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Evidence that the normal route of replication-allowed Red-mediated recombination involves double-chain ends

David S.Thaler, Mary M.Stahl and Franklin W.Stahl

Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA

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Recombination mediated by the Red pathway of bacteriophage λ is focused towards sites of double-chain cuts. Double-chain ends created either by type II restriction enzymes acting at unmodified recognition sites or by λ's packaging enzyme, terminase, acting at *cos* are utilized in a manner similar to the double-chain break repair pathway of recombination in yeast. When λ is allowed to recombine during replicative growth, spontaneous recombination is approximately evenly distributed along the chromosome. It has been proposed that replication-allowed recombination also is initiated by double-chain ends. In order to test this hypothesis we ask if the *in vivo* expression of the Mu *gam* protein is inhibitory to Red recombination. Mu *gam* has been shown *in vitro* to bind to linearized duplex DNA and to shield bound DNA from exonucleases. The expression of Mu *gam* is found to be inhibitory to Red recombination whether replication is blocked or allowed. As a control we ask if Mu *gam* inhibits Int-mediated recombination. It has been well documented that the Int pathway of recombination does not involve any double-chain breaks and, consistent with this, the Int pathway is not inhibited by Mu *gam*. We suggest that the *in vivo* expression of Mu *gam* or other similar activities may be a generally useful way to determine if those processes that respond to an artificially introduced double-chain cut normally involve double-chain ends.

Key words: *gam*/lambda/Mu/recombination/replication

Introduction

Generalized recombination pathways in several bacterial (Stahl *et al.*, 1985; Symington *et al.*, 1985), yeast (Orr-Weaver *et al.*, 1981; Orr-Weaver and Szostak, 1983) and mammalian systems (Thomas *et al.*, 1986; Wake *et al.*, 1985; Kucherlapati *et al.*, 1984) can proceed in harmony with Double-Chain-Break Repair (DCBR) models of recombination. When presented with a double-chain end of DNA these pathways use the end efficiently as a substrate in homologous recombination. The experimental evidence for DCBR activity has come from three approaches. The first involves the repair of chromosomes broken by radiation (Resnick and Martin, 1976; Kraisin and Hutchinson, 1977). The second involves the repair of linearized transforming DNA (Orr-Weaver *et al.*, 1981; Lin *et al.*, 1984; Symington *et al.*, 1985). A recent approach is the controlled action *in vivo* of enzymes that make double-chain cuts at defined sites (Stahl *et al.*, 1985; Kolodkin *et al.*, 1986; Thaler *et al.*, 1987). The contemporary question in several systems can be stated as follows: 'Is the normal mode of generalized recombination via double-chain-break repair?' (Szostak *et al.*, 1983; Rossignol *et al.*, 1984; Hastings, 1984; White *et al.*, 1985; Orr-Weaver and Szostak, 1985). In phage T4 a number of genetic and physical approaches have allowed the conclusion that normal replication and recombination are intimately related and do, in fact, involve conversion of double-chain ends into an invasive substrate for homologous recombination (Mosig, 1983).

In this work we introduce a methodology designed to complement approaches that deliberately produce double-chain breaks. Our method involves the controlled expression *in vivo* of the *gam* gene of phage Mu, which encodes for a protein possessing the *in vitro* activity of binding to double-chain ends and protecting them from exonuclease attack (Williams and Radding, 1981; Akroyd *et al.*, 1984). We show *in vivo* that Mu *gam* inhibits Red-mediated, replication-blocked, *cos*-stimulated lambda recombination, which has previously been shown to be initiated by a double-chain cut (Stahl *et al.*, 1985). We then ask if normal replication-allowed Red-mediated recombination is inhibited by the same protein. We have found this to be the case. To control for nonspecific effects of Mu *gam*, we compare the Red pathway of generalized recombination with the Int pathway of site-specific recombination, which certainly does not involve double-chain ends (Kitts *et al.*, 1984).

The Red pathway of bacteriophage λ catalyzes generalized recombination in the neighborhood of a double-chain cut. When replication is blocked, Red recombination is focused at *cos*, which is a double-chain cut site for the λ packaging enzyme terminase (Stahl *et al.*, 1985; Feiss and Becker, 1983). Recombination can be stimulated away from *cos* by delivering a double-chain cut, via *in vivo* type II restriction, to one of two parents in a λ × λ cross (Thaler *et al.*, 1987). When replication is blocked, double-chain-cut sites are the only initiators of recombination. Mechanistic models for the initiation of recombination by double-chain-cut sites have been presented for both *cos*- and restriction-stimulated recombination (Stahl *et al.*, 1985; Thaler *et al.*, 1987). These models are similar to DCBR models, which have been useful in studies on yeast and mammalian transformation.

