GENOME EDITING

Base editing goes into hyperdrive

CRISPR base editors can induce single-base-pair changes in the genome, although they are often inefficient. A study now shows that fusion of the DNA-binding domain of RAD51 to base editors enhances both the efficiency and the targeting range of optimized enzymes. These 'hyper-editors' offer effective tools for disease modeling and gene therapy.

Alyna Katti and Lukas E. Dow

Base editing is a relatively new genome-engineering technology that generates targeted single-base-pair changes without inducing double-strand breaks[1,2]. Base editors (BEs) consist of a DNA-modifying enzyme fused to a partially disabled Cas9 nuclease, thus facilitating chemical modifications of the nucleobases within a restricted targeting window of the Cas9-binding site. Numerous efforts have been made to optimize or expand this system, including modifications to (1) the deaminase enzyme, to increase expression and nuclear targeting, and increase or decrease the window of editing; (2) the Cas protein, to expand the range of recognized DNA motifs and restrict off-target events; or (3) the single guide RNA (sgRNA) sequence, to improve specificity and editing efficiency[3,4].

A new study from Zhang et al.[5], published in this issue of *Nature Cell Biology*, demonstrates that, rather than mutating the deaminase, Cas9 protein or sgRNA, BE activity can be quantitatively and qualitatively enhanced by altering the interaction of the BE enzyme with single-stranded (ss) DNA. The authors hypothesized that insertion of a native DNA-binding domain (DBD) with affinity for ssDNA into an existing BE construct would increase affinity for the DNA substrate and thus improve the editing activity. To test this idea, they screened ten non-sequence-specific ssDBDs from different human proteins fused at the N terminus of the optimized cytidine base editor BE4max[6] (Fig. 1a). Integration of the RAD51 ssDBD resulted in the greatest overall improvement in C-to-T editing efficiency at two endogenous loci in HEK293T cells and was maximized when the RAD51 ssDBD was repositioned between the cytidine deaminase and Cas9 'nickase' (Cas9n) protein (hyBE4max; Fig. 1b). In this configuration, hyBE4max showed increased editing within the canonical editing window (C4–C8) as well as outside (C9–C15) the typical range. Zhang et al.[5] further showed that the human cytidine deaminase APOBEC3A (A3A), previously shown to base-edit efficiently[7], fused to Cas9n and the RAD51 ssDBD (hyA3A-BE4max; Fig. 1b) further increased the activity and expanded the editing range in HEK293T cells and mouse embryos. Importantly, the hyperactive cytidine base editors (hyCBEs) did not display increased DNA off-target editing, indel generation or cellular toxicity. Whereas RNA editing was mildly increased with hyA3A-BE4max, the relative increases in target DNA editing were more dramatic.

