A *recA* Null Mutation May Be Generated in *Streptomyces coelicolor*

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The recombinase RecA plays a crucial role in homologous recombination and the SOS response in bacteria. Although *recA* mutants usually are defective in homologous recombination and grow poorly, they nevertheless can be isolated in almost all bacteria. Previously, considerable difficulties were experienced by several laboratories in generating *recA* null mutations in *Streptomyces*, and the only *recA* null mutants isolated (from *Streptomyces lividans*) appeared to be accompanied by a suppressing mutation. Using gene replacement mediated by *Escherichia coli*-*Streptomyces* conjugation, we generated *recA* null mutations in a series of *Streptomyces coelicolor* A3(2) strains. These *recA* mutants were very sensitive to mitomycin C but only moderately sensitive to UV irradiation, and the UV survival curves showed wide shoulders, reflecting the presence of a *recA*-independent repair pathway. The mutants segregated minute colonies with low viability during growth and produced more anucleate spores than the wild type. Some crosses between pairs of *recA* null mutants generated no detectable recombinants, showing for the first time that conjugal recombination in *S. coelicolor* is *recA* mediated, but other mutants retained the ability to undergo recombination. The nature of this novel recombination activity is unknown.

The biological importance of the recombinase gene *recA* is reflected by its ubiquity in nearly all free-living bacteria (absent in endosymbionts of the genera *Buchnera* and *Blochmannia*) and by its low evolution rate (34). RecA carries out a crucial step in homologous pairing that is common to all known recombination pathways in bacteria. Different bacteria have developed various recombination pathways to perform the housekeeping function of maintaining genome integrity and generating genetic variation (reviewed in reference 34). The best studied are the RecBCD and RecF pathways of *Escherichia coli*. While both are involved in genome recombination, the former repairs double-stranded breaks, and the latter repairs single-stranded gaps. While the RecF pathway is present in all genomes studied, the RecBCD pathway is replaced by the AddAB pathway in firmicutes and most α - and β -proteobacteria or is missing in others (34).

Aside from having its role in recombination, RecA is also the key player in regulating the SOS response, which increases the capacity to repair and tolerate DNA damage. It is therefore not surprising that *recA* mutations are remarkably pleiotropic.

Recombination frequencies during conjugation are reduced by about 10^5 -fold in *recA* null mutants of *E. coli* (9). Recombinational repair of double-stranded lesions (such as double-stranded breaks, daughter strand gaps, and interstrand cross-links) is defective in *recA* mutants. Because the SOS response is abolished in *recA* null mutants, they are extremely sensitive to UV and other DNA-damaging agents. Moreover, because of the crucial role of homologous recombination in chromosome replication, *E. coli recA* mutants display severe DNA degradation (4) and continuously generate up to 50% dead cells (5, 11) under normal growth conditions. These deleterious effects probably account for the slow growth of *recA* mutants relative to that of the wild type in all bacteria studied.

Gram-positive soil bacteria of the genus *Streptomyces*, with their full genetic repertoire of linear chromosomes, linear and circular plasmids, phages, and transposable elements, have been under extensive genetic and molecular study for decades (reviewed in reference 14). The linear chromosomes and linear plasmids of *Streptomyces* are capped by terminal proteins (TP) covalently bound to the 5' ends of the DNA. These linear replicons contain terminal inverted repeats (TIR) of tens of bp to hundreds of kb. Except for the terminal few tens of bp, the TIR sequences are not conserved. The widely divergent lengths and sequences of TIR result from frequent exchanges of chromosomal arms through homologous recombination and/or transposition (reviewed in reference 8). A dramatic case was observed with laboratory strains of the model organism *Streptomyces coelicolor* A3(2), many of which, including the sequenced, "standard" strain M145, have a short (22-kb) TIR, whereas the others have a long (1-Mb) TIR (38). It is not clear whether the short TIR was derived from shortening of one (right) arm or the long TIR was the result of duplication of a (left) arm sequence on the other arm through an unequal crossover between sister chromosomes. These variations in the size and sequence of TIR do not seem to affect the viability or stability of the linear chromosomes or plasmids, and the biological roles of TIR are not clear.

The TP, on the other hand, is clearly involved in patching the single-stranded gaps at the 3' ends of the linear replicon, which result from bidirectional replication initiated internally (reviewed in reference 7). Several models have been proposed for the "end patching," including one in which the single-stranded gaps are filled by homologous recombination using a new strand synthesized from the other telomere and primed on the TP (7).

