

Chromosomal Deletion and Rearrangement in *Streptomyces glaucescens*

ASHLEY BIRCH,^{†*} ALEX HÄUSLER,[‡] CORINNE RÜTTENER,[§] AND RALF HÜTTER

*Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum,
CH-8092 Zürich, Switzerland*

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The *Streptomyces glaucescens* genome frequently undergoes gross genomic rearrangement events which result in the deletion of extremely large segments of chromosomal DNA. The structure and origin of the DNA forming the novel junctions arising from five of these deletion events are described. Only one junction proved to be the result of a relatively simple event; the remainder were more complex, with one involving DNA which originated from at least five distinct loci. In three of the investigated cases, DNA sequences present in the junctions appeared to have resulted from the duplication of previously unique sequences, suggesting that duplication of chromosomal segments may be an important factor in genetic instability. The nucleotide sequences surrounding these junctions and their respective wild-type termini were determined.

Genetic instability is widespread within the genus *Streptomyces* (4, 20), a group of gram-positive, filamentous soil bacteria known chiefly for their biosynthetic versatility. This intriguing phenomenon characteristically involves the high-frequency loss (>0.1% of plated spores) of certain species-specific traits, several of which have been mapped to the chromosome (10, 21). When investigated, the instability has been shown to be the result of chromosomal deletions which remove the corresponding structural gene(s) (3, 5, 13, 18, 20, 30).

Investigation and analysis of genetic instability in *Streptomyces glaucescens* involved the construction of a contiguous *Bam*HI genomic map over 800 kb large of the relevant segment of the wild-type chromosome via alignment of overlapping cosmid clones (5, 16). Hybridization analyses of unstable mutant strains using these clones resulted in the characterization of extremely large chromosomal deletions (5, 16). These are directional and sequentially remove the hydroxystreptomycin phosphotransferase and tyrosinase structural genes, giving rise to the characteristic streptomycin-sensitive and melanin-negative unstable mutant phenotype. The deletions range from 270 to over 800 kb (Fig. 1) and terminate at numerous points within the intervening 530 kb (5). Furthermore, many of the more extensive deletions are accompanied by intense DNA amplifications which originate from a single 100-kb segment of the chromosome called the AUD (amplifiable unit of DNA) locus. The majority of the larger deletions terminate within this locus, which can therefore also be regarded as a hot spot for deletion termination (16). Cloning of a novel deletion junction fragment generated by termination of a deletion within the AUD locus of *S. glaucescens* GLA 161-2.22 (5) permitted the identification of sequences juxtaposed from the distal end of a large chromosomal deletion. These sequences originated

from a locus which was designated 08 H 06 (Fig. 1 and 2), which, intriguingly, proved to be the site of deletion termination in many other mutant strains (5). Thus, during the course of these studies, numerous deletion termini and their corresponding novel deletion junction fragments were identified. Because the molecular events and mechanisms responsible for triggering and generating such enormous rearrangements are poorly understood, analysis of the sequences involved in formation of these novel junctions would have the potential to shed further light on these intriguing events.

To date, only four small rearrangements and deletions have been analyzed at the sequence level in *Streptomyces* spp., two in a phage (38) and two in plasmid molecules (25, 34). These events took place in the absence of any extensive sequence homology, a situation which parallels that of the numerous rearrangement events analyzed in both *Escherichia coli* and *Bacillus subtilis* (2). These data have, however, been accumulated through the study of relatively small deletions involving extrachromosomal molecules or artificial constructs placed within the chromosome. Here we investigate the formation of deletions 2 to 3 orders of magnitude greater, which take place between unmanipulated sequences within the chromosome.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. *S. glaucescens* GLA 000 (ETH 22794) was used throughout this study as the wild-type strain. All strains carrying large chromosomal deletions (GLA 24, GLA 111, GLA 161-2.22, GLA 207, and GLA 600) have been previously described (5, 16). *S. glaucescens* cultivation in solid and liquid media was performed as detailed in references 5 and 17, respectively. *E. coli* ED 8767 (33) and JM 103 (31) were used as recipients in cloning experiments and for the propagation of cosmids. These strains were grown on either LB or M9 medium (28), and selection was carried out with tetracycline (20 µg/ml) and ampicillin (5 to 100 µg/ml). The plasmids used were pUC18/19 (35) and pBR322 (7). For the former, recombinant clones were identified by using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 40 µg/ml) and isopropyl-β-D-thiogalactopyranoside (IPTG; 50 mM). Transformation was performed as described previously (40).

* Corresponding author.

† Present address: Organisch-Chemisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

‡ Present address: Biology Department and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

§ Present address: Centre de Recherche Nestlé, Vers-chez-les-blanc, CH-1000 Lausanne 26, Switzerland.

