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Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional *recN* product

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Summary. Mutation of the *recN* gene of *Escherichia coli* in a *recBC sbcB* genetic background blocks conjugational recombination and confers increased sensitivity to UV light and mitomycin C. The basis for this phenotype was investigated by monitoring the properties associated with *recN* mutations in otherwise wild-type strains. It was established that *recN* single mutants are almost fully resistant to UV irradiation, and that there is no detectable defect in repair of UV lesions by excision, error-prone, or recombinational mechanisms. However, *recN* mutations confer sensitivity to mitomycin C and ionizing radiation both in wild-type and *recB sbcB* strains. The sensitivity to ionizing radiation is correlated with a deficiency in the capacity to repair DNA double-strand breaks by a UV inducible mechanism. Recombinant λ phages that complement the recombination and repair defects of *recN recBC sbcB* mutants have been identified, and the *recN* gene has been cloned from these phages into a low copy-number plasmid.

Introduction

The study of *Escherichia coli* mutants deficient in conjugational recombination has revealed that they are also defective in repair of damaged DNA (Clark 1973; Hanawalt et al. 1979). Mutation of any one of the seven recombination genes (*recA, recB, recC, recF, recJ, recN*[1], *ruv*) identified so far is associated with increased sensitivity to agents that cause damage to DNA. The repair phenotype varies with the gene concerned, the type of lesion introduced into the DNA, and the genetic background of the mutant strain (Clark and Margulies 1965; Willetts and Mount 1969; Horii and Clark 1973; Lloyd et al. 1983, 1984; Lovett S and Clark AJ – J Bacteriol, in press). *recF, recJ*, and *recN* mutations were identified originally in a *recBC*[2] *sbcB* genetic background where the recombination deficiency associated with *recB* and *recC* mutations is suppressed by mutation of *sbcB*, the structural gene for exonuclease I (Kushner et al. 1971, 1972; Clark 1973). The defect in repair associated with these mutations is much less extreme in a *recBC*$^+$ *sbcB*$^+$ strain, which is probably related to the fact that recombination proficiency is not significantly reduced in this background.

The *recA* gene is indispensible for recombination and has been shown to play an important role in the postreplicative repair of lesions introduced into DNA by ultraviolet (UV) light. Rupp et al. (1971) and Ganesan (1974) demonstrated that single-strand gaps present in DNA after replication of a UV-irradiated template could be closed by a *recA* dependent mechanism that involves the exchange of sister strands between damaged and undamaged regions of the daughter molecules. The *recA* product has been identified and characterized extensively in vitro (Radding 1982). It can bind to gaps in DNA and catalyses homologous pairing and strand exchange between a variety of DNA substrates, including molecules that share imperfect homology or which contain UV lesions (DasGupta and Radding 1982; Livneh and Lehman 1982; West et al. 1982).

Recombination has also been implicated in the repair of double-strand breaks in DNA following exposure of cells to ionizing radiation. The molecular basis for this type of repair is not yet clear, though it has been suggested that the integrity of an interrupted duplex could be restored by coupling strand exchange with DNA synthesis using an uninterrupted region of a homologous duplex as a template (Szostak et al. 1983). Repair of double-strand breaks in *E. coli* appears to be an inducible process, since resistance to ionizing radiation can be increased by prior irradiation of cells with UV light (Pollard et al. 1981). It requires the presence of a homologous duplex, *recA* protein, and possibly other gene products that are induced by UV light (Krasin and Hutchinson 1977, 1981).

We have recently identified a new recombination gene (*recN*) whose expression, like that of *recA* and several other genes involved in DNA repair (Little and Mount 1982), is induced following damage to DNA (Lloyd et al. 1983). In this paper, we describe the DNA repair phenotype of *recN* mutants in more detail and present evidence that the *recN* product is needed specifically for the repair of double-strand breaks. We also describe the isolation of phages and plasmid clones that restore recombination proficiency and radiation resistance to *recN* mutants.

Materials and methods

Strains. The *E. coli* K12 strains used are listed in Table 1a. *recN* is the locus defined by the *rec-259, rec-261*, and *rec-262*

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1 *recN* is the locus identified by *rec-259* (Lloyd et al. 1983)

2 *recBC*, with superscript + or −, is used throughout to denote strains that are wild-type or mutant, respectively, for both *recB* and *recC*

