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Host/vector interactions which affect the viability of recombinant phage lambda clones

(Recombinant DNA; RecBC enzyme; *chi* site; *recD*; genomic libraries; palindromes; Spi$^-$ phenotype; *rec*$^+$-inhibited phenotype)

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SUMMARY

A class of recombinant phage λ clones are recovered from human genomic libraries on *Escherichia coli recB*21 *recC*22 *sbcB*15 cells, which fail to form plaques on wild-type cells. We report experiments which address the mechanism of this inhibition. The introduction of the recombination-stimulating sequence *chi* into one such clone allows growth of this phage on Rec$^+$ cells. In addition, the insertion of λ *gam*$^+$ gene into a *rec*$^+$-inhibited clone results in the ability of the phage to form plaques on wild-type cells. Since λ Gam protein is an inhibitor of host RecBC enzyme, we tested a collection of such phage for growth on a variety of hosts altered in RecBC function. Host permissiveness correlated with the inactivation of the RecBC nucleolytic activities and not with the recombinational activities. These observations suggest that the inserted DNA sequences of these phage limit the production of packageable chromosomes. This conclusion is easily reconciled with our current knowledge of the interaction of the host recombination systems with λ replication and encapsidation. Based on these experiments we have constructed strains, both recombination-proficient and recombination-deficient, which serve as improved hosts for the recovery of genomic sequences which are otherwise inhibitory to the growth of phage λ.

INTRODUCTION

Phage λ is intolerant of perfect inverted repetitions (palindromes; Leach and Stahl, 1983). Such phage will form plaques on *E. coli* hosts that are mutant in the *recB*, *recC* and *sbcB* genes, but will not form plaques on conventional hosts. This host specificity is also seen for a significant fraction of recombinant λ phages containing human DNA (Wyman et al., 1985), or *Physarum* DNA (Nader et al., 1985).

The products of the *E. coli recB* and *recC* genes are two subunits of a heteromultimeric enzyme called exonuclease V (Telander-Muskavitch and Linn, 1981; hereafter referred to as the RecBC enzyme). This enzyme acts in concert with the product of the *recA* gene to catalyze essential steps in the RecBC recombination pathway. The RecBC enzyme demonstrates several activities: ATP-dependent

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Abbreviations: bp, base pair(s); *chi*, an 8 bp sequence which stimulates homologous recombination (*chi*^{0} = the absence, *chi*$^+$ = the presence of *chi*); Exo I, exonuclease I; kb, kilobase(s) or 1000 bp; pfu, plaque-forming units; SDS, sodium dodecyl sulfate.

exonucleolytic degradation of single and double-stranded DNA, low-level endonucleolytic cleavage of single-stranded DNA, DNA-dependent ATP degradation, site-specific cutting at *chi* sequences (Ponticelli et al., 1985; Taylor et al., 1985), and DNA unwinding activity (Taylor and Smith, 1980). None of these activities are detected in strains with null mutations in either *recB* or *recC*, and such cells are recombination-deficient (Emmerson and Howard-Flanders, 1967; Low, 1968; Willetts and Mount, 1969).

The introduction of a mutation in the structural gene for exonuclease I (*sbcB*; Kushner et al., 1971) restores the Rec$^+$ phenotype to *recB* or *recC* mutant cells (Barbour et al., 1970). In addition to the *recA* gene product, recombination in *recB*21 *recC*22 *sbcB*15 cells requires several other gene products that are components of the RecF pathway, a DNA-damage-inducible recombination system (Lloyd et al., 1983). Mutations in *sbcB* might 'unmask' the RecF pathway by increasing the transcriptional activity of the *recF* gene, and possibly other LexA-regulated genes (e.g., *recA*; Karu and Belk, 1982). In addition, the absence of Exo I may stabilize a DNA structure that is utilized by or within the RecF pathway (Clark, 1973).