In decades of genetic studies in *Streptomyces*, one of the major shortcomings has been the lack of useful *rec* mutants. Surprisingly, despite their competence in diverse homologous modes of recombination (such as repair, conjugation, or plasmid or phage integration), *Streptomyces* genome sequences (3, 19) lack the typical presynaptic proteins RecBC and AddAB (34). This suggests either that such proteins are not required or

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype/description	Source or reference
<i>S. coelicolor</i> strains		
3454	<i>pgl</i> SCP1 [−] SCP2 [−]	H. Kieser
T3454-1	$\Delta recA::aac(3)IV$ mutant of 3454	This study
T3454-3	$\Delta recA::aac(3)IV$ mutant of 3454	This study
3456	<i>pgl</i> SCP1 ^{NF} SCP2 [−]	38
T3456-20	$\Delta recA::aac(3)IV$ mutant of 3456	This study
T3456-97	$\Delta recA::aac(3)IV$ mutant of 3456	This study
J1508	<i>hisA1 uraA1 strA1 pgl-1</i> SCP1 ^{NF} SCP2 [−]	H. Kieser
T1508-5	$\Delta recA::aac(3)IV$ mutant of J1508	This study
T1508-17	$\Delta recA::aac(3)IV$ mutant of J1508	This study
M130	<i>hisA1 uraA1 strA1 pgl(Ts)</i> SCP1 [−] SCP2 [−]	18
T130-18	$\Delta recA::aac(3)IV$ mutant of M130	This study
T130-28	$\Delta recA::aac(3)IV$ mutant of M130	This study
M145	SCP1 [−] SCP2 [−]	18
T145-15	$\Delta recA::aac(3)IV$ mutant of M145	This study
T145-41	$\Delta recA::aac(3)IV$ mutant of M145	This study
<i>E. coli</i> strains		
BW25113/pIJ790	K-12 derivative; $\Delta araBAD \Delta rhaBAD/\lambda$ -RED (<i>gam bet exo</i>) <i>cat araC rep101(Ts)</i>	10
ET12567/pUZ8002	<i>dam-13::Tn9 dcm cat tet hsdM hsdR zjj-201::Tn10/tra neo RP4</i>	10
Plasmids		
pIJ773	<i>aac(3)IV oriT</i>	10
pLUS371	<i>recA</i> of St4H8 replaced with Apra ^r cassette	This study
pLUS221	pBluescript II KS(−) containing 1.3-kb BamHI terminal fragment of <i>S. coelicolor</i> chromosome	3
St4H8	<i>S. coelicolor</i> cosmid containing <i>recA</i>	33

that they are substituted by an unknown novel system. On the other hand, *Streptomyces* chromosomes do contain *recA* orthologs. *Streptomyces* RecA proteins strongly resemble those of other bacteria, except for a Lys- and Ala-rich extension of the C-terminal region (26), which appears to be involved in regulating the expression of *recA* (1).

Although *recA* null mutants of most bacteria are viable despite all their deficiencies, difficulties have been experienced by several laboratories in creating *recA* null mutations in *Streptomyces* (e.g., references 2 and 27). While 62 amino acids (aa) could be deleted from the C terminus of RecA in *S. ambofaciens* (2) and 87 aa from the C terminus of RecA in *S. lividans*, *S. coelicolor*, and several other species, deletions of 165 aa or more could not be achieved (27). *recA* was proposed by the authors of these findings to be indispensable for the viability of *Streptomyces*. Such difficulty in knocking out *recA* was also experienced with the spirochete *Borrelia burgdorferi* (31) and the proteobacterium *Myxococcus xanthus* (28). The former, like *Streptomyces*, possesses a linear chromosome. The latter, which, like *Streptomyces*, displays a complex differentiation and sporulation pathway, has two *recA* genes. One copy, *recA*₁, whose expression was not detected, could be disrupted, whereas the other copy, *recA*₂, whose expression was detected and damage inducible, could not be disrupted in spite of repeated attempts.

Later, an insertion mutation in *recA* was produced in *Streptomyces rimosus*, which contained a copy of full-length *recA* lacking its own promoter and another copy lacking about 60% of the C-terminal sequence (25). However, the supposed lack of transcription of the full-length *recA* was not confirmed. Complete deletion of a *recA* gene was achieved in *S. lividans* by

Vierling et al. (36), who first deleted the chromosomal *recA* gene in the presence of a wild-type *recA* gene on a plasmid and subsequently eliminated the expression plasmid by introducing an incompatible plasmid. Several mutants were isolated, all of which were UV sensitive, recombination deficient (by a plasmid integration test), and sporulation defective. The sporulation defect appeared to be due to a secondary mutation, because it could not be rescued by complementation of a wild-type *recA* gene. These authors suggested that the additional mutation might allow tolerance of a null *recA* mutation.