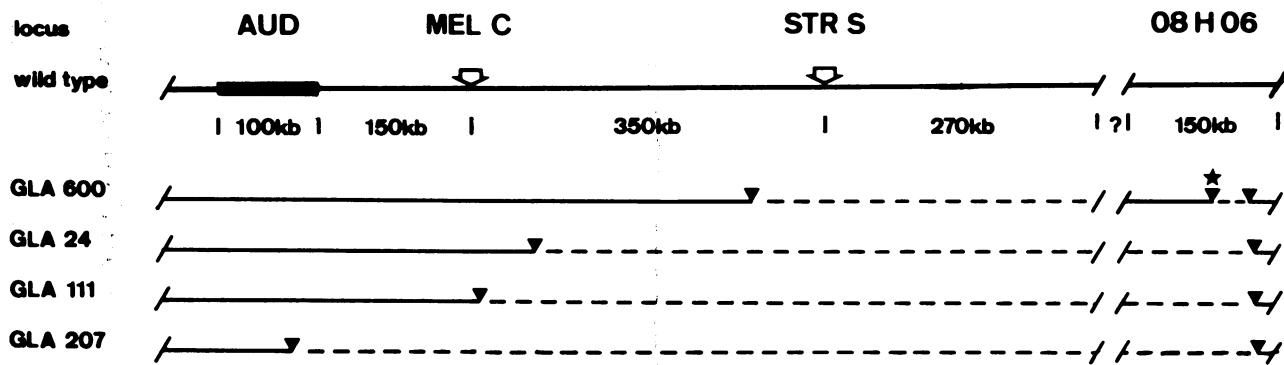


FIG. 1. Schematic representation of the segment of the *S. glaucescens* wild-type (strain GLA 000) chromosome which frequently undergoes deletion-rearrangement. The upper line depicts a genomic map which extends for over 1,000 kb and includes the following loci: AUD, the amplifiable unit of DNA (a locus which contains sequences that frequently undergo amplification); MEL C, the tyrosinase structural gene; STR S, the hydroxystreptomycin phosphotransferase structural gene; and 08 H 06, a deletion-termination hot spot. The lower part of the diagram depicts the deletion strains investigated during this study. Symbols: —, single-copy DNA; ---, deleted DNA; —▼—, deletion terminus (previously mapped); —★—, deletion terminus identified during this study; —/—, uncharacterized DNA.

DNA isolation and manipulation. All DNA isolation procedures were as detailed previously (5), except for lambda preparation, which was done by the method described by Maniatis et al. (28). DNA fragments required for shotgun cloning and for labeling were recovered from gels by using low-melting-point agarose (19) and GeneClean (BIO 101, La Jolla, Calif.), respectively, by the recommended procedure. Restriction endonucleases were purchased from Boehringer, Mannheim, Federal Republic of Germany, and Pharmacia, Uppsala, Sweden. T4 DNA ligase, phosphatase (calf intestinal alkaline phosphatase), DNA polymerase I, and Klenow fragment were all obtained from Boehringer; Sequenase was obtained from United States Biochemical Corp., Cleveland, Ohio; and *Taq* polymerase was obtained from Beckman, Fullerton, Calif. Standard procedures were used throughout for the manipulation of DNA (28). Blotting and hybridization were all as previously described (5). *E. coli* colony, cosmid, and lambda bank replicas were prepared by standard methods (28). DNA probes were labeled to high specific activity

by using the method of Feinberg and Vogelstein (12), with pd(N)⁶ hexanucleotides (Pharmacia) as previously detailed (5).

Construction of a lambda genomic library. Size-fractionated *Sau*3AI partially digested GLA 000 total DNA (15 to 20 kb) was ligated into *Bam*HI phosphatase-treated lambda EMBL4 (14), which was then packaged by using the Packagene system of Promega Biotec, Madison, Wis. This was subsequently used to infect *E. coli* NM538 and NM539 (14), sufficient plaques being obtained to yield a probability of >99.9% that any one particular sequence was actually represented in the library (19). The cosmid library was that used in previous studies (5).

DNA sequencing. DNA sequences were determined by the dideoxy-chain termination method (37) with M13mp18 and M13mp19 as vectors (42). Sequenase was used for the chain elongation reactions. Compressions caused by the high G+C content of streptomycete DNA were resolved by (i) substituting the nucleotide analog 7-deaza-dGTP for dGTP in the