Table 1. *Escherichia coli* K12 and strains

Strain	Genotype	Source or derivation
a) *E. coli* strains		
AB1157	F⁻ *thi-1 his-4 proA2 argE3 thr-1 leuB6 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31*	Bachmann 1972
JC7623	F⁻ as AB1157 but *recB21 recC22 sbcB15*	Kushner et al. 1971
JC12334	F⁻ as AB1157 but *tna-300::*Tn*10 recF143*	Ream et al. 1980
AB2463	F⁻ as AB1157 but *recA13*	Bachmann 1972
AB1886	F⁻ as AB1157 but *uvrB5*	Bachmann 1972
N3078	F⁻ *pheA13*::Tn*10*	[a]
N3141	F⁻ as AB1157 but *pheA13*::Tn*10*	P1.N3078 × AB1157
AT757	F⁻ as JC7623 but *thr⁺ leu⁺ lacI3 lacz118 ilvO⁻ recF143*	[b]
SP213	F⁻ as JC7623 but *pheA13*::Tn*10*	P1.N3078 × JC7623
SP215	F⁻ as JC7623 but *thyrA16*::Tn*10 recN261*	Lloyd et al. 1983
SP216	F⁻ as JC7623 but *tyrA16*::Tn*10 recN262*	Lloyd et al. 1983
SP226	F⁻ as JC7623 but *recN261*	P1.SP215 × SP213
SP231	F⁻ as JC7623 but *recN262*	P1.SP216 × SP213
SP253	F⁻ as AB1157 but *recN261*	SP255 to Tyr⁺(Tet^S)
SP254	F⁻ as AB1157 but *recN262*	SP256 to Tyr⁺(Tet^S)
SP255	F⁻ as AB1157 but *tyrA16*::Tn*10 recN261*	P1.SP215 × N3141
SP256	F⁻ as AB1157 but *tyrA16*::Tn*10 recN262*	P1.SP216 × N3141
SP263	F⁻ as AB1886 but *tyrA16*::Tn*10*	P1.SP256 × AB1886
SP264	F⁻ as AB1886 but *tyrA16*::Tn*10 recN262*	P1.SP256 × AB1886
SP267	F⁻ as AB1157 but *tna-300*::Tn*10*	P1.JC12334 × AB1157
SP268	F⁻ as AB1157 but *tna-300*::Tn*10 recF143*	P1.JC12334 × AB1157
SP269	F⁻ as JC7623 but *tna-300*::Tn*10*	P1.JC12334 × JC7623
SP270	F⁻ as JC7623 but *tna-300*::Tn*10 recF143*	P1.JC12334 × JC7623
SP271	F⁻ as SP253 but *tna-300*::Tn*10*	P1.JC12334 × SP253
SP272	F⁻ as SP253 but *tna-300*::Tn*10 recF143*	P1.JC12334 × SP253
SP273	F⁻ as SP254 but *tna-300*::Tn*10*	P1.JC12334 × SP254
SP274	F⁻ as SP254 but *tna-300*::Tn*10 recF143*	P1.JC12334 × SP254
SP308	F⁻ *car-98*::Tn*10 lacI3 lacZ118 proB48 metE90 trpA9605 his-29 gyrA19 rpsL171*	P1.N3050[a] × KL320 (Birge and Low 1974)
NH4104	F-prime(F42) *lac⁺*/*uvrA6 proA2 leu-8 thr-4 his⁻ thi⁻ ara-14 lac-1*	K.B. Low
KL548	F-prime(F128) *lacI3 lacZ813 proAB⁺*/*Δ(lac-pro)*$_{XIII}$ *rpsE⁻ xyl⁻ mtl⁻ recA1*	K.B. Low
KL226	Hfr(Cavalli) *relA1 tonA22*	K.B. Low
b) phage λ strains		
λPE11	*sbhI 1° Chi⁺ Anin5 cI857*	Arthur et al. 1982
λRL121	as λPE11 but *recN⁺* region replacing *int-cIII*	This work[c]
λRL122	as λPE11 but *recN⁺* region replacing *int-cIII*	This work[c]

[a] Tn*10* insertion mutants of W3110 (Bachmann 1972) isolated by the procedure of Berman et al. (1981)
[b] From JC7623 × KL334 (Birge and Low 1974), followed by transduction to valine resistance with P1 from an *ilvO recF143* strain
[c] Isolated from the λ library of Arthur et al. (1982)

mutations described by Lloyd et al. (1983). Strains were constructed by transduction or by conjugation and the genotype confirmed when necessary by appropriate backcrosses. F42 *lac⁺* was introduced into F⁻ strains by mating with strains NH4104 and selecting Lac⁺(*rpsL*) transconjugants. Phage λ strains are listed in Table 1b. The library of recombinant λ phages screened for *recN* transducing particles was that described by Arthur et al. (1982).

Media. Luria broth (LB) and agar, 56/2 minimal salts media and dilution buffer have been referred to previously, as have media for routine propagation of λ stocks (Lloyd et al. 1974; Berman et al. 1981). Media were supplemented with 20 μg/ml tetracycline, 25 μg/ml ampicillin, 40 μg/ml kanamycin, or 100 μg/ml streptomycin, when required.

Enzymes. Restriction endonucleases and T4 DNA ligase were obtained from commercial sources and used as directed by the suppliers.

Methods for measuring growth in liquid culture and sensitivity to UV light and gamma-radiation have been described previously, as have methods for mating F⁻ strains with Hfr or F-prime strains to measure recombination proficiency and F-prime repliconation (Lloyd et al. 1974; Lloyd and Barbour 1974; Lloyd and Low 1976). Broth medium for matings contained 10 g sodium chloride per litre. P1 transductions followed the method of Miller (1972), modified as described by Shurvinton and Lloyd (1982). The source of gamma radiation was ^{60}Co, with an output of 0.88 Krad at 5 cm from the source. Methods for working with phage λ and for preparing λ DNA were essentially as described by Berman et al. (1981). Plasmid DNA was extracted by the scaled up "miniprep" method of Ish-Horowicz and Burke (1981). Procedures for separation of DNA restriction fragments by agarose gel electrophoresis, ligation and transformation of plasmid DNA into calcium treated cells have been described elsewhere (Berman et al. 1981; Shurvinton et al. 1984).

Mitomcycin C sensitivity. Strains were grown at 37° C in LB broth to approximately 2×10^8 cells/ml, washed and resuspended in 56/2 minimal salts containing 2 μg mitomycin C per ml and incubated at 37° C. Samples removed at intervals were diluted and plated on LB agar. Colonies of survivors were scored after 24–48 hr incubation at 37° C. Plate assays were as described by Lloyd et al. (1974).

