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Genetics

Indirect stimulation of genetic recombination

(DNA damage/UV irradiation/λ crosses/*uvrA* gene/*ssb* gene)

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ABSTRACT **Recombination between *lacZ* alleles in crosses of λ*lacZ*$_1^-$ × λ*lacZ*$_2^-$ and F$^-$ *lacZ*$_1^-$ × λ*lacZ*$_2^-$ in *Escherichia coli* (λ) can be stimulated manyfold by UV irradiation of one of the λ*lacZ* phages [Porter, R. D., McLaughlin, T. & Low, B. (1979) *Cold Spring Harbor Symp. Quant. Biol.* 43, 1043–1048]. Analogous stimulation has now been observed by coinfection of the cells by UV-irradiated λ phage which carries no *lac* region. This indirect stimulation is not dependent on induction of the SOS system. The bacterial *uvr* system can effectively remove the damages on the λ DNA which cause the indirect stimulation. Among a number of mutations tested, only *ssb-1* was found to cause a drastic decrease in the indirect stimulation. Indirect stimulation was caused only by using phage that had a region of homology with the recombining phage. The homologous region can be separated from the recombining region by an extended nonhomologous region (>7.9 × 10^3 base pairs). This implies that damages to the DNA molecule, which stimulate recombination, can be located very far from the recombining region of the molecule.**

In spite of considerable progress achieved lately in the study of genetic recombination in microorganisms, in general this process is still replete with mystery and puzzles. One of these puzzles is the well-known phenomenon of the stimulation of recombination by DNA damage. For example, it was shown that UV irradiation can increase considerably the frequency of recombination in many organisms (1). This phenomenon, which has been known for several decades, is still without a clear explanation. Two natural hypotheses for partial explanation of stimulation are: (*i*) the production of damage in the region of recombination, which can facilitate recombination by breakage and reunion, and (*ii*) induction of the bacterial SOS system that leads to excess synthesis of *recA* protein or other proteins (or both) which could be involved in recombination. However, our results show that, at least in our systems, stimulation of recombination by UV light can occur in the absence of either of these two conditions.

We have studied recombination in the *lacZ* gene by crossing λp*lacZ*$^-$ with either a different λp*lacZ*$^-$ or a chromosomal *lacZ*$^-$ in bacteria lysogenic for phage λ. In both systems, UV irradiation of a λp*lacZ*$^-$ phage prior to infection leads to substantial increase in its subsequent recombination (2). To our surprise we find similar stimulation if UV-irradiated λ$^+$ infects bacteria simultaneously with unirradiated recombining (i.e., λ*lacZ*$^-$) phages. This indirect stimulation can be observed only when the damaged phage has a region of homology with the recombining phage. However, this region of homology may be removed from the recombining region by an extended region [7.9 kilobases (kb)] of nonhomology. We denote this long-range stimulation process "teleactivation."

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MATERIALS AND METHODS

Bacteria and Bacteriophages. The bacteria and bacteriophages used are listed in Table 1. Phages λlacZ36, λlacZ118, and λlacZ813 are derivatives of λp*lac5* (5, 9). Hybrid phages hy1 and hy5 (6) were kindly provided by C. Radding. Phages λTn5 (7) and λTn5-*112* (8) were kindly provided by D. Berg. To obtain phage λlacZ118-φ80, phages λlacZ118 and hy1 were used in a mixed infection of strain KL627, at a multiplicity of infection (moi) of 5. The lysate was plated on KL627 (λ) and plaques of hybrid phage λlacZ118-φ80 which carried the *lacZ118* region were detected by staining plaques with *o*-nitrophenyl-β-D-galactopyranoside (5).

Irradiation with 254-nm UV Light. Phages were diluted to 6 × 10^9–1 × 10^{10} per ml in 0.01 or 0.1 M $MgSO_4$ and were exposed to germicidal UV light of 254-nm wavelength at 1 J/m^{2}sec. Unless otherwise stated, the dose was 70 J/m^2. This represents the dose given to phages (before infection) in all tables in which the symbol (UV) is shown after the name of the phage.

