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Spatial organisation of *Streptomyces venezuelae* chromosome during sporulation and germination of spores - the role of condensing

ABSTRACT

Streptomyces are Gram-positive soil bacteria characterized by a complex morphological life cycle, which includes vegetative growth of multigenomic hyphae and sporulation, leading to the formation of monogenomic spores *Streptomyces* chromosome is linear, and its organization is dependent on the cell cycle stage. The presence of a large number of chromosomal copies in *Streptomyces* cells requires a precise and efficient process of their condensation and segregation during sporulation. The ParB protein is important for global chromosome organization and segregation. ParB binds to *parS* sequences and also binds and hydrolyzes CTP (cytidine-5′-triphosphate) molecules. Due to its interaction with CTP and nonspecific interactions with DNA, ParB accumulates along the DNA, leading to the formation of large nucleoprotein complexes. Interestingly, in several bacterial species, ParB has been shown to recruit SMC condensin. SMC recruitment promotes its interaction with DNA, providing spatial chromosome organization and increasing long-range chromosomal contacts, observed as chromosome arm cohesion. SMC-dependent chromosome arm cohesion has also been described in *Streptomyces*. However, it has not yet been investigated whether SMC recruitment affects ParB complexes, nor has it been studied whether SMC-dependent chromosome condensation in *Streptomyces* spores affects their germination or the positioning of the *oriC* region of the chromosome during germination. The aim of this PhD thesis was to address these questions.

As part of this PhD thesis, the importance of the ParB protein for the binding of SMC to the central part of the chromosome during sporogenesis was confirmed. Moreover, a previously undescribed effect of SMC on ParB complexes was revealed. *In vivo* analyses showed that the removal of SMC protein accelerates the disassembly of ParB complexes and increases ParB dynamics. Furthermore, it was confirmed that SMC promotes the binding of ParB to *parS* and its spreading from the binding site. *In vitro* analyses confirmed that SMC

inhibits the CTPase activity of the ParB protein in the presence of the *parS* sequence. The obtained results indicate the role of SMC in stabilizing the ParB complexes in *S. venezuelae*.

The effect of SMC on the organization of the chromosome during spore germination was also examined. It was shown that the elimination of SMC alters the organization of the chromosome and the location of the *oriC* region in relation to the nucleoid in vegetative hyphae. Moreover, the lack of the SMC protein was observed to slow down chromosome replication in *S. venezuelae*. These studies indicate the role of SMC in the positioning of the *oriC* region in the cell and in the proper progression of chromosome replication.