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RESEARCH HIGHLIGHT

A type III-E CRISPR Craspase exhibiting RNase and protease activities

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Type III-E CRISPR-Cas effector gRAMP/Cas7-11 is a RNA-guided RNA endonuclease associated with a putative caspase-like protease TRP-CHAT/Csx29, which targets endogenous protein substrate Csx30. In a recent paper published in *Science*, the Ailong Ke lab has captured cryo-EM snapshots of the nuclease–protease complex Craspase in both resting and different functional states; this paper together with four other related studies have elucidated the molecular mechanisms of both target RNA cleavage and RNA-mediated allosteric regulation of protease catalytic activity, thereby highlighting RNA-dependent protease-based programmed cell death.

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated genes) systems employ CRISPR RNA-guided effectors to cleave and eliminate invading mobile genetic elements, thereby providing adaptive immunity in bacteria and archaea.[1] CRISPR-Cas systems are divided into two classes and further classified into types I–VI based on the composition of effector complexes.[2] The diverse effectors have been leveraged for development of programmable DNA- or RNA-targeting tools, revolutionizing various fields ranging from genome editing to nucleic acid detection.[3] Class 1 type III effectors are sub-divided into subtypes A to F and are typically composed of multiple Cas proteins and a mature CRISPR RNA (crRNA). The effectors in subtypes III-A/D (Csm complex containing Csm1–Csm5) and III-B/C (Cmr complex containing Cmr1–Cmr6) exhibit two intertwined helical filaments that degrade single-stranded RNA (ssRNA) and DNA (ssDNA) from invading phages and mobile genetic elements using distinct subunits (Fig. 1a, middle).[4] Multiple Cas7 superfamily endoribonucleases Csm3/Cmr4 pack with each other to form a helical filament that cleave target ssRNA complementary to crRNA spacer segment at 6-nucleotide (nt) periodic intervals. In addition, Cas10 superfamily members Csm1/Cmr2 achieve both ssDNA hydrolysis and cyclic oligoadenylate (cAn) secondary messenger production, the latter for Csm6/Csx1 RNase activation (Fig. 1a, right). To avoid self-targeting of host antisense transcripts, self-RNA complementarity with crRNA repeat segment allosterically prohibits both Csm1/Cmr2 and Csm6/Csx1 activation (Fig. 1a, left).

The recently discovered type III-E system consists of an atypical genetic composition, given that its locus lacks the canonical signature *cas10* gene, but instead encodes a putative caspase-like protease TPR-CHAT (also called Csx29) together with accessory proteins Csx30, Csx31, and RpoE.[5,6] The encoded effector gRAMP (also named Cas7-11) is a large single fusion protein consisting of a predicted Cas11/Csm2-like domain, four Cas7 domains, and an insertion domain (named INS or BID) that physically associates with crRNA, as well as TPR-CHAT to form a stable complex termed Craspase (CRISPR-guided caspase), suggesting an alternative novel RNA-guided and protease-based immune response. Notably, Cas7-11 has been applied for programmable RNA knockdown in human cells with lower collateral RNA cleavage activity compared to established Cas13-based tools.[6] Additionally, cryo-electron microscopy (cryo-EM) studies of *Desulfonema ishimotonii* Cas7-11 (*Di*Cas7-11) in complex with crRNA and target RNA have provided structural insights into effector complex assembly and mechanisms underlying target RNA cleavage.[7] The four Cas7 domains (Cas7.1–Cas7.4) form a helical filament with the INS domain capping the end of the Cas7.4 domain, while the Cas11 domain interacts with the Cas7.2 and Cas7.3 domains (Fig. 1b, middle). The 5′-repeat segment of crRNA is wrapped by Cas7.1 and Cas7.2 domains, while the guide:target duplex is anchored by Cas7.2–Cas7.4 and INS domains. Structural and biochemical data reveal that *Di*Cas7-11 elegantly deploys multiple Cas7 domains for dual RNase activities on crRNA maturation and target RNA cleavage.[5,7] Cas7.1 domain recognizes and cuts its cognate pre-crRNA at the repeat segment, while Cas7.2, Cas7.3 and Cas11 domains cut the target RNA between the 3rd and 4th nucleotides (site 1) and between 9th and 10th nucleotides (site 2). Structure-guided engineering of Cas7-11 has resulted in reduction of the molecular size following removal of the INS and C-terminal regions that are dispensable for RNA cleavage, thereby enabling a single-vector adeno-associated viruse (AAV) packaging of the truncated Cas7-11 and its crRNA.[7]