The growth of phage λ is dependent upon the formation of chromosome multimers since only molecules with at least two complete cohesive end sequences in direct repetition are used for DNA packaging (Feiss and Becker, 1983). During the course of normal growth multimeric λ chromosomes are produced by σ, or rolling circle replication, which is dependent upon the inhibition of the RecBC enzyme by the phage Gam$^+$ function (Enquist and Skalka, 1973). Therefore, phage that lack Gam$^+$ function must recombine their chromosomes to form the multimers required for DNA packaging. These phage make small plaques, unless they acquire the recombination stimulating sequence, *chi* (Lam et al., 1974). The *chi*$^+$ derivatives of *red*$^-$ *gam*$^-$ phage make larger plaques, indicating that the recombination frequency limits the production of packageable chromosomes.

The experiments reported here address the recovery and propagation of recombinant λ clones that do not form plaques on conventional hosts. Specifically, we attempt to: (1) determine the RecBC activities that are responsible for blocking growth of these phage; (2) question the role of *sbcB* mutations in host permissiveness; and (3) identify phage traits that are able to suppress the non-plaquing phenotype. This information is used to construct better hosts strains for the propagation of recombinant λ clones.

MATERIALS AND METHODS

(a) Bacteria and phage strains

The strains of *E. coli* and bacteriophage λ used in these experiments are listed in Table I. Bacterial strains were constructed using bacteriophage P1*vir* or P1*cam*, *clr*100-mediated transduction (Miller, 1972). Transductants of *argA*81::Tn*10* were screened for coinheritance of *recD*1014 by testing for sensitivity to phage T4 gene*2*amN51 as described (Chaudhury and Smith, 1984b). Transductants of *zjj*-202::Tn*10* were screened for coinheritance of *hsdR*2 by cross-streaking isolates against unmodified and modified λ phage. Putative *recA* mutant transductants were identified on the basis of their extreme sensitivity to ultraviolet light. Bacteriophage recombination was performed as described (Davis et al., 1980).

(b) Conditions for plating and propagation tests

Bacteriophage stocks were prepared from plate lysates (Maniatis et al., 1982) on *E. coli* CES200 or DB1257 using NZC agar plates [per liter: 5 g NaCl, 2 g $MgCl_2 \cdot 7H_2O$, 10 g NZ-amine (Kraft Inc., Memphis, TN), 1 g Bacto casamino acids (Difco; Detroit, MI), 11 g Bacto-agar]. Plating bacteria were prepared as described (Maniatis et al., 1982).

Host/phage combinations were tested for the ability to form plaques by preadsorbing phage (0.1 ml) at 37°C for 10 min to 0.1 ml of plating cells, adding 2.5 ml of λ soft agar (Davis et al., 1980), and overlaying onto an NZC agar plate. All incubations were at 37°C. In addition, a qualitative spot test was used to test the ability of phage to propagate on strains of interest. These were performed as follows; single plaques were removed from the plate with a micropipette and were soaked in 2 ml of SM buffer containing a drop of chloroform for 2 h, at which