Repair of DNA double-strand breaks. The method used to monitor the conversion of low molecular weight DNA fragments, generated by exposing cells to ionizing radiation, to high molecular weight molecules was essentially that described by Krasin and Hutchinson (1981). Cells were grown for several generations in the presence of (methyl-^{3}H) thymidine (specific activity 25 Ci/mmol, Amersham) before irradiating with 10 J/m^2 UV light and incubating in the dark for 45 min at 37° C to allow synthesis of inducible proteins. Rifampicin was added to a final concentration of 75 μg/ml and the cells exposed to 12 Krad gamma radiation while being held on ice. Samples of the cells exposed to both UV and gamma radiation were divided into two portions, one of which was kept on ice while the other was incubated for 45 min at 37° C along with a control sample exposed to gamma radiation but which had not received an inducing dose of UV light. For each treatment, a sample of the cells was lysed on top of a linear 5–20% neutral (pH 7.4) sucrose gradient and centrifuged at 3,750 rpm for 40 hr at 20° C (SW41 Beckman rotor). Samples of equal volume were collected from the bottom of each gradient and the radioactivity determined by liquid scintillation.

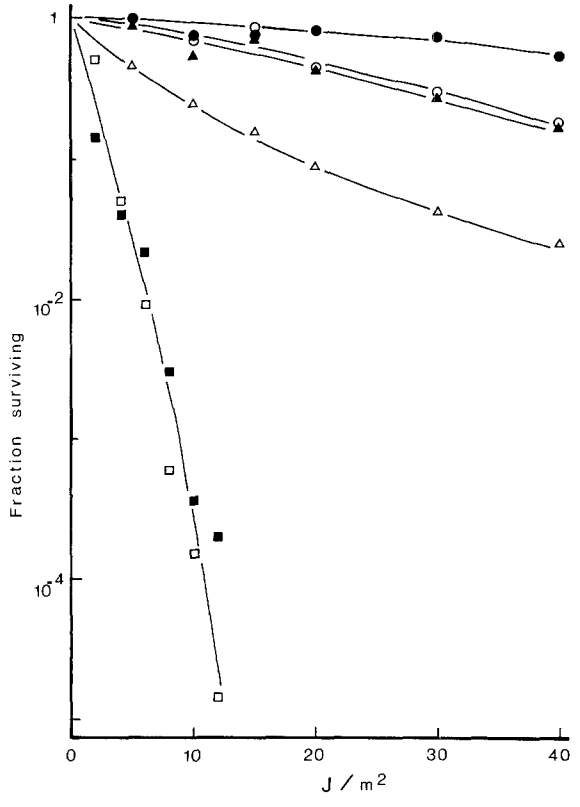

Fig. 1. Effect of genetic background on the UV survival of *recN* mutants. The strains used were: ● AB1157(wild-type); ○ SP254(*recN262*); ▲ JC7623(*recBC sbcB*); △ SP231(*recBC sbcB recN262*); ■ SP263(*uvrB5*); □ SP264(*uvrB5 recN262*)

Table 2. Conjugational recombination in *recN* mutants

	Recipient strain	Transconjugants per ml mating mixture[a]	
		× KL548(F-*lac*$^+$)	× Hfr KL226
a)	AB1157 (wild-type, *rec*$^+$)	1.1×10^7	2.3×10^7 (1.0)
	SP253 (*recN261*)	1.3×10^7	6.7×10^6 (0.25)
	SP254 (*recN262*)	7.6×10^6	8.7×10^6 (0.57)
	JC7623 (*recBC sbcB*)	7.9×10^6	5.9×10^6 (0.37)
	SP226 (*recBC sbcB recN261*)	4.6×10^6	8.8×10^4 (0.0097)
	SP231 (*recBC sbcB recN262*)	4.4×10^6	5.7×10^4 (0.0064)
b)	SP267 (wild-type, *rec*$^+$)	4.7×10^7	2.1×10^7 (1.0)
	SP268 (*recF143*)	5.5×10^7	1.7×10^7 (0.69)
	SP269 (*recBC sbcB*)	3.4×10^7	6.4×10^6 (0.42)
	SP270 (*recBC sbcB recF143*)	2.6×10^6	3.3×10^3 (0.0026)
	SP271 (*recN261*)	3.9×10^7	3.9×10^6 (0.22)
	SP272 (*recN261 recF143*)	3.5×10^7	3.7×10^6 (0.23)
	SP273 (*recN262*)	2.9×10^7	4.8×10^6 (0.37)
	SP274 (*recN262 recF143*)	4.0×10^7	3.5×10^6 (0.20)
c)	AB1157 (wild-type)(λPE11)$^+$	1.7×10^7	2.5×10^7 (1.0)
	AB1157 (λRL121)$^+$	1.2×10^7	1.7×10^7 (0.96)
	AB1157 (λRL122)$^+$	2.0×10^7	2.5×10^7 (0.85)
	JC7623 (*recBC sbcB*)(λPE11)$^+$	9.1×10^6	1.3×10^7 (1.0)
	JC7623 (λRL121)$^+$	1.6×10^7	1.1×10^7 (0.45)
	JC7623 (λRL122)$^+$	2.1×10^7	1.2×10^7 (0.39)
	SP231 (*recBC sbcB recN262*) (λPE11)$^+$	4.8×10^6	7.0×10^4 (0.011)
	SP231 (λRL121)$^+$	1.7×10^7	9.5×10^6 (0.38)
	SP231 (λRL122)$^+$	1.8×10^7	1.0×10^7 (0.37)