In this study, using a PCR-targeting gene replacement technique (10), we successfully generated *recA* null mutations in five different strains of *S. coelicolor*. The mutants grew poorly and were very sensitive to mitomycin C but only moderately sensitive to UV irradiation—in marked contrast to the UV supersensitivity seen for *recA* mutants of *E. coli*. The UV killing curves of the *Streptomyces recA* mutants display wide shoulders typical of repair of the damage, which are absent from *E. coli recA* mutants. The recombination competences of *recA* mutants were tested by three series of matings between pairs of *recA* null mutants. In two of the series, recombination was abolished. However, in the third series, significant recombination activity was still observed, suggesting the presence of a *recA*-independent recombination activity in these mutants.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains and plasmids used in this study are listed in Table 1. Basic microbiological and molecular biological procedures were done according to the work of Kieser et al. (20) and that of Sambrook et al. (35).

Gene replacement. The REDIRECT system of Gust et al. (10) was used for gene replacement in *Streptomyces*. The *recA*-disrupting cassette was generated by

PCR using a forward (GTGAAGCGATCGAATCAAGCAAACCGGGTGGGAACCATGatccgggagtcgacc; 59 nucleotides [nt]) and reverse (CGCGTACTCGGCCAGTCGGTTCGTCGGGTCACGGGTCAtgaggctggagctcttc; 58 nt) primer; each primer contained 36 nt homologous to the beginning and end of the *recA* coding sequences (uppercase; start and stop codons are in boldface) plus a unique priming site (lowercase) and pIJ773 as the template for *aac(3)IV* (apramycin resistance marker) and *oriT*. This cassette was used to transform *E. coli* BW25113/pIJ790 harboring cosmid clone St4H8 of *S. coelicolor* (33) containing the *recA* gene to create a $\Delta recA::aac(3)IV$ allele, which was subsequently used for gene replacement in *S. coelicolor* via conjugal transfer from *E. coli* ET12567/pUZ8002 (29).

UV and mitomycin C sensitivity. For testing UV sensitivity, spores spread on LB agar in 9-cm petri dishes were irradiated at various dosages with a UV Stratalinker 1800 instrument (Stratagene) and incubated at 30°C for 5 days in the dark (to prevent photoreactivation). For testing mitomycin C sensitivity, spores were spread on LB containing various concentrations of mitomycin C and incubated at 30°C for 5 days.

Streptomyces conjugation. Conjugation between *Streptomyces* strains was performed according to the methods of Kieser et al. (20). Spores of the mating pair were mixed and plated on R5 medium and incubated at 30°C for 14 days. The spores produced by the mating cultures were plated on selective media to score recombinant progeny and each of the two parents. The output of parental-type progeny in the mating was imbalanced in most of the crosses for the following reasons. Any pair of *recA*⁺ SCP1^{NF}/SCP1⁻ parents exhibited a drastic imbalance of outputs. This was further complicated by the fact that each *recA* mutation further decreased the output of the mutant strain in a mating. Attempts to offset the imbalance by inoculating the spores of the disadvantaged parent in larger portions or up to 1 day earlier were not successful. Therefore, assuming that most of the excess of the majority output parent had not been involved in active mating, we calculated the recombination frequencies based on the minority output parent.

Microscopic detection of anucleate spores. Aerial mycelium and spore chains were collected on sterile coverslips from day 7 cultures according to the methods of Kim et al. (21), fixed in methanol, stained with 5 µg/ml DAPI (4',6'-diamidino-2-phenylindole) in phosphate-buffered saline containing 50% glycerol, and then examined with a fluorescence microscope (Leica DMLB) with 360-nm excitation light and a 425-nm emission filter.

RESULTS

***recA* can be deleted in *S. coelicolor*.** We employed the REDIRECT gene-replacement procedure (10) to delete the *recA* gene (SCO5769) from three plasmidless (SCP1⁻) *S. coelicolor* strains (M145, M130, and 3454) and two donor strains, J1508 and 3456 (both containing an integrated SCP1^{NF} plasmid). The *recA* gene on cosmid St4H8 was replaced by an apramycin resistance *aac(3)IV* gene cassette in *E. coli* and then transferred to *S. coelicolor* by conjugation. Most of the apramycin-resistant (Apra^r) exconjugants were resistant to kanamycin (Kan^r; resistance was conferred by the *neo* gene on the cosmid vector) and contained the cosmid integrated through a single crossover. Kanamycin-sensitive (Kan^s) exconjugants, in which the chromosomal *recA* gene supposedly had been replaced by a double crossover, were readily isolated at frequencies of 1 to 6% from all the strains except M145. Kan^s segregants were isolated from M145 exconjugants only after two consecutive cycles of sporulation.

Southern hybridization of genomic DNA isolated from Apra^r Kan^s cultures showed replacement of the *recA* gene by the *aac(3)IV* cassette through a double crossover (Fig. 1). Two independent *recA* mutants from each strain were used for subsequent characterization: T145-15 and T145-41 from M145, T130-18 and T130-28 from M130, T3454-1 and T3454-3 from 3454, T1508-17 and T1508-5 from J1508, and T3456-20 and T3456-97 from 3456.

