STAHL (1977)
RM202	*recB21*	STAHL and STAHL (1977)
JM1	SuIII$^+$ indicator for Red$^-$Gam$^-$λ	STAHL *et al.* (1980)

MATERIALS AND METHODS

Bacterial strains: *Escherichia coli* strains used are listed in Table 1. Other strains were described previously (YAGIL *et al.* 1980; KOBAYASHI *et al.* 1983).

Phage genetic elements and nomenclature: The λ genetic elements used are listed in Table 2 and shown in Figure 3.

Nomenclature of *cos* is slightly modified from that used previously (KOBAYASHI *et al.* 1982; STAHL, KOBAYASHI and STAHL 1982; KOBAYASHI *et al.* 1983) (see also Table 2 and Figure 3). The ends of lambda's genetic map define the site for the primary *cos*. The middle of lambda's genetic map sometimes has a secondary, cloned *cos*. The cloned *cos* is a φ80-lambda hybrid *cos* (φ80 right end—lambda left end) but has cosλ specificity (that is, it is recognized by the λ packaging machinery). The primar *cos* is oriented rightward on the standard lambda genetic map (by definition). *cos* at the pimary *cos* site can be intact (*cos*$^+$) or inactivated by the *cos2* mutation (*cos*$^-$). The secondary *cos* is oriented leftward in the present work. When a *cos* is drawn as an arrowhead it points to its *front* side; the other side is its *back* side. We list the primary *cos* genotype first and then the secondary *cos* genotype.

Nomenclature of Chi is as follows (KOBAYASHI *et al.* 1982; KOBAYASHI *et al.* 1983): A given DNA segment either has (χ$^+$) or does not have (χ^0) a Chi sequence (SMITH *et al.* 1981). On the λ map χ can be oriented either leftward or rightward. χ is said to be oriented leftward in λ when 5′-GCTGGTGG is on the 1-strand. Chi's that arose by mutation in λ, for example, the χ$^+$C mutation, are leftward (STAHL, CRASEMANN and STAHL 1975; SPRAGUE, FAULDS and SMITH 1978). Tn*5* is oriented "leftward" on the λ map when its single *Sma*I site is in its left half. When the Tn*5* Chi mutant is leftward, its χ is leftward. The phenotype of Tn*5*χ$^+$ can be Chi$^+$ or Chi$^-$ depending on its orientation relative to that of *cos*. The antiparallel χ$^+$-*cos* combination shows Chi$^+$ phenotype, and the parallel one shows Chi$^-$ phenotype (KOBAYASHI *et al.* 1982). When Chi is drawn as an arrow, it points to its front side.

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; KOBAYASHI *et al.* 1983). The corroborating test was gel electrophoresis of restriction fragments from rapid DNA preparations or from purified DNA (KOBAYASHI *et al.* 1982; KOBAYASHI *et al.* 1983).

Lytic cross under replication block: A *dnaB* thermosensitive strain, FA77, was grown at 26° to 1.5 × 10^8/ml in λ tryptone broth (STAHL and STAHL 1971) supplemented with 0.2% maltose, 10 mM $MgSO_4$, 10 μg/ml of vitamin B1 and 20 μg/ml of thymine. The culture (0.5 ml) was incubated at 39° for 10 min, and a mixture of two phage strains (0.5 ml) was incubated at 39° for 2 min.

TABLE 2

Genetic elements employed

Genetic elements	Relevant properties	Reference
secondary *cos* (*cosM*)	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%	KOBAYASHI *et al.* (1982)
cos2	22-bp deletion removing nicking site of *cos*	KOBAYASHI *et al.* (1982); FEISS, KOBAYASHI and WIDNER (1983)
χ^+C*157*	Active Chi sequence within *cII* with cII$^-$ phenotype	STAHL, CRASEMANN and STAHL (1975); SPRAGUE, FAULDS and SMITH (1978)
Tn*5* χ^+	χ^+ mutant of Tn*5*	YAGIL *et al.* (1980)
b1453	Red$^-$ Gam$^-$ deletion	HENDERSON and WEIL (1975)
imm^{434}	Immunity region of phage 434 substituted into λ	KAISER and JACOB (1957)
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)
Sam7	Amber mutation in *S*, suppressible by SuIII$^+$ but not by SuII$^+$	GOLDBERG and HOWE (1969)
Ats14	*ts* mutation in gene *A*	BROWN and ARBER (1964)
Rts129	*ts* mutation in gene *R*	CAMPBELL and DEL CAMPILLO-CAMPBELL (1963)
gam::Tn5	Insertion of transposon Tn*5* in *gam210* allele	YAGIL *et al.* (1980)
ΔC	Deletion from *Eco*RI site 2 to site 3	CAMERON *et al.* (1975)

Bacteria and phage were then mixed to give a multiplicity of 10 for each phage. The mixture was aerated at 39° for 75 min. The cells do not lyse under these conditions. The infected culture was then poured into 20 ml of SM [0.5% NaCl, 1 mM $MgSO_4$ and 20 mM Tris-HCl (pH 7.4)] in a centrifuge tube on ice and centrifuged at 5 krpm for 10 min to remove unadsorbed phage. The pellet was suspended in 2 ml of broth. The resuspended cells were incubated with 50 μg/ml of egg white lysozyme (Sigma) at room temperature for 10 min and with a drop of $CHCl_3$ at room temperature for 10 min. The cell debris was removed by low-speed centrifugation.

An aliquot of the FA77 culture was incubated at 39° without phage. The *dnaBts* genotype was confirmed by the appearance of filaments.

Biased packaging experiments (restriction analysis): The cells (594 or JC8679) were grown at 37° in λ tryptone broth to the exponential phase. The phage was added at a multiplicity of 5. An equal volume of prewarmed LBH (0.5% NaCl, 0.5% yeast extract, 1% tryptone, 20 μg/ml of thymine, pH 7) was added, and the culture was aerated for 4 hr. The cells do not lyse because of the *S*$^-$ mutation. To remove unadsorbed phage the cells were pelleted by low-speed centrifugation and then resuspended in SM buffer. The cells were incubated with 500 μg/ml of lysozyme at 0° for 10 min and then with a drop of $CHCl_3$. The cells were frozen and thawed in two cycles. DNase (10 μg/ml) and RNase (10 μg/ml) were added. The cell debris was removed by low-speed centrifugation. From these phage preparations DNA was isolated directly by the rapid method or

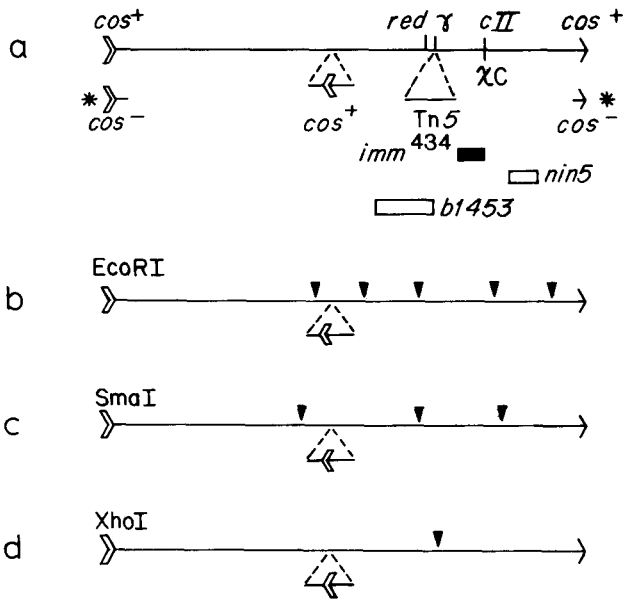

FIGURE 3.—a, Lambda genetic map. See Table 2. b–d, Lambda restriction cleavage maps.

by phenol extraction after CsCl banding (for MMS947 and MMS911) as described previously (KOBAYASHI *et al.* 1983).