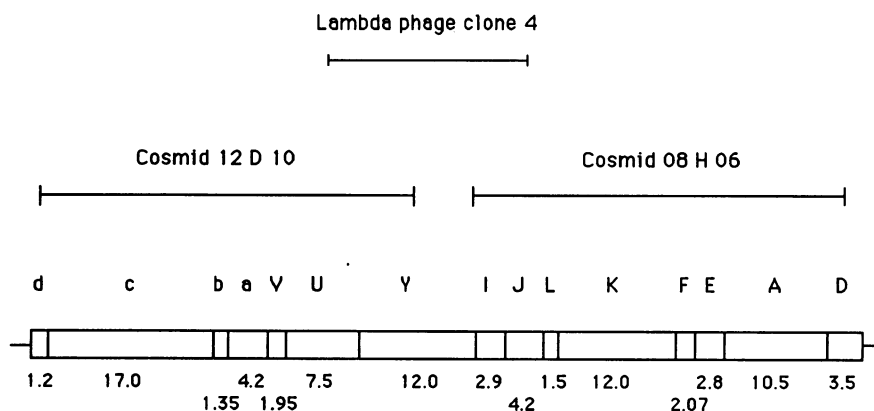


FIG. 2. Schematic representation of a segment of the 08 H 06 locus in which many of the presented deletions and rearrangements took place. This locus was initially identified as the source of sequences juxtaposed to the AUD locus by a large chromosomal deletion in strain GLA 161-2.22 (5). The designation 08 H 06 stems from cosmid 08 H 06, which contains wild-type DNA corresponding to these juxtaposed sequences. Although the map was initially 40 kb (the extent of the sequences present in cosmid 08 H 06), we have subsequently enlarged the map at this locus to 150 kb by chromosome walking. To avoid confusion, the original 08 H 06 designation has been retained. The open bar depicts a *Bam*HI chromosomal map of the *S. glaucescens* GLA 000 (wild-type) chromosome at this locus together with fragment designations and sizes in kilobases. The upper portion of the diagram shows the cosmid and phage clones which are mentioned in the text.

reaction mixes, (ii) using DNA polymerase I Klenow fragment and carrying out the reactions at 42 or 50°C, or (iii) using Taq polymerase as described previously (24). Products of the reactions were analyzed as described previously (5).

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper will appear in GenBank/EMBL/DDBJ nucleotide sequence data bases under the accession numbers: M62936 to M62951.

RESULTS

We chose to investigate four rearrangement events from among those already described, representing a variety of situations. These included the following cases (depicted schematically in Fig. 1): mutant strain GLA 600; GLA 111; GLA 207, and GLA 24 (5). None of these strains contained amplified DNA sequences, as we have previously found that such reiterations, when in close proximity to deletions, can preclude the identification and isolation of the corresponding deletion junctions (16). All the above deletion events resulted in the appearance of at least one easily identifiable novel junction fragment (in each case using probes specific for both deletion termini) which presumably represented the fusion of previously discrete sequences flanking the deleted DNA. To avoid repetition and involved presentation, only the analysis of the first event will be dealt with in detail; the remaining events will be merely described. These other events were, however, all analyzed in a manner directly analogous to that applied to the first event.

Rearrangement in strain GLA 600. Hybridization analyses (5, 16) of strain GLA 600 with a series of overlapping cosmid clones covering the 800-kb segment of the wild-type genome depicted in Fig. 1 (all those sequences to the left of the uncharacterized DNA designated ?) established that this mutant strain possessed an extensive chromosomal deletion, one terminus of which was situated in the neighborhood of the hydroxystreptomycin phosphotransferase structural gene (Fig. 1). Hybridization of cosmid DNA covering this locus (5) to *Bam*HI-digested genomic DNA failed to reveal an easily identifiable novel, junction-containing fragment. A second deletion terminus was, however, mapped within the 08 H 06 locus (Fig. 1, right). Hybridization of labeled cosmid 08 H 06 DNA against *Bam*HI-digested chromosomal DNA from strain GLA 600 revealed that a deletion in this strain terminated in the fragment designated K (Fig. 3A) and removed all sequences lying to the left of this terminus (according to the map orientation of Fig. 1). This can be seen as the disappearance of bands I, J, and L and the replacement of the 12.0-kb K fragment with a novel junction-containing *Bam*HI band of 7.3 kb (Fig. 3A and 4). This novel fragment was shotgun cloned into pBR322 by excision and purification of a window of *Bam*HI-digested DNA of ca. 7.3 kb from a preparative agarose gel. The desired clones containing this fragment were identified by using labeled K fragment in a colony hybridization experiment. Additionally, the K fragment was subcloned from cosmid 08 H 06 into pBR322. Restriction mapping and comparison of these two clones permitted a more precise localization of the deletion terminus and hence the identification of sequences within the junction clone which represented DNA juxtaposed from the distal (left-hand) end of the deletion by the deletion event (sequences to the left of the deletion junction in the junction-containing fragment [Fig. 3A, bottom]). A small DNA fragment representing this juxtaposed DNA (not depicted) was isolated from the junction clone, labeled, and used to screen a cosmid library containing wild-type DNA. The resulting

cosmid clones contained DNA corresponding to the wild-type locus from which the juxtaposed DNA originated and hence the distal terminus of the deletion event. Hybridization of the labeled 7.3-kb junction fragment to a cosmid representing this locus (12 D 10) showed that the juxtaposed sequences stemmed from a 7.5-kb *Bam*HI fragment (U [Fig. 3A]). Fragment U was subcloned into pBR322 and restriction mapped. Subsequently, this clone and those containing the K fragment and the deletion junction were used in control hybridization studies against genomic DNA from strains GLA 000 and GLA 600 to ensure that no secondary, cloning-induced rearrangements had occurred. Comparison of the restriction maps of the three clones permitted the identification of small fragments surrounding both the left and the right deletion termini and the deletion junction which were suitable for sequencing studies. A total of 329, 291, and 259 bp were determined surrounding the left deletion terminus, right deletion terminus, and deletion junction, respectively, to permit a more precise characterization of the rearrangement event and to analyze the sequences involved. Sequences obtained are depicted in Fig. 5.