FIG. 1. Creation of *recA* null mutants of *S. coelicolor*. (A) Maps of the *recA*⁺ and $\Delta recA::aac(3)IV$ alleles on the chromosome. The *recA* alleles are shown with their neighboring genes (arrows). The filled bar represents the *aac(3)IV* (white arrow) cassette used for replacement of the *recA* gene. The hybridization probe is indicated by the bar below. The PstI cutting sites (Ps) are marked, and the size of the probe-hybridizing PstI fragment is indicated. (B) Hybridization analysis of selected Apra^r Kan^s exconjugants. Genomic DNA was digested with PstI and subjected to Southern hybridization after electrophoretic separation in an agarose gel. Each panel shows the result of the *recA*⁺ parent and a representative mutant. The respective 1.3- and 0.85-kb hybridizing fragments expected in the parent and the mutant are indicated.

The chromosomes of the *recA* mutants remain linear. Because the previous difficulty in creating *recA* null mutations suggested that the *recA* gene might be essential (such as for replication of the linear chromosome), the chromosomes of our *recA* mutants were checked for possible circularization, which is mediated by recombination between the two arms of chromosomes and results in loss of the telomeres (24). Southern hybridization using the telomere DNA showed that the chromosomes of the *recA* mutants had the same terminal sequences as the wild type (Fig. 2), indicating that the mutant chromosomes had not become circular.

The *recA* mutants are moderately sensitive to UV. All of the *recA* mutants exhibited elevated sensitivity to UV (Fig. 3A). Inactivation of 99% of the mutant spores required only about 0.3 to 0.5 of the UV dosage needed for the *recA*⁺ parent. This increase in UV sensitivity is very moderate compared to the several thousandfold increases of UV sensitivity displayed by *E. coli recA* mutants (9).

The wide shoulders on the UV survival curves of all the *recA* mutants of *Streptomyces* indicate repair of UV damage, which would be *recA* independent and not subject to archetypical SOS regulation. No such shoulders are seen in the survival curves for *recA* mutants of *E. coli* (e.g., see reference 9), as

FIG. 2. Linearity of the chromosome in the *recA* mutants. (A) Restriction map of the terminal region of the *S. coelicolor* chromosome. The terminal protein is indicated by the circle. Restriction sites for BamHI (Ba) and PstI (Ps) are marked, with their distances from the chromosomal terminus indicated in kb. The 1.3-kb BamHI fragment (on pLUS221) was used as a hybridization probe (filled bar). (B) Genomic DNA was isolated from strains 3454 and 3456 and their *recA* mutants, digested with PstI, and subjected to Southern hybridization. The hybridizing 6.2-kb PstI fragments are indicated.

expected from the repression of most repair genes by a defective SOS response.

For comparison, two *recA* mutants (T130-18 and T145-15) were tested for sensitivity to the DNA interstrand cross-linking drug mitomycin C (Fig. 3B). At a mitomycin C concentration of 10 ng/ml, killing of the *recA*⁺ strains was barely detectable, but the survival of the two *recA* mutants dropped to 10^{-4} and to 10^{-3} . This was in great contrast to the moderate UV sensitivity of the mutants. The mitomycin C survival curves of the two mutants displayed shoulders at low concentrations. T130-18 has a wider shoulder than T145-15: at 5 ng/ml of mitomycin C, the survival of T130-18 is about 3 orders of magnitude higher than that of T145-15. This suggested the possible existence of stronger *recA*-independent recombinational repair in T130-18.

The *recA* mutants exhibit a growth defect and segregate into nonviable minute colonies. All of the *recA* mutants from all five strains displayed a growth defect. Their mycelial yields in liquid cultures were reduced, and protoplasts produced from the *recA* mutants showed very low viability on regeneration—at least 30-fold lower than that for wild-type cultures.

All the *recA* mutants grew more slowly than the *recA*⁺ strains on solid media and gave rise to many minute (20 to 40%) colonies of different sizes, which were rarely (1 to 4%) seen in the *recA*⁺ strains (Fig. 4A). A significant portion of the minute colonies of the *recA* mutants did not form spores. Ten to 50 percent of the minute colonies of the *recA* mutants did not form colonies upon streaking onto new plates.

The *recA* mutants produce more anucleate spores. To investigate the possible effect of the *recA* mutation on chromosome partitioning into spores, we examined spore chains of T145-15 and T3454-1 by fluorescence microscopy after staining with DAPI. The results (Fig. 4B) showed that the *recA* mutants produced more anucleate spores—about 19.0% for T145-15 and 11.6% for T3454-1—than M145 and 3454, which produced 8.8 and 5.6% anucleate spores, respectively. The larger fractions of anucleate spores for M145 and 3454 observed in this study relative to that (1.3%) found for M145 by Kim et al. (21) are probably due to the longer growth time (7 versus 4 days) in our case.