Recombination Systems. We used three systems of recombination between transducing phages, either with each other or with homologous regions on the bacterial chromosome. In all cases, a homoimmune resident prophage prevented replication and expression of phage functions. One system utilizes bacteria that are F$^-$ Δ*lac*(λcIind$^-$) (i.e., lysogenic for λcIind$^-$ which is noninducible by UV or UV-damaged DNA) infected with two transducing phages λ*lacZ*$^-$ which carry two different ochre mutations in *lacZ*. The efficiency of recombination is measured by the level of β-galactosidase (β-D-galactosidase; EC 3.2.1.23) found after 2–3 hr of incubation at 37°C after infection (2, 10). Earlier work showed that the mutations used do not complement each other (2, 10), and background levels of β-galactosidase activity found in control cultures lacking one of the λ*lac*$^-$ phages were 0.01 enzyme unit/ml or less. These background levels have been subtracted in calculating all of the values given in the tables. In the second system, F$^-$ *lacI*$^-$ *lacZ*$^-$(λcIind$^-$) bacteria are infected with phage λ*lacZ*$^-$ carrying a different ochre mutation. In this case recombination could be assayed either by measuring β-galactosidase levels as above or by measuring the frequency of Lac$^+$ colony formation. The reversion frequencies of the *lacZ*$^-$ mutations used here were all <10^{-8}. In the third system, F$^-$(λcIind$^-$) were infected by phage λTn5 or λTn5-*112* and we measured the frequency of production of kanamycin-resistant (Kan^R) colonies. If we infect a nonlysogenic strain with phage λTn5, the frequency of Kan^R colonies is ≤10^{-4} per infected cell due to transposition of the Tn5 into the bacterial chromosome. Phage λTn5-*112* carries the mutant transposon Tn5-*112* (8) which transposes at a frequency 1/50th that of Tn5 and produces correspondingly 1/50th as many Kan^R colonies after infection of our nonlysogenic bacteria. In contrast, when these phages are

Abbreviations: moi, multiplicity of infection; Kan^R, kanamycin-resistant; kb, kilobase(s).

Table 1. Bacterial and phage strains

Strain	Genotype*	Ref. or source†
Escherichia coli		
EG339	F⁻ *lacI3 lacZ813*$_{(oc)}$ *thyA*⁻ *deo*⁻ *ssb-1 str*⁻(λcIind⁻)	
EG340	As EG339 but *ssb*⁺	
EG350	F⁻ *lacI3 lacZ118 leu-6 metE70 cysJ43 lexA1 ara-14 xyl-5 mtl-1 supD*⁻ *nalA49 str-109*(λcIind⁻)	
EG357	As EG350 but *lex*⁺	
EG358	As EG350 but Δ(*pro-lac*)X111 *car-96*::Tn*10*	
EG359	As EG358 but *lex*⁺	
EG378	F⁻ Δ(*pro-lac*)X111 *thi-1 argE3 car-96*::Tn*10 his-4 galK2 mtl-1 xyl-5 str-31 supE44*(λcIind⁻)	
EG379	As EG378 but *uvrA6*	
EG384	As KL921 but (*ϕ*80)	
EG394	As EG378 but (*pro-lac*)⁺ *lacI3 lacZ813*$_{(oc)}$	
EG467	As KL931 but (*ϕ*80)	
KL627	F⁻ Δ(*pro-lac*)X111 *supC*⁻	
KL921	F⁻ Δ(*pro-lac*)X111 *thyA*⁻ *deo*⁻ *malE*⁻::Tn*10 str*⁻(λcIind⁻)	
KL922	As KL921 but *ssb-1*	
KL931	As KL921 but (*pro-lac*)⁺ *lacI3 lacZ813*$_{(oc)}$	
Phage		
λ	*cI857 S7*	2, 5
λlacZ36	plac5 - *lacZ36 cI857 S7*	R. Porter
λlacZ118	plac5 - *lacZ118 cI857 S7*	2, 3
λlacZ813	plac5 - *lacZ813*$_{(oc)}$ *cI857 S7*	2, 5
*ϕ*80		I. Herskowitz
hy1	*att*$_λ$ *int*$_λ$ *bet*$_{ϕ80}$ *imm*$_{ϕ80}$	6
hy5	*att*$_{ϕ80}$ *int*$_{ϕ80}$ *exo*$_{ϕ80}$ *bet*$_λ$ *imm*$_λ$	6
λlacZ118-*ϕ*80	*att*$_λ$ *int*$_λ$ *bet*$_{ϕ80}$ *imm*$_{ϕ80}$ plac5 - *lacZ118*	‡
λTn*5*	*b221 rex*::Tn*5 cI857*	D. Berg; ref. 7
λTn*5-112*	*b221 cIII*::Tn*5-112 cI857*	D. Berg; ref. 8
186		R. Calender
Plkc		J. Scott

