The Genetic Dependence of Recombination in *recD* Mutants of *Escherichia coli*

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ABSTRACT

RecBCD enzyme has multiple activities including helicase, exonuclease and endonuclease activities. Mutations in the genes *recB* or *recC*, encoding two subunbits of the enzyme, reduce the frequency of many types of recombinational events. Mutations in *recD*, encoding the third subunit, do not reduce recombination even though most of the activities of the RecBCD enzyme are severely reduced. In this study, the genetic dependence of different types of recombination in *recD* mutants has been investigated. The effects of mutations in genes in the RecBCD pathway (*recA* and *recC*) as well as the genes specific for the RecF pathway (*recF*, *recJ*, *recN*, *recO*, *recQ*, *ruv* and *lexA*) were tested on conjugational, transductional and plasmid recombination, and on UV survival. *recD* mutants were hyper-recombinogenic for all the monitored recombination events, especially those involving plasmids, and all recombination events in *recD* strains required *recA* and *recC*. In addition, unlike $recD^+$ strains, chromosomal recombination events and the repair of UV damage to DNA in *recD* strains were dependent on one RecF pathway gene, *recJ*. Only a subset of the tested recombination events were affected by *ruv*, *recN*, *recQ*, *recO* and *lexA* mutations.

THE *recB*, *recC* and *recD* genes of *Escherichia coli* encode the subunits for a complex enzyme (exonuclease V) which is a single- or double-stranded DNA exonuclease, single-stranded DNA endonuclease, and DNA helicase (reviewed in TELANDER-MUSKAVITCH and LINN 1981; TAYLOR 1988). Under certain conditions the enzyme will also nick linear duplex DNA at specific DNA sequences known as Chi sites (PONTICELLI *et al.* 1985; TAYLOR *et al.* 1985), sites which stimulate recombination *in vivo* (SMITH 1987). Mutations in the *recB* and *recC* genes lead to a reduction in many types of recombination events, a reduction in cell viability and a loss of repair capacity to DNA-damaging agents such as UV light (reviewed in CLARK 1973). Mutants in the *recD* gene, however, are hyper-recombinogenic in many types of genetic crosses, even though they lack detectable exonuclease and helicase activity *in vitro* and the ability to respond to Chi sequences *in vivo* (CHAUDHURY and SMITH 1984; AMUNDSEN *et al.* 1986; BIEK and COHEN 1986; SMITH 1987). To explain these results it has been suggested that the RecB and RecC subunits, even in the absence of the RecD subunit, retain some activity that is sufficient for normal recombination and repair processes (AMUNDSEN *et al.* 1986). *In vitro*, the RecD subunit can be stripped off the RecBCD complex: the RecD-less RecBC enzyme retains 10% of its ATPase activity (LIEBERMAN and OISHI 1974) and some endonuclease and helicase activity (PALAS and KUSHNER 1987). However, it has not been possible to demonstrate any of the RecBCD enzymatic activities in crude extracts of *recD* mutants, even using overproducers of the RecBC complex (A. TAYLOR and G. SMITH, personal communication). It has been assumed that the activities of the RecBCD enzyme which are negated by *recD* mutations must not be important for recombination, since *recD* mutants are recombination-proficient. However, recombination may proceed differently in *recD* mutants than in wild-type strains and use a different "pathway" to mediate recombination, perhaps involving the RecBC complex in addition to other gene products.

Recombination can be mediated without the *recB*, *recC* and *recD* genes by an alternate "pathway," the RecF pathway. This pathway can substitute efficiently for the RecBCD pathway in conjugational combination and P1 transduction if the gene for a single-stranded DNA specific exonuclease (exoI), *sbcB*, is mutant (KUSHNER *et al.* 1971). Additional mutations in one of several genes which together comprise the "RecF pathway" reduce or abolish recombination in *sbcB*-suppressed *recB recC* mutant strains; these genes include *recF*, *recJ*, *recO* (HORII and CLARK 1973; LOVETT and CLARK 1984; KOLODNER, FISHEL and HOWARD 1985) and the *lexA*-regulated genes *recN*, *recQ*, and *ruv* (LLOYD, PICKSLEY and PRESCOTT 1983; LLOYD, BENSON and SHURVINTON 1984; NAKAYAMA *et al.* 1984; IRINO, NAKAYAMA and NAKAYAMA 1986). The RecF pathway does not significantly contribute to conjugational recombination and P1 transduction

in wild-type *E. coli* strains. However, plasmid recombination in wild-type strains requires the products of several RecF pathway genes (*recF*, *recJ* and *recO*, in addition to *recA*) and does not require *recB* or *recC* (FISHEL, JAMES and KOLODNER 1981; LABAN and COHEN 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; KOLODNER, FISHEL and HOWARD 1985).

