Mechanism of chromosome replication initiation in *Streptomyces*

Abstract

DNA replication is a process critical for a cell and subject to strict regulation, mainly at the initiation stage. Mechanisms of bacterial chromosome replication regulation are well known for model species (*E. coli, B. subtilis*): the initiator protein DnaA cooperatively binds specific sequences (DnaA boxes) within a single origin of replication, *oriC*, forming the orisome and unwinding the DNA helix.

Streptomyces is an interesting model for exploring replication initiation, as they form multinucleoid aerial hyphae with up to 50 non-segregated chromosomes that undergo asynchronous replication.

This work shows that *Streptomyces oriC* contains 14 DnaA boxes of consensus sequence (5′-TT[G/C]TCCACA-3’) that differs in the third position from the one known from *E. coli*. *In silico* analysis of *oriC* regions from several *Streptomyces* species detected two putative DUEs (DUE1 and DUE2), located close to each other toward the 5’ end of *oriC* region. Using RIP mapping *in vivo* for *S. venezuelae* I confirmed one of them, DUE2.

Previous works showed that AdpA, one of the most pleiotropic transcription regulators in bacteria, inhibits chromosome replication at the initiation stage. As the role of AdpA in the new *Streptomyces* model species, *S. venezuelae*, was unknown, ChiP-qPCR experiment was conducted in order to identify which sequences are bound by AdpA in *S. venezuelae* chromosome. The interaction between *S. venezuelae* AdpA and *oriC* was not confirmed, which suggests that AdpA role as DNA replication regulator is not common Streptomyces. Interestingly, it was found that AdpA binds to *adpA* promoter and in result autoregulates its own gene transcription. What is more, I observed that *adpA* deletion mutant is unable to generate aerial hyphae and produces only occasional spores, although the growth rate of the mutant was comparable to wild type. The highest levels of both AdpA protein and its transcript were observed before the aerial hyphae formation. These results show that AdpA participates in *S. venezuelae* morphological differentiation.

The AdpA role in secondary metabolites production of *Streptomyces* is well known, also AdpA boxes were found in silico within the chloramphenicol biosynthesis gene cluster. Therefore I tested the antibiotic properties of liquid culture extracts of *S. venezuelae* WT and deletion mutant *ΔadpA*, and confirmed that the deletion mutant does not produce chloramphenicol, which shows the crucial role of AdpA in chloramphenicol biosynthesis in *S. venezuelae*.