* Genetic nomenclature is as in refs. 3 and 4.
† Strains whose sources are not shown are derived from the strains of Porter *et al.* (2, 5). Complete derivations are long in some cases and are available on request.
‡ See *Materials and Methods*.

used to infect lysogens [*E. coli* K-12 (λcIind⁻)], we find that the frequencies of formation of Kan^R colonies are the same for both phages (≥10^{-3}). Because, in this case, the frequency does not depend on transposition function, we assume that these frequencies are the frequencies of recombination between the λTn5 or λTn5-*112* phage and the λcIind⁻ prophage.

Cross Procedures. The recipient strain was grown to 1 × 10^8 cells per ml in "enriched 56/2 medium" (5) and then was centrifuged and resuspended in 1/10 vol of 0.01 M (or sometimes, 0.1 M) $MgSO_4$. In the same concentration of $MgSO_4$, phages then were added and adsorption was for 15 min at 37°C. This mixture was diluted 1:10 in prewarmed (37°C) enriched 56/2 medium and then was incubated with moderate shaking at 37°C. After 1 hr, cells were plated onto media to select for Lac⁺ or Kan^R recombinants. β-Galactosidase activity was assayed (2) after 2–3 hr. For Kan^R colony selection, cells were plated after 1 hr onto LB agar (11) which contained 30 μg of kanamycin per ml. The recombination frequency was calculated as the ratio of the titer of colonies that can grow on selective medium to the titer of infected cells.

RESULTS

Indirect Stimulation of Recombination: Its Level and Independence of the SOS System. UV irradiation of a transducing phage led to considerable increase in the efficiency with which it subsequently recombined (Table 2) in each of three systems under consideration. These results are consistent with earlier reports of UV stimulation in similar systems (12, 2). However, a great deal of stimulation could be seen even when UV-induced damage was introduced on phages that do not carry the recombining regions (Table 2). The maximum in the dose–response curve for this indirect stimulation is ≈70 J/m^2, which is the same dose as that for maximal direct stimulation (data not shown). The extent of indirect stimulation depends linearly on the moi of irradiated phage up to a moi of 15 (data not shown). At this moi, the extent of indirect stimulation can be >50% of the level of direct stimulation (however, observed with a lower moi) (Table 2 and below).

We observed indirect stimulation using three pairs of recombining *lacZ* markers (Table 3). The *lacZ118* mutation alters the 17th amino acid residue of the *lacZ* product (13) and *lacZ813* is ≈2,000 bases downstream from this (14). *lacZ36* is relatively close to *lacZ813* and lies in the same deletion interval (H125–H138) as does *lacZ813* (10, 11, 14). The amount of stimulation was approximately the same for all of these *lacZ*⁻ pairs, even though the distances between markers were different.

In λ phage–prophage crosses, treatment of the phage with 4,5′,8-trimethylpsoralen with 360-nm light increases recombination (15). Stimulation of recombination in our systems can be achieved also by similar treatment of the nonrecombining phage (data not shown).

The possibility of dependence of indirect stimulation on the bacterial SOS system was tested in two ways. The *lexA1* mutation, which prevents SOS induction (16, 17), was introduced into recipient bacteria, and it was found that most of the indirect stimulation was still observed (Table 4). For further confirmation of this observation, UV-irradiated nonhomologous phages (*ϕ*80, Plkc, or 186) were substituted for the UV-irradiated λ phage in experiments with corresponding prophages for immunity, and no UV stimulation of recombination was observed (Table 5 and data not shown). We conclude that (*i*) the induction of SOS does not play an essential role in the observed indirect stimulation of recombination and (*ii*) homology between the stimulating phage and the recombining phage is essential for stimulation.

