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Chromosomal Arm Replacement in *Streptomyces griseus*

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UV irradiation of *Streptomyces griseus* 2247 yielded a new chromosomal deletion mutant, MM9. Restriction and sequencing analysis revealed that homologous recombination between two similar lipoprotein-like open reading frames, which are located 450 and 250 kb from the left and right ends, respectively, caused chromosomal arm replacement. As a result, new 450-kb terminal inverted repeats (TIRs) were formed in place of the original 24-kb TIRs. Frequent homologous recombinations in *Streptomyces* strains suggest that telomere deletions can usually be repaired by recombinational DNA repair functioning between the intact and deleted TIR sequences on the same chromosome.

The filamentous soil bacteria, *Streptomyces* spp., are unusual in carrying an 8- to 9-Mb linear chromosome (8, 9, 11, 12–14, 17); the genome project for *Streptomyces coelicolor* A3 (2), a model strain for *Streptomyces* genetics, has been recently completed (1). *Streptomyces* species are also known to frequently possess linear plasmids, which range from 12 to 1,700 kb in size (6). *Streptomyces* linear chromosomes and plasmids have principally the same structural features: terminal inverted repeats are present at both ends, and the 5′ ends are linked covalently to terminal proteins. *Streptomyces* linear chromosomes easily undergo telomere deletions spontaneously and by various mutagenic treatments (7, 21). The deleted chromosomes display three types of rearrangements: circularization, arm replacement, and amplification. The last phenomenon has not been clarified well at the molecular level and may be an intermediate state before a final stable state is reached.

Chromosomal circularization in *Streptomyces* spp. was indicated by detecting a macrorestriction fusion fragment in deletion mutants of *Streptomyces lividans* (11, 18) and *Streptomyces ambofaciens* (8). It was finally confirmed in *Streptomyces griseus* by cloning and sequencing of the fusion junctions of the circularized chromosomes (5). The result suggested that chromosomal circularization occurs by nonhomologous recombination between the deletion ends. On the other hand, Fischer et al. (3) reported that homologous recombination between two sigma factor-like open reading frames (ORFs), which are located on both the left and the right arms, caused chromosomal arm replacement in *S. ambofaciens*. In addition, Rauland et al. (16) reported that in some of *S. lividans* mutants only one of the two telomeres was deleted, where generated new ends have not been analyzed.

To study what happens concomitant with telomere deletions in *Streptomyces* spp. in greater detail, we prepared additional mutants from *S. griseus* 2247. We report here a new deletion mutant, MM9, wherein homologous recombination between two similar lipoprotein-like ORFs caused chromosomal arm replacement and generated new long terminal inverted repeats (TIRs). Based on the accumulated data, a possible function of TIRs of *Streptomyces* linear replicons is proposed.

Preparation of new deletion mutants. Spore suspension of *S. griseus* 2247 (9) was UV irradiated at a dose of 99 to 99.9% killing to obtain new deletion mutants. Surviving colonies were randomly picked and reciprocally cultured in glucose-meat extract-peptone medium (9). Total DNAs were prepared as described previously (4), digested with *Bam*Hl, separated by agarose gel electrophoresis, and subjected to Southern hybridization analysis. Hybridization was carried out by using the DIG system (Roche Diagnostics GmbH, Mannheim, Germany) overnight at 70°C in standard buffer.

To distinguish deletions at each of the left and right telomeres, we used two different probes that are located outside of TIR-L (probe L, 7.9-kb *Bam*HI fragment) and TIR-R (probe R, 6.2-kb *Pst*I fragment) (Fig. 1A). Among 60 colonies tested, one colony, MM9, showed a positive signal when probe L was used but no signal when probe R was used (Fig. 1B). On the other hand, two colonies did not show any signal by either probe (data not shown). These results suggested that only the right telomere was deleted in MM9, whereas both telomeres were deleted in the two other mutants. Mutant MM9, which shows a bald morphology and normal growth, was chosen for further analysis.

Analysis of chromosomal deletion in mutant MM9. To determine the size of deletion in MM9, the ordered cosmids covering the right terminal region of the 2247 chromosome (Fig. 2A) (10) were hybridized to the *Bam*HI digest of the MM9 DNA and compared with the 2247 DNA. As shown in Fig. 2B, cosmid 6E12 at the right end showed hybridizing signals for the MM9 DNA due to the existence of the 24-kb TIR-L at the opposite left end. The detection of the four terminal *Bam*HI fragments (15, 3.8, 2.3, and 1.4 kb; see Fig. 1A) in the TIR region indicates that the left chromosomal arm was conserved in MM9. Seven inner cosmids from 10G12 to 6H3 did not give any signals (data not shown), whereas cosmid 10E12 showed fewer signals compared with the 2247 DNA. Thus, the deletion end was located on cosmid 10E12 in the right arm.

