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Non-reciprocal crossing over in phage λ

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Abstract. In replication-blocked crosses in phage λ, *cos*, λ's packaging origin, provokes Red-mediated recombination in its neighborhood. In crosses of wild-type × mutant *cos*, *cos*-stimulated crossover products can be packaged from *cos* sites cloned medially in the two parents (Stahl F W, Kobayashi I and Stahl M M 1985 *J. Mol. Biol.* 181: 199–209). We find that the complementary crossover products are not produced in equal numbers. In agreement with the model presented earlier (*ibid*), most of the recombinants inherit λ's right arm (*R* arm) from the cos^+ parent and λ's left arm (*A* arm) from the parent with the mutant *cos*.

Keywords. Recombination; Red system; *cos*, cohesive end site; double strand breaks.

1. Introduction

We have presented a model for a nonreciprocal break-join recombination reaction mediated by the Red system of phage λ acting at the double strand cut site *cos* (Stahl *et al* 1985). The model (figure 1) has the following features: (i) The terminase (Ter) of λ binds at *cosB* to the right of the cut site, *cosN* (figure 1a). (ii) Terminase introduces staggered cuts at *cosN* (creating "sticky ends") while remaining bound at *cosB* (figure 1b). (iii) An exonuclease, product of the *redα* gene of λ, digests the exposed right end of λ but cannot digest the left end of λ because of Ter binding. Since that nuclease digests in the 5′ to 3′ direction, its action removes the right hand sticky end and exposes a single stranded 3′ overhang (figure 1c). (iv) This overhang invades an uncut homologous duplex creating a stretch of biparental ("hybrid" or "heteroduplex") DNA (figure 1d). (v) Limited DNA synthesis, reconstitutes *cos* using the invading chain as primer and the complementary chain of the invaded duplex as template (figure 1e). (vi) The invasion is concluded by a break-join event at the left end of the hybrid region (figure 1f). (vii) When the *cos* of the invaded phage is wild-type, then the Ter that cut the invading chromosome can initiate a packaging act that is completed at the re-constituted *cos* (figure 1g). (viii) When the *cos* of the invaded duplex is mutant, and cannot participate in packaging, then a break-join event at the right end of the hybrid region completes a nonreciprocal act of splicing (figure 1h). The resulting product of nonreciprocal crossing over can then be packaged from accessory *cos* sites (figure 1i).

The roles for Ter and *cos* invoked in the model are based on demonstrated properties of that enzyme and its site of action (Feiss and Widner 1982; Feiss *et al* 1983; Murialdo and Fife 1984). The role of λ exonuclease is based on demonstrated *in vitro* activities of that enzyme (Little 1967; Carter and Radding 1971; Sriprakash *et al* 1975).

The model accounts for the following features of λ Red-mediated recombination in replication-blocked *recA* host cells. (i) In replication-blocked crosses marked at the near-terminal genes *A* and *R* (figure 2) exchanges (to produce $A^+ R^+$ recombinants) are clustered at the right end (*R* end) of the marked interval (Stahl *et al* 1974). (ii) These

