

Interaction of Linear Plasmid with *Streptomyces rimosus* Chromosome: Evidence for the Linearity of Chromosomal DNA

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Recent studies imply that a linear chromosome may be characteristic of the *Streptomyces* genus. *S. rimosus* R6, the oxytetracycline (OTC) producer, carries a linear plasmid pPZG101 that can interact with the bacterial chromosome to form integrants and plasmid-primers. Restriction analysis of one integrant (MV25) showed that pPZG101 sequence had been integrated into the chromosome together with its linear end(s). Another strain (MV17) carried a linear plasmid of increased size of about 1 Mb (pPZG103). It was shown that OTC-genes were carried on the pPZG103, indicating that the plasmid was pPZG101'-OTC. Restriction mapping of pPZG103 demonstrated that one plasmid end was derived from the parental pPZG101 while the other end was a linear chromosomal end. The linearity of *S. rimosus* R6 chromosomal DNA was further supported by the development of the chromosomal physical map.

INTRODUCTION

The *Streptomyces* genome possesses many unusual features that are important for both fundamental and applied research. First of all, *Streptomyces* strains contain about 8 Mb of DNA per haploid genome, nearly twice the value for *Escherichia coli*. Secondly, *Streptomyces* DNA is remarkable for its

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high G+C content of more than 70%, probably the highest of all eubacteria.¹ Thirdly, there is a tendency of the *Streptomyces* chromosome to undergo extensive deletions of long stretches of DNA, often accompanied by massive tandem amplifications of specific neighbouring DNA segments.^{2,3} And last but not least, a wide range of accessory genetic elements were found in streptomycetes, many resembling types previously documented in other prokaryotes, while some represent novel genetic elements.¹ Among the latter, linear plasmids showed the widest dissemination and are, therefore, most intriguing. Apart from the discovery of giant linear plasmids,⁴ the advent of pulsed-field gel electrophoresis allowed construction of physical maps of *Streptomyces* chromosomes and their comparison to already existing genetic maps.⁵ These studies and the studies of the interaction of linear plasmids and bacterial chromosome suggested that a linear chromosome may be characteristic of *Streptomyces* genus.^{6,7}

A class of genetic elements called »invertrons« has recently been defined by Kenji Sakaguchi.⁸ These elements occur in both eucaryotes and in prokaryotes. The ends of the elements are inverted repeats and there is a protein, covalently bound to the 5'-end of the DNA molecule, which is thought to act as a primer for replication. According to Sakaguchi, *Streptomyces* linear plasmids also belong to that class of genetic elements.⁸ The data accumulated during the last two years suggest that *Streptomyces* chromosomes themselves can also be regarded as »invertrons«. We will try to support this statement with some data using the example of *Streptomyces rimosus*.

RESULTS AND DISCUSSION

Our previous work showed the presence of a plasmid DNA molecule in *S. rimosus* R6, which migrated in pulsed-field gels like a linear molecule of 387 kb in size (Figure 1; line 3). We named it pPZG101.⁹ To confirm the linearity of the pPZG101, the agarose blocks containing total DNA were treated with exonuclease III. Under these conditions the plasmid DNA was subjected to degradation (Figure 1; line 4). This indicates that the molecules are indeed linear, since circular plasmid DNA is not sensitive to the exonuclease preparation used.

Restriction analysis and hybridization experiments, using individually isolated fragments as probes, allowed development of a linear restriction map of pPZG101 for the enzymes *Ase*I, *Bfr*I, *Dra*I and *Xba*I. These experiments also revealed the existence of long terminal inverted repeats of over 90 kb in length.⁹ Long inverted repeats have been observed in the linear plasmid SCP1 of *S. coelicolor*,¹⁰ but the example of SLP2 of *S. lividans*¹¹ shows that long inverted repeats are not a universal property.