Although the above studies were very informative, mechanistic details on discrimination of RNA invaders and TPR-CHAT activation during Craspase-mediated immunity remained poorly understood. The paper by Hu et al. directly addressed these challenges by structural characterization of a series of cryo-EM snapshots of *Candidatus* "*Scalindua brodae*" gRAMP (*Sb*-gRAMP) and Craspase with or without loading of target RNA.[8] *Sb*-gRAMP in complex with bound non-self-RNA exhibits a similar architecture to that reported previously for target RNA-bound *Di*Cas7-11 and generates two cleavage sites at the same positions. Structural comparisons of *Sb*-gRAMP in resting (apo), self-RNA-bound, non-self-RNA-bound and post-cleavage states suggest that the linker between Cas11 and Cas7.2 domains (defined as gating loop)

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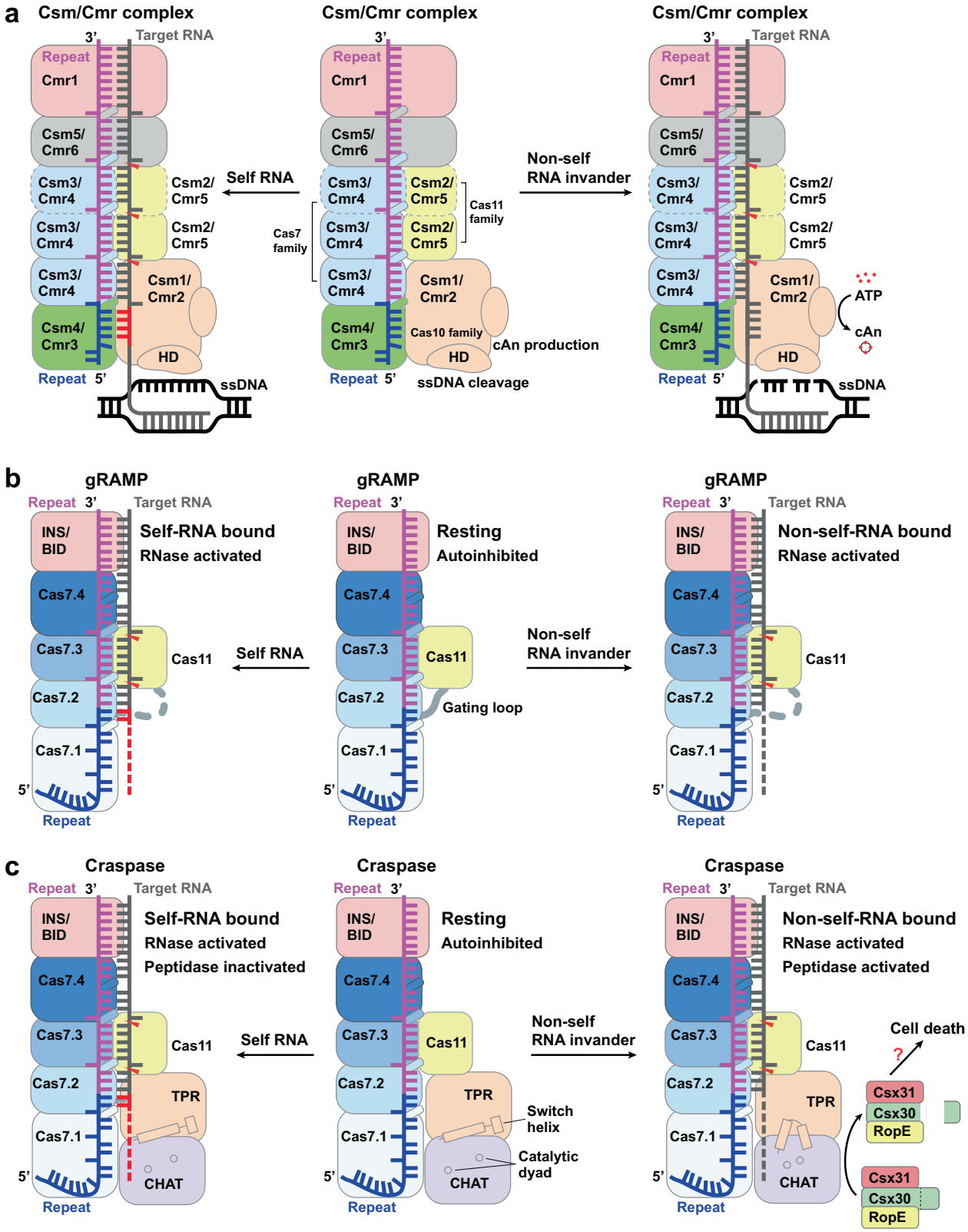