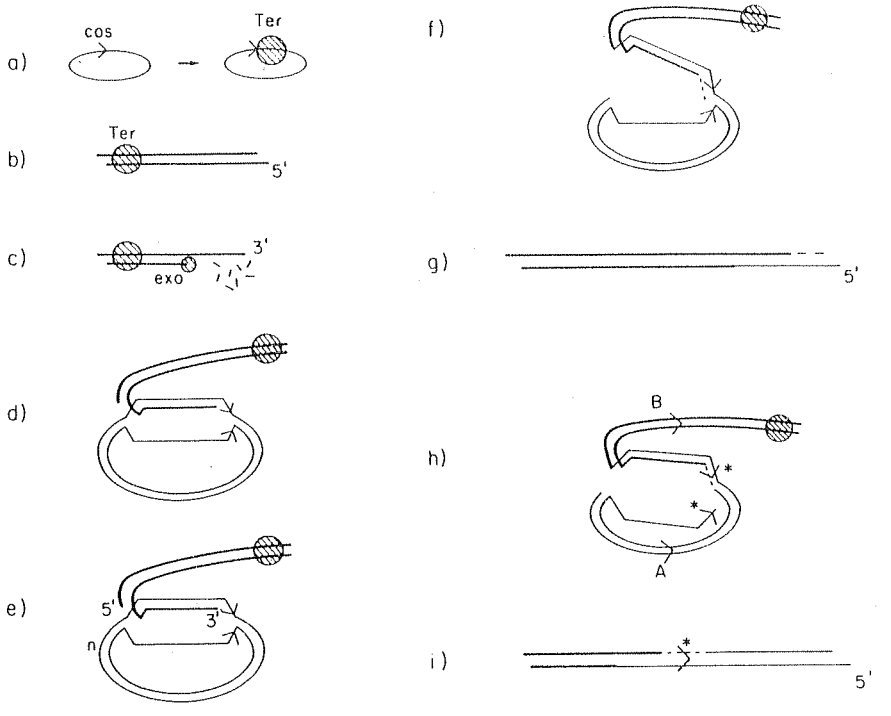

Figure 1. Model for *cos*-stimulated Red-mediated recombination between non-replicated chromosomes (after Stahl *et al* 1985).

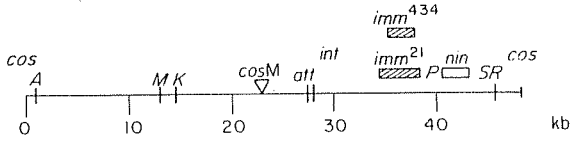

Figure 2. Map of λ showing features referred to. See table 2 for description of markers used.

recombinants are frequently heteroduplex for markers near the *R* end, including markers in *R* (Russo 1973). (iii) Such heteroduplexes have inherited most of their DNA from one parent but have a contribution from the other parent confined to the chain that ends 5′ on the right (Stahl and Stahl 1974). (iv) The recombination concentrated near the *R* end is dependent on a functional *cos* site located there. If the *cos* sites in the two parents are inactivated (and substitute sites are provided at a remote location), then there is no replication-independent recombination near *R* (Stahl *et al* 1982). (v) Recombination can occur near λ's *R* end when there is a functional *cos* at the standard location in only *one* of the parents. (In such crosses, the recombinants are packaged from the substitute *cos* sites at a non-standard location.) (vi) In crosses between markers near λ's *R* end the recombinants recovered (point 5) can be fully conserved; i.e., essentially all of their DNA is derived from the chromosomes of the infecting particles. (vii) These recovered, conserved recombinants have inherited the *A* arm of their chromosome from the parent with the nonfunctional standard *cos*.

(viii) The standard *cos* of these recombinants is always mutant (points 5–8 established by Stahl *et al* 1985).

In the model (figure 1) the nonreciprocal break-join splicing is presumed to be asymmetric because of Ter binding at λ's *A* end. That feature of the model depends on the observations that Ter can bind there (Feiss and Widner 1982; Feiss *et al* 1983; Miwa and Matsubara 1983) and that replication-blocked crosses between phages with normal *cos* sites yield no A^+R^+ recombinants arising from exchanges near gene *A* (Stahl *et al* 1974). However, the latter observation is subject to two interpretations: (a) Exchange in Red-mediated *recA* crosses is confined to λ's *R* end (as supposed by the model), or (b) exchange can occur at both ends of λ, but exchanges at the *A* end are confined to the region between *cos* and our marker in *A*. These two possibilities make different predictions regarding the recombination of markers that flank the standard *cos* locus when one of the two parents is mutant at *cos*. The prediction of interpretation (a) and of the model in figure 1 is that most or all of the recombinants will inherit the locus to the left of *cos* from the parent with the functional *cos* whereas the marker to the right of *cos* will come from the parent with the mutated *cos*. In other words, recombination in each act will be nonreciprocal, and it will always be the same one of the two conceivable crossover products that will be produced. In this paper we report the results of such crosses and conclude that the model presented in figure 1 remains an adequate and attractive view of Red-mediated exchange stimulated by *cos*.

2. Materials and methods

Bacterial strains employed are described in table 1. Phage mutations are described in table 2. Their map positions are shown in figure 2.

