The CRISPR Journal
Volume 2, Number 2, 2019

DOI: 10.1089/crispr.2019.0001

The CRISPR Journal

REVIEW ARTICLE

Development and Application of Base Editors

Bei Yang,[1] Li Yang,[2,3] and Jia Chen[3,4]

Abstract

Base editing is emerging as a potent new strategy to achieve precise gene editing. By combining different nucleobase deaminases with Cas9 or Cpf1 proteins, several base editors have recently been developed to achieve targeted base conversions in different genomic contexts. Importantly, base editors have been successfully applied in animals, plants, and bacteria to induce precise substitutions at the single-base level with high efficiency. In this review, we summarize recent progress in the development and application of base editors and discuss some of the future directions of the technology.

Introduction

Genome editing is a cohort of genetic engineering technologies to insert, delete, or modify the sequences of genome in a living organism.[1–3] Utilizing genome editing tools to genetically manipulate the genomic information of cells and living organisms has broad applications in life sciences research, development of biotechnology and agricultural technology, and pharmaceutical and clinical innovation and therapeutics.

Early genome editing tools mainly involved zinc finger nucleases and transcription activator-like effector nucleases.[1] Although these early gene editing tools enabled researchers to manipulate genomes programmably, their DNA targeting relies on protein–DNA interactions. For its high efficiency, convenience, and broad application in a vast array of living organisms, the CRISPR-Cas system has been a powerful genome editing tool since its conception.[4–6] Directed by a guide RNA (gRNA), a Cas nuclease can generate DNA double-strand breaks (DSBs) at targeted genomic sites. These DSBs are then repaired by the endogenous DNA repair system, which could be employed to perform desired genome editing. In general, two major DNA repair pathways can be activated by DSBs: nonhomologous end joining (NHEJ) and homology-directed repair (HDR).[7]

NHEJ can introduce random insertions or deletions (indels) in the genomic DNA regions surrounding these DSBs, thereby leading to open reading frame shifts and ultimately gene inactivation. In contrast, when HDR is triggered, the genomic DNA sequence at the target site can be replaced by the sequence of the exogenous donor DNA, resulting in precise editing and in principle the correction of genetic mutations. Although NHEJ-mediated gene knockouts are highly efficient, the efficiency of HDR-mediated precise editing in practice is generally low because the occurrence of homologous recombination requires more complicated machinery and is cell cycle-dependent. As a consequence, NHEJ is triggered much more frequently than HDR.[8]

Base editing is a recently developed gene-editing system that has been successfully applied in many species to induce targeted base substitutions in DNA and RNA with high precision and efficiency. By combining the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like)/AID (activation-induced cytidine deaminase) family of cytidine deaminases[9,10] with the CRISPR-Cas gene editing system,[5,6] Alexis Komor and David Liu first developed a series of cytosine base editors (BEs/CBEs) to induce targeted base editing. Linking different CRISPR-Cas proteins with different nucleobase deaminases further leads to a variety of CBEs or adenine base editors (ABEs), the latter developed first by Nicole Gaudelli in the Liu lab, which achieve conversions of

[1]*Shanghai Institute for Advanced Immunochemical Studies and* [3]*School of Life Science and Technology, ShanghaiTech University, Shanghai, China;* [2]*Chinese Academy of Sciences (CAS) Key Laboratory of Computational Biology, CAS-Max Planck Gesellschaft Partner Institute for Computational Biology, Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China;* [4]*CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China.*

Address correspondence to: *Bei Yang, Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, 393 Middle Huaxia Road, Shanghai 201210, China,* E-mail: yangbei@shanghaitech.edu.cn or *Li Yang, CAS Key Laboratory of Computational Biology, CAS-Max Planck Gesellschaft Partner Institute for Computational Biology, Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China,* E-mail: liyang@picb.ac.cn or *Jia Chen, School of Life Science and Technology, ShanghaiTech University, 393 Middle Huaxia Road, Shanghai 201210, China,* E-mail: chenjia@shanghaitech.edu.cn

cytosine to thymine (C to T)[11] or adenine to guanine (A to G)[12] in various cells, animals, plants, and bacteria.

Here, we discuss the early studies related to base editing, the development and the application of base editors, the comparison of base editing with Cas-mediated gene editing and the potential directions of base editing.

Unexpected Link Between APOBEC and CRISPR-Cas

Even before the development of BE, it was known that APOBECs induce C-to-T mutations in complementary DNA that is reverse transcribed from viral RNA genome to restrict viral activity.[13,14] Recent studies also indicate that APOBEC/AID can actually cause a broader array of mutations, which involves not only the genome of large DNA viruses[15] but also genomic DNA during replication,[16,17] repair,[18–21] carcinogenesis,[22–24] and antibody diversification.[25–27] As cytidine deaminases, APOBECs catalyze the deamination of cytosines in single-stranded (ss) nucleic acids including ssRNA and ssDNA.[9,28] Notably, most APOBECs prefer to deaminate the C in TpC dinucleotides except for APOBEC3G, which prefers the latter C of CpC.[10,29]

Indeed, a linkage between APOBECs and CRISPR-Cas9–mediated gene editing was appreciated in early studies that were attempting to reduce off-target (OT) indel formation. To reduce the unwanted DSBs and indels at OT sites, a double-nicking strategy was exploited by using a pair of gRNA and either Cas9 nickase (nCas9) or the catalytically dead Cas9 (dCas9) fused with a single *Fok*I nuclease domain.[30,31] Theoretically, the DNA single-strand break (SSB) at a particular OT site would be readily sealed by DNA ligases and thus not create indels. Nevertheless, unwanted indels were still induced by nCas9 monomer at some OT sites, albeit at lower frequency.[32,33] Surprisingly, nCas9 monomer was also found to induce unexpected C-to-T base substitutions at on-target sites.[34] By analyzing the sequence context, Tsai *et al.* found that most of the mutated cytosines were in TpC dinucleotides, manifesting a typical APOBEC mutational signature. This suggested that APOBECs might be involved in nCas9-triggered mutagenic process[34] (Fig. 1A). Around the same time, Chen *et al.* independently found that endogenous human APOBEC family members, including hAPOBEC3B (hA3B), hAPOBEC3C (hA3C) and hAPOBEC3F (hA3F), can induce C-to-T base substitutions during the repair of a preexisting DNA SSB in an episomal shutter vector[20] (Fig. 1B). These early studies implied that endogenous APOBECs were likely involved in the DNA repair process triggered by the nCas9-generated SSB and responsible for the base substitutions observed therein (Fig. 1). Notably, such notion was later proved by Lei *et al.* experimentally[35] (see below).

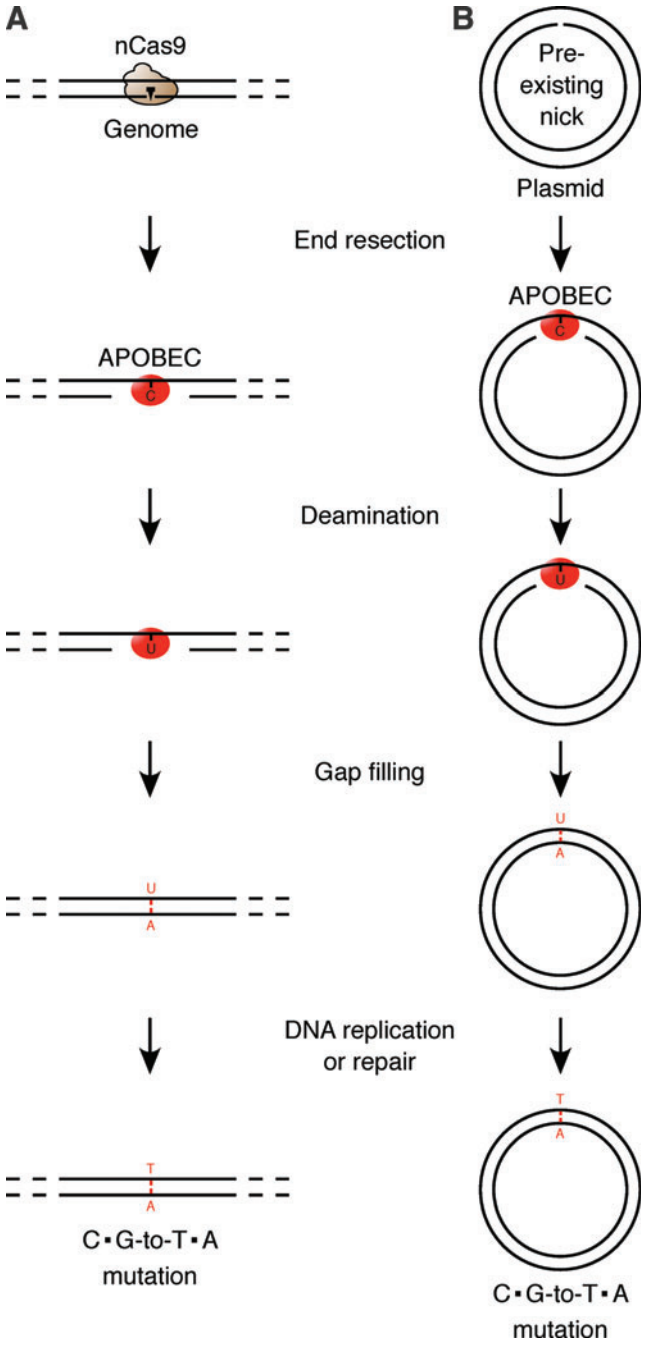

FIG. 1. Involvement of apolipoprotein B mRNA editing enzyme (APOBEC) in gene editing and DNA repair. **(A)** Schematic illustration of the involvement of APOBEC in Cas9 nickase (nCas9)–mediated gene editing. **(B)** Schematic illustration of the involvement of APOBEC in the repair of single-strand break in plasmid DNA.

Development of Cytosine Base Editing

Editing efficiency

In a landmark series of experiments that began by fusing rat APOBEC1 (rA1) and dCas9, Komor *et al.* developed the first generation of base editor (BE1).[11] Although BE1 can induce efficient C-to-T base editing *in vitro*, it yielded only low levels of base substitution in mammalian cells. As uracil DNA glycosylase (UDG) recognizes and removes uracils from genomic DNA,[36] Komor *et al.* fused a uracil DNA glycosylase inhibitor (UGI) into BE1 to inhibit endogenous base excision repair (BER) at the target site, producing a second-generation base editor (BE2), although the editing efficiency of BE2 was still relatively low at most loci.