When replication is allowed, spontaneous Red-mediated recombination becomes approximately evenly distributed along the λ chromosome (Stahl *et al.*, 1972). However, under these conditions the distribution of recombinants is still focused toward the site of a type II restriction cut (Thaler *et al.*, 1987).

There are three ways of thinking about the even distribution of spontaneous recombinants in Red-mediated recombination-allowed crosses. Replication itself may be the source of double-chain ends that are random in the genome. The tips of rolling circles have been suggested as the source of replication-dependent randomly distributed double-chain ends (Stahl *et al.*, 1985). Alternatively, there may exist a replication-dependent initiation of Red recombination not involving double-chain ends. In this view, although the Red system can efficiently utilize double-chain ends as substrates for recombination, these ends are not the normal substrate. The third view presumes break-copy recombination. According to the break-copy view, replication is needed for the completion, not the initiation, of recombination (Stahl *et al.*, 1982). Break-copy cannot be the only mode of Red recombi-

Table I. Bacteria, plasmids and λ genetic elements used in this study

	Relevant properties	Source, reference, or derivation
A. Bacteria		
594	Su^-	Weigle (1966)
JC12542	Δ(*rec*A−*srl*R)306::Tn10	A.J.Clark (Berkeley)
ED206	*rec*A56 Su^-	N.S.Willetts (Monash University)
594Δ(*rec*A−*srl*R)306::Tn10	*rec*A^- Su^-	Pl transduction of Tet^r from JC12542, this work
FA77Δ(*rec*A−*srl*R)306::Tn10	*rec*A^- dnaBts22 Su^-	Pl transduction of Tet^r from JC12542, this work
C600*rec*A56	*rec*A^- Su^+	Laboratory collection
DH1	*rec*A^- Su^+	Maniatis *et al.* (1982)
FS1585	*rec*D^- Su^+	Stahl *et al.* (1986)
B. Plasmids		
pJA21	Mu *gam* gene under λ cI857 control	Akroyd *et al.* (1984)
pXY228	Control plasmid, as above without Mu *gam*	Akroyd *et al.* (1984)
pLT11	*D* gene of λ cloned into pACYC184	This work, described in Materials and methods
C. λ genetic elements		
Jsus6	Suppressor-sensitive allele (amber) of *J*	Weil and Signer (1968)
int4	Unconditional *int* mutation	Gingery and Echols (1967)
imm^{434}	Immunity region of phage 434	Kaiser and Jacob (1957)
$imm^{434}cI$	Clear allele of imm^{434}	Laboratory collection
Rts2	Temperature-sensitive allele of *R* grows at 32 not at 37°C	Brown and Arber (1969)
bio1	Substitution rightward from att (5% net deletion)	Hradecna and Szybalski (1969)
b527	Deletion left of the attachment site of 8.3% of λ	Parkinson (1971)
Psus3	Amber in *P*	Campbell (1961)
Psus80	Amber in *P*	Campbell (1961)
Dsus123	Amber in *D*	Campbell (1961)

nation (Stahl *et al.*, 1985), but minor amounts of DNA synthesis do appear to be involved in completing replication-blocked recombinant formation (Stahl and Stahl, 1986). A major role for break-copy has not been ruled out when replication is allowed. Hybrid models are also possible, for example the randomly disposed tips of rolling circles may invade homologues, initiating events that are completed via DNA replication. A similar view of T4 DNA metabolism has been termed 'join-copy' (Mosig, 1983).

Bacteriophage Mu encodes a function that, *in vivo*, blocks activities attributable to *Escherichia coli's ExoV*. The function enhances the burst size of Red^-Gam^- λ growing in a $RecA^-$ cell, and it inhibits 'rec-less' DNA degradation (Van Vliet and Couturier, 1978). The activity has been partially purified from induced Mu lysogens cells and characterized as a generalized inhibitor of exonucleases (Williams and Radding, 1981). We shall refer to this inhibitor (and its gene) as Mu *gam*. The kinetics of protection imply that Mu *gam* shields the DNA rather than inactivating the nuclease. A given amount of the extract protects a given amount of DNA independently of the quantity of added nuclease. In contrast, a fixed amount of λ γ protein protects any amount of DNA from a certain quantity of ExoV. Lambda gamma protein functions by binding to, and inactivating ExoV. Williams and Radding (1981) found that Mu *gam* co-purifies with a DNA binding activity specific for double-chained linear DNA. They concluded that Mu *gam* functions by binding to the ends of duplex DNA, thereby shielding the bound ends from exonuclease attack.