To determine the deletion end more precisely, we constructed a *Bam*HI restriction map for cosmid 10E12 (Fig. 2A). Comparison of the hybridizing signals probed by cosmid 10E12

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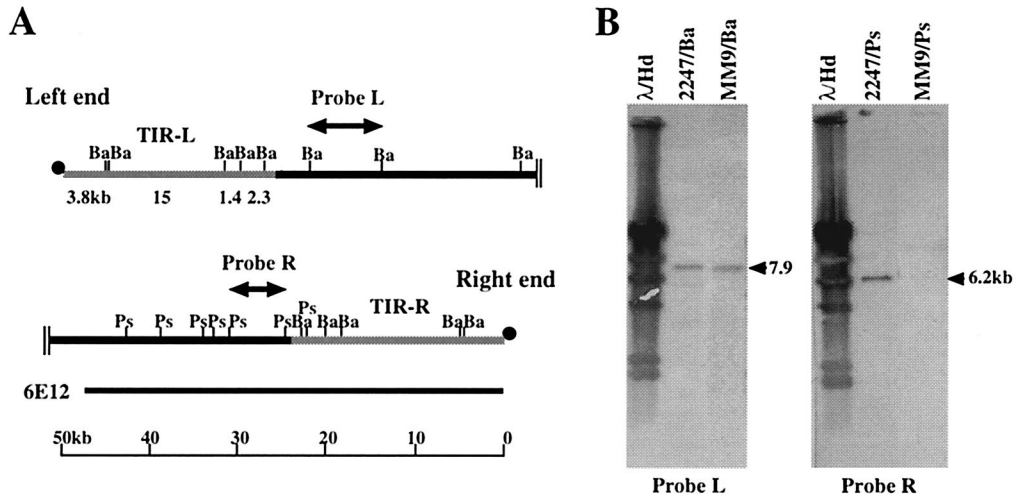

FIG. 1. Restriction maps at the left and right chromosomal ends and the locations of the two hybridization probes, probe L and probe R (A), and Southern hybridization analysis of mutant MM9 (B). Only *Bam*HI (Ba) and *Pst*I (Ps) sites are shown for the left and right ends, respectively, except for the TIR-R, where *Bam*HI sites are also shown. Black circles indicate terminal proteins, and shaded lines indicate TIR regions. The location of the end cosmid 6E12 is also shown. λ/Hd, λ DNA digested with *Hin*dIII.

revealed a new fusion *Bam*HI fragment at 6.6 kb in place of the 9.0-kb fragment (Fig. 2B). This result located the deletion end at the 9.0-kb *Bam*HI fragment in 10E12 and determined the size of deletion to be 250 kb from the right chromosomal end. The newly appeared 6.6-kb *Bam*HI fragment was cloned from the MM9 DNA, named p1D4, and used for further analysis.

Lipoprotein-like ORFs involved in chromosomal arm replacement. Next, we studied how the 6.6-kb *Bam*HI fusion fragment was generated. All of the ordered cosmids covering both the left and the right terminal regions of the 2247 chromosome (10) were digested with *Bam*HI plus *Eco*RI, separated, and probed by the fusion plasmid p1D4. *Eco*RI was added to separate an insert from the vector. As a matter of course, cosmid 10E12 at the right deletion end showed a positive signal at 9.0 kb whereas, unexpectedly, cosmid F2B4 in the opposite left arm gave a signal at 3.2 kb (Fig. 2C). This result suggested that homologous DNA regions are present in both the right and the left chromosomal arms. This fact reminded us that homologous recombination between two sigma factor-like ORFs caused chromosomal arm replacement in *S. ambofaciens* (3).

To test a similar possibility, the homologous region in F2B4 was analyzed and located on the rightmost *Bam*HI-*Eco*RI fragment of the cosmid, namely, 450 kb from the left chromosomal end. The homologous regions in cosmids, 10E12 and F2B4, and plasmid p1D4 were subcloned and finally sequenced (Fig. 3). Three similar ORFs directing toward inside of the chromosome were found; ORF-L (218 amino acids [aa]) in the left