Restriction enzyme cuts and gel electrophoresis: The enzymes *Sma*I and *Xho*I were from New England Biolab. The condition used to minimize cohesive end annealing was described (KOBAYASHI *et al.* 1983). After electrophoresis through 1% Seakem agarose, the gel was stained with 2 μg/ml of ethidium bromide and destained with water. Ultraviolet-illuminated gels were photographed with Kodak Royal X-Pan sheet film.

Quantitative densitometry: The densities in appropriate regions of the gel photographs were quantitated by first creating a digital image of the photograph on magnetic tape at 100 μm resolution (1.76×10^6 points/photograph). This digital image was then viewed on a color computer graphics terminal. Contrast-enhanced false color images were used to emphasize important parts of the photograph.

The total density inside an appropriate rectangular region of the photograph was calculated by adding the individual densities of each point inside the region. The net density of a given band was determined as the difference in density between a rectangular region containing the band of interest and a rectangular region of equivalent background intensity and equal area. The absolute number of DNA molecules in a given band can be determined from this density by using the molecular weight of the DNA and the density/base pair. However, for this paper, knowledge of the relative number of DNA molecules per band in different experiments is sufficient, and this is proportional to our quantitative measure.

In control experiments, the areas calculated by densitometry were determined to be linearly related to the amount of DNA in a band. Each gel photograph contained internal calibration standards to determine the linear response region of the film.

The magnetic tape image was created by an Optronics P 1000 digital microdensitometer under the control of a Varian V76 computer (MATTHEWS, KLOPFENSTEIN and COLMAN 1972). The density calculations were performed through a highly interactive computer program (written by DALE TRONRUD) which used an AED 767 color graphics display terminal and a VAX 11/780 computer. Further details will be published elsewhere.

Biased packaging experiments (genetic crosses): Host cells (RM199 or RM202) were grown to 1.5×10^8/ml in maltose-supplemented (0.2%) broth. They were infected at a multiplicity of 7 of each

parental type. The infected cells were diluted, usually about 100×, and aerated at 37° for 90 min, at which time the culture was sterilized by shaking with a few drops of chloroform. Unadsorbed phage were removed by filtration before the dilution step.

RESULTS

An alternative model for unequal recovery of complementary recombinants in crosses λ χ^+ × λ χ^o: The alternative view for the origin of the inequality in Figure 1e is illustrated in Figures 1f and 4a. An event at *cos* activates Chi on the same DNA molecule (Figure 4, a1). Chi action promotes an exchange to its front side producing a packageable dimer (a2). From this dimer packaging can proceed in either of two ways (EMMONS 1974; ROSS and FREIFELDER 1976). One way starts from the *cos* that has activated Chi, proceeds frontwards (class I) and produces class I recombinants (a3). The other starts from the *cos* (class II) on the χ^o parent and produces class II recombinants. The former is preferred to the latter because the Chi-activating event predisposes the participating *cos* to initiate packaging.

Uncoupling of Chi activation from packaging leads to disappearance of unequal recovery: A test to distinguish between the two models is illustrated in Figure 4b. Chi activation and packaging initiation can be uncoupled as follows: only the χ^+ parent carries an intact *cos* of the χ-activating orientation; the other (χ^o) parent carries a *cos*$^-$ deletion. Such as "unpaired *cos*" can activate Chi on the same DNA for exchange as effectively as a paired *cos* (Figure 4, b2) (KOBAYASHI *et al.* 1983). However, this *cos* cannot be used to initiate successful packaging because the terminating *cos* is defective (b3). The recombinant DNA can be packaged from the *cos* pair that is parallel to, and hence does not activate, Chi (b4 and b5). Packaging will start from the *cos* contributed by the λχ^+ or from that contributed by λχ^o with an equal likelihood. The nonreciprocality model predicts persistence of unequal recovery in this cross. The coupling model predicts disappearance of the inequality.

Figure 5 shows the crosses. The diagram in b(ii) is the cross of interest, in which only the χ^+-parent carries *cos* of Chi-activating orientation. The control is b(i), in which both DNA molecules carry *cos* of Chi-activating orientation. The marker combination is shown in Figure 5a. I and II are the complementary recombinants produced by the leftward action of χ^+C. III is the reference interval. We compared I/II from the χ^+ and χ^o crosses. As shown in Table 3, class I and II recombinants were unequal when both parents carried χ-activating and non-χ-activating *cos* (Table 3, first line).When the activating *cos* was present in only the χ^+ parent, this inequality disappeared (Table 3, second line). Chi action is as strong with unpaired *cos* as with paired *cos* as shown previously (KOBAYASHI *et al.* 1983) and as suggested here by comparison of recombinants II and III in combination (ii). These results favor the coupling model for the unequal recovery of the recombinant classes; the inequality is a reflection of the coupling of χ activation and packaging initiation rather than a property of Chi-stimulated exchange *per se*.

Chi action biases choice of cos *for packaging in lambda carrying two* cos: The coupling of Chi activation and packaging initiation inferred from the nonre-

