Genetic Analysis of Conjugational Recombination in *Escherichia coli* K12 Strains Deficient in RecBCD Enzyme

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Conjugational recombination in *Escherichia coli* was investigated by comparing the effects of *recN*, *recO*, *ruv* and *lexA* mutations on the formation of recombinants in crosses with strains lacking RecBCD enzyme. The results presented reveal that *recN* and *ruv* mutations do not abolish residual recombination in a *recB* mutant, and have only a rather modest effect on recombination in *recBC sbcA* strains; in these respects they are quite different from *recF*, *recJ* and *recO* mutations. The differences between these two groups of genes are discussed in relation to the molecular exchanges needed to produce viable recombinants.

INTRODUCTION

During conjugation between Hfr and F⁻ strains of *Escherichia coli*, a single strand of DNA with a 5' end leading from *oriT* of the integrated F plasmid is transferred to the recipient cell where it probably serves as a template for DNA synthesis (Willetts & Wilkins, 1984). Transfer usually terminates prematurely, releasing a DNA fragment composed of the proximally transferred half of the F plasmid linked to a variable length of the Hfr chromosome. The 3' terminus probably remains single-stranded due to the lagging-strand nature of complementary DNA synthesis. Recombination between the Hfr DNA and the recipient chromosome occurs with high efficiency and shows an absolute requirement for RecA protein (Clark & Margulies, 1965; Howard-Flanders & Theriot, 1966) which is known to catalyse homologous pairing and strand exchange between duplex DNA molecules provided at least one of these contains a region that is single-stranded (Radding, 1982).

The efficient recovery of recombinants also requires the activities of *recB* and *recC*. These genes provide subunits of RecBCD enzyme (Hickson *et al.*, 1985; Amundsen *et al.*, 1986), a DNA helicase and exonuclease that has the ability to unwind molecules with blunt, or nearly blunt, duplex ends and in doing so to nick the DNA at specific sequences called Chi (Taylor & Smith, 1985; Taylor *et al.*, 1985; Ponticelli *et al.*, 1985). The requirement for RecBC can be circumvented in certain lysogenic strains by mutations (*sbcA*) of the λ prophage that activate synthesis of exonuclease VIII, the product of *recE* (Barbour *et al.*, 1970; Kushner *et al.*, 1974; Willis *et al.*, 1985) or in the absence of λ by mutation of both *sbcB*, the structural gene for exonuclease I (Kushner *et al.*, 1971, 1972) and *sbcC* (Lloyd & Buckman, 1985). Recombination in *recBC sbcBC* strains depends on *recF* (Horii & Clark, 1973), *recJ* (Lovett & Clark, 1984), *recN* (Lloyd *et al.*, 1983), *recO* (Kolodner *et al.*, 1985), *recQ* (Nakayama *et al.*, 1984), and *ruv* (Lloyd *et al.*, 1984). Furthermore, it is reduced by mutations in *lexA* (Ind⁻) that prevent expression of the SOS response (Lloyd & Thomas, 1983; Lovett & Clark, 1983; Walker, 1984), an effect which is related to the fact that *recN*, *ruv* and *recQ*, are inducible genes regulated by LexA protein (Shurvinton & Lloyd, 1982; Lloyd *et al.*, 1983; Picksley *et al.*, 1984*b*; Irino *et al.*, 1986). *recBC sbcA* strains require at least *recF* and *recJ* (Lovett & Clark, 1984), in addition to *recE* (Gillen *et al.*, 1981). Mutation of any one of these genes has little or no effect on the efficiency of recombinant formation in matings with *recB*⁺ *recC*⁺ strains (op. cit.).

In this paper, we describe a further analysis of conjugational recombination in which we examined the effect of *recN*, *recO*, *ruv* and *lexA* mutations on the formation of recombinants in both *sbc*⁺ and *sbc*⁻ strains lacking RecBCD activity.

