COMMENTARY

Expanding the horizons of genome editing in the fruit fly with Cas12a

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For well over a century, geneticists have relentlessly bombarded the genome of the fruit fly *Drosophila melanogaster* with increasingly sophisticated mutagenic agents (1). Collectively, these loss-of-function studies have been astoundingly informative, providing fundamental breakthroughs in nearly all fields of biology (2, 3). Initially, such studies relied on mutagens that attack the genome in quasirandom locations, such as X-rays, mutagenic chemicals, and transposable elements. The application of RNA interference (RNAi) to *Drosophila* genetics made it possible to intentionally target the transcript of any specific gene of interest and, with the Gal4-UAS system, to do so with precise spatial and temporal control (4, 5). The field was revolutionized again 7 y ago, when CRISPR-Cas9–based genome editing was demonstrated in *Drosophila*, making it possible to mutate and rewrite the genome of the fruit fly with ease, specificity, and scalability that were previously unimaginable (6). Yet for all of the profound properties of Cas9, the powerhouse RNA-guided DNase at the center of this technical revolution, there are downsides to relying solely on this particular nuclease for genome editing. Indeed, a growing number of alternative CRISPR approaches have been described in recent years, based on both naturally occurring and laboratory-evolved CRISPR-family proteins. To date, however, none of these alternatives have been effectively adapted for in vivo studies in *Drosophila*. In PNAS, Port et al. (7) provide such a demonstration, focusing on the Cas12a enzyme (formerly known as Cpf1). Cas12a displays a number of intriguing properties that make it a broadly useful complement to Cas9 (Fig. 1) and a valuable tool to continue the collective interrogation on the *Drosophila* genome.

New genetic techniques spread through the research community like evolution by natural selection. Scientists continually create new techniques, and those that prove particularly effective, reliable, and affordable spread rapidly and serve as fodder for additional variation and specialization. In this analogy, CRISPR is the Cambrian explosion. In an incredibly short period of time, an adaptive radiation of CRISPR-based techniques has spread around the world, specializing to every new model organism and sparking further adaptation to ever-more specific niches. The Cas9 enzyme from *Streptococcus pyogenes* (SpCas9) has undeniably been the dominant branch of this evolutionary tree, yet a growing pool of alternatives has been developed in parallel, offering important technical complements to Cas9 that are particularly advantageous for certain uses.

One of these alternates, Cas12a, was first described by Zetsche et al. (8), and it possesses four relevant differences from Cas9. First, Cas9 and Cas12a can target distinct targets in the genome. Specifically,

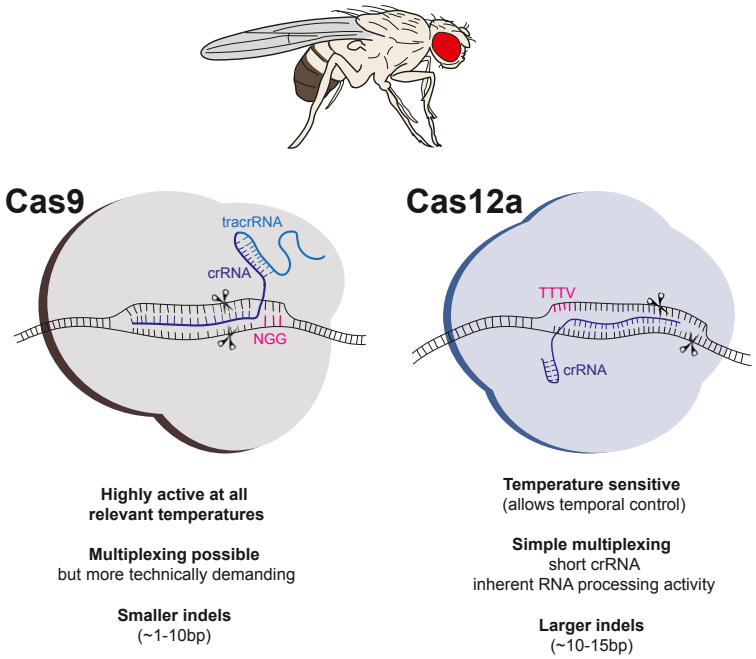

Fig. 1. In vivo genome editing with the Cas12a system offers several technical complements relative to standard Cas9-based editing in *Drosophila*. "NGG" and "TTTV" are the protospacer-adjacent motif (PAM) sequences required for cleavage by SpCas9 and LbCas12a, respectively, where N = A, C, T, or G, and V = A, C, or G.

