MOLECULAR BIOLOGY

Harnessing mutation: The best of two worlds

Combining vertebrate and bacterial immunity components enables targeted mutagenesis

By **Silvestro G. Conticello**[1] *and* **Cristina Rada**[2]

Rapid evolution requires high rates of mutation that come at the cost of reduced viability. This is true at the single-cell level in bacteria as well as in the highest complex vertebrates. Immunity relies on rapid evolution to enhance the recognition of external threats and to neutralize them, often by inflicting damage to the genomes of potentially harmful invaders. On page 1248 of this issue, Nishida *et al.* (*1*) report combining a polynucleotide cytosine deaminase (PmCDA1)—an enzyme involved in antigen receptor diversification in vertebrates—with the bacterial immune restriction system clustered regularly interspaced short palindromic repeat (CRISPR)–Cas9 to enhance point mutation with almost single-nucleotide precision to levels that, if occurring genome-wide, would otherwise result in lethality. With localized mutagenesis, also achieved through a similar approach by Komor *et al.* (*2*), comes the promise of gene correction by means of targeted editing. Both studies show that not only is targeted mutation feasible but that its efficiency, and even the type of mutation, can be driven by manipulating or engaging the various cellular pathways involved in repairing edited bases.

Targeted editing of large vertebrate genomes is a bioengineer's dream that has been on the horizon since the early 1970s. But the prospects of high throughput and high versatility have only become a tantalizingly imminent possibility since the RNA-guided bacterial Cas9 entered the game in 2012 (*3*–*5*). Bacteria and archaea acquire adaptive immunity by incorporating snippets of genetic material from foreign DNA into their own genome during the course of an infection or invasion. This piece of foreign DNA is used to produce RNA guides for the Cas9 endonuclease to recognize further attacks by the same threat. The small RNA molecules associate with Cas9 and use homology to displace the double helix of foreign DNA and direct cleavage of the two DNA strands. The stable displacement of one DNA strand by the RNA guide creates a perfect substrate for cytosine deaminases, such as AID (activation-induced cytidine deaminase) or the APOBECs (apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like), that act on single-stranded substrates (*6*).

The CRISPR-Cas9 complex has greatly facilitated targeted editing by means of homologous recombination (*4*), which requires exogenous addition of a DNA template bearing the correction. Although the frequency of recombination at a specific site is increased by locally induced DNA breaks, the process remains inefficient and at risk of creating genes marred with deletions or insertions. The coupling of a catalytically inactive Cas9 (dCas9) with a deaminase that can only act on single-stranded DNA triggers localized cytosine-to-uracil conversion but avoids collateral damage and insertions or deletions (indels) brought about by the repair of breaks.

For both Nishida *et al.* and Komor *et al.*, tricks learned from unraveling the mechanisms of AID (the mammalian homolog of PmCDA1) during antibody diversification proved useful (*7*): By inhibiting the

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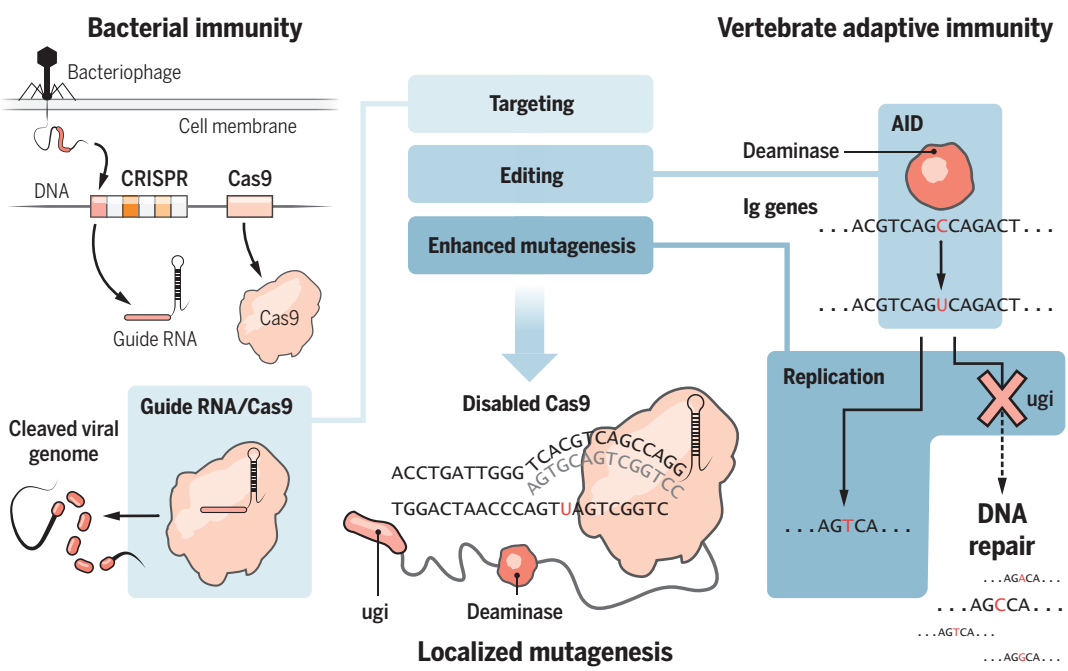
Localized mutagenesis tool