METHODS

The *E. coli* K12 strains used are listed in Table 1. Genotype designations are those of Bachmann (1983). UV, MC, Km, Tm and Tc are used to describe the response to ultraviolet light, mitomycin C, kanamycin, trimethoprim and tetracycline, respectively. The Tn10 insertions first identified in strains N3072, N3071 and N3074 were located to *recA*, *recB* and *recC*, respectively, by their cotransducibility with *srl* or *thyA* and by complementation studies using cloned *recA*⁺, *recB*⁺ and *recC*⁺ genes (unpublished work). *recC266*::Tn10 confers a more extreme recombination deficient phenotype than the classical *recC22* allele (Table 2). The presence of mutant *rec* alleles in the strains constructed was deduced from the sensitivity of these strains to UV light or mitomycin C, or by their recombination deficiency. The genotype of multiple *rec* mutants was confirmed by P1 transduction of the relevant alleles to *rec*⁺, *recBC sbcA* or *recBC sbcBC* recipients, as required for their detection.

LB broth and agar, and 56/2 minimal salts media have been described by Lloyd *et al.*, 1974. LB media contained sodium chloride at 0.5 g l⁻¹ except for matings when the concentration of salt was raised to 10 g l⁻¹. Methods for strain construction by P1vir transduction, and for detecting sensitivity to UV light and mitomycin C have been cited by Lloyd & Buckman, 1985 as have procedures for mating F⁻ strains with Hfr or F-prime donors in broth medium, measuring zygotic induction of prophage λ , determining the numbers of viable cells in the recipient cultures used, and for selecting recombinants or F-prime transconjugants.

RESULTS AND DISCUSSION

Recombination was investigated in three closely related genetic backgrounds, all of which lacked RecBCD enzyme. The first carried *recB268*, a Tn10 insertion that reduces recombination by about the same amount as the classical *recB21* allele (Table 2). *recB21* is an insertion of 1.5 kb in *recB* which is polar on the adjacent *recD* gene and therefore eliminates both the B and the D subunits of RecBCD enzyme (Amundsen *et al.*, 1986). We assume that a Tn10 insertion has the same effect. The second carried *recB21 recC22 sbcA23* and the third *recB21 recC22 sbcB15 sbcC201*. Recombination was measured by means of standard Hfr matings. Zygotic induction of prophage λ and recovery of F-prime transconjugants in crosses with strains Hfr GY2200 and the F128 donor, KL548, respectively, provided measures of DNA transfer. Matings with *recA*, *recN*, *recO*, *ruv* and *lexA* single mutants were included as controls. The data obtained are shown in detail in Table 2. The yields of recombinants obtained in crosses with Hfr strains KL226 and AB259 gave similar estimates of the recombination proficiency of mutant strains relative to the *rec*⁺ control strain, AB1157. Data from these two crosses were therefore pooled to provide a mean estimate which we present separately in Table 3 to make comparisons between recipient strains easier.

Residual recombination in recB strains

Mutations in *recB* or *recC* that inactivate RecBCD reduce the number of recombinants recovered from Hfr crosses to about 1% of the normal yield. Previous studies revealed that this residual capacity for recombination depends on *recF* and *recJ* (Gillen & Clark, 1974; Gillen *et al.*, 1981; Lovett & Clark, 1984). In our hands, *recF* and *recJ* mutations were found to reduce the ability of a *recB* strain to form recombinants by factors of about 8-fold and 20-fold, respectively (data not shown). The results presented in Table 3 show that a *recO* mutation has a similar effect. A *recB recO* double mutant was found to produce hardly any more recombinants than a *recA* strain (Table 2). In contrast, *recN* and *ruv* mutations appeared to have little or no effect on recombination in a *recB* strain. Given that *recN* and *ruv* single mutants are slightly deficient in recombination, it is clear from the results presented that *recB recN* and *recB ruv* double mutants are no more deficient than a *recB* single mutant. Since the production of recombinants was also largely unaffected by introducing a *lexA*(Ind⁻) mutation, it seems unlikely therefore that the residual recombination observed in a *recB* mutant is related to the activity of LexA regulated genes. This would be consistent with the fact that introduction of a *lexA*(Def) mutation to increase expression of LexA regulated genes (Walker, 1984) failed to increase recombination in