The *recA* mutants are defective in conjugal recombination. The *recA* mutants were tested for recombination competence during conjugation (Fig. 5; Table 2). Two SCP1^{NF} strains (J1508 and 3456) and their derivatives were used for the mating donors in the hope that the high fertility conferred by these donors (1 to 100% frequencies of donor marker transfer) would provide a strong contrast to any deficiency displayed by the *recA* mutants. In all the mating experiments, one parent was *hisA1 uraA1 strA1* and the other was a streptomycin-sensitive (*strA*⁺) plasmidless prototroph. Two types of recombinants, Ura⁺ Str^r (designated UxS) and His⁺ Str^r (designated HxS), were selected.

The *recA* mutants allowed three series of reciprocal matings (Fig. 5). The recombinant chromosomes were all expected to

FIG. 3. UV and mitomycin C sensitivity of the *recA* mutants. The *recA*⁺ strains (filled symbols) and their *recA* mutants (open symbols) were subjected to UV irradiation (A) and mitomycin C cross-linking (B), and the surviving spores were scored.

FIG. 4. Growth defects of the *recA* mutants. (A) Minute colonies (arrows) produced by the *recA* null mutants. Spores of the *recA* mutants were spread on MM (20) medium with appropriate supplements and incubated at 30°C for 6 days. (B) Anucleate spores produced by the *recA* mutants. Aerial mycelium and spore chains were collected on glass slides inserted into MM agar supplemented with mannitol (21), on which the *recA* mutants were grown at 30°C (21). The collected material was stained with DAPI and examined with a fluorescence microscope. The top photographs are phase-contrast images of the spore chains. The bottom photographs are fluorescence images of the spore chains. Examples of anucleate spores are indicated by the arrowheads. The numbers and percentages (in parentheses) of nucleate (+) and anucleate (–) spores from more than 15 fields for each strain are tabulated at the bottom.

be SCP1^{NF} (16, 17). Therefore, an additional crossover is required within the relatively short distance between SCP1^{NF} and *uraA1* in the UxS recombination in the J1508 × M145 and J1508 × 3454 series, but not in the 3456 × M130 series. As expected, the UxS recombination frequencies (designated RF[UxS]) in the first two series were mostly significantly lower than in the last series (Fig. 5; and see below). The HxS recombination, on the other hand, required only a crossover in the large chromosomal segment between *hisA1* and SCP1^{NF}. Also, as expected, the HxS recombination frequencies (designated RF[HxS]) were mostly higher than RF[UxS] (Fig. 5; also see below).

In *recA*⁺ SCP1^{NF} × *recA*⁺ SCP1[–] matings (‘Cross I’), both RF[HxS] and RF[UxS] were on the order of 10^{–1}, as expected for typical SCP1^{NF} × SCP1[–] crosses (16, 17). This provided the wild-type level of recombination competence against which to measure the effect of *recA* mutations.

To determine unambiguously the effect of *recA* mutations on chromosomal recombination, conjugation must be performed

between two *recA* mutant parents (cross II), because, unlike conjugation in *E. coli*, in which the *rec* allele of the donor is irrelevant to conjugal recombination activity (13), an NF donor of *S. coelicolor* appears to transfer chromosomal markers relatively efficiently (16, 17). The possibility that the complete donor chromosome may often be transferred during *Streptomyces* conjugation has been raised previously (15). The possibility that a *recA*⁺ allele in an NF strain may be transferred and expressed in the recipient was tested by isolating independent progenies of recipient genotype from a mating between an SCP1^{NF} *recA*⁺ donor and an SCP1[–] *recA* mutant recipient and performing Southern hybridization on their genomic DNA by using *recA* DNA as a probe. About 21% of the recipients showed the presence of *recA* sequence in their genomes (data not shown). Therefore, the transfer of a *recA*⁺ allele from SCP1^{NF} donors to the recipient was real and had to be avoided in measuring the effect of the *recA* mutation.

In *recA* → *recA* crosses (cross II) of the J1501 × M145 and J1508 × 3454 series, no recombinants were found, and RF[HxS]

FIG. 5. Scheme of recombination during conjugation. Chromosomal recombination in the *recA*⁺ SCP1^{NF} × *recA* mutant SCP1[−] crosses (cross IV) in the three series (Table 2) is illustrated. The linear chromosomes are depicted by the horizontal lines, and the long (1-Mb) and short (22-kb) TIR by the bars. The relative positions of the *hisA1* (h), *uraA1* (u), *strA1* (s), and *recA* alleles are marked, and the selection markers are labeled by triangles. The position of SCP1^{NF} (NF) is marked by diamonds. The approximate RF[UxS] and RF[HxS] are listed to the right. The expected crossovers required to produce Ura⁺ Str^r and His⁺ Str^r recombinants are indicated by the solid and dashed lines, respectively.

and RF[UxS] were more than 10^4 - and 10^6 -fold lower than the corresponding cross I (*recA*⁺ → *recA*⁺) recombination frequencies. This result clearly demonstrated that essentially all the conjugal recombination activity in these crosses was *recA* dependent. In cross II of the 3456 × M130 series, RF[HxS] and RF[UxS] were also reduced, but only by one and two orders of magnitude from the corresponding recombination frequencies from cross I. Thus, in this mating series, although the recombination activity was in part *recA* dependent, there was an unknown residual recombination activity present.

