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Recombination Levels of *Escherichia coli* K-12 Mutants Deficient in Various Replication, Recombination, or Repair Genes

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Escherichia coli strains containing mutations in *lexA, rep, uvrA, uvrD, uvrE, lig, polA, dam,* or *xthA* were constructed and tested for conjugation and transduction proficiencies and ability to form Lac$^+$ recombinants in an assay system utilizing a nontandem duplication of two partially deleted lactose operons (*lacMS286*ϕ80dII*lacBK1*). *lexA* and *rep* mutants were as deficient (20% of wild type) as *recB* and *recC* strains in their ability to produce Lac$^+$ progeny. All the other strains exhibited increased frequencies of Lac$^+$ recombinant formation, compared with wild type, ranging from 2- to 13-fold. Some strains showed markedly increased conjugation proficiency (*dam uvrD*) compared to wild type, while others appeared deficient (*polA107*). Some differences in transduction proficiency were also observed. Analysis of the Lac$^+$ recombinants formed by the various mutants indicated that they were identical to the recombinants formed by a wild-type strain. The results indicate that genetic recombination in *E. coli* is a highly regulated process involving multiple gene products.

The study of genetic recombination in *Escherichia coli* K-12 has been based primarily on observed alterations in either conjugation or transduction proficiency (6). Since recipient strains carrying mutations in *recA, recB,* or *recC* yield relatively few recombinant progeny after conjugation (100- to 1,000-fold reduction) (7, 32, 35), these gene products likely mediate reactions essential for recombinant formation. On the other hand, the *rep* and *lexA* genes appear to be more involved in repair or replication processes, since they exert only minor alterations in recombination after conjugation (4, 25).

The development by Konrad (15) of a strain of *E. coli* carrying a specially constructed duplication of the lactose operon (*lacMS286*ϕ80dII-*lacBK1*) has provided an additional means of studying recombination. Since the ϕ80dII-*lacBK1* contains a small deletion in the proximal portion of the *lacZ* gene, and *lacMS286* is deleted in the distal portion of *lacZ,* Lac$^+$ recombinants occur at low frequencies. Konrad and Lehman used this strain to isolate derivatives of *E. coli* with increased frequencies of Lac$^+$ recombinant formation ("hyper-Rec") (16, 17). Recently Zieg and Kushner (35) have demonstrated that the increases or decreases in the number of Lac$^+$ recombinants obtained in the presence of a specific mutation provide a sensitive measure of genetic recombination within the cell. This assay therefore appeared useful as a means of studying the regulation of genetic recombination in *E. coli.* Accordingly, a series of isogenic strains carrying mutations in *rep, lexA, xthA, uvrD, uvrE, polA, dam, lig,* and *uvrA* were constructed and tested for conjugation, transduction, and *lac* gene recombination proficiency. The results presented in this paper suggest that most of the above gene products are involved in genetic recombination either directly or indirectly.

MATERIALS AND METHODS

Materials. Reagents were obtained from the following sources: lactose, glucose-free, U.S. Biochemical Corp.; 2,3,5-triphenyl-2H-tetrazolium chloride, methyl methane sulfonate, and ethyl methane sulfonate, Eastman Kodak Co.; streptomycin sulfate and amino acids, Sigma. Spectinomycin sulfate was the generous gift of The Upjohn Co. All other chemicals were of reagent grade.

Bacterial strains and bacteriophages. Bacterial strains are listed in Table 1. Nomenclature conforms to that of Demerec et al. (9). Gene symbols are those used by Bachmann et al. (1). Figure 1 shows the locations of the various genes and Hfr's described in the paper. All strains were constructed by either conjugation or P1*vir* transduction. Inheritance of *uvrD, uvrE, lexA, uvrA,* and *dam* mutations was detected by sensitivity to UV light using the replica-plating techniques of Clark and Margulies (7). The presence of *rep* alleles was measured by the inability to support P2*vir*22. *polA* and *xthA* mutations were analyzed by sensitivity to 0.08% methyl methane sulfonate in Luria