DNA Damages That Cause Indirect Stimulation Can Be Removed by the Bacterial *uvr* System. It was found that infection by UV-damaged λ phage 30 min prior to infection by the recombining λ*lacZ*⁻ phages resulted in complete loss of indirect stimulation. However, there was no such loss when a *uvrA*⁻ strain was used (Table 6). Evidently, there is a bacterial system, dependent on *uvrA*⁺, which can remove, within 30 min, all damages that are necessary for indirect stimulation.

DNA Damage That Causes Recombination Stimulation Can Be Separated from the Recombining Region by an Extended Stretch of Nonhomologous DNA. Because UV irradiation of nonhomologous phages does not cause indirect stimulation (see above), one can suppose that UV-induced damage in the stimulating phage must interact with a homologous region of the recombining phage. Therefore, the question arises: how far away from the recombining region can the area of interaction with the stimulating phage be located? To answer this question we carried out stimulation experiments with hybrid λ-*ϕ*80 phages. In one set of experiments, strain EG467 [F⁻ *lacZ813*(λcIind⁻)] was infected simultaneously with λlacZ118 for recombination and also UV-irradiated hy5 for stimulation.

Table 2. Direct and indirect UV-induced stimulation of recombination in three different systems

Cross number	Cross and recipient strain	Phages	β-Galactosidase formation: Enzyme units/ml	β-Galactosidase formation: Stimulation factor	Lac⁺ colonies: Frequency	Lac⁺ colonies: Stimulation factor	Kan^R colonies: Frequency	Kan^R colonies: Stimulation factor
I	λlacZ118 × λlacZ813;	λlacZ118 + λlacZ813	4.0	1				
		λlacZ118 + λlacZ813 (UV)	340	85				
	KL921	λlacZ118 + λlacZ813 + λ	1.8	0.5				
		λlacZ118 + λlacZ813 + λ (UV)	160	40				
II	F^- *lacZ813* × λlacZ118;	λlacZ118	0.045	1	3.7×10^{-5}	1		
		λlacZ118 (UV)	1.9	42	5.0×10^{-4}	14		
	KL931	λlacZ118 + λ	0.048	1.0	5.0×10^{-5}	1.4		
		λlacZ118 + λ (UV)	0.460	10	1.4×10^{-4}	4		
III	F^-(λcIind⁻) × λTn*5-112*;	λTn*5-112*					1.5×10^{-3}	1
		λTn*5-112* (UV)					1.4×10^{-2}	9
	EG357	λTn*5-112* + λ					1.4×10^{-3}	0.9
		λTn*5-112* + λ (UV)					1.2×10^{-2}	7

moi for cross I is: λlacZ118, 2.5; λlacZ813, 2.5; λ, 10. moi for cross II is: λlacZ118, 1.2; λ, 15. moi for cross III is: λTn*5-112*, 5; λ, 10.

The hy5 phage consists of φ80 DNA, except for the region corresponding to the interval from 64.5 to 90.6 on the λ genome (6) (see Fig. 1*a*). Irradiation of this phage leads to approximately the same degree of indirect stimulation as seen with irradiated λ (Table 5). The closest junction of λ and φ80 DNA in hy5 (near *exo*) is located at least 7.9 kb away from the *lacZ* gene on λlacZ813. Therefore, we conclude that the region of interaction between the stimulating phage DNA and the recombining phage DNA can be located at least 7.9 kb from the region of recombination.

This conclusion was supported by a second set of experiments in which phage λlacZ813 was crossed with hybrid phage λlacZ118-φ80, which has the left part of the genome (genes *A* to *exo*) from λlacZ118 and the right portion (*exo* to *R*) from φ80 (see Fig. 1*b*). Recombination between these phages was stimulated to approximately the same extent by either λ or φ80 (Table 5). As in the above case, the closest junction of the λlacZ118 DNA with φ80 DNA in the λlacZ118-φ80 hybrid is at least 7.9 kb from the *lacZ* region. Thus, in this cross between two λ*lacZ*⁻ transducing phages, we come to the same conclusion as in the cross F^- $lacZ_1^-$ × λ$lacZ_2^-$ given above—i.e., the UV-induced stimulation site can be located at least 7.9 kb distant from the region of recombination.

