RESEARCH HIGHLIGHT

First structural insights into CRISPR-Cas-guided DNA transposition

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The transposition mechanism of the *Vibrio cholerae* Tn6677 transposon (VcTn6677) is based on the unique synergy between a classical transposition machinery (TnsABC-TniQ complex) and a nuclease-deficient type I-F CRISPR-associated complex for antiviral defense (VcCascade). Four independent studies, three of which appeared in *Cell Research*, recently reported structures of the VcCascade, VcCascade-TniQ, and/or VcCascade-TniQ-dsDNA complexes as well as a TniQ dimer complex, providing the first insights into the assembly and DNA targeting mechanism of a CRISPR-associated transposase complex.

The CRISPR (Clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated proteins) systems in bacteria and archaea are best known for their role as RNA-guided adaptive immune systems that cleave and eliminate invasive mobile genetic elements to protect their hosts.^{1,2} The guided nuclease modules of various CRISPR-Cas systems have been repurposed as tools for programmable introduction of double stranded DNA breaks, revolutionizing the field of genome editing.^{3,4} Alternative natural functions of the CRISPR-Cas systems have been described, including involvement in regulation of gene expression to control sporulation, biofilm formation and virulence.⁵ Recently, yet another variation on the CRISPR theme has been reported. Several Tn7-like transposons have been described to contain short CRISPR arrays accompanied by nuclease- and adaptation-deficient type I-B, type I-F, and type V-K CRISPR-Cas systems.⁶ It was anticipated that these CRISPR-Cas systems guide the transposition of these Tn7-like transposons. Indeed, two experimental studies have demonstrated that CRISPR-associated transposons are guided by crRNA to complementary genomic target sites, after which the transposon cargo is inserted there as the result of a functional interaction between the nuclease-deficient CRISPR-Cas systems and the canonical transposase complexes that they express.^{7,8} The potential of these CRISPR-associated transposons to control the efficient introduction of large DNA fragments (at least up to 10 kb in length) in a genome, without the restrictive need for homologous recombination flanks and selection markers, can provide the basis for a new generation of genome engineering tools, both in prokaryotes and in eukaryotes.

Vibrio cholerae Tn6677 (VcTn6677) is one of the first characterized Tn7-like CRISPR-associated transposons.⁷ VcTn6677 encodes a nuclease-deficient, DNA-targeting type I-F Cascade complex (VcCascade) and a minimal CRISPR array. VcCascade is composed of Cas6, Cas7 and fused Cas8-Cas5 (Cas8/5) protein subunits. It was previously demonstrated that the Cas6 of the VcCascade complex is a ribonuclease that catalyzes the pre-crRNA

maturation, a key step for the assembly of the VcCascade complex around the resulting 60-nt long mature crRNA, forming the VcCascade/crRNA complex.⁷ The crRNA is comprised of an 8-nt 5'-handle, a central 32-nt spacer sequence, and a 20-nt palindromic repeat at the 3'-end. The VcTn6677 transposase complex is composed of the TnsA, TnsB, TnsC and TniQ protein subunits. The anticipated first step towards the formation of the complete CRISPR-Cas-associated Tn6677 transposition complex is the binding of VcCascade/crRNA to the TniQ transposase subunit. After binding of this complex to a DNA target, TniQ is expected to recruit the Tn6677-DNA loaded TnsABC complex. Eventually, the VcCascade/crRNA-TniQ-TnsABC complex allows for insertion of the Tn6677 transposon in the DNA region approximately 50 bp downstream the crRNA-determined target sequence.⁷

Three independent studies, published in *Cell Research* by Li et al.⁹ Jia et al.¹⁰ and Wang et al.,¹¹ together with an additional study by Halpin-Healy et al.,¹² confirm the VcCascade^{crRNA}-TniQ interaction and report the high-resolution cryo-EM structures of three different crRNA-bound complexes: VcCascade/crRNA,⁹ VcCascade/crRNA-TniQ,^{9–12} VcCascade/crRNA-TniQ-dsDNA,^{10–12} in addition to the crystal structure of a dimer of the TniQ transposase subunit.¹¹ All studies report that the crescent architecture and the subunit stoichiometry of the VcCascade^{crRNA} complex (Cas6:Cas7:Cas8/5, 1:6:1) (Fig. 1a) is similar to that of the *Pseudomonas aeruginosa* (Pa) Cascade complex.¹³ In addition, the Cas6 ribonuclease subunit binds to the 3'-hairpin of the crRNA, forming the head of VcCascade, the six Cas7 subunits form a right-handed helical backbone around the spacer part of the crRNA, while the fused Cas8/5 subunit binds and stabilizes the 5'-handle of the crRNA, forming the tail of VcCascade. The VcCascade head is connected to the VcCascade backbone via a Cas6-Cas7.1 interaction in both PaCascade and VcCascade complexes; nonetheless, Li et al.⁹ report that the interaction in PaCascade complex is based on the ferredoxin-like domain (also known as RNA-recognition motif, RRM) of Cas6, whereas the interaction in VcCascade complex is based on the Cas6 thumb domain. Hence, the ferredoxin-like domain of Cas6 in the VcCascade remains available for interaction with TniQ, indicating a subtle change in the VcCascade structure to facilitate an interaction with the DNA transposition system.