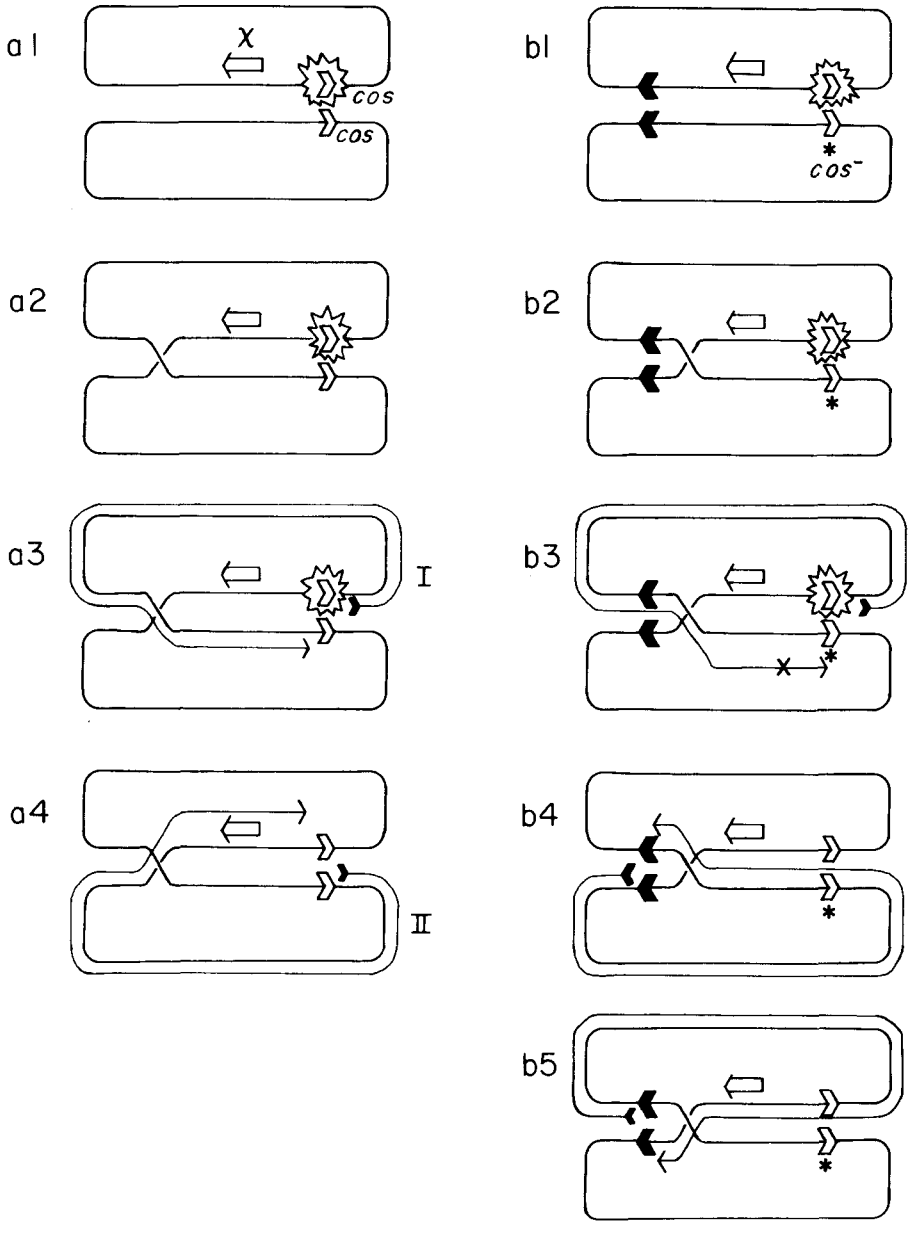

FIGURE 4.—a, An alternative model for unequal recovery of complementary recombinants. See text RESULTS. b, A test to distinguish the two models for inequality.

ciprocality experiments can be demonstrated directly by analysis of DNA in phage particles. In these experiments, the cells were infected with phages carrying two *cos* sequences of opposite orientation (see Figure 2b). DNA from the phage particles produced in that infection was analyzed with restriction enzymes to determine which *cos* had been used for packaging. The action of

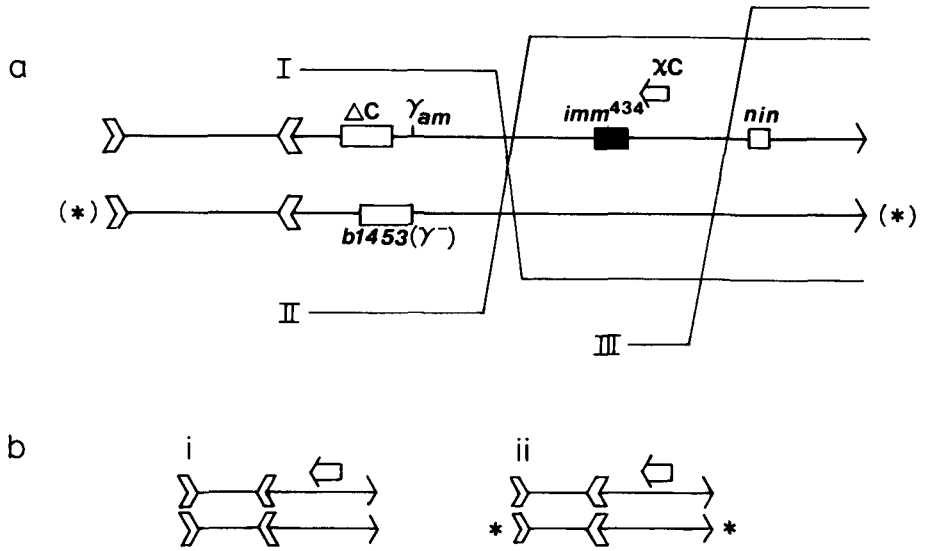

FIGURE 5.—Tests of the two models for inequality. a, By the leftward action of χ^+C, the exchange in the interval *b1453-imm*^{434} is stimulated. The complementary recombinants I and II were compared. III is the reference interval to assess Chi activity. The upper phage carries either χ^+C*157* or *cII2002*. The primary *cos* is either active or mutant (*cos2*). Both phages carry secondary *cos* in leftward orientation. Abbreviations of phage genotypes are *γam*, *gam210*; *nin*, *nin5* deletion. The recombinant genotype and the selective indicator bacteria to detect recombinants are I: ΔC *γam imm*$^{\lambda}$, FS1189; II: *b1453 imm*^{434}, FS690; III: *imm*$^{\lambda}$ *nin5*, FS1430. Order, but not size or position, of markers is accurate. b, Crosses with different *cos* combinations: (i) Rightward *cos*, which can activate leftward χ, is active in both the χ-carrying parent and the non-χ-carrying parent. This *cos* can be used for packaging a recombinant. (ii) Rightward *cos* is active only in χ-carrying parent. This *cos* can activate leftward Chi but cannot be cleaved during packaging to become the ends of phage recombinant particles.

TABLE 3

Effect of cos *on recovery of complementary recombinants*

Cross	Output ratio	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Fig. 5b (i)	$\frac{\text{I/II in } \chi^+ \text{ cross}}{\text{I/II in } \chi^0 \text{ cross}}$	$\frac{2.77}{1.40} = 1.98$	$\frac{3.14}{1.54} = 2.04$	$\frac{2.01}{1.14} = 1.76$	$\frac{3.46}{1.61} = 2.15$
Fig. 5b (ii)		$\frac{1.94}{1.83} = 1.06$	$\frac{1.27}{1.26} = 1.01$	$\frac{1.15}{1.71} = 0.67$	$\frac{1.66}{1.91} = 0.87$
Figure 5b (ii)	$\frac{\text{II/III in } \chi^+ \text{ cross}}{\text{II/III in } \chi^0 \text{ cross}}$			$\frac{1.61}{0.299} = 5.4$	$\frac{1.13}{0.181} = 6.2$

See Figure 5.

Chi will cause bias in the choice of *cos* as illustrated in Figure 6. The event at the *cos* that is antiparallel to Chi activates Chi on the same chromosome (Figure 6a). The Chi action leads to formation of a dimer (Figure 6b), from which a chromosome can now be packaged. The *cos* that activated Chi is more likely

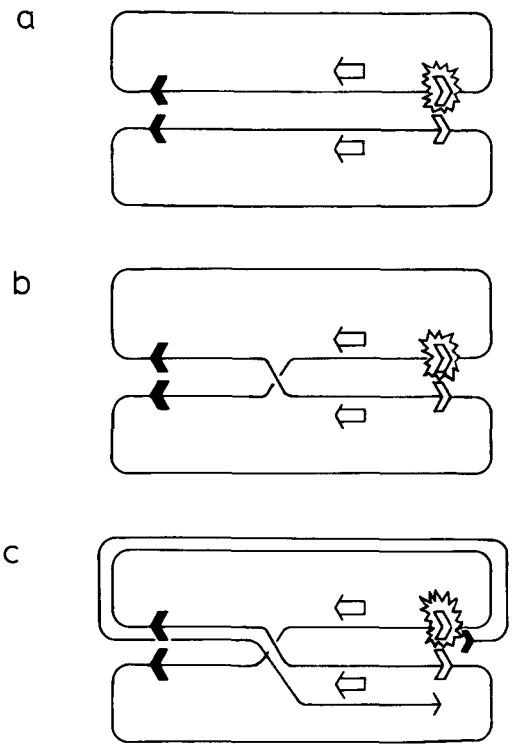

FIGURE 6.—Model for biased packaging. An event at white *cos* (a) can activate an antiparallel Chi and thereby promote dimerization (b). On the resulting dimer that white *cos* is used to initiate packaging (c) more often than is any other *cos*. Among the phage particles produced, most of the DNA molecules result from packaging from the white (Chi-activating) *cos*.

to be used for packaging initiation than are the other *cos* sites (Figure 6c). Thus, the phage particle DNA results more often from packaging from the white *cos* (Figure 6) than from the black *cos*. Since most of the particles in the lysate result from Chi-promoted dimerization, most of the particles should be packaged from the antiparallel *cos*.