TABLE 1. *Bacterial strains*

Strain	Sex	*arg*	*his*	*thr*	*leu*	*pro*	*thi*	*rpsE*[a]	*rpsL*[b]	*sup*	*lac*	Other markers	Source or derivation
AB1157	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*		A. J. Clark
AB1360	F⁻	*E3*	*4*	+	+	*A2*	*1*	+	+	?	−	*aroD6*	A. J. Clark
AB2500	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*uvrA6 thyA deoB*	R. Cole
AB2569	F⁻	−	*4*	+	+	*A2*	*1*	+	+	?	*Y1*	*metA28*	B. Bachmann
AB3292	F⁻	*E3*	*4*	+	+	*A2*	−	+	−	?	−	*ilv pabA1*	G. Tritz
BW9091	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*xthA1*	B. Weiss
BW9101	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*ΔxthA ΔpncA*	B. Weiss
DM49	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*lexA3*	D. Mount
DY81	F⁻	+	+	+	+	+	+	+	−	?	+	*trp uvrD3*	D. Young
E3	F′	+	+	+	+	+	+	+	+	?	+	*F′lac*	A. J. Clark
ES271	F⁻	+	+	+	+	+	+	+	+	*19*	−	*ilvD188 uvrE4*[c]	R. Cole
GM33	F⁻	+	+	+	+	+	−	+	+	?	+	*dam-3*	M. Marinus
JC158	Hfr	+	+	+	+	+	−	+	+	+	−	*serA*	A. J. Clark
JC2915	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*cysC43*	A. J. Clark
JC4729	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*metE46 recB21 recC22 sbcB15*	A. J. Clark
JC8403	F⁻	*E3*	*4*	*1*	*6*	*A2*	*1*	+	*31*	*37*	*Y1*	*ilvA*	A. J. Clark
KL96	Hfr	+	+	+	+	+	*1*	+	+	+	+		B. Bachmann
KL209	Hfr	+	+	+	+	+	+	+	+	+	+		B. Bachmann
KMBL1789	F⁻	*A101*	+	+	+	+	+	+	+	+	+	*pheA97 bio-87 endA101 polA107*	B. Glickman
KMBL1493	F⁻	*A101*	+	+	+	+	+	+	+	−	+	*pheA97 uvrD101 bio-87 endA101*	B. Glickman
KS244	Hfr	+	+	+	+	+	*1*	+	+	+	+	*lig-7*(Ts)	E. B. Konrad
KS391	Hfr	+	+	+	+	+	+	+	+	+	*BK1*[d]		E. B. Konrad
PM5	F⁻	+	+	+	+	+	+	+	+	+	+	*rep-5*	R. Calendar
Ra-2	Hfr	+	+	+	+	+	+	+	+	+	+		B. Bachmann
Rep-3	F⁻	+	+	+	+	+	+	+	+	−	+	*rep-3*	A. J. Clark
RS9	F⁻	+	+	+	+	+	+	+	−	?	*BK1*	*uvrE100*	E. B. Konrad
RS5033a	F⁻	+	+	+	+	+	+	+	−	+	*BK1*	*dam-4*	E. B. Konrad
SK212	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	*BK1*	*ilvA*	JC8403 × KS391 Pro⁺ conjugant
SK217	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*uvrE100*	SK212 × ES271 Ilv⁺ transductant
SK236	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*metE46*	SK212 × JC4729 Ilv⁺ transductant
SK246	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*uvrD3*	SK212 × DY81 Ilv⁺ transductant
SK274	F⁻	*E3*	*4*	+	+	*A2*	*1*	−	+	?	−	*aroD6*	RpsE⁻ derivative of AB1360
SK276	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*polA107*	SK236 × KMBL1479 MetE⁺ transductant
SK277	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*		SK236 × KMBL1479 MetE⁺ transductant
SK285	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*rep-3*	SK212 × Rep-3 Ilv⁺ transductant
SK287	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*		SK212 × Rep-3 Ilv⁺ transductant
SK329	F⁻	*E3*	*4*	+	+	+	+	−	+	−	+	*BK1*	*pabA1*	AB3292 × KS391 Pro⁺ conjugant
SK336	F⁻	+	*4*	+	+	+	+	−	+	*31*	+	*BK1*	*ilvA metA28*	SK212 × AB2569 ArgE⁺ transductant
SK343	F⁻	*E3*	*4*	+	+	+	+	*1*	−	+	+	*BK1*	*aroD6*	SK274 × KS391 ProA conjugant
SK366	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	+	*BK1*	*lexA3 ilvA*	SK336 × DM49 MetA⁺ transductant
SK368	F⁻	*E3*	*4*	+	+	+	+	*1*	+	*31*	⁺	*BK1*	*ilvA*	SK336 × DM49 MetA⁺ transductant
SK377	F⁻	*E3*	*4*	+	+	+	+	*1*	−	+	+	*BK1*		SK343 × BW9091 AroD⁺ transductant

Table 1—*Continued*

Strain	Sex	*arg*	*his*	*thr*	*leu*	*pro*	*thi*	*rpsE*[a]	*rpsL*[b]	*sup*	*lac*	Other markers	Source or derivation
SK383	F⁻	*E3*	*4*	+	+	+	−	+	−	+	*BK1*	*dam-4*	SK329 × RS5033a PabA⁺ transductant
SK385	F⁻	*E3*	*4*	+	+	+	−	+	−	+	*BK1*	*dam-3*	SK329 × GM33 PabA⁺ transductant
SK395	F⁻	+	*4*	+	+	+	*1*	+	*31*	+	*BK1*	*uvrA6 ilvA*	SK336 × AB2500 MetA⁺ transductant
SK703	F⁻	*E3*	+	+	+	+	*1*	+	*31*	+	*BK1*	*metE46 lig-7*(Ts)	SK236 × KS244 His⁺ conjugant
SK705	F⁻	*E3*	+	+	+	+	*1*	+	*31*	+	*BK1*	*metE46*	SK236 × KS244 His⁺ conjugant
SK866	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	*BK1*	*uvrE4*	SK212 × ES271 IlvA⁺ transductant
SK1175	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	*BK1*	*rep-5*	SK212 × PM5 Ilv⁺ transductant
SK1314	F⁻	*E3*	*4*	+	+	+	*1*	−	+	+	*BK1*	*xthA1*	SK343 × BW9091 AroD⁺ transductant
SK1328	F⁻	*E3*	*4*	+	+	+	*1*	−	+	+	*BK1*	Δ*xth*	SK343 × BW9101 AroD⁺ transductant
SK1455	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	−		Lac⁻ revertant from Lac⁺ recombinant of SK287
SK1456	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	−		Lac⁻ revertant from Lac⁺ recombinant of SK287
SK1458	F⁻	*E3*	*4*	+	+	+	*1*	+	*31*	+	−	*rep-3*	Lac⁻ revertant from Lac⁺ recombinant of SK255
SK1459	F⁻	*E3*	*4*	+	+	+	+	+	*31*	+	−	*uvrD3*	Lac⁻ revertant from Lac⁺ recombinant of SK246

