THE ROLE OF TARGET SEQUENCE LENGTH FOR DNA INTERFERENCE IN THE TYPE I-F CRISPR-CAS SYSTEM

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) – Cas (CRISPR Associated) is the immune system of bacteria or archaea that provides resistance against invasive genetic elements [1]. Proteins encoded by *cas* genes together with a mature crRNA molecule that carries a sequence (spacer) of extracellular nucleic acid origin form an effector complex, which destroys foreign nucleic acids [2]. In type I CRISPR-Cas systems, ribonucleoprotein complex, termed Cascade (CRISPR-associated complex for antiviral defence), recognises and binds intruding DNA, which has (i) a spacer-complementary sequence, named protospacer, and (ii) a protospacer adjacent motif (PAM). Cascade binding to DNA target triggers a Cas3 helicase/nuclease, which destroys the intruder [3].

In type I-E systems, Cascade-DNA target interaction is destabilised by mutations at the PAM distal end of the protospacer [4]. Otherwise, the influence of mutations at the protospacer distal end for a type I-F system, which is the phylogenetically closest for type I-E, was not yet investigated [5]. In this work, we show the importance of these nucleotides for interference in the type I-F system from *Aggregatibacter actinomycetemcomitans* D7S-1 bacteria. Mutations at the PAM distal end of the protospacer do not inhibit interactions between the Cascade complex and DNA target. Cascade complexes have a similar affinity for truncated protospacers and form R-loops the length of which depends on spacer-protospacer complementarity. However, R-loop of at least 32nt is necessary to trigger Cas2/3 protein for target DNA destruction.

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