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Indirect Stimulation of Recombination in *Escherichia coli* K-12: Dependence on *recJ*, *uvrA*, and *uvrD*

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Direct and indirect UV-stimulated homologous genetic recombination was investigated in *Escherichia coli* strains blocked in several host-encoded functions. Genetic recombination was assayed by measuring β-galactosidase produced after recombination between two noncomplementing *lacZ* ochre alleles. Both types of stimulation (direct and indirect) were found to be primarily RecF pathway-mediated. In a *rec*$^+$ background, both direct and indirect stimulation were found to be dependent on *uvrD* (coding for helicase II). In a *recB21 sbcB15* background, direct and indirect stimulation were *uvrD* dependent only when the strain was additionally deficient in the UvrABC excision repair pathway. Indirect but not direct stimulation was also dependent on *recJ* (coding for a 5′-to-3′ exonuclease specific for single-stranded DNA) regardless of *sbcA* or *sbcB* configuration. The methyl-directed mismatch repair system (*mutSLH*) also appeared to play an important role in stimulation. On the basis of these findings, we suggest that excision of UV-induced DNA damage is a prelude to UV-mediated stimulation of genetic recombination.

The stimulatory effect of UV irradiation and other forms of DNA damage on genetic recombination in various systems has been the subject of some study, but this phenomenon remains poorly understood (4). For example, it is not known what types of damage sites are most stimulatory nor what the nature of recombination intermediates is. Another complicating variable concerns the relationship between the location of the DNA damage site and the location of the ensuing recombination event. For example, Porter et al. (17) reported that recombination between λ*lacZ* with a noncomplementing *lacZ* mutation on the *Escherichia coli* chromosome (Fig. 1A) is greatly stimulated (10- to 50-fold) if the λ*lacZ* phage is UV irradiated before infection. Upon further analysis of this process, Golub and Low (7) found that even if the λ*lacZ* phage is not UV damaged but is coinfected with a second, irradiated phage (e.g., λ or a λ-φ80 hybrid), recombination between the chromosomal *lacZ* gene and the (unirradiated) λ*lacZ* phage is stimulated (Fig. 1B). This stimulation does not depend on a functional SOS system but does require some homology between the damaged phage (λ or λ-φ80) and the recombining phage (λ*lacZ*) (7). This type of stimulation, characterized by a stimulatory interaction at a site located far from the region of recombination, has been termed indirect stimulation or teleactivation (7).

In a somewhat analogous system in yeast cells, in vivo homologous recombination is stimulated between noncomplementing heteroalleles by the introduction of HO-mediated double-chain breaks in *trans* at distal sites (18). Though these results are not easily explained by conventional recombination theories, to explain the latter observation Ray et al. (18) have proposed a discontinuous heteroduplex recombination model in which extensive exonucleolytic degradation of the damaged molecule produces single-stranded regions which can, through distal gene conversion or mismatch repair followed by DNA synthesis, be repaired to yield a recombinant allele.

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Presumably, indirect stimulation involves at least two stages: interaction between the damage site and one of the recombining molecules and subsequent recombination between the two mutant alleles. In this study, we have continued the analysis of indirect stimulation by testing some of its functional requirements in F$^-$ *lacZ* × λ*lacZ* crosses and report the dependence of this process on elements of recombination (*recJ*), excision (*uvrABCD*), and methyl-directed mismatch repair (*mutSLH uvrD*) pathways.

MATERIALS AND METHODS

Bacterial and bacteriophage strains and media. Strains used in this study are listed in Table 1. P1-mediated transductions and conjugational matings were performed by standard techniques (15). All strains employed in recombination assays were F$^-$ *lacI lacZ813*(Oc) and were lysogenized with λ*c*Iind$^-$ to repress infecting-phage replication and recombination functions. Cultures were routinely grown on enriched 56/2 medium (17) supplemented with 0.5% glycerol. Phage stocks were prepared by thermal induction of appropriate lysogens (16), purified by equilibrium centrifugation through CsCl gradients (21), dialyzed, and diluted in TMG buffer (21) to working stock concentrations of 10^{10} to 10^{13} PFU/ml. For transductions and conjugations, antibiotics were employed at concentrations of 1.0, 15, 50, and 100 μg/ml for mitomycin, tetracycline, kanamycin, and rifampin, respectively.