To accurately determine the extent of the deletion and the status of the DNA between the two termini, we carried out cosmid walking from the left-hand (map orientation, Fig. 1) extremity of cosmid 08 H 06 (fragment I, Fig. 2) and the right-hand extremity of cosmid 12 D 10 (fragment Y, Fig. 2). Unfortunately, no new cosmid clones could be isolated, indicating that there was a gap in the cosmid bank at this point. To circumvent this problem, we carried out a second chromosome walk, screening a lambda bank containing wild-type DNA. Several clones were isolated, of which clone 4 (Fig. 2) is a representative example. This clone allowed us to connect the two disparate cosmid groupings and thus construct a contiguous chromosomal map of the region under study. Hybridization of cosmid and lambda clones spanning the entire interval between the two deletion termini against *Bam*HI-digested GLA 600 chromosomal DNA showed that the deletion was a straightforward event which resulted in the loss of the intervening 32.6 kb of DNA (Fig. 1 and 3A). The previously identified deletion terminus in the neighbourhood of the *strS* locus (Fig. 1) must therefore represent another deletion event, indicating that there are at least two distinct deletions in this strain.

Rearrangement in strain GLA 207. This strain contains a large deletion for which two deletion termini have been mapped (Fig. 1), one in the 08 H 06 locus and one in the AUD locus. The novel junction fragments identified by hybridization with probes specific for each mapped terminus are, however, of differing size, indicating that these mapped termini must in fact represent the ends of two discrete deletions and not a single event. As both deletion termini resulted in the appearance of easily identifiable novel junctions in *Bam*HI genomic hybridizations, we decided to proceed with the investigation of both events.

The deletion terminating in the 08 H 06 locus lies within a 2.07-kb *Bam*HI fragment (F [Fig. 3B]), the event removing all sequences to the left of this end (map orientation) and producing a novel junction fragment of 2.9 kb (Fig. 3B and 4). Sequences present in the junction fragment corresponding to the distal end of the deletion proved, surprisingly, to originate from a 3.5-kb *Bam*HI fragment (D [Fig. 3B]) which lies 13 kb to the right of the F fragment. Remarkably, this fragment lies on the opposite side of the deletion terminus to the deletion (Fig. 3B). Interestingly, the D fragment and the entire interval between it and the F fragment remain intact in strain GLA 207 (Fig. 3B and 4) despite the fact that se-