[a] Formerly *strA*.
[b] Formerly *spc*.
[c] Formerly *mutU4*.
[d] *BK1* indicates presence of *lacMS286*φ80dIII*lacBK1*.

agar plates. The duplication of the lactose operon used in this work was that of Konrad (15) and Konrad and Lehman (16) and was detected as described previously (35). For introduction of the lactose operon duplication, 2-h matings were employed.

Bacterial viruses were obtained from the following sources: φ80*am*2, Ethan Signer; P1*vir*, A. J. Clark; T4D, T4B22, and T4N82, Bruce Alberts; φ80*vir*, Max Gottesman; and P2*vir*22, Richard Calendar. The presence of amber suppressors was tested by the ability of strains to plate T4B22 and T4N82 amber mutants.

Media. The complete medium used was Luria broth as described previously (19). For solid medium, 2% agar was added. Lactose tetrazolium agar indicator plates were prepared as described by Miller (24). The minimal medium used for plates (M56/2) has been described by Willetts et al. (31). M9 medium (30) supplemented with amino acids to a final concentration of 50 μg/ml was used for liquid cultures. Lactose minimal agar plates contained lactose as the sole carbon source.

Conjugation and transduction. The procedures used for conjugation and transduction were those of Willetts et al. (31). In testing for the ability of cells to take up an F′ plasmid, F′*lac* was used. Ninety-minute incubation times were used for these experiments. For determination of quantitative transduction frequencies, P1*vir* grown on JC158 was used as the donor. Transduction frequencies are expressed as number of transductants per 10^7 plaque-forming units. In determining conjugation frequencies, Hfr donors were chosen which would transfer selected markers early but which would not transfer the wild-type alleles of the mutations being examined within the 60-min mating period. Matings were interrupted by mechanical agitation after 60 min.

Lactose recombination assay. Five single colonies of each strain to be tested were grown individually in 7-ml Luria broth cultures to cell densities of approximately 10^8 cells per ml at 37°C. Viable counts were determined on each isolate using Luria agar plates, and Lac⁺ recombinants were measured on lactose minimal agar plates. Between 10^6 and 10^7 cells (in Luria broth) were plated on each minimal agar plate. All plates were incubated at 37°C (Luria agar plates for 24 h, minimal agar plates for 48 h). Unless otherwise noted, all plates were overlaid with soft agar. In some cases the cells were washed three times with M56/2 buffer prior to plating on the minimal agar plates.

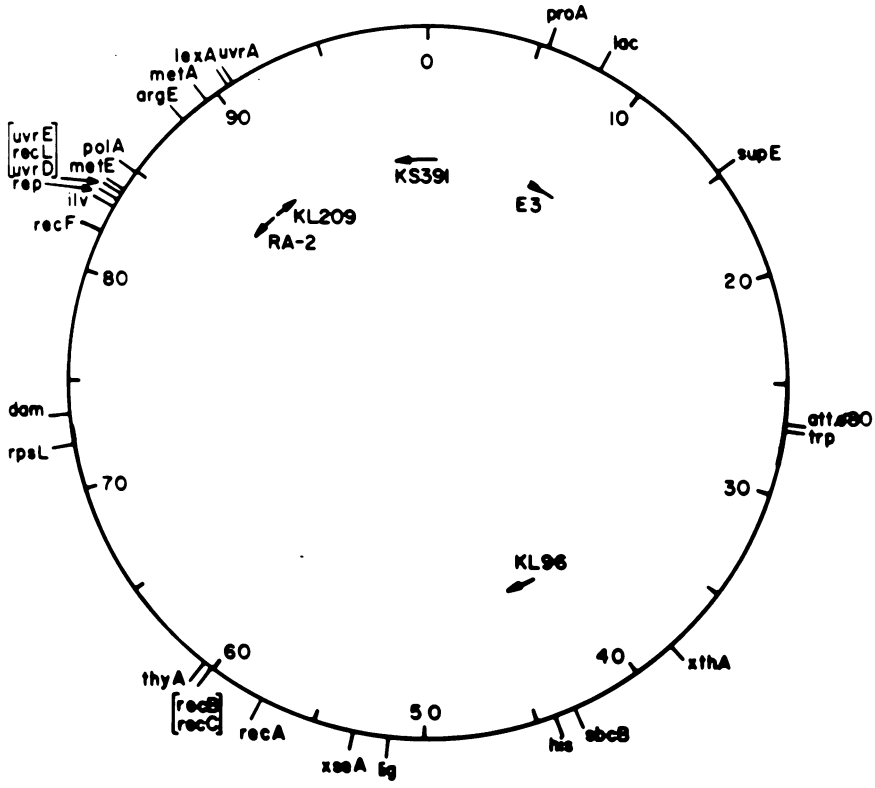

FIG. 1. *E. coli genetic map. Locations of genes and Hfr's described in text are presented according to Bachmann et al. (1).*

Other methods. Lac$^+$ recombinants were analyzed as described by Zieg and Kushner (35). Respreading experiments were carried out as outlined previously (35). Cell growth was followed densitometrically with a Klett-Summerson photometer (no. 42 green filter). For UV sensitivity analysis, plates were exposed to approximately 1,000 ergs/mm^2 from a 15-W GE germicidal lamp.