Figure 7 shows two isogenic series of phage strains for this biased packaging experiment. The phage carries two *cos* sites. If the primary, rightward *cos* is cleaved during packaging, in series a, the phage particle DNA will have a 12.2-kb fragment. If the leftward *cos* is used for packaging, the phage DNA will have 6.8-kb fragment. The three strains differ in the presence and orientation of Chi. Figure 8 shows analysis of *XhoI* digests of phage particle DNA from *rec*$^+$ infection by these strains. The presence of Chi biases the choice of *cos* for packaging. When χ is leftward (MMS856), use of rightward *cos* is more favored than in χ°. When Chi is rightward (MMS857), use of the leftward *cos* is favored. In *recB* cells there was no large difference in *cos* usage between λ χ° and λ χ$^+$.

Figure 9 shows another example. The isogenic phage strains are illustrated in Figure 7b. The leftward Chi in MMS911 favors packaging from the rightward *cos* in *rec*$^+$ cells. In contrast Chi introduces no bias in *recB* cells, in which

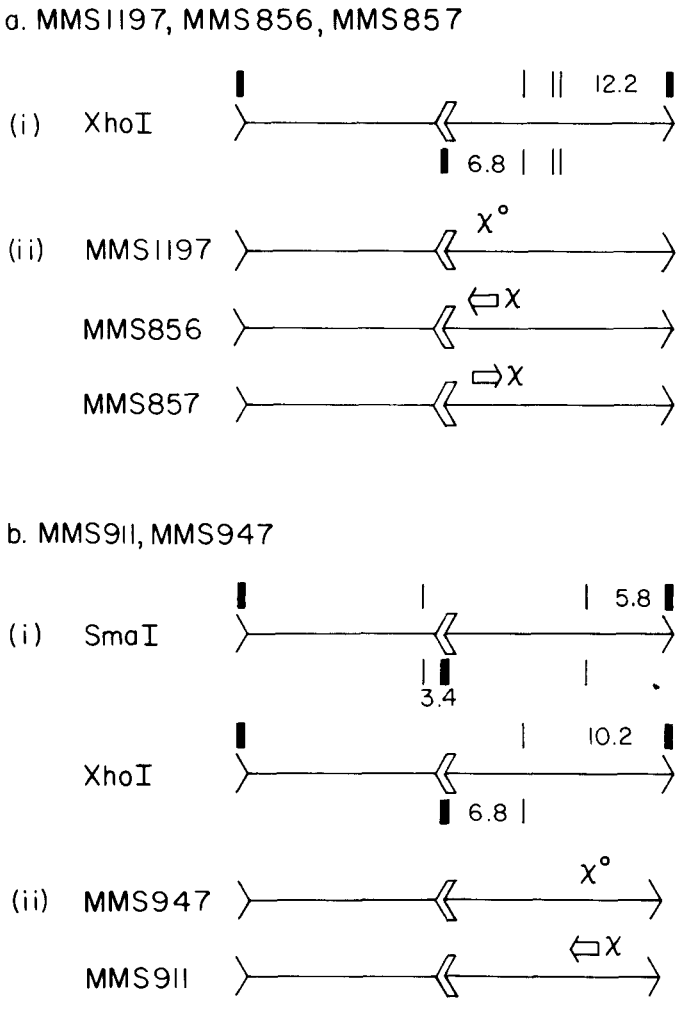

FIGURE 7.—The double *cos* phages for biased packaging experiments. The thick vertical lines show the cleavage at *cos* by *in vivo* packaging. The thin vertical lines show the cleavage by restriction enzymes. a, The common genotype is *cosM* ΔC *gam*::Tn*5* *cI857 nin5 Sam7*. (i) The phage DNA that was packged from the primary, rightward *cos* will give the 12.2-kb fragment. The phage DNA that was packaged from the secondary, leftward *cos* will give the 6.8-kb fragment. (ii) χ^+ is on Tn*5*. b, The common genotype is *cosM* ΔC *gam210 imm*^{434} *nin5 Sam7*. (i) Packaging from the rightward *cos* gives the 5.8-kb fragment (for *Sma*I) and the 10.2-kb fragment (for *Xho*I). Packaging from the leftward *cos* will give the 3.4-kb fragment (for *Sma*I) and the 6.8-kb fragment (for *Xho*I). (ii) MMS947 is *cII2002*. MMS911 is χ^+*C157*.

Chi is inactive by recombination tests (GILLEN and CLARK 1974; STAHL and STAHL 1977).

The pictures of the gel electrophoresis were analyzed by densitometry to quantitate DNA in each band. We calculated the frequency with which each *cos* site is used for packaging. The result is shown in Tables 4 and 5. We see that Chi favors packaging from antiparallel *cos* in *rec*$^+$ infection but not in *recB*$^-$ infection.

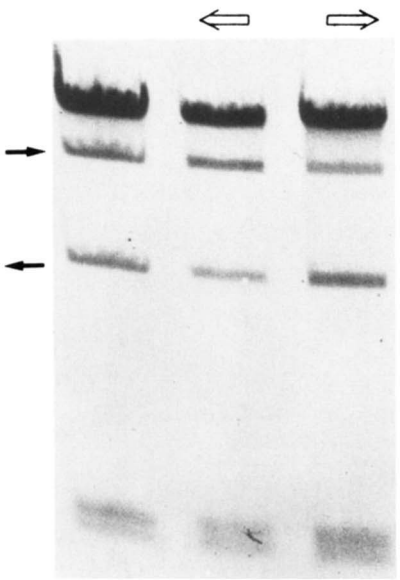

FIGURE 8.—Packaging biased by Chi. The *rec*$^+$ bacterium, 594, was infected with the phages shown in Figure 7a. DNA from the packaged phage particles was analyzed with *Xho*I. One fragment (12.2 kb) corresponds to cleavage at the rightward *cos*. Another (6.8 kb) corresponds to cleavage at the leftward *cos*. Chi changes the ratio of the two fragments by biasing usage of the two *cos* sites. Leftward Chi favors rightward *cos*. Rightward Chi favors leftward *cos*. From left to right: no χ (MMS1197), leftward χ (MMS856), rightward χ (MMS857).

Genetic crosses indicating Chi-influenced choice of cos *for packaging*: If a phage carries two *cos*, genetic linkage data may reveal whether one or the other is preferentially used for DNA packaging. Genes *A* and *R* of λ are at opposite ends of the linkage map (and virion chromosome) because, in circular lambda, *cos* is located on the short arc between them (see Figure 10a). The cleavage at *cos* during packaging unlinks *A* and *R*. Were *cos* located elsewhere, *A* and *R* would be closely linked (Figure 10b). The principle of our linkage test for biased packaging is to provide λ with two *cos* in opposite orientation, one in the standard position and one in the long arc between *A* and *R* (Figure 10c). Packaging at standard *cos* helps to unlink *A* and *R* (Figure 10c–d). Packaging at the other *cos* will link them (Figure 10c–e). The influence of Chi orientation on the linkage of *A* and *R* is then determined. The crosses are diagrammed in Figure 11.

