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Genetic instability and its possible evolutionary implications on the chromosomal structure of *Streptomyces*

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Summary — Recent progress in the understanding of the chromosomal rearrangements in *Streptomyces* has allowed us to envisage the possible involvement of genetic instability in the evolution of the chromosomal structure. The characterization of recombinational events in the terminal parts of the *S. ambofaciens* chromosome, as well as the observation by other groups that linear plasmids and chromosomes are able to interact, would provide an explanation for the very high levels of polymorphism seen in the terminal inverted repeats of different strains of *Streptomyces*.

***Streptomyces* / genetic instability / chromosomal rearrangements**

Genetic instability in *Streptomyces*

Bacteria belonging to the genus *Streptomyces* exhibit a very high degree of genetic instability, which was defined as the appearance of spontaneous mutations with frequencies higher than 10^{-3} . Early observations revealed that this instability was confined to particular stages of the *Streptomyces* cycle of development [1]. On solid medium, spores germinate to form the vegetative mycelium which produces aerial hyphae as nutrients become limiting. Then the growing mycelium subdivides by septation into many compartments destined to become spores. The secondary metabolism, associated to the late stages of differentiation, is of considerable industrial importance in particular for the production of antibiotics. When grown in liquid culture, the synthesis of these metabolites is concomitant with the onset of the stationary phase [2]. Most phenotypic characteristics involved in differentiation and expression of secondary metabolism are genetically unstable, whilst only few traits of the primary metabolism are affected. Hence, genetic instability affects specific genes indicating that this phenomenon is positionally localized. At first it was not known if the genes affected by the genetic instability were located on the chromosome or associated with plasmids. The increase in the mutation rates after treatment with plasmid-curing agents was initially interpreted as the loss of plasmid born characters [3, 4]. However, several unstable traits were unambiguously

mapped to the chromosome. Later, the appearance of mutations was correlated with the occurrence of gross chromosomal rearrangements such as large scale deletions and amplifications of particular sequences called AUD (amplifiable unit of DNA) [5–7]. More recently, the development of the pulsed-field gel electrophoresis (PFGE) technique has allowed these rearrangements to be assigned to a particular region of the chromosome called the unstable region [8, 9].

Chromosomal structure in natural environments

Physical mapping experiments revealed that the unstable region corresponded to both ends of the linear chromosomal DNA [9, 10]. Chromosomal linearity was first reported in *S. lividans* by Lin *et al* [11]. At present, all WT *Streptomyces* strains where the chromosomal structure is known have a linear DNA molecule of approximately 8 Mb containing TIRs (terminal inverted repeat) and proteins covalently bound to the ends [10–14]. In *S. ambofaciens*, the physical mapping of three geographic isolates was achieved by ordering the *AseI* restriction fragments resolved by PFGE. The two terminal *AseI*-fragments exhibited a retarded PFGE mobility when proteinase treatment was omitted, demonstrating that proteins were covalently bound to both of them. Repeated sequences present at both ends of the chromosome were mapped as long TIRs stretching over 210 kb and cloned as eight overlapping cosmids. The deletable region of *S. ambofaciens* was localized at the chromosomal ends (fig 1) [10]. To investigate the hypothetical relationship between chromosomal linearity and genetic instability, the

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Abbreviations: AUD, amplifiable unit of DNA; DNA, deoxyribonucleic acid; kb, kilobases; Mb, megabase; PFGE, pulsed-field gel electrophoresis; TIR, terminal inverted repeat; WT, wild type.