Recombination assays. To measure recombination between two heteroalleles, we employed two mutant noncomplementing *lacZ* alleles as previously described (7). The *lacZ118* and *lacZ813*(Oc) alleles contain ochre mutations which revert at a very low rate. In addition, negligible complementation is observed between these alleles under the assay conditions used, allowing for sensitive determination of any transcribable recombination products. Two types of recombination could be measured by this system. Direct stimulation was assayed by infecting unirradiated *lacZ813*(Oc) *E. coli* cells with irradiated λ*lacZ118*. Indirect stimulation was assayed by infecting unirradiated *lacZ813*(Oc) *E. coli* cells with a mixture of unirradiated λ*lacZ118* and irradiated λ (which contains no *lac* DNA).

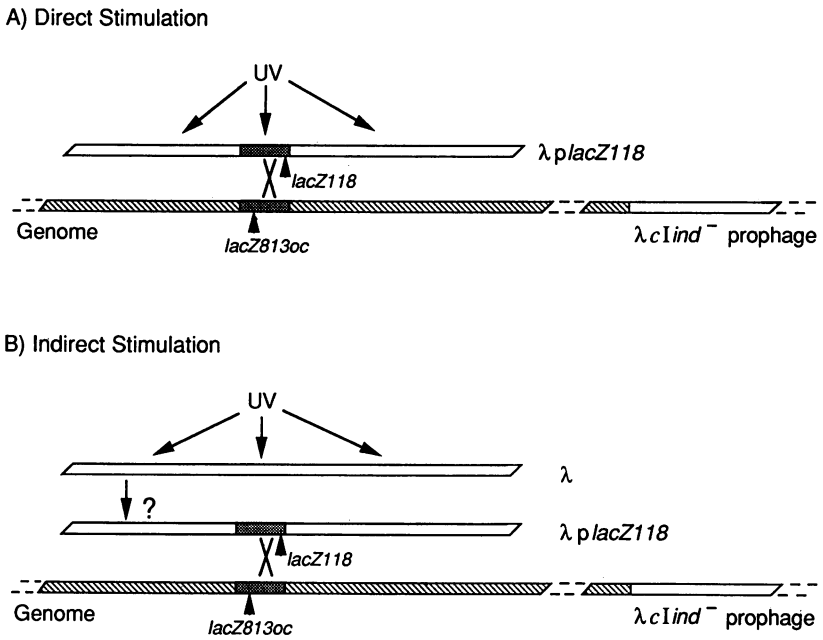

FIG. 1. Direct and indirect stimulation of genetic recombination by the repressed infection assay. The schematic indicates how each of the two types of recombination are thought to occur in the in vivo assays. In panel A, the UV irradiated λlac is a substrate for both DNA damage-processing enzymes and recombination mechanisms. In the case of indirect stimulation, these two steps are physically discontiguous, as shown. Because it is not known how UV damage stimulates recombination at distal sites, this step is denoted by a question mark. ▨, *E. coli* DNA; □, λ DNA; ▦, *lac* DNA.

UV irradiation procedures. To stimulate F⁻ *lacZ* × λ*lacZ* recombination, phage were irradiated at a dose of 70 J/m² unless indicated otherwise. This dose, producing the equivalent of approximately 50 thymine dimers per irradiated phage genome (8), has been previously shown to cause maximal stimulation in direct and indirect recombination (7).

Infection procedures. Except as indicated in figure legends, the following assay parameters were used. Overnight recipient cultures were diluted 1 in 50 into enriched 56/2 medium, grown to density of 10^8 cells per ml, harvested by centrifugation (6,000 × *g*, 10 min, 4°C), and resuspended in 1/10 volume of 10 mM $MgSO_4$. Recombining (λ*lacZ118*) and stimulating (λ) phage were added at multiplicities of infection of 3 and 10, respectively, unless indicated otherwise. After 15 min of adsorption at 37°C, the phage and cell mixture was diluted 10-fold with prewarmed enriched 56/2 medium and incubated with shaking at 37°C. After 2 h, the optical density at 590 nm was determined, the reaction volumes were centrifuged (10,000 × *g*), and the pellets were assayed for β-galactosidase activity. In some cases, aliquots were removed prior to β-galactosidase assay to determine the number of Lac⁺ recombinants per ml by dilution and plating on lactose minimal medium. Lac⁺ colonies were enumerated after 2 days of incubation at 37°C.

β-Galactosidase assays. β-Galactosidase activities were determined as previously described (2). Activities are normalized to an optical density of 1.0 (590 nm) and are expressed as enzyme units per ml. Uninfected control samples yielded background control values of 0.01 or less enzyme unit per ml which were subtracted from experimental values.