To further improve the editing efficiency, Komor *et al.* replaced the dCas9 with nCas9 (D10A), which nicks the gRNA-complementary DNA strand (target strand, T-strand), to develop the third generation of base editor (BE3) (Fig. 2A). BE3 generates a U/G mismatch with a flanking nick in the T-strand, which serves as a preferred substrate for cellular mismatch repair (MMR).[37] Being

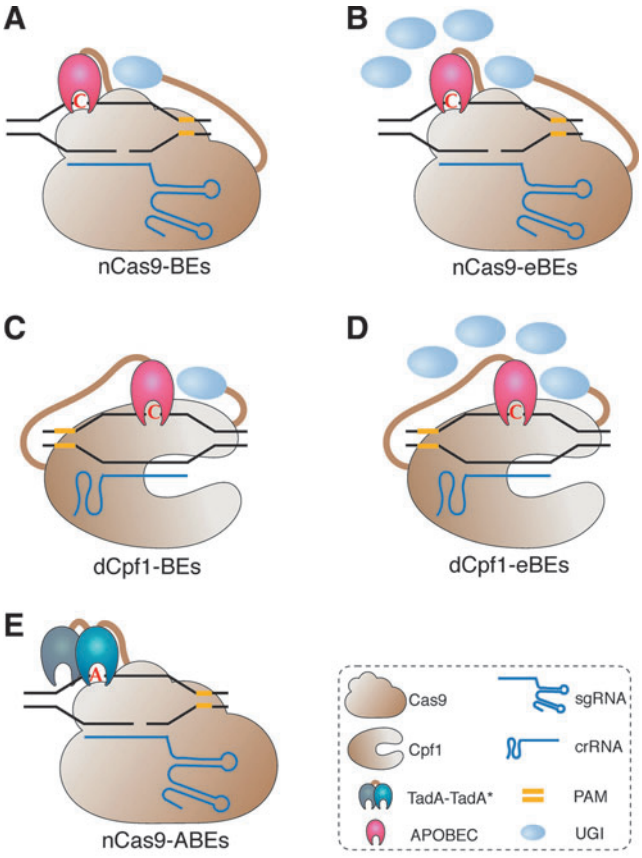

FIG. 2. Base editors (BEs) with Cas9 or Cpf1 proteins. Schematic illustration of nCas9-BEs **(A)**, nCas9-eBEs **(B)**, dCpf1-BEs **(C)**, dCpf1-eBEs **(D)**, and nCas9-ABEs **(E)**.

an endogenous DNA repair system, MMR recognizes mismatched bases during DNA replication and then excises the newly synthesized DNA strands according to the existence of nicks. Thus, MMR can also recognize the U/G mismatch and the flanking nick generated by BE3 in the T-strand, and excise the T-strand that contains the G of U/G mismatch. As a consequence, subsequent DNA resynthesis will use the remaining U-containing nontarget strand (NT-strand) as a template to install a U/A pair, which will be converted to a T/A pair after DNA replication or repair. Hence, by taking advantage of the endogenous MMR system, BE3 leads to C-to-T base substitution at higher frequency than BE2 in mammalian cells.[11]

As another cellular DNA repair system, BER starts by removing damaged bases with DNA glycosylases.[38] Although one copy of UGI is fused in BE3 to prevent the abasic site (AP site) formation catalyzed by UDG, unintended by-products derived from AP sites (e.g., C-to-A or C-to-G conversions) were still detected at some loci.[11] These results suggested that the U in the editing intermediate (U/G pair) can still be excised by UDG somehow, thereby leading to compromised C-to-T editing efficiency. To keep the U in place, Komor *et al.* fused another copy of UGI into BE3 to develop BE4, which manifested higher editing efficiency than BE3.[39]

In an alternative approach, Wang *et al.* fused 2A (self-cleaving peptide)-UGI sequences into BE3 to develop an enhanced base editor (eBE) (Fig. 2B), which can express free UGI to intensively inhibit BER and thus improve base editing efficiency.[40] Of note, while some studies showed that expressing free UGI could increase the mutation frequency of a shuttle vector plasmid in thymine DNA glycosylase–deficient cells and shift the mutational pattern of a hypermutating chicken cell line, DT40,[41,42] free UGI expression was reported not to generate either spontaneous or induced mutations in the mitochondrial DNA of human cells.[43] Considering the redundancy of mammalian DNA repair system for uracil repair, for example, MMR[37] or alternative uracil glycosylases that are insensitive to UGI (such as thymine DNA glycosylase[44]), whether expressing free UGI increases random C to T mutations in the genome of normal mammalian cells needs to be further investigated.

More recently, Koblan *et al.*[45] and Zafra *et al.*[46] sought to increase the editing efficiency of base editors by optimizing the codon of BEs to improve their expression in mammalian cells. These codon-optimized BEs (e.g., BE4max) appreciably increased base editing frequency (~1.7- to 9-fold) especially when transfection efficiency is limited. Furthermore, Koblan *et al.* used ancestral sequence reconstruction to develop AncBE4max, which showed even higher editing efficiency than BE4max at some loci.[45]

DNA modification is another factor that might restrain base editing efficiency. DNA methylation at CpG sites greatly suppresses the cytosine deamination catalyzed by mouse APOBEC1 (mA1).[47] By modulating local DNA methylation, Wang *et al.* showed that CpG methylation indeed has a generally negative effect on the C-to-T editing efficiency mediated by rA1-based BE3.[48] To develop a BE that can efficiently induce base editing in highly-methylated regions, Wang *et al.* screened a dozen of BEs, each containing a distinct APOBEC/AID cytidine deaminase family member. Among those tested, the human hA3A-derived BE (hA3A-BE3) induced the highest editing efficiency,[48] which is consistent with reports that hA3A can catalyze the deamination of methylated cytosine efficiently.[49,50] Through fusing three copies of 2A-UGI sequences to hA3A-BE, Wang et al, also developed hA3A-eBE to further enhance base editing efficiency.[48]

Thus, via manipulating cellular DNA repair systems, codon optimization, ancestral-sequence reconstruction and screening different APOBECs family members, the efficiency of CBEs has been significantly improved in various contexts in the relatively short time since their initial description.

Targeting scope

In base editing, Cas9 or Cpf1 (also known as Cas12a) proteins generally work as locators to guide cytidine deaminases to the target sites. One important feature of Cas9 or Cpf1 is that they require a protospacer adjacent motif (PAM) sequence for target-site recognition.[51] For example, *Streptococcus pyogenes* Cas9 (SpCas9) specifically recognize a G-rich PAM.[52] As such, base editing mediated by SpCas9-derived BEs was generally limited to G/C-rich regions. In contrast, Cpf1 recognizes a T-rich PAM sequence,[53] requires only a short gRNA (crRNA), and generally has a higher targeting specificity than Cas9.[54,55] These characteristics and particularly the T-rich PAM preference pointed to Cpf1 as a promising gene editing tool complementary to Cas9. However, Cpf1 was not used initially in base editors, largely because it uses only a single nuclease domain to cleave the NT-strand and T-strand sequentially.[56–58] Thus it is difficult to engineer Cpf1 into a nickase that solely cleaves T-strand as nCas9 in BE3 does. Having tested a few published Cpf1 proteins, Li *et al.* found that the catalytically dead *L. bacterium* Cpf1 can be used instead to perform base editing[59] (Fig. 2C). Though the original version of dCpf1-based BE induced relatively low levels of base editing, the efficiency was significantly improved by adding more nuclear localization sequences (NLS) to the N-terminus and even in the middle of dCpf1-BE.[59] Notably, the additional NLS strategy is also effective in improving the efficiencies of nCas9-based BEs.[45,46,60]

In parallel, Kim *et al.* replaced the SpCas9 in BE3 with engineered SpCas9 proteins that recognize altered PAM sequences, *Staphylococcus aureus* Cas9 (SaCas9) or an engineered SaCas9 with altered PAM specificity to develop VQR-BE3, EQR-BE3, VRER-BE3, SaBE3, and SaKKH-BE3.[61] These new BEs bypass the requirement of an NGG PAM sequence. Recently, Hu *et al.* took advantage of phage-assisted continuous evolution (PACE)[62] to evolve a SpCas9 variant (xCas9) that can recognize a much broader range of PAM sequences than wild-type SpCas9.[63] Alternatively, Nishimasu *et al.* used rational design to engineer SpCas9 and also obtained a variant (SpCas9-NG) that recognizes an NG instead of an NGG PAM sequence.[64] xCas9 and SpCas9-NG were then respectively fused with rA1 and AID to induce base editing at expanded target sites.[63,64] Hence, by making use of Cas9 and Cpf1 proteins that exhibit different PAM preferences, the editing repertoire of BEs have been greatly expanded.

Editing precision

Cas9 and Cpf1 proteins are guided by gRNAs to the appropriate target sites. However, the binding or editing at OT sites, where the sequences are similar to that of the on-target (ON) site, is always one of the major concerns in CRISPR-related research.[65,66] The OT effects of Cas9 or Cpf1 have been determined in many studies.[55,67,68] Similar to Cas9 and Cpf1 nucleases, BEs are also reported to cause OT effects, which raise concerns about the precision of BEs.[69–72] Interestingly, in a genome-wide study about the OT effects of BE, Kim *et al.* reported that Cas9 and the Cas9-driven BE did not edit the same OT sites,[73] suggesting that the Cas9-mediated DNA double-strand cleavage and the BE-mediated cytidine deamination may exhibit a different tolerance of the mismatches between gRNA and target genomic DNA. By fusing rA1 with high fidelity (HF)-Cas9, which has an improved targeting specificity, Rees *et al.* developed HF-BE3 to reduce the unwanted C-to-T mutations at OT sites.[70] In the same study, the delivery of ribonucleoprotein (RNP) complex instead of plasmid DNA further decreased the unwanted mutations at OT sites while maintaining the editing efficiency at ON site.