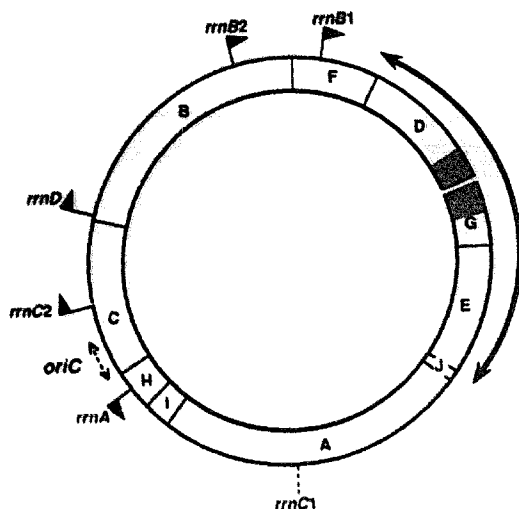


Fig 1. Structural organization of the chromosome of *S. ambifaciens* DSM40967. The *Ascl* fragments are lettered according to Leblond *et al* [10]. The localization of *oriC* (dotted arrow), the transcriptional orientation of the *rrn* operons (symbolized by a black flag, not determined for *rrnC1*) as well as the localization of the unstable region (double arrow) are according to Berger *et al* [25]. The TIRs are represented by light-gray boxes at the extremities of the *Ascl*-G and -D fragments.

chromosomal structure of mutant strains which had arisen by genetic instability was studied.

Alterations of the chromosomal structure associated to genetic instability

Different types of chromosomal structure were identified in mutant strains selected under laboratory conditions. In the first group, deletions internal to one chromosomal arm were characterized using PFGE and sequences cloned from the unstable region. In two strains analyzed, the deletion terminus were found to be within the same chromosomal arm leaving a linear DNA structure ending in TIRs. In both cases, one deletion terminus mapped within the TIR. This resulted in the shortening of the TIRs, from 210 kb in the WT strain, to approximately 100 kb in one strain and 200 kb in the other (Fig 2). This shortening was not due to the degradation from one end of the chromosome but to a recombination event between two points within the same chromosomal arm [15]. This case is similar to the description of *S. glaucescens* where an internal deletion of 32.6 kb lay in a region cloned in overlapping cosmids [16]. However, the

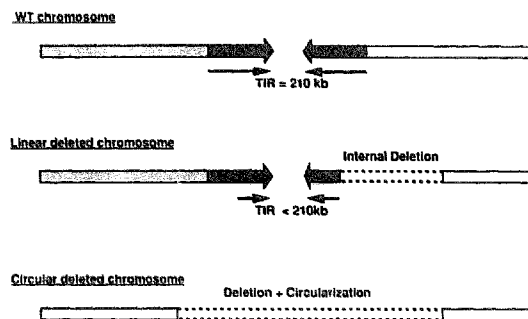


Fig 2. Schematic representation of the chromosomal rearrangements in *S. ambifaciens* DSM40697. The left and right chromosomal extremities (according to the figure orientation) are drawn in light-gray and white, respectively, whilst the TIRs are represented as black arrows. The sizes of the TIRs are indicated. Dotted lines symbolized the deleted sequences.

chromosomal structure of this species was not described. These deletion events demonstrated that the chromosomal ends are not directly implicated in the formation of the deletion.

A second group of mutants was characterized by a deletion encompassing the entire TIRs on both chromosomal arms. In each of the four cases analyzed, the deletion was approximately 2000 kb. The chromosome of these strains, shown to be circularized as a junction fragment, homologous to sequences originating from internal regions of both arms, was characterized (Fig 2). The phenotypic instability in the progeny of spontaneous circular DNA mutants was estimated to be 10-fold higher than in the wild type strain. The chromosomal stability of such a structure was assessed in the progeny of these circular DNA mutants. Additional deletion events were detected in 11 out of the 13 mutant strains isolated, demonstrating that the occurrence of a deletion is independent of the presence of chromosomal ends [15]. Similar results were described in *S. lividans* where artificial circularization of the chromosome was shown to enhance genetic instability by 20-fold as well as increasing associated genome rearrangements [17, 18].

