ABSTRACT

A highly processive topoisomerase I from Streptomyces coelicolor – DNA relaxation dynamics and biological role of the enzyme.

Chromosome segregation in bacteria occurs accordingly to strategies characteristic for particular types of microorganisms. In most bacterial species chromosome segregation, which precedes cell division, is initiatitad during their onging repliaction and requires activity of segregation proteins ParAB, however the some aspects of the segregation process are different for different groups of bacteria. In *Streptomyces* - a soil filamentous bacteria, chromosome are actively segregated during sporulation induced by stress conditions when multigenomic hyphae differentiates into chains unigenomic spores. In *Streptomyces* ParA forms filaments that spread along the hyphae, while ParB binds *parS* sequences located in the *oriC*-proximal region (*origin of chromosome replicatio*n). (Fig. 1).

In search of new elements involved in chromosome segregation in *Streptomyces coelicolor* a novel protein *Sc*TopA (SCO3543) was identified. *In vitro* and *in vivo* studies confirmed that *Sc*TopA binds selectively in the vicinity of ParB-DNA complexes (segrosomes). Based on the homology to other bacterial proteins, *Sc*TopA was classified as topoisomerase of type IA , although it differs remarkably from its homologues from other bacteria in its C-terminal domain which is longer and lacks Zn-finger motifs. Enzymatic assays show that *Sc*TopA acts as type IA topoisomerase - it relaxes negatively supercoiled DNA in the presence of magnesium ions but in the absence of ATP. Using single-molecule magnetic tweezer the dynamics of DNA relaxation by *Sc*TopA protein was characterized and also the number of supercoils removed in a single burst (150 supercoils/burst) was calculated. Moreover, I showed that for the DNA relaxation *Sc*TopA requires single stranded regions on DNA molecule. The results suggest that *Sc*TopA removes negative supercoils with extremely high processivity, not described previously for any other topoisomerases of type IA and its processivity is correlated with the presence of untypical C-terminal domain. Analyses of *S. coelicolor* mutant strains show that deletion *Sc*TopA is lethal and depletion of its level leads to blockage of differentiation process and inhibition of sporulation pathway. Interestingly, fluorescent microscopy

revealed that in *Sc*TopA-depletion strain the localization of ParB-EGFP complexes is disrupted and formation of septa is affected.

Summarizing, the results allowed me to develop the model of chromosome segregation in *S. coelicolor* (Fig. 1) and specify the role of ScTopA in this process, namely decreasing the level of topological stress accompanying the formation of ParB-DNA complexes. Moreover, the high processivity of *Sc*TopA seems to be crucial for efficient segregation of dozens of the chromosomes at the same time.

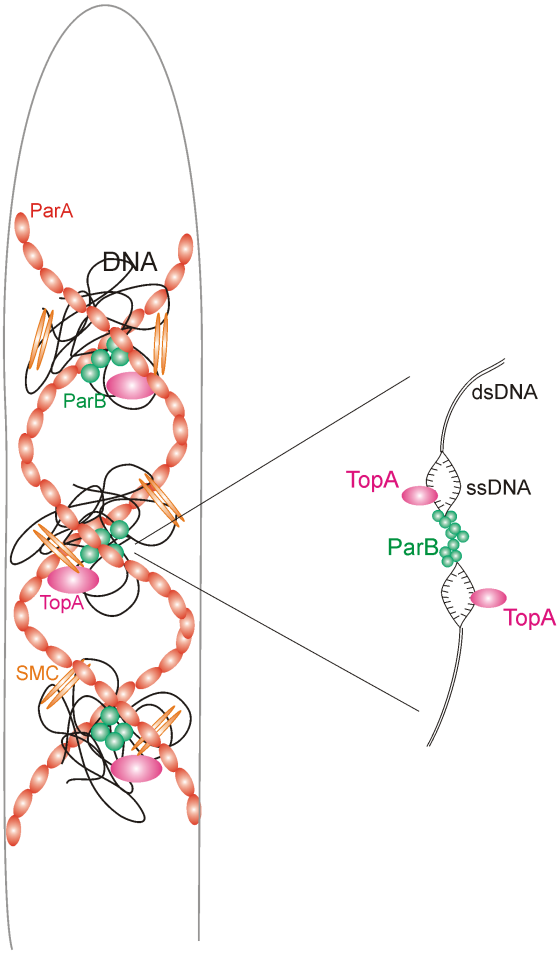

Fig. 1. Model of chromosome segregation in *S. coelicolor*. In the aerial hyphae ParA filaments act as a scaffold for ParB-DNA complexes (segrosomes) distributed along the hyphae. Assembling of segrosomes generates topological stress in the vicinity of ParB-binding sites. Since *Sc*TopA requires ssDNA for its activity, it is recruited to ParB-DNA complex and decreases the DNA tension. Properly separated chromosomes are condensed in the next step during septa formation.

PhD Thesis

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2. **Szafran M.**, Zakrzewska-Czerwińska J., Jakimowicz D. Bakteryjne topoizomerazy typu I – rola biologiczna i zastosowanie jako potencjalnych celów dla antybiotyków (2013), Postepy Hig. Med. Dosw. 67:130-142

3. **Szafran MJ.**, Strick T., Strzałka A., Zakrzewska-Czerwińska J, Jakimowicz D. (2014) A highly processive topoisomerase I: studies at the single-molecule level. Nucleic Acids. Res. doi: 10.1093/nar/gku494

4. **Szafran MJ.**, Trojanowski D., Skut P., Hołówka J. (2014) W poszukiwaniu nowych antybiotyków - inhibitory replikacji chromosomów bakteryjnych. Postepy Hig. Med. Dosw., 68