NOTES

Streptomyces coelicolor Undergoes Spontaneous Chromosomal End Replacement^{∇†}

Elizabeth M. Widenbrant,^{1*} Hsiu-Hui Tsai,² Carton W. Chen,² and Camilla M. Kao¹

Department of Chemical Engineering, Stanford University, Stanford California 94305,¹ and Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Shih-Pai, Taipei 112, Taiwan²

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We report a previously unobserved form of genetic instability for *Streptomyces coelicolor*, the replacement of one chromosome end by the other end. These genetic changes occurred spontaneously in both a wild-type strain and strains harboring a foreign transposon. Deleted and duplicated DNA comprises up to 33% of the genome.

The linear chromosome of a model actinomycete, *Streptomyces coelicolor*, whose complete sequence appeared in 2002, extends more than 8 MB (1). An accompanying paper describes our analysis, using DNA microarrays, of 50 *S. coelicolor*

mutants that experienced genetic instability (6). The microarray data suggested that a few of these mutants underwent spontaneous replacement of one chromosome end by the other end. Figure 1 shows microarray data for three such mutants.

FIG. 1. DNA microarray data for *S. coelicolor* mutants 87/45 (A) and CO25 (B), which harbor the foreign transposon Tn4560, and mutant S26 (C), derived from the wild-type parent M145. The $\log_2$ scale shows red/green ratios. $\log_2(\text{red/green})$ values of 0 represent gene copy numbers equal to the wild type. (D) Color representation of the same microarray data from the left and right chromosome ends. In the color scale, green, black, and red represent gene copy numbers less than, equal to, and greater than the wild-type copy numbers, respectively.

* Corresponding author. Mailing address: Department of Chemical Engineering, Stanford University, Stanford, CA 94305. Phone: (650) 723-4906. Fax: (650) 723-9780. E-mail: ewidenbrant@gmail.com.

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FIG. 2. Restriction maps of the *S. coelicolor* chromosome. (A) Left and right chromosome ends of M145, a wild-type strain lacking natural plasmids, and restriction sites of AseI (top lines), HindIII (bottom short lines), and SspI (bottom long lines). (B and C) Deletion of the left chromosome end (black X) and duplication of the right chromosome end (2x) of mutant strains 87/45 and CO25, respectively. (D and E) New structures of the left chromosome ends of 87/45 and CO25, respectively. See text for details.

For two strains with Tn4560, 87/45 and CO25, the microarray data showed ~383-kb and ~372-kb deletions of the left chromosome end, respectively, and ~670-kb and 2,529-kb duplications of the right end, respectively (Fig. 1A and B and 2B and C). The locations of the deletions and duplications failed to correlate with the insertion sites of the foreign transposon (data not shown). For strain S26, derived from M145 and thus lacking the foreign transposon Tn4560, the microarray data showed a ~411-kb deletion of the left chromosome end and a ~1,303-kb gene duplication of the right end (Fig. 1C). Figure 1D shows microarray data on a color scale for these strains.

Since microarray data provide no information about the physical location of duplicated DNA, we examined strains 87/45, CO25, and S26 with pulsed-field gel electrophoresis (PFGE). Genomic DNA preparation in agarose gel plugs, restriction enzyme digestion, and PFGE were performed as previously described (4). Genomic DNA analyzed with the restriction enzymes AseI, HindIII, and SspI showed, together, (i) loss of the left chromosome end, (ii) retention of the right chromosome end, and (iii) for 87/45 and CO25, duplication of the right chromosome end at the left end. Analysis of undigested DNA showed no extra-chromosomal DNA fragments (data not shown).

87/45 PFGE. PFGE of AseI-digested DNA showed that strain 87/45 lost the left chromosome end and retained the right end. Here, the left and right chromosome ends of wild-type *S. coelicolor* contain AseI sites 240 kb and 1,601 kb from the termini, respectively (Fig. 2A). Digestion with AseI gener-

ates the “J” restriction fragment and the “A” restriction fragment, respectively (3). Gels probed with a 1.3-kb BamHI fragment containing the chromosome ends, located within the terminal inverted repeats and therefore identical for both ends, showed the presence of both the “J” and “A” fragments in genomic DNA of the parent strain M145 and loss of the “J” fragment and maintenance of the “A” fragment in the mutant strain (Fig. 3A).

PFGE with HindIII also showed loss of the left chromosome end and retention of the right end. Here, the left and right chromosome ends of wild-type *S. coelicolor* contain HindIII sites 45 kb and 84 kb from the termini, respectively. Digestion with HindIII generates terminal restriction fragments 45 kb and 84 kb in size, respectively (Fig. 2A). Gels probed with the terminal 1.3-kb BamHI fragment showed the presence of both fragments in genomic DNA of the parent strain M145 and loss of the 45-kb fragment and maintenance of an 84-kb fragment in the mutant strain (Fig. 3B).