In addition to editing at OT sites, indels and non-C-to-T conversions (i.e., C to A or C to G) at ON sites are also unexpected byproducts of base editing. Replacing dCas9 with Cas9 nickase D10A significantly enhanced the editing efficiency, yet also increased the formation of unintended indels and non-C-to-T conversions.[11,74] During BE3-induced editing, MMR was triggered to resect the nicked T-strand, thereby leaving the 'U'-containing NT-strand as ssDNA (Fig. 3). The cleavage of T-strand will

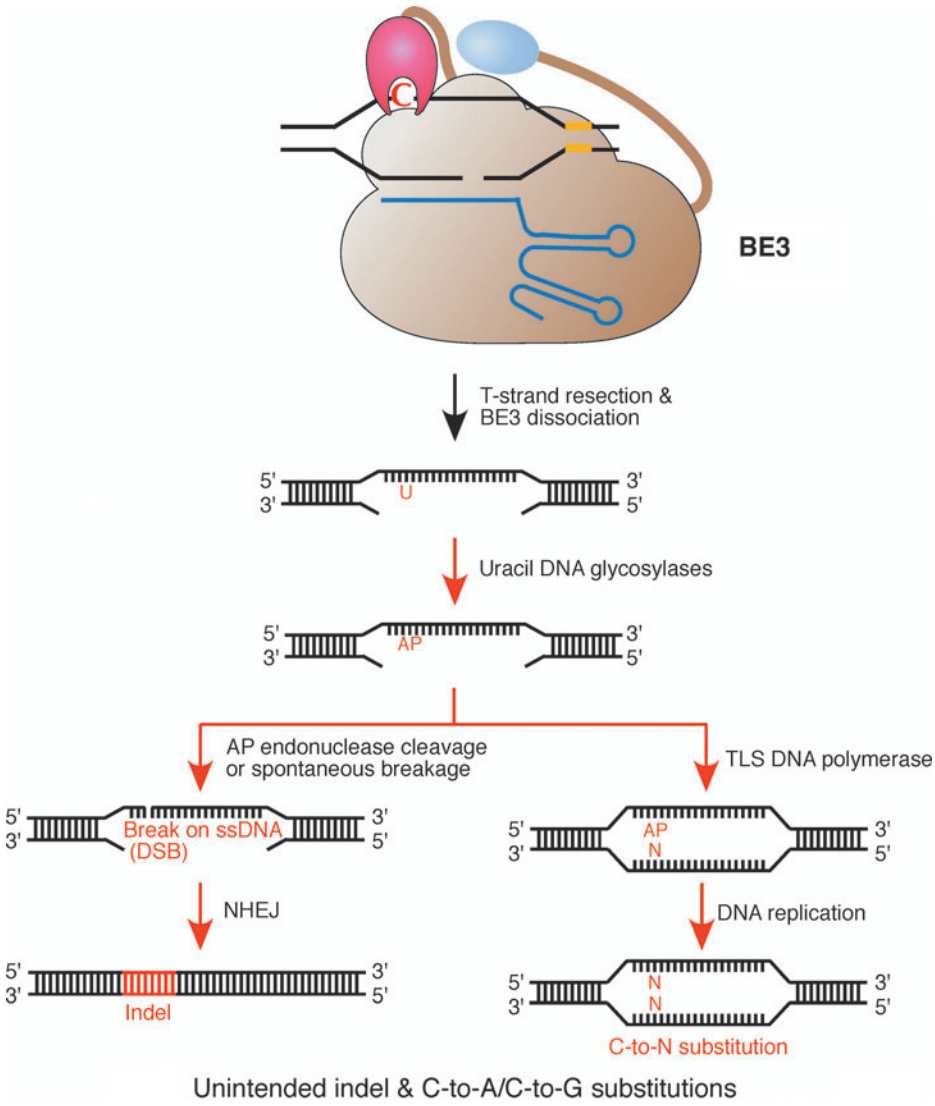

FIG. 3. Unintended products of nCas9-containing cytosine base editors. The pathways by which the third generation of base editor generates indels (left) and non-C-to-T substitutions (right) are shown.

lead to the disassociation of BE3 and thus the BE3-fused UGI from the target site, which in turn renders the 'U' in the NT-strand accessible by UDG. Then endogenous UDG will be able to excise the 'U' and generate an AP site in the single-stranded NT-strand. The cleavage by AP endonuclease or a spontaneous breakage at AP site of the NT-strand will produce a DSB and induce indels around ON site[35] (Fig. 3). Alternatively, copying AP site by translesion DNA synthesis polymerase[75,76] during the resynthesis of T-strand will result in non-C-to-T conversions (Fig. 3). Through fusing 2A-UGI sequences to the C-terminus of BE3, Wang *et al.* developed eBE, which expresses free UGI in addition to the BE3-fused one, to induce higher editing efficiency. Moreover, eBE also reduced the formation of unwanted indels and non-C-to-T conversions, owing to its better preservation of the 'U' in NT-strand. Because of a similar mechanism, dCpf1-eBE (Fig. 2D) also induces purer editing products than does dCpf1-BE.[59]

Editing window

The editing window is the range of nucleotides in the gRNA target region, wherein all the cytosines have the chance in theory to be converted to thymines. If an editing window is too big, multiple cytosines in the same editing window would be edited simultaneously, resulting in the generation of undesired ''bystander'' byproducts.[77] Hence, the editing window is an important parameter to consider when the goal is to edit a particular cytosine by BE.

In order to narrow the 5-bp editing window of BE3, Kim *et al.* engineered rA1 by introducing amino acid changes in the deaminase domain to develop YE1-BE3, YE2-BE3, EE-BE3, and YEE-BE3, all of which showed constricted editing windows compared to BE3.[61] Using the same strategy, Li *et al.* developed dCpf1-BE-YE and dCpf1-BE-YEE, which manifested narrower editing windows as well (albeit compromising editing efficiency).[59] After analyzing the structural information of hA3A, Wang *et al.* substituted amino acid changes into the substrate recognition domain of hA3A to develop hA3A-BE3-Y130F and hA3A-BE3-Y132D, both of which showed narrowed editing windows and retained high editing efficiency.[48] Recently, Tan *et al.* truncated the C-terminus of sea lamprey cytidine deaminase (pmCDA1) and changed the length of rigid linkers between pmCDA1 and nCas9, which leads to new BEs with narrowed editing windows.[78] Alternatively, Gehrke *et al.* screened several hA3A mutants and developed A3A-BE3-N57G (engineered A3A-BE3, eA3A-BE3) to stringently edit the C within a context of TpC dinucleotides.[79]

Although considerable effort has been made to narrow the editing window, a larger window is sometimes desirable, especial for applications such as making premature stop codons to knock out genes (iSTOP or CRISPR-STOP)[80,81] or identifying the effects of new point mutations.[82] Ma *et al.* fused AIDx (an engineered AID with enhanced activity) with dCas9 to develop targeted AID-mediated mutagenesis,[83] which leads to an editing window of around 25 bp and has been effectively applied to modulate RNA splicing.[84] Meanwhile, Hess *et al.* used the MS2 loop-containing gRNAs to recruit multiple copies of AID*Δ (a hyperactive AID mutant) fused with MS2-loop binding protein, and the resulting CRISPR-X expands the editing window to around 100 bp.[85] Moreover, Jiang *et al.* fused SunTag with dCas9 to recruit multiple copies of APOBEC-UGI fusion proteins to develop BE-PLUS,[86] which has an editing window around 13 bp. Recently, Liu *et al.* also took advantage of MS2 loop–containing gRNA to recruit stable monomers of AID or hA3A, and developed diversifying base editors to achieve antibody affinity maturation *ex vivo*.[87]

Taken together, the multifaceted improvement of BEs (Table 1) greatly enriches the genome editing toolbox.

Cytosine Base Editing Applications

Animals

Base editing has been successfully applied in various animals (Table 2). Kim *et al.* started by microinjecting or electroporating gRNAs and the mRNA of BE3 into mouse embryos to make nonsense mutations in two genes, *Dmd* (dystrophin) and *Tyr* (tyrosinase),[88] mutations that result in Duchenne muscular dystrophy and albinism, respectively. The corresponding phenotypes (e.g., loss of dystrophin expression and ocular albinism) were observed in the edited mice as expected. Liang *et al.* fused rA1 with dCas9-HF2 to develop HF2-BE2 and then successfully applied HF2-BE2 in mouse embryos to induce missense mutations in *Tyr*.[89] Similarly, Yang *et al.* used the iSTOP strategy to introduce stop codons into V-set immunoregulatory receptor (*VISTA*) and CD160 antigen (*CD160*) in mouse embryos, and obtained homozygous mutants in F1 mice.[90] Yang *et al.* also utilized BE3 to make point mutations (R17H in *Hist1H3* and *Hist2H3*) or premature stop codons (*Carm1*) in genes involved in chromatin modification to study the epigenetic regulation in mouse embryo development.[91]

Meanwhile, Sasaguri *et al.* compared the efficiencies of BE3 and Target-AID at *Psen1* (presenilin 1) in mice and found that BE3 induced higher editing frequency than Target-AID.[92] In addition, they also found that VQR-BE3 can generated efficient base editing at the target sites with altered PAM sequences.[92] Recently, Li *et al.* compared the editing of BE3 and hA3A-BE3-Y130F at eight genomic loci, i.e., *Tyr*, *Hoxd13* (homeobox D13), *Ar* (androgen receptor), *Gfap* (glial fibrillary acidic protein), *Dmd*, *Lmna* (Lamin A/C), *Mecp2* (methyl CpG binding protein 2), *Tnni3* (troponin I3) and *Abcd1* (ATP Binding Cassette Subfamily D Member 1) in mice. They found that hA3A-BE3-Y130F induced higher editing efficiency in G/C-rich regions,[93] consistent with the finding in mammalian cells.[48] The mice with *Ar* mutation displayed the AIS (androgen insensitivity syndrome)-like sex reversal phenotype. Of note, Li *et al.* also did whole-genome sequencing for the *Ar*-mutant mice to determine OT effects but no significant base substitution was found at the potentials OT sites, which share similar sequence with ON site.[93]

Instead of editing zygotes, Chadwick *et al.* packaged BE3 into an adenoviral vector and delivered it into the livers of adult mice to generate premature stop codons in *Pcsk9* (proprotein convertase subtilisin/kexin type 9) and found that both plasma PCSK9 and cholesterol levels were significantly reduced in the edited mice.[94] Moreover, Chadwick *et al.* used the same strategy to mutate *ANGPTL3*, another gene involved in lipid metabolism, and reduced blood lipid levels in the mutant mice.[95] In utero gene editing is another strategy to potentially treat genetic diseases that manifest significant morbidity or mortality. By using BE3 in utero, Rossidis *et al.* mounted an early stop codon in *Pcsk9* in wild-type mice and a nonsense mutation in *Hpd* (4-hydroxyphenylpyruvate dioxygenase) in a mouse model of hereditary tyrosinemia type 1.[96] Successful base editing of these genes reduced the cholesterol level