The region of Mu responsible for exonuclease inhibition has been cloned on a controlled expression vector, pJA21 (Akroyd *et al.*, 1984, 1986; Akroyd and Symonds, 1986). We have used this expression vector *in vivo* to assay the effects of Mu's exonuclease inhibitor on Red-mediated recombination. If this protein, which binds to the ends of double-chain DNA, interferes with Red-mediated recombination, the idea that the normal mode of Red-mediated recombination involves double-chain ends will be supported.

Results

Bacterial strains, plasmids and λ genetic elements are described in Table I. Bacteria containing the Mu *gam* plasmid pJA21 or the control plasmid pXY228 were grown at 26−30°C. Each of these plasmids contains the temperature-sensitive λ repressor gene cI857 and the cI-repressed operator λpL. In pJA21 the Mu exonuclease inhibitor is under λpL control. Phage crosses were carried out in these strains at 30°C and at 40−41°C. Only at the higher temperature is Mu *gam* activity expressed. In the case of *dnaBts* strains, raising the temperature has two effects: replication is inhibited and Mu *gam* activity is expressed.

The relative contributions of Red- and Int-mediated recombination can be assessed in crosses of this form:

Jam	att	c^+	+
+	att	c	Rts

which are conducted with Int^+ and Int^- phages respectively. If the removal of Int function has little effect on the c^+/c ratio among ++ recombinants, the generalized recombination activity is judged to be high. Generalized recombination activity is low if the Int^+ cross gives a much higher c^+/c ratio among ++ recombinants than does the Int^- cross. The two previous sentences may be expressed algebraically as a quantity here defined as Int activity:

$$\frac{c^+/c \quad Int^+ \text{ cross}}{c^+/c \quad Int^- \text{ cross}}$$

If Int activity is high, generalized recombination is weak; con-

Table II. Attenuation of Red recombination by the Mu *gam* activity on pJA21

Cross host	Cross temperature (°C)	Int activity[a]	Mu *gam* hotspot value[b]
ED206[pJA21]	40.5	5.8	6.0
ED206[pXY22Y]	40.5	0.96	
ED206[pJA21]	30.0	2.0	0.95
ED206[pXY228]	30.0	2.1	
594Δ(*rec*A−*srl*R)306::Tn10[pJA21]	40.5	15	13
594Δ(*rec*A−*srl*R)306::Tn10[pXY228]	40.5	1.2	
594Δ(*rec*A−*srl*R)306::Tn10[pJA21]	30.0	1.4	1.0
594Δ(*rec*A−*srl*R)306::Tn10[pXY228]	30.0	1.4	
FA77Δ(*rec*A−*srl*R)306::Tn10[pJA21]	41.0	18	4.4
FA77Δ(*rec*A−*srl*R)306::Tn10[pXY228]	41.0	4.1	
FA77Δ(*rec*A−*srl*R)306::Tn10[pJA21]	30.0	2.5	1.4
FA77Δ(*rec*A−*srl*R)306::Tn10[pXY228]	30.0	1.8	

[a] $\frac{\text{turbid/clear Int}^+ \text{ cross}}{\text{turbid/clear Int}^- \text{ cross}}$

[b] $\frac{\text{Int activity in pJA21}}{\text{Int activity in pXY228}}$

The Int$^+$ crosses are Jsus6 imm^{434} × imm^{434} cI Rts2.
The Int$^-$ crosses are Jsus6 int4 imm^{434} × imm^{434} cI Rts2.
Clears and turbids were scored on 594 after incubation at 37°C.

versely, if Int activity is equal to one, generalized recombination is strong (Stahl and Stahl, 1977).

The phage used in this study are all *imm*^{434}. *imm*^{434} phage are not affected by the λ cI repressor, which is expressed in cells harboring the Mu *gam* expression plasmid pJA21 or the control plasmid pXY228.

