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Interaction Between the *sbcC* Gene of *Escherichia coli* and the *gam* Gene of Phage λ

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Manuscript received April 15, 1989
Accepted for publication July 7, 1989

ABSTRACT

gam mutants of phage λ carrying long palindromes fail to form plaques on wild-type *Escherichia coli* but do grow on strains that are mutant in the *sbcC* gene. gam^+ λ carrying the same palindrome grow on both hosts and on a host deleted for the *recB*, *C* and *D* genes. These results suggest that the Gam protein of λ, known to interact also with *E. coli*'s *recBCD* protein, can interact with the product of the *sbcC* gene.

THE Gam protein of phage λ influences both recombination and replication of the phage. Phage λ mutant in its viral recombination genes, *redα* and *redβ*, as well as for *gam*, recombines primarily by the RecBCD pathway of recombination. Expression of *gam* renders *Escherichia coli* phenotypically $recBC^-$ (UNGER, ECHOLS and CLARK 1972). Gam is also required for λ's transition from the theta to the sigma mode of replication (SKALKA 1977; GREENSTEIN and SKALKA 1975). This requirement is abolished in a *recBC* mutant (ENQUIST and SKALKA 1973). The inhibitory actions of Gam on RecBCD recombination and on the transition in modes of λ replication are probably due to a biochemical inhibition of the *E. coli* RecBCD enzyme by the λ Gam protein (KARU *et al.* 1975; UNGER and CLARK 1972).

Phage λ carrying a DNA sequence with palindromic symmetry grows poorly on wild-type strains of *E. coli*, relative to its growth on a *recBC sbcB* multiple mutant (LEACH and STAHL 1983). The absence of the *Exo*V (RecBCD) and *Exo*I (SbcB) enzymes in the *recBC sbcB* mutant was thought to stabilize palindromic sequences. LLOYD and BUCKMAN (1985) showed that a commonly used *recBC sbcB* strain, that allowed the efficient growth of the palindrome-containing phage, also contained an additional mutation, named *sbcC201*. The *sbcC* mutation is necessary to confer full expression of the RecF pathway recombination in a *recBC sbcB* mutant. CHALKER, LEACH and LLOYD (1988) showed that the *sbcC* mutation is necessary and sufficient for the plating of the palindrome-containing phage. This observation argues that the SbcC product rather than *Exo*V and/or *Exo*I is the key player in the killing of λ-carrying palindromic sequences. We have investigated the role of *gam* in the stable propagation of the palindrome-containing phage, and report evidence for interaction of the λ *gam* gene with the recombination-related *sbcC* gene.

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MATERIALS AND METHODS

Construction of λ*palS gam210 cI857*: A 1.1-kb fragment flanked by two *Sst*I sites was removed from within the *Eco*RI B segments of λDRL 32 (*Asus32 gam210 cI857*) and λMMS 999 (*B′ ΔC gam210 cII68 Psus80*) to yield DRL 32′ and MMS 999′, each containing a unique *Sst*I restriction site (Figure 1) (LEACH and STAHL 1983). DRL 32′ has a normally oriented, shrunken *Eco*RI B fragment (*delB*), whereas MMS 999′ has an inverted *delB*. High titer stocks of these two phages were prepared by plate lysates. The phages were concentrated by high speed centrifugation and then purified by banding in a CsCl equilibrium density gradient. The phage DNA was isolated by treatment with EDTA, SDS and phenol, followed by extensive dialysis against TE (10 mM Tris, 1 mM EDTA, pH 8.0). The DNA from DRL 32′ and MMS 999′ was cut with *SSt*I (Bethesda Research Laboratory) in a buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and 50 mM NaCl at 37° for 1 hr. The two digests were pooled, and segments were joined with T4 DNA ligase in 50 mM Tris (pH 7.4), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM spermidine, 1 mM ATP and 0.1 mM bovine serum albumin at 4° overnight. The ligated DNA was packaged *in vitro* as described by ROSENBERG (1987). The packaged phages were plated on JC9387 (Su^- *recBC sbcB sbcC*), and plaques were tested for the presence of the palindrome (*palS*) by the criterion of poor plating on a Su^- rec^+ $sbcC^+$ strain.