Indirect Stimulation Depends on *ssb* Function. We have carried out a partial survey of dependence of indirect stimulation on various gene functions. Using λ*lac*⁻ × λ*lac*⁻ infection followed by β-galactosidase assay, we found no significant effect of the following single mutations (see ref. 3) on either the basal level of recombination or the degree of indirect stimulation: *recB21*, *sbcA8*, *uvrA6*, *polA1*, *gyrA43*, *gyrB* (ts). In a *recB21 sbcA8* double mutant, the basal level of β-galactosidase was 10 times higher than wild type but indirect stimulation caused the same factor increase as in the *rec*⁺ case. Using the Kan^R colony formation assay for recombination, we found no significant effect of the following mutations (see refs. 3, 18, and 19) on the basal level or indirect stimulation: *dinA1*, *dinB1*, *dinD1*, *dinE1*, *dinF4*, *mutD5*, *mutH34*, *mutL13*, *mutT1*, *mutU4*, *recF143*, *umuC36*, *ung-1*, or *xthA*. A *recB21* mutation led to a reduction to 1/10th of the basal recombination level with this system, but the degree of indirect stimulation above this basal level was as for the *rec*⁺ case (data not shown).

The only mutation that we found to have an effect on indirect stimulation was *ssb-1* (20). This mutation decreased drastically (to 1/8th of) the magnitude of indirect stimulation [by λ (UV)] in crosses of λlacZ118 and λlacZ813 in an *ssb-1* host (KL922) compared to an *ssb*⁺ host (KL921). In crosses of λlacZ118 with F^- *lacZ813*, the *ssb-1* mutation (strain EG339) eliminated completely the indirect stimulation. Recombination in these experiments was carried out at 43°C to inactivate *ssb* function (20).

Thus, the product of only one gene, *ssb* (referring to single-stranded DNA-binding protein), is implicated so far in indirect stimulation. A possible involvement for *recA* function in stimulation could not be checked because in our system we saw no recombination in a *recA*⁻ strain (even with additional *sbcA*⁻ or *sbcA*⁻ *recB*⁻ mutations) either with or without UV stimulation (data not shown).

Table 3. Independence of indirect stimulation on distance between recombining markers

Cross	Stimulating phage	β-Galactosidase formation: Enzyme units/ml	β-Galactosidase formation: Stimulation factor
λlacZ36 × λlacZ813	None	0.3	
	λ (UV)	6.0	20
λlacZ36 × λlacZ118	None	1.7	
	λ (UV)	33	19
λlacZ118 × λlacZ813	None	3.5	
	λ (UV)	60	17

moi: λlacZ36, 2.5; λlacZ118, 2.5; λlacZ813, 2.5; λ, 15. Recipient strain, EG384.

DISCUSSION

Our results show that the proficiency of recombination between two phages or between a phage and a homologous chromosomal region, under repressed conditions, is greatly increased if a UV-irradiated third phage that does not carry the recombining region is introduced simultaneously. This stimulation was observed only if the irradiated stimulating phage has a region of homology with the DNA of a recombining phage. Evidently, damage in the DNA of the stimulating phage can induce changes in homologous DNA of the recombining phage. One possible mechanism for such changes might involve initial steps in recombination (or, a complete recombination event). For example, local denaturation of the DNA of the stimulating phage might lead to interaction of one strand of the damaged duplex with the homologous region of the DNA of the recombining phage. With the help of branch migration (21, 22), changes in duplex DNA structure of the recombining phage might occur far from the initial site of interaction. However, branch migration cannot progress through even a small region of nonhomology, at least