Elements from bacterial and vertebrate adaptive immunity can be combined in a new approach. The bacterial endonuclease Cas9 contributes the targeting module by using a homology-guided small RNA and by creating a single-stranded substrate for a vertebrate polynucleotide cytosine deaminase (AID) to initiate editing through a C-to-U change. Addition of a bacteriophage inhibitor of uracil glycosylase (ugi) prevents the removal of the edited base, thus increasing mutagenesis efficiency. The use of a disabled Cas9 that lacks endonuclease activity prevents unwanted collateral mutagenesis in the form of insertions and deletions. Ig, immunoglobulin.



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removal of uracil with a bacteriophage peptide (ugi) that inactivates the cellular repair enzyme uracil glycosylase, mutation frequencies are enhanced to levels that obviate the need for additional selection (see the figure). The sequence of such targeted yeast genomes contains negligible off-target changes, but up to 10% localized mutation. By using rat APOBEC1, a stronger DNA-RNA deaminase than lamprey PmCDA1 (*8*), Komor *et al.* obtained mutation frequencies up to 20% in human cells, with a further increase to 37% achieved by biasing the cellular mismatch repair toward correction of the unedited DNA strand—at the slight cost of increasing indels (from <0.1 to 1.1%). APOBEC1, like the related human APOBEC3 deaminases, is a powerful genome mutator in cancer cells (*9*, *10*), and even in physiological conditions, AID off-target activity is a frequent cause of oncogenic mutations and translocations (*11*).

"With localized mutagenesis...comes the promise of gene correction..."

Although testing known off-target sites of CRISPR-Cas9 for APOBEC-dependent mutations in the study of Komor *et al.* suggests low collateral damage, whole-genome or -exome sequencing will still be a necessary proposition for human gene therapy. Physically tethering the deaminase to Cas9 might be the key strategy that allows both groups to minimize unwanted editing activity in mammalian cells, where transient single-stranded DNA is unavoidably associated with transcription. It could well be that "less is more" in this case and that the less efficient deaminase AID might be a better choice in mammalian cells.

Transient use of dCas9-PmCDA1-ugi practically eliminates off-target mutations and indels but limits the genes amenable for correction because only transitions from GC to AT pairs can be reliably achieved. This is not a major limitation to technological applications; the obvious combination of dCas9-AID with yeast display immediately comes to mind as a tool for accelerated antibody evolution, but many other examples are ripe for its use, from genome evolution to conventional genetics.

Aside from the technical innovation, the methods described by Nishida *et al.* and Komor *et al.* could provide insights into the physiology of these deaminases. Coexpression of dCas9 and PmCDA1 is sufficient to increase the frequency of localized mutation even in trans; indeed, the main requirement for deamination is the presence of a small but persistent single-stranded region of DNA (*12*). In vivo, other factors contribute to editing efficiency, such as the sequence context preferences of the edited cytosine and its position within the open DNA. In both the Nishida *et al.* and Komor *et al.* studies, distal sites were more frequently edited, a difference that was exacerbated for cytosines that did not fall within the sequence consensus of the deaminase. As exemplified by Nishida *et al.*, a more relaxed sequence context favored by AID [WRC (where W is either A or T, and R is either A or G) versus NTC (where N is any base)] can be an advantage for mutagenesis applications, whereas hybrid deaminases with different sequence context preferences could be part of the targeted mutation toolkit (*13*).