The χ^0 strain contains the transposon Tn*5* "hopped" into the *gam* gene of λ. Chi was then introduced into the transposon by spontaneous mutation. Since *cos* in that λ was standard, the Chi that was found was a leftward Chi. An inversion of Tn*5*, giving us a rightward Chi, was detected as a small plaque variant of that phage. Further details of the derivation of these Chi mutants are in YAGIL *et al.* (1980). By standard crosses, the χ^0 and the leftward and rightward χ^+ Tn*5* insertion mutations of *gam* were introduced into the marked strains of Figure 11.

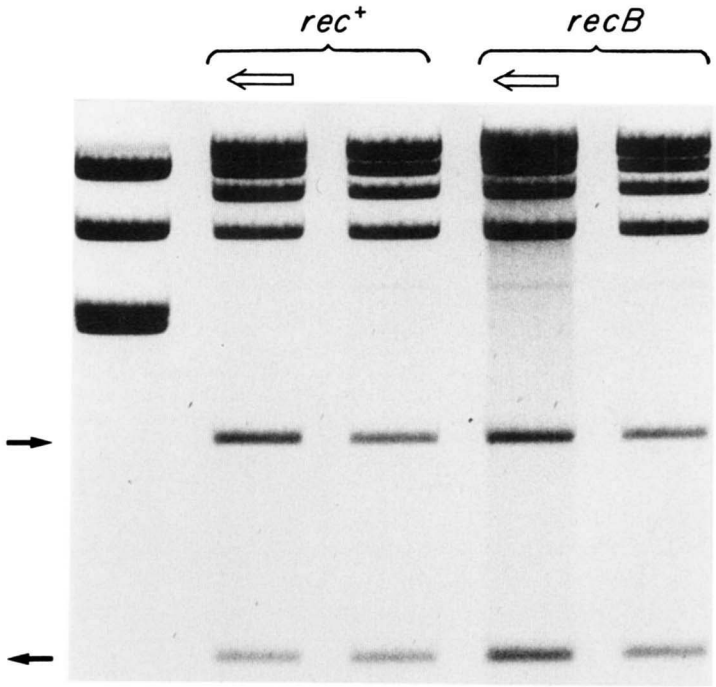

FIGURE 9.—Packaging biased by Chi. The *rec*$^+$ bacterium, 594, or *recB* bacterium, JC8679, were infected with the phages in Figure 7b. DNA from the packaged phage particles was analyzed with *Sma*I. One fragment corresponds to packaging at rightward *cos* and another to packaging at leftward *cos*. From left to right: marker (λ^+) DNA, leftward χ (MMS911) from *rec*$^+$, no χ (MMS947) from *rec*$^+$, leftward χ from *recB*, no χ from *recB*.

TABLE 4

Effect of Chi on choice of cos *for packaging*

	rec$^+$			*recB*		
	No χ	χ leftward	χ rightward	No χ	χ leftward	χ rightward
Ratio of band density (12.2-kb *Xho*I fragment/6.8-kb *Xho*I fragment)	1.49	2.99	1.00	1.58	1.39	1.43
Molar ratio of the two fragments	0.83	1.67	0.56	0.88	0.78	0.80
Fraction of phage packaged from rightward *cos* (%)	45	63	36	47	44	44

The *rec*$^+$ bacterium, 594, or the *recB* bacterium, JC8679, were infected with the phages in Figure 7a. DNA from the packaged phage particles was cut with *Xho*I and run through 1% agarose. One band (12.2 kb) corresponds to packaging at rightward *cos* and another (6.8 kb) to packaging at leftward *cos* (Figure 7a). The density of the two bands in the film was measured as described in MATERIALS AND METHODS. The ratio of the density was converted to the ratio of the number of the molecules based on their size.

TABLE 5

Effect of Chi on choice of cos *for packaging*

	rec$^+$		*recB*	
	χ	No χ	χ	No χ
Ratio of band density (10.2-kb *Xho*I fragment/6.8-kb *Xho*I fragment)	5.6	1.37	3.2	3.0
Mole ratio of the two fragments	3.7	0.91	2.1	2.0
Fraction of phage packaged from rightward *cos* (%)	79	48	68	67

The *rec*$^+$ bacterium, 594, or the *recB* bacterium, JC8679, were infected with the phages in Figure 7b. DNA from the packaged phase particles was cut with *Xho*I and run through 1% agarose. One band (10.2 kb) corresponds to packaging at rightward *cos* and another (6.8 kb) to packaging at leftward *cos* (Figure 7b). The density of the two bands in the film was measured as described in MATERIALS AND METHODS. The ratio of the density was converted to the ratio of the number of the molecules based on their size.

Each of three crosses was conducted in parallel on two different hosts. For each cross, a single mixture of the two parents was made, and aliquots were then adsorbed to a *rec*$^+$ and to an isogenic *recB* strain. Unadsorbed phage were removed by filtration. Total progeny were enumerated on bacterial strain JM1 at 34°, and Ts$^+$ recombinants were enumerated on the same indicator at 42°. Each such set of crosses was conducted on four different days. The results are in Table 6. The data have the following features: (1) In each *rec*$^+$ trial (Table 6A) leftward χ$^+$ gave the largest, χ° the next, and rightward χ$^+$ the smallest A^+R^+ recombination frequency. This is the result predicted if the antiparallel *cos*, which is responsible for activating Chi, is preferentially used to package from the dimers that result from Chi-stimulated exchange. (2) In the corresponding *recB* crosses (Table 6B) the range of these values is less than that observed in the *rec*$^+$ crosses, and there is no consistent rank order in the recombination frequencies.

Since all known Chi-related phenomena are dependent on the wild-type allele of the *recB* gene, we may take the variability in a given set of *recB* crosses to represent the reproducibility of the measured recombination frequencies. Some of the variability is expected to be due to nonidealities in the parental phage mixes and some in variability in media or other conditions that fluctuate from day to day. These fluctuations are removed by calculating the ratios of the recombination frequencies in the *rec*$^+$ crosses to those in the corresponding *recB* crosses (Table 6C). For each trial, these ratios retain the rank order seen in Table 6A. The averages of the values represent our best estimate of the degree to which the *cos*-Chi interaction influences the choice of *cos* (antiparallel over parallel) to be used for packaging.