RESULTS

Dependence of UV-stimulated recombination on *recB*, *sbcB*, and *recJ*. UV-mediated direct stimulation of recombination in λ*lacZ* phage × F⁻ *lacZ* crosses has been shown to be mostly RecF pathway mediated (4, 8, 16). We examined indirect stimulation in several *recB21* derivatives to determine whether a functional *recB* gene (coding for part of exonuclease V) was required for this type of recombination. Consistent with earlier work (17), direct stimulation did not depend on exonuclease V (compare results for strains KL781 and KL782 in Table 2) and was, in fact, much higher in *recB21* strains which carried the *recB* suppressor *sbcA8* or *sbcB15*. A similar pattern was observed in the case of indirect recombination (Table 2), although the absolute levels of recombination were somewhat lower. The *sbcA8* allele activates transcription at *recE* coding for exonuclease VIII (turning on the RecE pathway), while *sbcB15* inactivates exonuclease I and turns on the RecF pathway (for a review, see reference 4). To confirm this apparent dependence of both direct and indirect stimulation on RecE and RecF pathway functions, a *recJ*::Tn*10* mutation was transduced (P1*vir* mediated) into the tested strains. Since the *recJ* gene product is required by both the RecE and RecF pathways for efficient conjugational recombination (12), a mutation in *recJ* might be expected to lead to a severe reduction in the amount of recombination observed in *recB sbcA* or *recB sbcB* strains. Substantial stimulated recombination, however, was found in the direct case for all tested *recJ* strains (i.e., when the infecting λ*lacZ118* phage was irradiated), while virtually no stimulation was observed in the indirect experiments (Table 2). In view of the known catalytic activity of RecJ (5′-to-3′ exonuclease specific for single-stranded DNA [13]), we wondered whether the indi-

TABLE 1. Bacterial and bacteriophage strains employed

Strain	Relevant markers	Other characteristics	Source or reference
Bacterial strains			
SK3451	*uvrD282*::Tn*5*	F⁻ *argH1 his-4 ilvD188 lacMS286* φ80d*lacZBK1*	14
ES1481	*mutS215*::Tn*10*	F⁻ *lacZ53*(Am) λ⁻ *thyA36 rha-5 met-1 deoC2* IN(*rrnD-rrnE*)*1*(?)	20
ES1484	*mutL218*::Tn*10*	F⁻ *lacZ53*(Am) λ⁻ *thyA36 rha-5 met-1 deoC2* IN(*rrnD-rrnE*)*1*(?)	20
JC12123	*recJ284*::Tn*10*	As AB1157	A. J. Clark via B. Bachmann
KL765	*rec*⁺	F⁻ *lacZ813*(Oc) *lacI3 his-29 metE70 pro-48 trpA605* λcIind⁻	KL318[a]
KL787	*rec*⁺	As KL765 but lysogenized with φ80	This work
KL788	*mutS215*::Tn*10*	As KL787	P1(ES1481) × KL787 → Tet[r]
KL789	*mutL218*::Tn*10*	As KL787	P1(ES1484) × KL787 → Tet[r]
KL790	*uvrD282*::Tn*5*	As KL787	P1(SK3451) × KL787 → Kan[r] (mitomycin[s] UV[s])
KL690	*sbcA8*	F⁻ *lacZ813*(Oc) *lacI22 his-29 metE70 pro-48 gyrA19* Str[r] λcIind⁻	KL318[a]
KL691	*recB21 sbcA8*	As KL690	KL318[a]
KL635	*recB21 sbcB15 sbcC250*	As KL690 but *his*⁺ *trpA605*	KL318[a]
KL781	*recB*⁺	As KL690 but *trpA605*	KL318[a]
KL782	*recB21*	As KL781	KL318[a]
KL784	*sbcA8 recJ284*::Tn*10*	As KL690	P1(JC12123) × KL690 → Tet[r] (mitomycin[s])
KL785	*recB21 sbcA8 recJ284*::Tn*10*	As KL691	P1(JC12123) × KL691 → Tet[r] (mitomycin[s])
KL786	*recB21 sbcB15 sbcC250 recJ284*::Tn*10*	As KL635	P1(JC12123) × KL635 → Tet[r] (mitomycin[s])
KL783	*recB*⁺ *recJ284*::Tn*10*	As KL781	P1(JC12123) × KL781 → Tet[r] (mitomycin[s])
KL794	*recB21 sbcB15 sbcC250 uvrA6*	As KL635	KL318[a]
KL791	*recB21 sbcB15 sbcC250 mutS215*::Tn*10*	As KL635	P1(ES1481) × KL635 → Tet[r]
KL792	*recB21 sbcB15 sbcC250 mutL218*::Tn*10*	As KL635	P1(ES1481) × KL635 → Tet[r]
KL795	*recB21 sbcB15 sbcC250 mutS215*::Tn*10 uvrA6*	As KL635	P1(ES1481) × KL794 → Tet[r]
KL796	*recB21 sbcB15 sbcC250 mutL218*::Tn*10 uvrA6*	As KL635	P1(ES1484) × KL794 → Tet[r]
KL793	*recB21 sbcB15 sbcC250 uvrD282*::Tn*5*	As KL635	P1(SK3451) × KL635 → Kan[r]
KL797	*recB21 sbcB15 sbcC250 uvrD282*::Tn*5 uvrA6*	As KL635	P1(SK3451) × KL794 → Kan[r]
Phage strains			
λ	*c*I857 *S7*		16, 17
λ*lacZ118*	*plac5-lacZ118 c*I857 *S7*		16, 17
λ*lacZ813*	*plac5-lacZ813*oc *c*I857 *S7*		16, 17
λ*lacZ*	*placZ c*I857 *S7*		16, 17