Crosses of the type outlined above were conducted in RecA$^-$ cultures at 30 and at 40°C. At 30°C, a temperature at which there is no expression from pL on these plasmids, pJA21- and pXY228-harboring cultures yielded similar results. At 40°C, a temperature that induces expression from the λpL promoter (and therefore of Mu *gam* in pJA21), crosses in cultures containing the Mu *gam* plasmid pJA21 show a higher Int activity than crosses in cultures containing the control plasmid pXY228. The effect of Mu *gam* on Int activity can be expressed as a single number by dividing the 40°C Int activity value for crosses in cultures harboring pJA21 by the Int activity value from the corresponding crosses conducted in parallel in a culture harboring pXY228. These values (Int activity as affected by Mu *gam*), shown in Table II, are between 4.4 and 12.5 for crosses conducted in various strains of *E. coli* at 40.5°C. These data imply that Mu *gam* expression from pJA21 attenuates Red-mediated λ recombination.

Full induction of Mu *gam* from pJA21 is harmful to cells that harbor the plasmid. A temperature of 42°C is required for a full replication block of P$^+$ phage in the *dnaBts* hosts used in this study. At 42°C we were unable to obtain phage yields from *dnaBts* hosts harboring pJA21. It was therefore possible that Mu *gam* inhibits Red only among phage that have replicated. In order to show unambiguously that Mu *gam* inhibits *cos*-stimulated Red recombination among unreplicated phage, we repeated the Int$^+$ and Int$^-$ crosses with density-labeled phage. When crosses are performed in unlabeled cells, replicated and unreplicated progeny can be distinguished via cesium formate density gradient centrifugation. The results of these crosses are shown in Figure 1. The left-most peak in each panel, which is the major fraction of the yield for all crosses, consists of genetically recombinant phage whose DNA was not replicated in the cross. The Int activity value, as affected by Mu *gam*, can be calculated from the unreplicated peak in these crosses. Such a calculation gives a value of 4. Thus Mu *gam* diminishes Red relative to Int recombination even among phage that have not replicated.

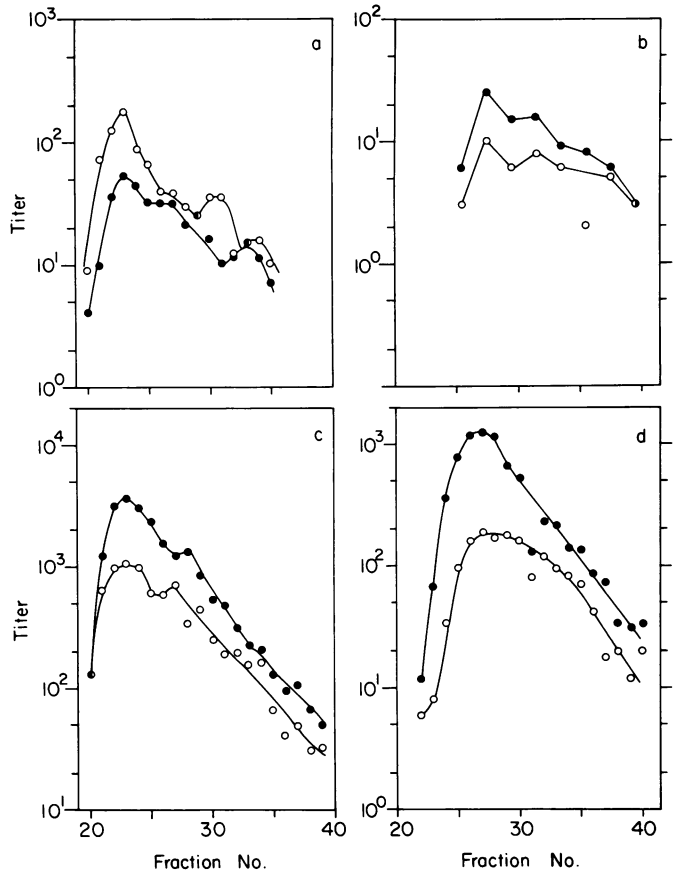

Fig. 1. (a) and **(b)**: density-labeled crosses conducted in FA77Δ(*rec*A−*srl*R)306::Tn10 [pJA21]; **(c)** and **(d)**: density-labeled crosses conducted in FA77Δ(*rec*A−*srl*R)306::Tn10 [pXY228]. The crosses are all *Jsus6 imm*^{434} × *imm*^{434} *cI Rts2*. In (a) and (c) the Int as well as the Red system is operative. In (b) and (d) both phage are Int$^-$ (int4). The yield of J^+R^+ recombinants was scored on plates seeded with 594 and incubated at 37°C. For (b) two fractions were pooled for each point. Open circles indicate the titer of turbid plaques, and closed circles indicate the titer of clear plaques.