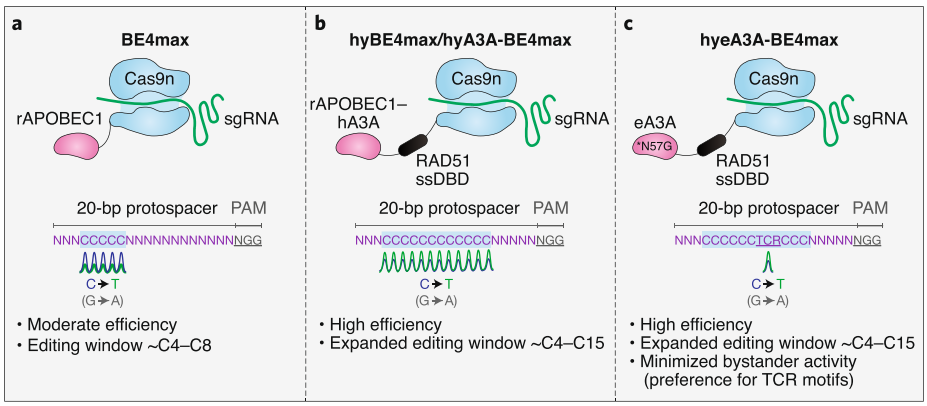

Fig. 1 | Enhanced efficiency and expanded editing range of hyper-base-editing enzymes engineered to include a non-specific ssDNA-binding domain of the human RAD51 protein. a, Koblan et al.[6] generated an optimized cytosine base editor (BE4max) consisting of rat (r) APOBEC1 fused to a codon-optimized Cas9n that can be directed to catalyze C to T (G to A) single-base-pair changes. BE4max deaminates nucleotides within a targeting window of 4–8 base pairs (bp) of the 20-bp protospacer when targeted by a complementary sgRNA. **b**, The addition of the RAD51 ssDBD to BE4max between rAPOBEC1 and Cas9n (hyBE4max) enhances the editing activity at cytosines inside and outside the typical editing range of BE4max. A hyBE4max equivalent incorporating human (h) A3A rather than rAPOBEC1 (hyA3A-BE4max) is similarly hyperactive in an expanded window of editing[5]. **c**, An engineered N57G mutant hA3A protein (eA3A) fused to RAD51 ssDBD and Cas9n to generate hyeA3A-BE4max[5]. The known preferential editing of the eA3A base editor for TC motifs (specifically TCR motifs)[9] improves the selectivity of hyeA3A-BE4max for cytosines within TCR motifs, thus minimizing bystander mutations while maintaining hyperactive editing efficiency at target cytosines. PAM, protospacer adjacent motif.

Most applications of BE technology have focused on modeling or correcting specific disease-associated missense mutations. The required level of precision to achieve this goal results in two counteracting limitations: flexibility in the editing range and the necessary restriction of editing to only target nucleotides. Previous work has identified specific deaminase modifications that narrow the window of BE activity, thus providing more editing precision[8,9]. However, many cases exist in which no functional protospacer adjacent motif is present to position the target C within the standard activity window. Such cases require BEs with an expanded targeting range, as described by Zhang et al.[5] and others[10–12]. Yet expanding the window can also come at a cost, inducing additional bystander or

collateral editing of non-target nucleotides. Zhang et al.[5] cleverly addressed this issue by pairing the hyperactive BE with an engineered mutant (eA3A-BE3) previously shown to limit bystander activity through preferential conversion of TC motifs, with the highest activity at TCR(A/G) sites (together, hyeA3A-BE4max; Fig. 1c)[9]. As expected, hyeA3A-BE4max showed preferential editing at TC motifs within the expanded editing window, thus effectively limiting bystander editing while maximizing activity at the desired cytosine. In a powerful demonstration of the utility of this approach, Zhang et al.[5] used hyeA3A-BE4max to induce a specific targeted mutation that has therapeutic potential for the treatment of β-hemoglobinopathies[13,14]. They showed that, unlike hyA3A-BE4max, hyeA3A-BE4max induced a precise C11 (C to T) edit within a cognate TCR motif without bystander editing at surrounding cytosines C3 or C16. The specific C11 in this context corresponds to position 117 in the ^{A}γ-globulin promoter, which activates γ-globulin expression, thus resulting in functional formation of fetal hemoglobin. Together with α-globin, fetal γ-globulin can substitute for adult hemoglobin and thereby alleviate the symptoms of β-hemoglobinopathies[15]. Importantly, the authors show that preventing associated collateral editing has important functional consequences. All cells with editing at C11 showed improved γ-globulin expression, but those containing additional editing at C3 and C16 had significantly lower induction. Together, these experiments provide a clear example of how sequential engineering of BE variants to optimize target editing within specific mutational contexts can have important functional and potentially clinical implications. With the continuing emergence of many different BE variants, the generation of specific hybrid enzymes will provide increased flexibility and precision for modeling and therapeutic BE.

The mechanism underlying how the insertion and specific positioning of the RAD51 ssDBD between APOBEC and Cas9n leads to hyperactivity and expanded reach has yet to be elucidated. Zhang et al.[5] suggest that increased binding affinity is the likely reason for the enhanced activity of the CRISPR machinery at the locus of interest. Specific interaction with the unwound ssDNA might also enable more efficient or prolonged access of APOBEC deaminase to the target nucleotide. In a similar study, Zafra et al. previously generated '2X', a base editor with expanded editing activity, which shows enhanced activity at positions C3–C11 of the protospacer[10]. Interestingly, the 2X enzyme has two nuclear-localization sequences located between APOBEC and Cas9n; these sequences do not increase nuclear trafficking but do contain runs of positively charged residues. Given the data described by Zhang et al.[5], the properties of nuclear-localization sequences themselves, through positive charges, might potentially facilitate interaction with the negatively charged DNA backbone, thus enhancing editing efficiency and expanding the reach of 2X, similarly to the ssDBD in hyBE4max. The findings from Zhang et al.[5] may enhance understanding of the protein–DNA–RNA mechanics during base editing and drive the rational design of editors that enhance specific features of precise BE. This work also raises the interesting possibility that augmenting ssDNA-binding affinity could affect other CRISPR-related technologies, almost all of which rely on binding genomic targets.