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SpCas9 can only cleave target sites flanked by "NGG" (where N can be A, C, G, or T); this is the strictly required "protospacer-adjacent motif" (PAM). In contrast, Cas12a requires a T-rich PAM sequence, and thus, each system can target genomic regions inaccessible to the other. Second, while Cas9 requires two RNA components, a transactivating RNA (tracrRNA) and a CRISPR RNA (crRNA), typically fused in the laboratory into a chimeric short guide RNA (sgRNA), Cas12a requires only the crRNA. Third, while both enzymes display DNase activity, Cas12a is additionally an RNase capable of processing multiple crRNAs from a single precursor, which can be exploited for multiplexed editing (9, 10). Fourth, the indels created by Cas12a are often larger than those created by Cas9. Together, these differences make Cas12a an attractive complement to Cas9.

New CRISPR-based genome editing approaches are typically first assessed in vitro and/or in cell culture. Adapting any system for in vivo studies in a multicellular model organism requires a great deal of time and a dedication to optimization against unforeseen biological complexity. Indeed, Port and Bullock (11) first tested Cas12a in *Drosophila* in 2016, where they focused on Cas12a isolated from *Acidaminococcus* bacteria (AsCas12a). In that study, they observed exceedingly low rates of editing compared with SpCas9. Subsequent studies in vertebrates suggested that this particular Cas12a variant only functions optimally at temperatures above 30 °C, which is above the long-term thermal limits for *Drosophila*.

In the present study, Port et al. (7) thus begin with a head-to-head comparison of AsCas12a vs. a Cas12a variant isolated from *Lachnospiraceae bacterium* (LbCas12a), which has been previously shown to function at lower temperatures. Using constitutively expressed Cas12a transgenes, the authors found that LbCas12a dramatically outperforms AsCas12a at temperatures *Drosophila* can tolerate and is highly active for 7 of the 11 crRNAs tested (64%). While a success rate of 64% is lower than that observed for panels of sgRNAs tested with Cas9 [e.g., a previous study from Port et al. (12) found that 65 of 66 Cas9 sgRNAs were active in vivo], there is reason to hope that this success rate will improve as the field learns more about design principles for Cas12a crRNAs. The authors confirm via sequencing that Cas12a typically creates 10- to 15-bp deletions, larger than commonly observed for Cas9, which may correspond to an increased mutagenicity.

Port et al. (7) also confirm that Cas12a is highly temperature sensitive in *Drosophila*: 7 of 11 crRNAs were active with LbCas12a at 29 °C, but only 1 was reliably active at 18 °C. As the authors emphasize, this temperature sensitivity can be exploited for experimental purposes: for example, to bypass early lethal phenotypes by limiting mutagenesis to later stages. While this inherent temporal control may indeed be an asset, it is important to note that the control is not absolute, and the effect is at least partially crRNA specific, meaning that researchers will need to carefully characterize the specific crRNAs they use.

One of the key advantages of Cas12a is a technical one for researchers: crRNAs are far shorter than the sgRNAs used with Cas9 and are inherently amenable to multiplexing due to the RNase activity of Cas12a. For example, to generate a Cas9-compliant 6× sgRNA construct, one must perform five independent PCR reactions, a subsequent low-efficiency cloning step, and colony PCR to identify putative successes (11). In addition, the repeated tracrRNA sequences and transfer RNA sequences (necessary between sgRNAs to cleave individual sgRNAs from the precursor transcript) make it nearly impossible to synthesize these fragments commercially. In contrast, Port et al. (7) demonstrate that the small size and self-processing of Cas12a crRNAs make it possible to order up to an 8× crRNA from a commercial vendor and that one such array successfully drives editing at five of the eight intended sites. While there is room for improvement in the reliability of crRNAs, this represents a major advantage over Cas9 for multiplexed experiments. Such multiplex experiments will be particularly useful for studies of genetic interactions between multiple sets of genes and to target multiple redundant genes at once.