Table 1. Base Editors with Various Characteristics

Base editor	Deaminase	Cas	PAM	NLS	Fused UGI	Free UGI	Codon optimization	Editing window (nt)	Reference
BE1	rA1	dCas9	NGG	1×	–	–	–	4–8	11
BE2	rA1	dCas9	NGG	1×	1×	–	–	4–8	11
BE3	rA1	nCas9	NGG	1×	1×	–	–	4–8	11
HF-BE3	rA1	HF-nCas9	NGG	1×	1×	–	–	4–8	70
HF2-BE2	rA1	dCas9-HF2	NGG	1×	1×	–	–	4–8	89
BE4 & BE4-Gam	rA1	nCas9	NGG	1×	2×	–	–	4–8	39
eBE-S1	rA1	nCas9	NGG	1×	1×	1×	–	4–8	40
eBE-S3	rA1	nCas9	NGG	1×	1×	3×	–	4–8	40
BE4max	rA1	nCas9	NGG	2×	2×	–	+	4–8	45
AncBE4max	rA1	nCas9	NGG	2×	2×	–	+	4–8	45
YE1-BE3	rA1-YE1	nCas9	NGG	1×	1×	–	–	4–7	61
YE2-BE3	rA1-YE2	nCas9	NGG	1×	1×	–	–	5–6	61
EE-BE3	rA1-EE	nCas9	NGG	1×	1×	–	–	5–6	61
YEE-BE3	rA1-YEE	nCas9	NGG	1×	1×	–	–	5–6	61
VQR-BE3	rA1	nVQRCas9	NGA	1×	1×	–	–	4–11	61
VRER-BE3	rA1	nVRERCas9	NGCG	1×	1×	–	–	3–10	61
SaBE3	rA1	nSaCas9	NNGRRT	1×	1×	–	–	3–12	61
SaBE4 & SaBE4-Gam	rA1	nSaCas9	NNGRRT	1×	2×	–	–	3–12	39
SaKKH-BE3	rA1	nSaKKHCas9	NNNRRT	1×	1×	–	–	3–12	61
dCpf1-BE	rA1	dCpf1	TTTV	3×	1×	–	–	8–13	59
dCpf1-BE-YE	rA1-YE1	dCpf1	TTTV	3×	1×	–	–	10–12	59
dCpf1-eBE	rA1	dCpf1	TTTV	3×	1×	3×	–	8–13	59
dCpf1-eBE-YE	rA1-YE1	dCpf1	TTTV	3×	1×	3×	–	10–12	59
xBE3	rA1	nxCas9	NG, GAA, GAT	1×	1×	–	–	4–8	63
BE-PLUS	rA1-scFv	nCas9	NGG	1×	1×	–	–	4–14	86
hA3A-BE3	hA3A	nCas9	NGG	1×	1×	–	–	2–13	48
hA3A-BE3-Y130F	hA3A-Y130F	nCas9	NGG	1×	1×	–	–	3–8	48
hA3A-BE3-Y132D	hA3A-Y132D	nCas9	NGG	1×	1×	–	–	3–7	48
hA3A-eBE-Y130F	hA3A-Y130F	nCas9	NGG	1×	1×	3×	–	3–8	48
hA3A-eBE-Y132D	hA3A-Y132D	nCas9	NGG	1×	1×	3×	–	3–7	48
eA3A-BE3	hA3A-N57G	nCas9	NGG	1×	1×	–	–	4–8 (TpC)	79
eA3A-HF1-BE3-2xUGI	hA3A-N57G	nCas9-HF1	NGG	1×	2×	–	–	4–8 (TpC)	79
eA3A-Hypa-BE3-2xUGI	hA3A-N57G	nHypaCas9	NGG	1×	2×	–	–	4–8 (TpC)	79
DBE-A3A	hA3A-MS2	nCas9	NGG	1×	1×	–	–	2–17	87
Target-AID	pmCDA1	nCas9	NGG	1×	1×	–	–	2–8	74
Target-AID-NG	pmCDA1	nCas9-NG	NG	1×	1×	–	–	2–8	64
TAM	hAIDx	nCas9	NGG	1×	–	1×	–	4–8	83
CRISPR-X	hAIDΔ-MS2	nCas9	NGG	1×	–	–	–	∼ -50–50	85
DBE-AIDmono	hAID-mono-MS2	nCas9	NGG	1×	–	–	–	2–17	87
ABE7.9	TadA-TadA*	nCas9	NGG	1×	–	–	–	4–9	12
ABE7.10	TadA-TadA*	nCas9	NGG	1×	–	–	–	4–7	12
ABEmax	TadA-TadA*	nCas9	NGG	2×	–	–	+	4–7	45
xABE	TadA-TadA*	nxCas9	NG, GAA, GAT	1×	–	–	–	4–7	63
VQR-ABE	TadA-TadA*	nVQRCas9	NGA	1×	–	–	–	4–8	113, 106
VRER-ABE	TadA-TadA*	nVRERCas9	NGCG	1×	–	–	–	4–8	106
ABEsa	TadA-TadA*	nSaCas9	NNGRRT	1×	–	–	+	8–14	114
SaKKH-ABE	TadA-TadA*	nSaKKHCas9	NNNRRT	1×	–	–	–	8–13	113, 106

and rescued the lethal phenotype of hereditary tyrosinemia type 1 in mice. Another BE, SaKKH-BE3, has also been used to treat a metabolic liver disease in adult mice. Importantly, Villiger *et al.* leveraged a split-intein moiety to split SaKKH-BE3 into two parts, both of which can be packaged into a single adeno-associated virus (AAV) vector, and the split-BE was applied to correct the *Pah* mutation in adult Pah^{enu2} mice.[60] As expected, the *Pah* mRNA expression was restored and the blood L-phenylalanine was reduced to physiological levels.

In rabbits, Liu *et al.* used BE3 to induce mutations in *Mstn* (Myostatin), *Dmd*, *Tia1* (cytotoxic granule-associated RNA binding protein 1), *Tyr*, and *Lmna*.[97] The rabbits with *Mstn*, *Tyr*. or *Lmna* mutations showed corresponding phenotypes of double-muscle, albino, or Hutchinson–Gilford progeria syndrome, respectively. Liu *et al.* also compared the editing efficacy induced by BE3 and BE4-Gam at the loci of *Dmd* and *Tia1* and found that BE4-Gam induced higher editing frequency and product purity.[97] In pigs, Li *et al.* used BE3 to mount a pathogenic E75K mutation in twist-related protein 2 (*Twist2*) in porcine fetal fibroblast and then performed somatic cell nuclear transfer to generate edited embryos.[98] A premature stop codon in *Tyr* was also induced in the similar way. The mutations in *Twist2*

Table 2. Application of Base Editing in Animals, Plants, and Bacteria

Species	*Base editor*	*Target site*	*Reference*
Mouse	BE3	*Dmd*, *Tyr*, *Vista*, *Cd160*, *CTNNB1*, *Hist1H3*, *Hist2H3*, *Carm1*, *Pcsk9*, *Hpd*, *Psen1*, *Hoxd13*, *Ar*, *Gfap*, *Lmna*, *Mecp2*, *Tnni3*, *Abcd1*, *ANGPTL3*	88, 90, 91, 92, 136, 94, 96
Mouse	SaBE3	*Tyr*	116
Mouse	SaKKH-BE3	*Pah*	60
Mouse	HF2-BE2	*Tyr*	89
Mouse	Target-AID	*Psen1*	92
Mouse	VQR-BE3	*Psen1*	92
Mouse	hA3A-BE3-Y130F	*Tyr*, *Hoxd13*, *Ar*, *Gfap*, *Dmd*, *Lmna*, *Mecp2*, *Tnni3*, *Abcd1*	93
Rabbit	BE3	*Mstn*, *Dmd*, *Tia1*, *Tyr*, *Lmna*	97
Rabbit	BE4-Gam	*Dmd*, *Tia1*	97
Pig	BE3	*Twist2*, *Tyr*	98
Zebrafish	BE3	*Twist2*, *Gdf6*, *Ntl*, *Tyr*	99
Zebrafish	VQR-BE3	*Twist2*, *Tial1*, *Urod*	99
Rice	PBE (BE3)	*OsCDC48*, *OsNRT1.1B*, *OsSPL14*	100
Rice	A3A-PBE (hA3A-BE3)	*OsAAT*, *OsCDC48*, *OsDEP1*, *OsNRT1.1B*, *OsOD*,*OsEV*, *OsHPPD*	101
Rice	Target-AID	*ALS*, *FTIP1e*	104
Rice	BE3	*OsPDS*, *OsSBEIIb*	102
Rice	BE3-ΔUGI	*NRT1.1B*, *SLR1*	103
Rice	CBE-P1 (BE3)	*SNB*	106
Rice	CBE-P3 (VQR-BE3)	*PMS3*	106
Rice	rBE9 (Target-AID)	*OsAOS1*, *OsJAR1*, *OsJAR2*, *OsCOI2*	105
Potato	A3A-PBE (hA3A-BE3)	*StALS*, *StGBSS*	101
Wheat	PBE (BE3)	*TaLOX2*	100
Wheat	A3A-PBE (hA3A-BE3)	*TaALS*, *TaMTL*, *TaLOX2*, *TaDEP1*, *TaHPPD*, *TaVRN1-A1*	101
Maize	PBE (BE3)	*ZmCENH3*	100
Tomato	Target-AID	*DELLA*, *ETR1*	104
Bacteria (*Escherichia coli*)	Target-AID	*galK*, *rpoB*, *xylB*, *manA*, *pta*, *adhE*, *tpiA*	109
Bacteria (*Escherichia coli*)	BE3	*tetA*, *rppH*	110
Bacteria (*Brucella melitensis*)	BE3	*virB10*	110
Bacteria (*Staphylococcus aureus*)	BEC (BE3-ΔUGI)	*agrA*, *cntA*, *esaD*	111
Bacteria (*Pseudomonas* species)	BEC (BE3-ΔUGI)	*rhlR*, *rhlB*, *cadR*, *ompR*, *per*, *aspC*, *gacA*, *hrpL*	112
Mouse	ABE7.10	*Tyr*, *Dmd*, *AR*, *Hoxd13*, *Hbb-bs*, *Fah*,	115–117
Mouse	VQR-ABE	*Hbb-bs*	113
Mouse	SaKKH-ABE	*Otc*	113
Rat	ABE7.10	*Gaa*	113
Rabbit	ABE7.10	*Dmd, Otc, Sod1,*	97
Zebrafish	zABE7.10	*ddx17-g1*, *musk*, *rps14*, *atp5b*, *wu:fc01d11*	118
Zebrafish	zABE7.10max	*musk*, *rps14*, *atp5b*, *wu:fc01d11*	118
Zebrafish	zABE7.10-GE	*musk*, *rps14*, *atp5b*, *wu:fc01d11*	118
Rice	ABE-P1 (ABE7.10)	*OsSPL14*, *SLR1*, *OsSPL16*, *OsSPL18*, *LOC_Os02g24720,*	114
Rice	ABE-P2 (ABEsa)	*OsSPL14*, *OsSPL17*	114
Rice	ABE-P3 (VQR-ABE)	*OsSPL14*, *OsSPL16*, *OsSPL17*, *OsSPL18*	106
Rice	ABE-P4 (VRER-ABE)	*OsTOE1*, *OsIDS1*	106
Rice	ABE-P5 (SaKKH-ABE)	*SNB*	106
Rice	rBE14 (ABE7.10)	*OsMPK6*, *OsMPK13*, *OsSERK2*, *OsWRKY45*	119
Rice	PABE (ABE7.10)	*OsACC*, *OsALS*, *OsCDC48*, *OsAAT*, *OsEV*, *OsOD*, *OsDEP1*, *OsNRT1.1B*	120
Wheat	PABE (ABE7.10)	*TaDEP1*, *TaEPSPS*, *TaGW2*	120
Arabidopsis	pcABE7.10	*AtALS*, *AtPDS*, *AtFT*, *AtLFY*	121
Rapeseed	pcABE7.10	*BnALS*, *BnPDS*	121

and *Tyr* led to phenotypes of ablepharon macrostomia syndrome and albinism, respectively. These studies thus served as principles of proof that BEs can also induce efficient base editing in big animals such as pigs and rabbits.