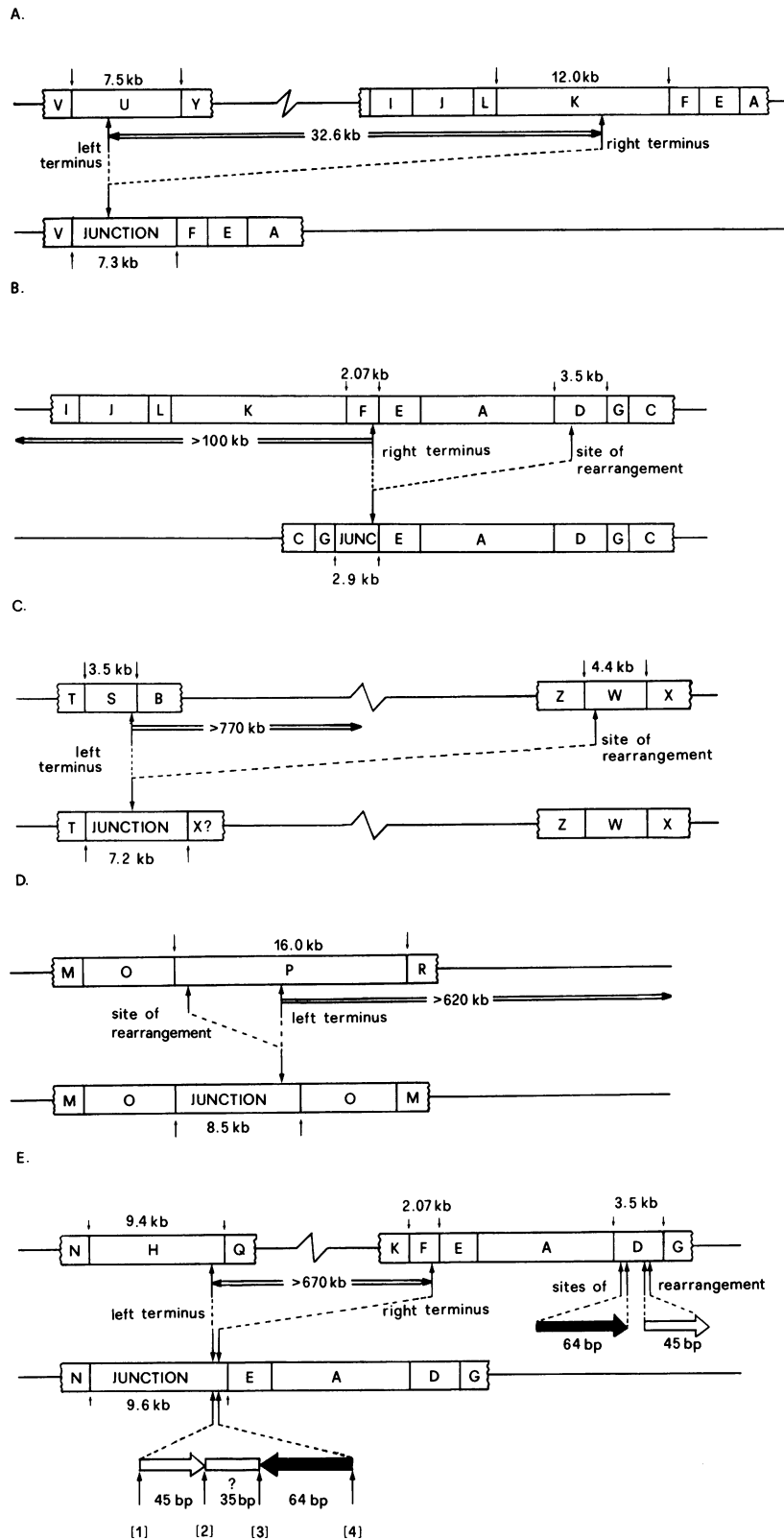


FIG. 3. Schematic representation of the five deletion-rearrangement events investigated during this study. The upper half of each diagram depicts a simplified chromosomal DNA map of the *S. glaucescens* (GLA 000) wild-type loci which participate in the deletion-rearrangement events leading to formation of a novel junction fragment in the corresponding mutant strain (lower half of each diagram). To avoid overcomplication, only those *Bam*HI fragments necessary for an understanding of the events are drawn (represented by open bars). Each of these *Bam*HI maps represents a DNA segment surrounding a deletion terminus depicted in Fig. 1. The small vertical arrows delineate the

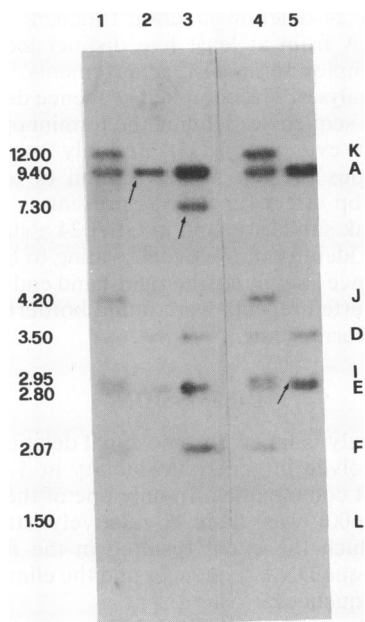


FIG. 4. Southern blot hybridization analyses of *Bam*HI-digested chromosomal DNA of three *S. glaucescens* mutant strains which possess a deletion terminus within the 08 H 06 locus. Hybridization with labeled cosmid 08 H 06 DNA. Lanes: 1, GLA 000; 2, GLA 24; 3, GLA 600; 4, GLA 000; 5, GLA 207. The size and designation of the *Bam*HI bands are shown. For the chromosomal order of the fragments, consult Fig. 2. The arrows indicate the position of novel junction-containing bands.

quences originating from the D fragment are present at a new location (the deletion junction fragment). Furthermore, the segment of D which is present in the junction fragment is inverted compared with its orientation in the wild-type strain (Fig. 3B). This would suggest that the D fragment has been duplicated prior to or during the deletion event leading to the formation of the junction fragment. Although the precise extent of the duplication was not determined, it encompasses at least the adjacent C fragment. A total of 365, 308, and 346 bp were determined surrounding the left deletion terminus, the site of rearrangement in the D fragment, and the deletion junction, respectively. These are shown in Fig. 5.