[a] Donor and recipient strains were grown in LB broth to approximately 2×10^8 cells/ml, mixed in a ratio of 1:4 and mated for 30 min (F-prime donor) or 60 min (Hfr donor) before terminating mating and imposing selection for Pro$^+$(*rpsL*) transconjugants. Mating was at 37° C (a and b) or 32° C (c). Colonies were scored after 48–72 hrs incubation. Values in parenthesis are recombination frequencies relative to the wild-type control corrected for any deficiency in F-prime transfer

Identification of λ recN transducing phages. The λ library of Arthur et al. (1982) was screened for phage particles that transduced a *recN* mutant to mitomycin C resistance. Strain SP216 $(\lambda cI857)^+$ was infected with a sample of the library at a multiplicity of 0.01–0.1 phage per cell and after 20 min at 32° C to allow adsorption the infected cells were spread on LB agar containing 0.5 μg mitomycin C per ml. After 24 hr incubation at 32° C, large colonies of mitomycin C resistant transductants were purified and used to prepare phage lysates by thermal induction. The lysates were purified to single plaques and $\lambda recN^+$ phages identified by cross-streaking against strain SP216 on agar plates containing mitomycin C (Shurvinton et al. 1984).

Results and discussion

The *recN* gene is located at minute 56.5 on the standard genetic map of *E. coli* (Bachmann 1983) and was identified as an inducible gene, mutation of which severely reduces the efficiency of conjugational recombination and confers increased sensitivity to UV light and mitomycin C in a *recBC sbcB* genetic background (Lloyd et al. 1983). However, when $recBC^+$ function was restored, or the *recN* mutation transferred to a $recBC^+$ $sbcB^+$ strain, the level of recombinant formation was found to be within 30–50% of normal and the strains produced were almost fully resistant to UV light, although they remained sensitive to mitomycin C (Lloyd et al. 1983; Figs. 1, 5, Table 2a).

Most of the lesions introduced into DNA by irradiating cells with UV light are normally removed in the dark by an excision mechanism that relies on the *uvrA,B,C* products (Hanawalt et al. 1979; Sancar and Rupp 1983). *uvr* mutants are highly sensitive to UV light, and repair is restricted to postreplicative recombinational and error prone mechanisms that requires *recA* function (Witkin 1976; Hall and Mount 1981). *recN262* was introduced into *uvrA, uvrB,* and *uvrC* mutants and the UV sensitivity of the double mutants was determined. In all three cases, there was no significant increase in UV sensitivity compared with the *uvr* single mutants, which suggests that postreplicative repair is unaffected. The results obtained with *uvrB* are shown in Fig. 1. Since *recN* mutants also reactivate UV irradiated λ phage with normal efficiency, and are as UV mutable as a $recN^+$ strain (data not shown), we conclude that *recN* has no major role in the repair of UV lesions.

Although $recBC^+$ function alleviates the UV sensitivity of *recN recBC sbcB* mutants, the results shown in Fig. 2a demonstrate that it does not restore resistance to mitomycin C. A *recN* single mutant is as sensitive to mitomycin C as a *recN recBC sbcB* strain when compared with the otherwise isogenic $recN^+$ control strain. Mitomycin C is a cross-linking agent which produces lesions affecting both strands of the DNA duplex simultaneously. The repair of cross-linked DNA is thought to involve *uvr* dependent incision of one strand followed by a recombinational exchange with an undamaged homologous duplex (Linn et al. 1977; Sinden and Cole 1978). Since *recN* mutants appear proficient in recombinational exchange of single DNA strands, we decided to examine their sensitivity to ionizing radiation to see if they could be deficient in the repair of double-strand breaks in DNA. The results (Fig. 2b) revealed that loss of *recN* function severely reduces resistance to ionizing radiation, both in wild-type and *recBC sbcB* backgrounds.

Krasin and Hutchinson (1981) demonstrated that the repair of double-strand breaks is an inducible process whose efficiency can be enhanced by prior exposure to UV light, and that following this inducing treatment the repair of double-strand breaks introduced by subsequent exposure to gamma-radiation can be monitored by lysing the cells and sedimenting the DNA through a neutral sucrose gra-