[a] These strains are derived from KL318 (2, 17). The *uvrA6* allele was introduced from AB1886 by cotransduction with *malE*::Tn*10*, followed by reversion of *malE* to regain Tet[s].

TABLE 2. Direct and indirect stimulation of recombination in *recB*⁺ and *recB21* strains of *E. coli*[a]

Strain	Relevant genotype	β-Galactosidase (U/ml)[b]					
		Direct stimulation			Indirect stimulation		
		No UV	+UV	Ratio	No UV	+UV	Ratio
KL781	*rec*⁺	0.09 (0.01)	5.06 (0.97)	58.0	0.10 (0.02)	0.45 (0.08)	4.5
KL690	*sbcA8*	0.09 (0.01)	4.18 (1.16)	48.9	0.05 (0.01)	0.28 (0.02)	5.2
KL691	*recB21 sbcA8*	0.38 (0.01)	12.6 (2.4)	33.4	0.29 (0.07)	1.37 (0.45)	4.7
KL782	*recB21*	0.08 (0.02)	4.91 (1.13)	64.1	0.07 (0.02)	0.45 (0.08)	6.8
KL635	*recB21 sbcB15 sbcC250*	1.49 (0.26)	32.8 (2.6)	22.1	0.47 (0.25)	3.51 (0.37)	7.5
KL783	*recJ284*	0.07 (0.01)	1.50 (0.26)	21.4	0.05 (0.02)	0.06 (0.02)	1.1
KL784	*sbcA8 recJ284*	0.08 (0.02)	1.26 (0.21)	16.7	0.07 (0.01)	0.10 (0.02)	1.4
KL785	*recB21 sbcA8 recJ284*	0.13 (0.04)	1.33 (0.25)	10.4	0.10 (0.02)	0.10 (0.01)	1.0
KL786	*recB21 sbcB-15 sbcC250 recJ284*	0.04 (0.01)	1.16 (0.42)	30.4	0.04 (0.01)	0.06 (0.01)	1.5

[a] Cultures were grown and assayed for both direct and indirect stimulation as described in Materials and Methods. The multiplicities of infection of the recombining (λ*placZ118*) and stimulating (λ) phage were 3 and 10, respectively.

[b] Each value is the average from three independent experiments, with the standard error of the mean indicated in parentheses.

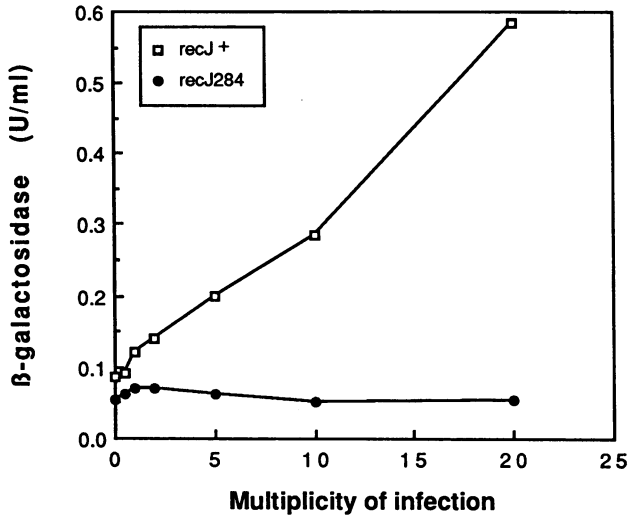

FIG. 2. Dependence of indirect stimulation of genetic recombination on *recJ*. Strains KL781 (*recJ*$^+$) and KL783 (*recJ284*) were infected with λ*lacZ118* and λ (UV) as described in Materials and Methods. Activities were corrected for low levels of β-galactosidase activity observed in samples incubated without added λ phage (see Materials and Methods).