Recombination in *recD* mutants has some of the characteristics of RecF pathway recombination. These include: high frequency of exchange between close markers (MAHAJAN and DATTA 1977; CHAUDHURY and SMITH 1984; LLOYD and THOMAS 1984) and the localization of exchange near ends during recombination of λ *red gam* (D. THALER, F. STAHL, E. SAMPSON, I. SADDIQI, S. ROSENBERG and M. STAHL, personal communication). To determine if genes in the RecF pathway are required for recombination and repair of UV damage in *recD* mutant strains, a series of strains carrying a *recD* mutation and a mutation in one of the RecF pathway genes (*lexA*, *recF*, *recJ*, *recO*, *recN*, *recQ* and *ruv*) were constructed and compared to strains carrying mutations in *recD* and *recA* or *recC* (genes required for the RecBCD pathway). The results indicated that efficient recombination and repair of UV damage in *recD* mutants required the RecBC complex and often required one RecF pathway gene, *recJ*. In addition, depending on the recombination or repair event examined, a limited number of other RecF pathway gene products were required.

MATERIALS AND METHODS

Strains and plasmids: The strains tested in this study were derived from AB1157 and are described in Table 1. Intermediates in strain constructions requiring two or more steps are listed in Table 1. Strains were constructed by P1 transduction as in WILLETTS, CLARK and LOW (1969). To score *rec* mutations patch tests for conjugational recombination-proficiency and UV-sensitivity were employed (CLARK and MARGULIES 1965). Other strains, not derived from AB1157, used as donors in conjugation or P1 transductions are given in Table 1. The dimer plasmid pRDK41 was used to study recombination of circular plasmids and the dimer plasmid pRDK69 was used to study recombination of linear dimer plasmids. A circular dimer of pBR322, pRDK41 contains one copy each of the *tet-10* and *tet-14* mutant alleles (DOHERTY, MORRISON and KOLODNER 1983); pRDK69 is a derivative of pRDK41 containing an *Xba*I linker insertion in the *Bam*HI site closest to the *tet-14* mutation of pRDK41 (SYMINGTON, MORRISON and KOLODNER 1985). Both plasmids were maintained in *recA304 E. coli* strain JC10287 (CSONKA and CLARK 1978).

Media: Strains were grown routinely in LB medium (WILLETTS, CLARK and LOW 1969). Plate minimal medium consisted of 56/2 salts (WILLETTS, CLARK and LOW 1969) with 0.2% glucose, 0.5 μg/ml thiamine, and 50 μg/ml amino acid supplements. Streptomycin (Sm), nalidixic acid (Nal), kanamycin (Km), ampicillin (Ap) and tetracycline (Tc) were added to 100, 20, 30, 100, and 20 μg/ml concentrations, respectively. P1 was plated on R medium (MILLER 1972) with 50 μg/ml thymine added.

Chromosomal recombination and viability tests: Each double mutant combination was assayed for chromosomal recombination events at least two times, with *rec*$^+$ and *recD* control strains in parallel. Single mutants were assayed one or more times; values obtained are in agreement with previous genetic characterization of these mutants. Values reported are representative of these determinations, except for those of the *rec*$^+$ and *recD* strains, which are averages of 6–10 determinations. Separate determinations did not differ from each other by more than two-fold. Matings were performed for 1 hr at 37° with a 10:1 recipient to donor ratio and used recipient and donor cells that had been grown to an OD_{590} of 0.4. Hfr JC11033 was used to assay inheritance of both a chromosomal marker, *leuB*, by recombination and a plasmid marker, Km^r, conjugally transferred in the same cross. Contraselection in both cases was Ser$^+$ Sm^r in minimal medium. In strains already carrying a gene for Km^r, inheritance of an F′ factor carrying *leu*$^+$ was assayed in an independent cross with RDK1376. A different test of conjugation efficiency measured zygotic induction of a λ prophage during conjugation with RDK1911. After mating, cells were diluted in 56/2 buffer and the number of infective centers determined by plating with CGSC5760 (Nal^r Str^r) with L + 0.7% agar to L Sm Nal plates. As controls, the donor lysogen culture alone was titered with the plating bacteria and the number of free phage in each mating culture was also determined. Since these values were <0.1% of the values obtained for "infective centers" in the mating cultures, the titers presented represent the number of infected transconjugant cells, and not merely zygotic induction in the plating culture or free phage from already lysed cells. The frequency of inheritance has been expressed relative to the *rec*$^+$ control strain, AB1157.