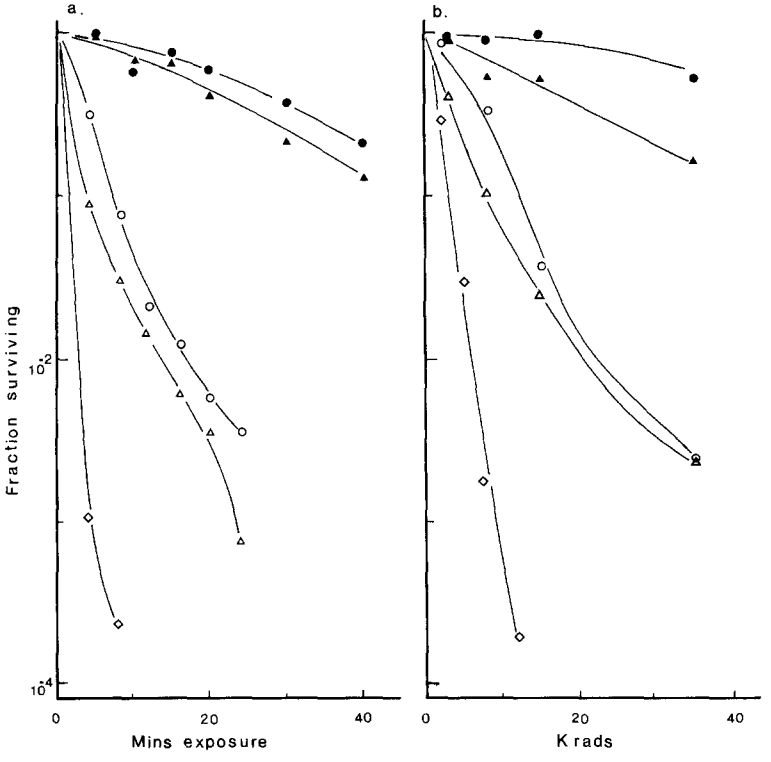

Fig. 2a, b. Survival of *recN* and *recN recBC sbcB* mutants after exposure to **a**, mitomycin C, and **b**, gamma-radiation. ● AB1157(wild-type); ▲ JC7623(*recBCsbcB*); ◇ AB2463(*recA13*); △ SP231(*recBC sbcB recN262*); ○ SP254(*recN262*)

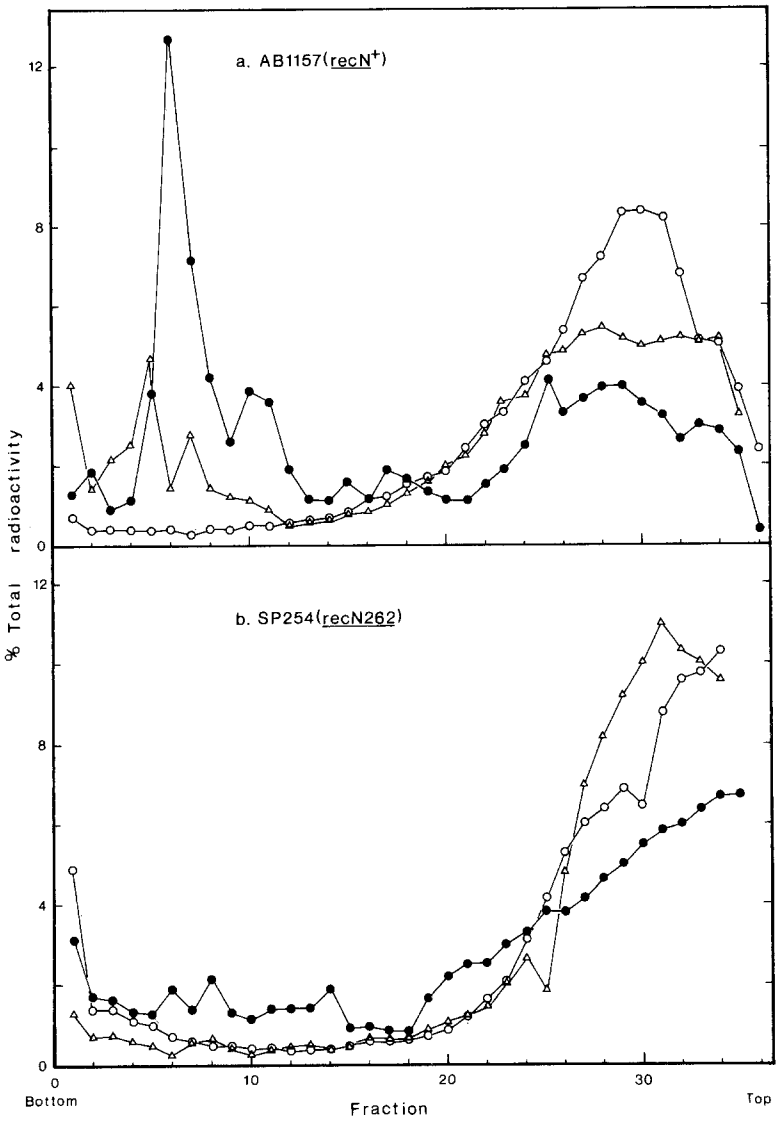

Fig. 3a, b. Neutral sucrose sedimentation of (^{3}H)thymidine-prelabelled DNA from gamma-irradiated cells with or without prior UV irradiation. Symbols: o UV irradiated but no post gamma irradiation incubation; • UV irradiated and incubated for 45 min after gamma irradiation; ▵ no UV irradiation but incubated for 45 min after gamma irradiation. **a** AB1157(*recN*$^+$); **b** SP254(*recN262*)

dient. We have used this method to gain a more direct measure of whether *recN* mutants are deficient in repair of double-strand breaks. The results shown in Fig. 3a demonstrate that when a *recN*$^+$ strain is irradiated with UV light and then incubated for a 45 min period to allow synthesis of UV-inducible proteins (Little and Mount 1982) before exposing the cells to ionizing radiation, most of the DNA is found to sediment near the top of the gradient, and is presumably in the form of low molecular weight fragments. However, if the cells were incubated for 45 min after exposure to the ionizing radiation a peak of DNA was found to sediment near the bottom of the gradient, suggesting that some of the DNA fragments had been joined together to give high molecular weight molecules. According to Krasin and Hutchinson (1981), this shift in molecular weight of gamma-irradiated DNA is due to repair of double-strand breaks. There was much less evidence of this repair activity in the absence of an inducing dose of UV light. The results in Fig. 3b show that repair is absent in a *recN* mutant. This cannot be due to a failure to derepress UV inducible genes since expression of *recN* itself is induced by damage to DNA in a strain carring a Mu-*d*(Ap^R *lac*) insertion in *recN* (Lloyd et al. 1983), including UV-induced damage (unpublished work). Furthermore, *recN* mutants filament profusely after damage to their DNA, a feature which is associated with increased expression of inducible genes involved in repair (Huisman and D'Ari 1981; Little and Mount 1982). We conclude that the *recN* product itself must be necessary for the repair of double-strand breaks. Whether it participates directly in the repair process or is needed simply to protect the damaged DNA cannot be decided on the basis of these results.