Evolutionary implications of terminal recombination

Based on the above evidence, the occurrence of deletions does not result from the degradation of the chromosomal ends but may be the consequence of illegitimate recombination, as no DNA homology was found between the deletion termini [16]. The resulting chromosomal structure depended on the location of the two recombination points; these may be on the same chromosomal arm or on different

arms. An example of recombinational interactions between both arms of the chromosome can be seen in *S. ambifaciens* and in *S. lividans* 66 [9, 15]. This recombination has caused the deletion of all TIR sequences and the circularization of the chromosomal DNA. However, these mutant chromosomes were shown to be more unstable than the WT chromosomes. It can not be ruled out that this structural instability resulted from the loss of functions encoded by the deleted sequences. Nevertheless, artificially circularized chromosomes which did not have any large deletion were also highly unstable [17, 18]. This increased level of genetic instability might be caused by the inability of circular chromosomes to terminate replication and to partition as they probably lack specific terminator sequences. The increased level of recombination may also be due to negative supercoiling of the circular DNA.

On the other hand, the linearity of the chromosomal DNA seems to be a general trait of *Streptomyces* (although all were selected for their ability to produce antibiotics). These data suggest that chromosome circularization is probably counter-selected in natural environments, and that there might be an advantage to *Streptomyces* having a linear chromosomal DNA.

Contrasting with the common invertion structure in *Streptomyces* species, the TIRs are highly variable in size and also in sequences.

The TIR sizes ranged from 24 kb in *S. griseus* to 550 kb in *S. rimosus* [12, 14]. In addition, despite a close phylogenetic relationship [19] and a common genetic organization between *S. ambifaciens*, *S. lividans* 66 and *S. coelicolor* A (32), the TIRs are of different sizes: 210 kb, 25 kb and 61 kb, respectively [10, 11, 13]. In *S. ambifaciens*, internal deletions within one chromosomal arm generated TIR length polymorphism within the same species [15].

Intra-specific polymorphism was also seen between two WT isolates of *S. ambifaciens*, DSM40697 and ATCC23877. TIR sequences flanking the ends of the chromosome in strain DSM40697, cloned in cosmid AD91 [10], were not homologous to any DNA sequence from strain ATCC23877. In addition, there was no homology between the TIR of the two related species *S. lividans* 66 and *S. ambifaciens* (unpublished results). It can be concluded that TIR sequences are highly variable, although short palindromes are partially conserved at the ends of the linear replicons [11, 20, 21], suggesting that the mechanism of their evolution is different from that of the rest of the chromosome.

The terminal recombination events described here may have played a role in this evolutionary mechanism. Terminal recombination events have been described between linear plasmids and the chromosome of *Streptomyces*. For example in *S. rimosus*, a producer of the antibiotic oxytetracycline, one end of the chromosomal DNA was shown to have been replaced by terminal sequence of the 387 kb linear plasmid pZG101 [22]. Therefore, linear chromosomes could gain a new end by interacting with linear plasmids. In *S. lividans*, the terminal 16 kb of the 25 kb TIRs

were identical to one end of the 50 kb linear plasmid SLP2 [11, 23]. These examples suggest that in the ancestors of the modern *Streptomyces*, interaction occurred between the chromosome and the ends of the linear plasmids (or chromosomal DNA ends) and that these interactions may have been involved in the mechanism which caused chromosomal linearity.

Such recombination events generate hybrid chromosomes with two different ends. However, *Streptomyces* linear replicons (plasmids or chromosomes) belong to the structurally related class of genetic elements called invertions [24]. These linear DNA molecules are characterized by identical sequences in inverted orientation at their termini.

To account for this phenomenon we must assume the existence of a mechanism which leads to the homogenization of the terminal sequences on the hybrid chromosomes. A simple way for this to happen would be the recombination of two replicated copies of the hybrid chromosome. The report of recombinational exchanges between chromosomes and linear plasmids support the idea that interactions between two copies of the chromosome are possible. This recombination event could take place either after replication when both copies are physically close to each other or between the multiple copies of the chromosome present in the hyphal compartments of the mycelium.

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