Construction of gam^+ and gam^- versions of *palS*: gam^+ and gam^- derivatives of λ *palS* were isolated from the progenies of standard crosses performed in a *recBC sbcB sbcC* (JC9387) cross host. gam^+ derivatives were made by crossing *palS* into a wild-type or an *int4 red3* background, while the gam^- derivatives were made by crossing *palS* into an *int4 red3 gam210* or a *b1453* background. *b1453* is a deletion that removes the *int*, *xis*, *redα*, *redβ* and *gam* genes (HENDERSON and WEIL 1975). The presence of the palindrome in the gam^- constructions was verified by the inability of the recombinant phage to plate on Su^- $sbcC^+$ (DL128) host. In the gam^+ derivatives, the presence of the palindrome was verified by crossing the candidates with λ containing

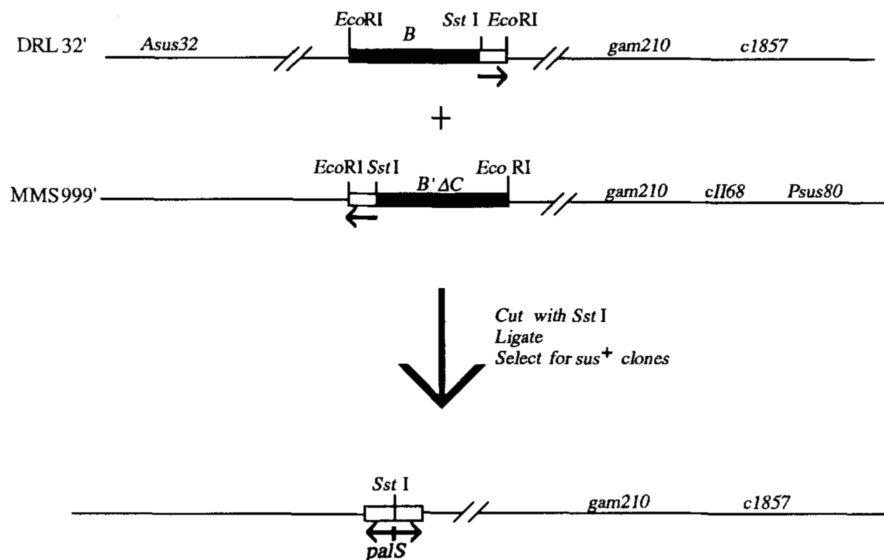

FIGURE 1.—A 1.1-kb fragment flanked by *Sst*I sites was removed from phage DRL 32 and MMS 999, resulting in phage DRL 32′ and MMS 999′. DRL 32′ has a normally oriented shrunken *Eco*RI B fragment, while MMS 999′ has an inverted B fragment. The *Sst*I restriction site divides the B fragment into 3600- and 227-bp fragments. After an *Sst*I digestion of each phage, the two phages were ligated together, *in vitro* packaged and plated on a Su^- lawn to select for sus^+ clones. These clones invariably contained a palindrome of length 2 × 227-bp, *palS*, which was verified genetically and by restriction analysis.

TABLE 1

***Escherichia coli* and phage λ strains**

Bacterial strains	Relevant genotype	Source
JC9387	*recB21 recC22 sbcB15 sbcC201*	A. J. CLARK
DL128	$recBC^+$ $sbcC^+$	D. R. F. LEACH
DL184	*recB21 recC22* $sbcC^+$	LEACH and STAHL (1983)
N2364	$recBC^+$ *sbcC201* (linked to Tn*10*) *supE44*	LLOYD and BUCKMAN (1985)
FS2657	$recBC^+$ *sbcC201* sup^+ (N2364 Su^-)	This study
FS2821	$recBC^+$ *sbcC201*	This study
FS2820	*recB21 recC22 sbcC201*	This study
AC113	Δ(*thyA recBCD argA*) $sbcC^+$	CHAUDHURY and SMITH (1984)
FS2837	Δ(*thyA recBCD argA*) *sbcC201*	This study