Bonura and Smith (1975) and Wang and Smith (1982) have shown that double-strand strand breaks are occasionally introduced into DNA during enzymic repair of UV lesions. This may account for the slight UV sensitivity of *recN* single mutants. The more extreme sensitivity of *recN recBC sbcB* mutants suggests that double-strand breaks may arise more frequently in this genetic background or that in the absence of *recBC* function, repair is restricted to a mechanism dependent on the *recN* product. In a *recBC sbcB* strain, recombination and DNA repair are both dependent on the *recF* product as well as the *recA, recJ, recN,* and *ruv* products. *recF* mutations differ from *recN* mutations in that they confer a more extreme sensitivity to UV light in a wild-type background and increase the sensitivity of *uvr* mutants (Horii and Clark 1973; Rothman

Table 3. Chromosome mobilization by F42-*lac*$^+$ from *recBC sbcB* mutants carrying *recN* or *recF* mutations

F-prime donor strain (F42)	Relative frequency of transconjugants[a]	
	Lac$^+$	Car$^+$
AB1157 (wild-type, *rec*$^+$)	1.0	1.0
JC7623 (*recBC sbcB*)	0.27	0.16 (0.59)
SP215 (*recBC sbcB recN261*)	0.49	0.16 (0.33)
SP216 (*recBC sbcB recN262*)	0.20	0.046 (0.23)
AT757 (*recBC sbcB recF143*)	0.23	0.0041 (0.018)

[a] Donor and recipient strains were grown in LB broth to approximately 2×10^8 cells/ml, mixed in a ratio of 1:4 and mated for 30 min before sampling for Lac$^+$, F-prime repliconants, and for 60 min before sampling for Car$^+$ recombinants. The recipient strain was SP308 (*lacZ car-98*::tn*10*) and counterselection of the donor on the selective agar plates was achieved by the absence of required aminoacids. Incubation was at 37° C and colonies were scored after 48 h. Values in parenthesis are corrected for any deficiency in F-prime repliconation

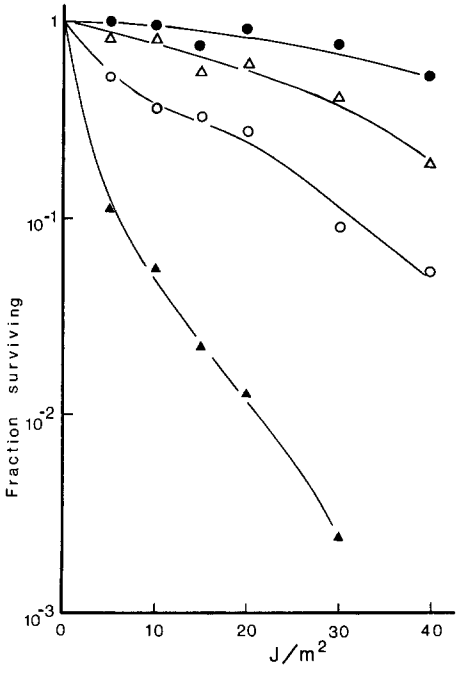

Fig. 4. Effect of *recN262* on the UV survival of a *recF143* strain. ● SP267(*rec*$^+$); ○ SP268(*recF143*); △ SP273(*recN262*); ▲ SP274(*recF143 recN262*)

and Clark 1977). Furthermore, *recF* and *recN* mutations appear to block recombination at different stages. The results shown in Table 3 demonstrate that a *recF* mutation prevents F-prime mobilization of the chromosome from a *recBC sbcB* strain whereas a *recN* mutation does not. The generally accepted minimum requirement for chromosome mobilization is the capacity to break and rejoin single DNA strands. The fact that chromosome mobilization occurs with near normal frequency from a *recN* mutant is therefore consistent with the idea that recombinational repair by exchange of single DNA strands is able to proceed in the absence of *recN* product. Presumably, therefore, the molecular basis for the UV sensitivity of a *recN*(*recBC sbcB*) mutant differs from that of *recF* mutants. This is supported by the results shown in Fig. 4 which reveal that the *recN* and *recF* defects act synergistically to increase sensitivity to UV light in a *recBC*$^+$ *sbcB*$^+$ strain even though their combined effect on recombination is no more than additive (Table 2b).

