Review

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Precise genome editing with base editors

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Abstract: Single-nucleotide variants account for about half of known pathogenic genetic variants in human. Genome editing strategies by reversing pathogenic point mutations with minimum side effects have great therapeutic potential and are now being actively pursued. The emerge of precise and efficient genome editing strategies such as base editing and prime editing provide powerful tools for nucleotide conversion without inducing double-stranded DNA breaks (DSBs), which have shown great potential for curing genetic disorders. A diverse toolkit of base editors has been developed to improve the editing efficiency and accuracy in different context of application. Here, we summarized the evolving of base editors (BEs), their limitations and future perspective of base editing-based therapeutic strategies.

Keywords: adenine base editor; base editing; clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein; cytosine base editor.

Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas) system, an adaptive immune system originally found only in bacteria with the capability to direct the silencing of invading nucleic acids [1, 2], has greatly enhanced the toolkit for genome editing. CRISPR gene editing technology has been rapidly applied to precise editing of bacterial [3], plants and mammalian genomes [4]. CRISPR-Cas9 gene editing technology employs artificially designed single guide RNA (sgRNA) to identify targeted DNA sequence in the genome, which guides Cas9 protein to the specific site and cut the DNA helix to generate DNA double strand breaks (DSBs) [5]. DSBs activate two main endogenous cellular repair pathways, non-homologous end joining (NHEJ) and homology-directed repair (HDR) [6], which creates insertions and deletions (indels) or accurate site-specific repairmen, respectively [6]. However, the DSBs most often generates undesired mutations such as indels, translocations, large deletion and chromothrypsis, which can lead to serious consequences such as cancer development [6].

In 2016 David Liu's team at Harvard University developed a base editing system that enables precise nucleotide conversion without the need of DNA double-strand breaks [7]. The base editing system fuses cytosine deaminase to CRISPR/dead Cas9 (dCas9). Under the guidance of gRNA, the cytidine base editor (CBE) directly converts cytosine (C) to uracil (U) by deamination without causing DNA double strand break. The following DNA replication further replaces U with thymine (T), resulting C-to-T conversion. Based on this concept, adenine base editor (ABE) that catalyzed adenine (A)-to-T nucleotide conversion were also developed by David Liu's group [43]. Multiple optimized versions of CBEs and ABEs have been developed to overcome different technical bottlenecks. Here we summarized the development and evolving of the CBE and ABE systems (Tables 1 and 2), limitations and discussed future perspectives of applications.

Cytosine base editor (CBE)

Development of CBE

Although deaminases in nature predominantly act on RNA, apolipoprotein B mRNA editing enzyme, catalyticpolypeptide-like (APOBEC) family retains the capability of deamination on single-stranded DNA (ssDNA). The common characteristic of this class of deaminase is a conserved cytidine deaminase (CDA) domain that can deaminate base C. The resultant U from deaminated of C will be further converted to T through DNA repair and replication, achieving C-to-T conversion [7]. With this concept, in April 2016, David Liu's laboratory at

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Table 1: CBE with various characteristics.