Phage strains	Genotype	Phage strains	Genotype
SKK1	*palS gam210 cI857*	SKK11	*delB b1453*
SKK3	*palS int4 red3 gam210*	SKK12	*delB int4 red3*
SKK8	*palS b1453*	MMS 664	*delB cI857*
SKK9	*palS b1453* χ^D	DRL 32	*Asus32 gam210 cI857*
SKK10	*palS int4 red3*	MMS 999	*B′ ΔC gam210 cII68 Psus80*
SKK15	*palS*	DRL 32′	*Asus32 gam210 cI857* shrunken B fragment
SKK14	*delB gam210*		
SKK6	*delB int4 red3 gam210*	MMS 999′	*B′ ΔC gam210 cII68 Psus80*, shrunken B′ fragment

See MATERIALS AND METHODS.

b1453 or *spi6*, another deletion removing the *red* and *gam* genes. Phages from the cross lysate were banded in a cesium formate gradient to separate populations according to their densities, which reflect the amount of DNA deleted. Fractions from the gradient were spotted on a palindrome-permissive host (JC9387) that gives large plaques for Red^- Gam^- λ. Putative *palS b1453* recombinants were isolated from a low density fraction and were tested for the palindrome by their inability to plate on a $sbcC^+$ host. All the *delB* phages were constructed by standard genetic crosses. The presence of *delB* was verified by comparison of the density of the phage with a reference phage, on a cesium formate density gradient. The genotypes of phages used in this paper are given in Table 1.

Physical verification of palindrome presence and stability: Stocks of the *palS gam210 cI857* phage were grown on JC9387. DNA was extracted and digested with either *Eag*I and *Sst*I, *Sca*I and *Sst*I, or *Eco*RI. The resulting fragments were separated on a 0.7% agarose gel and stained with EtBr. Presence of only the expected fragments and of the central *Sst*I site confirmed the presence of the palindrome and its stability on JC9387.

Construction and verification of bacterial strains: Unless otherwise specified, the strains used for the platings described in RESULTS are Su^- derivatives of *E. coli* AB1157 (TEMPLIN, MARGOSIAN and CLARK 1978). Isogenic sets of $sbcC^+$ and $sbcC^-$ strains were constructed following a standard P1 transduction protocol (A. F. TAYLOR, personal communication). P1 grown on *E. coli* FS2657, a Su^- derivative of N2364 which has Tn*10* linked to *sbcC201*, was used to transduce DL128, DL184 and AC113 to tetracycline resistance. Cotransductants of *sbcC201* (FS2821, FS2820, and FS2837, respectively) were identified by the ability of the strains to plate Gam^- palindrome phage. Strain AC113, which is in a 594 background, contains a deletion from *thyA* to *argA* which removes the *recB*, *recC* and *recD* genes (CHAU-

DHURY and SMITH 1984). The *recBCD* genotype of the deletion mutant and point mutant strains was tested by their ability to give equally large plaques when plating λ Red$^-$ Gam$^-$ χ^+ phage and λ Red$^-$ Gam$^-$ χ^0 phage and by their UV sensitivity.

Plating of phages: Phages were diluted to yield between 100 and 700 plaques per plate on a *recBC sbcB sbcC* strain. Plating cultures were grown to late log phase in tryptone broth supplemented with maltose (0.2%), 50 mM magnesium sulfate, thymine (0.01 mg/ml) and vitamin B1 (0.025 mg/ml). Subsequently, 0.25 ml of each culture was mixed with 0.1 ml of phage dilution. After 10 min the mixtures were pour-plated on trypticase (Baltimore Biological Laboratory) plates and incubated overnight at 34°.

