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Stirring Up the Type V Alphabet Soup

Daan C. Swarts

Novel Cas12 Effectors Underscore the Diversity of Type V CRISPR-Cas Systems

Type II and type V CRISPR-Cas effectors are multidomain enzymes that utilize a guide RNA to bind and cleave complementary DNA targets.[1] The facile programmability of these effectors allows for their exploitation as genome editing tools. While the type II effector Cas9 is undoubtedly the best-known genome editing tool, the more recently characterized type V effectors (i.e., Cas12a and Cas12b) have also been repurposed for genome editing.[1–3]

Despite their highly similar functionality, Cas9 and Cas12 have distinct domain architectures and mechanisms, making them complementary tools for genome editing.[1] Strikingly, there is a high diversity in sequence and domain organization among the different type V effectors.[4,5] Therefore, characterization of these functionally diverse type V effectors could lead to the expansion of the CRISPR toolbox. Writing in *Science*,[6] Yan *et al*. from Arbor Biotechnologies and the National Center for Biotechnology Information have extended the type V alphabet by identifying and characterizing four novel type V effectors: type V-C Cas12c type V-G Cas12g, type V-H Cas12h, and type V-I Cas12i.

The Type V Alphabet

All type V effectors contain a C-terminal RuvC domain but appear to have evolved via distinct pathways from different transposase-encoded TnpB genes.[4] Consequently, type V effectors have low sequence identities and differentiated domain architectures, making their identification and subsequent characterization tricky. Nevertheless, intensified data-mining efforts have resulted in a steady letter-by-letter expansion of the type V family.[6–9]

As a consequence of following distinct evolutionary paths, type V effectors developed a variation of functional mechanisms (Fig. 1). Some type V effectors utilize single crRNA guides, but others use dual-RNA (tracrRNA:crRNA) guides.[2,6–9] While most type V effectors target dsDNA with T-rich protospacer adjacent motifs (PAMs), some effectors target ssDNA only.[9] All type V effectors rely on a single catalytic site in the RuvC domain for target cleavage. In Cas12a, this site becomes solvent exposed upon crRNA-target DNA hybridization, which allows for sequential *cis*-cleavage of the both target DNA strands.[10–12] For at least some type V effectors, the catalytic site remains solvent exposed after target cleavage, and consequently these effectors display collateral *trans*-cleavage of non-target single-stranded DNA.[12–14]

Stirring the Soup

Yan *et al*. have identified and characterized type V-C, V-G, V-H, and V–I effectors and thereby highlight the divergent interference mechanisms of type V

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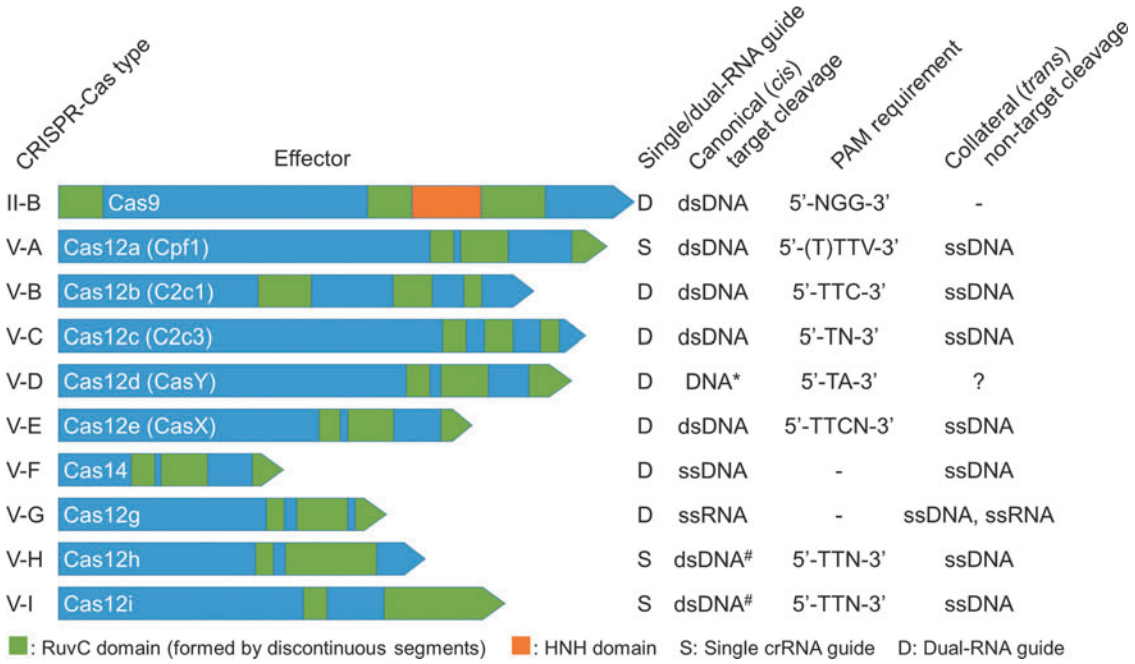