3. Results

We have performed two types of crosses to test the prediction of the model (figure 1) that recombinants flanking *cos* are produced nonreciprocally. Markers flanking the standard *cos* locus are chosen to allow for selective plating of the complementary recombinants arising by exchange at standard *cos*. In one cross, the standard *cos* of one parent is nonfunctional by virtue of a 22 base pair deletion (*cos*2) that removes the cut site. In the other cross it is the other parent that is so deleted. In both crosses we expect the recombinants to be packaged from the substitute, cloned *cos* sites. The model

Table 1. Bacterial strains

Designation	Relevant genotype	Reference
FZ14	Su^- *dnaBts*22 *recA*56	Stahl *et al* 1972
ED206	Su^- *recA*56	Henderson and Weil 1975
K12SH-28	Su^+	Fangman and Novick 1966
594	Su^-	Weigle 1966
FS1646	K12SH-28 (λ *Mts* imm^{434})	—
FS1802	594 (λ *Ksus* imm^{21})	—
FS1664	Su^+ *dnaBts*22 Δ(*recA-srl*)::Tn10	—

Table 2. Phage markers

Marker	Properties	Reference
imm^{21}	immunity region of phage 21	Campbell (1971)
imm^{434}	immunity region of phage 434	Kaiser and Jacob (1957)
*Ksus*768	*sus* in *K*	Parkinson (1968)
*Mts*5	*ts* in *M*	Brown and Arber (1964)
*cos*2	22-bp deletion removing nicking site of *cos*	Kobayashi *et al* (1982) Feiss *et al* (1983)
*cos*ML	*cos* cloned (leftward) in *Bgl* II site at 47%	Kobayashi *et al* (1982)
*int*29	*sus* in *int*	Enquist and Weisberg (1976)
*nin*5	5% deletion in right arm	Fiandt *et al* (1971)

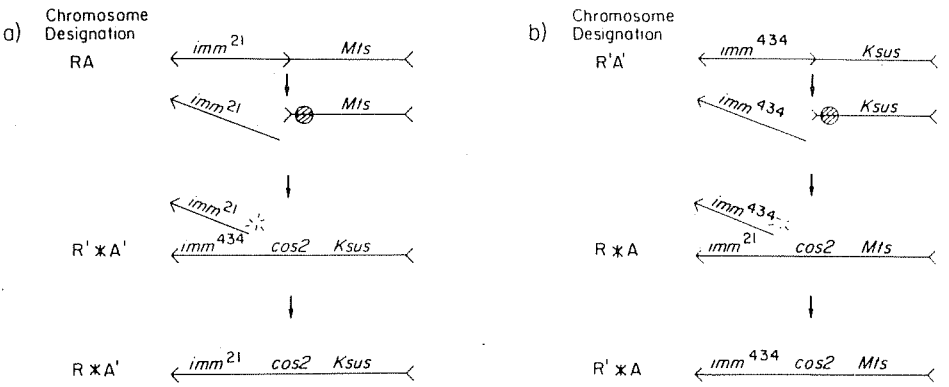

Figure 3. Crosses to detect nonreciprocality in Red-mediated *cos*-stimulated crossing over. In each cross, the model of figure 1 specifies that cos (>) in the upper parent is cut. The immunity fragment (corresponding to λ's right arm) then invades the lower parent and a nonreciprocal act of crossing over produces a recombinant that inherits the uncuttable *cos* site (*cos*2). Phage chromosomes are displayed in the linear (open at *cos*ML) form that the recombinant will have in the virion. *R* and *A* refer to the arms of λ. * designates *cos*2.

predicts that complementary recombinants will be recovered in unequal numbers and that the sign of the inequality will be reversed for the two crosses (figure 3).

Crosses were performed in the *dnaBts*22 *recA*56 host FZ14 at high temperature (39° or 42°) to impose the DNA replication block that reveals *cos*-stimulated exchange (Stahl *et al* 1972). Some of the crosses were Int$^+$, while others were Int$^-$. The Int system, acting only on *att*, is not expected to contribute packageable recombinants. Cross lysates were plated selectively for the complementary recombinant classes on lysogens FS1646 and FS1802. Data are in table 3; they show that the recombination is nonreciprocal in all crosses, with one recombinant at least six times more frequent than the other. In each cross the more frequent recombinant is the one inheriting λ's *R* arm (marked by the immunity region) from the parent with the wild-type standard *cos*.

The data in table 3 reveal the following features of secondary importance: (i) As expected, the Int system has little, or no influence. (ii) There are marker effects. The magnitude of the nonreciprocality always appears greater in the crosses in which the *cos*2 mutation is in the *imm*^{21} parent. The values of average index (table 3) are estimates

Table 3. Nonreciprocality of *cos*-stimulated, Red-mediated recombination.