TABLE I

Bacterial and phage strains

Designation	Genotype/properties	Source/reference
Escherichia coli		
594	F$^-$, λ^-, Lac$^-$, *galK*2, *galT*22, *rpsL*179	G. Smith
594(P2)	P2 lysogen of 594	F. Stahl
AB1157	F$^-$, λ^-, *thr*-1, *ara*-14, *Δ(gpt-proA)*62, *lacY*1, *tsx*-33, *supE*44, *galK*2, *hisG*4, *rfbD*1, *rpsL*31, *kdgK*51, *xyl*-5, *mtl*-1, *argE*3, *thi*-1, *leuB*6	B. Bachmann[a]
BNN45	F$^-$, λ^-, *thi*, *met*, *supE*44, *supF*, *hsdR*	Davis et al. (1980)
CES200	as AB1157 but also; *recB*21, *recC*22, *sbcB*15, *hsdR*	F. Stahl[b]
DB1154	*hsdR*2, *zjj*-202::Tn*10*, *supE*44	Lab strain
DB1170	F$^-$, λ^-, *end*, *recB*21, *recC*22, *sbcB*15, *hsdR*	Wyman et al. (1985)
DB1257	as CES200 but also; *supF*58	Lab strain[b]
DB1316	*recD*1014, *hsdR*2, *zjj*-202::Tn*10*	This work[b]
DB1317	*recD*1014, *hsdR*2, *zjj*-202::Tn*10*, *supF*58, *trp*-89::Tn*5*	This work[b]
DB1318	*recD*1014, *hsdR*2, *zjj*-202::Tn*10*, *recA*::*cam*^{R}	This work[b]
DB1319	*recD*1014, *hsdR*2, *zjj*-202::Tn*10*, *recA*::*cam*^{R}, *supF*58, *trp*-89::Tn*5*	This work[b]
DE294	*recA*730, *srlC*300::Tn*10*, *lexA*51, *sfiA*11, *mtl*-1, *rpsL*31, *Δ(lac-pro)*XIII	Ennis et al. (1985)
DM49	as AB1157 but also; *lexA*3	Little and Mount (1982)
JC9387	as AB1157 but also; *met*, *recB*21, *recC*22, *sbcB*15	J. Clark (via G. Smith)
MC1061	*araD*139, *Δ(ara-leu)*7697, *Δ(lac)*X74, *galU*$^-$, *galK*$^-$, *hsdR*$^-$, *rpsL*	M. Casadaban (1980)
MG1655	F$^-$, λ^-	B. Bachmann
S933	as 594 but also; *thy*, *recB*21	G. Smith
S972	as 594 but also; *recC*22	G. Smith
V186	*Δ(argA-thyA)*232, IN(*rrnD-rrnE*)1	Chaudhury and Smith (1984a)
V324	as 594 but also; *recD*1009	Chaudhury and Smith (1984b)
V355	as 594 but also; *recD*1014	Chaudhury and Smith (1984b)
V356	*Δ(argA-recB)*241, IN(*rrnD-rrnE*)1	Chaudhury and Smith (1984a)
V375	as 594 but also; *recC*1010	Chaudhury and Smith (1984b)
W3110	*thyA*36, *deoC*2, IN(*rrnD-rrnE*)1	B. Bachmann
WM1200	as MC1061 but also; *recB*21, *recC*22	B. Seed
WM1300	as MC1061 but also; *recB*21, *recC*22, *Δ(recA)*306::Tn*10*	B. Seed
Phage λ strains		
Charon 30	Cloning vector	Rimm et al. (1980)
684-4	Rec$^+$ non-plaquing Charon 30 derivative	Wyman et al. (1985)
EMBL3	Cloning vector	Frischauf et al. (1983)
MMS316	*int*4 *red*3 *gam*210 *c*I857 *R*5	M. Stahl
MMS405[c]	*chiC*151	M. Stahl
MMS529	*int*-4 *red*-3 *gam*-210 *c*I857 *R*5 *chiA*131	M. Stahl
MMS564[c]	*chiA*131	M. Stahl
Sus684-4	*A*(am) and/or *B*(am) derivative of λ684-4	This work
684-4RC	*gam*$^+$, *ral*$^+$, derivative of λ684-4	This work

[a] B. Bachmann, *E. coli* Genetic Stock Center, Department of Biology 255 OML, Yale University, P.O. Box 6666, New Haven, CT 06511-7444, U.S.A.

[b] Available upon request from the *E. coli* Genetic Stock Center.

[c] *chiC*151 and *chiA*131 indicate mutations conferring a Chi$^+$ phenotype.

time 0.01-ml aliquots were spotted onto fresh tester lawns. This yields different degrees of lawn clearing as a function of host/phage permissiveness. Plating for single plaques appears to be the more critical test: for example, phage which do not form plaques on a given host will cause slight clearing in the spot test (i.e., turbid spots).