The Cas8/5^{HB} domain is demonstrated to interact with Cas6 in the TniQ-free VcCascade structure, and this interaction is disrupted by the association between the head-to-tail TniQ homodimer ((TniQ)₂) and VcCascade⁹ (Fig. 1a). TniQ.1 binds to Cas6 and TniQ.2 interacts with Cas7.1 (Fig. 1b). One of the available structures indicates that TniQ.2 forms additional contacts

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Fig. 1 Schematic representation of CRISPR-guided transposition. **a** The VcCascade/crRNA and the apo-(TniQ)₂ dimer structures before their association. **b** The VcCascade/crRNA-(TniQ)₂ structure, and **c** the VcCascade/crRNA-(TniQ)₂-dsDNA structure. In panels **a–c** the crRNA guide (red) is superimposed for clarity. **d** A stretched version of the structure in **c**, to clarify some relevant details on the guide-target recognition.

with Cas6, the thumb domain of Cas7.2, and the helical bundle domain of Cas8/5 (Cas8/5^{HB})⁹ (Fig. 1b). Hence, it was suggested that the Cas8/5^{HB} domain of VcCascade is involved in the recruitment of TniQ₂, whereas the Cas8^{HB} domain of PaCascade recruits Cas2/3 for DNA cleavage,¹³ a function that the VcCascade lacks.^{9,12} Altogether, these data strongly suggest the functional adaptation of the Cas8/5^{HB} domain of VcCascade for DNA transposition, while the remaining Cas8/5 subunit is still responsible for PAM identification and the onset of the R-loop formation via opening of the double helix and displacement of the non-target strand^{10–12} (Fig. 1c). Nonetheless, because of the reported intrinsic inflexibility of the Cas8/5^{HB} domain,^{9,12} the reported Cas8/5^{HB} related observations remain to be confirmed.

Collectively, the data presented in the discussed studies provide valuable insights into the subtle structural changes induced by the interaction between VcCascade/crRNA and (TniQ)₂, and they do support the highly structured nature of the VcCascade and VcCascade/crRNA-(TniQ)₂ complexes, which involves a large number of protein-protein interactions. The specificity of these interactions probably reflects the reported difficulty to achieve DNA transposition when substituting the VcCascade of the Tn6677 transposase complex with the effector modules of other (even closely related) CRISPR-Cas systems.⁷ Moreover, the detailed comparison of the VcCascade structure with the structure of the well-studied, canonical type I-F PaCascade complex,¹³ has allowed for the identification of functionally-relevant adaptations of the two systems. A currently missing piece of the puzzle is the molecular details that allow for the subsequent recruitment of the TnsABC complex by the VcCascade/crRNA-(TniQ)₂ complex. It

is anticipated that future studies will focus on the in-depth functional and structural characterization of this and other CRISPR-associated transposon systems. Finally, the acquired information is expected to stimulate the development of a new class of accurate and efficient genomic editing tools.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

1. Mohanraju, P. et al. *Science* **353**, aad5147 (2016).
2. Makarova, K. S. et al. *Nat. Rev. Microbiol.* **18**, 67–83 (2020).
3. Wu, W. Y., Lebbink, J. H. G., Kanaar, R., Geijsen, N. & van der Oost, J. *Nat. Chem. Biol.* **14**, 642–651 (2018).
4. Mougikakos, I., Bosma, E. F., de Vos, W. M., van Kranenburg, R. & van der Oost, J. *Trends Biotechnol.* **34**, 575–587 (2016).
5. Westra, E. R., Buckling, A. & Fineran, P. C. *Nat. Rev. Microbiol.* **12**, 317–326 (2014).
6. Peters, J. E., Makarova, K. S., Shmakov, S. & Koonin, E. V. *Proc. Natl Acad. Sci. USA* **114**, E7358–E7366 (2017).
7. Klompe, S. E., Vo, P. L. H., Halpin-Healy, T. S. & Sternberg, S. H. *Nature* **571**, 219–225 (2019).
8. Strecker, J. et al. *Science* **365**, 48–53 (2019).
9. Li, Z., Zhang, H., Xiao, R. & Chang, L. *Cell Res.* **30**, 179–181 (2020).
10. Jia, N., Xie, W., de la Cruz, M. J., Eng, E. T. & Patel, D. J. *Cell Res.* **30**, 182–184 (2020).
11. Wang, B., Xu, W. & Yang, H. *Cell Res.* **30**, 185–187 (2020).
12. Halpin-Healy, T. S., Klompe, S. E., Sternberg, S. H. & Fernández, I. S. *Nature* **577**, 271–274 (2020).
13. Chowdhury, S. et al. *Cell* **169**, 47–57 (2017).