Host	Temperature	Int	Parents	Recombinants RA′	Recombinants R′A	Index of nonreciprocality	Average index
FZ14	42°	−	RA × R′*A′	219(20)	310(1)	14·1	25·7
			R′A′ × R*A	80(1)	149(20)	37·3	
FS1664	42°	+	RA × R′*A′	179(20)	347(1)	10·3	22·2
			R′A′ × R*A	61(1)	104(20)	34·1	
FS1664	39°	+	RA × R′*A′	1264(1)	215(1)	5·9	10·8
			R′A′ × R*A	97(1)	1523(1)	15·7	
FS1664	39°	+	RA × R′*A′	474(1)	65(1)	7·3	23·2
			R′A′ × R*A	15(1)	587(1)	39·1	

RA is the imm^{21} *Mts* parent. R′A′ is the imm^{434} *Ksus* parent. * signifies the *cos*2 mutation in the appropriate parent. See figure 3.

Entries under Recombinants are plaque counts on the appropriate selective indicator followed, in parentheses, by the relative dilution factor. Index of nonreciprocality is RA′/R′A for the first cross of each pair and is R′A/RA′ for the second.

of the magnitudes of the nonreciprocalities corrected for the marker effect. They indicate that, at 42°, which imposes the strongest block to DNA replication, the discrepancies in the complementary recombinant classes are 20 fold or greater. (iii) The nonreciprocalities are probably less pronounced at 39° than at 42° (the exceptional value rests on a plaque count of only 15). Since the block to DNA replication is stronger at 42° than at 39°, this suggests that the nonreciprocality is a feature only of recombination between replication-blocked chromosomes, a point substantiated below.

Our previous work (Stahl *et al* 1985), which led to the model in figure 1, showed that replication-blocked Red-mediated recombinants in λ's right arm between genes *P* and *S* (figure 2), inherited *cos* from whichever of the two parents was mutant for *cos*. From the cross RA × R′*A′ in table 3, we isolated 10 recombinants of the favored class and determined the allelic state of the standard *cos* locus (Stahl *et al* 1985). All 10 were mutant (i.e., R*A′), in accord with the model (figure 1i).

When DNA replication is permitted, recombination occurs throughout the λ chromosome in Red^+ $RecA^-$ crosses (Stahl and Stahl 1971; Stahl *et al* 1972). We examined recombination across *cos* in crosses with unrestricted DNA replication (table 4). All crosses were Int^-. With the host (FZI4) carrying the temperature-sensitive DNA synthesis mutation, infection at high temperature (42°) introduced nonreciprocality like that seen in table 3. An aliquot of the same culture infected at 34° showed no nonreciprocality. The *recA* host ED206, which is wild-type for DNA synthesis genes, showed no nonreciprocality at either temperature.

4. Discussion

Our results support a model for Red-mediated recombination presented previously (Stahl *et al* 1985). In particular, they demonstrate that exchanges initiated by the asymmetric double chain cut site *cos* are frequently nonreciprocal. One of the two

Table 4. Effect of replication on nonreciprocality.

Host	Temperature	Condition	Cross	Recombinants RA′	Recombinants R′A	Index of nonreciprocality	Average index
ED206	34°	DNA$^+$	RA × R′*A′	460(400)	553(400)	0·832	1·1
			R′A′ × R*A	346(400)	444(400)	1·28	
ED206	42°	DNA$^+$	RA × R′*A′	59(400)	134(400)	0·440	0·95
			R′A′ × R*A	185(400)	268(400)	1·45	
FZ14	34°	DNA$^+$	RA × R′*A′	108(20)	142(20)	0·761	1·5
			R′A′ × R*A	44(20)	95(20)	2·16	
FZ14	42°	DNA°	RA × R′*A′	104(1)	28(1)	3·7	55
			R′A′ × R*A	1(1)	107(1)	107	

For explanation, see table 3.

possible crossover products is recovered more than the other. The bias seen is the one predicted by the notion that λ's terminase, which cuts *cos*, remains bound to the right of the cut site, thereby inhibiting λ's *A* end from invading an uncut homologue.

The failure to see nonreciprocality in these crosses when DNA replication is allowed could imply that replication promotes dissociation of terminase from *cos*. Alternatively, the replication-dependent part of the Red recombination pathway may be independent of any recombinogenic role for *cos* (Stahl *et al* 1972, 1982). Experiments with Chi, a recombinator in the RecBC pathway may bear on the former possibility. We have noted (Stahl *et al* 1983) that a Chi that is inactive due to its parallel orientation with *cos* becomes detectably active when chromosome replication is permitted. An economical interpretation for this observation is that terminase, whose binding at cosB is presumed to be responsible for the *cos*-Chi interaction (Kobayashi *et al* 1984), can be dislodged by replication, thereby depolarizing *cos* with respect to entry into the duplex by the RecBC protein. On the other hand there is support for the latter possibility. When replication is allowed, Red-mediated recombinants in λ's right arm frequently inherit wild-type *cos* in $cos^+ \times cos2$ crosses.