The deletion terminating in the AUD locus lies within a

Strain/event	DNA sequence adjacent to junctions
GLA 600	<u>TGGCTAGCCGTGTTGGCGCTGTAGACCTTGTCCCTCGCGCTCAGCAAGCGTCCACGGGGAGGCG</u>
GLA 207 (AUD-locus)	<u>CTTCGACCTCGGCCCTCGGACGCTCGGGCGGCCATGATGAACATGCCAATGGCCAGGTAGGGAGA</u>
GLA 207 (08 H 06)	<u>ACCGGGCCCTTCGTTGCTTCGGTGAAGCTCTCGCGGGCAACCCACCAAGCAATGCCCTCTACCAA</u>
GLA 111	<u>GGGGCGTACGGCCAGTTCCTGCCTGGTCTCTGCCGGACGGTGATCTCGTGGGACGGGTCTGGAGCT</u>
GLA 24 [1]	<u>TGCACGAGCATCGTTCGGTCCCGTGGCCGTGTGCCCGTACCTCAGCGAACCCGGAGACACAGCC</u>
GLA 24 [2]	<u>GGCTTCAAGGAGCTGCTGCAGCAGGCTTGCCICCGGTGTAGGCCACTTA</u>
GLA 24 [3]	<u>CATGAGTGGCAGCAACAAAACCACAGCCCTGCGGACTGCCCATGC</u>
GLA 24 [4]	<u>ACGCCGCCACCCCTCAACCCAGGACCGCATCCAGCCGTACCATCGACACCGCCCTGCCCTGGGCCAGG</u>

FIG. 5. DNA sequences surrounding the left (upper line) and right (lower line) termini of eight deletion-rearrangement events. Solid lines indicate the sequence of the resulting junction fragments, and the vertical broken lines indicate the position of the junctions. The position of junctions which occur within a repeated sequence (boxed) can be anywhere within the depicted repeat. As the origin of the 35-bp segment between junctions [2] and [3] (Fig. 3E) in strain GLA 24 was not determined, the wild-type sequences which participated in the rearrangement events leading to the formation of part of the GLA 24 junction were not available for comparison. Therefore, the sequences shown as originating from the right terminus of event [2] and the left terminus of event [3] are derived from those present in the junction fragment. The nonavailability of the wild-type progenitor sequences also precludes a precise determination of the site of the rearrangements. Letters in bold type show positions of identity between the two aligned termini.

3.5-kb *Bam*HI fragment S, the deletion removing all sequences to the right (map orientation) and resulting in its replacement by a novel *Bam*HI junction fragment of 7.2 kb (Fig. 3C). Sequences present in the junction fragment corresponding to the distal end of the deletion originated from a 4.4-kb *Bam*HI fragment designated W (Fig. 3C), which does not lie in any mapped region of the chromosome. Unexpectedly, this fragment and sequences surrounding it remain intact in mutant strain GLA 207 and are thus apparently unaffected by the deletion event. These results strongly suggest either that the W fragment has been duplicated

fragments in which the event(s) took place. The large vertical arrows show the sites of deletion-rearrangement (upper) which gave rise to the novel junction (lower). Broken lines connect the junction and its respective progenitor termini. The horizontal arrows represent DNA sequences which are deleted in the corresponding mutant strains as a result of these rearrangement events. Where these arrows possess two arrowheads, the event has led to the fusion of the sequences delineated by the small vertical arrows and deletion of all DNA known to lie between the two ends; where these arrows possess only a single arrowhead, the rearrangement event has taken place between sequences at the depicted terminus and a second site without elimination of the intervening DNA but accompanied by a deletion with the depicted directionality and minimum ascertained extent. (A) Rearrangement leading to the formation of a novel junction and the deletion of 32.6 kb of chromosomal DNA in mutant strain GLA 600. The illustrated termini correspond to the termini within the 08 H 06 locus depicted in Fig. 1. (B) Rearrangement affecting the 08 H 06 locus in mutant strain GLA 207 (right-hand deletion terminus in Fig. 1). (C) Rearrangement affecting the AUD locus in mutant strain GLA 207 (left-hand deletion terminus in Fig. 1). (D) Rearrangement in the neighbourhood of the tyrosinase structural gene in mutant strain GLA 111 (left-hand deletion terminus in Fig. 1). (E) Rearrangement leading to the formation of a complex junction fragment in mutant strain GLA 24 (involving the left and right deletion termini shown in Fig. 1). The large solid and open horizontal arrows in panel E depict the extent and orientation of sequences present in the novel junction fragment (lower diagram in panel E) and their progenitor sequences in the D fragment (upper diagram in panel E). The open bar sandwiched between these two arrows in the lower part of the diagram depicts a 35-bp sequence whose origin was not determined. The numbers in brackets below these arrows designate the junctions between these sequences (see also Fig. 5). To gain a full understanding of these complicated events, it is recommended that this figure be consulted in conjunction with Fig. 1 and 2 and the text.

before or during participation in the deletion event or that it exists in more than one copy in the chromosome. A total of 162, 187, and 131 bp were determined surrounding the left deletion terminus, the site of rearrangement (W fragment), and deletion junction, respectively, and are depicted in Fig. 5.