Table 1. *E. coli* K12 strains

Strain	Relevant genotype*	Other markers	Source or derivation
AB1157	<i>rec⁺ sbc⁺</i>	†	Bachmann (1972)
AB2470	<i>recB21</i>	†	Bachmann (1972)
JC5489	<i>recC22</i>	†	Bachmann (1972)
JC7623	<i>recBC sbcBC</i>	†	Kushner <i>et al.</i> (1971)
JC8679	<i>recB21 recC22 sbcA23</i>	†	Gillen <i>et al.</i> (1981)
RDK1541	<i>recO1504::Tn5</i>	†	Kolodner <i>et al.</i> (1985)
DM49	<i>lexA3(lnd⁻)</i>	†	D. W. Mount
DM1420	<i>lexA51(Def)</i>	†, but <i>sfiA11 arg⁺</i>	D. W. Mount
HI24	<i>ruvA4</i>	†	Otsuji <i>et al.</i> (1974)
CS85	<i>ruv-53</i>	†, <i>eda-51::Tn10</i>	Shurvinton <i>et al.</i> (1984)
CS86	<i>ruv-54</i>	as CS85	Shurvinton <i>et al.</i> (1984)
CS140	<i>ruv-53</i>	as CS85 but <i>eda[?](Tc^s)</i>	Shurvinton <i>et al.</i> (1984)
SP254	<i>recN262</i>	†	Picksley <i>et al.</i> (1984a)
SP256	<i>tyrA16::Tn10 recN262</i>	†	Picksley <i>et al.</i> (1984a)
FB288	<i>recBC sbcA23 recAo254</i>	†	†
N3100	<i>recA269::Tn10</i>	†	P1.N3072 × AB1157 to Tc ^r
N3071	<i>recB268::Tn10</i>	IN(<i>rrnD-rrnE</i>)I	§
N3072	<i>recA269::Tn10</i>	as N3071	§
N3074	<i>recC266::Tn10</i>	as N3071	§
N1434	<i>recAo254</i>	F ⁻ <i>lexA3</i>	Thomas & Lloyd (1980)
N1627	<i>recA200 srl-1300::Tn10</i>	†, but <i>arg⁺ his⁺</i>	
N1630	<i>lexA3 malE::Tn5</i>	†	P1.(<i>malE::Tn5 lexA3</i>) × AB1157 to Km ^r
N1642	<i>lexA3 malE::Tn10</i>	as DM49	P1.(<i>malE::Tn10</i>) × DM49 to Tc ^r
N1824	<i>lexA51</i>	as DM1420 but <i>thyA</i>	Tm selection on DM1420
N1844	<i>lexA51</i>	as DM1420	P1.AB2470 × N1824 to Thy ⁺
N1845	<i>lexA51 recB21</i>	as DM1420	P1.AB2470 × N1824 to Thy ⁺
N2101	<i>recB268::Tn10</i>	†	P1.N3071 × AB1157 to Tc ^r
N2103	<i>recC266::Tn10</i>	†	P1.N3074 × AB1157 to Tc ^r
N2105	<i>lexA3 recB268::Tn10</i>	as DM49	P1.N3071 × DM49 to Tc ^r
N2113	<i>recN262 recB268::Tn10</i>	†	P1.N3071 × SP254 to Tc ^r
N2117	<i>ruvA4 recB268::Tn10</i>	†	P1.N3071 × HI24 to Tc ^r
N2133	<i>ruv-53 recB268::Tn10</i>	as CS140	P1.N3071 × CS140 to Tc ^r
N2232	<i>recBC sbcBC lexA3</i>	† and <i>malE::Tn10</i>	P1.N1642 × JC7623 to Tc ^r
N2237	<i>recBC sbcBC recN262</i>	† and <i>tyrA16::Tn10</i>	P1.SP256 × JC7623 to Tc ^r
N2242	<i>recBC sbcA23 ruv-53</i>	† and <i>eda-51::Tn10</i>	P1.CS85 × JC8679 to Tc ^r
N2244	<i>recBC sbcA23 ruv-54</i>	as N2242	P1.CS86 × JC8679 to Tc ^r
N2249	<i>recBC sbcA23 lexA3</i>	† and <i>malE::Tn5</i>	P1.N1630 × JC8679 to Km ^r
N2254	<i>recBC sbcA23 recN262</i>	† and <i>tyrA16::Tn10</i>	P1.SP256 × JC8679 to Tc ^r
N2283	<i>recBC sbcBC recAo254</i>	†	¶
N2286	<i>recBC sbcBC recAo254 lexA3</i>	† and <i>malE::Tn10</i>	P1.N1642 × N2283 to Tc ^r
N2291	<i>recBC sbcBC recAo254</i>	† and <i>tyrA16::Tn10</i>	P1.SP256 × N2283 to Tc ^r
N2292	<i>recBC sbcBC recAo254 recN262</i>	as N2291	P1.SP256 × N2283 to Tc ^r
N2445	<i>recO1504::Tn5</i>	†	P1.RDK1541 × AB1157 to Km ^r
N2447	<i>recO1504::Tn5 recB268::Tn10</i>	†	P1.N3071 × RDK1541 to Tc ^r
N2510	<i>recBC sbcA23 recO1504::Tn5</i>	†	P1.RDK1541 × JC8679 to Km ^r
N2551	<i>recBC sbcA23 recAo254</i>	† and <i>malE::Tn10</i>	P1.N1642 × FB288 to Tc ^r
N2552	<i>recBC sbcA23 recAo254 lexA3</i>	as N2251	P1.N1642 × FB288 to Tc ^r
N2554	<i>recBC sbcA23 recAo254 recN262</i>	† and <i>tyrA16::Tn10</i>	P1.SP256 × FB288 to Tc ^r
KL548	F(128) <i>proAB⁺ lacI3 lacZ813/Δ(pro-lac)χ111</i>	<i>recA rpsE</i>	K. B. Low
KL226	HfrC (PO2A)	<i>relA1 tonA22</i>	K. B. Low
AB259	Hfr (Hayes, PO1)	<i>relA1 thi-1</i>	K. B. Low
GY2200	Hfr (Hayes) (<i>lnd⁻</i>) ⁺	<i>thi-1 mal</i>	R. Devoret

