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# ACTIVATION AND REGULATION OF THE TYPE-III CRISPR-CAS ASSOCIATED SIGNALING CASCADE

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Prokaryotes employ various defense mechanisms to protect themselves against foreign nucleic acids, including viruses. Several of these mechanisms rely on a signaling pathway that uses cyclic nucleotide derivatives to activate specific effectors. Examples of such defense systems include CBASS [1], Thoeris [2], Pycsar [3], and the type III CRISPR-Cas system [4]. In the latter, upon detecting viral RNA, the interference complex generates cyclic oligoadenylates ($cA_n$), which activate effector proteins through a sensory CARF or SAVED domain [5]. To date, predominantly single-protein CARF effectors have been characterized [6]. However, the existence of type III CRISPR-Cas-associated multi-component effector systems that can function as CRISPR-activated signaling cascades has been proposed [7-9].

This study focuses on the type III-A CRISPR-Cas-associated tripartite CalpL-CalpT-CalpS effector system from *Candidatus* Cloacimonas acidaminovorans strain Evry. CalpS, which functions as an ECF-like sigma-factor, forms a stable heterodimer with its anti-sigma factor CalpT. When activated by $cA_4$, the SAVED-Lon protease fusion protein CalpL specifically cleaves CalpT, releasing CalpS for gene expression regulation. In this study, we used structural and biochemical assays and experiments in *E. coli* to elucidate the molecular mechanism of the activation and regulation of the CRISPR-Cas-activated CalpL-CalpT-CalpS signaling cascade.

[1] Slavik KM, Kranzusch PJ. CBASS to cGAS-STING: the origins and mechanisms of nucleotide second messenger immune signaling. Annual Review of Virology. 2023 Sep 29;10:423-53.
[2] Ofir G, Herbst E, Baroz M, Cohen D, Millman A, Doron S, Tal N, Malheiro DB, Malitsky S, Amitai G, Sorek R. Antiviral activity of bacterial TIR domains via immune signalling molecules. Nature. 2021 Dec 2;600(7887):116-20.
[3] Tal N, Morehouse BR, Millman A, Stokar-Avihail A, Avraham C, Fedorenko T, Yirmiya E, Herbst E, Brandis A, Mehlman T, Oppenheimer-Shaanan Y. Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. Cell. 2021 Nov 11;184(23):5728-39.
[4] Kazlauskiene M, Kostiuk G, Venclovas Č, Tamulaitis G, Siksnys V (2017) A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science, 357(6351), 605-609.
[5] Makarova KS, Timinskas A, Wolf YI, Gussow AB, Siksnys V, Venclovas Č, Koonin EV. Evolutionary and functional classification of the CARF domain superfamily, key sensors in prokaryotic antivirus defense. Nucleic acids research. 2020 Sep 18;48(16):8828-47.
[6] Stella G, Marraffini L. Type III CRISPR-Cas: beyond the Cas10 effector complex. Trends in Biochemical Sciences. 2023 Nov 8.
[7] Rouillon C, Schneberger N, Chi H, Blumenstock K, Da Vela S, Ackermann K, Moecking J, Peter MF, Boenigk W, Seifert R, Bode BE. Antiviral signalling by a cyclic nucleotide activated CRISPR protease. Nature. 2023 Feb 2;614(7946):168-74.
[8] Strecker J, Demircioglu FE, Li D, Faure G, Wilkinson ME, Gootenberg JS, Abudayyeh OO, Nishimasu H, Macrae RK, Zhang F. RNA-activated protein cleavage with a CRISPR-associated endopeptidase. Science. 2022 Nov 25;378(6622):874-81.
[9] Altae-Tran H, Kannan S, Suberski AJ, Mears KS, Demircioglu FE, Moeller L, Kocalar S, Oshiro R, Makarova KS, Macrae RK, Koonin EV., Zhang F. Uncovering the functional diversity of rare CRISPR-Cas systems with deep terascale clustering. Science. 2023 Nov 24;382(6673):eadi1910.