doi 10.18699/bgrs2024-8.4-18

Insights into the natural activation mechanism of the CRISPR-Cas immune system in *Escherichia coli* from a computational modeling analysis

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Key words: CRISPR-Cas derepression; H-NS; modeling expression dynamics, machine learning

Motivation and Aim: CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR-Associated proteins) systems provide bacteria with defense against viruses by utilizing spacers, fragments of viral DNA stored within the CRISPR array, a locus in a bacterial genome. These spacers are transcribed and processed into crRNAs, which guide Cas proteins to target and eliminate matching viral DNA sequences. Despite the extensive biotechnological applications of CRISPR-Cas in gene editing, its natural functioning within bacteria remains incompletely understood. Particularly in *Escherichia coli*, the most extensively studied system, CRISPR-Cas activity is typically silenced under experimental conditions. This silencing is attributed to the repression of promoters governing the expression of *cas* genes and the CRISPR array, facilitated by cooperative binding of the global regulatory protein H-NS (Histone-like Nucleoid-Structuring). Moreover, a fraction of H-NS proteins might become sequestered by viral DNA if it possesses a higher AT content compared to bacterial DNA [1]. Additionally, the pleiotropic transcriptional regulator, LeuO, whose expression is modulated by BglJ (encoded by the β-glucoside operon), can alleviate H-NS-mediated repression [2]. This study aimed to investigate whether a modest reduction in the cellular H-NS level could initiate a positive feedback loop, activating the CRISPR-Cas system through BglJ and LeuO proteins, thereby leading to rapid crRNA generation for effective cellular defense against fast-replicating bacteriophages.

Methods and Algorithms: We developed a mathematical model to predict the temporal dynamics of crRNA levels during the activation of the CRISPR-Cas system upon infection of a bacterial cell by foreign DNA. This model, utilizing principles of statistical thermodynamics and non-linear dynamics, incorporates known regulatory features of the system as well as our proposed mechanism of combined activation, by: 1) derepression of the *cas* genes promoter due to sequestration of H-NS by foreign DNA, and 2) activation of *cas* genes expression through positive feedback performed by LeuO and BglJ-RcsB. We systematically searched for parameter combinations at which the increase in the crRNA amount, indicative of the activation of the CRISPR-Cas system, can occur during the first 30 min upon entry of the foreign, AT-rich DNA. To identify the parameters exerting the most significant influence on eliciting this response from the system, we applied the Random Forest machine learning technique. Additionally, we conducted a bioinformatics analysis where 16,388 viruses were matched to their respective host bacteria harboring CRISPR-Cas using the Virus-Host DB database and

tools for identifying CRISPR arrays in bacterial genomes. For each bacterial genus, we assessed the disparity in genomic AT content between bacterial and viral DNA.
Results: Our analysis revealed a consistent, small elevation in AT content within viral genomes compared to host genomes, supporting a potential reduction in available H-NS levels for transcriptional repression upon bacteriophage DNA introduction into a cell. The Random Forest analysis showed that the most important parameters are: 1) the baseline cellular H-NS level, 2) the change in accessible H-NS concentration as a consequence of its interaction with foreign DNA, and 3) the degree of cooperativity associated with H-NS binding to promoter DNA. Our findings indicate that substantial reductions in the available H-NS levels can readily induce crRNA expression. On the other hand, achieving timely crRNA generation from a minor reduction in H-NS levels necessitates high H-NS binding cooperativity and a baseline H-NS level close to its equilibrium dissociation constant for binding to DNA.
Conclusion: Our modeling approach suggests that CRISPR-Cas systems possess the capability to mount a rapid response in the face of fast-replicating bacteriophages, generating sufficient crRNAs for effective defense. The identified kinetic features essential for this response, which should be the focus of future experimental work, contribute to understanding the intrinsic behavior of the system, crucial for the development of biotechnological tools leveraging bacterial hosts for compound production and the design of antiviral platforms.
Funding: This work is supported by the Science Fund of the Republic of Serbia (projects No. 7750294, q-bioBDS, and No. 6417603, CRISPR modelling).

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