RESULTS AND DISCUSSION

Phage λ carrying a 2 × 227-bp palindrome, *palS*, and an amber allele of *gam*, *gam210* (ZISSLER, SIGNER and SCHAFFER 1971), was constructed *in vitro* using the method of LEACH and STAHL (1983) (Figure 1). The palindrome, which has an *Sst*I restriction site at the center of symmetry, replaces the 4.9-kb long *Eco*RI B fragment of λ (from 21.2 to 26.1 kb; see DANIELS *et al.* 1983). The palindrome is stabily maintained, by the criterion of the retention of the central *Sst*I restriction site, upon growth of λ*palS gam210* on a *recBC sbcB sbcC* mutant strain. The *palS* phage also displays the highly diagnostic characteristic of λ carrying a large (> about 30 bp) palindrome, by plating at drastically reduced efficiencies on wild-type (nonsuppressing) host compared to a *recBC sbcB sbcC* mutant strain (see MATERIALS AND METHODS).

A *gam*$^+$ derivative of λ*palS gam210* was produced by standard genetic methods (see MATERIALS AND METHODS). Plating properties of λ*palS* and λ*palS gam210 cI857* were compared with *gam*$^+$ and *gam210* versions of λ deleted for the *Eco*RI B fragment (*delB*) (Table 2). All the strains used were Su$^-$ to silence the *gam* function. The titers of the *palS* and *delB* phages on the various hosts were normalized to their titers measured on the same day on the *recBC sbcB sbcC* strain. The effects of the palindrome are reported as the average values of normalized titers of the *palS* phage divided by the normalized titers of the control phage. The platings of each phage on each host were carried out six independent times.

The *delB* phages plated well on all hosts. The *gam*$^+$ *palS* phage, too, plated well on all hosts. The *gam210 palS* phage plated well only if the host was mutant for *sbcC*. We draw attention to the observation that the *gam210 palS* plates poorly on *recBC* hosts, while the *gam*$^+$ derivative of *palS* plates efficiently on the same host (lines 3 and 6, Table 2). The surprising result is that *gam* has a phenotype in a *recBC* mutant, even when that mutant is deleted for the *recB*, *C* and *D* genes. This suggests that *gam* is interacting with a gene other than *recBC*. The *sbcC* gene is a likely candidate for an interaction with *gam* since the *gam* phenotype in a *recBC* strain is abolished when the strain carries in addition an *sbcC* mutation. These data confirm the importance of the *sbcC* mutation for allowing the growth of λ palindrome phage. They confirm, also, that the classical target for Gam function, RecBC(D), does not hinder the growth of λ *pal* (CHALKER, LEACH and LLOYD 1988).

The data also show that mutations in λ's recombination functions Int and Red have no effect on the *gam*-dependent growth of the *palS* phage (Table 3). *palS* derivatives containing mutations in the λ recombination functions, Int (*int4*, GINGERY and ECHOLS 1967) and Red (*red3*, SIGNER and WEIL 1968), and a *b1453* deletion (HENDERSON and WEIL 1975) derivative which removes *int*, *xis*, *red*α, *red*β and *gam* (Table 1) were also tested. The palindrome-containing phages plated in the same *gam*-dependent manner in the absence or presence of the Int and Red functions. Thus, our data fail to reveal any requirement of λ's recombination functions for growth of the palindrome-containing phage except for the presence of Gam or the absence of SbcC functions.

The importance of Gam can also be evaluated by comparing the growth of *palS gam210 cI857* on suppressing and nonsuppressing hosts, λ *palS gam210 cI857* plates with unit efficiency on our isolate of the widely used Su$^+$ *rec*$^+$ strain, C600. It also plates better on suppressing versions of the *sbcC*$^+$ hosts used in this study, but the plaques are variable in both size and number (data not shown). We take this variability to mean that these Su$^+$ AB1157 derivatives do not fully suppress the *gam210* mutation.

TABLE 2

***gam*-dependent plating of the palindrome-containing phage**

Strain	Relevant genotype	e.o.p. of *pal* phage relative to *delB* phage	
		gam^{+a}	*gam210*^{a,b}
JC9387	*recBC* (*sbcB*) *sbcC*	1.00	1.00
DL128	*recBC*$^+$ *sbcC*$^+$	0.98 ± 0.14	$4.95 \times 10^{-3} \pm 7.00 \times 10^{-4}$
DL184	*recBC sbcC*$^+$	1.11 ± 0.13	$7.28 \times 10^{-3} \pm 2.80 \times 10^{-3}$
FS2821	*recBC*$^+$ *sbcC*	0.99 ± 0.16	0.94 ± 0.11
FS2820	*recBC sbcC*	1.00 ± 0.14	0.78 ± 0.10
AC113	Δ*recBC* *sbcC*$^+$	0.86 ± 0.33	$7.67 \times 10^{-3} \pm 4.25 \times 10^{-3}$
FS2837	Δ*recBC sbcC*	0.89 ± 0.39	0.63 ± 0.15