* *recBC* is used to denote *recB21 recC22*, and *sbcBC* to denote *sbcBC15 sbcC201* (Lloyd & Buckman, 1985).

† F⁻ *thi-1 his-4 Δ(gpt - proA)62 argE3 thr-1 leuB6 kdgK51 rfbD1? ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31*.

‡ P1.N1627 × JC8679 to *recA200 srl-1300::Tn10*, then × P1.N1434 to *srl⁺* and Rec⁺ at 42 °C.

§ Isolated as Tn10 insertion mutants of strain W3110 (Bachmann, 1972) by following the procedure of Silhavy *et al.* (1984) and screening for sensitivity to UV.

|| An *srl-1300::Tn10* derivative of strain N1802 (Lloyd & Thomas, 1983).

¶ Derived from JC7623 as for FB288.

Table 2. *Transconjugant formation and viability of recipient strains in F-prime and Hfr crosses with F⁻ strains carrying combinations of rec, ruv, lexA and sbc mutations*

Mating was for 30 min ($\times$ KL548), 40 min ($\times$ KL226 and $\times$ AB259) or 60 min ($\times$ GY2200). Strain AB1157 was used as the *rec*⁺ control and was mated along with the mutant strains to be tested in all cases. Matings were done in parallel sets, with samples of the same recipient culture being mated with samples of each donor culture and vice versa. The number of viable cells in the recipient cultures used was determined by plating samples on non-selective 56/2 agar. Yields of transconjugants per ml of mating mixture for strain AB1157 are means of 30–40 independent matings. Viability and transconjugant yields for other strains are given relative to AB1157 and are the means of the relative values obtained in at least two, but usually between three and five, independent matings.