Our data can be used to estimate the fraction of time that the antiparallel *cos*, which was responsible for activating the Chi, was chosen for packaging. The numbers stand in the approximate ratios of ¾ (for χ$^+$ Left): ½ (for χ°) to ¼ (for χ$^+$ Right). Let us assume that *A* and *R* are essentially inseparable

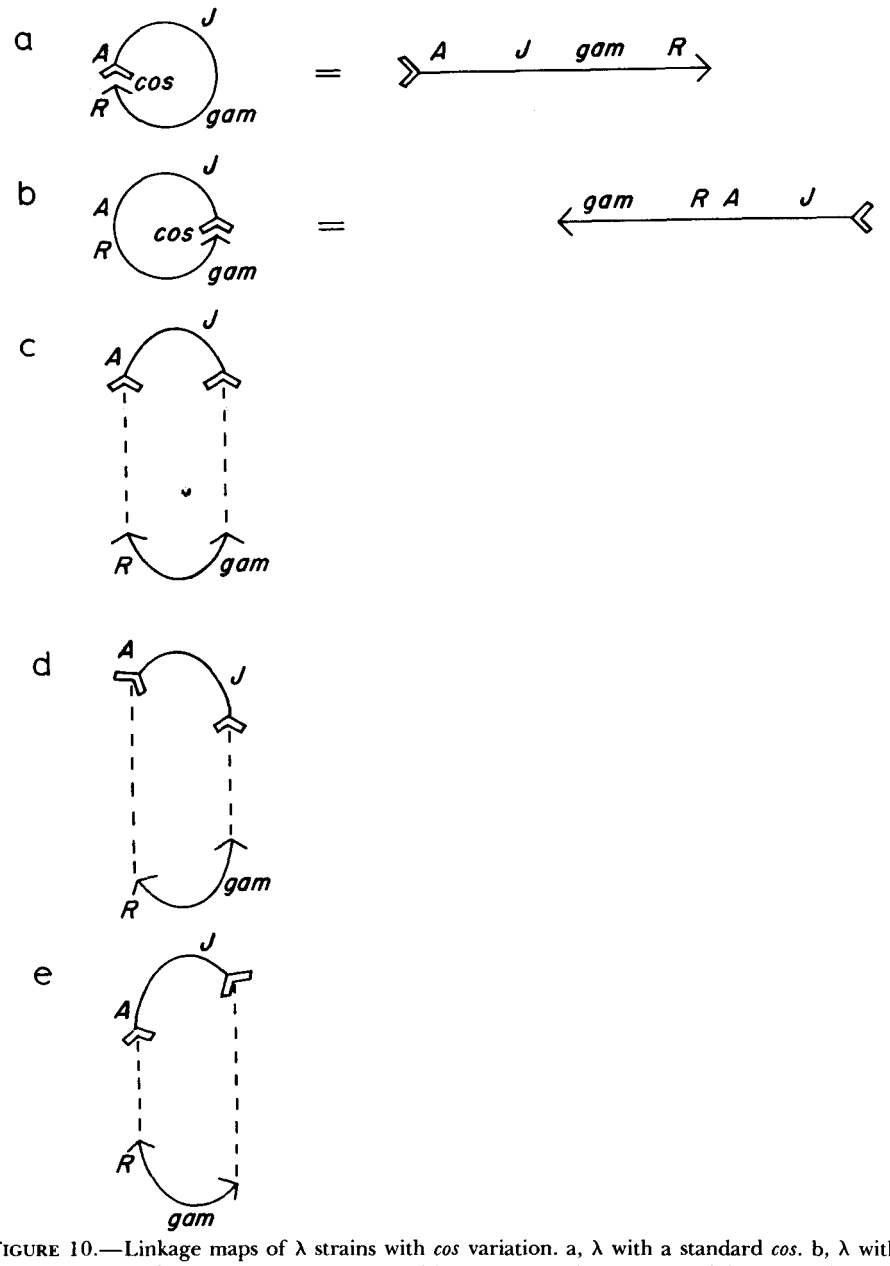

FIGURE 10.—Linkage maps of λ strains with *cos* variation. a, λ with a standard *cos*. b, λ with a *cos* in the middle of the standard map. c, λ with two *cos*. Both *cos* are used for packaging at an equal frequency. d, λ with two *cos*. The standard *cos* is used more often than the central *cos*. e, λ with two *cos*. The central *cos* is used more often than the standard *cos*.

whenever the cloned *cos* is used for packaging. We presume, further, that each *cos* is used equally in the χ° cross, so that ½ is the fraction of time that the standard *cos* is used. Then, when Chi is left, that *cos* is used ¾ of the time, and when Chi is right, it is used ¼ of the time. These estimates compare

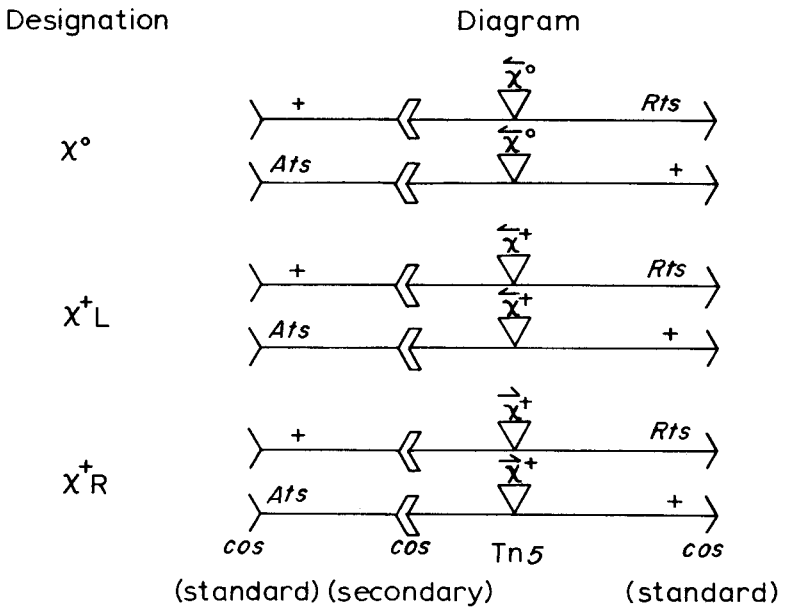

FIGURE 11.—Crosses to detect preferential *cos* utilization as a function of the orientation of Chi. The secondary *cos* is in the *Eco*RI B fragment. Tn*5* is in *gam*. All phages were deleted from *Eco*RI site 2 to site 3 (ΔC) and were *cI857* and *nin5*.

TABLE 6

Results of crosses shown in Figure 11

	Experiment				
	1	2	3	4	
A. A^+R^+ frequency (%) in *rec*$^+$ host					
χ^0	0.97	2.20	2.01	2.09	
χ^{+L}	1.87	3.23	3.58	3.38	
χ^{+R}	0.31	1.05	0.96	1.01	
B. A^+R^+ frequency (%) in *recB* host					
χ^0	0.20	0.34	0.53	0.48	
χ^{+L}	0.21	0.46	0.49	0.34	
χ^{+R}	0.10	0.41	0.37	0.45	
C. A/B					Average
χ^0	4.9	6.5	3.8	4.4	4.9
χ^{+L}	8.9	7.0	7.3	9.9	8.3
χ^{+R}	3.1	2.6	2.6	2.2	2.6

reasonably well with those obtained from gel electrophoresis of restriction digests of DNA isolated from mature phage progeny (Tables 4 and 5).

DISCUSSION

When Chi is active on lambda carrying two *cos* of opposite orientation, the phage particle DNA is more often the result of packaging from one *cos* than

from the other. The favored *cos* is the one whose orientation activates Chi (KOBAYASHI *et al.* 1982; KOBAYASHI *et al.* 1983). We infer that packaging at *cos* and Chi-activation by *cos* are coupled. This coupling is not obligatory for Chi action since Chi activation can be uncoupled from packaging without decreasing Chi activity (KOBAYASHI *et al.* 1983).

