Clostridioides difficile CRISPR-Cas regulation by biofilm-related secondary messenger c-di-GMP

P-01.2-22

A. Maikova[I], N. Berezenkova[I], J. Peltier[II], K. Severinov[III,IV,V], O. Soutourina[II]

[I]Peter the Great St. Petersburg Polytechnic University, Saint-Petersburg, Russia, [II]Institute for Integrative Biology of the Cell (I2BC), CNRS, Université Paris-Saclay, Gif-sur-Yvette, France, [III]Skolkovo Institute of Science and Technology (Skoltech), Skolkovo, Moscow Region, Russia, [IV]Waksman Institute for Microbiology, Piscataway, NJ, United States of America, [V]Institute of Molecular Genetics Russian Academy of Scieces, Moscow, Russia

Clostridioides difficile is an anaerobic spore-forming bacterium that is the major cause of nosocomial diarrhea associated with antibiotic therapy. Many aspects of *C. difficile* pathogenesis and its adaptation to changing conditions inside the host are poorly understood. Our deep-sequencing data previously published in: Soutourina et al. (2013) PLoS Genet. 9(5), e1003493 strongly suggests the importance of RNA-based mechanisms for the control of gene expression and infection cycle in *C. difficile*. More than 200 regulatory RNAs were identified, including abundant CRISPR RNAs for the prokaryotic adaptive immune system against foreign invaders. *C. difficile* possesses an unusual CRISPR-Cas system characterized by a large set of CRISPR arrays, multiple type I-B *cas* operons, and the toxin-antitoxin type I systems, linked to several arrays. In present study we investigated the role of biofilm-related secondary messenger c-di-GMP in *C. difficile* CRISPR-Cas system regulation. We explored the global effect of the c-di-GMP on the expression of CRISPR-Cas system components. Real-time PCR experiments showed expression induction of both *cas* operons and several CRISPR arrays in *C. difficile* 630Δ*erm* strain by high c-di-GMP levels. Additionally, we found a c-di-GMP-dependent riboswitch associated with the CRISPR12 array in *C. difficile* 630Δ*erm* strain, which can indicate the direct impact of c-di-GMP-dependent regulation on this array function. Plasmid conjugation efficiency experiments revealed a slight induction of interference in *C. difficile* 630Δ*erm* CRISPR12 array by high levels of c-di-GMP. Obtained results demonstrate *C. difficile* CRISPR-Cas regulation in biofilm conditions and show the possible role of this system in *C. difficile* survival during its infection cycle. This work was supported by the Russian Science Foundation (project No. 20-74-00052).