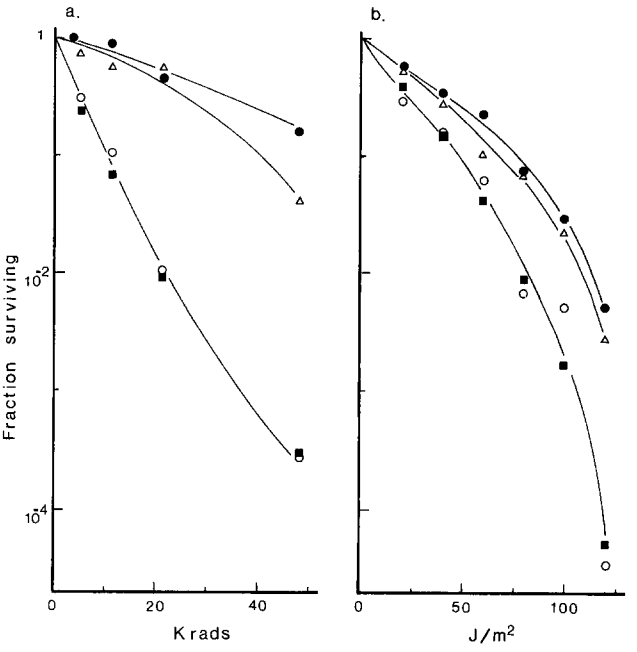

Fig. 5a, b. Effect of pSP100 on the radiation sensitivity of *recN262* mutants. **a** gamma-radiation, **b** UV-radiation. The strains used were AB1157(*rec*$^+$) and SP254(*recN262*). ● AB1157/pHSG415; ○ SP254/pHSG415; △ SP254/pSP100; ■ SP254/SP100 *recN*::$\gamma\delta$

As a preliminary to the identification and characterization of the *recN* product, we have isolated recombinant λ phages and plasmid clones that repair the defects associated with mutations in *recN*. λRL121 and λRL122 were identified by their ability to transduce strain SP216(*recN262*) to mitomycin C resistance (see Methods). These carried different but overlapping segments of *E. coli* DNA as judged by restriction endonuclease analysis. Strain SP231(*recN recBC sbcB*) was lysogenized with λRL121 or λRL122 and crossed with an Hfr donor to see if the phage was also able to restore normal recombination proficiency. The results (Table 2c) show that the level of recombinant formation was a high as with the otherwise isogenic *recN*$^+$ strain. We conclude that both phages carry the *recN*$^+$ region of the *E. coli* chromosome. This is supported by the fact they are able to integrate near *tyrA*.

An approximately 7.0 Kb *Hind*III fragment of λRL121 was cloned into the unique *Hind*III site of the low copy number plasmid pHSG415 (Timmis 1981). The recombinant plasmid was designated pSP100 and was found to restore resistance to both gamma- and UV-radiation in a *recN262* strain (Fig. 5). It also complemented the mitomycin C sensitivity and UV sensitivity of a *recN259* strain, but had no effect on the sensitivity caused by *recA13* in strain AB2463 (data not shown). pSP100 was transformed into NH4104(F-*lac*$^+$) and then mobilised into strain SP254(*recN262*) to mutate the plasmid by $\gamma\delta$ insertion (Guyer 1978). Approximately 8% of the ampicillin resistant clones selected were found to be sensitive to mitomycin C and carried a larger plasmid in which $\gamma\delta$ was inserted within

the 7.0 Kb *E. coli* DNA fragment. One of these mutant plasmids was re-transformed into strain SP254. Figure 5 shows that it no longer complemented radiation sensitivity. Presumably the $\gamma\delta$ insertion has inactivated *recN* or prevents its expression. From these studies, we conclude that *recN* mutations are recessive to $recN^+$.

In conclusion, we have shown that the *recN* product is involved in the repair of double-strand breaks in DNA but is not required otherwise for postreplicative recombinational repair of UV lesions. In this respect, the *recN* product appears to have a similar role to that played by the RAD52 gene product in yeast. RAD52 mutants of yeast are also deficient in the repair of double-strand breaks and are sensitive to ionizing radiation as well as being defective in recombination (Resnick and Martin 1976; Prakash et al. 1980). The identification of a similar mutant in *E. coli*, where methods for detailed examination of recombination in vitro have been established, together with the availability of the cloned gene, should provide a good basis for investigating the molecular mechanism of DNA double-strand break repair.

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References

Arthur HM, Bramhill D, Eastlake P, Emmerson PT (1982) Cloning of the *uvrD* gene of *E. coli* and identification of the product. Gene 19:285–295

Bachmann BJ (1972) Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol Rev 36:525–557

Bachmann BJ (1983) Linkage map of *Escherichia coli* K12, edition 7. Microbiol Rev 47:180–230

Berman ML, Enquist LW, Silhavy TJ (1981) Advanced bacterial genetics. Cold Spring Harbor Laboratory, New York

Birge EA, Low KB (1974) Detection of transcribable recombination products following conjugation in Rec^+, $RecB^-$, and $RecC^-$ strains of *Escherichia coli* K12. J Mol Biol 83:447–457

Bonura T, Smith KC (1975) Enzymic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K-12. J Bacteriol 121:511–517

Clark AJ (1973) Recombination deficient mutants of *E. coli* and other bacteria. Ann Rev Genet 7:67–86

Clark AJ, Margulies AD (1965) Isolation and characterisation of recombination – deficient mutants of *Escherichia coli* K12. Proc Natl Acad Sci USA 53:451–459

DasGupta C, Radding CM (1982) Polar branch migration promoted by *recA* protein: Effect of mismatched base pairs. Proc Natl Acad Sci USA 79:762–766

Ganesan AK (1974) Persistence of pyrimidine dimers during postreplication repair of ultraviolet light-irradiated *Escherichia coli* J Molec Biol 87:103–119

Guyer MS (1978) The $\gamma\delta$ sequence of F is an insertion sequence. J Mol Biol 126:347–365

