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Things at the Ends of the Linear *Streptomyces* Replicons

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The chromosomes of the soil bacteria *Streptomyces* are linear DNA molecules of 7 - 9 Mb that contain terminal inverted repeats of variable lengths and terminal proteins covalently bound to the 5' ends. Linear plasmids, which are abundant in *Streptomyces,* share the same basic structural features. Replication of the linear chromosomes and plasmids is initiated at an internal origin and proceeds toward the telomeres, resulting in single-stranded gaps at the 3' ends. The length of the single-stranded gaps found on different linear *Streptomyces* replicons ranges from 250 to 300 nt.

The telomere sequences in the single-stranded gaps are conserved among all the *Streptomyces* chromosomes and linear plasmids studied - except SCP1 plasmid (in *Streptomyces coelicolor).* These sequences contain tightly packed palindromic sequences, which would form extensive secondary structures on the 3' single-stranded overhangs.

The TPs of several *Streptomyces* chromosomes and linear plasmids have been identified. They are very conserved in size and sequences. Recently we isolated the TP of SCP1 (TpgSCP1) from a recombinant mini-SCP1 plasmid, and identified its sequence by mass spectrometry. The TpgSCP1 turned out to be very different from the other know TPs and formed a class of its own. Therefore, TpgSCP1 in combination with its cognate telomere sequence of SCP1 constitutes a novel class in linear *Streptomyces* replicons of a different evolutional origin.

The single-stranded gaps at the 3' ends during replication are proposed to be patched by DNA synthesis primed by TP catalyzed by a cellular DNA polymerase. To test this postulate and to investigate the biochemical mechanisms of such terminal patching, we have recently developed an *in vitro* deoxyribonucleotidylation system containing ATP, Mg^{+2}, [^{32}P]-labeled deoxyribonucleotides, TP expressed in *E. coli,* and *Streptomyces* extract (providing the linear DNA template and enzyme activities). The extract from a *polA* mutant could successfully substitute that from a wild-type strain, suggesting that the DNA polymerase I does not catalyze the deoxyribonucleotidylation reaction.

In the *in vitro* system, dCMP, the first nucleoside of the telomere sequence, was specifically incorporated on to the TP. Hydrolysis and chromatographic analysis show that the dCMP is attached to a Thr residue

in the C-terminal region of the TP. The extension synthesis observed in this reaction gives hope to investigation terminal patching using this *in vitro* system.

There are several lines of genetic and biochemical evidence that indicate the two telomeres of a linear *Streptomyces* replicon are associated with each other. For example, despite the linearity of the chromosomes, the genetic maps of *Streptomyces* chromosomes have been shown to be *circular*. The circular genetic maps are automatically generated by a strong bias toward even number of crossovers during recombination between *Streptomyces* chromosomes. In turn, the even numbers of crossover is attributed to the formation of a circular configuration by the chromosomes through telomere-telomere interactions. This notion is supported by (*i*) immobilization of the TP-capped terminal DNA fragments during electrophoresis without protease digestion or SDS denaturation, and (*ii*) proximity of the two chromosomal termini *in vivo* as detected by in situ fluorescence hybridization.

We demonstrated the interactions between TPs on the telomeres *in vitro* by chemical cross linking using disuccinimidyl glutarate (DSG). Terminal DNA fragments treated with DSG were retarded during electrophoresis even in the presence of SDS. Cross-linked DNA fragments were seen under an atomic force microscope. These results support the notion that the linear *Streptomyces* replicons form a circular configuration *in vivo*. Such circular forms would make supercoiling possible at least for the telomere regions, which is important for essential DNA transaction activities, such as replication, transcription, and recombination.