The effect of the transcriptional repressor StpA on CRISPR-Cas activity in E. coli cells lacking H-NS

P-01.1-05

D. Mitić[I], M. Radovčić[I], D. Markulin[I], I. Ivančić Baće[I]

[I]University of Zagreb, Faculty of Science, Zagreb, Croatia

Clustered regularly interspaced short palindromic repeats – CRISPR associated genes (CRISPR-Cas) is a prokaryotic defence system that protects against phage infection and foreign DNA, such as plasmids. It is comprised of *cas* protein genes and the CRISPR locus which consists of repeat arrays interspaced with sequences originating from invading DNA, that are transcribed and processed into CRISPR RNA (crRNA). *E. coli* has a type I-E system, in which foreign DNA targets are recognized by Cascade, a crRNA-guided complex comprised of five proteins (CasA, CasB, CasC, CasD, CasE) and degraded by Cas3. In *E. coli* the CRISPR-Cas type I-E system is repressed by the histone-like nucleoid-structuring protein H-NS, a global transcriptional repressor. In *wt* cells H-NS repression can be relieved by elevated levels of LeuO transcription factor which induce higher transcript levels of *cas* genes than was observed for Δ*hns* cells. This suggested that derepression in Δ*hns* cells is incomplete and that an additional repressor could be involved in silencing. We wanted to test if StpA, a paralogue of H-NS with similar DNA binding preferences as H-NS, is another repressor of *cas* genes. By overexpressing *stpA* from the plasmid we have managed to abolish resistance to phage in naturally resistant Δ*hns* cells. To confirm the exact mechanism of this phenomenon, we determined *cas3* and *casA* transcript levels in different mutant cells (*wt*, Δ*hns*Δ*stpA*, Δ*hns*, and Δ*hns* cells overexpressing *stpA*). Our results show that in the absence of H-NS, the StpA protein is another repressor of *cas* genes.