BEs have also been successfully applied in nonmammals. Zhang *et al.* injected gRNA and the codon-optimized mRNA of BE3 into the zebrafish embryos at one-cell stage to induce mutations in *Twist2*, *Gdf6* (growth differentiation factor 6), *Ntl* (no tail), and *Tyr*.[99] The amino-acid change resulted from the *Tyr* mutation led to a phenotype that is similar to human ocular albinism. They also used VQR-BE3 to induce mutations in *Twist2*, *Tial1* (cytotoxic granule associated RNA binding protein like 1), and *Urod* (uroporphyrinogen decarboxylase) and demonstrated that VQR-BE3 indeed expanded the editing scope *in vivo*.

Plants

As gene editing can help to improve the traits of crops, base editors have also been applied in various plants

(Table 2). Zong *et al.* transfected the plasmids expressing codon-optimized BE3 (plant base editor, PBE) and individual gRNAs into protoplasts to mutate three rice genes (*OsCDC48*, *OsNRT1.1B*, and *OsSPL14*), a wheat gene (*TaLOX2*) and a maize gene (*ZmCENH3*). The editing of these genes succeeded, albeit with relatively low frequencies.[100] The *OsCDC48*-edited rice, the *TaLOX2*-edited wheat and the *ZmCENH3*-edited maize were obtained by *Agrobacterium*-mediated transformation or plasmid delivery into immature plant embryos. More recently, Zong *et al.* also optimized hA3A-BE3 to develop the corresponding plant version (A3A-PBE).[101] A3A-PBE induced very efficient base editing in wheat (*TaALS*, *TaMTL*, *TaLOX2*, *TaDEP1*, *TaHPPD* and *TaVRN1-A1*), rice (*OsAAT*, *OsCDC48*, *OsDEP1*, *OsNRT1.1B*, *OsOD*, *OsEV*, and *OsHPPD*) and potato (*StALS* and *StGBSS*) genes. In particular, A3A-PBE induced efficient editing at GpC sites across seven genomic regions in wheat and rice, whereas PBE induced virtually no editing events. The editing window of A3A-PBE spans from the first base to the seventeenth in protospacer region, which is also bigger than that of PBE. These results are consistent with the previous study in mammalian cells.[48] The big editing window of A3A-PBE (17-nt width) is suitable for mutagenesis-oriented editing such as iSTOP but may lead to more bystander mutations when precise editing is needed. Thus, it is interesting to know whether the hA3A-derived BEs that have narrowed editing windows (e.g., hA3A-BE3-Y130F and hA3A-BE3-Y132D[48]) can also induce precise editing while maintaining efficiency in plants.

Independently, Li *et al.* and Lu *et al.* used BE3 and BE3-ΔUGI (rA1-nCas9 fusion) to induce base editing in rice genes (*OsPDS*, *OsSBEIIb*, *NRT1.1B*, and *SLR1*).[102,103] By using AID as the deaminating module, Shimatani *et al.* and Ren *et al.* respectively developed the plant version of Target-AID (PmCDA-nCas9 fusion)[104] and rBE9 (a BE containing nCas9, UGI and AID*Δ, a hyperactive AID mutant),[105] which can induce base editing in rice genes (*ALS* and *FTIP1e*, *OsAOS1*, *OsJAR1*, *OsJAR2*, and *OsCOI2*). Hua *et al.* also expanded the base editing scope in plants by replacing the Cas9 nickase of BE3 (CBE-P1 as the plant version) with VQR-Cas9 nickase and the latter BE (CBE-P3) induced base editing at the expanded targets site (*PMS3*).[106]

Bacteria

Being a part of a prokaryotic immune system, CRISPR-Cas was first found to cleave targeted DNA in bacteria.[107,108] Whether BEs can also be applied in bacteria has also been tested (Table 2). Banno *et al.* adopted the Target-AID system in bacteria to generate point mutations in various genes (*galK*, *rpoB*, *xylB*, *manA*, *pta*, *adhE*, and *tpiA*) in *E. coli*.[109] Notably, Target-AID simultaneously induced 41 targeted mutations with four gRNAs against multicopy transposable elements.[109] By using BE3, Zheng *et al.* also induced stop codons and missense mutations in *rppH*.[110] In addition to *E. coli*, three reports generated early stop codons in bacterial strains *Brucella melitensis* (*virB10*), *S. aureus* (*agrA*, *cntA*, and *esaD*) and *Pseudomonas* (*rhlR*, *rhlB*, *cadR*, *ompR*, *per*, *aspC*, *gacA*, and *hrpL*) by using BE3 and BE3-ΔUGI, and showed that base editing system can be widely applied in various prokaryotic species.[110–112]

Development and Application of Adenine Base Editors

Development and improvement of adenine base editors

In addition to CBEs, ABEs have been developed recently to induce A-to-G base editing. After seven rounds of directed evolution *in vitro*, Gaudelli, Komor, and colleagues evolved a tRNA adenosine deaminase (TadA) of *E. coli* into a deoxyadenosine deaminase (TadA*) that can induce adenine to inosine (A-to-I) deamination on ssDNA.[12] In order to improve the binding of TadA* to ssDNA substrate, a wild-type TadA monomer was fused at the N-terminus of TadA*. Then the TadA-TadA* heterodimer was further linked with nCas9 (D10A) to develop a series of ABEs (Table 1). Compared to CBEs, ABEs do not need a DNA glycosylase inhibitor, probably because inosines in DNA cannot be removed efficiently by any known mammalian DNA glycosylase. The subsequent MMR or DNA replication will use the inosine-containing strand as the template and then insert a cytosine opposite to the inosine, which eventually installs an A/T-pair to G/C-pair conversion at the target site.[12]

Several versions of ABE have been developed since the milestone report by Gaudelli *et al.* (Table 1). By replacing the nCas9 with nxCas9 in ABE7.10, Hu *et al.* developed xABE,[63] which expands the targeting scope of ABEs to the genomic regions containing NG, GAA or GAT PAM sequences. Other Cas9 variants with altered PAM specificity (i.e., VQR and VRER) have also been used to develop new ABEs, which recognize the PAM sequences NGA and NGCG respectively.[106,113] In addition to the SpCas9-containing ABEs, the SaCas9-containing ABE and its engineered form SaKKHCas9 have also been constructed to induce editing in the regions containing NNGRRT and NNNRRT PAM sequences.[106,113,114] Recently, Koblan *et al.* also applied the codon-optimization and additional NLS strategy on ABE7.10, and the resulting ABEmax has an even higher editing efficiency compared to ABE7.10.[45]

Application of adenine base editors

Several versions of ABEs have been applied in animals successfully (Table 2). Ryu *et al*. first used ABE7.10 to induce a missense mutation in *Tyr* gene in mice,[115] which modeled the phenotype of the Himalayan mouse. Importantly, Ryu *et al*. also packaged ABE7.10 into a dual trans-splicing AAV system to correct a nonsense mutation (CAG to TAG) of *Dmd* gene in a mouse model of Duchenne muscular dystrophy, which they generated with BE3 previously.[88] The expression of dystrophin was partially restored in the *Dmd*-corrected mice,[115] suggesting the therapeutic potential of ABE. Liu *et al*. also used ABE7.10 to install mutations in *AR* and *Hoxd13* genes in mice and the relevant phenotypes were observed.[116] Of note, Liu *et al*. demonstrated that the SpCas9-derived ABE and the SaCas9-derived SaBE3 can induce efficient base editing at different target sites orthogonally.[116] Liang *et al*. employed ABE7.10 to introduce mutations at the splicing sties of *Tyr* and *Dmd* genes and Duchenne muscular dystrophy-like phenotypes were observed in the *Dmd*-edited mice.[117] Independently, Yang *et al*. generated mutations in *Hbb-bs* and *Fah* genes with ABE7.10 and the phenotypes of tyrosinemia type I were correspondingly observed in the mice containing the *Fah* mutation.[113] Furthermore, Yang *et al*. used two new ABEs (SaKKH-ABE and VQR-ABE) they developed to induce mutations in *Otc* and *Hbb-bs* genes in mice.[113] In rats, Yang *et al*. installed mutations in *Gaa* with ABE7.10 and the abnormal accumulation of large lysosomes filled with glycogen in multiple tissues, a typical phenotype of Pompe disease, was found in multiple tissues of $Gaa^{D645G/I646V}$ F_1 offspring.[113] In rabbits, Liu *et al*. utilized ABE7.10 to efficiently induce mutations in *Dmd*, *Otc*, and *Sod1* genes, and the rabbits with T279A mutant of *Dmd* displayed typical clinical symptoms similar to human X-linked dilated cardiomyopathy.[97] Together, these studies demonstrated that ABEs are efficient in editing mammals.[97,113]

In addition to mammals, ABEs have been utilized in zebrafish. Qin *et al*. used the zebrafish-compatible zABE7.10 to introduce mutations in *musk*, *rps14*, *atp5b*, and *wu:fc01d11* genes and the mutation of *rps14* recapitulate the typical mutant phenotypes.[118] And through applying the strategy succeeded in ABEmax (i.e. further codon-optimization and introducing more NLS[45]) Qin *et al*. also developed zABE7.10max.[118]

Meanwhile, many plant-compatible ABEs have been rapidly developed and applied as well (Table 2). Hua *et al*. generated the ABE-P1 (ABE7.10) and ABE-P2 (ABEsa) to induce mutations in six rice genes (*SLR1*, *OsSPL14*, *OsSPL16*, *OsSPL17*, *OsSPL18*, and *LOC_Os02g24720*)[114] and Yan *et al*. constructed rBE14 (ABE7.10) to introduce mutations in four rice genes (*OsMPK6*, *OsMPK13*, *OsSERK2* and *OsWRKY45*)[119]. Around the same time, Li *et al*. developed seven versions of PABE that have different TadA-TadA*/nCas9/NLS configurations and found that PABE-7, which has the same configuration of ABE7.10 but with three copies of NLS at C-terminus, induced the highest editing efficiency.[120] Then, Li *et al*. used PABE-7 to introduce mutations in eight rice genes (*OsACC*, *OsALS*, *OsCDC48*, *OsAAT*, *OsEV*, *OsOD*, *OsDEP1*, *OsNRT1.1B*) and three wheat genes (*TaDEP1*, *TaEPSPS*, *TaGW2*), and one resulted rice strain bearing C2186R substitution in OsACC is resistant to herbicide.[120] More recently, Kang *et al*. used the plant-compatible ABE7.10 (pcABE7.10) to induce mutations in four *Arabidopsis* genes (*AtALS*, *AtPDS*, *AtFT*, and *AtLFY*) and two rapeseed genes (*BnALS* and *BnPDS*).[121] The *AtFT*-targeted plants displayed a late-flowering phenotype and the *AtPDS3*-targeted plants showed a range of dwarfism and mosaic albino phenotypes, both confirming that ABE can be used to alter the phenotype of *Arabidopsis*. In order to expand the editing scope in plants, Hua *et al*. developed ABE-P3 (VQR-ABE), ABE-P4 (VRER-ABE), and ABE-P5 (SaKKH-ABE) and utilized these plant versions of ABEs to install mutations in seven rice genes (*OsSPL14*, *OsSPL16*, *OsSPL17*, *OsSPL18*, *OsTOE1*, *OsIDS1*, and *SNB*).[106]

Base Editing or CRISPR-Cas?