Table 4. Effect of *lexA* on indirect stimulation

Cross number	Cross and recipient strain	*lex* genotype	Phages	β-Galactosidase formation		Kan^R colonies	
				Enzyme units/ml	Stimulation factor	Frequency	Stimulation factor
I	λlacZ118 × λlacZ813						
	EG359	*lex*$^+$	λlacZ118 + λlacZ813	1.0	1		
			λlacZ118 + λlacZ813 + λ (UV)	17	17		
	EG358	*lexA1*	λlacZ118 + λlacZ813	1.0	1		
			λlacZ118 + λlacZ813 + λ (UV)	12	12		
II	F$^-$ lacZ118 × λlacZ813						
	EG357	*lex*$^+$	λlacZ813	0.03	1		
			λlacZ813 + λ (UV)	0.28	9		
	EG350	*lexA1*	λlacZ813	0.02	1		
			λlacZ813 + λ (UV)	0.13	6		
III	F$^-$(λcIind$^-$) × λTn*5*						
	EG357	*lex*$^+$	λTn*5*			6×10^{-3}	1
			λTn*5* + λ (UV)			7×10^{-2}	12
	EG350	*lexA1*	λTn*5*			5×10^{-3}	1
			λTn*5* + λ (UV)			3×10^{-2}	6

moi for cross I is: λlacZ118, 2.5; λlacZ813, 2.5; λ, 10. moi for cross II is: λlacZ813, 5; λ, 15. moi for cross III is: λTn*5*, 5; λ, 5.

in vitro (23). Because of this, our results with hybrid phages, in which the regions of homology between the stimulating phage and the recombining phage are >7.9 kb from the recombining region, are probably not due to a mechanism that simply requires branch migration. This conclusion is unavoidable particularly for the system depicted in Fig. 1*a*. For this system, the region of homology between the UV-damaged phage and the *lac*-bearing phage is separated from the *lac* region by DNA that is not homologous to either the damaged phage DNA or the other (chromosomal) *lac*-bearing DNA.

In what other possible ways could UV-induced DNA damages affect recombination that occurs a great distance from the site of alteration resulting from the damage? One theoretically possible explanation is that the increase in recombination proficiency is due to a conformational change in the recombining DNA resulting from interaction with the damaged DNA. It was reported that recombination in *lacZ* occurs much more frequently between λ*lac* and F*lac* than between λ*lac* and the chromosome, although the higher recombination rate is *recB*-dependent (2). Conditions that repress fertility, and hence nicking activity of the F factor, lead to substantial loss of the ability of the F*lac* factor to undergo recombination (24). Therefore, it is possible that nicking of F in F*lac* leads to a conformational change and results in increased time spent in a relaxed or partially relaxed state. If unwinding of superhelical DNA can really cause increased recombination frequency, UV-induced stimulation of recombination might be correlated with a decrease in superhelical density of the molecule of recombining DNA in our system. A decrease in superhelicity of λ DNA after UV irradiation has been shown (25, 26). In a partial test of the possible role of superhelicity, we studied the effect of temperature-sensitive mutations of DNA gyrase [*gyrA43*(Ts) or *gyrB*(Ts)] (3) on recombination between two λ*lac* phages and recombination of λ*lac*$^-$ with F*lac*$^-$, with subsequent transfer of F*lac*$^+$ to a (Gyr$^+$) recipient strain at the nonpermissive temperature. No effects of *gyr*$^-$ were observed (data not shown).

A second possible general explanation for indirect induction stems from the existence of certain nucleotide sequences (χ sites) that can stimulate general recombination within ≈10 kb of their location. Here also, an intervening nonhomologous region does

Table 5. Indirect stimulation: hybrid phages

Cross number	Cross and recipient strain	Stimulating phage	β-Galactosidase formation					
			Exp. 1		Exp. 2		Exp. 3	
			Enzyme units/ml	Stimulation factor	Enzyme units/ml	Stimulation factor	Enzyme unit/ml	Stimulation factor
I	F$^-$ *lacZ813* ×	None	0.04	1	0.09	1	0.04	1
	λlacZ118;	hy5	0.05	1.2				
	EG467	hy5 (UV)	0.46	11	1.1	12	0.25	6
		λ	0.07	1.7				
		λ (UV)	0.43	11	1.0	11	0.5	12
		φ80					0.07	1.7
		φ80 (UV)					0.06	1.5
II	λlacZ813 ×	None	1.6	1	1.1	1		
	λlacZ118-φ80;	λ (UV)	33	20	11	10		
	EG384	φ80	2.0	1.2	1.6	1.4		
		φ80 (UV)	11	7	8.7	8		
	λlacZ813 × λlacZ118;	None	1.5	1				
	EG384	λ (UV)	24	16				
		φ80 (UV)	1.3	0.9				

moi for cross I is: λlacZ118, 1.2; hy5, 15; λ, 15; φ80, 15. moi for cross II is: λlacZ813, 2.5; λlacZ118-φ80, 2.5; λlacZ118, 2.5; λ, 15; φ80, 15.