Genotype tested*	Strain no.	Viability	Transconjugants per ml relative to strain AB1157			
			$\times$ KL548 (Pro ⁺)	$\times$ GY2200 (λ plaques)	KL226 (Pro ⁺)	$\times$ AB259 (Thr ⁺ Leu ⁺)
<i>rec</i> ⁺	AB1157	$1.9 \times 10^8 = 1.0$	$1.5 \times 10^7 = 1.0$	$5.6 \times 10^6 = 1.0$	$6.7 \times 10^6 = 1.0$	$7.6 \times 10^6 = 1.0$
<i>sbc</i> ⁺ strains						
<i>recA269::Tn10</i>	N3100	0.50	0.34	0.46	0.000011	0.000037
<i>recB21</i>	AB2470	0.32	0.31	1.05	0.0027	0.0012
<i>recB268::Tn10</i>	N2101	0.31	0.24	0.93	0.0017	0.00094
<i>recC266::Tn10</i>	N2103	ND	0.28	ND	0.0023	0.0012
<i>recN262</i>	SP254	1.10	0.71	0.95	0.47	0.53
<i>recN262 recB268</i>	N2113	0.41	0.26	0.95	0.0014	0.00073
<i>recO1504::Tn5</i>	N2445	1.07	0.77	1.16	0.88	0.84
<i>recO1504 recB268</i>	N2447	0.30	0.13	ND	0.0002	0.000067
<i>ruvA4</i>	H124	0.57	0.33	0.93	0.15	0.29
<i>ruvA4 recB268</i>	N2117	0.22	0.13	0.85	0.0019	0.0015
<i>ruv-53</i>	CS140	0.45	0.41	0.69	0.27	0.13
<i>ruv-53 recB268</i>	N2133	0.33	0.16	1.0	0.001	0.00049
<i>lexA3(Ind⁻)</i>	DM49	0.93	0.43	0.75	0.24	0.33
<i>lexA3 recB268</i>	N2105	0.39	0.22	1.07	0.00095	0.0006
<i>lexA51(Def)</i>	N1844	ND	0.45	0.97	1.13	1.00
<i>lexA51 recB21</i>	N1845	ND	0.07	0.27	0.00081	0.0015
<i>recB21 recC22 sbcA23</i> strains						
(<i>recBC sbcA</i>)	JC8679	0.67	0.88	1.23	0.52	0.82
<i>recN262</i>	N2254	0.58	0.83	1.33	0.14	0.25
<i>recO1504::Tn5</i>	N2510	0.34	0.22	1.17	0.0039	0.0076
<i>ruv-53</i>	N2242	0.31	0.013	1.02	0.061	0.038
<i>ruv-54</i>	N2244	0.44	0.038	1.42	0.13	0.25
<i>lexA3</i>	N2249	0.62	0.54	1.24	0.032	0.082
<i>recAo254</i>	N2551	0.57	0.50	1.22	0.47	0.73
<i>recAo254 recN262</i>	N2554	0.43	0.56	0.98	0.21	0.26
<i>recAo254 lexA3</i>	N2552	0.40	0.24	1.27	0.11	0.15
<i>recB21 recC22 sbcB15 sbcC201</i> strains						
(<i>recBC sbcBC</i>)	JC7623	0.60	0.92	0.63	0.60	0.70
<i>recN262</i>	N2237	0.46	0.52	1.02	0.014	0.016
<i>lexA3</i>	N2232	0.39	0.37	1.18	0.002	0.004
<i>recAo254</i>	N2291	0.60	0.95	0.91	0.71	0.69
<i>recAo254 recN262</i>	N2292	0.31	0.74	0.82	0.012	0.017
<i>recAo254 lexA3</i>	N2286	0.64	0.47	0.93	0.014	0.013

* For ease of presentation, transposon insertions are sometimes abbreviated to show the gene symbol and mutant allele number only.

ND, Not determined.

a *recB* strain (Table 3), as was reported by Lovett & Clark (1983). A similar series of strains constructed with *recB21* or *recC266::Tn10* to inactivate RecBCD gave essentially identical findings (data not shown).

Recombination in *recBC sbc* strains

The efficiency of recombination in crosses with *recBC sbcA* strains approaches that of a *rec*⁺ *sbc*⁺ strain. Previous studies (Gillen *et al.*, 1981; Lovett & Clark, 1984) established that

Table 3. Effect of *recN*, *recO*, *ruv*, *lexA* and *recAo* mutations on recombination in *recB* and *recBC sbc* genetic backgrounds

The mean values and standard errors (in parentheses) shown are based on the data obtained from an equal number of crosses with Hfrs KL226 and AB259 (see Table 2 for details) and are not corrected for any deficiency in DNA transfer or viability. Numbers of crosses involved are given as subscripts.

