REGULATION OF CRISPR-CAS TYPE III ACTIVITY BY MEMBRANE-ASSOCIATED PROTEINS WITH CARF AND SAVED DOMAINS

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Introduction. The type III CRISPR-Cas system is a prokaryotic adaptive immune system that confers immunity against invading mobile genetic elements (MGEs). This system involves the use of a multi-subunit effector complex to target foreign DNA transcripts complementary to a guide/CRISPR RNA (crRNA). Type III CRISPR-Cas has an additional layer of protection that includes the production of signaling molecules and effector proteins that respond to them. This system provides a wide range of potential defense pathways involving nucleases, transcription factors, proteases, deaminases, and membrane-associated proteins [1]. Therefore, tight and specific regulation of the activation of accessory proteins has evolved, exhibiting properties such as signal amplification, autoregulation, and tuning the concentration of signaling molecules.

The affiliation of various associated proteins containing CARF and SAVED domains to type III CRISPR-Cas systems, which bind cyclic adenylates, was investigated and their potential role in the mechanism of immunity was determined. Most of the associated proteins studied so far have been shown to be non-specific nucleases. Multiple transmembrane proteins with CARF or SAVED domains (CARF/SAVED-TM) were identified among them [2]. However, their function in the mechanism of immunity is the least understood. This study aimed to review and generalize current hypotheses regarding CARF/SAVED-TM.

A genomic neighborhood analysis of type III CRISPR-Cas loci revealed numerous CARF/SAVED-TMs, among other ancillary proteins, suggesting multiple potential cOA-based defense pathways [2].

Materials and methods. Several methods were employed in this study. Firstly, a review and generalization of current hypotheses regarding CARF/SAVED-TM were performed. Secondly, schematic representations were created to illustrate the possible regulatory mechanisms of CARF/SAVED-TM in the type III CRISPR-Cas systems (Fig. 1.).

Results and discussion. The results of the work allow us to assume the possible functions of CARF/SAVED-TM. The following hypotheses are considered:

1. CARF/SAVED-TM in type III CRISPR-Cas systems may play a significant role in the degradation of cyclic oligoadenylate second messengers (cOAn) through ring nuclease activity, weakening or disabling ancillary proteins-mediated immune response (Fig. 1. A) [3].

2. CARF-TM proteins can potentially lead to altruistic cell death, possibly mediated by cell hyperpolarization through the opening or formation of ion channels (Fig. 1. B) [1].

3. SAVED-TM proteins may be involved in intracellular signaling, transmitting the presence of infection to other bacteria in the population to strengthen an immune response (Fig. 1. C) [3].

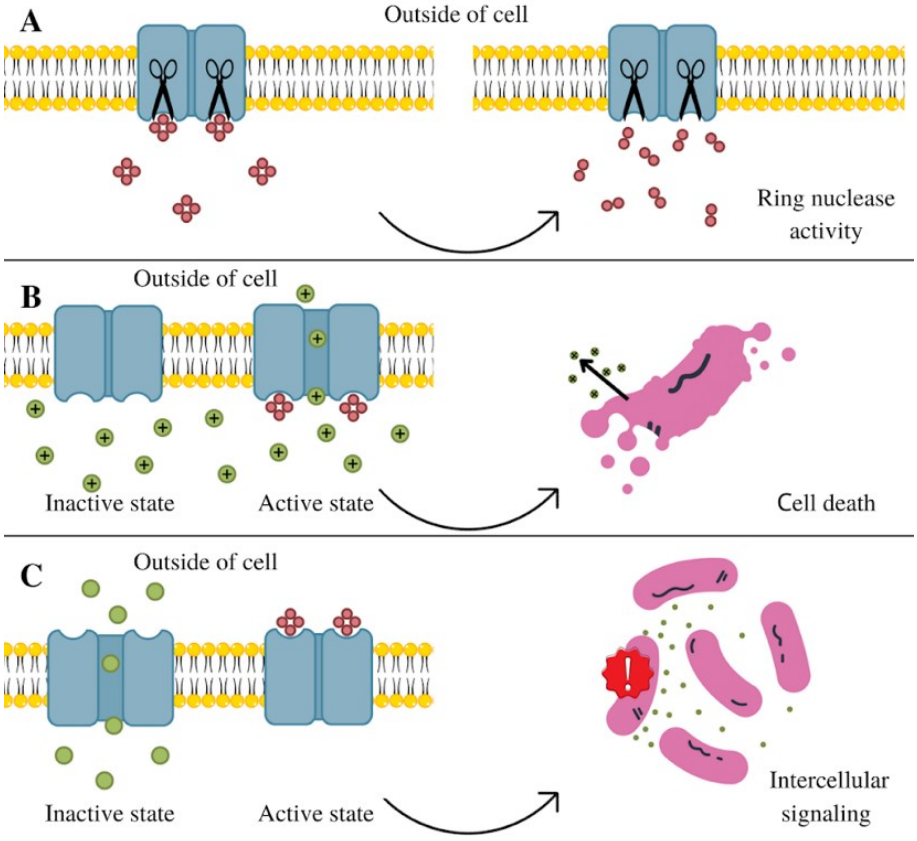

Fig. 1. Schematic representation of possible regulations mechanism of CARF/SAVED-TM in type III CRISPR-Cas systems. (A) The degradation of cOAn molecules through ring nuclease activity can lead to additional regulation of other ancillary proteins, further modulating the CRISPR-Cas immune response. (B) The activation of CARF/SAVED-TM by cOAn, results in opening of ion channels, causing cell death. (C) The released signaling molecules are initiating signal transduction, which involves the transmission of signals to other cells about the presence of an infection.

Conclusions. Understanding the regulation of type III CRISPR-Cas activity by membrane-associated proteins may lead to new biotechnological applications and expand our knowledge of cOA-dependent intermolecular communication pathways. The discovery of cOA-based signaling in type III CRISPR-Cas systems and the production of cyclic oligoadenylate second messengers are potential targets for new biotechnological developments.

In this study, we defined three of the most promising hypothesis about the possible functions of CARF/SAVED-TM and the regulation of CRISPR-Cas type III activity by these proteins.

References:

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