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The development of the class 2 CRISPR-cas based cellular system for studying the genome packaging of influenza viruses

Mindaugas Juozapaitis

Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

The main objective of this study is to develop the cellular system for the targeting RNA genome patches which are exposed on influenza virus ribonucleoproteins (vRNP) and responsible for the coordinated genome packaging of influenza viruses. In order to achieve this, it was conceived to take advantage of the recently discovered class 2 CRISPR-Cas systems which are targeting RNA. The essence of the proposed system is to target influenza viral RNA sequences which are exposed when packed into vRNPs. The targeting must be achieved while vRNPs are transported form the perinuclear region to the cytoplasmic membrane.

In order to achieve the main objective of this study, selected CRISPR-Cas effector was successfully integrated into a MDSK cellular genome. Two stable MDCK cell lines with the following features were established: the expression of the selected RNA targeting enzyme can be regulated and it does not significantly affect the replication efficiency of influenza viruses. To keep the newly produced RNA targeting enzyme in the cytoplasm, the enzyme was fused to a nuclear export signal. Next, the most efficient method for the delivery of the single-component programmable RNA-guide was tested and selected. Currently, as a proof of concept, the established system is tested for its ability to function as expected on the selected RNA target during an influenza virus infection. And if the proof of concept is successful, this system will be used to target influenza viral RNA sequences which are exposed when packed into vRNPs and responsible for the coordinated genome packaging.

mindaugas.juozapaitis@gmc.vu.lt