P1 transductions to Leu$^+$ were performed (WILLETTS, CLARK and LOW 1969) using an m.o.i. of 0.1. The P1 transducing lysate was propagated on JC158. The relative transduction frequency is the number of transductants obtained for the strain in question relative to the number obtained for AB1157 transduced in parallel. For each transduction, the number of infected cells was determined by diluting the washed cells after the period of adsorption and plating with a titering culture of AB1157 on R plates. The number of infected cells for the strain in question relative to the number obtained for AB1157 is given as the "relative P1 infectivity" and is included in order to evaluate the P1 transduction data in case the failure to transduce is due to failure of a P1 particle to gain entry into the cell. However, this is an imperfect control since some strains can be infected with P1 yet do not support normal plating of the virus; many Rec$^-$ strains show a concomitant decrease in P1 plating efficiency for unknown reasons, perhaps due to improper metabolism of P1 DNA.

Viability of cells in L broth cultures (OD_{590} = 0.4) was determined by comparing the number of viable cells (determined by diluting and plating to duplicate LB plates) to the total number of cells (determined by counting cells directly in Petroff-Hausser counting chamber using a phase-contrast microscope).

UV survival assays: Cells were grown in L broth and diluted in 56/2 buffer. Dilutions were plated on L medium and the plates were immediately irradiated with a 20 J/m^2 dose of UV irradiation. The plates were then incubated in the dark. Values obtained from this procedure did not vary significantly from those previously obtained by irradiation of the cells in buffer suspension with subsequent dilution and plating.

Plasmid recombination: Fluctuation test analysis was used to determine the frequency of production of Tc^r prog-

eny after transformation of a strain with 44 ng of pRDK41, as described (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982) with the following modifications. Transformants were selected on LB + Ap at 37°. Entire single Ap^r colonies were resuspended in 1 ml of minimal salts, the cell suspension was then serially diluted and 25 μl of each dilution was spotted onto LB+Tc and LB+Ap plates. For each strain, 11 individual Ap^r transformants were analyzed in this way. The fluctuation test was performed one to three times for each strain tested. Recombination rates were calculated by the method of LEA and COULSON (1949). Using this method, $r_0 = M(1.24 + \ln M)$ where r_0 is the median number of Tc^r recombinants among the 11 cultures and M is the average number of recombinants per culture. M was solved by interpolation and then used to calculate the recombination rate, $r = M/N$ where N is the final average concentration of Ap^r transformants in each 1 ml cell suspension.

Linear dimer plasmid transformation efficiencies were determined using *Xba*I-digested pRDK69. Following extensive digestion with *Xba*I, the plasmid DNA was further purified by HPLC chromatography using a Nucleogen DEAE-4000 column (Rainin Instruments, Woburn, Massachusetts) and the chromatography conditions provided by the manufacturer. Fractions containing linear dimers were pooled, dialyzed against 10 mM Tris pH 8.0/1 mM EDTA, extracted with phenol, precipitated with ethanol, and resuspended in 10 mM Tris pH 8.0/1 mM EDTA. Undigested pRDK69 was used as the circular dimer control in the transformations. Competent *E. coli* cells were prepared and transformed essentially as described (WENSINK *et al.* 1974) with each transformation mix containing 56 ng of linear or circular plasmid DNA. The number of Ap^r transformants per ml of culture was determined relative to the total number of viable cells per ml of culture. The efficiency reported is the transformation efficiency of the linear dimer relative to that of the circular dimer analog. The efficiency of transforming different strains by the circular dimer plasmid did not vary more than 10-fold.

RESULTS

Chromosomal recombination events, cell viability and UV survival: The results for conjugational inheritance, viability, P1 transduction and UV survival are given in Table 2. Values relative to wild-type are given for inheritance of a chromosomal marker, *leuB*, by recombination and the conjugal inheritance of a ColE1-derivative plasmid, pML2, carrying Km^r, in the same cross. For those strains already Km^r, inheritance of F′ factor carrying *leuB* was also assayed in a separate cross. Since the plasmid inheritance does not require homologous recombination, it can be used to control for the efficiency with which a strain undergoes conjugation. However, it should be noted that these inheritance values can be lowered by problems in metabolism of plasmid DNA (LOVETT and CLARK 1983; LLOYD, BENSON and SHURVINTON 1984) and also by a lowered viability of the strain. As a different measure of the ability to undergo conjugation, these strains were assayed for zygotic induction of a λ prophage transferred in an Hfr cross, which does not demand stable maintenance of any genetic element in the strain. As a control for P1 transduction, data regarding the relative ability of the strain to produce P1 after infection is also included.