(c) Phage DNA preparation

Phage lysates (35 ml), in a SW28 (Beckman Ultraclear) centrifuge tube, were underlaid with 3 ml of SM buffer containing 40% glycerol and centrifuged for 2.5 h at 120000 × *g*. The bacteriophage pellet was resuspended in 0.5 ml of SM buffer, pancreatic DNase I (5 μg) and RNase A (25 μg) were added and the mixture was incubated at 37°C for 20 min. Then Na · EDTA was added (50 μl of 0.5 M), and the mixture was incubated at 65°C for 5 min, at which time 10 μg of proteinase K and 25 μl of 10% SDS were added. After 15 additional min. at 65°C, the mixture received 100 μl of 3 M K · acetate, was mixed by inverting, and transferred to ice for 20 min. The mixtures were then cleared by centrifugation in a microfuge for 15 min. Isopropanol (0.6 ml) was added to the supernatant, mixed by inverting, and the flocculent precipitate was recovered with a fine glass rod. This DNA was washed in 1 ml of 70% ethanol and dissolved in 100 μl of TE buffer (Maniatis et al., 1980).

(d) In vitro constructions

All recombinant DNA manipulations were performed by standard procedures (Maniatis et al., 1982) using conditions prescribed by the manufacturer (New England Biolabs, Beverly, MA). The construction of the *Bam*HI/*Eco*RI library in λCharon 30 has been previously described (Wyman et al., 1985). The *Sau*3A library 685 was constructed as described elsewhere (Wyman et al., 1986). The products of phage constructions were packaged in vitro using commercial extracts (Promega Biotech, Madison, WI).

RESULTS AND DISCUSSION

(a) The nature of the *rec*$^+$ restriction of phage growth

Wyman et al. (1985) had previously studied several human recombinant clones in phage λ that form plaques on *recB*21 *recC*22 *sbcB*15 hosts, but are unable to plaque on *recB*$^+$ *recC*$^+$ *sbcB*$^+$ hosts (hereafter referred to as wild type). However, if wild-type cells are infected with these phage in liquid culture at low multiplicity of infection, one can detect a small amplification of phage (i.e., an average burst size of 10) as a consequence of a single round of infection. From such experiments one cannot distinguish between two interpretations: (1) all infected cells produce a small number of phage, or (2) a fraction of infected cells produce normal size phage bursts.

In order to determine the fraction of infected wild-type cells that release phage, we mixed a low number of infected wild-type cells with a 20-fold excess of *recB*21 *recC*22 *sbcB*15 cells, and plated the mixture for single plaques. The results of such experiments with five different *rec*$^+$-nonplaquing clones are shown in Table II. These results demonstrate that all or most infected wild-type cells do indeed produce progeny phage, but at burst sizes too low for plaque formation. Therefore, we will subsequently refer to clones that form plaques on *recB*21 *recC*22 *sbcB*15 hosts, but not on wild-type hosts, as *rec*$^+$-inhibited isolates.

TABLE II

Relative plating efficiencies and transmission of phage by *rec*$^+$ cells to *recB*$^-$ *recC*$^-$ *sbcB*$^-$ lawns

Phage[a]	Relative plating efficiencies[b] (Adsorb to / Plated on)		
	CES200 / CES200	BNN45 / BNN45	BNN45 / CES200
λ684-4	100	$<10^{-2}$	112
λ685-1-24	100	<0.5	148
λ685-1-30	100	<0.5	104
λ685-1-32	100	<0.5	78
λ685-1-45	100	<0.5	105

[a] See Table I.

[b] Phage were adsorbed (approx. 2×10^2 for CES200, or 1×10^4 for BNN45) for 30 min at 37°C to 1×10^7 cells in 0.2 ml. The adsorption mixtures then received 1×10^9 plating lawn cells, and the mixtures were plated as described in section **b** of MATERIALS AND METHODS. The number of pfu were corrected for unadsorbed phage, which was determined by treating an aliquot of the adsorption mixtures with chloroform before plating on CES200 lawns. These numbers were then normalized to the number of pfu under permissive conditions (adsorbed to CES200: plated on CES200) which was set to 100.

(b) *Chi* rescue of *rec*$^+$-inhibited isolates

As previously mentioned, most λ recombinant clones are incapable of rolling-circle replication on wild-type hosts, and are therefore dependent upon recombination for the production of packageable chromosomes. These considerations led us to test if the insertion of the recombination-stimulating sequence *chi* into such phage would increase their burst size sufficiently to allow plaque formation on wild-type cells.