The comparisons of Int versus generalized recombination cited above show that Mu *gam* diminishes the strength of Red-mediated recombination relative to the strength of Int-mediated recombination. The algebra is such that the same results would be obtained whether Mu *gam* decreases the absolute level of Red recombination or increases the absolute level of Int recombination. Two other types of experiments demonstrate that the *in vivo* expression of Mu *gam* results in an absolute depression in Red-mediated recombination. Table III shows the results of experiments in which Red recombination is used to recover wild-type information for λ's *D* gene from a plasmid resident in a cell in which Mu *gam* is, or is not, expressed. Mu *gam* has an approximately 10-fold effect, depressing the proportion of the phage yield that has picked up the D^+ allele from a plasmid resident in the cross host.

Another type of experiment that demonstrates Mu *gam* inhi-

Table III. Inhibition of marker rescue from a plasmid

Temperature (°C)	Mu *gam* plasmid	Proportion of yield that is D^+		
		$Red^+ Int^{+a}$	$Red^+ Int^{-a}$	$RED^- Int^{+b}$
30	pXY228	$(2.4 \pm 0.53)10^{-3}$	$(1.1 \pm 1.0)10^{-3}$	$<7 \times 10^{-6}$
30	pJA21	$(2.0 \pm 0.54)10^{-3}$	$(1.1 \pm 0.1)10^{-3}$	$<2 \times 10^{-6}$
38.5	pXY228	$(4.8 \pm 0.86)10^{-3}$	$(2.1 \pm 0.1)10^{-3}$	$<6 \times 10^{-6}$
38.5	pJA21	$(1.7 \pm 0.82)10^{-4}$	$(1.1 \pm 0.9)10^{-4}$	$<2.5 \times 10^{-6}$

[a]Average and standard deviation of two crosses.
[b]Upper limit of the 95% confidence interval (Poisson) based on the plaque count for the cross that gave the larger value.
One-step growth experiments were carried out in DH1[pXY228] [pLT11] and in DH1[pJA21] [pLT11] at 30° and at 38.5°C. The yield was plated on FS1585 for total phage and on 594Δ(*rec*A−*srl*R)306::Tn10 for D^+ recombinants. The $Red^+ Int^+$ phage is of the genotype Dsus123 imm^{434}. The Int^- phage is Dsus123 imm^{434}. The Red^- phage is Dsus123 red3 imm^{434}. Data are corrected for unadsorbed phage.

bition of Red is shown in Figure 2. In this approach two phage are crossed, each of which has a different deletion with one end point at the attachment site. Only Int can act to recombine these deletions. Phage that have recombined via Int have a unique density that can be used to purify the products of Int-mediated recombination. Among phage that have recombined via Int, which are purified on the basis of density, what proportion have also recombined via Red? The expression of Mu *gam* diminishes the proportion of Int-recombined phage that have also enjoyed generalized recombination. The strategy is illustrated in Figure 2. The experiment and its results are shown in Figure 3. In Figure 3 the left-most peak in each panel represents phage that have undergone Int-mediated recombination. Total phage titer across the gradient is shown in filled circles; titer for phage that have experienced generalized recombination is shown in open circles. The cross was conducted at 38.5°C, a temperature at which partial induction of Mu *gam* is expected from pJA21. This experiment allows the unqualified conclusion that the effect of the expression of Mu *gam* is to decrease Red-mediated — rather than to increase Int-mediated — recombination.