The unequal recovery of complementary recombinants produced by Chi action was previously taken as evidence for nonreciprocality in Chi-stimulated exchange (STAHL *et al.* 1980). Since the inequality disappears when packaging is uncoupled from Chi action, however, the usual coupling of packaging and Chi activation can explain the inequality. Furthermore, the inequality argues that *initiation* of rightward packaging at *cos* is associated with activation of Chi on the same DNA molecule under coupling conditions (See Figure 4a). The involvement of initiating *cos*, as opposed to terminating *cos*, and the uncoupling suggest that some early step of packaging at *cos* activates Chi. From this and the other lines of evidence (KOBAYASHI, STAHL and STAHL 1984), we infer that reversible cutting at *cos* by terminase is relevant (KOBAYASHI *et al.* 1983). This inference is in harmony with the activation of Chi by the action of endonuclease *Eco*RI (STAHL *et al.* 1983b). That terminase cutting at *cos* can occur separately from packaging *in vivo* has been demonstrated by H. MURIALDO and W. L. FIFE (personal communication).

How does the *cos* event or the *Eco*RI action activate Chi at a distance? We infer that the double-stranded end provides an entry site for a recombinase that travels along DNA searching for a Chi of proper orientation (KOBAYASHI *et al.* 1983; STAHL *et al.* 1983; KOBAYASHI, STAHL and STAHL 1984). The orientation dependence is explained by asymmetric interaction of the packaging machinery, in particular, terminase, with *cos* (FEISS and WIDNER 1982; FEISS, KOBAYASHI and WIDNER 1983; MIWA and MATSUBARA 1983). The simplest picture is that terminase binding on the front side of *cos* blocks entry of the putative recombinase. The recombinase is forced to travel in the other direction as schematized in Figure 12. The travel of the recombinase in this direction is in good harmony with the unequal recovery of the complementary recombinants as illustrated in Figure 4a.

Chi activity has been demonstrated in transduction mediated by phage P1 (DOWER and STAHL 1981). The results of the crosses, which involved λ prophages and adjacent *E. coli* chromosome intervals, revealed asymmetries that were interpreted as manifestations of intrinsic bias ("directionality") and "nonreciprocality" operating together. The present work argues that nonreciprocality, instead of being a fundamental property of Chi activity, is a result of coupling between Chi activation and λ packaging. Since the P1 transductions do not involve λ packaging, the explanation offered for those asymmetries is in doubt.

There is no longer any evidence for nonreciprocality of Chi-stimulated exchange. In the accompanying paper a different protocol is employed to detect any asymmetry that might be associated with Chi action (STAHL, LIEB and STAHL 1984).

DAVID HAGEN kindly provided a *nusA1* strain from IRA HERSKOWITZ's collection. We are grate-

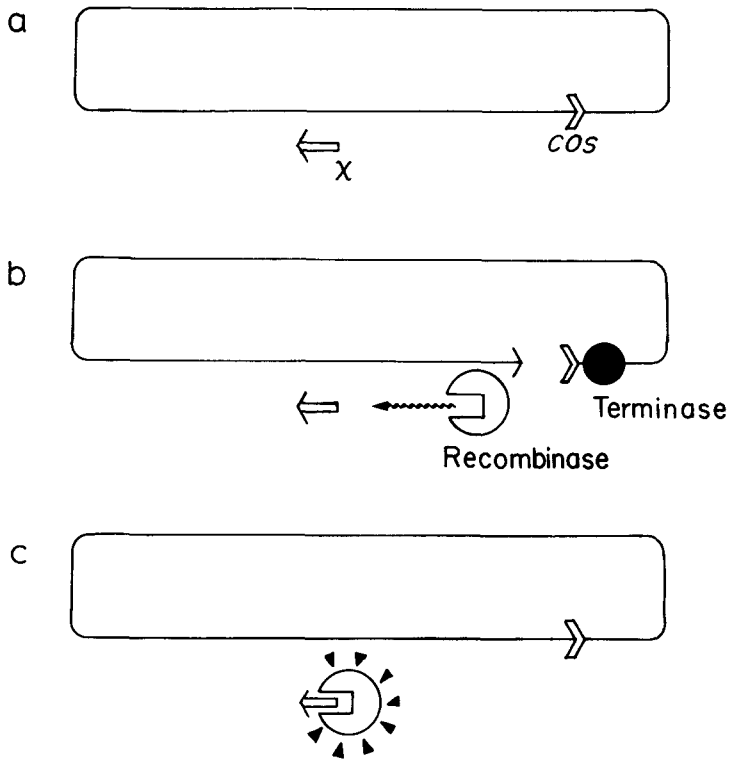

FIGURE 12.—A plausible mechanism of activation of Chi by *cos*. a, Chi and antiparallel *cos* are the same DNA. b, Terminase introduces a cut at *cos*. A recombinase enters DNA at the right end and travels leftward. Terminase blocks entry at the left end. c, When the recombinase meets Chi of proper orientation, it stimulates recombination.

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LITERATURE CITED

APPLEYARD, R. K., 1954 Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39:** 440–452.

BROWN, A. and W. ARBER, 1964 Temperature-sensitive mutants of coliphage lambda. Virology **24:** 237–239.

CAMERON, J., R. S. PANASENKO, I. R. LEHMAN and R. W. DAVIS, 1975 *In vitro* construction of bacteriophage λ carrying segments of the *Escherichia coli* chromosome: selection of hybrids containing the gene for DNA ligase. Proc. Natl. Acad. Sci. USA **72:** 3416–3420.

CAMPBELL, A. and A. DEL CAMPILLO-CAMPBELL, 1963 Mutant of lambda bacteriophage producing a thermolabile endolysin. J. Bacteriol. **85:** 1202–1207.

DOWER, N. A. and F. W. STAHL, 1981 χ activity during transduction-associated recombination. Proc. Natl. Acad. Sci. USA **78:** 7033–7037.

EMMONS, S. W., 1974 Bacteriophage lambda derivatives carrying two copies of the cohesive end site. J. Mol. Biol. **83:** 511–525.

FAULDS, D., N. DOWER, M. M. STAHL and F. W. STAHL, 1979 Orientation-dependent recombination hotspot activity in bacteriophage λ. J. Mol. Biol. **131:** 681–695.

FEISS, M. and A. BUBLITZ, 1975 Polarized packaging of bacteriophage lambda chromosomes. J. Mol. Biopl. **94:** 583–594.

FEISS, M., R. A. FISHER, D. A. SIEGELE, B. P. NICHOLS and J. E. DONELSON, 1979 Packaging of the bacteriophage lambda chromosome: a role for base sequence outside *cos*. Virology **92:** 56–67.

FEISS, M., I. KOBAYASHI and W. WIDNER, 1983 Separate sites for binding and nicking of bacteriophage lambda DNA by terminase. Proc. Natl. Acad. Sci. USA **80:** 955–959.

FEISS, M. and W. WIDNER, 1982 Bacteriophage lambda DNA packaging: scanning for the terminal cohesive end site during packaging. Proc. Natl. Acad. Sci. USA. **79:** 3498–3502.

FIANDT, M., Z. HRADECNA, H. A. LOZERON and W. SZYBALSKI, 1971 Electron micrographic mapping of deletions, insertions, inversion, and homologies in the DNAs of coliphage lambda and phi 80. pp. 329–354. In: *The Bacteriophage Lambda*, Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

GILLEN, J. and A. J. CLARK, 1974 The RecE pathway of bacterial recombination. pp. 123–136. In: *Mechanisms in Recombination*, Edited by R. F. GRELL. Plenum Press, New York.

GOLDBERG, A., and M. HOWE, 1969 New mutations in the S cistron of bacteriophage lambda affecting host cell lysis. Virology **38:** 200–202.

