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.

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