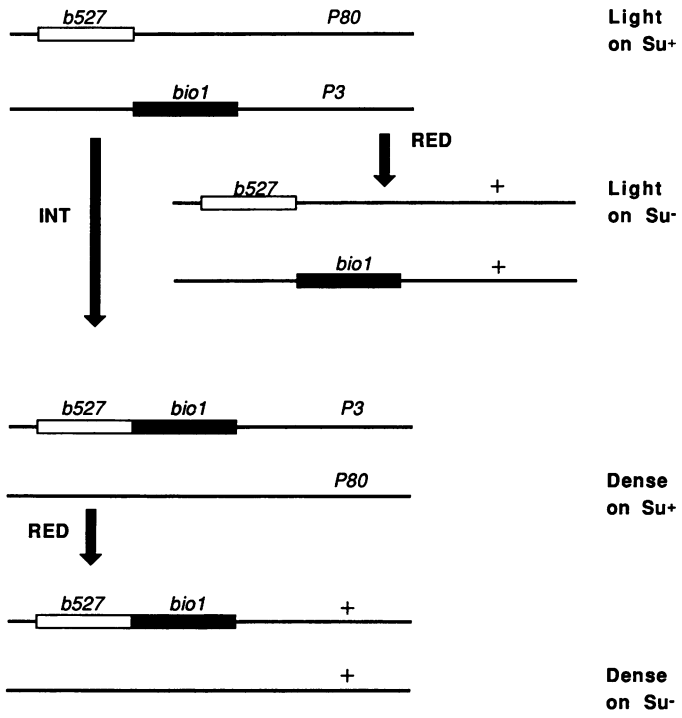

Fig. 2. An experimental strategy to isolate phage that have undergone Int-mediated recombination and to ask what proportion of these phage have also recombined via Red. bio1 and b527 are respectively a substitution with its left end abutting the λ attachment site and a deletion with its right end abutting the attachment site. The only homology in the region between bio1 and b527 is the attachment site core, ~12 bp. The core is the specific substrate for the Int system but is too small a region of homology to be acted upon by Red. bio1 is a net 5% deletion of DNA. Because the bio1 substitution covers the λ *red* and γ genes, λ bio1 phage are not able to form plaques on a $RecA^-$ lawn. When the gradient is plated on a $RecA^-$ host, the denser peak consists of phage that have recombined via Int to become wild-type in size. Only the Red system can recombine the two amber *P* alleles and generate a phage that can plate on a Su^- lawn. The ratio of titer on Su^- to Su^+ lawns of a *rec*A bacterium in the left (i.e. the denser) peak is a direct measure of the fraction of Int-mediated recombinants that have also undergone Red-mediated recombination.

Discussion

Previous work has shown that the Red pathway of genetic recombination is capable of acting as a DCBR pathway. The experimental evidence that Red is capable of acting as a DCBR pathway has come from two types of studies reviewed by Stahl (1986). The first line of evidence came from studies in which the distribution of recombinants along the length of the λ chromosome was scored under replication-blocked conditions in a *rec*A host. In these studies recombination was found to be focused at the right end of the standard λ map. The relevant feature at the right end of the λ map was demonstrated to be *cos* (the cohesive end site), i.e. the site at which the λ chromosome is linearized as part of the packaging reaction (Stahl *et al.*, 1982). The key role of *cos* was for some years interpreted in terms of a break-copy model of recombination (e.g. Stahl, 1979; but see Russo, 1973). According to the proposed break-copy mechanism, *cos* was a focus for recombination among phage recovered from a replication-blocked cross as the consequence of *cos* being the terminus of λ, i.e. the last site required to complete a viable phage chromosome. The break-copy model proposed that DNA synthesis from the point of recombination initiation out to the right end of a chromosome was required in order to complete a viable recombinant. In the break-copy model *cos* was proposed to act as the terminus, not the initiator, of events leading to recoverable recombinants.

The break-copy model predicted that a given *cos* would be a focus for recombination only if that *cos* could also be the terminus of a viable phage. This prediction was tested by asking if a *cos* that could be opened normally by terminase, but could not be the terminus of a viable recombinant, would also act as a recombination hotspot (Stahl *et al.*, 1985). It was found that a *cos* could stimulate Red recombination in its neighborhood even if the stimulating *cos* was forbidden from becoming the terminus of a viable recombinant. This finding led to the proposal that *cos* is a focus for Red recombination by virtue of being a double-chain-cut site and that the Red pathway would in general focus recombination to the tip of a DNA double helix.