Table 6. Role of *uvrA* in removing damages that cause indirect stimulation (cross λlacZ118 × λlacZ813)

Recipient strain	*uvr* genotype	Stimulating phage	β-Galactosidase formation: λ (UV) addition simultaneous with λlacZ118 and λlacZ813 — Enzyme units/ml	Stimulation factor	λ (UV) addition 30 min before λlacZ118 and λlacZ813* — Enzyme units/ml	Stimulation factor
EG378	*uvr*$^+$	None	0.66	1	0.51	1
		λ (UV)	7.8	12	0.30	0.6
EG379	*uvrA6*	None	0.42	1	0.48	1
		λ (UV)	13	31	13	27

moi: λlacZ118, 2.5; λlacZ813, 2.5; λ, 15.
* After absorption of λ (UV), cells were diluted 1:20 in enriched 56/2 medium, incubated 30 min at 37°C with aeration, and then concentrated 20 times and infected with λlacZ118 and λlacZ813.

not affect the stimulation (27, 28). To explain this stimulation it was suggested that χ sequences are recognized by a protein that operates at a rate-limiting step in the *recBC* pathway of *E. coli* and can slide away from its initial binding site before nicking the DNA (28, 29). By analogy, we can suggest that an enzyme that recognizes a damage site formed during indirect stimulation could migrate far from the site of interaction to a recombination region and act to initiate recombination. A third conceivable general scheme for indirect induction could involve interaction of a recombining DNA (e.g., *λlacZ*$^-$) with damaged DNA, followed by extensive degradation of the recombining DNA to a point where its end is homologous to the other (*lac*$^-$) recombining partner.

Two major aspects of the phenomenon of indirect stimulation of genetic recombination that we have described are apparent: (*i*) interaction of a damaged DNA molecule with a homologous undamaged one which makes the latter a more active recombination substrate and (*ii*) the induction of recombination events at sites far removed from these sites of interaction. Both of these processes could play an important role in any recombination system that involves damaged DNA.

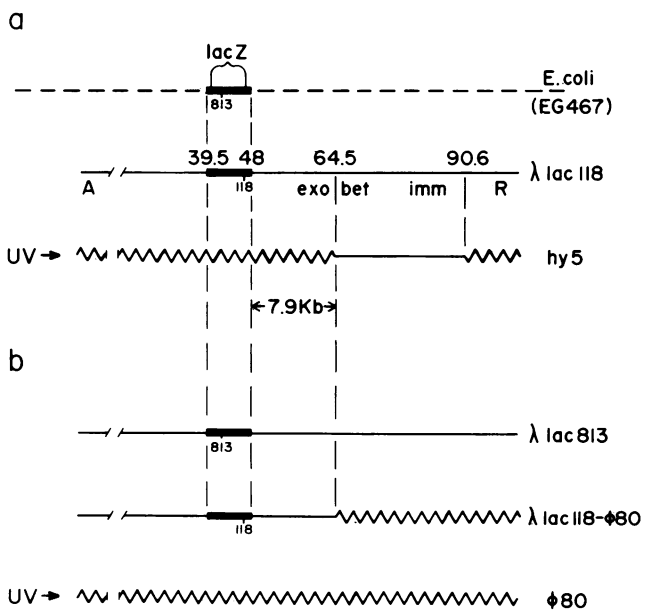

FIG. 1. Genetic maps and *lac* configuration for the two crosses involving hybrid phages. The numbers above the phage map are percentages of mature λ DNA length, starting at the A end (4). Regions —— and ^^^ depict portions of phage λ and φ80, respectively. The borders between λ and φ80 regions in hybrid phages are shown according to ref. 6, assuming that the borders in phage λlacZ118-φ80 correspond to the one in phage hy1 (6). Region --- depicts *E. coli* DNA and region ▬ depicts the portion of the *lac* operon that is included on the λ*plac5* phage used for these experiments (9).

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