Additional mutation(s) tested	Yield of recombinants relative to the <i>rec⁺ sbc⁺</i> control strain AB1157			
	<i>rec⁺ sbc⁺</i>	<i>recB268::Tn10</i>	<i>recBC sbcA</i>	<i>recBC sbcBC</i>
None	1.0	0.0013(0.0002) ₆	0.73(0.08) ₁₀	0.65(0.05) ₁₀
<i>recO1504::Tn5</i>	0.856(0.06) ₁₀	0.00013(0.00004) ₄	0.006(0.001) ₄	
<i>ruvA4</i>	0.22(0.04) ₆	0.0012(0.0004) ₄		
<i>ruv-53</i>	0.19(0.05) ₄	0.0008(0.0002) ₄	0.05(0.007) ₈	
<i>ruv-54</i>			0.19(0.04) ₄	
<i>recN262</i>	0.49(0.07) ₆	0.0011(0.0003) ₄	0.19(0.03) ₈	0.015(0.002) ₄
<i>lexA3(Ind⁻)</i>	0.29(0.03) ₆	0.0008(0.0001) ₄	0.057(0.015) ₄	0.003(0.0008) ₆
<i>lexA51(Def)</i>		0.0042(0.0005) ₂ *		
<i>recAo254</i>			0.63(0.12) ₄	0.70(0.1) ₈
<i>recAo254 recN262</i>			0.23(0.018) ₆	0.02(0.002) ₄
<i>recAo254 lexA3</i>			0.13(0.018) ₈	0.014(0.0006) ₄

* The strain used in this case (N1845) carried *recB21* and not *recB268*.

recombination in this background requires *recA*, *recF* and *recJ*. The data presented in Table 3 show that *recO* is also needed. Mutation of *recO* reduces the yield of recombinants by more than 200-fold, which is very similar to the effect of a *recF* or *recJ* mutation (op. cit.; R. G. Lloyd & C. Buckman, unpublished work). However, the actual deficiency in recombination may be nearer 100-fold since there is some 3- to 5-fold reduction in cell viability and in the formation of F-prime transconjugants (Table 2). Mutations in *recN* and *ruv* also reduce recombination, but in each case the effect produced is not severe (Table 3). A *recBC sbcA recN* mutant produced 25% as many recombinants as the *recN⁺ (recBC sbcBC)* control strain. With *recBC sbcA ruv* mutants, the reduction in the yield of recombinants ranged from 4-fold (*ruv-54*) to 15-fold (*ruv-53*). Studies with strains carrying *ruv-52* and *ruv::Tn10* (Shurvinton *et al.*, 1984) gave similar results, with the yield of recombinants from Hfr crosses reduced by about 3-fold and 10-fold, respectively (data not shown). It is clear from these results that the recombination deficiency of *recBC sbcA recN* and *recBC sbcA ruv* strains is only some 2- to 3-fold greater than the corresponding *recN* and *ruv* single mutants.

We discovered that mutation of *ruv* in a *recBC sbcA* genetic background interfered with the recovery of F-prime transconjugants. Thus, the yield of Pro⁺ colonies obtained in crosses between KL548 (F128-*pro*⁺) and *recBC sbcA ruv* recipients strains was at least 25-fold lower than with the corresponding *ruv⁺* recipient (Table 2). Since this deficiency can be alleviated entirely by making the *recBC sbcA ruv* strain *recA* (unpublished work), we suspect that it reflects abortive recombination between the transferred F-prime and the homologous region of the recipient chromosome in the absence of *ruv* function. A *recA* mutation would be expected to prevent the initiation of such recombination and thereby allow the F-prime to become established in the transconjugant cell.