Conjugational recombination was reduced 50- to 100-fold in *recB* and *recC* mutants, and appeared to be increased 2-fold in *recD* mutants. P1 transduction was reduced at least 300-fold in *recB* or *recC* mutants and was increased 2.3-fold in *recD* mutants. The slightly hyper-rec phenotype of *recD* mutants was seen in each of greater than ten independent determinations. The *recB* and *recC* mutants were UV-sensitive; whereas, *recD* mutants were as resistant as wild type. These phenotypes are in agreement with those cited previously (EMMERSON and HOWARD-FLANDERS 1967; WILLETTS and MOUNT 1969; CHAUDHURY and SMITH 1984; BIEK and COHEN 1986). The apparent additivity of *recB* and *recC* mutations for UV survival was unexpected, given the interpretation that they make up subunits of the same enzyme, but the result was reproducible and has been similarly noted by others (DYKSTRA, PRASHER and KUSHNER 1984). The *recB* and *recC* mutations tested can be considered null mutations: *recB21* is a large insertion mutation (AMUNDSEN *et al.* 1986) and *recC22* a nonsense mutation (TEMPLIN, MARGOSSIAN and CLARK 1978).

In *recD* mutant strains, addition of a mutation in *recA* reduced conjugational or transductional recombination to extremely low levels. Addition of a *recC* mutation to a *recD* mutant strain reduced conjugational recombination 140-fold and transductional recombination 70-fold relative to single *recD* mutants—levels which are comparable to *recB* or *recC* mutant strains. The low level of residual recombination in *recD recC* mutant strains is presumably a result of alternate *recB recC*-independent (although *recA*-dependent) recombination pathways. The requirement for *recB* in *recD* strains was not tested since the two genes are tightly linked and construction of the appropriate strain was unsuccessful. However, it is likely that *recB* is required for conjugational and transductional recombination and UV survival in *recD* mutants since the *recB21* mutation examined in this study is polar on *recD* expression (AMUNDSEN *et al.* 1986) and therefore could be considered phenotypically $RecD^-$. These results are consistent with the expectation that in *recD* mutants, like wild-type strains, the RecA and RecBC complex were required to complete these types of recombination.

However, unlike wild-type strains, *recD* mutants required a functional *recJ* gene to mediate recombination after conjugation and for survival after UV-irradiation. Loss of *recJ* function in *recD* strains led to a phenotype that was virtually identical to *recB recC* mutants with respect to conjugation and UV survival. The transposon insertion mutation, *recJ284*::Tn*10*, produced a slightly more extreme phenotype than the *recJ77* allele, the strongest of the remaining known

TABLE 1

Bacterial strains

Strain	Relevant genotype	Source or derivation
A. *recD* derivatives and control strains in AB1157 background[a]		
AB1157	*rec*$^+$	BACHMANN (1972)
DM49	*lexA3*	MOUNT, LOW and EDMISTON (1972)
JC2924	*recA56*	CLARK and MARGULIES (1965)
JC5489	*recC22*	WILLETS and MOUNT (1969)
JC5519	*recB21 recC22*	WILLETS, CLARK and LOW (1969)
JC9239	*recF143*	HORII and CLARK (1973)
JC12123	*recJ284*::Tn*10*	LOVETT and CLARK (1984)
JC13030	*recJ77*	LOVETT and CLARK (1984)
RDK1540	*recN1502*::Tn*5*	KOLODNER, FISHEL and HOWARD (1985)
RDK1541	*recO1504*::Tn*5*	KOLODNER, FISHEL and HOWARD (1985)
RDK1542	*ruvB9*	KOLODNER, FISHEL and HOWARD (1985)
RDK1791	*recD1013 recJ77*	—[b]
RDK1792	*recD1013*	—[c]
RDK1796	*recD1013 recO1504*::Tn*5*	—[d]
RDK1798	*recD1013 recJ284*::Tn*10*	—[e]
RDK1863	*recD1013 recQ1*	—[f]
RDK1864	*recD1013 recA56*	—[g]
RDK1865	*recD1013 lexA3*	—[h]
RDK1868	*recD1013 recF143*	—[i]
RDK1869	*recD1013 ruvB9*	—[i]
RDK1870	*recD1013 recN1502*::Tn*5*	—[d]
RDK1880	*recD1013 recC22*	—[j]
RDK1900	*recQ1*	—[f]
B. Intermediates in strain constructions (related to AB1157)[a]		
RDK1580	*thyA*	—[k]
RDK1631	*metE163*::Tn*10*	—[l]
RDK1788	*thyA serA1 zgb-224*::Tn*10*	—[m]
RDK1789	*thyA recJ77*	—[n]
RDK1858	*thyA recD1013 argA*::Tn*10*	—[o]
RDK1859	*thyA argA*::Tn*10*	—[o]
RDK1860	*recD1013 malE*::Tn*10*	—[p]
RDK1861	*recD1013 srl-300*::Tn*10*	—[q]
RDK1862	*recD1013 metE163*::Tn*10*	—[l]
RDK1866	*thyA recF143 argA*::Tn*10*	—[r]
RDK1867	*thyA ruvB9 argA*::Tn*10*	—[r]
RDK1878	*thyA recD1013*	—[k]
RDK1901	*recD1013 recC22 argA*::Tn*10*	—[s]
C. Miscellaneous strains		
CGSC5760	F506 *aroD argE lac gal man rpsL nalA recA1*	B. BACHMANN
JC158	Hfr PO1 *serA6 thi-1 relA1 lacI22*	CLARK (1963)
JC11033	Hfr PO1 pML2 *serA6 thi-1 relA1 lacI22*	LOVETT and CLARK (1983)
RDK1376	F104::Tn*5*Δ*112 thyA hsdR recA1 rif*	—[t]
RDK1911	Hfr PO1 (λ *c*Iind) *serA6 thi-1 relA1 lacI22*	—[u]
V778	*recD1013 argA*::Tn*10 hsdR supF trpR*? *met*	G. SMITH