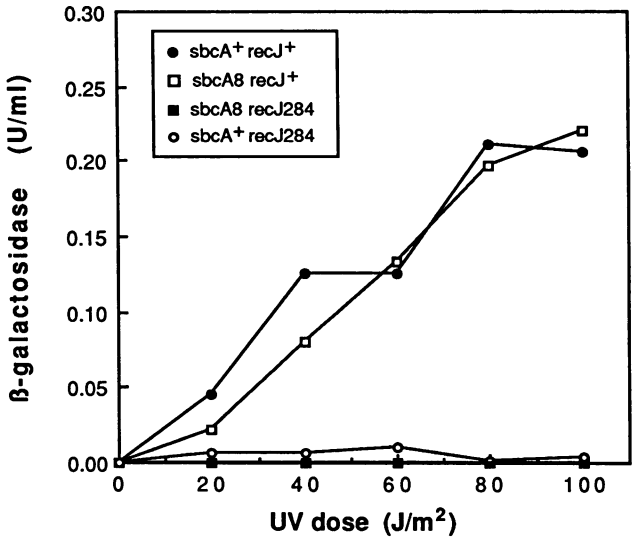

FIG. 3. Dependence of indirect stimulation on *recJ* as a function of UV dose. Strains KL781 (*sbcA*$^+$ *recJ*$^+$), KL690 (*sbcA8 recJ*$^+$), KL783 (*sbcA*$^+$ *recJ284*), and KL784 (*sbcA8 recJ284*) were grown and assayed as described in Materials and Methods. The multiplicities of infection of recombining (λ*lacZ118*) and stimulating [λ(UV)] phage were 3 and 10, respectively. Samples were corrected for basal recombination observed in samples containing unirradiated λ*lacZ118* phage.

rect-stimulation deficiency in the *recJ* mutants could be partially suppressed by the presence of another intracellular nuclease, exonuclease VIII, the product of the *recE* gene. To test this, *recB21 sbcA*$^+$ and *recB21 sbcA8* strains were tested with a constant multiplicity of infection of recombining phage and with various amounts of UV-irradiated stimulating phage. Results indicate that indirect stimulation is linearly dependent on the multiplicity of added stimulating phage (Fig. 2) and, more importantly, that *sbcA8* does not suppress the lack of indirect stimulation in a *recJ284* background (Fig. 3).

Dependence of UV-stimulated recombination on mismatch repair functions. Because of the nature of the damage produced by UV light (thymine dimers and 6,4-photolesions, etc.) (22), it seemed plausible that DNA repair functions such as mismatch and excision repair enzymes might play an important role in UV-stimulated recombination. For example, *uvrD* is hyperrecombinogenic (6, 25) and hypermutagenic (6) and plays an important role in removing incised oligomers at DNA damage sites during both methyl-directed mismatch repair and UvrABC-mediated excision repair (3, 23). Insertion mutations in genes for several mismatch functions (*mutS215*, *mutL218*, and *uvrD282*) were transduced into a *rec*$^+$ background and assayed for both directly and indirectly stimulated recombination. In direct-stimulation assays, strains KL788 (*mutS215*) and KL789 (*mutL218*) were found to be hyperrecombinogenic with respect to strain KL787 (*mut*$^+$) (Fig. 4 and Table 3). However, no detectable UV-stimulated recombination (above unstimulated levels) was observed in strain KL790 (*uvrD282*) in either direct (Fig. 4) or indirect (Fig. 5) assays. This striking result was somewhat surprising because mutations in *uvrD* are among the most hyperrecombinogenic alleles known (6, 25). Indeed, the unstimulated level of recombination observed in strain KL790 (*uvrD282*) was usually severalfold higher than that of the parental strain KL787 (*uvrD*$^+$) (Table 3). However, this higher basal level did not increase even when the stimulating phage was heavily irradiated (500 J/m^2) (data not shown). Samples of these infected cells were also plated on lactose minimal plates to measure viable recombinant formation. In these assays, UV irradiation of the λ*lacZ118* phage increased the number of Lac$^+$ recombinants obtained 10- and 5-fold in direct and indirect-stimulation experiments, respectively, performed with strain KL787 (*uvrD*$^+$), while