Pickley *et al.* (1984b) observed that expression of *recN* is undetectable in the presence of a *lexA(Ind⁻)* mutation. If this is true for a *recBC sbcA lexA(Ind⁻)* strain, the ability to form recombinants should be reduced to the level of a *recBC sbcA recN* strain at the very least, given that a *lexA(Ind⁻)* mutation is also expected to limit expression of *recA*, *recQ* and *ruv*. The data presented in Table 3 reveal that recombination is in fact reduced by a factor approaching 20-fold. To see if this additional effect was due to reduced expression of *recA*, we compared the effects produced by *lexA(Ind⁻)* and *recN* mutations in *recBC sbcA* strains carrying a *recA* operator mutation (*recAo254*) to increase synthesis of RecA. The results (Table 3) reveal that the level of recombination was unaltered in the *recN* derivative but was increased in the *lexA(Ind⁻)* derivative to the extent that the efficiency of recombinant formation is now essentially the same

in both cases. The same observation was made in a *recBC sbcBC* genetic background using a similar series of *recAo*, *recN* and *lexA*(Ind⁻) mutant derivatives, though in this case both *recN* and *lexA* mutations cause a more severe deficiency in recombinant formation. A preliminary report of studies with these *recBC sbcBC* derivatives was published by Picksley *et al.* (1984*b*). We conclude that a *lexA*(Ind⁻) mutation prevents efficient recombination in both the *recBC sbcA* and *recBC sbcBC* genetic backgrounds by abolishing expression of *recN* and reducing expression of *recA*, and that in this respect any reduction in the expression of *ruv* or *recQ* does not have a major additional effect.

Recombination in a *recBC sbcBC* strain is different from that operating in a *recBC sbcA* strain in that it is activated by loss of exonuclease I rather than by synthesis of exonuclease VIII. The reduced requirement for *recN* and *ruv* in the *recBC sbcA* background is presumably a reflection of this difference. Clark *et al.* (1984) and Lloyd & Thomas (1984) suggested that the single-strand overhang expected to be present at the 3' terminus of the DNA transferred in Hfr crosses is normally removed by exonuclease I, which is known to act specifically on single-stranded DNA in the 3'-5' direction. This would eliminate a substrate that might otherwise be used directly by RecA to initiate recombination. However, in *rec*⁺ strains, RecBCD helicase is available to regenerate a substrate for RecA and allow recombination to proceed efficiently. The absence of exonuclease I or synthesis of exonuclease VIII to counter its effect (exonuclease VIII degrades linear DNA duplexes in the 5'-3' direction, Joseph & Kolodner, 1983) would then enable RecA to initiate an exchange at the 3' terminus without prior intervention of RecBCD. Therefore, the discovery that *recN* and *ruv* are essential for recombination in *recBC sbcBC* strains but much less so in a *recBC sbcA* strain suggests that the products of these genes may operate to promote exchanges at the 3' terminus of the Hfr DNA. One possibility consistent with their known involvement in DNA repair (Lloyd *et al.*, 1984; Picksley *et al.*, 1984*a*) is that they help to protect single-stranded DNA from further nuclease digestion. A lack of *recN* or *ruv* function would be less of a problem for *recBC sbcA* strains since single-stranded DNA could always be replenished by exonuclease VIII.

recF, *recJ*, and *recO* are indispensable for conjugational recombination in the absence of RecBCD enzyme. Since these genes are also needed to promote recombination between circular plasmid molecules, whereas *recBC*, *recN* and *ruv* are not (Kolodner *et al.*, 1985), we assume that their products are not restricted to linear DNA substrates with duplex ends as seems to be the case with RecBCD enzyme (Taylor & Smith, 1985). In this sense, the RecFJO proteins may provide an alternative to RecBCD which during conjugation is able to initiate genetic exchanges well away from duplex DNA ends, possibly at transient gaps in the newly synthesized complementary strand, as suggested by Lloyd & Thomas (1984). A recent investigation of β -galactosidase synthesis in conjugational crosses between *lacZ* mutants has revealed that the products of *recFJO* do provide an alternative to RecBCD that can generate recombinant DNA with high efficiency (Lloyd *et al.*, 1987).

This interpretation of the genetics of conjugational recombination leads naturally to the conclusion that the course of recombination in general in *E. coli* will be dictated by the substrates involved, and in particular on whether or not the DNA molecules have ends.

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