[a] Efficiencies of plating (e.o.p.) of the *palS* and *delB* phages were calculated by dividing the titers obtained on the above strains for each phage by titers obtained on JC9387. The e.o.p. of the *palS* phages was divided by the e.o.p. of the *delB* phages. The platings were carried out six independent times. The mean ratios are given above along with their standard deviations.

[b] The *palS gam210* and *delB gam*$^+$ phages also contain a *cI857* mutation. We omit this mutation in this table for the sake of clarity. The *cI857* mutation has no consequence on the *gam*-dependent nature of plating, since the *gam*-dependent plating is present at low temperature, when the *cI857* mutation is not expressed. Also, *palS int4 red3 gam210* showed the same *gam*-dependent plating property as *palS gam210 cI857* (Table 3).

TABLE 3

Lack of effect of Int and Red functions on the *gam*-dependent plating of palindrome-containing phages

		e.o.p. of *pal* phage relative to e.o.p. of *delB* phage			
Strain	Relevant genotype	*int4 red3 gam*$^+$	*int4 red3 gam210*	*b1453*	*b1453* χ^D
JC9387	*recBC (sbcB) sbcC*	1.00	1.00	1.00	1.00
DL128	*recBC*$^+$ *sbcC*$^+$	1.03	1.80×10^{-3}		6.00×10^{-3}
DL184	*recBC sbcC*$^+$	0.99	2.20×10^{-3}	8.00×10^{-4}	8.00×10^{-4}
FS2821	*recBC*$^+$ *sbcC*	1.21	0.50		0.91
FS2820	*recBC sbcC*	1.09	0.90	1.08	0.93
AC113	Δ*recBC sbcC*$^+$	0.87	1.92×10^{-3}	1.85×10^{-4}	1.14×10^{-3}
FS2837	Δ*recBC sbcC*	0.97	0.56	0.79	0.72

A χ sequence (STAHL, CRASEMANN and STAHL 1975) was crossed into *palS b1453* to increase plaque size on *recBC*$^+$ hosts. The χ had no effect on the plating properties of the palindrome-containing phage. The *palS b1453* χ^D titers are normalized to those for *delB b1453*. From 100 to 700 plaques were counted on each indicator shown above. e.o.p. = efficiency of plating.

LEACH, LINDSEY and OKELY (1987) observed a dependence on *gam* in the plating of palindrome-containing phage on a *rec*$^+$ host. CHALKER, LEACH and LLOYD (1988) noted that a Red$^-$ Gam$^-$ λ *pal* plated on an *sbcC* mutant bacterial strain. By demonstrating that an interaction of *gam* and *recBCD* is not involved in the plating properties of λ *palS*, our work suggests a direct interaction of *gam* and *sbcC* gene products. Alternatively, suppression of *sbcC*$^+$ by *gam*$^+$ may involve two different pathways of plaque formation, one activated by the presence of Gam and the other by the absence of SbcC.

λ cloning vectors carrying "hard-to-clone" DNA fragments have been shown to be dependent on Gam for efficient growth on *recBC*$^+$ *E. coli* (WERTMAN, WYMAN and BOTSTEIN 1986).

Hence, we conclude the mechanism of suppression of the *sbcC* gene by *gam* to be an interaction of the *sbcC* gene and the *gam* gene. An alternative hypothesis that does not require the direct interaction of *gam* and *sbcC* to explain the suppression proposes two different pathways of plaque formation, one activated by the presence of Gam and the other by the absence of SbcC. The conclusions of this hypothesis are also supported by our data and cannot be ruled out.