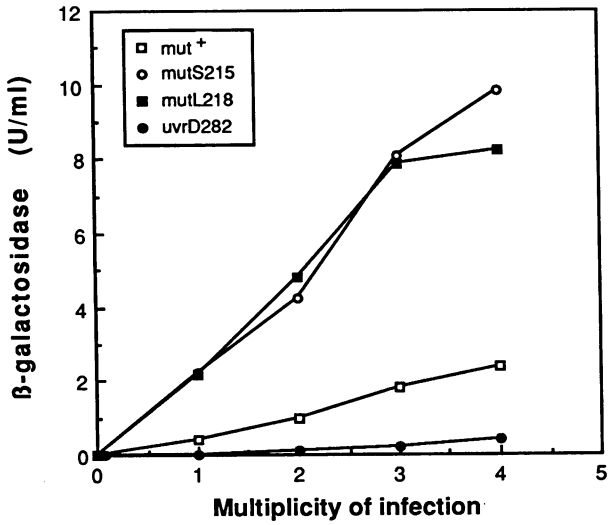

FIG. 4. Dependence of direct stimulation on *mutS*, *mutL*, and *uvrD* alleles. Strains KL787 (*mut*$^+$), KL788 (*mutS215*), KL789 (*mutL218*), and KL790 (*uvrD282*) were grown and assayed as described in Materials and Methods. Each value is the average from three independent infection experiments. Samples were corrected for basal recombination observed in samples containing unirradiated λ*lacZ118* phage.

TABLE 3. Direct and indirect stimulation in *rec*$^+$ mismatch repair deficient strains[a]

Strain	Relevant genotype	β-Galactosidase (U/ml)					
		Direct stimulation			Indirect stimulation		
		No UV	+UV (SEM)	Ratio	No UV	+UV (SEM)	Ratio
KL787	*mut*$^+$	0.05	1.88 (0.17)	37.6	0.13	0.45 (0.13)	4.1
KL788	*mutS215*	0.39	8.45 (0.32)	21.6	0.18	0.42 (0.03)	2.3
KL789	*mutL218*	0.19	8.17 (0.10)	43.1	0.69	0.89 (0.01)	1.3
KL790	*uvrD282*	0.27	0.48 (0.02)	1.8	0.45	0.52 (0.12)	1.1

[a] Cultures were grown and assayed as described in Materials and Methods. The multiplicities of infection and calculations are similar to those described in the footnotes to Table 2.

no increases above the background level were observed in the case of strain KL790 (*uvrD282*) (data not shown). This complete lack of stimulated recombination at the transcribable intermediate stage (measured by β-galactosidase assays) suggests that UvrD is involved in the early steps of UV-stimulated recombination.

Dependence of stimulated recombination on mismatch and excision functions in a *recBC sbcB* background. Because the absolute levels of recombination were found to be highest in a *recB21 sbcB15* background (Table 2), it seemed prudent to also examine the effects of mutations in mismatch and excision functions in this genetic background to increase the sensitivity of determination. In addition, the lack of a functional exonuclease V in this genetic background simplifies the interpretation of results, since this nuclease plays an important role in recombination between double-stranded substrates (4).

Recombination was found to be much higher in the *recB sbcB* strain and its derivatives (Table 4) than the *recB*$^+$ strains (Table 2). Mutations in *mutS* and *mutL* led to modest increases in both unstimulated and stimulated recombination in direct-stimulation experiments (compare strains KL791 and KL792 with strain KL635, Table 4). A mutation in *uvrD*, which abolished stimulated recombination in both direct (Fig. 4) and indirect (Fig. 5) stimulation experiments in a *recB*$^+$ background, did not completely block stimulation in a *recB sbcB* strain (compare strain KL793 with strain KL635, Table 4). However, in a *recB sbcB uvrD uvrA* strain, little stimulation was observed in either direct- or indirect-stimulation experiments (strain KL797, Table 4). The effects of mutations in other mismatch repair functions were variable. In a *uvrA*$^+$ background, both *mutS215* and *mutL218* increased the absolute levels of directly stimulated recombination slightly, while in most other crosses they had little effect (Table 4). The *mutS215* mutation, however, reduced indirect stimulation fivefold in a *recB sbcB uvrA*$^+$ background (Table 4). A mutation in *uvrA* resulted in a three- to eightfold decrease in direct recombination in otherwise isogenic strains (Table 4). In indirect-stimulation experiments, *uvrA6* reduced recombination only in a *uvrD* background (compare KL797 and KL793, Table 4).

