FIRST CUTS

CRISPR Surveillance Turns Transposon Taxi

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Four reports capture structures of TniQ in association with a crRNA-guided surveillance complex.

Like so many “CRISPR stories,” this one starts with an observation by Eugene Koonin and collaborators. In 2017, Peters *et al.* identified variants of the type I CRISPR system (i.e., I-B and I-F) that lack adaptation machinery (*cas1–2*) and the nuclease (*cas3*) necessary for target degradation (Fig. 1).¹ It is not uncommon to find degenerate CRISPR systems. So, an observation such as this might not interest most of us, but Peters *et al.* doubled down. Using comparative genomics and phylogenetics, they showed that these minimal CRISPR-Cas variants associate with distinct families of Tn7-like transposable elements.

Unlike most Tn7 transposons, which include *tnsABC*, *tniQ*, and *tnsE*, these particular Tn7-like elements lack the *tnsE* gene, which is responsible for transposition into plasmid DNA. In place of *tnsE*, these Tn7-like transposons encode *cas* genes that are characteristic of a type I CRISPR RNA (crRNA)-guided surveillance complex (Fig. 1).² Intriguingly, the spacers associated with the CRISPRs of these truncated systems most frequently match sequences derived from plasmids and other mobile genetic elements. This suggests that the crRNA-guided surveillance complex functionally complements TnsE, although it is not clear if or how this system provides an advantage over TnsE-mediated transposition. It is possible that crRNA-guided insertion of the transposon may inhibit replication of the incoming mobile genetic element, which could provide a defensive function for the host, but may limit the spread of the transposon.

In short, it is unclear whether the transposon domesticated the CRISPR, the CRISPR domesticated the transposon, or if the partnership is somehow mutually beneficial.

The Peters *et al.* paper concludes provocatively, noting that “transposon encoded CRISPR-Cas systems could be harnessed for genome-engineering applications.” This

statement resonated with Samuel Sternberg’s group. Working with a Tn7-like transposon encoding a variant I-F system, Sternberg’s group showed last year that the crRNA-guided surveillance complex targets DNA transposition 47–51 bp downstream of the crRNA guide.³ Showcasing its versatility, they engineered this system to deliver various genetic payloads to 16 different chromosomal locations with 95% or better on-target integration.

Hell Breaks Loose

Eric Sontheimer was recently quoted as saying, “Like everything else in the CRISPR world, the thin edge of the wedge comes in, and the next thing you know all hell breaks loose.”⁴ Sontheimer’s statement was in reference to the use of anti-CRISPRs for applications in genome engineering, but his words apply to most research in this field, including crRNA-guided transposons. In the past few months, four papers have been published on structures of transposon-encoded CRISPR-Cas systems. These include Sternberg’s group in collaboration with Israel Fernández in *Nature* last December⁵ (5 months after their preprint was posted on bioRxiv) and three other teams writing in *Cell Research*.^{6–8}

Collectively, these papers report a total of 10 structures including crystal structures of TniQ, and cryo-EM structures of TniQ in association with the I-F crRNA-guided surveillance complex from *Vibrio cholerae* (i.e., VcCascade-TniQ), both before and after binding to a DNA target. The minimal I-F crRNA-guided surveillance complex maintains a crescent-shaped backbone, comprised of six Cas7 subunits, and is capped on either end by a Cas6 “head” and a Cas8/5 “tail” (Fig. 1B). This overall shape is similar to what has been observed in structures of I-F surveillance complexes involved in phage defense (Fig. 1B).^{9–13} Moreover, crRNA-guided DNA binding triggers an elongation in the backbone.^{10,12} In the I-F defense systems, elongation of the backbone

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FIG. 1. Comparison of the I-F CRISPR defense system to the I-F Tn7-like system. **(A)** Genetic organization of type I-F CRISPR-Cas locus from *Pseudomonas aeruginosa* PA14 (top). Transposition genes in Tn7 transposon from *Escherichia coli* (middle). Genetic organization of Tn7-like I-F CRISPR-Cas locus from *Vibrio cholerae* strain HE-45 (bottom). **(B)** Structures of the type I-F surveillance complex before (PDB: 5UZ9) and after (PDB: 6NE0) binding dsDNA. The R-loop induced conformational change reveals a recruitment signal for Cas2-3 transacting nuclease (top). TniQ-VcCascade undergoes conformational changes on target hybridization (PDB: 6UVN, 6PIJ; bottom). The mechanism of recruitment remains unknown.

coincides with a dramatic rearrangement of a helical bundle on Cas8.¹² This rearrangement locks the complex onto the DNA target and presents a landing pad for nuclease recruitment.

In the Tn7-like I-F CRISPR system, Cas8 and Cas5 are fused (Cas8/5) and the corresponding helical bundle is positioned like a bridge that connects the Cas6 head to the tail of the complex. Density for this region of the protein is weak, and the importance of this position remains unclear, but its location suggests that it may be involved in stabilizing the displaced strand of DNA (R-loop) and/or coordinated recruitment of transposon-associated proteins. Contacts between the helical bundle of Cas8/5 and TniQ, which forms a homodimer that is prominently positioned at the head of the complex, offer additional support for a role in coordinated recruitment of transposon-associated proteins (Fig. 1B).

While this work has revealed specific interactions between VcCasacade and TniQ, additional structures that include TnsABC will help clarify the mechanism of transposon recruitment. We anticipate that additional mechanistic insights will help develop these systems for creative applications in genome engineering. Once these and other transposon-associated CRISPR systems¹⁴ are

used to deliver a therapeutic payload to a specific genetic address in the human genome, then “all hell” is really going to break loose.

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Cancer Screens: Better Together

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A formal comparison of the two largest published pan-cancer CRISPR-Cas9 knockout screens finds good concordance between data sets.

In the postgenomic era, it is maddening that most currently available cancer therapies are still broad-based chemotherapy treatments designed to target rapidly dividing cells whether normal or malignant cells. The mutations that cause cells to become oncogenic also confer specific vulnerabilities that normal cells lack and can hopefully be exploited for targeted cancer therapies.

In order to expand precision oncology treatments, we must gain a better understanding of these cancer dependencies and the underlying genetic alterations. To this end, several groups have conducted large-scale screens to identify cancer dependencies and hopefully novel therapeutic targets.^{1–7}

As part of the cancer dependency map effort,^{8,9} the Broad and Sanger Institutes have independently conducted pooled CRISPR-Cas9 genome-wide dropout screens in hundreds of cancer cell lines.^{1,2,4} Writing in *Nature Communications*,¹⁰ Joshua Dempster *et al.* conducted a formal comparison of these two pan-cancer CRISPR-Cas9 screens in order to assess the reproducibil-

ity of these experiments and the concordance of resulting data sets.

These screens were conducted by transducing Cas9-expressing cancer cell lines with a library of single guide RNAs (sgRNAs) targeting most genes in the genome for knockout. The sgRNAs were delivered via lentiviral transduction and at a low multiplicity of infection such that only one sgRNA was integrated per cell. Because the sgRNAs are integrated into the genome, they can also be used as barcodes to identify and quantify if sgRNAs are enriched or depleted during the screen.

Once expressed, each sgRNA will, in principle, complex with Cas9, find its target, and induce a knockout of the target gene (one gene knocked out per cell). Cells are subsequently collected and genomic DNA harvested and sequenced to identify which sgRNAs have been depleted. If a cancer cell line is dependent on a given gene for survival, cells containing a sgRNA targeting that gene will have a growth disadvantage and/or die, and correspondingly that sgRNA will be depleted or drop out.

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