PFGE with SspI also showed loss of the left chromosome end and retention of the right end. Here, the left and right chromosome ends of wild-type *S. coelicolor* contain SspI sites 266 kb and 997 kb from the termini, respectively (Fig. 2A). Digestion with SspI generates restriction fragments 266 kb and 997 kb in size, respectively. Gels probed for the terminal 1.3-kb BamHI fragment showed the presence of both fragments in genomic DNA of the parent strain M145 and loss of the 266-kb

FIG. 3. Pulsed-field gel electrophoresis of *S. coelicolor* mutants 87/45, CO25, and S26, which underwent chromosomal end replacement. Genomic DNA of 87/45 and CO25 digested with AseI (A), HindIII (B), and SspI (C) and the same gels probed with a 1.3-kb fragment from the chromosome end are shown. (D and E) PFGE of genomic DNA digested with the same enzymes and probed with SCO0601, which detects the AseI-F fragment. S26 genomic DNA digested with HindIII (F) and SspI (G) and the same gels probed with a 1.3-kb fragment from the chromosome end are shown. See text for details.

fragment and maintenance of the 997-kb fragment for the mutant strain (Fig. 3C).

For strain 87/45, the AseI and SspI gels also suggested a duplication of the right end at the left chromosome. In this

strain, a 372-kb fragment from the left end that includes the AseI "J" fragment is replaced by a 635-kb fragment from the right end that lacks an AseI site (Fig. 2D). Digestion with AseI would generate a longer 1,135-kb fragment from the left chro-

FIG. 4. DNA sequences of duplication junctions of *S. coelicolor* strains. (A and B) Black bars denote DNA from the left chromosome end. Gray bars denote DNA from the right chromosome end. Diagonal hashes represent sequences identical between DNA from both ends. The new junction in strain 87/45 joins the partial coding regions of SCO7194 and SCO0372 at a 4-bp overlapping sequence (A). The new junction in strain S26 joins the partial coding regions of SCO0389 and SCO6657 at a 5-bp overlapping sequence (B). New chromosomal structures of *S. coelicolor* strains 87/45 (C), CO25 (D), and S26 (E) are shown. Black bars denote regions of the chromosome present in single copy. Gray bars denote duplications of the right chromosome end.

mosome end. The corresponding gel probed with the terminal 1.3-kb BamHI fragment showed a new band of the expected size, just below the AseI “A” fragment (Fig. 3A).

Similarly, for strain 87/45, the 372-kb fragment lost from the left end includes the 266-kb SspI fragment, while the 635-kb fragment duplicated from the right end lacks an SspI site (Fig. 2D). Digestion with SspI would generate a longer 656-kb fragment from the left chromosome end. The corresponding gel probed with the terminal 1.3-kb BamHI fragment showed a new band of the expected size, just below the 997-kb SspI fragment (Fig. 3C).

Finally, a larger size of the AseI “F” fragment also indicates replacement of the left chromosome end by DNA duplicated

from the right end. For strain 87/45, a PFGE gel of AseI fragments was probed with SCO0601, a gene within the 632-kb “F” fragment (Fig. 2A). The “F” fragment would increase to 1,135 kb (Fig. 2D). A gel probed with SCO0601 shows the presence of the 632-kb fragment in genomic DNA of the parent strain M145 and a new fragment of the expected sizes for 87/45 (Fig. 3D).

PFGE gels of HindIII and SspI fragments, probed with SCO0601, showed the same fragments for the wild-type and mutant strains, as expected (Fig. 3E). Digestion with HindIII yields a 172-kb fragment that includes SCO0601, a fragment that chromosomal end replacement leaves intact (Fig. 2A). Similarly, digestion with SspI yields a 596-kb fragment that

includes SCO0601, a fragment that chromosomal end replacement leaves intact (Fig. 2A). Thus, strain 87/45 has a wild-type organization of DNA for this part of the chromosome.

CO25 and S26 PFGE. In a similar fashion, PFGE gels for mutant CO25, which contains Tn4560, and mutant S26, derived from M145, showed loss of the left chromosome end, duplication of the right chromosome end, and replacement of the left end with the right end (CO25) (Fig. 3F and G; see also the supplemental material).

PCR sequencing. To further characterize the mutants, we amplified and sequenced newly formed DNA junctions with primers based on microarray data and PFGE results. The new junctions resided within the coding regions of genes and possessed, as data for 87/45 and S26 showed, 4-bp and 5-bp overlapping sequences, respectively (Fig. 4A and B). Figures 4C to E summarize the mutants of this study. Note that, after four generations of growth on a solid medium, microarray data showed the retention of altered chromosomes by progeny of strain S26 (6).

To our knowledge, this work provides the first evidence of one chromosome end replaced by the other end in *S. coelicolor*. The largest duplication observed in this study (>2,500 kb) also exceeds by more than twofold the limit previously reported for *Streptomyces* bacteria (1,061 kb) (5). A previous report described two strains of another streptomycete, *S. ambofaciens*, that replaced the left or the right chromosome end, respectively, with the other end (2). Both replacements, which possibly arose through intermolecular or intramolecular homologous recombination, involved 480 kb of the left and 850 kb of the right chromosome ends (2). Certain laboratory strains of *S. coelicolor* have an extension of their terminal inverted repeats from 22 kb to 1,061 kb, which might have arisen from site-specific transposition of a native insertion element and homologous recombination (5). In this study, the DNA junctions lacked large overlapping sequences, suggesting a mechanism of

nonhomologous end joining. However, the proximity of a native transposase, SCO0368, to the DNA junctions in strains with Tn4560 suggests a possible role of the insertion element in the replacement mechanism. Examination of larger numbers of mutants derived from a wild-type strain might indicate whether transposons promote chromosomal end replacement.

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