In the first of such experiments we introduced *chi* into a previously characterized *rec*$^+$-inhibited phage (λ684-4; Wyman et al., 1985) by in vivo recombination. This was done by coinfecting CES200 with λ684-4 and one of several *chi*$^+$ *red*$^+$ *gam*$^+$ phage, and plating the progeny on wild-type cells lysogenized with bacteriophage P2. This strategy takes advantage of the inability of *red*$^+$ *gam*$^+$ phage to form plaques on P2 lysogens, thus selecting against the *chi*$^+$ parents (Table IIIA). Of course, this host also selected against λ684-4 (due to *rec*$^+$-inhibition). However, if *chi*$^+$ recombinants of λ684-4 were no longer *rec*$^+$-inhibited then they should form plaques on this strain. As can be seen (Table IIIB), λ684-4 recombinants were recovered when crosses were performed with any of several *chi*$^+$ phage (λEMBL3, λMMS405, or λMMS564), but not when crosses were made with *chi*^{0} phage (λCharon 30).

The results in the preceding section indicate derivatives of λ684-4 which arise by recombination with phage that contain *chi* are able to form plaques on wild-type cells. However, we were concerned that this result was not simply due to the general improvement in phage burst size enjoyed by any Spi$^-$ phage upon acquisition of *chi*, but instead that *chi*-stimulated recombination may have promoted the deletion or rearrangement of the inserted sequences. To test this possibility, we constructed *chi*$^+$A derivatives in vitro and directly tested the in vitro-packaged products for plating on wild-type cells. We began by constructing an *A*(am) *B*(am) derivative of λ684-4 (λsus684-4) by in vivo recombination with a λCharon 4A derivative on DB1257. Chromosomes from this amber phage were cut at the unique *Eco*RI site (at 21.5 kb) and religated in the presence of purified *A*$^+$ *B*$^+$ left-arm fragments bearing either

TABLE III

In vivo recombination of λ684-4 with assorted Spi$^+$ phage bearing *chi*^{0} or *chi*$^+$

Phage[a]	Phage properties			Relative plating efficiency[c]	
	Spi	*chi*	Rec$^+$[b]	*recB recC sbcB* host[d]	Rec$^+$(P2) host[e]
(A) Parents					
λ684-4	−	−	−	100	$<10^{-3}$
λCharon 30	+	−	+	100	$<10^{-3}$
λEMBL3	+	+	+	100	$<10^{-3}$
λMMS405	+	+	+	100	$<10^{-3}$
λMMS564	+	+	+	100	$<10^{-3}$
(B) Crosses					
λ684-4 × λCharon 30				100	$<10^{-3}$
λ684-4 × λEMBL3				100	2.6
λ684-4 × λMMS405				100	0.3
λ684-4 × λMMS564				100	0.3

[a] See Table I.

[b] Rec$^+$ refers to the ability to form plaques on Rec$^+$ cells (BNN45).

[c] Titer (pfu) of the lysates on the indicated host was normalized to the titer (pfu) on *recB*21 *recC*22 *sbcB*15 (set to 100).

[d] DB1170 for platings of λCharon 30 or λ684-4 × λEMBL3; CES200 for λMMS405 or λMMS564.

[e] 594(P2).

TABLE IV

In vitro replacement of the left arm of λsus684-4 with an arm containing either *chi*^{0} or *chi*$^+$*A*.