[a] Other alleles include *argE3 his-4 proA2 leuB6 thr-1 thi-1 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 supE44 kdgK51*.

[b] Thy$^+$ UV^s Tc^s transductant of RDK1789 using P1 grown on V778.

[c] Thy$^+$ transductant of RDK1580 with P1 grown on RDK1791. *recD* mutation was confirmed by plating phenotype of P2 and λ*red gam*.

[d] Km^r transductant of RDK1792; RDK1796 from P1 grown on RDK1541, RDK1870 from P1 grown on RDK1540.

[e] Tc^r transductant of RDK1792 with P1 grown on JC12123.

[f] Met$^+$ transductant of RDK1862 (for RDK1863) or RDK1631 (for RDK1900) with P1 grown on KD2196 (*recQ1 thyA12 thyR14* + markers of AB1157; from K. NAKAYAMA). The *recQ1* mutation was confirmed by backcrossing to strain RDK1630 (*metE163*::Tn*10 recB21 recC22 sbcB15* + AB1157 markers) giving cotransduction of a UV^s Rec$^-$ phenotype with Met$^+$.

[g] Srl$^+$ UV^s transductant of RDK1861 with P1 grown on JC2924.

[h] Mal$^+$ UV^s transductant of RDK1860 with P1 grown on DM49.

[i] Thy$^+$ Tc^s transductant of RDK1866 (for RDK1868) or RDK1867 (for RDK1869) from P1 grown on RDK1792. (*recD1013* scored by P2 and λ*red gam* plating phenotypes.) RDK1869 is additionally *kdgK*$^+$.

[j] UV^s Tc^s transductant of RDK1792 from P1 grown on RDK1901.

[k] Spontaneous *thyA* mutant of AB1157 (for RDK1580) or RDK1792 (for RDK1878), selected by plating on minimal media with trimethoprim (STACEY and SIMSON 1965).

[l] Tc^r Met$^-$ transductant of RDK1792 (for RDK1862) or AB1157 (for RDK1631) from P1 grown on RK4349 (from B. BACHMANN; *metE163*::Tn*10*).

TABLE 2

Chromosomal recombination events and UV survival

Genotype	Relative conjugational inheritance frequency[a]				Viability (%)	Relative P1 transd.[b]	Relative P1 infectivity	UV (20 J/m²) survival (%)
	Hfr cross		F′ cross plasmid	Hfr cross λ				
	Chromosomal	Plasmid						
rec$^+$	1	1	1	1	80	1	1	51
recD1013	2.0	1.1	1.5	1.1	100	2.3	0.95	40
recB21	0.0059	0.10		0.95	50	<0.002	0.34	0.30
recB21 recC22	0.015	0.13		0.88	20	<0.002	0.27	0.026
recC22	0.020	0.52		0.32	30	0.0029	0.47	0.14
recD1013 recC22	0.014	0.42		0.38	30	0.033	0.54	0.24
recA56	0.000069	0.52		0.17	60	<0.001	0.20	0.0039
recD1013 recA56	0.000043	0.40		0.42	70	<0.001	0.28	0.00050
recJ77	0.80	0.91		0.23	100	2.2	0.067	40
recD1013 recJ77	0.054	0.74		0.69	100	0.43	0.60	2.2
recJ284::Tn*10*	0.59	0.72		0.83	90	1.4	1.0	24
recD1013 recJ284::Tn*10*	0.024	0.20		0.74	100	0.16	0.63	0.028
recF143	1.0	1.1		0.39	60	0.86	0.91	8.6
recD1013 recF143	0.88	1.1		0.39	70	0.80	1.1	4.7
recO1504::Tn*5*	0.53		0.78	0.88	50	1.0	0.69	2.1
recD1013 recO1504::Tn*5*	0.44		0.16	0.64	50	3.0	0.69	0.93
recN1502::Tn*5*	0.53		0.89	0.78	100	0.29	0.47	42
recD1013 recN1502::Tn*5*	0.13		0.060	0.67	90	0.80	0.84	18
recQ1	0.88	1.7		0.65	90	2.8	0.90	54
recD1013 recQ1	1.4	1.1		0.72	100	2.9	0.56	31
ruvB9	0.25	0.59		0.95	100	0.12	0.95	0.14
recD1013 ruvB9	0.25	0.20		0.58	60	0.095	0.39	0.11
lexA3	0.20	1.4		1.8	80	0.27	0.94	0.0057
recD1013 lexA3	0.050	0.62		1.7	40	0.35	0.39	0.0056