Name	Deaminase	Cas nuclease	PAM	UGI	NLS	Editing window	References
BE1	rAPOBEC1	dCas9	NGG	–	1×	4–8	[7]
BE2	rAPOBEC1	dCas9	NGG	1×	1×	4–8	[7]
BE3	rAPOBEC1	nCas9 (H840A)	NGG	1×	1×	4–8	[7]
Target-AID	PmCDA1	dCas9	NGG	1×	1×	3–7	[8]
AIDx	hAIDx	dCas9	NGG	–	1×	5–9	[9]
dCas9-AIDx	hAIDx	dCas9	NGG	1×	1×	5–9	[9]
CRISPR-X	hAIDΔ-MS2	dCas9	NGG	–	1×	−50–50	[10]
ZF-AID	hAID	ZF	NGG	–	1×	–	[11]
TALE-AID	hAID	TALE	–	1×	1×	–	[11]
YE1-BE3	rAPOBEC1-YE1	nCas9 (H840A)	NGG	1×	1×	4–7	[12]
YE2-BE3	rAPOBEC1-YE2	nCas9 (H840A)	NGG	1×	1×	5–6	[12]
EE-BE3	rAPOBEC1-EE	nCas9 (H840A)	NGG	1×	1×	5–6	[12]
YEE-BE3	rAPOBEC1-YEE	nCas9 (H840A)	NGG	1×	1×	5–6	[12]
VQR-BE3	rAPOBEC1	nVQRCas9 (H840A)	NGAN	1×	1×	4–11	[12]
EQR-BE3	rAPOBEC1	nEQRCas9 (H840A)	NGAG	1×	1×	4–11	[12]
VRER-BE3	rAPOBEC1	nVRERCas9 (H840A)	NGCG	1×	1×	3–10	[12]
SaBE3	rAPOBEC1	nSaCas9 (H840A)	NNGRRT	1×	1×	3–12	[12]
SaKKH-BE3	rAPOBEC1	nSaKKHCas9 (H840A)	NNNRRT	1×	1×	3–12	[12]
PBE	rAPOBEC1	nCas9 (D10A)	NGG	1×	2×	3–9	[13]
HF2-BE2	rAPOBEC1	HF2-dCas9	NGG	1×	1×	4–8	[14]
HF-BE3	rAPOBEC1	HF1-nCas9 (H840A)	NGG	1×	1×	4–8	[15]
eBE-S3	rAPOBEC1	nCas9 (H840A)	NGG	4×	1×	4–8	[16]
CDA1-BE3	PmCDA1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
AID-BE3	hAID	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
APOBEC3G-BE3	hAPOBEC3G	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
BE3B	rAPOBEC1	nCas9 (D10A)	NGG	–	1×	4–8	[17]
SSB-BE3	rAPOBEC1	nCas9 (D10A)	NGG	–	1×	4–8	[17]
N-UGI-BE3	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
BE3-2 × UGI	rAPOBEC1	nCas9 (D10A)	NGG	2×	1×	4–8	[17]
BE3C	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
BE3D	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
BE3E	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
BE4	rAPOBEC1	nCas9 (D10A)	NGG	2×	1×	4–8	[17]
SaBE4	rAPOBEC1	nSaCas9 (D10A)	NNGRRT	2×	1×	3–12	[17]
BE3-Gam	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	4–8	[17]
SaBE3-Gam	rAPOBEC1	nSaCas9 (D10A)	NNGRRT	1×	1×	3–12	[17]
BE4-Gam	rAPOBEC1	nCas9 (D10A)	NGG	2×	1×	4–8	[17]
SaBE4-Gam	rAPOBEC1	nSaCas9 (D10A)	NNGRRT	2×	1×	3–12	[17]
xBE3	rAPOBEC1	nxCas9 (D10A)	NG	1×	1×	4–8	[18]
dCpf1-eBE	rAPOBEC1	dCpf1	TTTV	4×	3×	8–13	[19]
eAID-BE4max	hAID	nCas9 (D10A)	NGG	2×	2×	4–8	[20]
Spy-macnCas9-BE3	rAPOBEC1	Spy-macnCas9 (D10A)	NAA	1×	1×	4–8	[21]
BE-PLUS	rAPOBEC11-scFV	nCas9 (D10A)	NGG	1×	1×	4–16	[22]
eA3A-BE3	hAPOBEC3G-N57G	nCas9 (D10A)	NGG	1×	1×	4–8	[23]
eA3A-HF1-BE3-2 × UGI	hAPOBEC3G-N57G	HF1-nCas9 (D10A)	NGG	2×	1×	4–8	[23]
eA3A-Hypa-BE3-2 × UGI	hAPOBEC3G-N57G	nHypaCas9 (D10A)	NGG	2×	1×	4–8	[23]
hA3A-BE3	hAPOBEC3A	nCas9 (D10A)	NGG	1×	1×	2–13	[24]
hA3A-BE3-Y130F	hAPOBEC3A-Y130F	nCas9 (D10A)	NGG	1×	1×	3–8	[24]
Target-AID-NG	PmCDA1	nCas9 (D10A)-NG	NG	1×	1×	2–8	[25]
BE3-NG	rAPOBEC1	nCas9 (D10A)-NG	NG	1×	1×	4–8	[25]
A3A-PBE	hAPOBEC3A	nCas9 (D10A)	NGG	1×	2×	1–17	[26]
BE4-max	rAPOBEC1	nCas9 (D10A)	NGG	2×	2×	4–8	[26]
AncBE4-max	rAPOBEC1	nCas9 (D10A)	NGG	2×	2×	4–8	[26]
FNLS-BE3	rAPOBEC1	nCas9 (D10A)	NGG	1×	2×	3–8	[26]
DBE-A3A	hAPOBEC3A-MS2	nCas9 (D10A)	NGG	1×	1×	2–17	[27]
DBE-AIDmomo	hAID-momo-MS2	nCas9 (D10A)	NGG	1×	1×	2–17	[27]