Left arm donor	Titer of packaging reactions for:		
	Total phage[a]	A^+B^+ phage on *recB*$^-$ *recC*$^-$ *sbcB*$^-$ host[b]	A^+B^+ phage on Rec$^+$ host[c]
None added	1.4×10^5	$<2 \times 10^2$	$<2 \times 10^2$
MMS316 (*chi*^{0}*A*)	2.0×10^5	4.6×10^4	3.5×10^3
MMS529 (*chi*$^+$*A*)	3.5×10^5	1.9×10^5	1.9×10^5

[a] DB1257 (*recB*21 *recC*22 *sbcB*15 *supF*58)
[b] CES200 (*recB*21 *recC*22 *sbcB*15)
[c] AB1157 (*recB*$^+$ *recC*$^+$ *sbcB*$^+$)

chi^0 (MMS316) or *chi*$^+$A (MMS529). It can be seen in Table IV that all A^+ B^+ recombinants that have acquired *chi*$^+$*A* do plate on wild-type cells as efficiently as they do on *recB*21 *recC*22 *sbcB*15 cells. Restriction endonuclease analysis of DNA from *chi*$^+$A recombinants after growth on wild-type cells indicated that the phage were not deleted or rearranged relative to the parent (limit of detection approx. 50 bp).

(c) Gam$^+$ rescue of λ684-4

The key difference in the growth of Spi$^-$ phages on *rec*$^+$ vs. *recB*21 *recC*22 cells is the inhibition of rolling-circle replication on the former host. For this reason we asked whether restoration of rolling circle replication would be sufficient to permit λ684-4 to plaque on *rec*$^+$ cells. If so, then problematic cloned sequences should be innocuous when borne on *gam*$^+$ phage, since Gam induces a RecBC$^-$ phenocopy (Enquist and Skalka, 1973; Friedman and Hayes, 1986) and allows rolling-circle replication. This was tested by construction of a *gam*$^+$ derivative of λ684-4 (λ684-4RC) that is colinear with λ684-4 except for the inclusion of 2.8 kb of λDNA to the right of the insert. This fragment bears the wild-type *gam*$^+$ and *ral*$^+$ genes, and places them in their normal position for expression from the p_L promoter. Table V shows that this phage plates with the same efficiency on wild-type cells as on *recB*21 *recC*22 *sbcB*15 cells, thus indicating that *gam*-mediated inhibition of RecBC enzyme is sufficient for the suppression of the *rec*$^+$-inhibited phenotype of λ684-4.

(d) Screening for permissive hosts

To assess the generality of these conclusions, we tested the ability of several independent *rec*$^+$-inhibited isolates to grow on a variety of mutant strains (Table VI). In general, mutations which inactivated RecBC enzyme were sufficient to allow for growth of these phage.

Strains containing an *sbcB*15 mutation in combination with *recB*21 and *recC*22 mutations (CES200 or JC9387), appeared to be better hosts than those lacking only RecBC enzyme activity (V186, V356,

TABLE V

Relative plating efficiency of λ684-4 and the *gam*$^+$ derivative λ684-4RC.[a]

Phage	Host strain		
	CES200	594	594(P2)
Wild type[b]	100	117	$<1 \times 10^{-3}$
MMS586	100	124	77
684-4	100	$<2 \times 10^{-3}$	$<2 \times 10^{-3}$
684-4RC	100	112	$<1 \times 10^{-3}$

[a] The number of pfu was normalized to the pfu number using CES200 as a host, which was set to 100.
[b] λ$^+$.

TABLE VI

Testing strain permissiveness with various Rec$^+$-inhibited isolates.