Hall JD, Mount DW (1981) Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacteria and mammalian cells. Prog Nucleic Acid Res Mol Biol 25:54–126

Hanawalt PC, Cooper PK, Ganesan AK, Smith CA (1979) DNA repair in bacterial and mammalian cells. Ann Rev Biochem 48:783–836

Horii ZI, Clark AJ (1973) Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterisation of mutants. J Mol Biol 80:327–344

Ish-Horowicz D, Burke JF (1981) Rapid and efficient cosmid cloning. Nucleic Acids Res 9:2989–2998

Krasin F, Hutchinson F (1977) Repair of DNA double-strand breaks in *Escherichia coli* which requires *recA* function and the presence of a duplicate genome. J Mol Biol 116:81–98

Krasin F, Hutchinson F (1981) Repair of DNA double strand breaks in *Escherichia coli* cells requires synthesis of proteins that can be induced by UV light. Proc Natl Acad Sci USA 78:3450–3453

Kushner SR, Nagaishi H, Tomplin A, Clark AJ (1971) Genetic recombination in *Escherichia coli*: The role of Exonuclease I. Proc Natl Acad Sci USA 68:824–827

Kushner SR, Nagaishi H, Clark AJ (1972) Indirect suppression of *recB* and *recC* mutations by Exonuclease I deficiency. Proc Natl Acad Sci USA 69:1366–1370

Lin PF, Bardwell E, Howard-Flanders P (1977) Initiation of genetic exchanges in phage-prophage crosses. Proc Natl Acad Sci USA 74:291–295

Little JW, Mount DW (1982) The SOS regulatory system of *Escherichia coli*. Cell 29:11–22

Livneh Z, Lehman IR (1982) Recombinational bypass of pyrimidine dimers promoted by the recA protein of *Escherichia coli*. Proc Natl Acad Sci USA 79:3171–3175

Lloyd RG, Barbour SD (1974) The genetic location of the *sbcA* gene of *Escherichia coli*. Mol Gen Genet 134:157–171

Lloyd RG, Low B (1976) Some genetic consequence of changes in the level of *recA* gene function in *Escherichia coli* K12. Genetics 84:675–694

Lloyd RG, Low B, Godson GN, Birge EA (1974) Isolation and characterisation of an *Escherichia coli* K12 mutant with a temperature-sensitive $RecA^-$ phenotype. J Bacteriol 120:407–415

Lloyd RG, Picksley SM, Prescott C (1983) Inducible expression of a gene specific to the RecF pathway for recombination in *Escherichia coli* K12. Mol Gen Gent 190:162–167

Lloyd RG, Benson FE, Shurvinton CE (1984) Effect of *ruv* mutations on recombination and DNA repair in *Escherichia coli*. Mol Gen Genet 194:303–309

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbour Laboratory, New York

Pollard EC, Fluke DJ, Kazanis D (1981) Induced radioresistance: an aspect of induced repair. Mol Gen Genet 184:421–429

Prakash S, Prakash L, Burke W, Montelone BA (1980) Effects of the RAD52 gene on recombination in *Saccharomyces cerevisiae*. Genetics 94:31–50

Radding C (1982) Homologous pairing and strand exchange in genetic recombination. Ann Rev Genet 16:405–437

Ream LW, Margossian L, Clark AJ, Hansen FG, von Meyenburg K (1980) Genetic and physical mapping of *recF* in *Escherichia coli* K-12. Mol Gen Genet 180:115–121

Resnick MA, Martin P (1976) The repair of double strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol Gen Genet 143:119–129

Rothman RH, Clark AJ (1977) The dependence of postreplication repair on *uvrB* in a *recF* mutant of *Escherichia coli* K12. Mol Gen Genet 155:279–286

Rupp WD, Wilde CE, Reno DL, Howard-Flanders P (1971) Echanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. J Mol Biol 61:25–44

Sancar A, Rupp WD (1983) A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. Cell 33:249–260

Shurvinton CE, Lloyd RG (1982) Damage to DNA induces expression of the *ruv* gene of *Escherichia coli*. Mol Gen Genet 185:352–355

Shurvinton CE, Lloyd RG, Benson FE, Attfield PV (1984) Genetic analysis and molecular cloning of the *Escherichia coli ruv* gene. Mol Gen Genet 194:322–329

Sinden RR, Cole RS (1978) Topography and kinetics of genetic recombination in *Escherichia coli* treated with psoralen and light. Proc Natl Acad Sci USA 75:2373–2377

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW (1983) The double-strand break repair model for recombination. Cell 33:25–35

Timmis KN (1981) Gene manipulation in vitro. In Glover SW, Hopwood DA (eds), Genetics as a tool in microbiology, Cambridge University Press pp 49–109

Wang TV, Smith KC (1982) Effects of the *ssb-1* and *ssb-113* mutations on survival and DNA repair in UV-irradiated *uvrB* strains of *Escherichia coli* K-12

West SC, Cassuto E, Howard-Flanders P (1982) Post-replication repair in *E. coli*: Strand exchange reactions of gapped DNA by RecA protein. Mol Gen Genet 187:209–217

Willetts NS, Mount DW (1969) Genetic analysis of rec^- mutants of *Escherichia coli* carrying *rec* mutations cotransducible with *thyA*. J Bacteriol 100:923–934

Witkin EM (1976) Ultraviolet mutagenesis and inducible deoxyribonucleic acid repair in *Escherichia coli*. Bacteriol Rev 40:869–907

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