RESULTS

Recombination proficiency of strains deficient in UV repair. Mutations in *polA, uvrA, uvrD, uvrE,* and *lexA* result in increased sensitivity to UV light, but have not generally been associated with genetic recombination (8, 11, 25, 26, 28, 29, 34). The *uvrA* gene product is thought to introduce single-strand breaks in the vicinity of pyrimidine photoproducts (3). *polA* (deficient in DNA polymerase I) or *uvrD* strains can excise pyrimidine dimers normally or at a reduced rate, but appear deficient in repair synthesis or some other later function (2, 14, 26). *uvrD* and *uvrE* mutants exhibit increased spontaneous mutation rates as well as enhanced sensitivity to UV light (28). *lexA* is thought to be the structural gene for a repressor protein which controls a number of cellular repair functions (13).

A series of isogenic strains were constructed carrying *polA107, uvrA6, uvrD3, uvrD101, uvrE4, uvrE100,* or *lexA3.* A summary of Lac$^+$ recombinant formation and conjugation and transduction proficiencies for these strains is shown in Table 2. With the exception of *lexA3,* mutations affecting the repair of UV-irradiated DNA led to a 2- to 10-fold stimulation in the formation of Lac$^+$ recombinants over wild-type strains. In the case of SK246 (*uvrD3*), SK217 (*uvrE4*), and SK866 (*uvrE100*), conjugation and transduction proficiencies were also enhanced. After conjugation the extent of increase appeared dependent both on the marker selected and the Hfr donor employed. Ability to take up an F'*lac* plasmid also increased in the *uvrD3* mutant. SK246 (*uvrD3*) exhibited the largest increase in transduction proficiency of any strain tested. In contrast, SK276 (*polA107*) consistently demonstrated a 90 to 95% drop in conjugation proficiency, while its efficiency of transduction was not affected appreciably. As shown previously, *lexA3* strains (SK366) appeared almost as conjugally proficient as a wild-type control (25). On the other hand, the formation of Lac$^+$ recombinants was reduced dramatically, as was transduction proficiency.

TABLE 2. *Recombination proficiency of various strains*

	Strain	Relevant genotype	No. of Lac$^+$ colonies/plate	Conjugal deficiency index[a]			F'*lac* inheritance deficiency index[a]	Transductants/10^7 PFU[b]		
				KL96	Ra-2	KL209		Arg$^+$	His$^+$	Ilv$^+$
A										
	SK368	Wild type	118	1		1	1		81	1,868
	SK395	*uvrA6*	237	0.7		1.2	1		105	2,182
	SK366	*lexX3*	24	1.4		1.2	1		18	1,060
	SK276	*polA107*	396	20	12		4	292	60	
	SK277	*polA*$^+$	97	1	1		1	222	30	
	SK287	*uvrD*$^+$	99 (46)[c]	1	1		1	323	71	
	SK246	*uvrD3*	479 (237)[c]	0.6	0.1		0.4	850	154	
	SK1493	*uvrD101*	446 (286)[c]							
	SK218	*uvrE*$^+$	172 (12)[c]	1	1		1	149	53	
	SK217	*uvrE4*	421 (154)[c]	0.7	0.2		1	147	76	
	SK866	*uvrE100*	420 (130)[c]	0.9	0.8		1	224	99	
B										
	SK287	*rep*$^+$	99	1	1		1	32	22	
	SK285	*rep-3*	22	3.1	1.8		0.4	50	18	
	SK1175	*rep-5*	29	1.3	3.0			58	22	
C										
	SK387	*dam*$^+$	185 (50)[c]	1	1		1	362	110	4,849
	SK385	*dam-3*	633 (190)[c]	0.33	0.16		1.1	368	141	1,284
	SK383	*dam-4*	854 (288)[c]	0.12	0.05		1	245	121	761
D										
	SK377	*xthA*$^+$	103 (56)[c]	1			1	105	33	
	SK1314	*xthA1*	1,264 (638)[c]	1			1	103	30	
	SK1328	Δ*xthA*	1,341 (743)[c]	2			1	350	60	
E										
	SK703	*lig-7*(Ts)	17 (191)[d]			1,000 (2)[d]	(1)[d]	(56)[d]	(58)[e]	
	SK705	*lig*$^+$	60 (49)[d]			1 (1)[d]	(1)[d]	(46)[d]	(50)[e]	

[a] Conjugal deficiency indexes represent wild-type frequencies divided by mutant frequencies for 60-min matings selecting [His$^+$] [Sm^r] recombinants with KL96 or [Arg$^+$] [Sm^r] recombinants with either Ra-2 or KL209. F'*lac* inheritance was determined by mating E3 with appropriate recipients for 90 min. Deficiency index is as defined for conjugal crosses. All transductions were carried out with P1*vir* grown on JC158. Results represent the average of two determinations.