Parent mutant	Relevant genotype	Phage clones[a]												
		A	B	C	D	E	F	G	H	I	J	K	L	wt
AB1157	*recB*$^+$ *recC*$^+$ *sbcB*$^+$	−	−	−	±	±	±	±	+	−	−	+	+	+
CES200	*recB*21 *recC*22 *sbcB*15	+	+	+	+	+	+	+	+	+	±	+	+	+
JC9387	*recB*21 *recC*22 *sbcB*15	+	+	+	+	+	+	+	+	+	+	+	+	+
DM49	*lexA*3	−	−	−	−	−	−	±	−	−	−	±	±	+
DE294	*recA*730 *lexA*51	−	−	−	±	±	±	±	+	−	−	+	+	+
W3110	*recB*$^+$ *recC*$^+$ *sbcB*$^+$	−	−	−	−	±	±	±	±	−	−	±	+	+
V186	*Δ(argA-thyA)*232	±	−	±	+	+	±	+	+	+	−	+	±	+
V356	*Δ(argA-recB)*241	+	±	±	+	+	+	+	+	+	±	+	+	+
594	*recB*$^+$ *recC*$^+$ *sbcB*$^+$	−	−	−	±	±	±	±	±	−	−	±	±	+
S933	*recB*21	+	+	±	+	+	+	+	+	+	+	+	+	+
S972	*recC*22	±	±	±	+	+	±	+	+	±	±	+	+	+
V324	*recD*1009	+	+	+	+	+	+	+	+	+	+	+	+	+
V355	*recD*1014	+	+	+	+	+	+	+	+	+	+	+	+	+
V375	*recC*1010	+	+	+	+	+	+	+	+	+	+	+	+	+
MC1061	*recB*$^+$ *recC*$^+$ *sbcB*$^+$	−	−	−	−	−	−	±	−	−	−	−	−	+
WM1200	*recB*21 *recC*22	+	±	+	+	+	+	+	+	+	+	+	+	+
WM1300	*recB*21 *recC*22 *Δ(srlC-recA)*306	+	±	+	+	+	+	+	+	+	+	+	+	+

[a] Phage listed are as follows: A, λ684-4; B–L are 685 library isolates (Wyman et al., 1986). wt, λ$^+$.
(+), Complete spot clearing; (−), no clearing; (±), turbid spot (see MATERIALS AND METHODS, section **b**).

S933, or S972). Since *recB*21 *recC*22 *sbcB*15 cells are moderately derepressed for the SOS-regulated *recA* gene (Karu and Belk, 1982), we examined strains in which the expression of SOS activities is either constitutive (DE294), or non-inducible (DM49). These tests suggest that SOS expression contributes to host permissiveness, but that SOS derepression alone is insufficient.

Recently, Chaudhury and Smith (1984b) described a novel class of RecBC enzyme mutants. These strains lack detectable RecBC enzyme-dependent exonuclease activity and *chi*-stimulated recombination, but, unlike other RecBC mutants, remain recombination-proficient, do not segregate inviable daughter cells, and have normal growth rates. Further studies have shown that one class of these mutants, which were believed to have altered RecB function, actually identify a new gene (*recD*) that encodes a third subunit of the RecBC enzyme (Amundsen et al., 1986). These strains (V324, V355) proved to be exceptionally permissive for all *rec*$^+$-inhibited clones tested, as did a strain with a phenotypically similar *recC* mutation (V375).

(e) Conclusions

(1) Our results suggest that mutations that eliminate the RecBC-enzyme-mediated inhibition of rolling-circle replication result in hosts that are permissive for λ clones containing a class of inhibitory sequences. We conclude that some human nucleotide sequences limit the ability of Spi$^-$ λ phages to produce sufficient quantities of packageable chromosomes. This conclusion is supported by the observation that two distinct mechanisms, either of which increase chromosome multimer formation, permit plaque formation by these phages: (i) enhanced phage recombination via *chi*$^+$, or (ii) restoration of rolling-circle replication by Gam$^+$ function, or RecBC enzyme modification. This interpretation provides an understanding of the permissiveness of *recB*21 *recC*22 *sbcB*15 cells, since the RecBC enzyme normally blocks rolling-circle replication.

(2) The importance of the *sbcB*15 mutation is not yet clear. There are several mechanisms by which *sbcB*15 may improve host permissiveness. For example, these phage may be directly sensitive to the