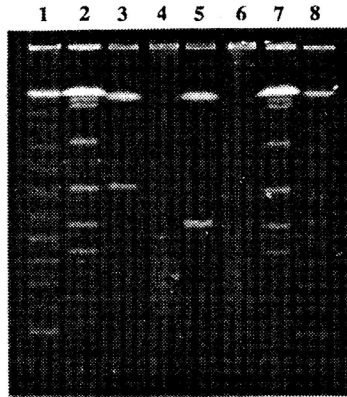


Figure 1. Linear plasmids of *Streptomyces rimosus* R6-500 and R7 seen after PFGE of undigested DNA. Tracks 3 and 5: R6-500 and R7 DNA. Tracks 4 and 6: R6-500 and R7 DNA digested *in situ* with *ExoIII*. Tracks 2 and 7: *Saccharomyces cerevisiae* chromosomes. Tracks 1 and 8: λ ladder. The gel was run in a Bio-Rad CHEF-DRII system in 0.5 x TBE at 14 °C with 200 V for 24 hrs, ramp of pulse times 20–40 s.

In order to study the interaction of pPZG101 with the *S. rimosus* chromosome, independently isolated mutants were screened by pulsed-field gel electrophoresis for the loss of plasmid molecules.⁹ Six of them had lost the pPZG101 band. A further mutant (MV17) contained a larger linear plasmid molecule, which will be described later. Two of the six mutants showed a total loss of plasmid DNA sequences, while in the other four parts of pPZG101 were integrated into the bacterial chromosome. Comparison of one of them (MV25) with the parent strain shows, in particular, that bands indistinguishable in size from the *AseI*-B fragment, the *BfrI*-C fragment and the *XbaI*-B fragment are still present in MV25. These fragments are the end fragments of the plasmid and these data suggest strongly that the integration event has preserved one or both ends of the plasmid as free ends.⁹ These data would be most easy to explain if the original *S. rimosus* chromosome were linear and the integration resulted from replacement of one (or both) chromosome ends by the plasmid ends.

As mentioned above, a further mutant (MV17) carried a plasmid DNA molecule of about 1 Mb in size instead of the 387 kb normal pPZG101.⁹ This strain produces more oxytetracycline (OTC) than the parent strain. It, therefore, seemed possible that the linear plasmid may have acquired chromosomal sequences from the OTC-region. This was tested by hybridizing a Southern transfer with a probe containing genes from the OTC-cluster.¹² It was shown that the plasmid in MV17 hybridizes with the *otrB* probe whereas the original pPZG101 shows no signal. The DNA of pPZG101 was

eluted from a pulsed-field gel, labeled and also used as a hybridization probe. The data showed that the larger linear plasmid hybridizes. Thus, the 1 Mb molecule is a pPZG101 prime carrying chromosomal sequences from the OTC region. We named it pPZG103.⁹

The question arises as to how plasmid-primed strains are formed. Several possibilities can be envisaged for their formation:¹³

Firstly, the OTC region may be carried on a transposable element, allowing transposition into the linear plasmid. The presence of specific recombinationally active sequences in this region was supported by the occurrence of reproducible deletions and amplifications of the region in spontaneous mutants. The deletions would be easy to explain if the OTC region was flanked by direct repeats allowing homologous recombination to delete the region between the repeats.¹⁴