[a] Frequencies for conjugational inheritance for the *rec*$^+$ strain ranged from 10 to 50%.
[b] P1 transduction frequencies for the *rec*$^+$ strain were $1–2 \times 10^{-5}$ per plaque-forming units in all determinations.

recJ alleles (LOVETT and CLARK 1984). P1 transduction frequencies in *recD recJ* mutants, however, were reduced only 5- to 14-fold. In contrast, *recB* or *recC* strains showed 70- to greater than 1000-fold less recombination than in *recD* strains. It appears then that *recB recC*-dependent UV survival and conjugational recombination have acquired an almost complete dependence on *recJ* in *recD* mutants, whereas P1 transductional inheritance was only partially dependent on *recJ* in *recD* mutants.

None of the remaining mutations had a clear differential effect on conjugational or transductional recombination in a *recD* mutant versus a *recD*$^+$ genetic background. Some mutations had moderate effects in both backgrounds and some affected stable inheritance of DNA by recombination and extrachromosomally equally. For instance, the *ruv* mutation had a 10- to 20-fold effect on conjugation and P1 transduction in *recD* strains; however, it affected these processes in *recD*$^+$ strains almost equally and produced a similar defect in plasmid inheritance and had a lowered P1 plating efficiency. Likewise, the *lexA* mutation lowered conjugational, and perhaps transductional recombination in *recD* strains but it lowered these events in wild-type strains as well. *recN* mutations may have lowered conjugational recombination somewhat

[m] Tc^r Ser$^-$ transductant of RDK1580 with P1 grown on RDK1445 (*serA1 zgb-224*::Tn*10* derivative of AB1157).
[n] Ser$^+$ transductant of RDK1788 using P1 grown on JC13030.
[o] Tc^r transductants of RDK1580 from P1 grown on V778; RDK1858 is RecD$^-$ and RDK1859 is RecD$^+$.
[p] Tc^r Mal$^-$ transductant of RDK1792 from P1 grown on TST1 (*malE*::Tn*10 araD139 ΔlacU169 rpsL relA fibB deoC* from T. SILHAVY).
[q] Tc^r Srl$^-$ transductant of RDK1792 from P1 grown on RDK1489 (*srl-300*::Tn*10* derivative of AB1157).
[r] Thy$^-$ Tc^r transductant of RDK1542 (for RDK1867) or JC9239 (for RDK1866) with P1 grown on RDK1859. RDK1542 and RDK1867 are additionally *kdgK*$^+$.
[s] Thy$^+$ UV^s transductant of RDK1858 with P1 grown on JC5489; the *recD* mutation was verified by a backcross to RDK1792 with all Tc^r UV^r transductants showing the RecD$^-$ diagnostic phenotype for P2 and λ*red gam* plating.
[t] Transposition of Tn*5Δ112* into F104 in strain KL725 (from B. BACHMANN; F104 *recA13* + AB1157 markers) selected by mating Km^r into HMS187 (*thyA hsdR recA1 rif*) (from J. CAMPBELL).
[u] λ *cI*-ind lysogen of JC158. (λ *cI*-ind provided by F. STAHL.)

TABLE 3

Plasmid dimer recombination in *recD* strains

Genotype	Linear dimer transformation efficiency (relative to circles)	Circular dimer recombination rate (to Tc^r) × 10^4
rec$^+$	0.038	0.015
recB21 recC22	0.020	0.84
recD1013	0.43	2.7
recD1013 recC22	0.017	0.46
recD1013 recA56	0.0064	≤0.044
recD1013 recJ77	0.25	1.0
recD1013 recJ284::Tn*10*	0.060	NT[a]
recD1013 recF143	0.31	3.9
recD1013 recO1504::Tn*5*	0.073	4.8
recD1013 recN1502::Tn*5*	0.022	1.2
recD1013 recQ1	0.33	0.52
recD1013 ruvB9	0.24	3.1
recD1013 lexA3	0.090	0.75

[a] Not tested.