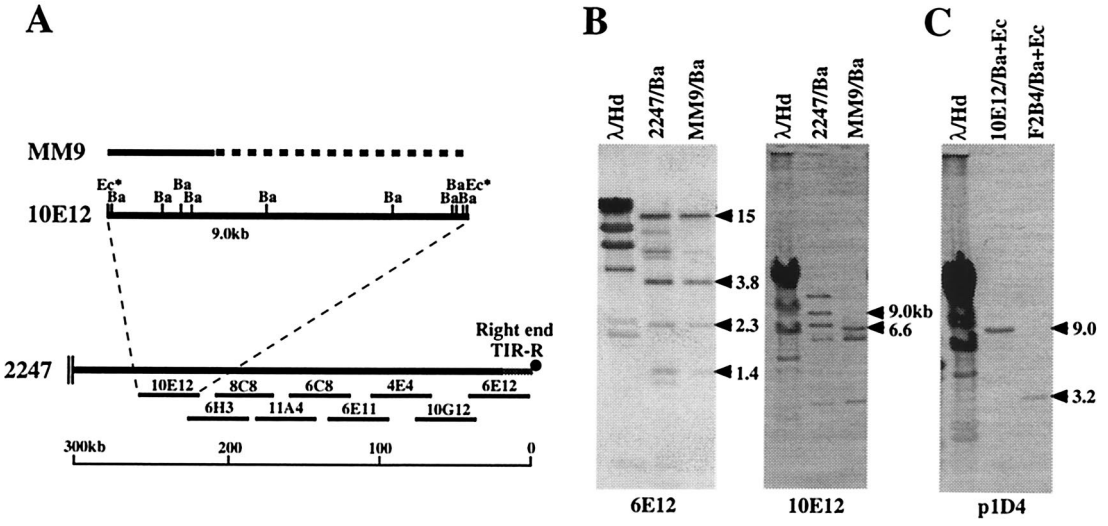

FIG. 2. Ordered cosmid map at the right chromosomal end of strain 2247 and *Bam*HI restriction map of the deletion end cosmid 10E12 (A) and Southern hybridization analysis of mutant MM9 (B) and the cosmids 10E12 and F2B4 (C). The deleted region in MM9 is indicated by a broken line. Ba, *Bam*HI; Ec*, *Eco*RI in the vector.

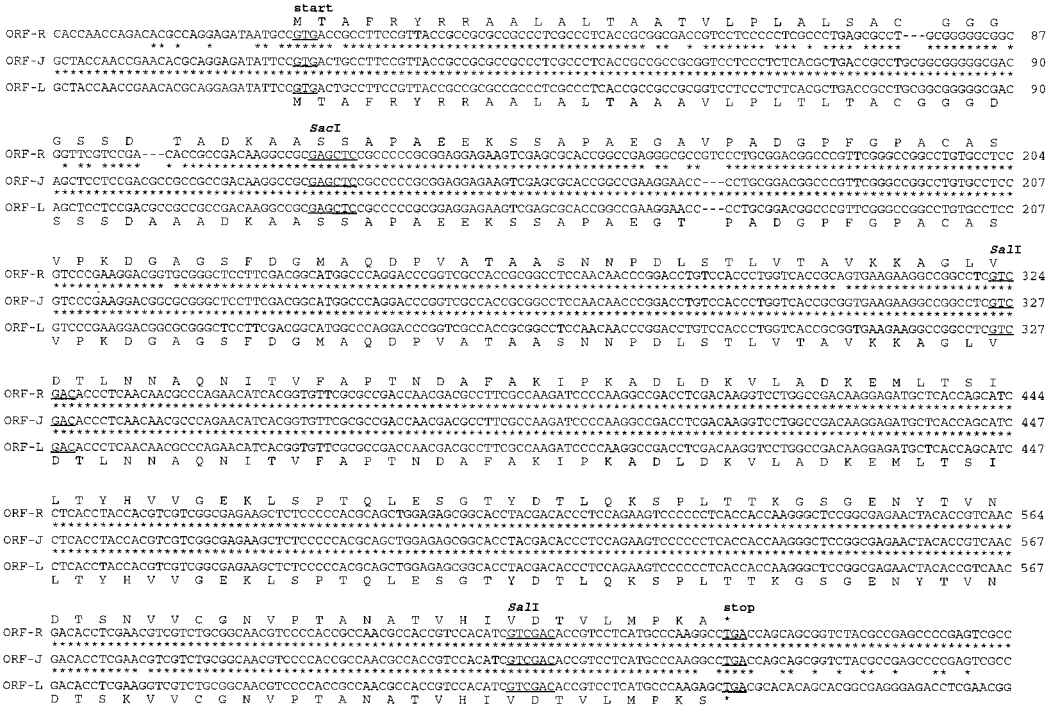

FIG. 3. Nucleotide and amino acid sequences of the three lipoprotein-like ORFs located on the left and right chromosomal arms in strain 2247 (ORF-L and ORF-R) and on the fusion junction in MM9 (ORF-J). The nucleotides of each ORF are numbered from the start codon to the stop codon. Identical nucleotides are indicated by asterisks between two sequences.