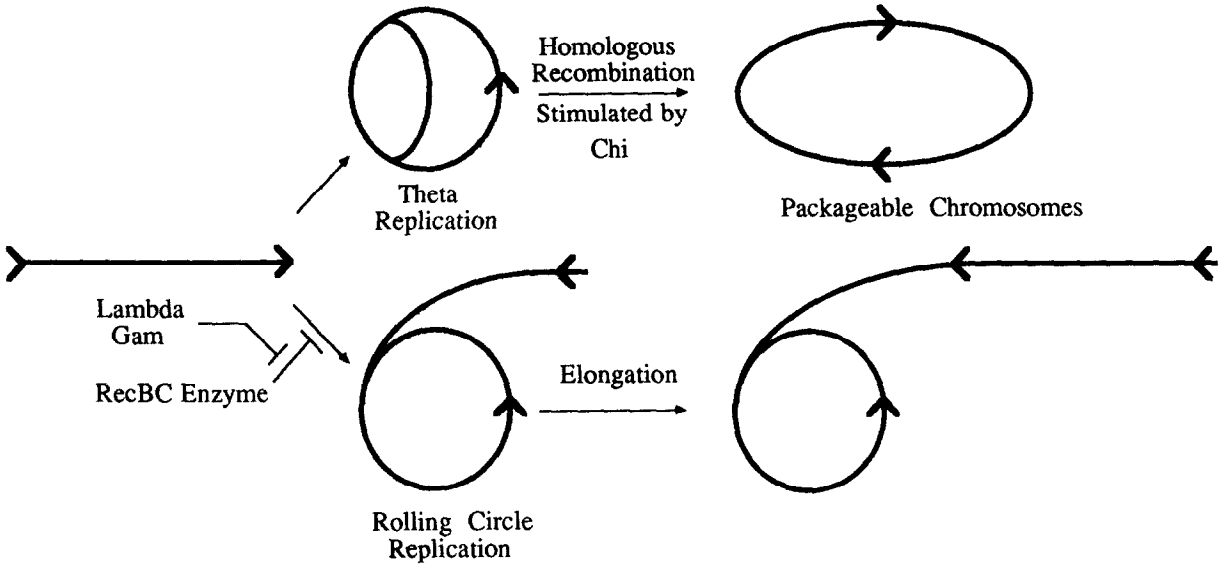

Fig. 1. Schematic representation of the alternate pathways for the formation of packageable λ chromosomes. Phage λ chromosomes are represented by heavy lines with arrowheads depicting the cohesive ends (*cos*). Cross bars indicate the inhibitory actions of Gam protein and RecBC enzyme.

activity of Exo I, or the improvement in phage growth may be due to the restoration of recombination proficiency and/or health of RecBC mutant cells by the *sbcB* mutation. Lloyd et al. (1984) have recently identified a second mutation (*sbcC*) in *sbcB*15 strains. Neither of these mutations alone is sufficient for suppression of *recB* or *recC* mutations, and their relative contributions to host permissiveness is currently unknown.

Further evidence that the *sbcB*15 mutation is of secondary importance is found in our studies of *recD*$^-$ hosts. The permissiveness of these strains indicates that RecBC-catalyzed recombination is not per se responsible for the *rec*$^+$-inhibition. This supports our contention that the primary action of RecBC enzyme is the elimination of rolling-circle replication. Consistent with this, *recD* mutations allow rolling-circle replication in the absence of Gam$^+$ function, as indicated by the ability of *red*$^-$ *gam*$^-$ phage to plaque on *recD recA* double mutants (K.F.W., unpublished observation). Based on this conclusion, we have constructed a set of host strains using the *recD*1014 allele (Table I): DB1316, DB1317, DB1318, and DB1319.

(3) Although our results do not directly address the mechanism by which the inserted sequences reduce the production of packaging substrate, we suspect they act as replication blocks, thereby reducing the production of monomer circles. A reduction in the concentration of monomeric chromosomes would be expected to greatly reduce the frequency of the bimolecular recombination event that is necessary for the formation of chromosome multimers by λ *gam*$^-$ phage. These considerations offer an explanation for an apparent contradiction between our results with *chi* and those of Nader et al. (1985), who found that a large fraction of *Physarum* clones in the *chi*$^+$ vector λEMBL3 failed to grow on wild-type cells. It is possible that the sequences encountered in *Physarum* libraries inhibit the synthesis of monomer circles so severely that *chi*-stimulated recombination is limited for substrate. Finally, evidence that palindromes do indeed create replication difficulties is provided by the results of Shurvinton et al. (1985), who have demonstrated a replication requirement for the deletion events associated with large palindromes in phage λ.

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