(20-fold) but were also defective in plasmid inheritance, and unaffected for transductional recombination. *recF*, *recO* and *recQ* had only minor effects, if any, on both these recombination events in either genetic background.

None of the mutations tested except *recJ* had any substantial synergism with *recD* with respect to UV survival, although *recA*, *recC*, *recF*, *recO*, *ruv* and *lexA* were required for optimal UV survival in both *rec*$^+$ and *recD* mutant genetic backgrounds. In all cases, the values for the RecF pathway mutations in the *recD*$^+$ background were consistent with previous reports (OTSUJI, IYEHARA and HIDESHIMA 1974; LLOYD, PICKSLEY and PRESCOTT 1983; LOVETT and CLARK 1983, 1984; LLOYD, BENSON and SHURVINTON 1984; NAKAYAMA *et al.* 1984; KOLODNER, FISHEL and HOWARD 1985).

Plasmid recombination: Two types of plasmid recombination events were assayed in *recD* mutant derivatives and the results are presented in Table 3. The first was the efficiency of transformation by a linear dimer plasmid compared to an analogous circular dimer. This transformation efficiency reflects the efficiency of intramolecular recombination of the linear dimer to yield recombinant circular monomers that can be stably maintained in cells (SYMINGTON, MORRISON and KOLODNER 1985). In wild-type strains the transformation efficiency of linear dimers is normally low (about 4% relative to the analogous circular dimer). The low efficiency in wild-type strains appears to reflect the failure of linear dimers to recombine rather than the simple degradation of the linear dimers by RecBCD nuclease or other nucleases. This is because mutations in *xthA* (exoIII) or *recB* and *recC* (exoV) had no effect on the efficiency of transformation of wild-type strains by linear dimers (SYMINGTON, MORRISON and KOLODNER 1985; C. LUISI-DELUCA and R. D. KOLODNER, in preparation) and because *recB* and *recC* were not required for recombination of circular dimer plasmids in wild-type strains (*xthA* was not tested) (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982; and Table 3). In *recD* mutant strains, the transformation efficiency of linear dimers was elevated at least 10-fold over that seen in wild type (Table 3). Restriction mapping verified that the transformants contained recombinant forms (data not shown). The efficient recombination of linear molecules in *recD* strains was reduced by mutations in *recA*, *recC* and *recN*, and to a lesser extent by mutations in *recJ*, *recO* and *lexA*. This reduced recombination of linear dimer plasmids does not reflect a general instability of plasmids in these strains because the linear dimer transformation efficiencies have been normalized to those obtained for an analogous circular dimer plasmid. None of these mutations, or other RecF pathway mutations, reduced the transformation efficiency of wild-type strains by linear dimers below the low level that is normally seen for wild-type strains (SYMINGTON, MORRISON and KOLODNER 1985; unpublished results of C. LUISI-DELUCA and R. D. KOLODNER).

In a second assay of plasmid recombination, the rate of a circular dimer plasmid containing two different mutant *tet* alleles to yield Tc^r recombinants was measured. This rate was elevated 70-fold in *recD* mutants relative to *rec*$^+$ (Table 3). This elevated rate was dependent on *recA* (at least 50-fold reduced) and was partially dependent on *recQ*. *recC* mutations reduced the frequency of recombination in *recD* mutants to the level observed in *recB recC* double mutants. The other mutations tested did not affect this type of recombination. (The *recJ*::Tn*10* insertion mutation, consistently more extreme in phenotype than *recJ77*, could not be tested since the strain is already Tc^r.) In comparison, recombination of circular dimer plasmids in wild-type strains requires the products of the *recA*, *recF*, *recJ* and *recO* genes (FISHEL, JAMES and KOLODNER 1981; LABAN and COHEN 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; KOLODNER, FISHEL and HOWARD 1985; unpublished results of C. LUISI-DELUCA and R. D. KOLODNER).