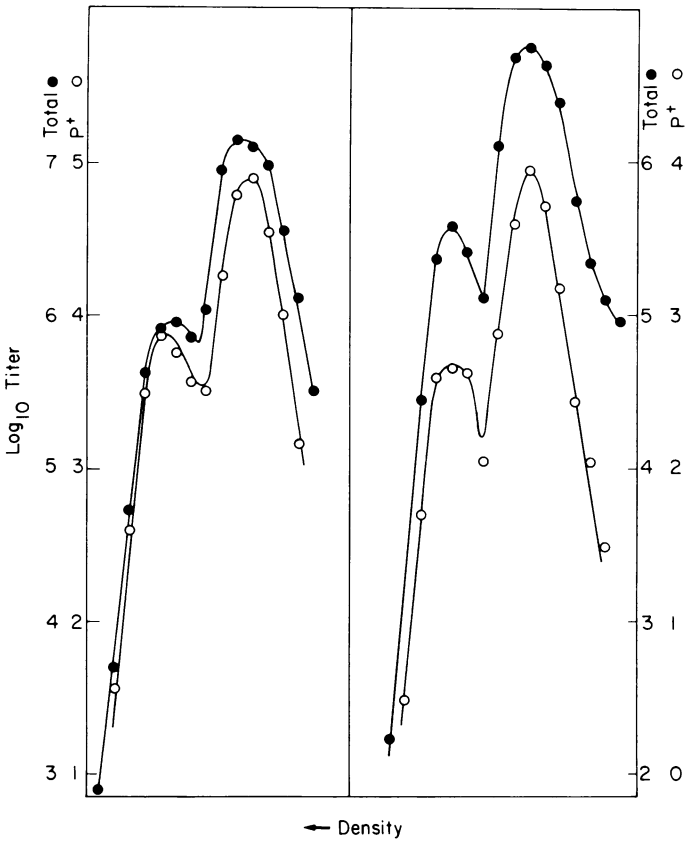

Fig. 3. Crosses to determine the proportion of those phage that have undergone Int-mediated recombination that have also undergone Red-mediated recombination. The phage cross is: bio1 imm^{434}*P*sus3 × b527imm^{434} cI *P*sus80. Cross **(a)** was carried out at 38.5°C in DH1[pXY228]; cross **(b)** was in DH1[pJA21]. The cross lysates were spun in cesium formate density gradients. The gradients were plated on C600*recA*56 (a Su$^+$ indicator, filled circles) and on 594Δ(*recA*−*srlR*)306::Tn10 (a Su$^-$ indicator, open circles).

A prediction of the DCBR model is that a double-chain end created by other means would also act as a focus for recombination. This prediction has been tested by asking if the site of action of a type II restriction enzyme becomes a focus for recombination mediated by Red (Thaler *et al.*, 1987). It has been demonstrated that type II restriction enzymes do indeed focus Red recombination to the site of their action. The restriction effect is general, i.e. restriction cuts at several locations in the λ chromosome are foci of recombination. A type II restriction cut is the focus for recombination whether or not replication is allowed; in contrast, *cos* is a detectable focus of recombination only among phage that have not replicated.

The distribution of recombinants among phage that have replicated is no longer focused at *cos*; it is distributed approximately evenly across the entire λ chromosome. Stahl *et al.* (1985) made an explicit proposal, in accordance with a DCBR mechanism for Red, for this replication-dependent recombination. The proposal made by Stahl *et al.* (1985) is that some aspect of replication creates double-chain ends that are randomly dispersed along the λ chromosome. The tips of rolling circles were suggested to be these replication-dependent double-chain ends. The results of the experiments reported in this paper offer support for the proposal of Stahl *et al.* (1985) by showing that the activity of Mu *gam* inhibits Red recombination whether replication is blocked or allowed. These results are consistent with the proposal that the normal initiator of replication-dependent Red recombination is the replication-dependent creation of double-chain ends.

The *in vivo* binding activities of Mu *gam* are the subject of some controversy. Williams and Radding (1981) reported that the exonuclease inhibitor isolated from induced Mu lysogens had a strong binding preference for linearized double-stranded DNA. On the other hand, Akroyd *et al.* (1986) found that Mu *gam* could bind to single-stranded and to covalently closed circular duplex DNA as well as to linear duplex DNA. Unfortunately, Akroyd *et al.* (1986) did not assay protection of any substrates other than linear duplex DNA. Akroyd and Symonds (1986) explored the exonuclease inhibiting activity of Mu *gam* and found that double-chain ends were protected equally well whether the ends had 3′ or 5′ protruding ends, flush ends, or had the 5′ terminal phosphate removed. All studies do agree that protection of DNA by Mu *gam* does not require any Mu sequences on the protected DNA, and that Mu *gam* protects from several types of exonucleases. Our interpretation that Mu *gam* inhibits Red recombination by blocking double-chain ends is valid to the extent that Mu *gam* binds specifically to double-chain ends *in vivo*.