With both serving as precise and efficient gene editing tools, base editing overlaps with CRISPR-Cas in certain applications. Choosing between them in such circumstances becomes a sweet burden. Base substitution is by definition the kind of gene editing that is well suited to BE. As BE directly catalyzes the deamination of cytosine to install C-to-T conversions in targeted DNA, the process of base editing is generally independent of DSB or a DNA donor. With recent improvements, the editing efficiencies of the latest versions of BE can reach ~50–70% in various mammalian cell lines.[45,48] In contrast, the base substitutions installed by CRISPR-Cas9-mediated HDR rely on homologous recombination, which is cell-cycle/type dependent and generally manifests low efficiency in most mammalian cells.[7,122] Thus, BE is an appropriate tool to be chosen when targeted base substitution is desired.

Another type of gene editing, gene knockout, can be performed with either Cas or BE. When combined with one or more gRNA, Cas can generate DSB to trigger NHEJ, which will result in indels at DSB sites.[7,123] This method can be broadly used to knockout various genes and DNA fragments. Yet, it is worth noting that in some studies, the generation of DSB triggered a p53-mediated DNA damage response[124,125] and/or cause

unintended large deletions, translocations or DNA rearrangements.[126,127] In contrast, BEs can be used to terminate the production of a functional protein by creating premature stop codons (iSTOP or CRISPR-STOP), which avoids the generation of DSB.[80,81] However, the iSTOP or CRISPR-STOP strategy is hard to be used in knocking out genes that do not express protein products (e.g., noncoding RNA)[128] and Cas9 or Cpf1 would therefore be a better choice in these situations.

Insertion or deletion of a specific DNA fragment at a specific target site is another common type of gene editing, which can be achieved with Cas though HDR, but not with BE. The NHEJ repair outcome of DSB can be predicted or even designed with high accuracy, depending on the sequence of cleavage site.[129–131] Thus, some specific deletions and insertions can be directly generated by Cas9 without the need of a donor DNA, which may be used to overcome the limitation of HDR efficiency in some cases.

Delivery is another factor to consider when weighing BE and Cas for *in vivo* editing. Though various viral system has been used to deliver Cas,[132–134] the size of most Cas is reaching the packaging limit of AAV, a popular vector for *in vivo* editing.[135] Because a nucleobase deaminase module needs to be fused to a Cas in BE, the size of BE is even bigger than that of Cas. Although BEs can be split and efficiently packaged into two separate AAVs,[60] the nonviral delivery system seems more promising for BEs, especially when considering the future addition of more functional modules into BEs. Recently, Yeh *et al.* packaged the gRNA/BE3 RNP in cationic lipid nanoparticles and injected the RNP-containing nanoparticles in the inner ears of mouse pups. The post-mitotic editing installed an S33F mutation in β-catenin (*CTNNB1*), which induces cell reprogramming.[136] In the future, the specificity and efficiency of nonviral delivering system[137] can be further improved to potentiate clinic-related applications.[138]

Perspective

Since the development of the first BE just a few years ago, base editing has undergone rapid expansion.[77,139,140] Current BE systems perform the transition of bases (i.e., a purine to a purine or a pyrimidine to a pyrimidine).[11,12] In the future, the development of new BEs that can induce transversions—a purine to a pyrimidine or vice versa—will enrich the repertoire of base editing. Currently, base editing at the ''single'' base level has not been realized in most cases. The development of new base editors with a 1-bp editing window, while still keeping high editing efficiency, will further improve editing precision. Similar to the OT effects of Cas-mediated gene editing, unintended base substitutions induced by BEs at OT sites will remain a concern for basic research and potential therapeutic applications. Besides, mutations at other unexpected sites (e.g., the sites that gRNAs do not bind to) may also be induced by the nucleobase deaminase fused in BEs.[71,72,141] Thus, the development of new methods for the genome-wide detection of BE-induced mutations in human somatic cells and new BEs with even higher editing specificity will further expand the application of base editing system, particularly in therapeutics.

Author Disclosure Statement

J.C. is supported by funding from Ministry of Science and Technology (grant number 2018YFC1004602) and National Natural Science Foundation of China (NSFC) (grant numbers 31822016, 81872305, and 31600654). B.Y. is supported by NSFC (grant number 31600619). L. Y. is supported by NSFC (grant number 31730111).

References

1. Kim JS. Genome editing comes of age. *Nat Protoc* 2016;11:1573–1578. DOI: 10.1038/nprot.2016.104.
2. Bak RO, Gomez-Ospina N, Porteus MH. Gene editing on center stage. *Trends Genet* 2018;34:600–611. DOI: 10.1016/j.tig.2018.05.004.
3. Urnov FD. Genome Editing B.C. (Before CRISPR): Lasting LESSONS from the "Old Testament". *CRISPR J.* 2018;1:34–46. DOI: 10.1089/crispr.2018.29007.fyu.
4. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 2014;157:1262–1278. DOI: 10.1016/j.cell.2014.05.010.
5. Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. *Cell* 2017;168:20–36. DOI: 10.1016/j.cell.2016.10.044.
6. Knott GJ, Doudna JA. CRISPR-Cas guides the future of genetic engineering. *Science* 2018;361:866–869. DOI: 10.1126/science.aat5011.
7. Ceccaldi R, Rondinelli B, D'Andrea AD. Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol* 2016;26:52–64. DOI: 10.1016/j.tcb.2015.07.009.
8. Hustedt N, Durocher D. The control of DNA repair by the cell cycle. *Nat Cell Biol* 2016;19:1–9. DOI: 10.1038/ncb3452.
9. Harris RS, Liddament MT. Retroviral restriction by APOBEC proteins. *Nat Rev Immunol* 2004;4:868–877. DOI: 10.1038/nri1489.
10. Yang B, Li X, Lei L, et al. APOBEC: From mutator to editor. *J Genet Genomics* 2017;44:423–437. DOI: 10.1016/j.jgg.2017.04.009.
11. Komor AC, Kim YB, Packer MS, et al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016;533:420–424. DOI: 10.1038/nature17946.
12. Gaudelli NM, Komor AC, Rees HA, et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* 2017;551:464–471. DOI: 10.1038/nature24644.
13. Harris RS, Petersen-Mahrt SK, Neuberger MS. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. *Mol Cell* 2002;10:1247–1253.
14. Harris RS, Bishop KN, Sheehy AM, et al. DNA deamination mediates innate immunity to retroviral infection. *Cell* 2003;113:803–809.
15. Cheng AZ, Yockteng-Melgar J, Jarvis MC, et al. Epstein-Barr virus BORF2 inhibits cellular APOBEC3B to preserve viral genome integrity. *Nat Microbiol* 2019;4:78–88. DOI: 10.1038/s41564-018-0284-6.
16. Haradhvala NJ, Polak P, Stojanov P, et al. Mutational strand asymmetries in cancer genomes reveal mechanisms of DNA damage and repair. *Cell* 2016;164:538–549. DOI: 10.1016/j.cell.2015.12.050.
17. Seplyarskiy VB, Soldatov RA, Popadin KY, et al. APOBEC-induced mutations in human cancers are strongly enriched on the lagging DNA strand during replication. *Genome Res* 2016;26:174–182. DOI: 10.1101/gr.197046.115.