CRISPR-mediated DNA cleavage is remarkably efficient, whereas we and others have noted that BE activity is somewhat unpredictable. Indeed, Zhang et al.[5] report some examples of unexpectedly low levels of base editing, even with optimized enzymes. Remarkably, editing at these loci was improved up to 250-fold with the use of hyeA3A-BE4max. Although anecdotal at this point, these data suggest that whereas some sgRNAs and/or target loci may show extremely low levels of base editing in standard assays, using alternative enzymes may overcome this limited activity. It will be important to determine whether such dramatic improvements in editing are rare occurrences or can be easily identified, and whether they are specific to guide RNAs and/or loci. In other words, is editing improved at all difficult-to-target sites, or are there still other factors relevant to BE efficiency that are not yet understood? Unmasking these properties will enable more rational and reliable design of future base-editing strategies.

Together, the work of Zhang et al.[5] extends a growing body of BE tools and applications, demonstrating the ability to generate base-editing mutations with improved efficiency and range as well as precise missense mutations with therapeutic potential in models of disease. The authors highlight the utility of enhanced BE in cells and in vivo for the purpose of model generation and therapy. Beyond the applications shown here, these hyper-editors may also advance knowledge of BE mechanics and the factors that govern editing efficiency, and may lead to improved rational design of new editors for research and clinical translation. ❐

Alyna Katti[1,2] and Lukas E. Dow[1,2,3] ✉

[1]*Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA.*
[2]*Graduate School of Medical Sciences, Weill Cornell Medicine, New York, NY, USA.*
[3]*Department of Medicine, Weill Cornell Medicine, New York, NY, USA.*
✉*e-mail: lud2005@med.cornell.edu*

Published online: 11 May 2020
https://doi.org/10.1038/s41556-020-0521-0

References

1. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. *Nature* **533**, 420–424 (2016).
2. Gaudelli, N. M. et al. *Nature* **551**, 464–471 (2017).
3. Rees, H. A. & Liu, D. R. *Nat. Rev. Genet.* **19**, 770–788 (2018).
4. Schatoff, E. M., Paz Zafra, M. & Dow, L. E. *Methods* **164–165**, 100–108 (2019).
5. Zhang, X. et al. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-020-0518-8 (2020).
6. Koblan, L. W. et al. *Nat. Biotechnol.* **36**, 843–846 (2018).
7. Wang, X. et al. *Nat. Biotechnol.* **36**, 946–949 (2018).
8. Kim, Y. B. et al. *Nat. Biotechnol.* **35**, 371–376 (2017).
9. Gehrke, J. M. et al. *Nat. Biotechnol.* **36**, 977–982 (2018).
10. Zafra, M. P. et al. *Nat. Biotechnol.* **36**, 888–893 (2018).
11. Jiang, W. et al. *Cell Res.* **28**, 855–861 (2018).
12. Hess, G. T. et al. *Nat. Methods* **13**, 1036–1042 (2016).
13. Martyn, G. E. et al. *Nat. Genet.* **50**, 498–503 (2018).
14. Dedoussis, G. V., Sinopoulou, K., Gyparaki, M. & Loutradis, A. *Eur. J. Haematol.* **65**, 93–96 (2000).
15. Wienert, B., Martyn, G. E., Funnell, A. P. W., Quinlan, K. G. R. & Crossley, M. *Trends Genet.* **34**, 927–940 (2018).

Competing interests

L.E.D. is an inventor on a patent that describes base-editing enzymes with increased efficiency and editing range: patent application PCT/US2019/040358 (filed 2 July 2019), international publication number WO2020/033083 (publication date 13 February 2020).