We have made some further *in vivo* observations that bear on the issue of Mu *gam* specificity. The expression of Mu *gam* from pJA21 allows λ *red* γ (i.e. Fec$^-$) to plate with unit efficiency on a *rec*A host at 37°C (data not shown). Lambda must concatamerize in order to package. Concatamerization can be accomplished either by recombination or by rolling circle replication. We have found that λ *red* γ does not recombine in a RecA$^-$ host even in the presence of Mu *gam* (Table III). Therefore, pJA21 must allow for rolling circle replication of λ, presumably by shielding the terminus of a rolling circle.

Lambda phage that contain large palindromes do not form plaques on *recBCD*$^+$ bacteria (Leach and Stahl, 1983). We have found that the expression of Mu *gam* from pJA21 does not facilitate the plating of phage with palindromes that are ~300 bp on an arm. Phage that contain palindromes of this size are sensitive to *recBCD* function but do not require that the host be mutant in *sbc*B in order to form plaques (Thaler and Shurvinton, unpublished observations; Leach *et al.*, 1987). These observations are consistent with the following interpretation: Mu *gam* protects the linear duplex end of a rolling circle from attack by gp*recBCD*, yet Mu *gam* does not protect the substrate offered by a large palindrome from attack by the same enzyme. In contrast we have found that λ's γ function does allow phage containing the 2(300)-bp palindrome to form plaques on *recBCD*$^+$ hosts. It is understandable that λ's γ function should inhibit the effect of *recBCD* on all substrates because the action of λ's γ protein is to bind to, and inactivate, the enzyme. The finding that Mu *gam* protects some, but not all, substrates from gp*recBCD* is consistent with a substrate specificity for binding and protection by the product of the Mu *gam* gene.

The Mu *gam* experiments presented in this paper were all conducted with pJA21. pJA21 contains a piece of Mu DNA which is larger than the *gam* gene. It is conceivable that some other Mu DNA included in the pJA21 clone is responsible for some of the *in vivo* effects of pJA21. The work of Akroyd and Symonds (1986) makes it likely that Mu *gam* alone is responsible for all the properties of pJA21. Akroyd and Symonds (1986) found that maxicells containing pJA21, when induced, produced only one Mu-encoded polypeptide. Further, they found that all of the induced exonuclease inhibitor activity copurified with this single polypeptide. Subclones of pJA21 that include little DNA besides the sequenced Mu *gam* gene contain all of the exonuclease inhibitor properties of pJA21.

Previous work has shown that all Red recombination in replication-blocked crosses is initiated by double-chain ends. Mu *gam*

is a protein that binds to double-chain ends. Thus, inhibition of the replication-blocked Red reaction by Mu *gam* seems reasonable. Mu *gam* also inhibits Red when replication is allowed. We take this observation to indicate that double-chain ends play a key role in replication-allowed Red-mediated recombination. We explicitly acknowledge the possibility that some substrate other than double-chain ends is also being blocked by Mu *gam* and that inhibition of this unknown substrate is the actual reason for the inhibition of Red recombination by Mu *gam*. Whatever the hypothetical substrate is, it must not occur as part of Int recombination or of the lethal event experienced by palindromes in a *recBCD*$^+$ host. The results presented in this paper, and those of others, permit the conclusion that whatever else, if anything, Mu *gam* may be doing *in vivo*, the protein does act *in vivo* (as *in vitro*) to protect the double-stranded terminus of a DNA helix from exonuclease attack. Mu *gam*, or other proteins that bind double-chain ends *in vivo*, may be useful in other analyses.

Materials and methods

Phage strains were constructed and stocks grown by standard techniques (Arber *et al.*, 1983). Isotopic labeling of phage stocks and density gradient centrifugation have been recently described (Shurvinton *et al.*, 1987). The Mu *gam* plasmid pJA21 and the control plasmid pXY228 were maintained with 100 μg/ml of ampicillin and were grown at 26–30°C. The cross protocol was as follows: cultures were grown at low temperature (26–30°C) until they were in mid-log phase (~2 × 10^8/ml). The cultures were then split, and half was incubated at the elevated temperature required to induce Mu *gam* for 10 min before addition of the phage mix. Phage to be crossed were added at a multiplicity of infection of seven of each parent.

pLT11 was constructed for this study and is the *Ssp*I–*Sph*I fragment of λ, from 2212 to 8469 bp, cloned into the *Eco*RV and *Sph*I sites of pACYC184 (Chang and Cohen, 1978). pLT11 contains the wild-type *D* gene of λ. pACYC184 is of a different compatibility group and can be easily maintained in cells that also harbor the ColE1 based plasmids that are used in pJA21 and pXY228.

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