18. Roberts SA, Sterling J, Thompson C, et al. Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Mol Cell* 2012;46:424–435. DOI: 10.1016/j.molcel.2012.03.030.
19. Taylor BJ, Nik-Zainal S, Wu YL, et al. DNA deaminases induce break-associated mutation showers with implication of APOBEC3B and 3A in breast cancer kataegis. *Elife* 2013;2:e00534. DOI: 10.7554/eLife.00534.
20. Chen J, Miller BF, Furano AV. Repair of naturally occurring mismatches can induce mutations in flanking DNA. *Elife* 2014;3:e02001. DOI: 10.7554/eLife.02001.
21. Chen J, Furano AV. Breaking bad: The mutagenic effect of DNA repair. *DNA Repair (Amst)* 2015;32:43–51. DOI: 10.1016/j.dnarep.2015.04.012.
22. Burns MB, Lackey L, Carpenter MA, et al. APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* 2013;494:366–370. DOI: 10.1038/nature11881.
23. Burns MB, Temiz NA, Harris RS. Evidence for APOBEC3B mutagenesis in multiple human cancers. *Nat Genet* 2013;45:977–983. DOI: 10.1038/ng.2701.
24. Starrett GJ, Luengas EM, McCann JL, et al. The DNA cytosine deaminase APOBEC3H haplotype I likely contributes to breast and lung cancer mutagenesis. *Nat Commun* 2016;7:12918. DOI: 10.1038/ncomms12918.
25. Revy P, Muto T, Levy Y, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* 2000;102:565–575.
26. Pham P, Bransteitter R, Petruska J, et al. Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. *Nature* 2003;424:103–107. DOI: 10.1038/nature01760.
27. Alt FW, Zhang Y, Meng FL, et al. Mechanisms of programmed DNA lesions and genomic instability in the immune system. *Cell* 2013;152:417–429. DOI: 10.1016/j.cell.2013.01.007.
28. Salter JD, Smith HC. Modeling the Embrace of a Mutator: APOBEC Selection of Nucleic Acid Ligands. *Trends Biochem Sci* 2018;43:606–622. DOI: 10.1016/j.tibs.2018.04.013.
29. Salter JD, Bennett RP, Smith HC. The APOBEC Protein Family: United by Structure, Divergent in Function. *Trends Biochem Sci* 2016;41:578–594. DOI: 10.1016/j.tibs.2016.05.001.
30. Ran FA, Hsu PD, Lin CY, et al. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell* 2013;154:1380–1389. DOI: 10.1016/j.cell.2013.08.021.
31. Shen B, Zhang W, Zhang J, et al. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. *Nat Methods* 2014;11:399–402. DOI: 10.1038/nmeth.2857.
32. Mali P, Aach J, Stranges PB, et al. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat Biotechnol* 2013;31:833–838. DOI: 10.1038/nbt.2675.
33. Fu Y, Sander JD, Reyon D, et al. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nat Biotechnol* 2014;32:279–284. DOI: 10.1038/nbt.2808.
34. Tsai SQ, Wyvekens N, Khayter C, et al. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. *Nat Biotechnol* 2014;32:569–576. DOI: 10.1038/nbt.2908.
35. Lei L, Chen H, Xue W, et al. APOBEC3 induces mutations during repair of CRISPR-Cas9-generated DNA breaks. *Nat Struct Mol Biol* 2018;25:45–52. DOI: 10.1038/s41594-017-0004-6.
36. Tarantino ME, Dow BJ, Drohat AC, et al. Nucleosomes and the three glycosylases: High, medium, and low levels of excision by the uracil DNA glycosylase superfamily. *DNA Repair (Amst)* 2018;72:56–63. DOI: 10.1016/j.dnarep.2018.09.008.
37. Kunkel TA, Erie DA. Eukaryotic Mismatch Repair in Relation to DNA Replication. *Annu Rev Genet* 2015;49:291–313. DOI: 10.1146/annurev-genet-112414-054722.
38. Carter RJ, Parsons JL. Base excision repair, a pathway regulated by posttranslational modifications. *Mol Cell Biol* 2016;36:1426–1437. DOI: 10.1128/MCB.00030-16.
39. Komor AC, Zhao KT, Packer MS, et al. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. *Sci Adv* 2017;3:eaao4774. DOI: 10.1126/sciadv.aao4774.
40. Wang L, Xue W, Yan L, et al. Enhanced base editing by co-expression of free uracil DNA glycosylase inhibitor. *Cell Res* 2017;27:1289–1292. DOI: 10.1038/cr.2017.111.
41. Radany EH, Dornfeld KJ, Sanderson RJ, et al. Increased spontaneous mutation frequency in human cells expressing the phage PBS2-encoded inhibitor of uracil-DNA glycosylase. *Mutat Res* 2000;461:41–58.
42. Di Noia J, Neuberger MS. Altering the pathway of immunoglobulin hypermutation by inhibiting uracil-DNA glycosylase. *Nature*. 2002;419:43–48. DOI: 10.1038/nature00981.
43. Kachhap S, Singh KK. Mitochondrial inhibition of uracil-DNA glycosylase is not mutagenic. *Mol Cancer* 2004;3:32. DOI: 10.1186/1476-4598-3-32.
44. Cortazar D, Kunz C, Saito Y, et al. The enigmatic thymine DNA glycosylase. *DNA Repair (Amst)* 2007;6:489–504. DOI: 10.1016/j.dnarep.2006.10.013.
45. Koblan LW, Doman JL, Wilson C, et al. Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. *Nat Biotechnol* 2018;36:843–846. DOI: 10.1038/nbt.4172.
46. Zafra MP, Schatoff EM, Katti A, et al. Optimized base editors enable efficient editing in cells, organoids and mice. *Nat Biotechnol* 2018;36:888–893. DOI: 10.1038/nbt.4194.
47. Nabel CS, Jia H, Ye Y, et al. AID/APOBEC deaminases disfavor modified cytosines implicated in DNA demethylation. *Nat Chem Biol* 2012;8:751–758. DOI: 10.1038/nchembio.1042.
48. Wang X, Li J, Wang Y, et al. Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. *Nat Biotechnol* 2018;36:946–949. DOI: 10.1038/nbt.4198.
49. Carpenter MA, Li M, Rathore A, et al. Methylcytosine and normal cytosine deamination by the foreign DNA restriction enzyme APOBEC3A. *J Biol Chem* 2012;287:34801–34808. DOI: 10.1074/jbc.M112.385161.
50. Ito F, Fu Y, Kao SA, et al. Family-wide comparative analysis of cytidine and methylcytidine deamination by eleven human APOBEC proteins. *J Mol Biol* 2017;429:1787–1799. DOI: 10.1016/j.jmb.2017.04.021.
51. Hille F, Richter H, Wong SP, et al. The biology of CRISPR-Cas: backward and forward. *Cell* 2018;172:1239–1259. DOI: 10.1016/j.cell.2017.11.032.
52. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012;337:816–821. DOI: 10.1126/science.1225829.
53. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 Is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015;163:759–771. DOI: 10.1016/j.cell.2015.09.038.
54. Kim D, Kim J, Hur JK, et al. Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells. *Nat Biotechnol* 2016;34:863–868. DOI: 10.1038/nbt.3609.
55. Kleinstiver BP, Tsai SQ, Prew MS, et al. Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. *Nat Biotechnol* 2016;34:869–874. DOI: 10.1038/nbt.3620.
56. Stella S, Alcon P, Montoya G. Structure of the Cpf1 endonuclease R-loop complex after target DNA cleavage. *Nature* 2017;546:559–563. DOI: 10.1038/nature22398.
57. Swarts DC, van der Oost J, Jinek M. Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a. *Mol Cell* 2017;66:221–233 e224. DOI: 10.1016/j.molcel.2017.03.016.
58. Stella S, Mesa P, Thomsen J, et al. Conformational activation promotes CRISPR-Cas12a catalysis and resetting of the endonuclease Activity. *Cell* 2018;175:1856–1871 e1821. DOI: 10.1016/j.cell.2018.10.045.
59. Li X, Wang Y, Liu Y, et al. Base editing with a Cpf1-cytidine deaminase fusion. *Nat Biotechnol* 2018;36:324–327. DOI: 10.1038/nbt.4102.
60. Villiger L, Grisch-Chan HM, Lindsay H, et al. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. *Nat Med* 2018;24:1519–1525. DOI: 10.1038/s41591-018-0209-1.
61. Kim YB, Komor AC, Levy JM, et al. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat Biotechnol* 2017;35:371–376. DOI: 10.1038/nbt.3803.
62. Esvelt KM, Carlson JC, Liu DR. A system for the continuous directed evolution of biomolecules. *Nature* 2011;472:499–503. DOI: 10.1038/nature09929.
63. Hu JH, Miller SM, Geurts MH, et al. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature* 2018;556:57–63. DOI: 10.1038/nature26155.
64. Nishimasu H, Shi X, Ishiguro S, et al. Engineered CRISPR-Cas9 nuclease with expanded targeting space. *Science* 2018;361:1259–1262. DOI: 10.1126/science.aas9129.
65. Fu Y, Foden JA, Khayter C, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. *Nat Biotechnol* 2013;31:822–826. DOI: 10.1038/nbt.2623.