We are emboldened by the success of our model for *cos*-stimulated, Red-mediated recombination (in the absence of DNA replication) to elaborate it further. In the model as described in figure 1, the enzymatic bases for the chain-breaking activities postulated in figures 1f and 1h are not specified. For each of these reactions, however, well-qualified candidate enzymes are available, and experiments are in progress to test the strengths of the candidacies.

4.1 *The break far from the invading end*

In a $cos^+ \times cos^+$ cross, completion of the recombinant requires breaking a chain on the invaded duplex at the point marked 5′ in figure 1e. The extreme length and variability of the heteroduplexes characteristic of these crosses (Stahl *et al* 1974; Russo 1973) argue against a well-coordinated enzyme machine as terminator of heteroduplex extension. Instead, the required break-join at 5′ occurs at a more or less exponentially distributed distance from the 3′ end, as if an "accident" triggers the event. We propose this accident

to be an unrelated nick on the invaded chromosome (*n* in figure 1e). We postulate that digestion at 5′ by λ's exonuclease continues with accompanying enlargement of the D-loop until that loop has enlarged to *n*. The reaction then stops due to the self-limiting nature of the strand-assimilation reaction (see below).

The strand-assimilation reaction of Red's exonuclease as described by Cassuto *et al* (1971) is summarized in figure 4a. Exonuclease processively digests (5′ to 3′) the chain to which it is bound. The reaction stops when the homologous chain (thin line in figure 4a) has become fully paired by spontaneous annealing. Stoppage of digestion results from the inability of exonuclease to bind (stay bound) to a simple nick. In our model the events at 5′ can be diagrammed (figure 4b) in a manner that reveals their analogy to the strand assimilation reaction of Cassuto *et al*. Note that the strand to be assimilated already has a pairing partner; its spontaneous assimilation cannot be expected to proceed rapidly. We propose that Red beta protein, a helix destabilizing protein (Kmiec and Holloman 1981) that can be isolated as a complex with the Red exonuclease (Radding *et al* 1971), promotes the assimilation.

4.2 *The break near the invading end*

When the invaded chromosome contains a functional *cos* at the same locus as the recombination-initiating *cos*, then completion of a packageable recombinant can be accomplished by chain extension at 3′ extending as far as *cos*. However, when the invaded chromosome is mutant at this *cos*, then more complex events at 3′ are required to produce the unreplicated recombinants. In particular, chain extension at 3′, if any, must be followed by breaking and joining as shown in figure 1h. The resulting recombinant (produced nonreciprocally) can then be packaged using the cloned *cos* present in each parent. We propose that the 5′ and 3′ exonuclease activity of polymerase

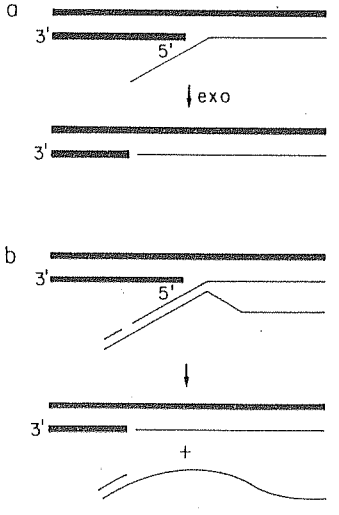

Figure 4. **a** Strand-assimilation by Red's exonuclease, **b** strand-assimilation in the model of figure 1.

I is responsible for that cut. The postulate is economical because of the likely involvement of pol I in the immediately preceding chain extension step. Furthermore, there is *in vitro* evidence in support of this postulated endonucleolytic activity of pol I. Liu and Wang (1975) showed that pol I will cut one chain of a circular duplex that has taken up a bit of homologous single chain DNA to form a D-loop. They speculated that the chain complementary to the invading chain was cut, and that it was cut near the 3′ end of that chain via pol I's 5′ to 3′ (nick-translating) exonuclease activity. More recently, Lundquist and Olivera (1982) have reported that pol I-mediated nick translation involves cutting of chains that have been displaced by polymerase-catalyzed chain extension. These two observations make pol I's 5′ to 3′ exonuclease a good candidate for the cut at 3′ required in our model.

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