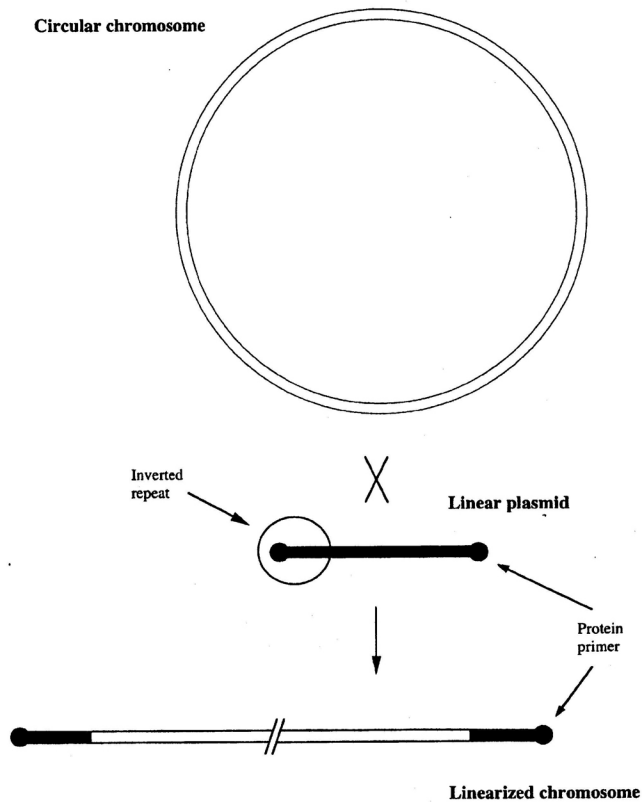


Figure 2. A simple model to explain a single crossing over between a circular chromosome and a linear plasmid.

A second possibility is that the linear plasmid may integrate into the chromosome in the OTC region and imprecise excision would result in the formation of a plasmid-prime. Such events are responsible for the formation of F' plasmids in *E. coli*. However, there would be some topological differences when a linear rather than a circular plasmid is involved. A single crossing over between a circular chromosome and a linear plasmid would integrate the plasmid and linearize the chromosome (Figure 2). As discussed above, derivatives of R6 have indeed been found in which pPZG101 is integrated and where free plasmid ends appear to be still present.

A third possibility arises, if the chromosome of *S. rimosus* were linear rather than circular. Although this is not yet clear for *S. rimosus*, the chromosome of *S. lividans* is linear, and the chromosomal ends are indistinguishable from one end of the linear plasmid SLP2.^{6,7,15} Integration of a linear plasmid into a linear chromosome by a single crossing over would produce two linear molecules with heterologous ends. If they were of unequal size, they might appear, in initial analysis, to be the chromosome and a plasmid-prime (Figure 3).

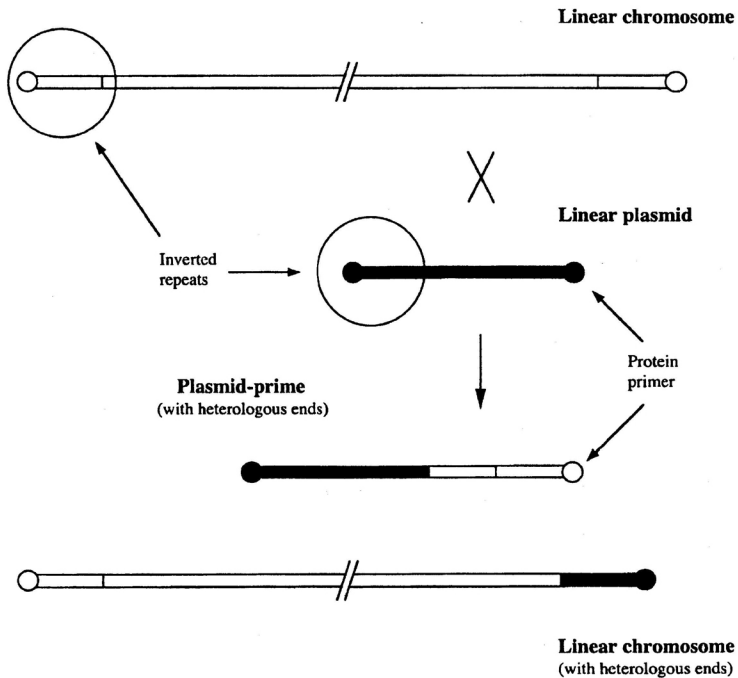


Figure 3. A simple model to explain integration of a linear plasmid into a linear chromosome by a single crossing over.