Rearrangement in mutant strain GLA 111. Two deletion termini have been mapped in this strain; however, only that adjacent to the *melC* locus produced an easily recognizable novel *Bam*HI junction fragment in hybridization analyses. The deletion terminates in a 16.0-kb *Bam*HI fragment P which is replaced by a novel *Bam*HI band of 8.5 kb (Fig. 3D). Sequences corresponding to the distal (right-hand) end of the deletion proved, surprisingly, to also originate from the P fragment but 6.9 kb to the left of the mapped deletion terminus and on the opposite side of the P fragment from the deletion (Fig. 3D). The distal sequences are inverted in the novel junction fragment compared with their original orientation. Furthermore, these sequences are unaffected by the deletion in strain GLA 111. This indicates that the sequences at the left of the P fragment have undergone inverse duplication before or during deletion formation. The precise extent of the duplication was not determined but includes at least the adjacent O and M fragments. A total of 128, 125, and 172 bp were determined surrounding the left deletion terminus, the site of rearrangement, and the novel junction, respectively. Unfortunately, this did not allow direct comparison of the sequences involved in the rearrangement event as an additional two A residues are present in the junction fragment which do not originate from either end of the deletion-rearrangement (Fig. 5). This result indicates that the novel junction fragment in strain GLA 111 is the result of at least two deletion-rearrangement events.

Rearrangement in mutant strain GLA 24. In strain GLA 24 two deletion termini have been mapped (Fig. 1), both of which give rise to novel *Bam*HI junction fragments of 9.6 kb. At the left terminus (*melC* locus) the deletion replaces a *Bam*HI band of 9.4 kb (H) and removes sequences lying to the right (map orientation), whereas at the 08 H 06-locus the deletion terminus (right-hand end) lies within a *Bam*HI fragment (F) of 2.07 kb, resulting in the elimination of this fragment and those lying to the left (Fig. 3E and 4). These data suggested that these ends defined the termini of a single deletion.

Detailed restriction mapping, however, revealed this to be an oversimplification of the situation. Excluding the sequences which originate from the left and right termini of the deletion, there is an additional central stretch of approximately 150 bp, consisting of DNA originating from two distinct loci within the D fragment (Fig. 3E). These loci and the entire interval between the D and the F fragments are unaffected by the events which have taken place in GLA 24, once again implicating duplication of DNA segments. The sequences flanking and including the entire intervening sequences in the junction fragment were determined (334 bp), as well as the left (218 bp) and right (379 bp) wild-type termini and the relevant segments of the D fragment (536 bp). These data revealed the situation depicted in Fig. 3E. The junction fragment not only consists of DNA originating from the deletion termini and sequences from the D fragment, but also contains a segment of DNA of unknown origin sandwiched between the D-fragment DNA. The DNA from one of the D-fragment loci (Fig. 3E) is in inverse orientation compared with the wild-type situation, whereas the other is in the same orientation with respect to the wild-type situation. From these data, it can be clearly seen

that the GLA 24 deletion junction fragment is composite, involving DNA from at least five distinct loci, and is the result of a complex series of rearrangements.

Sequence analyses. The compiled sequence data allowed us to analyze the sequences defining the termini of six deletion-rearrangement events (Fig. 5). In only two cases were microhomologies present at the termini of a deletion. In GLA 600 a 4-bp direct repeat was present as well as some additional weak similarity, and in GLA 24 a stretch of 10 of 16 bp showed identity in the event leading to the rearrangement of sequences involving the right-hand end (event 4). No significant inverted repeats were found bordering any of the investigated rearrangements.

DISCUSSION

The previously mapped chromosomal deletions which are intimately involved in genetic instability in *S. glaucescens* are of different complexities. In only one of the investigated cases (GLA 600) was there a relatively straightforward deletion in which the event resulted in the fusion of two previously unique DNA sequences and the elimination of the intervening sequences.

In three of the cases (both GLA 207 events, GLA 111, and some sequences involved in the GLA 24 event) the wild-type progenitor loci of sequences juxtaposed by the deletion are surprisingly unaffected by the rearrangement event in their respective mutant strains. This interesting result can be explained by postulating either that the sequences involved are duplicated before or during deletion or that they are already present in the chromosome at two distinct loci. The former is supported by the following facts: (i) in all cases, only a single set of overlapping wild-type cosmid clones could be isolated by using the probes containing the juxtaposed junction sequences; (ii) restriction and sequence analyses showed 100% identity between the DNA in the junction clones and their respective progenitor wild-type sequences; and (iii) in GLA 111, the duplication event involves the well-characterized tyrosinase gene in which point mutations are known to abolish activity, implying that a single locus is responsible in the wild-type strain. Interestingly, in GLA 207 (08 H 06 locus rearrangement), GLA 111, and a fragment in the GLA 24 event, the sequences which have undergone duplication are present in the junction fragment in the inverse orientation compared with their original situation. Furthermore, these sequences originate from loci which are situated on the opposite side of the mapped deletion terminus to the deletion itself. Although this inverse and direct duplication (seen in GLA 24) is not found in all cases, it could in fact have taken place and been subsequently disguised by the deletion events themselves. The creation of novel junctions as a result of such rearrangements suggests that duplication of chromosomal segments may be an important feature or trigger in deletion formation. Inverted duplications are known to be a common feature of amplification in eukaryotic organisms (36).