[b] PFU, Plaque-forming units.

[c] Numbers in parentheses indicate values obtained by washing cells with M56/2 buffer prior to plating on lactose minimal agar plates.

[d] Values obtained at 30°C. Although *lig-7*(Ts) is a conditionally lethal mutation, no loss in viability at 37°C was detected in these experiments.

[e] Met$^+$ transductants determined at 30°C.

Recombination proficiency of strains with various defects in DNA metabolism. The *rep* protein has been shown to be involved in the replication of certain *E. coli* bacteriophages such as P2 and φX174 (4, 10). Additionally, *rep* strains show slightly enhanced sensitivity to UV light. Both *rep* alleles tested here showed significantly reduced formation of Lac$^+$ progeny and small reductions in conjugation proficiency. Transduction efficiencies appeared unaffected (Table 2).

Polynucleotide ligase (*lig*) is required for cell viability presumably because of the need to generate covalently closed DNA (12, 18). Konrad has shown that ligase-deficient mutants yield higher levels of Lac$^+$ recombinants (15). As shown in Table 2, an interesting phenomenon was observed in the case of the temperature-sensitive allele *lig-7*(Ts). At 30°C, Lac$^+$ progeny formation was stimulated, while conjugation and transduction proficiencies remained unaltered. However, at 37°C, both Lac$^+$ progeny formation and conjugation proficiency dropped dramatically (Table 2), although cell viability was not affected at this temperature.

The absence of DNA adenosine methylase in *E. coli* leads to an accumulation of single-strand breaks in the cellular DNA (20). As shown in Table 2, not only was Lac$^+$ progeny formation increased, but conjugation proficiency was also increased dramatically by 3- to 20-fold. The extent of stimulation appeared to be a function of the donor Hfr. Transduction proficiency appeared to be marker dependent. The number of Ilv$^+$ (a gene close to the origin of DNA replication) transductants was reduced in both *dam-3* (SK385) and *dam-4* (SK383) mutants, while the frequencies of Arg$^+$ and His$^+$ transductants were unaffected.

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demonstrate increased sensitivity to methyl methane sulfonate but not to UV light (22, 33). Recently it has been shown that *xthA* mutants are also missing endonuclease II (33). Although conjugation proficiency was unchanged, Lac$^+$ recombinant formation increased over 10-fold with both the point mutant (SK1314 *xthA1*) and the deletion (SK1328 Δ*xthA*) (Table 2). Transduction proficiency also increased in the strain (SK1328) carrying the deletion (Table 2).

Nature of Lac$^+$ recombinants. Konrad (15) and Zieg and Kushner (35) have shown that in wild-type strains of *E. coli*, a recombinational event takes place such that an intact lactose operon is generated on the defective φ80 lysogen. A similar analysis of Lac$^-$ revertants derived from Lac$^+$ recombinants produced by either a "hyper-Rec" strain (SK246 *uvrD3*) or a "hypo-Rec" strain (SK285 *rep-3*) is presented in Table 3. Although the absolute number of spontaneously arising Lac$^-$ colonies is related to whether the strain is "hyper-Rec" or "hypo-Rec," the fraction of those Lac$^-$ derivatives retaining the φ80 prophage is identical within experimental error for all three strains. Those few Lac$^-$ revertants that papillated produced Lac$^+$ colonies independent of the mutation present within the cell (Table 4) and may have arisen from some type of chromosomal rearrangement.

Previous results have suggested that in wild-type strains Lac$^+$ recombinants occur as the cells enter late-log-phase growth (35). As shown in Fig. 2, the formation of Lac$^+$ progeny in a "hyper-Rec" strain (SK246 *uvrD3*) was similar to that of a wild-type control (SK287). Additionally, the ratio of mutation rates could be calculated from these curves, yielding an average value of 13.3 ± 4.0 over the first 6-h period. This number contrasts with the 5.2-fold increase in the total number of Lac$^+$ recombinants measured by the standard plate assay (Table 2).

DISCUSSION

From the results presented above it appears that Lac$^+$ recombinants arising in "hyper-Rec" or "hypo-Rec" mutants are formed by a mechanism similar to that which occurs in wild-type strains. This would account for the absence of this event in *recA* strains (35). Additionally, recombinant formation in "hyper-Rec" strains appears to occur when the cells enter late-log-phase growth. Since the assay can measure both increases or decreases in recombination proficiency, the *lacMS286*φ80dII*lacBK1* duplication provides a powerful method for studying recombination in *E. coli*.

The data presented in Table 2 and by Zieg and Kushner (35) represent the analysis of genetic recombination employing three independent assays. The differences in the methods should be noted. Formation of Lac$^+$ recombinants does not require an extracellular DNA donor. The information necessary is always present, but the process may be regulated, since it occurs at a very low frequency during logarithmic growth of the cell. On the other hand, both conjugation and transduction require an external source of donor DNA. Transfer from stable Hfr donors is highly efficient, and recombinational proficiencies close to the theoretical limit can be observed in these cases. In contrast, generalized transducing particles make up a small percentage of the total phage population, and many of these lead to abortive events. As a result, transduction proficiency is typically quite low and can be dependent upon the particular markers selected.