arm, ORF-R (217 aa) in the right arm, and ORF-J (218 aa) at the fusion junction. Three ORFs showed 95.3 to 99.5% nucleotide identities and 94.0 to 99.1% amino acid identities to each other. A homology search of databases identified two similar ORFs in *S. coelicolor* A3 (2) coding a putative lipoprotein (219 aa, 69.4% identity with ORF-L; accession number AL133424-20) and a putative secreted protein (220 aa, 45.3% identity; accession number AL031182-10). These ORFs are located at 680 kb from the left end and at 163 kb from the right end, respectively, in the *S. coelicolor* A3 (2) chromosome.

Sequence comparison of the three ORFs indicated that homologous recombination between ORF-L and ORF-R occurred at a point between nucleotides 307 and 578 according to the ORF-J numbering. Since the amino acid sequence of ORF-J is different only at two positions from ORF-L, the fused protein may function in mutant MM9.

Restriction analysis of chromosomal arm replacement. To demonstate further the chromosomal arm replacement, macrorestriction fragments of the 2247 and MM9 DNAs were compared by pulsed-field gel electrophoresis. Gel samples were prepared as described previously (9), digested with *Ssp*I, separated by use of contour-clamped homogeneous electric fields (2), and subjected to Southern hybridization. When cosmid F2B4 (Fig. 4A) was used as a probe, the 2247 DNA showed a 250-kb fragment (the 200-kb fragment on the right arm was not detected because of a short homology between F2B4 and 10E12), whereas the MM9 DNA indicated a 310-kb fusion fragment in addition to the 250-kb fragment (Fig. 4B). When the *Ssp*I linking cosmid 16C1 was used, the 2247 DNA gave hybridizing signals at 250 and 270 kb (the left end fragment), whereas the MM9 DNA gave signals at 250, 270, and 310 kb. The signal at 270 kb is stronger than other signals due to the duplication of this end fragment. All of these results support the chromosomal arm replacement in MM9.

To test whether the extreme chromosomal ends are conserved in MM9, total DNAs of 2247 and MM9 were digested with *Sac*I, *Alu*I, and *Kpn*I; separated in 2.0% agarose gel; and subjected to Southern hybridization by using the 865-bp *Sac*I fragment of pSGE1 that carries the extreme end of the 2247 chromosome (4). As shown in Fig. 4C, both the 2247 and the MM9 DNAs revealed the end fragments at the exact positions expected from the terminal sequence (4), confirming that mutant MM9 keeps the extreme chromosomal ends.

Possible function of TIRs in recombinational DNA repair. In the present study, we obtained a new deletion mutant MM9 by UV irradiation of *S. griseus* 2247. Restriction and sequencing analysis revealed that homologous recombination between two similar lipoprotein-like ORFs caused chromosomal arm replacement and generated new 450-kb TIRs in place of the original 24-kb TIRs. This is a second example of chromosomal arm replacement like that of *S. ambofaciens*, wherein homologous recombination of two sigma factor-like ORFs generated two mutants with 480- or 850-kb TIRs in place of the original 210-kb TIRs (3).

Rauland et al. (16) reported that some of *S. lividans* mutants suffered deletions only at one telomere. Pang et al. (14) also reported similar mutants in *Streptomyces hygroscopicus*. However, it is difficult to distinguish mutants deleted at one telomere and arm-replaced mutants. From the data presented in those studies, we speculated that both mutants in question