Restriction mapping and hybridization studies suggested that only one end of pPZG103 was derived from the original linear plasmid, and it was hypothesized that the other end was a chromosome end.¹³ The DNA of pPZG103 was purified from pulsed-field gel, digested with rarely cutting restriction enzymes *AseI* and *DraI* and subjected to a second round of pulsed-field gel electrophoresis. A Southern transfer of the gel was hybridized with the DNA of pPZG101. These data allowed construction of the *AseI* and *DraI* restriction maps. It is interesting to note that one end fragment (*AseI*-B) does not hybridize with the parental plasmid pPZG101 but comigrates with and hybridizes to the chromosomal *AseI*-J fragment (G. Biuković *et. al.*, in preparation). This was the second evidence for the existence of linear chromosomal ends in *S. rimosus*.

We simultaneously started a series of experiments that aimed at the construction of the chromosomal physical map for *S. rimosus* (K. Pandža *et. al.*, in preparation). The *AseI* digestion of *S. rimosus* chromosomal DNA revealed thirteen fragments, while digestion with *DraI* showed seven fragments. The sizes of these fragments were determined by pulsed-field gel electrophoresis analysis at various pulse times that were chosen to expand portions of the molecular size range for optimal resolution. The molecular size of *S. rimosus* chromosome can be determined by adding the sizes of all fragments. Agreement between the chromosome size separately determined from *AseI* and *DraI* digests was reasonably good and established about 8 Mb as the best measurement to date.

We screened a cosmid gene bank of *S. rimosus* for linking clones carrying sites for the rarely cutting restriction enzymes *AseI* and *DraI*. These cosmids were used as hybridization probes against Southern blots of pulsed-field gel electrophoresis of chromosomal DNA cut with these restriction enzymes. Further information on the linking of the fragments was obtained by double digests, and by the location of known sequences to restriction fragments. These sequences include the OTC-cluster (N. Perić *et. al.*, in preparation), the RP2 and RP3 prophages¹⁶ and the rRNA operons.¹⁷ This established a provisional physical map, which was consistent with a linear *S. rimosus* chromosome, whose ends are inverted repeats of over 300 kb, including the whole *AseI*-J fragment present on both ends of the chromosome. However, the linearity of *S. rimosus* chromosome has still to be proved by cloning, sequencing and direct comparison of the chromosomal and the pPZG101 linear ends.

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SAŽETAK

**Međusobno djelovanje linearnog plazmida s kromosomom
bakterije *Streptomyces rimosus*: dokaz za linearnu
strukturu kromosomske DNA**

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Nedavna su istraživanja nagovijestila da su linearni kromosomi možda svojstveni vrstama roda *Streptomyces*. Soj *S. rimosus* R6, proizvođač antibiotika oksitetra-ciklina (OTC), ima linearni plazmid pPZG101 koji može međusobno djelovati s bakterijskim kromosomom. Zbog toga se pojavljuju izolati u kojih se plazmid ugrađuje u bakterijski kromosom ili se pojavljuju tzv. pPZG101' sojevi. Restriksijska analiza

jednog izolata (MV25) pokazala je da se DNA plazmida pPZG101 ugradila u bakterijski kromosom zajedno s njegovim linearnim krajevima. Drugi soj (MV17) ima veći linearni plazmid, oko 1 Mb (pPZG103). Utvrđeno je da DNA plazmida pPZG103 hibridizira s DNA pPZG101 te da sadržava kromosomske OTC gene, što pokazuje da je on pPZG101'-OTC. Restrikcijским se mapiranjem dokazalo da jedan plazmidni kraj potječe od roditeljskog plazmida pPZG101, dok mu je drugi, kraj linearnog bakterijskog kromosoma. Izradbom fizičke mape kromosoma potvrđena je linearnost kromosoma soja *S. rimosus* R6.