Investigation of DNA rearrangements in *E. coli* and *B. subtilis* has shown that deletion events take place mainly in the absence of extensive sequence homology and are therefore examples of illegitimate recombination. Sequence analyses have, however, revealed that deletion formation frequently occurs between small perfect or imperfect directly repeated sequences (1, 11, 27) which have been termed microhomologies and, to a lesser extent between quasipalindromic sequences (9, 15). It has also been shown that DNA gyrase is capable of mediating illegitimate DNA rearrange-

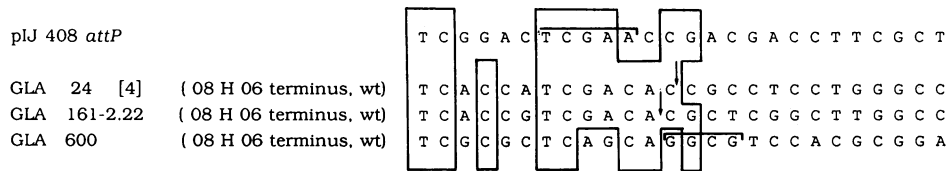


FIG. 6. Similarity between sequences surrounding the termini of three deletion-rearrangement events at the 08 H 06 locus in *S. glaucescens*. The positions of the respective termini are indicated by a vertical arrow for GLA 161-2.22 and GLA 24 and by brackets for GLA 600. GLA 161-2.22 and GLA 24 show a high degree of similarity, 12 of 13 or 19 of 26 bp. For comparative purposes, sequences present at the attP site of the *S. glaucescens* integrative plasmid pIJ408 are presented. The bracketed sequence 5'-TCGAA-3' is the putative crossover site and is common to many actinomycete integrative plasmids. Similarities between the first 14 bp of these sequences are boxed.

ments (22, 23, 32), and studies with both *E. coli* (29) and *B. subtilis* (27) have suggested that topoisomerase-like enzymes either promote or carry out certain deletion events. Many of the DNA sequences surrounding deletion termini do not, however, fall into any of the above categories, and it seems highly probable that as yet undefined sequence motifs and subtle features of chromosome structure (9) are involved in, or influence, deletion formation.

We have previously characterized a deletion in strain GLA 161-2.22 (5) in which the termini were precisely delineated by a quasipalindrome. Of the events investigated during this study, two involved imperfect direct repeat sequences (Fig. 4, GLA 600 and GLA 24, event 4), whereas the remainder showed neither direct nor indirect repeats at their termini. Interestingly, the complexity of the events in *S. glaucescens* is reminiscent of events proposed to be the result of gyrase-induced illegitimate recombination (29). The low sequence specificity of DNA gyrase, the absence of gyrase sequence data for streptomycetes, and the difference in G+C content between streptomycetes and these organisms would, however, preclude identification of a putative gyrase consensus sequence by comparison with those from other organisms (23). Furthermore, comparison of the sequences surrounding the deletion-rearrangement termini did not reveal any convincing similarity which could be interpreted as a consensus recognition sequence.

Striking similarity was, however, observed for GLA 24 (event 4) and the previously characterized GLA 161-2.22, where 12 of 13 or 19 of 26 bp were identical surrounding the right-hand termini and, to a lesser extent, the GLA 600 right-hand terminus (Fig. 6). Interestingly, these termini also share some similarity with sequences within the chromosomal attachment site of the indigenous plasmid pIJ408 (Fig. 6). This plasmid is present in a chromosomally integrated state within *S. glaucescens* and is site-specifically excised and transferred into recipient cells on mating with *S. lividans*, integration and excision taking place between identical 43-bp att sites present in the chromosome and on the plasmid (39). The stretch of DNA within the att site which displays similarity to the aforementioned deletion termini is the putative crossover site for the site-specific recombination event. Sequences at this site are strongly conserved between the att sites of several actinomycete integrative plasmids (39) and are thought to serve as the recognition site for a plasmid-encoded recombinase (6, 8, 26). It would be interesting to speculate on the involvement of the putative plasmid-encoded recombinase of pIJ408 in some of the gross genomic rearrangement events in *S. glaucescens*. Under conditions of stress, this recombinase may possibly be overexpressed or recognize other similar but degenerate sequences in addition to the attachment sites. Recognition of secondary sites with relaxed specificity has already been

reported for the phage lambda integrase (41). Unfortunately, despite exhaustive attempts to cure *S. glaucescens* of pIJ408, a plasmid-free strain could not be obtained (38a). It was therefore not possible to establish a correlation between the presence of the plasmid-encoded recombinase and the events described here.

These findings demonstrate that the extremely large prokaryotic chromosomal deletions in *S. glaucescens* are the result of complex illegitimate recombination events, several of which involve chromosomal duplication. They also suggest that future effort may be profitably focused on elucidating, in addition to DNA structures, the biochemical processes involved in these events, e.g., recombinase activities. The presence of such powerful recombinational activities in *S. glaucescens* and probably other *Streptomyces* species has significant implications for genome fluidity in this important genus.

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