Cross III, *recA*⁺ SCP1^{NF} × *recA* mutant SCP1[−], was performed in all of the series to determine the extent of involvement of the donor *recA*⁺ allele in conjugal recombination. All the matings showed measurable recombination frequencies from 10^{-4} to 10^{-1} , confirming the transfer and expression of the donor *recA*⁺ allele during conjugation. The levels of this recombination activity contributed by the donors varied among the three mating series. RF[HxS] and RF[UxS] for the J1508 × M145 series were the lowest (10^{-4} to 10^{-3}), followed by the corresponding recombination frequencies for the J1508 × 3454 series (10^{-2} to 10^{-1}); those for the 3456 × M130 series were at the wild-type level (10^{-1}). Cross IV, *recA* mutant SCP1^{NF} × *recA*⁺ SCP1[−], gave rise to a wild-type level of recombination in all matings, as expected.

DISCUSSION

Why did previous *recA* deletion attempts fail? Why was a straightforward *recA* null mutation not achieved in *S. lividans* previously? The *recA* null mutants of TK64 were created indirectly through several selection steps, during which a compensating mutation was deduced to have been acquired (36). The *recA* null mutations in this study were created in *S. coelicolor*, a closely related species. The 2.6-kb sequences spanning *recA* in these two species are 99% identical, and their RecA proteins differ by only one amino acid near the C terminus.

Difficulty was also encountered in our attempt to create *recA* null mutants of M145. Double-crossover segregants could not be found among the M145 exconjugants. Only after two more

cycles of sporulation and selection could *recA* null mutants arising from double crossovers be isolated. One cannot rule out the possibility that a rare compensating mutation had occurred during the propagation, allowing the otherwise untolerated *recA* mutation to survive, as in the *recA* mutants of TK64 created by Vierling et al. (10). However, unlike the TK64 mutants, all the *recA* mutants created in this study were sporulation proficient.

In contrast to what was seen for TK64 and M145, *recA* null mutants emerged readily among the primary exconjugants of three strains of *S. coelicolor* without the need for further propagation. It is virtually impossible that these mutants all contain a secondary mutation that suppresses any lethal effect of a *recA* null mutation. The genetic trait(s) contributing to the discrepancy in accommodating a *recA* null mutation can only be speculated on at present.

What is the nature of the *recA*-independent recombination activity? The most surprising result in this study was that recombination still occurred—although at a reduced frequency—in crosses in the 3456 × M130 series. Strain 3456 was derived from a mating between 3454 and J1508. A distinct difference between the M130-J1508 and the M145-3454 lineages is in the sizes of their TIR. M145 and 3454 have a short (22-kb) TIR (38), whereas M130 and J1508 have a long (1-Mb) TIR (our unpublished PCR and/or microarray results). 3456 also has a short TIR (38), which appears to have been inherited from 3454, the recipient in the mating with J1508.

Is it possible that M130 and its mutants contain some additional genetic elements that are not present in the other recipient strains? Indeed, M130 and its *recA* mutants were the only recipients with the 1-Mb TIR (Fig. 5), which consists of an extra copy of the 1-Mb left arm at the right end of the chromosome. Microarray studies indicated an absence of gross overexpression of genes in the 1-Mb TIR (38). However, chromosomes with long TIR might contain additional sequences that cannot be detected by microarrays, which were based on the sequence of the M145 chromosome (short TIR).

recA-independent generalized recombination activities previously have been shown to be encoded by lysogenic bacterio-

TABLE 2. Conjugational recombination frequencies of mating involving the *recA* mutants