DISCUSSION

The genetic results presented here indicate that recombination in *recD* mutants is fundamentally different than in wild-type strains, although both types of recombination use the *recB* and *recC* gene products. All of the chromosomal recombination and repair events tested in *recD* mutants showed a dependence on *recC* function to the same extent as seen in *recD*$^+$ strains. In contrast to these chromosomal events, *recB* and *recC* do not normally contribute to recombination of circular plasmids in wild-type strains and linear

dimer plasmids appear to recombine at low levels or not at all in wild-type and *recB recC* mutant strains (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982; SYMINGTON, MORRISON and KOLODNER 1985; Table 3). Rather, a subset of RecF pathway genes appear to mediate recombination of circular plasmids in both wild-type and *recB recC* strains (FISHEL, JAMES and KOLODNER 1981; LABAN and COHEN 1981; JAMES, MORRISON and KOLODNER 1982; COHEN and LABAN 1983; KOLODNER, FISHEL and HOWARD 1985; unpublished results of C. LUISI-DELUCA and R. D. KOLODNER). In this study, plasmid recombination frequencies were elevated in *recD* mutants (as seen previously for circular plasmids by BIEK and COHEN 1986) and they were *recC*-dependent. The smallest effect of *recC* observed was a fivefold reduction in the rate of circular plasmid recombination, to a level comparable to that seen in *recB recC* mutant strains. It appears that *recD* mutations allow both linear and circular plasmids to recombine by the RecBC pathway, which in the case of circular plasmids, is observed above a background of RecF pathway recombination (FISHEL, JAMES and KOLODNER 1981; JAMES, MORRISON and KOLODNER 1982). Because *recB21* is polar on *recD* (AMUNDSEN *et al.* 1986), all of the *recC*-dependent events that occur in *recD* mutants most likely have a dependence on *recB* as well. These results indicate that *recB* and *recC*, in *recD* mutants, have some activity that can act in recombination. Unfortunately, it is not yet clear what biochemical function RecBC has in the absence of the RecD subunit.

The RecBC complex in the absence of the RecD subunit required other gene products that were not required in the presence of the RecD subunit. The most striking requirement was for *recJ* which was required for all of the events detected, with the possible exception of the recombination of circular dimer plasmids where it was not possible to test the tightest *recJ* allele (*recJ284*::Tn*10*). Recently, we have overproduced the RecJ protein and shown that accumulation of RecJ protein in cells is accompanied by the accumulation of a new exonuclease activity (unpublished results of S. T. LOVETT and R. KOLODNER). This observation suggests that some exonuclease activity that is normally provided by the RecBCD complex may be required for RecBC-dependent recombination. Further analysis of the RecJ protein should provide additional insights into the activities of the RecBCD complex that are normally required for recombination.

In addition to *recJ*, other RecF pathway gene products were required for recombination and repair in *recD* mutants. The degree of dependence on a given gene product varied depending on the recombination or repair event tested. Our results are consistent with a small dependence of conjugational recombination on *recN*, *ruv* and *lexA*, and a small dependence on *ruv* and *lexA* for transductional recombination. *ruv* and *lexA* were also partially required for both conjugational and transductional recombination in wild-type strains. Recombinational repair in *recD* mutants, as well as in wild-type strains, required *recF*, *recO*, *ruv* and *lexA*. Recombination of linear dimer plasmids in *recD* mutants required *recO*, *recN* and *lexA*, whereas the recombination of circular dimer plasmids in *recD* mutants did not require any of the RecF pathway gene products, with the possible exception of *recQ*. In addition to *recC*, all of the events also required *recA*. And, all events that required *lexA* also required a *lexA* regulatable gene like *ruv* or *recN*. That such a variety of effects was observed is not surprising because each event involves a different substrate and probably recombines by a different mechanism. Furthermore, for any given recombination event, the substrates that are available for recombination in *recD* mutants are most likely different from the substrates that are available for recombination in wild-type strains. It is clear that further biochemical analysis of the different proteins and further mechanistic analysis of the different recombination events will be required to explain these different effects. The results presented here provide information about the types of recombination events that will be useful for studying these proteins and provide clues as to the roles that some proteins may play in recombination and repair.

The difference between the genetic requirements for the recombination of linear plasmid dimers and the recombination of circular dimer plasmids is striking. *recD* mutant strains are known to accumulate linear concatemers of plasmid DNA and recombination of circular plasmids may really represent recombination of this linear concatemeric DNA (COHEN and CLARK 1986). The results reported here suggest that the mechanism of recombination of these two types of substrates may be different, and/or that the *recJ*, *recN*, *recO* and *lexA* gene products may be required to stabilize linear DNA prior to circularization in the cell.

The fact that the effects of mutations in RecF pathway genes vary greatly depending on the recombination event tested in *recD* mutants points out that recombination "pathways" are much more fluid than previously appreciated. Among the 10 or so recombination genes known in *E. coli*, only two, *recB* and *recC*, have identical phenotypic effects. Mutations in each of the other genes are unique with respect to the types of recombination events affected, the severity of the mutant defect, and their interaction with other genes. It is clear that among recombination genes there must be some redundancy of function and the

genetic dependence of recombination will vary with the substrates provided.

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