Given the dizzying pace of discovery and improvement in the CRISPR field, it can be a genuine challenge for model organism researchers to decide when the time is right to make a meaningful investment in a given CRISPR strategy. The decision of Port et al. to invest in creating a working tool kit for Cas12a in *Drosophila* seems to have been a wise decision.

Perhaps even more crucially, many thousands of multiplex crRNA arrays can now be commercially synthesized on a chip and cloned into a vector in a batch "one-pot" reaction. For the purpose of creating a large transgenic *Drosophila* resource, this is an important technical advantage over Cas9. Additionally, as Port and colleagues (11, 12) have done in their previous CRISPR manuscripts, they provide a clear, easy-to-follow protocol for cloning crRNA plasmids (7). These protocols are very much appreciated by others in the field and are likely one of the reasons that the reagents they generate have been so widely adopted.

Having shown that ubiquitously expressed Cas12a is effective in vivo, the authors next showed that this system can be adapted for tissue-specific use via the Gal4-UAS system. Previous work has shown that Cas9 has two technical drawbacks when expressed in a tissue-specific manner. First, high levels of Cas9 expression using Gal4-UAS are often toxic and cause significant apoptosis and tissue malformation (13). Second, leaky expression of Cas9 outside of the intended tissue can lead to high levels of unintended target cleavage, especially when sgRNAs are expressed ubiquitously (11). Both of these drawbacks have required clever work-arounds to allow for tissue-specific Cas9 mutagenesis (11, 13). In the present manuscript, the authors show that Gal4-UAS–driven LbCas12a is well tolerated in various tissues, does not appear to drive target cleavage outside the intended Gal4+ domain, and is very effective for somatic, tissue-specific knockout in a number of tissues including the germline (7). The ability to perform tissue-specific Cas12a experiments greatly increases the likelihood that this system will be a valuable orthogonal approach to Cas9 and RNAi for tissue-specific genetic manipulations.

Lastly, the authors show that there is almost certainly room for improvement in this system. They test a single point mutation in LbCas12a (D156R) that has been previously shown to enhance cleavage efficiency in the plant *Arabidopsis* and find that this mutation functions similarly in *Drosophila*. Given the advantages conferred by this point mutation, one can assume there may be additional ways to improve the efficiency of the system. They then compare mutagenesis efficiencies of a panel of 39 target genes using Cas12a, Cas12a^{D156R}, and Cas9 and find that the efficiency of Cas12a^{D156R} approaches that of Cas9, a high bar. As the authors discuss, it is unlikely Cas12a will replace Cas9 for in vivo genome editing in *Drosophila*, especially given the large transgenic sgRNA collections that are currently being

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generated by a number of laboratories and resource centers. However, given the impressive mutagenic performance of Cas12a, and the notable technical advantages of batch cloning crRNAs, it is likely to become a widespread and important orthogonal approach in the field.

The discovery of CRISPR-Cas9 has been a gold mine, and scientists around the world continue to dig at an outrageous pace. In the past few weeks alone, a "hypercompact" enzyme termed CasΦ was described that is half the molecular weight of Cas9/Cas12a yet possesses similar genome-editing properties (14), and "prime editing" has been successfully adapted for in vivo studies in *Drosophila,* allowing for precise genome editing without DNA double-strand breaks (15). Given the dizzying pace of discovery and improvement in the CRISPR field, it can be a genuine challenge for model organism researchers to decide when the time is right to make a meaningful investment in a given CRISPR strategy. The decision of Port et al. (7) to invest in creating a working tool kit for Cas12a in *Drosophila* seems to have been a wise decision.

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