Cross ^a	J1508 × M145 series				J1508 × 3454 series				3456 × M130 series			
	Donor	Recipient	RF[UxS] ^b	RF[HxS] ^c	Donor	Recipient	RF[UxS] ^b	RF[HxS] ^c	Donor	Recipient	RF[UxS] ^b	RF[HxS] ^c
I (<i>recA</i> ⁺ → <i>recA</i> ⁺)	J1508	M145	1.6 × 10 ⁻¹	5.4 × 10 ⁻¹	J1508	3454	2.2 × 10 ⁻¹	3.3 × 10 ⁻¹	M130	M130	1.3 × 10 ⁻¹	5.7 × 10 ⁻²
II (<i>recA</i> → <i>recA</i>)	T1508-5	T145-15	<4.7 × 10 ⁻⁵	<4.7 × 10 ⁻⁵	T1508-5	T3454-1	<2.1 × 10 ⁻⁷	3.7 × 10 ⁻⁵	T130-18	T130-18	1.6 × 10 ⁻²	3.0 × 10 ⁻³
	T1508-5	T145-41	<3.4 × 10 ⁻⁵	<3.4 × 10 ⁻⁵	T1508-5	T3454-3	<1.9 × 10 ⁻⁷	5.8 × 10 ⁻⁷	T130-28	T130-28	1.6 × 10 ⁻²	3.3 × 10 ⁻³
	T1508-17	T145-15	<5.2 × 10 ⁻⁵	<5.2 × 10 ⁻⁵	T1508-17	T3454-1	<1.6 × 10 ⁻⁷	4.8 × 10 ⁻⁷	T130-18	T130-18	9.1 × 10 ⁻³	8.0 × 10 ⁻⁴
	T1508-17	T145-41	<2.6 × 10 ⁻⁵	<2.6 × 10 ⁻⁵	T1508-17	T3454-3	<1.6 × 10 ⁻⁷	4.8 × 10 ⁻⁷	T130-28	T130-28	1.7 × 10 ⁻²	4.9 × 10 ⁻³
	J1508	T145-15	6.6 × 10 ⁻⁵	1.6 × 10 ⁻³	J1508	T3454-1	1.1 × 10 ⁻²	9.5 × 10 ⁻²	T130-18	T130-18	4.1 × 10 ⁻¹	2.8 × 10 ⁻¹
III (<i>recA</i> ⁺ → <i>recA</i>)	J1508	T145-41	2.4 × 10 ⁻⁴	2.6 × 10 ⁻³	J1508	T3454-3	1.2 × 10 ⁻²	9.8 × 10 ⁻²	T130-28	T130-28	6.3 × 10 ⁻¹	5.8 × 10 ⁻¹
	T1508-5	M145	2.2 × 10 ⁻¹	3.7 × 10 ⁻¹	T1508-5	3454	1.5 × 10 ⁻¹	2.1 × 10 ⁻¹	M130	M130	2.5	5.0 × 10 ⁻¹
IV (<i>recA</i> → <i>recA</i> ⁺)	T1508-17	M145	3.8 × 10 ⁻¹	8.8 × 10 ⁻¹	T1508-17	3454	2.0 × 10 ⁻¹	3.1 × 10 ⁻¹	T3456-20	T3456-20	1.8	3.6 × 10 ⁻¹

^a The arrows indicate the direction of conjugal transfer between the parents whose *recA* status is indicated.

^b Recombination frequency of Ura⁺ Str^r.

^c Recombination frequencies were calculated by dividing the number of recombinants by the number of output of the minority parental type. Frequencies of spontaneous mutation to the recombinant phenotype in the parental strains were at least 2 orders of magnitude lower than the recombination frequencies for all the reported matings.

phages or prophages, such as lambda and Rac in *E. coli* (22). However, no homolog of a phage-type recombinase has been identified in the *S. coelicolor* chromosome (3, 6). On the other hand, at least 10 site-specific recombinase genes and about 100 transposase genes were annotated in the *S. coelicolor* chromosome. There exists the possibility that the *recA*-independent recombination is mediated by transposition or by site-specific recombination between two homologous mobile elements through the action of a resolvase. In this scenario, the transposase or resolvase gene(s) would be derepressed during conjugation in order to achieve the relatively high recombination frequencies.

Recombination activity provided by the NF donor. Conjugation in *Streptomyces* is drastically different from that in the well-studied *E. coli* system, not only in the way that the mating partners are brought together for genetic exchange but also in the way that the genetic material is transferred. For example, during *E. coli* conjugation, DNA is transferred from the donor to the recipient in a linear single-stranded form, whereas DNA transfer during *Streptomyces* conjugation appears to be in a double-stranded form (30). In fact, chromosomal transfer as a double-stranded loop is an attractive model to account for the apparent bidirectional transfer of chromosomal markers from an NF strain to an SCP1⁻ recipient (15). Frequent spontaneous disruptions during chromosomal transfer greatly limit the extent of transfer in *E. coli*, and the Hfr status is rarely transferred to the recipient. In *Streptomyces*, transfer of whole donor chromosomes is observed in certain modes of conjugation (e.g., those mediated by linear plasmids [37]) and may even be the norm (15).

The *recA* gene is about 2.5 Mb from the SCP1^{NF} sequence (Fig. 5), which is inherited at a 100% frequency during conjugation (16, 17). Two *recA*-flanking markers, *leuB* and *cysD*, are inherited by recombinants in NF × SCP1 crosses at 0.4 and 0.2% frequencies, respectively (17). Thus, an inheritance frequency of 0.3% might be estimated for *recA* during SCP1^{NF} × SCP1⁻ mating (17). In this study, about 20% of the recipient-type progeny in an SCP1^{NF} × SCP1⁻ mating had inherited the *recA*⁺ allele from the SCP1^{NF} donor, perhaps because of the faster growth and better sporulation of the *recA*⁺ recombinants.