What is the physiological significance, if any, for the interaction of *gam* and *sbcC*? λ's *ori* contains several sequences with palindromic symmetry and, therefore, has potential to form secondary structures (FURTH and WICKNER 1983). Although we see no difference in plaque size of λ *gam210* on a Su$^-$ *sbcC* as compared to a Su$^-$ *sbcC*$^+$ host, under some conditions these structures may be substrates for a debilitating action of the *sbcC* gene product.

Our results suggest that the products of the *E. coli sbcC* and *recBCD* genes bind the same protein, Gam. Evidence from other studies suggest that the two bacterial proteins may also act on similar substrates. The survival of palindrome-carrying phage in *sbcC* mutant cells suggests that the SbcC product attacks palindromic DNA. SHURVINTON, STAHL and STAHL (1987) showed that λ carrying a large palindrome is at risk (in an *sbcC*$^+$ cell) only if that phage replicates its DNA (and see LEACH, LINDSEY and OKELY 1987). This argues that the action of the SbcC product on the palindrome takes place during replication. The product may attack hairpins arising when the complementary chains are separated for replication. A. F. TAYLOR and C. E. SHURVINTON (personal communication) have shown that purified RecBCD protein can cut cruciform DNA (symmetric hairpins resulting from extrusion of a palindrome) on linear λ *in vitro*. Thus it seems likely that SbcC and RecBCD both have domains that specifically recognize and cut DNA hairpins.

RILEY and ANILIONIS (1978) have suggested that *E. coli* has undergone two genome duplications resulting in homologous genes lying either one quarter or half way around *E. coli*'s circular chromosome relative to each other. The *sbcC* gene maps 180° from *recBCD* (LLOYD and BUCKMAN 1985), supporting the possibility that *sbcC* is homologous to one or more of the genes of the *recBCD* locus.

sbcC mutations were recognized as spontaneously arising suppressors of *recBC sbcB* mutants (LLOYD and BUCKMAN 1985). The acquisition of an *sbcC* mutation conferred upon these strains essentially wild-type rates of growth and genetic recombination and resistance to DNA damaging agents; *i.e.*, an *sbcC* mutation confers full RecF pathway activity upon a *recBC sbcB* mutant strain. RecBCD protein has been identified as an inhibitor of the RecF pathway of *E. coli* (LUISI-DELUCA, LOVETT and KOLODNER 1989). We suggest that the SbcC protein (assuming such exists) shares a similar inhibitory activity so that its removal fully opens the RecF pathway. THALER *et al.* (1989) suggested that RecBCD protein is antirecombinogenic until it loses its RecD subunit. We speculate that SbcC protein is functionally equivalent to (part of?) the RecBCD protein but, unlike that protein, is unable to be converted to a recombinase.

We are indebted to DAVID LEACH, ROBERT LLOYD, ANDY TAYLOR, MARY STAHL and JOHN CLARK for strains and to ANN HAGEMANN, BETH SAMPSON, JIM SAWITZKE, LYNN THOMASON, M-PASCALE DOUTRIAUX and IMRAN SIDDIQI for their encouragement and criticism. Comments from ANNA MARIE SKALKA, JOHN CLARK, DAVID LEACH and ROBERT LLOYD improved the manuscript. The work was supported by National Science Foundation grant PCM8409843 and National Institutes of Health (NIH) grant GM33677. S.K.K. was supported by NIH Training Grant GM07759. F.W.S. is American Cancer Society Research Professor of Molecular Genetics.

LITERATURE CITED

CHALKER, A., D. R. LEACH and R. G. LLOYD, 1988 *sbcC* mutations permit stable propagation of a long DNA palindrome in *Escherichia coli.* Gene **71:** 201–205.

CHAUDHURY, A. M., and G. R. SMITH, 1984 *Escherichia coli recBC* deletion mutants. J. Bacteriol. **160:** 788–791.

DANIELS, D. L., J. L. SCHROEDER, W. SZYBALSKI, F. SANGER and F. R. BLATTNER, 1983 A molecular map of coliphage lambda, pp. 469–517 in *Lambda II*, edited by R. HENDRIX, J. ROBERTS, F. STAHL and R. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

ENQUIST, L. W., and A. SKALKA, 1973 Replication of bacteriophage λ DNA dependent on the function of host and viral genes. I. Interaction of *red*, *gam* and *rec*. J. Mol. Biol. **75:** 185–212.