Although powerful, the system is still short of the promised targeted base editing. One reason is that mutations are biased to GC pairs and are promiscuous within the single-stranded bubble created by the Cas9-RNA guide. More importantly, the short homology required for RNA-guided recognition is an adaptation that provides bacterial CRISPR defense the flexibility to accumulate immunity against a large number of viruses or foreign plasmids while retaining a compact genome, but results in promiscuous targeting in larger genomes. As in the case of the deaminase component, a less efficient Cas9 with a more fastidious and longer homology requirement would be a desirable development.

Harnessing mutation, a dream long anticipated by geneticists and pioneered by early molecular biologists, was still haunting Michael Smith in his 1993 Nobel Prize lecture: "The ignis fatuus of genetics has been the specific mutagen, the reagent that would penetrate to a given gene, recognize it, and modify it in a specific way." He was quoting Joshua Lederberg's 1959 Nobel Prize lecture. By the looks of it, the dreamt future of genetics is now. ■

REFERENCES

1. K. Nishida *et al.*, *Science* **353**, aaf8729 (2016).
2. A. C. Komor, Y. B. Kim, M. S. Packer, J. A. Zuris, D. R. Liu, *Nature* **533**, 420 (2016).
3. M. Jinek *et al.*, *Science* **337**, 816 (2012).
4. L. Cong *et al.*, *Science* **339**, 819 (2013).
5. P. Mali *et al.*, *Science* **339**, 823 (2013).
6. S. G. Conticello, *Genome Biol.* **9**, 229 (2008).
7. J. Di Noia, M. S. Neuberger, *Nature* **419**, 43 (2002).
8. I. B. Rogozin *et al.*, *Nat. Immunol.* **8**, 647 (2007).
9. G. Saraconi *et al.*, *Genome Biol.* **15**, 417 (2014).
10. C. Swanton, N. McGranahan, G. J. Starrett, R. S. Harris, *Cancer Discov.* **5**, 704 (2015).
11. L. Pasqualucci *et al.*, *Nature* **412**, 341 (2001).
12. B. J. Taylor, Y. L. Wu, C. Rada, *eLife* **3**, e03553 (2014).
13. M. Wang, C. Rada, M. S. Neuberger, *J. Exp. Med.* **207**, 141 (2010).

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VACCINES

Vaccine trust and the limits of information

Understanding trust in local contexts is key to communication about vaccination

By **Heidi J. Larson**

Over the past decade, there has been growing recognition and increasing research around the phenomenon of vaccine reluctance and refusal (*1, 2*). More recently, there has been a flurry of articles on what is being referred to as "vaccine hesitancy," depolarizing the earlier characterization of individuals or groups as being outright pro- or antivaccine, and instead recognizing the liminal state between becoming aware of, and deciding whether or not to accept, vaccination. Episodes of waning public confidence around vaccines have become so global that the World Health Organization's Strategic Advisory Group of Experts on Immunization convened a working group (*3*) to better understand and recommend actions to address this growing challenge of vaccine hesitancy, which the group defined as "delay in acceptance or refusal of vaccination despite availability of vaccination services." Indeed, vaccine hesitancy is complex and context specific (*4*). How can we better understand the circumstances that influence this state to ensure more effective uptake of vaccines and secure public health?

Faced with an ever-growing portfolio of new vaccines and combinations of vaccines, parents—and society more broadly—are becoming more questioning as they assess whether vaccines for themselves or their children are too many or too new, better given individually or in combination, or worth even the smallest risk. The landscape of information and misinformation about vaccines, as well as the varied and sometimes divisive views of legitimate and self-espoused experts, is further complicating the public's genuine interest in making the right decision. A broader environment of distrust in institutions (*5*) and "experts" (*6*) additionally prompts pub-

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