CHARACTERIZATION OF A NON-CANONICAL TYPE IV-A1 CRISPR-CAS SYSTEM

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CRISPR-Cas systems are used to provide adaptive immunity against mobile genetic elements in bacteria and archaea. Among these, type IV-A1 CRISPR-Cas systems are distinguished from other CRISPR types by the absence of canonical DNA or RNA nuclease activity. Instead, target DNA is unwound through the recruitment of a DinG helicase by these multisubunit complexes, resulting in transcriptional interference rather than cleavage and in this way preventing the infection [1][2].

Structural and biochemical studies have primarily been conducted on canonical type IV-A1 systems, in which a Cas8 homolog is employed in combination with Cas5 to mediate protospacer adjacent motif (PAM) recognition [3][4]. In contrast, a recently reclassified non-canonical type IV-A1 system – formerly designated type IV-A2 – has remained largely unexplored [1]. These systems were long thought to lack a Cas8 homolog, suggesting that an alternative mechanism of target recognition was used [5]. However, it has been shown that non-canonical type IV-A1 systems consistently co-occur with a small, conserved, *cas8-like* gene located adjacent to the *cas* operon and transcribed in the opposite orientation. Understanding of the function of the protein encoded by this gene may reveal a previously unrecognized component of type IV-A1 interference and expand current understanding of CRISPR-Cas systems diversity.

Here, initial insights into the structure and mechanism of a representative non-canonical type IV-A1 CRISPR-Cas system are presented.

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