TABLE 4. *Analysis of papillating Lac$^-$ revertants obtained from Lac$^+$ recombinants*[a]

Strain	Genotype	Origin	No. of Lac$^+$ colonies/plate
Parental strains			
SK287	Wild type		99
SK285	*rep-3*		22
SK246	*uvrD3*		479
Lac$^-$ revertants			
SK1455	Wild type	SK287	6
SK1456	Wild type	SK287	5
SK1458	*rep-3*	SK285	7
SK1459	*uvrD3*	SK246	16

[a] Lac$^+$ recombinants were isolated from SK287, SK285, and SK246 as described previously (35). Strains SK1455, SK1456, SK1458, and SK1459 are representatives of the class of papillating Lac$^-$ revertants obtained from Lac$^+$ recombinants (see Table 3).

TABLE 3. *Analysis of spontaneously occurring Lac$^-$ revertants arising from Lac$^+$ recombinants*[a]

Strain	Genotype	Avg no. of Lac$^-$ colonies/10^4 Lac$^+$ cells	No. of Lac$^-$ colonies tested	Fraction of Lac$^-$ colonies retaining φ80dII*lac*	Fraction of Lac$^-$ colonies papillating
SK246	*uvrD3*	11.3	382	0.08	0.02
SK287	Wild type	1.4	128	0.05	0.02
SK285	*rep-3*	0.5	63	0.10	0.02

[a] Twenty independently isolated Lac$^+$ recombinants were isolated from each strain and grown overnight in L broth. The cells were plated on lactose tetrazolium agar plates. Spontaneously arising Lac$^-$ colonies were analyzed as described previously (35).

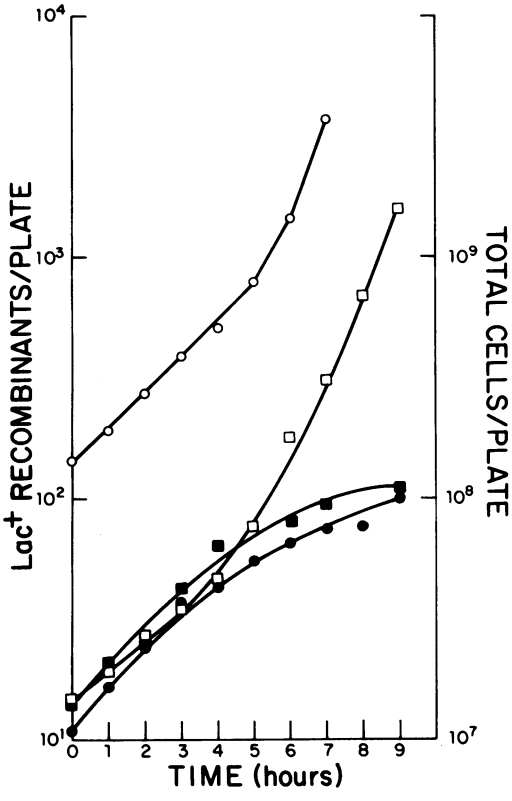

FIG. 2. *Growth of total cell mass and Lac⁺ recombinants on lactose minimal agar plates. Strains were grown in K medium as described in the text. At times indicated, four plates were removed from the 37°C incubator and respread with 0.1 ml of prewarmed M56/2 buffer. Additional plates were washed with 2.0 ml of M56/2 buffer for determination of total cells per plate.* (○) *Lac⁺ recombinants obtained with SK246 (uvrD3);* (□) *Lac⁺ recombinants obtained with SK287 (uvrD⁺);* (●) *total SK246 cells on lactose minimal agar plates;* (■) *total SK287 cells on lactose minimal agar plates.*

Based on these inherent differences, it would not be surprising if the control of the presynaptic steps for these three recombination systems differed considerably whereas postsynaptic events (e.g., elongation of the paired regions and generation of an intact recombinant molecule) might be more closely related. Accordingly, analysis of a variety of mutants employing the three assays might uncover such divergences.

As shown in Table 5, the *recA* gene product is absolutely required for all forms of homologous recombination in *E. coli*. However, the effects of *recB* and *recC* mutations on Lac⁺ progeny formation are much less striking than they are on transduction and conjugation. It is unlikely that the decrease observed in the *recB* and *recC* mutants can be explained on the basis of decreased cell viability, since the Lac⁺ recombinant assay is independent of the number of cells plated. Exonuclease V may play only a marginal role in this type of recombinational event. On the other hand, the lethal sectoring observed in *recB* and *recC* strains (23) may be of much greater significance when an external DNA donor is involved.

lexA and *rep* mutations reduced Lac⁺ progeny formation to levels similar to *recB* and *recC* strains, but did not appear to appreciably affect recombinant yields after conjugation or transduction. This result is of particular interest, since *lexA* mutants phenotypically resemble *recA* strains in all characteristics except for conjugation proficiency (25). Results presented here suggest, however, that the *lexA* protein is required for some types of genetic recombination. Support for this hypothesis is provided by the fact that strand rejoining during repair of psoralen cross-linked DNA is reduced markedly (R. Sinden and R. Cole, personal communication).

