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„ The role of Lsr2 in chromosome organization and regulation of gene expression in *Mycobacterium smegmatis* ”

Abstract

Nucleoid-associated proteins (NAPs) are responsible for maintaining highly organized and dynamic chromosome structure in bacteria. The genus *Mycobacterium* possesses a unique set of NAPs, including Lsr2, which is a functional homolog of *E. coli* H-NS. Lsr2 is a DNA-bridging protein that plays a role in the chromosome organization and transcriptional regulation.

I demonstrated that deletion of *lsr2* gene has a pleiotropic effect on the cells morphology of *Mycobacterium smegmatis*, a saprophytic bacterium, which is a model organism for studies of mycobacterial cell biology. Cells lacking Lsr2 are shorter, wider, and more rigid than the wild-type cells. Since the morphological disorders are presumably the result of altered transcriptional landscape, I analyzed the role of Lsr2 as a transcription factor. A combination of RNA-seq and ChIP-seq data revealed that Lsr2 acts mainly as a repressor, controlling gene expression either directly by binding promoter regions or indirectly through DNA-loop formation and DNA coating. One of the Lsr2-repressed genes encodes polyketide synthase (MSMEG_4727), which is involved in the synthesis of lipooligosaccharides (LOSs), a component of the mycobacterial outer membrane. *M. smegmatis* strain deprived of Lsr2 produces more LOSs, which is reflected by changes in the smoothness of the cells and their susceptibility to antibiotics.

Lsr2 also takes a part in maintaining chromosome organization and influences chromosome replication. Time-lapse fluorescent microscopy experiments showed that fluorescently tagged Lsr2 forms large and dynamic nucleoprotein complexes and that the N-terminal oligomerization domain of Lsr2 is indispensable for the formation of nucleoprotein complexes *in vivo*. Moreover, *lsr2* deletion exerts a significant effect on the replication time and replisome dynamics. Thus, the Lsr2 nucleoprotein complexes may contribute to maintaining the proper organization of the newly synthesized DNA and therefore influencing mycobacterial cell cycle.

Unlike *M. tuberculosis*, *M. smegmatis* additionally encodes a paralogue of Lsr2, MSMEG_1060, which is a novel member of the mycobacterial NAP family. The Lsr2 and

MSMEG_1060 proteins exhibit different DNA binding specificities and chromosomal localizations. My results suggest that these proteins help *M. smegmatis* cells cope with stress conditions, including hypoxia and exposure to antibiotics.