Table 1: (continued)

Name	Deaminase	Cas nuclease	PAM	UGI	NLS	Editing window	References
BE3-PAPAPAP	rAPOBEC1	nCas9 (D10A)	NGG	1×	1×	5–6	[28]
nCDA1-BE3	PmCDA1Δ	nCas9 (D10A)	NGG	1×	1×	3–4	[28]
enAsBE1.1–1.4	rAPOBEC1	denAsCas12a	TTTV	1×	1 × -4×	5–25	[29]
BE3-R33A	rAPOBEC1-R33A	nCas9 (D10A)	NGG	1×	1×	5–7	[30]
BE3-R33A/K34A	rAPOBEC1-R33A/K34A	nCas9 (D10A)	NGG	1×	1×	5–6	[30]
CP-CBEmax	rAPOBEC1	CP-nCas9 (D10A)	NGG	2×	1×	4–11	[31]
$BE3^{W90Y/R126E}$	rAPOBEC1-W90Y/R126E	nCas9 (D10A)	NGG	1×	1×	4–8	[32]
$hA3A\text{-}BE3^{R128A}$	hAPOBEC3A-R128A	nCas9 (D10A)	NGG	1×	1×	3–9	[32]
$hA3A\text{-}BE3^{Y130F}$	hAPOBEC3A-Y130F	nCas9(D10A)	NGG	1×	1×	3–9	[32]
evoAPOBEC1-BE4max	rAPOBEC1	nCas9(D10A)	NGG	2×	2×	3–8	[33]
evoFERNY-BE4max	FERNY	nCas9 (D10A)	NGG	2×	2×	1–8	[33]
evoCDA1-BE4max	PmCDA1	nCas9 (D10A)	NGG	2×	2×	1–13	[33]
N-1-BE	PmCDA1L1_4 (1)	nCas9 (D10A)	NGG	1×	2×	9–13	[34]
C-1-BE	PmCDA1L1_4 (1)	nCas9 (D10A)	NGG	1×	2×	10–13	[34]
N-2-BE	LpCDA1 (2)	nCas9 (D10A)	NGG	1×	2×	1–8	[34]
C-2-BE	LpCDA1 (2)	nCas9 (D10A)	NGG	1×	2×	−1,3–5	[34]
N-4-BE	LjCDA1L1_4 (4)	nCas9 (D10A)	NGG	1×	2×	−1–13	[34]
N-7-BE	LjCDA1 (7)	nCas9 (D10A)	NGG	1×	2×	2–6	[34]
N-8-BE	LpCDA1L1_1 (8)	nCas9 (D10A)	NGG	1×	2×	1–14	[34]
C-8-BE	LpCDA1L1_1 (8)	nCas9 (D10A)	NGG	1×	2×	9–14	[34]
N-9-BE	LjCDA1L1_1 (9)	nCas9 (D10A)	NGG	1×	2×	1–15	[34]
N-10-BE	LpCDA1L1_3 (10)	nCas9 (D10A)	NGG	1×	2×	1–13	[34]
N-12-BE	LjCDA1L2_1 (12)	nCas9 (D10A)	NGG	1×	2×	7–12	[34]
N-13-BE	LpCDA1L1_4 (13)	nCas9 (D10A)	NGG	1×	2×	1–12	[34]
BE-PIGS	rAPOBEC1	nCas9 (D10A)	NGG	2×	1×	4–14	[35]
STEME-1	hAPOBEC3A-ecTadA-ecTadA7.10	nCas9 (D10A)	NGG	1×	2×	1–17	[36]
STEME-2	ecTadA-ecTadA7.10-hAPOBEC3A	nCas9 (D10A)	NGG	1×	2×	1–17	[36]