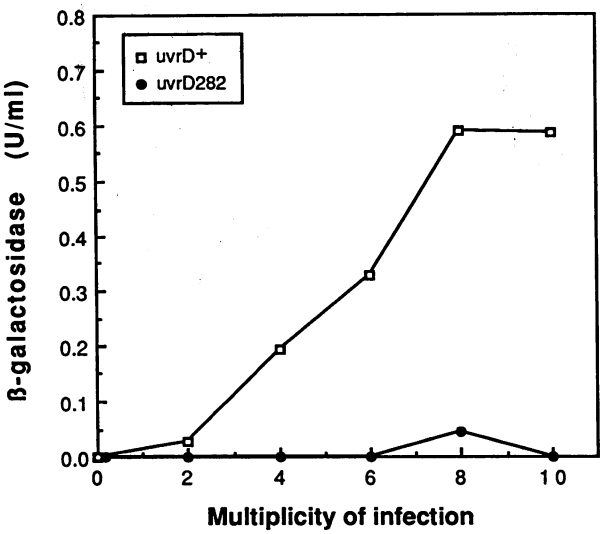

FIG. 5. Dependence of indirect stimulation on *uvrD* in a *rec*$^+$ background. Strains KL787 (*uvrD*$^+$) and KL790 (*uvrD282*) were grown and assayed as described in Materials and Methods. The recombining phage (λ*lacZ118*) was used at a multiplicity of infection of 3, while the multiplicity of infection of the stimulating phage (λ) was varied as indicated. Each value is the average from three independent experiments. Samples were corrected for basal recombination observed in samples containing unirradiated λ*lacZ118* phage.

DISCUSSION

In this study we have tested genetic blocks in several recombination and repair functions in *E. coli* to determine their possible roles in UV-stimulated recombination.

The effect of *recJ284* in λ*lacZ* × F$^-$ *lacZ* crosses reported in this work suggests a dependence somewhat different from that found in conjugation or plasmid systems in which recombination is strongly RecJ dependent (11, 12). In contrast, we observe considerable unstimulated recombination and direct stimulation but almost no indirect stimulation (teleactivation) in *recJ284* strains with the λ*lacZ* × F$^-$ *lacZ* system. This is the first indication that the two modes of stimulated recombination (direct and indirect) have different enzymological requirements. In view of the 5′-to-3′ activity of the RecJ exonuclease on single-stranded DNA (13), these results suggest that the processing of damaged molecules by the RecJ exonuclease may produce long single-stranded regions which can pair and recombine with homologs (e.g., λ to λ*lac*) during indirect stimulation. Alternatively, RecJ may play a later role in UV-stimulated recombination by processive degradation of distal damage sites, producing long single-stranded regions extending from the UV damage site to the recombining site (*lac* region). This would eliminate the need for branch migration during repair of UV-damage sites which does not proceed through even short regions of nonhomology (5).

Both mismatch repair (*mutSL*) and excision repair (*uvrA*) functions were found to be required for normal levels of UV-stimulated recombination (Table 5). A striking result

TABLE 4. Direct and indirect stimulation in *recB21 sbcB15* strains defective in mismatch and excision repair functions[a]

Strain	Relevant genotype	β-Galactosidase (U/ml)					
		Direct stimulation			Indirect stimulation		
		No UV	+UV (SEM)	Ratio	No UV	+UV (SEM)	Ratio
KL635	*mut*$^+$ *uvrA*$^+$	1.21	21.8 (1.9)	17.9	0.42	4.30 (0.4)	10.2
KL791	*mutS215 uvrA*$^+$	1.78	43.4 (4.2)	24.4	0.28	0.76 (0.09)	2.7
KL792	*mutL218 uvrA*$^+$	3.47	40.2 (1.8)	13.5	0.59	2.49 (0.40)	4.2
KL793	*uvrD282 uvrA*$^+$	1.50	15.1 (0.4)	10.9	0.34	1.13 (0.25)	3.4
KL794	*mut*$^+$ *uvrA6*	1.65	7.1 (0.2)	4.2	0.37	6.42 (0.80)	17.3
KL795	*mutS215 uvrA6*	1.85	6.1 (0.3)	3.3	0.61	3.58 (0.31)	5.7
KL796	*mutL218 uvrA6*	3.40	7.1 (0.3)	2.1	0.89	3.49 (0.15)	3.9
KL797	*uvrD282 uvrA6*	1.72	3.2 (0.1)	1.8	0.36	0.32 (0.19)	0.9

[a] Cultures were grown and assayed as described in Materials and Methods. The multiplicities of infection and calculations are similar to those described in the footnotes to Table 2.