HENDERSON, D. and J. WEIL, 1975 Recombination-deficient deletions in bacteriophage λ and their interaction with *chi* mutations. Genetics **79:** 143–174.

HOHN, B., 1975 DNA as substrate for packaging into bacteriophage lambda *in vitro*. J. Mol. Biol. **98:** 93–106.

KAISER, A. D. and F. JACOB, 1957 Recombination between temperate bacteriophages and the genetic control of immunity and prophage localization. Virology **4:** 509–521.

KATSURA, I., 1983 Tail assembly and injection, pp. 331–346. In: *Lambda II*, Edited by R. W. HENDRIX, J. W. ROBERTS, F. W. STAHL and R. A. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

KOBAYASHI, I., H. MURIALDO, J. M. CRASEMANN, M. M. STAHL and F. W. STAHL, 1982 Orientation of cohesive end site (*cos*) determines the active orientation of χ sequence in stimulating RecA·RecBC-mediated recombination in λ lytic infections. Proc. Natl. Acad. Sci. USA **79:** 5981–5985.

KOBAYASHI, I., M. M. STAHL, D. LEACH and F. W. STAHL, 1983 The interaction of *cos* with Chi is separable from DNA packaging in *recA-recBC*-mediated recombination of bacteriophage lambda. Genetics **104:** 549–570.

KOBAYASHI, I., M. M. STAHL and F. W. STAHL, 1984 The mechanism of the Chi-*cos* interaction in RecA-RecBC-mediated recombination in phage λ. Cold Spring Harbor Symp. Quant. Biol. In press.

LAM, S. T., M. M. STAHL, K. D. MCMILIN and F. W. STAHL, 1974 Rec-mediated recombinational hot spot activity in bacteriophage lambda II. A mutation which causes hot spot ativity. Genetics **77:** 425–433.

LISSOUBA, P., J. MOUSSEAU, G. RIZET and J.-L. ROSSINGNOL, 1962 Fine structure of genes in the ascomycete *Ascobolus immersus*. Adv. Genet. **1:** 343–380.

MARKHAM, P. and H. L. K. WHITEHOUSE, 1982 A hypothesis for the initiation of genetic recombination in eukaryotes. Nature **295:** 421–423.

MATTHEWS, B. W., C. E. KOPFENSTEIN and P. M. COLMAN, 1972 A computer controlled film scanner for X-ray crystallography. J. Phys. E. Sci. Instrum. **5:** 353–359.

McMILIN, K. D. and V. E. A. RUSSO, 1972 Maturation and recombination of bacteriophage lambda DNA molecules in the absence of DNA duplication. J. Mol. Biol. **68:** 49–55.

MIWA, T. and K. MATSUBARA, 1983 Lambda phage DNA sequences affecting the packaging process. Gene **24:** 199–206.

MURRAY, N. E., 1963 Polarized recombination and fine structure within the *me-2* gene of *Neurospora crassa*. Genetics **48:** 1163–1183.

ROSS, D. G. and D. FREIFELDER, 1976 Maturation of a single λ phage particle from a dimeric circular λ DNA. Virology **74:** 414–425.

SIGNER, E. R. and J. WEIL, 1968 Recombination in bacteriophage λ. I. Mutants deficient in general recombination. J. Mol. Biol. **34:** 261–271.

SMITH, G. R., 1983 General recombination. pp. 175–209. In: *Lambda II*, Edited by R. HENDRIX, J. ROBERTS, F. STAHL and R. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

SMITH, G. R., S. M. KUNES, D. W. SCHULTZ, A. TAYLOR and K. L. TRIMAN, 1981 Structure of Chi hotspots of generalized recombination. Cell **24:** 429–436.

SPRAGUE, K. U., D. H. FAULDS and G. R. SMITH, 1978 A single basepair change creates a Chi recombinational hotspot in bacteriophage λ. Proc. Natl. Acad. Sci. USA **75:** 6182–6186.

STAHL, F. W., 1979a *Genetic Recombination, Thinking About It in Phage and Fungi*. W. H. Freeman, San Francisco.

STAHL, F. W., 1979b Special sites in generalized recombination. Annu. Rev. Genet. **13,** 7–24.

STAHL, F. W., J. M. CRASEMANN and M. M. STAHL, 1975 Rec-mediated hotspot activity in bacteriophage λ. III. Chi mutations are site mutations stimulating Rec-mediated recombination. J. Mol. Biol. **94:** 203–212.

STAHL, F. W., I. KOBAYASHI and M. M. STAHL, 1982 Distance from *cos* determines the replication requirement for recombination in phage λ. Proc. Natl. Acad. Sci. USA **79:** 6318–6321.

STAHL, F. W., I. KOBAYASHI and M. M. STAHL, 1983 Chi is activated by a variety of routes. pp. 773–783. In: *Mechanisms of DNA Replication and Recombination*, Edited by N. R. COZZARELLI. Alan R. Liss, Inc., New York.

STAHL, F. W., M. LIEB and M. M. STAHL, 1984 Does Chi give or take? Genetics **108:** 795–808.

STAHL, F. W. and M. M. STAHL, 1975 Rec-mediated recombinational hotspot activity in bacteriophage λ. IV. Effect of heterology on Chi-stimulated crossing over. Mol. Gen. Genet. **140:** 29–37.

STAHL, F. W. and M. M. STAHL, 1977 Recombination pathway specificity of Chi. Genetics **86:** 715–726.

STAHL, F. W., M. M. STAHL, R. E. MALONE and J. M. CRASEMANN, 1980 Directionality and nonreciprocality of Chi-stimulated recombination in phage λ. Genetics **94:** 235–248.

STAHL, F. W., M. M. STAHL, L. YOUNG and I. KOBAYASHI, 1983a Injection is not essential for high level of Chi activity during recombination between λ vegetative phages. pp. 27–40. In: *Genetic Rearrangements*, Edited by K. F. CHATER, C. A. CULLIS, D. A. HOPWOOD, A. W. B. JOHNSTON and H. W. WOOLHOUSE. Croom Helm, London.

STAHL, M. M., I. KOBAYASHI, F. W. STAHL and S. K. HUNTINGTON, 1983b Activation of Chi, a recombinator, by the action of an endonuclease at a distant site. Proc. Natl. Acad. Sci. USA **80:** 2310–2313.

STAHL, M. M. and F. W. STAHL, 1971 DNA synthesis associated with recombination. I. Recombination in a DNA-negative host. pp. 431–442. In: *The Bacteriophage Lambda*, Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

STERNBERG, N. and R. WEISBERG, 1975 Packaging of prophage and host DNA by coliphage λ. Nature **256:** 97–103.

SUSSMAN, R. and F. JACOB, 1962 Sur un systeme de repression thermosensible chez le bacteriophage λ d'*Escherichia coli*. C. R. Acad. Sci. (Paris) **254:** 1517–1519.

WEIGLE, J., 1966 Assembly of phage lambda *in vitro*. Proc. Natl. Acad. Sci. USA **55:** 1462–1466.

YAGIL, E., N. A. DOWER, D. CHATTORAJ, M. STAHL, C. PIERSON and F. W. STAHL, 1980 Chi mutation in a transposon and the orientation dependence of Chi phenotype. Genetics **96:** 43–57.

ZISSLER, J., E. SIGNER and F. SCHAEFER, 1971 The role of recombination in growth of bacteriophage lambda. I. The gamma gene. pp. 455–468. In: *The Bacteriophage Lambda*, Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

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