With the exception of *recF143*, mutations in any of the genes examined led either to a stimulation of or a reduction in Lac⁺ progeny formation (Table 5). The increases varied from 1.6 (*xseA7*) to 13 (Δ*xthA*). As has been pointed out previously (16), the formation of single-strand breaks in the chromosome is apparently a rate-limiting step in the formation of Lac⁺ recombinants, since strains which accumulate such breaks (*polA, lig, dam,* and *sof* [15–17]) show increased Lac⁺ recombinant formation. Although strand breaks may be required for the initiation of these events, inability to repair them might also dramatically reduce the yield of recombinants. The results obtained at 37°C with the *lig-7*(Ts) strain (Table 2) seem to support this hypothesis.

It is not clear, however, whether strand breaks are rate limiting or even required for conjugation or transduction. With SK703 *lig-7*(Ts), conjugation and transduction were unaffected at 30°C. On the other hand, in the *polA107* strain (SK276), conjugation was actually reduced 90 to 95% while transduction was stimulated slightly. *dam* mutants showed a dramatic increase in conjugation proficiency, particularly when unstable Hfr donors (Ra-2) were employed. Marinus and Konrad have shown changes in the unselected marker inheritance in such crosses (20). In contrast, the number of transductants for a marker very close to the origin of DNA replication (*ilv*) was significantly reduced, while the frequencies for other more distant markers were unaffected.

The greatest stimulation in Lac⁺ recombinant

TABLE 5. *Effect of various mutations on recombination proficiency*

Relevant genotype	Increase (decrease)[a] in recombination proficiency					Source
	Lac$^+$ progeny formation	Conjugation		Transduction		
		Ra-2	KL96	Arg$^+$	His$^+$	
recA13	(>100)	(5,900)	(3,000)	(100)	(32)	35
recA142	(47)	(5,000)	(428)	(42)	(19)	35
recB21 recC22	(5.5)	(121)	(358)	(62)	(17)	35
recC22	(5.0)	(113)	(251)	(36)	(100)	35
recB21	(5.0)	(96)	(176)	(548)	(365)	35
lexA3	(5.0)		(1.2)		(4.5)	This paper
rep-3	(4.4)	(1.8)	(3.1)	(1.3)	(1.3)	This paper
rep-5	(3.4)	(2.9)	(1.2)	(1.2)	(1.2)	This paper
recF143	(1.1)	(1.5)	1.1	1.1	(1.8)	35
xseA7	1.6					5
uvrA6	2.0		1.2		1.3	This paper
sbcB15	2.4	1.2		2.7		35
recL152	2.7	3.1	1.7	1.4	1.8	35
polA480 (polAex1)	3.1					5
recB21 sbcB15	3.5					35
dam-3	3.8	6.3	3.3	1.0	1.3	This paper
lig-7(Ts)	3.9	(1.2)		1.2	1.2	This paper
polA107	4.1	(12.2)	(20)	1.3	2.0	This paper
uvrD3	5.2	12.7	1.8	2.6	2.2	This paper
dam-4	5.8	20.3	8.6	(1.5)	1.1	This paper
uvrD101	6.2					This paper
uvrE4	10.8	1.2	1.1	1.5	1.8	This paper
uvrE100	12.0	4.6	1.4	1.7	1.8	This paper
xthA1	12.3		1.0	1.0	1.0	This paper
Δ*xthA*	13.0		(2.0)	3.0	1.8	This paper

[a] Increases were determined by dividing the mutant frequency by the wild-type frequency. (Decreases) represent the ratio of wild-type to mutant frequency.

formation was observed in strains where accumulation of single-strand breaks has not as yet been observed (*uvrD, uvrE, xthA1,* Δ*xthA*). Although the function of the *uvrD* and *uvrE* gene products remains to be determined, the substrate specificity of exonuclease III (*xthA*) is well characterized (27). Its ability to form single-strand gaps in nicked duplex DNA molecules makes it an appealing candidate for an enzyme mediating recombination. Although its absence has little or no effect on conjugation or transduction, Lac$^+$ recombinant formation is increased markedly. Differences in the presynaptic events for the three processes could account for the observations reported above.

From the data shown in Table 5, it is clear that genetic recombination is a complex process in *E. coli*. Many gene products may serve to limit the nature and extent of genetic recombination within the cell presumably by competing for common intermediates or by preventing the formation of DNA structures which would facilitate genetic exchanges. For example, the lactose duplication system may provide an analog for recombination events which can take place between daughter strands or multi-chromosomes within the cell, such as may be involved in recombinational repair. Although these events would normally go undetected because of a lack of selection method, their occurrence could in fact interfere with DNA replication, chromosome folding, or cell division.

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LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
2. **Boyle, J. M., M. C. Paterson, and R. B. Setlow.** 1970. Excision-repair properties of an *Escherichia coli* mutant deficient in DNA polymerase. Nature (London) **226**:708–710.
3. **Braun, A., and L. Grossman.** 1974. An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from *uvrA* and *uvrB* mutants. Proc. Natl. Acad. Sci. U.S.A. **71**:1838–1842.
4. **Calendar, R., B. Sironi, G. Lindquist, and A. J. Clark.** 1970. Characterization of REP$^-$ mutants and their interaction with P2 phage. Virology **40**:72–83.
5. **Chase, J. W., and C. C. Richardson.** 1977. *Escherichia coli* mutants deficient in exonuclease VII. J. Bacteriol. **129**:934–947.