STEME-3	hAPOBEC3A-ecTadA-ecTadA7.10	nCas9 (D10A)	NGG	2×	3×	1–17	[36]
STEME-4	ecTadA-ecTadA7.10-hAPOBEC3A	nCas9 (D10A)	NGG	2×	3×	1–17	[36]
STEME-NG	hAPOBEC3A-ecTadA-ecTadA7.10	nCas9-NG (D10A)	NG	1×	2×	1–17	[36]
A3A-PBE-NG	hAPOBEC3A	nCas9-NG (D10A)	NG	1×	2×	1–17	[36]
YE1-BE4	rAPOBEC1-YE1	nCas9 (D10A)	NGG	2×	2×	4–7	[37]
YE2-BE4	rAPOBEC1-YE2	nCas9 (D10A)	NGG	2×	2×	5–6	[37]
EE-BE4	rAPOBEC1-EE	nCas9 (D10A)	NGG	2×	2×	5–6	[37]
YEE-BE4	rAPOBEC1-YEE	nCas9 (D10A)	NGG	2×	2×	5–6	[37]
R33A-BE4	rAPOBEC1-R33A	nCas9 (D10A)	NGG	2×	2×	5–7	[37]
R33A+ K34A-BE4	rAPOBEC1-R33A/K34A	nCas9 (D10A)	NGG	2×	2×	5–6	[37]
eA3A-BE4	hAPOBEC3G-N57G	nCas9 (D10A)	NGG	2×	2×	4–9	[37]
A3A-BE4	hAPOBEC3A	nCas9 (D10A)	NGG	2×	2×	3–11	[37]
AID-BE4	hAID	nCas9 (D10A)	NGG	2×	2×	4–8	[37]
CDA-BE4	PmCDA1	nCas9 (D10A)	NGG	2×	2×	1–13	[37]
FERNY-BE4	FERNY	nCas9 (D10A)	NGG	2×	2×	1–8	[37]
BE4-NG	rAPOBEC1	nCas9-NG (D10A)	NG	2×	2×	3–14	[37]
YE1-BE4-NG	rAPOBEC1-YE1	nCas9-NG (D10A)	NG	2×	2×	5–8	[37]
YE2-BE4-NG	rAPOBEC1-YE2	nCas9-NG (D10A)	NG	2×	2×	5–8	[37]
EE-BE4-NG	rAPOBEC1-EE	nCas9-NG (D10A)	NG	2×	2×	5–8	[37]
YEE-BE4-NG	rAPOBEC1-YEE	nCas9-NG (D10A)	NG	2×	2×	5–8	[37]
R33A+ K34A-BE4-NG	rAPOBEC1-R33A/K34A	nCas9-NG (D10A)	NG	2×	2×	5–8	[37]
YE1-BE4-CP1028	rAPOBEC1-YE1	nCas9 (D10A)	NGG	2×	2×	3–12	[37]
YE2-BE4-CP1028	rAPOBEC1-YE2	nCas9 (D10A)	NGG	2×	2×	3–12	[37]
EE-BE4-CP1028	rAPOBEC1-EE	nCas9 (D10A)	NGG	2×	2×	3–12	[37]
YEE-BE4-CP1028	rAPOBEC1-YEE	nCas9 (D10A)	NGG	2×	2×	5–12	[37]
R33A+ K34A-BE4-CP1028	rAPOBEC1-R33A/K34A	nCas9 (D10A)	NGG	2×	2×	5–12	[37]
AALN-BE4	rAPOBEC1-R33A/K34A/H122L/D124 N	nCas9 (D10A)	NGG	2×	2×	3–12	[37]
hyBE4max	rAPOBEC1	nCas9 (D10A)	NGG	2×	2×	4–12	[38]
hyA3A-BE4max	hAPOBEC3A	nCas9 (D10A)	NGG	2×	2×	3–15	[38]