FIG. 1. Catalytic domain organization and functionality of type V effectors. *Left*: CRISPR-Cas type and corresponding Cas effector with the RuvC domain (formed by discontinuous segments) indicated in green. *Right*: Functionality of type V effectors in terms of guide RNA requirement, canonical targets, protospacer adjacent motif requirement, and collateral activity. S, single crRNA guide; D, dual-RNA guide. *Demonstrated *in vivo* only; #predominant nicking of dsDNA targets.

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systems.[6] Three characterized Cas12c effectors utilize dual-RNA guides to target and cleave dsDNA targets, similar to Cas12b.[7] While two of these Cas12c effectors require a 5′-TG-3′ PAM for double-stranded DNA targeting, the third Cas12c requires a short 5′-TN-3′ PAM only.

Similar to previously characterized type V-F Cas14 effectors,[9] Cas12g is phylogenetically clustered with predicted type V-U effectors and transposon-encoded TnpB. These effectors are thought to have evolved recently and are generally much smaller than other CRISPR effectors.[4,5] While both Cas14 and Cas12g utilize dual-RNA guides to target single-stranded nucleic acids, Cas12g targets RNA rather than DNA. Thus, for the first time, RNA-guided RNA interference by a type V effector has been described. In addition to *cis*-cleavage of target ssRNA, Cas12g displays collateral *trans*-cleavage of both non-target ssDNA and ssRNA.

Cas12h and Cas12i cluster phylogenetically with Cas12b, but their functionality is more similar to Cas12a; they utilize a single crRNA to target dsDNA with a 5′-TTN-3′ PAM. Although Cas12a rapidly cleaves both strands of the target DNA sequentially,[10–12] Cas12i predominantly cleaves one of the target DNA strands only (DNA nicking).

Forward Path

These new findings pave the way for numerous new lines of research that investigate how and why functional diversity has evolved in type V systems. Are type V effectors ''immature'' products of evolution with developmental flaws, or do their distinct functionalities provide certain evolutionary benefits? Furthermore, one wonders what the mechanistic explanations are for these distinct functionalities. How do type V effectors distinguish between RNA and DNA targets? What features are required to be able to target and cleave double-stranded targets efficiently? Presumably, future biochemical studies will shed light on the structural basis for the differentiated mechanisms exploited by type V effectors.

As Yan *et al*. note, each of the peculiar activities of the novel effectors could be exploited for certain applications.[6] The short PAM requirement of Cas12c variants expands the number of sites that can be targeted by CRISPR effectors, the nicking activity of Cas12i might allow for high-fidelity genome editing,[15] and the RNA target-activated *trans*-cleavage activity of Cas12g can be exploited for highly sensitive nucleic acid detection.[16] Nevertheless, experimental support for these applications

is crucial to demonstrate their added value to the currently used type II and V effectors.

Lastly, this study demonstrates that the expanding quantity of available sequence data is far from fully exploited. Undoubtedly, rapidly developing metagenomics will yield an increasing amount of novel (putative) CRISPR-Cas systems that await characterization and classification. This might pose a new challenge for type V systems, as previously predicted by Makarova *et al*. who wrote: ''a liberal approach in type V subtype assignment can easily exhaust the Latin alphabet within a few years.''[5] Therefore, careful consideration—or consultation—is required to avoid unrestrained proliferation of CRISPR-Cas (sub)families.

While this First Cut item was in preparation, a detailed characterization of type V-E Cas12e (CasX) by Liu *et al.* was published in *Nature*.[17] Not only do the authors confirm previously established Cas12e-crRNA mediated *cis*-cleavage of dsDNA targets by *in vitro* assays, they also demonstrate that Cas12e mediates *trans*-cleavage of non-target ssDNA (albeit at much lower efficiencies than other type V effectors). Liu *et al*. furthermore show that Cas12e can be repurposed for both gene silencing and genome editing, thereby expanding the genome editing toolbox with a fourth Cas effector. While its activity and mode of action appears to be similar to other Cas effectors, Cryo-EM structures of Cas12e reveal that only certain domains of Cas12e are homologous to other type V effectors (e.g. the RuvC domain). While other Cas12e domains show functional analogy to the domains of different type V effectors, they are formed by distinct folds. In conclusion, the studies by Yan *et al*. and Liu *et al*. underscore the diversity of Type V CRISPR-Cas systems.

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Author Disclosure Statement

The author declares no conflict of interest.

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