What is the recombination pathway in *Streptomyces* conjugation? The identity of *recA*-dependent homologous recombination pathway involved in conjugal recombination is also not clear. It is not mediated by the RecBCD (the major pathway in *E. coli*) or AddAB (functional equivalent of RecBCD) pathways, because the presynaptic genes *recBC* and *addAB* (helicase/nucleases) are absent in sequenced *Streptomyces* genomes (3, 19, 34). The RecF pathway genes are present in *Streptomyces* genomes, and a *recF* mutant of *S. lividans* TK64 showed increased UV sensitivity, as expected (23), but the effect of the mutation on conjugal recombination has not been tested. The RecF pathway remains a candidate for the major activity in conjugal recombination in *Streptomyces*.

What do UV and mitomycin C sensitivities tell us about the *recA* mutants? The moderate increase of sensitivity to UV of the *recA* mutants indicates that a large portion of UV damage is repaired in one or more *recA*-independent pathways in *Streptomyces*. In contrast, the drastic increases in the mitomycin C sensitivity of the *recA* mutants indicate that the interstrand

cross-linking caused by the drug is repaired mainly by homologous recombination in *Streptomyces*. Interestingly, the *recA* mutant of M130, T130-18, displayed a significant shoulder in the survival curve (survival 10^3 -fold higher than that of T145-15 at 5 ng/ml) plus a gradual increase in slope (Fig. 3B), which also indicated repair activities.

Previously, Harold and Hopwood (12) attempted but failed to find conjugal recombination-deficient mutants among UV-sensitive mutants of *S. coelicolor*. All the UV-sensitive mutants tested were recombination proficient during conjugation. The failure might be ascribed either to an insufficient size of the test sample or to possession by the strain used of a *recA*-independent recombination activity. The mutants of Harold and Hopwood (12) exhibited about the same degree of UV sensitivity as our *recA* mutants as well as similar shoulders in the UV killing curves. The shoulders and the shallow slopes of the UV inactivation curves exhibited by the *recA* mutants of *Streptomyces* are in marked contrast to those of *recA* mutants of *E. coli*. This contrast suggests the presence of one or more repair systems of substantial efficiency that are not under the archetypal RecA-dependent SOS regulation.

Two Ku-like genes, SCO0601 and SCO5309, are present in the chromosome of *S. coelicolor*. The latter lies next to a primase-like gene, SC5308, a typical functional association with a Ku protein in eukaryotic nonhomologous end-joining systems. Ku-like proteins have been shown to be involved in nonhomologous end joining in *Bacillus subtilis* (39). However, these Ku-like genes apparently are not involved in repair of UV damage, because Ku-like gene deletion mutants did not show increased UV sensitivity (Y.-J. Chen, unpublished results).

Why do *recA* mutants produce minute colonies and empty spores? The segregation into nonviable minute colonies and the increased appearance of anucleate spores in the *recA* mutants probably reflect some defect in the processing of the chromosomes during vegetative growth and sporulation. About 10% of *recA* cells contain no DNA in *E. coli* (4). This is comparable to the frequency of anucleate spores in *Streptomyces*. It is likely that chromosome degradation occurs continuously in the *recA* mutants of *Streptomyces* because of the lack of recombinational repair for broken replication forks. Accumulation of broken chromosomes in the mycelium would not be immediately lethal, but the growth of the colonies would suffer and eventually stop when no viable chromosomes remained. The spectrum of colony sizes, from just smaller than that seen for wild-type colonies to barely visible, presumably represents stochastic loss of mycelial viability at various stages of growth. The relatively high incidence of sporulation defects among the minute colonies is probably caused by loss of genes required for sporulation from the chromosomal sequences. Many of the spores from the sporulating minute colonies were not viable, presumably because they lacked an intact chromosome.

Is recombination involved in end patching during replication? Homologous recombination has been proposed as a possible mechanism for the patching of the single-stranded gaps generated at the telomere of the *Streptomyces* chromosome during replication (7). In this model, recombination takes place between the single-stranded 3' overhang (the result of lagging strand synthesis) and a fully duplex telomere (the result of leading strand synthesis). Qin and Cohen (32) rejected this

model because no homogenotization was detected between the two arms of linear plasmids, which is expected if recombination occurs between the two telomere DNAs in each replication cycle. However, the proposed recombination in the "patching by recombination" model could be between the same ends of two daughter molecules. On the other hand, the this model predicts that homologous recombination is essential for the replication of linear chromosomes and plasmids in *Streptomyces*. The fact that the chromosomes of the *recA* mutants in this study remained linear rules out end patching by *recA*-mediated generalized recombination. Nevertheless, it remains possible that a *recA*-independent recombination pathway is involved in this process.

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