Fig. 1 Schematic representation of RNA-guided and protease-based immunity by Craspase. a Model of type III-A/B Csm/Cmr complex relies on target ssRNA to regulate ssRNA/ssDNA cleavage and cAn synthesis. **b** Model for gRAMP RNase activation upon target RNA loading. **c** Model for Craspase-mediated immunity. Invading RNA induces conformational changes of TPR-CHAT and thereby allosterically triggers peptidase activity on Csx30, resulting in cell death. For **a**–**c**, pairings with self and non-self RNAs are shown in left and right panels, respectively, while red wedges represent cleavage sites.

controls Cas7-11 RNase activity (Fig. 1b). In the resting state, *Sb*-gRAMP adopts an autoinhibitory state, given that the N-terminal region of the gating loop occupies cleavage site 1, thereby sterically blocking the formation of guide:target duplex in this region (Fig. 1b, middle). Upon RNA loading, target RNA pairs with the crRNA spacer segment and propagates along Cas7.4 towards Cas7.2, which results in the moving away of gating loop to release the catalytic activity at site 1 (Fig. 1b). Both self-RNA (Fig. 1b, left) and non-self-RNA (Fig. 1b, right) switch on the Cas7-11 RNase activity, implying that self/non-self discrimination could not be acquired at the RNA cleavage level. Impressively, Hu et al. carried out further work to probe for the nuclease–protease functional relationship by determining a set of Craspase structures in different functional states. TPR-CHAT is located at the side of crRNA repeat segment and mainly interacts with Cas7.2 and the gating loop (Fig. 1c, middle). In the resting and self-RNA-bound states, the catalytic dyad in the CHAT protease domain is far apart from each other, suggesting an autoinhibited conformation (Fig. 1c, left and middle). Non-self-RNA binding induces significant conformational changes in the CHAT domain, as well as in a long helix between the TPR and CHAT domains (defined as switch helix). The switch helix rotates towards and packs against CHAT domain, subsequently triggering the closure of the catalytic dyad to form a proteolytically active conformation through widening of the cleft between *Sb*-gRAMP and TPR-CHAT for protein substrate access (Fig. 1c, right). The accessory protein Csx30 was found to be the natural substrate of Craspase and may enable cell toxicity after hydrolysis. By contrast, self-RNA binding did not result in a conformational change in either the CHAT domain or the switch helix, preventing it from adopting a proteolytically active conformation (Fig. 1c, left).

The paper by Hu et al.,[8] together with four additional papers by Kato et al.,[9] Liu et al.,[10] Cui et al.[11] and Wang et al.,[12] highlight insights into the molecular mechanism underlying crRNA-guided and non-self-RNA-dependent control of nuclease and peptidase activation through capture of insightful structural snapshots. Furthermore, Kato et al. found that N-terminal segment of Csx30, Csx31 and RpoE form a stable complex regardless of Craspase cleavage and possibly mediate diverse downstream signaling events.[9]

Collectively, autoinhibited Craspase degrades ssRNA complementary to spacer and switches on peptidase activity only when bound to non-self-transcripts for stimulation of potential cell death or growth arrest. A currently missing piece of the puzzle is the functional relevance and molecular details of Csx30-induced cell toxicity. Future exploration of structural and functional characterization of Craspase systems could pave the way for the development of new programmable RNA-guided RNA- and protein-targeting technologies.

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ADDITIONAL INFORMATION

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