66. Hsu PD, Scott DA, Weinstein JA, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol* 2013;31:827–832. DOI: 10.1038/nbt.2647.
67. Kim D, Bae S, Park J, et al. Digenome-seq: genome-wide profiling of CRISPR-Cas9 off-target effects in human cells. *Nat Methods* 2015;12:237–243, 231 p following 243. DOI: 10.1038/nmeth.3284.
68. Tsai SQ, Zheng Z, Nguyen NT, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol* 2015;33:187–197. DOI: 10.1038/nbt.3117.
69. Li G, Liu Y, Zeng Y, et al. Highly efficient and precise base editing in discarded human tripronuclear embryos. *Protein Cell* 2017;8:776–779. DOI: 10.1007/s13238-017-0458-7.
70. Rees HA, Komor AC, Yeh WH, et al. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. *Nat Commun* 2017;8:15790. DOI: 10.1038/ncomms15790.
71. Jin S, Zong Y, Gao Q, et al. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science* 2019. DOI: 10.1126/science.aaw7166.
72. Zuo E, Sun Y, Wei W, et al. Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. *Science* 2019. DOI: 10.1126/science.aav9973.
73. Kim D, Lim K, Kim ST, et al. Genome-wide target specificities of CRISPR RNA-guided programmable deaminases. *Nat Biotechnol* 2017;35:475–480. DOI: 10.1038/nbt.3852.
74. Nishida K, Arazoe T, Yachie N, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* 2016;353. DOI: 10.1126/science.aaf8729.
75. Lange SS, Takata K, Wood RD. DNA polymerases and cancer. *Nat Rev Cancer* 2011;11:96–110. DOI: 10.1038/nrc2998.
76. Yang W, Gao Y. Translesion and repair DNA polymerases: diverse structure and mechanism. *Annu Rev Biochem* 2018;87:239–261. DOI: 10.1146/annurev-biochem-062917-012405.
77. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet* 2018;19:770–788. DOI: 10.1038/s41576-018-0059-1.
78. Tan J, Zhang F, Karcher D, et al. Engineering of high-precision base editors for site-specific single nucleotide replacement. *Nat Commun* 2019;10:439. DOI: 10.1038/s41467-018-08034-8.
79. Gehrke JM, Cervantes O, Clement MK, et al. An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. *Nat Biotechnol* 2018;36:977–982. DOI: 10.1038/nbt.4199.
80. Billon P, Bryant EE, Joseph SA, et al. CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons. *Mol Cell* 2017;67:1068–1079 e1064. DOI: 10.1016/j.molcel.2017.08.008.
81. Kuscu C, Parlak M, Tufan T, et al. CRISPR-STOP: gene silencing through base-editing-induced nonsense mutations. *Nat Methods* 2017;14:710–712. DOI: 10.1038/nmeth.4327.
82. Yilmaz A, Peretz M, Aharony A, et al. Defining essential genes for human pluripotent stem cells by CRISPR-Cas9 screening in haploid cells. *Nat Cell Biol* 2018;20:610–619. DOI: 10.1038/s41556-018-0088-1.
83. Ma Y, Zhang J, Yin W, et al. Targeted AID-mediated mutagenesis (TAM) enables efficient genomic diversification in mammalian cells. *Nat Methods* 2016;13:1029–1035. DOI: 10.1038/nmeth.4027.
84. Yuan J, Ma Y, Huang T, et al. Genetic modulation of RNA splicing with a CRISPR-guided cytidine deaminase. *Mol Cell* 2018;72:380–394 e387. DOI: 10.1016/j.molcel.2018.09.002.
85. Hess GT, Fresard L, Han K, et al. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. *Nat Methods* 2016;13:1036–1042. DOI: 10.1038/nmeth.4038.
86. Jiang W, Feng S, Huang S, et al. BE-PLUS: a new base editing tool with broadened editing window and enhanced fidelity. *Cell Res* 2018;28:855–861. DOI: 10.1038/s41422-018-0052-4.
87. Liu LD, Huang M, Dai P, et al. Intrinsic nucleotide preference of diversifying base editors guides antibody ex vivo affinity maturation. *Cell Rep* 2018;25:884–892 e883. DOI: 10.1016/j.celrep.2018.09.090.
88. Kim K, Ryu SM, Kim ST, et al. Highly efficient RNA-guided base editing in mouse embryos. *Nat Biotechnol* 2017;35:435–437. DOI: 10.1038/nbt.3816.
89. Liang P, Sun H, Sun Y, et al. Effective gene editing by high-fidelity base editor 2 in mouse zygotes. *Protein Cell* 2017;8:601–611. DOI: 10.1007/s13238-017-0418-2.
90. Yang G, Zhu TY, Lu ZY, et al. Generation of isogenic single and multiplex gene knockout mice by base editing-induced STOP. *Sci Bull* 2018;63:1101–1107. DOI: 10.1016/j.scib.2018.07.002.
91. Yang G, Zhou C, Wang R, et al. Base-editing-mediated R17H substitution in histone H3 reveals methylation-dependent regulation of Yap signaling and early mouse embryo development. *Cell Rep* 2019;26:302–312 e304. DOI: 10.1016/j.celrep.2018.12.046.
92. Sasaguri H, Nagata K, Sekiguchi M, et al. Introduction of pathogenic mutations into the mouse Psen1 gene by Base Editor and Target-AID. *Nat Commun* 2018;9:2892. DOI: 10.1038/s41467-018-05262-w.
93. Li J, Liu Z, Huang S, et al. Efficient base editing in G/C-rich regions to model androgen insensitivity syndrome. *Cell Res* 2019. DOI: 10.1038/s41422-018-0133-4.
94. Chadwick AC, Wang X, Musunuru K. In vivo base editing of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) as a therapeutic alternative to genome editing. *Arterioscler Thromb Vasc Biol* 2017;37:1741–1747. DOI: 10.1161/ATVBAHA.117.309881.
95. Chadwick AC, Evitt NH, Lv W, et al. Reduced blood lipid levels with in vivo CRISPR-Cas9 base editing of ANGPTL3. *Circulation* 2018;137:975–977. DOI: 10.1161/CIRCULATIONAHA.117.031335.
96. Rossidis AC, Stratigis JD, Chadwick AC, et al. In utero CRISPR-mediated therapeutic editing of metabolic genes. *Nat Med* 2018;24:1513–1518. DOI: 10.1038/s41591-018-0184-6.
97. Liu Z, Chen M, Chen S, et al. Highly efficient RNA-guided base editing in rabbit. *Nat Commun* 2018;9:2717. DOI: 10.1038/s41467-018-05232-2.
98. Li Z, Duan X, An X, et al. Efficient RNA-guided base editing for disease modeling in pigs. *Cell Discov* 2018;4:64. DOI: 10.1038/s41421-018-0065-7.
99. Zhang Y, Qin W, Lu X, et al. Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system. *Nat Commun* 2017;8:118. DOI: 10.1038/s41467-017-00175-6.
100. Zong Y, Wang Y, Li C, et al. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat Biotechnol* 2017;35:438–440. DOI: 10.1038/nbt.3811.
101. Zong Y, Song Q, Li C, et al. Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC3A. *Nat Biotechnol* 2018. DOI: 10.1038/nbt.4261.
102. Li J, Sun Y, Du J, et al. Generation of Targeted Point Mutations in Rice by a Modified CRISPR/Cas9 System. *Mol Plant* 2017;10:526–529. DOI: 10.1016/j.molp.2016.12.001.
103. Lu Y, Zhu JK. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. *Mol Plant* 2017;10:523–525. DOI: 10.1016/j.molp.2016.11.013.
104. Shimatani Z, Kashojiya S, Takayama M, et al. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. *Nat Biotechnol* 2017;35:441–443. DOI: 10.1038/nbt.3833.
105. Ren B, Yan F, Kuang Y, et al. Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-guided hyperactive hAID mutant. *Mol Plant* 2018;11:623–626. DOI: 10.1016/j.molp.2018.01.005.
106. Hua K, Tao X, Zhu JK. Expanding the base editing scope in rice by using Cas9 variants. *Plant Biotechnol J* 2018. DOI: 10.1111/pbi.12993.
107. Marraffini LA, Sontheimer EJ. Self versus non-self-discrimination during CRISPR RNA-directed immunity. *Nature* 2010;463:568–571. DOI: 10.1038/nature08703.
108. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science* 2008;322:1843–1845. DOI: 10.1126/science.1165771.
109. Banno S, Nishida K, Arazoe T, et al. Deaminase-mediated multiplex genome editing in Escherichia coli. *Nat Microbiol* 2018;3:423–429. DOI: 10.1038/s41564-017-0102-6.
110. Zheng K, Wang Y, Li N, et al. Highly efficient base editing in bacteria using a Cas9-cytidine deaminase fusion. *Commun Biol* 2018;1:32. DOI: 10.1038/s42003-018-0035-5.
111. Gu T, Zhao S, Pi Y, et al. Highly efficient base editing in *Staphylococcus aureus* using an engineered CRISPR RNA-guided cytidine deaminase. *Chem Sci* 2018;9:3248–3253. DOI: 10.1039/c8sc00637g.
112. Chen W, Zhang Y, Zhang Y, et al. CRISPR/Cas9-based genome editing in *Pseudomonas aeruginosa* and cytidine deaminase-mediated base editing in *Pseudomonas* species. *iScience* 2018;6:222–231. DOI: 10.1016/j.isci.2018.07.024.

113. Yang L, Zhang X, Wang L, et al. Increasing targeting scope of adenosine base editors in mouse and rat embryos through fusion of TadA deaminase with Cas9 variants. *Protein Cell* 2018;9:814–819. DOI: 10.1007/s13238-018-0568-x.
114. Hua K, Tao X, Yuan F, et al. Precise A.T to G.C Base editing in the rice genome. *Mol Plant* 2018;11:627–630. DOI: 10.1016/j.molp.2018.02.007.
115. Ryu SM, Koo T, Kim K, et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. *Nat Biotechnol* 2018;36:536–539. DOI: 10.1038/nbt.4148.
116. Liu Z, Lu Z, Yang G, et al. Efficient generation of mouse models of human diseases via ABE- and BE-mediated base editing. *Nat Commun* 2018;9:2338. DOI: 10.1038/s41467-018-04768-7.
117. Liang P, Sun H, Zhang X, et al. Effective and precise adenine base editing in mouse zygotes. *Protein Cell* 2018;9:808–813. DOI: 10.1007/s13238-018-0566-z.
118. Qin W, Lu X, Liu Y, et al. Precise A*T to G*C base editing in the zebrafish genome. *BMC Biol* 2018;16:139. DOI: 10.1186/s12915-018-0609-1.
119. Yan F, Kuang Y, Ren B, et al. Highly Efficient A.T to G.C Base editing by Cas9n-guided tRNA adenosine deaminase in rice. *Mol Plant* 2018;11:631–634. DOI: 10.1016/j.molp.2018.02.008.
120. Li C, Zong Y, Wang Y, et al. Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. *Genome Biol* 2018;19:59. DOI: 10.1186/s13059-018-1443-z.
121. Kang BC, Yun JY, Kim ST, et al. Precision genome engineering through adenine base editing in plants. *Nat Plants* 2018;4:427–431. DOI: 10.1038/s41477-018-0178-x.
122. Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. *Nat Med* 2015;21:121–131. DOI: 10.1038/nm.3793.
123. Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell* 2012;47:497–510. DOI: 10.1016/j.molcel.2012.07.029.
124. Haapaniemi E, Botla S, Persson J, et al. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. *Nat Med* 2018;24:927–930. DOI: 10.1038/s41591-018-0049-z.
125. Ihry RJ, Worringer KA, Salick MR, et al. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. *Nat Med* 2018;24:939–946. DOI: 10.1038/s41591-018-0050-6.
126. Kosicki M, Tomberg K, Bradley A. Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol* 2018;36:765–771. DOI: 10.1038/nbt.4192.
127. Cullot G, Boutin J, Toutain J, et al. CRISPR-Cas9 genome editing induces megabase-scale chromosomal truncations. *Nat Commun* 2019;10:1136. DOI: 10.1038/s41467-019-09006-2.
128. Zhu S, Li W, Liu J, et al. Genome-scale deletion screening of human long non-coding RNAs using a paired-guide RNA CRISPR-Cas9 library. *Nat Biotechnol* 2016;34:1279–1286. DOI: 10.1038/nbt.3715.
129. Allen F, Crepaldi L, Alsinet C, et al. Predicting the mutations generated by repair of Cas9-induced double-strand breaks. *Nat Biotechnol* 2018. DOI: 10.1038/nbt.4317.
130. Shou J, Li J, Liu Y, et al. Precise and predictable CRISPR chromosomal rearrangements reveal principles of Cas9-mediated nucleotide insertion. *Mol Cell* 2018;71:498–509 e494. DOI: 10.1016/j.molcel.2018.06.021.
131. Shen MW, Arbab M, Hsu JY, et al. Predictable and precise template-free CRISPR editing of pathogenic variants. *Nature* 2018;563:646–651. DOI: 10.1038/s41586-018-0686-x.
132. Maggio I, Holkers M, Liu J, et al. Adenoviral vector delivery of RNA-guided CRISPR/Cas9 nuclease complexes induces targeted mutagenesis in a diverse array of human cells. *Sci Rep* 2014;4:5105. DOI: 10.1038/srep05105.
133. Ran FA, Cong L, Yan WX, et al. In vivo genome editing using *Staphylococcus aureus* Cas9. *Nature* 2015;520:186–191. DOI: 10.1038/nature14299.
134. Choi JG, Dang Y, Abraham S, et al. Lentivirus pre-packed with Cas9 protein for safer gene editing. *Gene Ther* 2016;23:627–633. DOI: 10.1038/gt.2016.27.
135. Mingozzi F, High KA. Overcoming the host immune response to adeno-associated virus gene delivery vectors: the race between clearance, tolerance, neutralization, and escape. *Annu Rev Virol* 2017;4:511–534. DOI: 10.1146/annurev-virology-101416-041936.
136. Yeh WH, Chiang H, Rees HA, et al. In vivo base editing of post-mitotic sensory cells. *Nat Commun* 2018;9:2184. DOI: 10.1038/s41467-018-04580-3.
137. Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. *Nat Rev Drug Discov* 2017;16:387–399. DOI: 10.1038/nrd.2016.280.
138. Yin H, Xue W, Anderson DG. CRISPR-Cas: a tool for cancer research and therapeutics. *Nat Rev Clin Oncol* 2019. DOI: 10.1038/s41571-019-0166-8.
139. Hess GT, Tycko J, Yao D, et al. Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. *Mol Cell* 2017;68:26–43. DOI: 10.1016/j.molcel.2017.09.029.
140. Ranzau BL, Komor AC. Genome, epigenome, and transcriptome editing via chemical modification of nucleobases in living cells. *Biochemistry* 2019;58:330–335. DOI: 10.1021/acs.biochem.8b00958.
141. Laughery MF, Mayes HC, Pedroza IK, et al. R-loop formation by dCas9 is mutagenic in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2018. DOI: 10.1093/nar/gky1278.