was the nearly absolute dependence of stimulation on helicase II (encoded by *uvrD*) in a wild-type (with respect to other recombination functions) background. Since this enzyme plays an important role in turning over both mismatch and excision repair complexes (23), it appears that in a *rec*$^+$ background, almost all of the UV-stimulated recombination depends on either the mismatch repair or excision repair pathway. Presumably, such processing would involve UV damage recognition (thymine dimers and 6,4-photolesions, etc.) by these repair mechanisms prior to recombinational repair. The importance of helicase action in UV damage repair processes may extend to other organisms. Recently, it has been demonstrated that RADH, a yeast gene involved in UV sensitivity in *Saccharomyces cerevisiae*, has some similarity to helicases on the basis of predicted protein structure (1). In humans, a defective ERCC allele, encoding a putative helicase, may be responsible for some type of UV sensitivity (24).

The UvrD protein is believed to play a late role in excision repair by recycling the UvrABC excision complex (for a review, see reference 23). To be reconciled with this repair model, the dependence of UV-stimulated recombination on *uvrD* reported in this work would require that the UvrD protein play an early role in the recombination process. The *lacZ* × *lacZ* assay (2, 17) used in this work allows independent measurement of transcribable recombination products (as measured by β-galactosidase assays) and viable recombinants (as determined by Lac$^+$ colony formation), to distinguish such early factors from later-acting factors. Since *uvrD282* was found to block UV-stimulated β-galactosidase production, it must act prior to the formation of a transcribable intermediate(s). The recent finding (19) that UvrD can effect DNA unwinding in the absence of ancillary proteins, provided that the DNA is already nicked, suggests that the requirement for incising enzymes of the mismatch or excision pathways for DNA unwinding during repair and recombination is not absolute. It is possible that nicks generated in vivo by other repair enzymes (e.g., *N*-glycosylases) during repair could also serve as substrates for UvrD action.

Hays and coworkers (8–10) have identified many of the host functions which are involved in UV-stimulated recombination by using a tandem prophage system. They found that stimulation is mostly RecF mediated and is, in addition, partially dependent on excision functions (22). Our results also indicate that λ*lacZ* × F$^-$ *lacZ* recombination is RecF (or RecE) pathway dependent but also requires processing of the damage sites by UvrABC (excision dependent) and some other unidentified mechanism(s) (excision independent). The results of this report indicate that all UvrABC-independent processing appears to be dependent on UvrD. Since the mismatch mutations (*mutS* and *mutL*) tested in this work were also found to be hyperrecombinogenic in the direct-stimulation assay (Table 5), it is not surprising that *uvrD* has a greater effect than *uvrA* alone in reducing UV-stimulated recombination.

The failure of the *uvrD* mutation to completely block stimulated recombination in a *recB sbcB* background may be the result of either (i) destruction of potential prerecombinant structures in the *rec*$^+$ background that are not processed by the UvrD helicase or (ii) a change in the nature of the RecE or RecF pathway which obviates the need for the

TABLE 5. Summary of functional dependence for direct and indirect stimulation of recombination from data reported in this paper

Background type	Type of recombination[a] with:	
	Direct stimulation	Indirect stimulation
rec$^+$		
recB21	+	+
recJ284	+	−
sbcA8	+	+
recJ284 sbcA8	−	−
mutS215	+*	−
mutL218	+*	−
uvrD282	−	−
recB21		
sbcA8	+	+
recJ284 sbcA8	+	−
sbcB15	+	+
recJ284 sbcB15	+	−
sbcB15 sbcC250 mutS215	+	(+)
sbcB15 sbcC250 mutL218	+	(+)
sbcB15 sbcC250 uvrD282	+	(+)
sbcB15 sbcC250 uvrA6	(+)	+
sbcB15 sbcC250 uvrA6 mutS215	(+)	(+)
sbcB15 sbcC250 uvrA6 mutL218	(+)	(+)
sbcB15 sbcC250 uvrA6 uvrD282	(+)	−

[a] *, hyperstimulated; +, stimulated; (+), weakly stimulated; −, not stimulated.

UvrD protein during recombinational repair. At present, we are unable to distinguish between these two possibilities but note that both unstimulated and stimulated levels of recombination are elevated only in *sbcA* or *sbcB* derivatives of *recB* strains (Table 2), favoring the latter hypothesis.

Although the data presented in this paper indicate a central role of UvrD in the recombination system used, many questions remain. We do not, for example, know what types of damage sites are most stimulatory, nor can we reconcile the observed effects of *mutS* and *mutL* with current models of mismatch repair. Future studies will include examining the nature of recombinants produced in the crosses described in this work and, in addition, tests of the role of *uvrD* in UV-stimulated conjugational recombination.

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