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The approaches for CRISPR/Cas9 regulation on the guide RNA level

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Motivation and Aim: The increase of effectivity and accuracy of gene editing system is the actual task of modern molecular biology and gene engineering. Design of regulated CRISPR/Cas systems and development of approaches for controlling their activity by physico-chemical stimuli are especially interesting in the context of gene editing. Among such approaches, activity regulation on the level of guide RNAs by introducing chemical modifications, changing the sequences or adding the blocking oligonucleotide are seem to be very promising. The aim of this research is design and testing of CRISPR/Cas9 system that can be regulated by irradiation and small molecules.

Methods and Algorithms: For the synthesis of guide RNA, we used solid phase automatic phosphoramidate method and corresponding modified or non-nucleotide synthons. Two variants of regulation were tested in model system: irradiation by the light with definite wavelength and allosteric regulation by theophylline.

Results: Linear and cyclic photocleavable guide RNA and blocking oligonucleotide were synthetized. Linear crRNAs and sgRNAs contained the photocleavable linkers which permit to destroy guide RNA in concrete moment, thus inhibiting DNA cleavage by Cas9 nuclease. Cyclic crRNAs do not fit well for CRISPR/Cas9 system, therefore before irradiation the DNA cleavage is ineffective. After irradiation, crRNA system became active due to the RNA linearization. Linear photocleavable blocking oligonucleotides form the duplex with guide RNA and inhibit the DNA cleavage before irradiation. Cyclic photocleavable blocking oligonucleotides linearize upon irradiation and deactivate CRISPR/Cas9 system. Using of azobenzene non-nucleotide linker within the guide RNA structure allows switching the activity of the system by irradiation with UV (365 nm) or visible (505 nm) light. The theophylline-binding RNA aptamer was introduced into guide RNA structure for allosteric regulation of the CRISPR/Cas9 system, making it possible to turn it on or off by changing the concentration of theophylline.

Conclusion: We proposed a set of approaches for design of CRISPR/Cas9 systems which can be regulated by irradiation or by small molecules.

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