6. **Clark, A. J.** 1973. Recombination deficient mutants of *Escherichia coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
7. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **53**:451–459.
8. **DeLucia, P., and J. Cairns.** 1969. Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1164–1166.
9. **Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman.** 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
10. **Denhardt, D. T., D. H. Dressler, and A. Hathaway.** 1967. The abortive replication of φX174 DNA in a recombination-deficient mutant of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **57**:813–820.
11. **Glickman, B. W., C. A. van Sluis, H. L. Heijneken, and A. Rörsch.** 1973. A mutant of *Escherichia coli* K-12 deficient in the 5′-3′ exonucleolytic activity of DNA polymerase I. Mol. Gen. Genet. **124**:69–82.
12. **Gottesman, M. M., M. L. Hicks, and M. Gellert.** 1973. Genetics and function of DNA ligase in *Escherichia coli*. J. Mol. Biol. **77**:531–547.
13. **Gudas, L. J., and A. B. Pardee.** 1975. Model for regulation of *Escherichia coli* DNA. Proc. Natl. Acad. Sci. U.S.A. **72**:2330–2334.
14 **Kanner, L., and P. Hanawalt.** 1970. Repair deficiency in a bacterial mutant defective in DNA polymerase. Biochem. Biophys. Res. Commun. **39**:149–155.
15. **Konrad, E. B.** 1977. Method for the isolation of *Escherichia coli* mutants with enhanced recombination between chromosomal duplications. J. Bacteriol. **130**:167–172.
16. **Konrad, E. B., and I. R. Lehman.** 1974. A conditional lethal mutant of *Escherichia coli* K-12 defective in the 5′-3′ exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. **71**:2150–2154.
17. **Konrad, E. B., and I. R. Lehman.** 1975. Novel mutants of *Escherichia coli* that accumulate very small DNA replicative intermediates. Proc. Natl. Acad. Sci. U.S.A. **72**:2150–2154.
18. **Konrad, E. B., P. Modrich, and I. R. Lehman.** 1973. Genetic and enzymatic characterization of a conditional lethal mutant of *Escherichia coli* K-12 with a temperature-sensitive DNA ligase. J. Mol. Biol. **77**:519–529.
19. **Kushner, S. R.** 1974. *In vivo* studies of temperature-sensitive *recB* and *recC* mutants. J. Bacteriol. **120**:1213–1218.
20. **Marinus, M. G., and E. B. Konrad.** 1976. Hyper-recombination in *dam* mutants of *Escherichia coli* K-12. Mol. Gen. Genet. **149**:273–277.
21. **Marinus, M. G., and N. R. Morris.** 1974. Biological function for 6-methyl adenine residues in the DNA of *Escherichia coli* K-12. J. Mol. Biol. **85**:309–322.
22. **Milcarek, C., and B. Weiss.** 1972. Mutants of *Escherichia coli* with altered deoxyribonucleases. I. Isolation and characterization of mutants for exonuclease III. J. Mol. Biol. **68**:303–318.
23. **Miller, J. E., and S. D. Barbour.** 1977. Metabolic characterization of the viable, residually dividing and non-dividing cell classes of recombination-deficient strains of *Escherichia coli*. J. Bacteriol. **130**:160–166.
24. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Mount, D. W., K. B. Low, and S. J. Edmiston.** 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**:886–893.
26. **Ogawa, H., K. Shimada, and J. I. Tomizawa.** 1968. Studies on radiation-sensitive mutants of *E. coli*. I. Mutants defective in the repair synthesis. Mol. Gen. Genet. **101**:227–244.
27. **Richardson, C. C., I. R. Lehman, and A. Kornberg.** 1964. A deoxyribonucleic acid phosphatase-exonuclease from *Escherichia coli*. J. Biol. Chem. **239**:251–258.
28. **Siegel, E. C.** 1973. Ultraviolet-sensitive mutator strain of *Escherichia coli* K-12. J. Bacteriol. **113**:145–160.
29. **Smirnov, G. B., and A. G. Skavronskaya.** 1971. Location of *uvrE502* mutation on the chromosome of *Escherichia coli* K-12. Mol. Gen. Genet. **113**:217–221.
30. **Vapnek, D., and W. D. Rupp.** 1971. Identification of individual sex-factor DNA strands and their replication during conjugation in thermosensitive DNA mutants of *Escherichia coli*. J. Mol. Biol. **60**:413–424.
31. **Willetts, N. S., A. J. Clark, and K. B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**:244–249.
32. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. J. Bacteriol. **100**:923–934.
33. **Yajko, D. M., and B. Weiss.** 1975. Mutations simultaneously affecting endonuclease II and exonuclease III in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:688–692.
34. **Youngs, D. A., and K. C. Smith.** 1976. Genetic control of multiple pathways of post-replicational repair in *uvrB* strains of *Escherichia coli* K-12. J. Bacteriol. **125**:102–110.
35. **Zieg, J., and S. R. Kushner.** 1977. Analysis of genetic recombination between two partially deleted lactose operons of *Escherichia coli* K-12. J. Bacteriol. **131**:123–132.