FURTH, M. E., and S. H. WICKNER, 1983 Lambda DNA replication, pp. 145–173 in *Lambda II*, edited by R. HENDRIX, J. ROBERTS, F. STAHL and R. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

GINGERY, R., and H. ECHOLS, 1967 Mutants in bacteriophage λ unable to integrate into the host chromosome. Proc. Natl. Acad. Sci. USA. **58:** 1507–1514.

GREENSTEIN, M., and A. SKALKA, 1975 Replication of bacteriophage λ DNA: *in vivo* studies of the interaction between the viral gamma protein and the host *recBC* DNAase. J. Mol. Biol. **97:** 543–559.

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

KARU, A. E., S. YOSHIYUKI, H. ECHOLS and S. LINN, 1975 The γ protein specified by bacteriophage λ. J. Biol. Chem. **250:** 7377–7387.

LEACH, D. R. F., J. LINDSEY and E. OKELY, 1987 Genome interactions which influence DNA palindrome mediated instability and inviability in *Escherichia coli.* J. Cell. Sci. Suppl. **7:** 33–40.

LEACH, D. R. F., and F. W. STAHL, 1983 Viability of λ phages carrying a perfect palindrome in the absence of recombination nucleases. Nature **305:** 448–451.

LLOYD, R. G., and C. BUCKMAN, 1985 Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K-12. J. Bacteriol. **164:** 836–844.

LUISI-DELUCA, C., S. T. LOVETT and R. D. KOLODNER, 1989 Genetic and physical analysis of plasmid recombination in *recB recC sbcB* and *recB recC sbcA* in *Escherichia coli* K12 mutants. Genetics **122:** 269–278.

RILEY, M., and A. ANILIONIS, 1978 Evolution of the bacterial genome. Annu. Rev. Microbiol. **32:** 519–560.

ROSENBERG, S. M., 1987 Improved *in vitro* packaging of λ DNA. Methods Enzymol. **153:** 95–103.

SHURVINTON, C. E., M. M. STAHL and F. W. STAHL, 1987 Large palindromes in the λ phage genome are preserved in a rec^+ host by inhibiting λ DNA replication. Proc. Natl. Acad. Sci. USA **84:** 1624–1628.

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

SKALKA, A. M., 1977 DNA replication-bacteriophage lambda. Curr. Top. Microbiol. Immunol. **78:** 201–237.

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

THALER, D. S., E. SAMPSON, I. SIDDIQI, S. M. ROSENBERG, F. W. STAHL and M. M. STAHL, 1988 A hypothesis: *chi*-activation of RecBCD enzyme involves removal of the RecD subunit, pp. 413–422 in *Mechanisms and Consequences of DNA Damage Processing*, edited by E. C. FRIEDBERG and P. C. HANAWALT. Alan R. Liss, New York.

TEMPLIN, A., L. MARGOSIAN and A. J. CLARK. 1978 Suppressibility of *recA*, *recB*, and *recC* mutations by nonsense suppressors. J. Bacteriol. **134:** 590–596.

UNGER, R. C., and A. J. CLARK, 1972 Interaction of the recombination pathways of bacteriophage λ and its host *Escherichia coli* K-12: effects on exonuclease V activity. J. Mol. Biol. **70:** 539–548.

UNGER, R. C., H. ECHOLS and A. J. CLARK, 1972 Interaction of the recombination pathways of bacteriophage λ and host *Escherichia coli*: effects on λ recombination. J. Mol. Biol. **70:** 531–537.

WERTMAN, K. F., A. R. WYMAN and D. BOTSTEIN, 1986 Host/vector interactions which affect the viability of recombinant phage λ clones. Gene **49:** 253–262.

ZISSLER, J., E. SIGNER and F. SCHAFFER, 1971 The role of recombination in the growth of bacteriophage λ: I. The gamma gene, pp. 455–468 in *The Bacteriophage Lambda*, edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Communicating editor: G. MOSIG