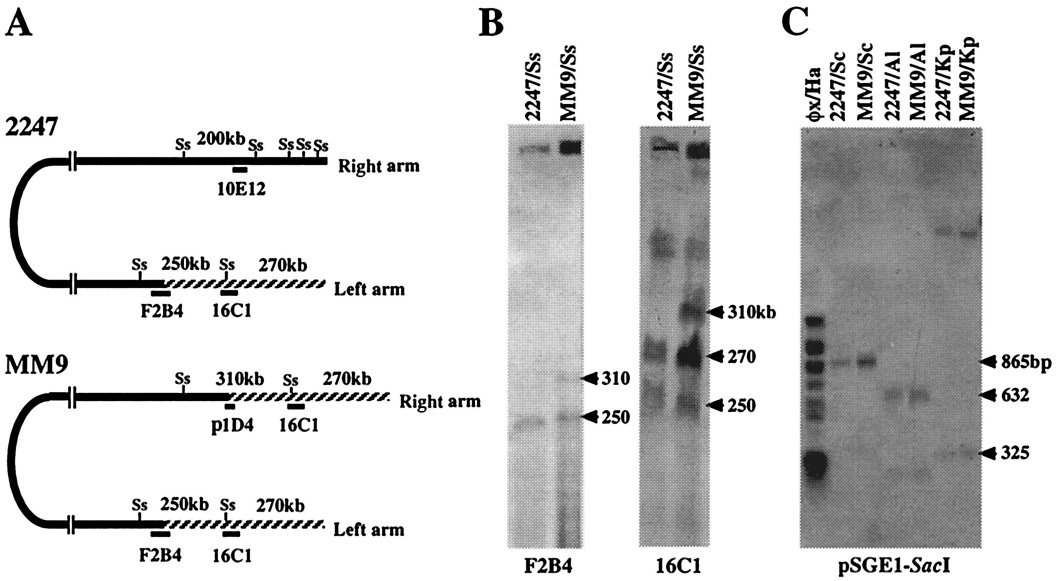

FIG. 4. Gross chromosomal structures of strains 2247 and MM9 (A) and Southern hybridization analysis of macrorestriction fragments (B) and extreme end fragments (C). The hatched region in strain 2247 generated the new long TIRs in MM9. A 2% agarose gel was used in panel C in place of the usual 0.7% gel. Al, *Alu*I; Kp, *Kpn*I; Sc, *Sac*I; Ss, *Ssp*I; ϕx/Ha, ϕx174 DNA digested with *Hae*III.

were arm-replaced mutants. We found mutant MM9 from 60 survived colonies after UV irradition. Therefore, chromosomal arm replacement is not a rare phenomenon in *Streptomyces* spp.; namely, two similar ORFs in the left and right arms can cause homologous recombination relatively frequently. If so, homologous recombination between the left and right TIR regions might occur much frequently because their nucleotide sequences are completely identical.

Volff and Altenbuchner (20, 21) proposed that telomere deletions are repaired by recombinational DNA repair based on the fact that *recA* mutants of *S. lividans* showed a high level of genetic instability (20). As shown in Fig. 5A, a deletion end may invade an intact replicated chromosome by homologous recombination, reproduce a replication fork, and complete the replication to the end. However, in this case the telomere on the same side is reproduced, and therefore arm replacement does not occur. On the other hand, Fischer et al. (3) suggested that direct homologous recombination between two nonallelic sigma factor-like ORFs in two sister chromosomes caused chromosomal arm replacement because multichromosomes are common in a filamentous mycelium of *Streptomyces*.

Based on the accumulated data, we propose that recombinational DNA repair may also function between the deleted and intact arms in the same chromosome (Fig. 5B). If this intrachromosomal repair occurs inside the TIR regions, we do not detect it because an identical TIR structure is recovered; this event may occur frequently in *Streptomyces* cells. Only when recombination occurred outside the TIRs can we recognize it as a chromosomal arm replacement. Thus, TIRs may guarantee homologous sequences for recombinational DNA repair even in a mononuclear state. Sakaguchi (19) proposed the racket frame model for linear DNA replicons that possess TIRs at both ends. This idea has been supported by Yang and Losick (22), who detected by in situ hybridization an association of two chromosomal ends in *S. coelicolor* A3 (2). The racket frame structure may facilitate homologous recombination between the left and right TIR sequences.

While the present study was in review, Qin and Cohen (15) reported that an introduction of deletion into one telomere of the linear plasmid pSLA2 caused repair of the damaged telomere, circularization, and formation of long palindromic linear plasmids. Based on these data, they proposed survival mechanisms for *Streptomyces* linear replicons after telomere damage.

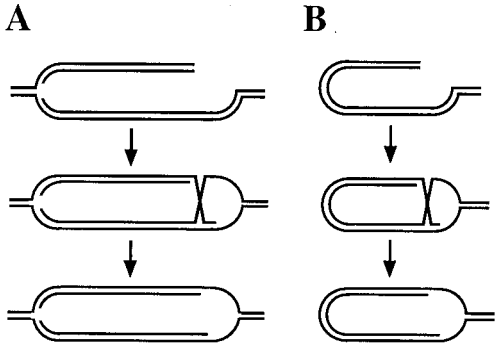

FIG. 5. Interchromosomal (A) and intrachromosomal (B) recombinational DNA repairs, which reproduce an identical telomere and causes chromosomal arm replacement, respectively.

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