Table 1: (continued)

Name	Deaminase	Cas nuclease	PAM	UGI	NLS	Editing window	References
hyeA3A-BE4max	hAPOBEC3G-N57G	nCas9 (D10A)	NGG	2×	2×	4–15	[38]
Target-ACE	PmCDA1-ecTadA7.10	nCas9 (D10A)	NGG	2×	1×	4–8	[39]
Target-ACEmax	PmCDA1-ecTadA7.10	nCas9 (D10A)	NGG	2×	2×	2–9	[39]
Target-AIDmax	hAID	nCas9 (D10A)	NGG	2×	2×	2–9	[39]
ACBEmax	rAPOBEC1-ecTadA7.10	nCas9 (D10A)	NGG	2×	2×	4–9	[39]
A3G-BE4max	hAPOBEC3-CTD	nCas9 (D10A)	NGG	2×	2×	4–10	[40]
TadCBE	TadA[a]	nCas9 (D10A)	NGG	2×	2×	3–8	[41]
TadDE	TadA-CDS	nCas9 (D10A)	NGG	2×	2×	3–8	[41]
Td-CGBE	TadA[a]-N46L	nCas9 (D10A)	NGG	2×	2×	5–6	[42]
Td-CBEs	TadA[a]-N46L	nCas9 (D10A)	NGG	–	2×	6–7	[42]

Table 2: ABE with various characteristics.

Name	Deaminase	Cas nuclease	PAM	UGI	NLS	Editing window	References
ABE0.1	TadA	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE1.1	TadA	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE1.2	TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE2.1	TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE2.9	TadA*-TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE3.1	TadA*-TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE4.3	TadA*-TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE5.1	TadA*-TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE5.3	TadA*-TadA*	nCas9 (D10A)	NGG	–	1×	–	[43]
ABE6.3	TadA-TadA*	nCas9 (D10A)	NGG	–	1×	4–9	[43]
ABE6.4	TadA-TadA*	nCas9 (D10A)	NGG	–	1x	4–9	[43]
ABE7.9	TadA-TadA*	nCas9 (D10A)	NGG	–	1×	4–9	[43]
ABE7.10	TadA-TadA*	nCas9 (D10A)	NGG	–	1×	4–7	[43]
ABE-P1	TadA-TadA*	nCas9 (D10A)	NGG	–	1×	4–10	[44]
ABESa	TadA-TadA*	Sa-Cas9 (D10A)	NNGRRT	–	1×	6–12	[45]
xABE	TadA-TadA*	x-nCas9 (D10A)	NG	–	1×	4–7	[18]
SaKKH-ABE	TadA-TadA*	SaKKH-nCas9 (D10A)	NNNRRT	–	1×	3–14	[46, 47]
PABE-7	TadA-TadA*	nCas9 (D10A)	NGG	–	3×	4–8	[48]
ABEmax	TadA-TadA*	nCas9 (D10A)	NGG	–	2×	4–7	[49]
VQR-ABE	TadA-TadA*	VQR-nCas9 (D10A)	NGA	–	1×	3–8	[46]
VRER-ABE	TadA-TadA*	VRER-nCas9 (D10A)	NGCG	–	1×	4–8	[47]
NG-ABEmax	TadA-TadA*	nCas9-NG (D10A)	NG	–	2×	4–8	[25]
ScCas9-ABE (7.10)	TadA-TadA*	Sc-nCas9 (D10A)	NNGN	–	1×	4–7	[50]
ABE-NG	TadA-TadA*	nCas9-NG (D10A)	NG	–	1×	4–7	[51]
ABEmaxAW	TadA-E59A-TadA*-V106W	nCas9 (D10A)	NGG	–	2×	4–8	[52]
ABEmaxQW	TadA-E59Q-TadA*-V106W	nCas9 (D10A)	NGG	–	2×	4–8	[52]
SaKKH-ABEmax	TadA-TadA*	SaKKH-nCas9 (D10A)	NNNRRT	–	2×	4–14	[31]
VQR-ABEmax	TadA-TadA*	VQR-nCas9 (D10A)	NGA	–	2×	4–8	[31]
VRER-ABEmax	TadA-TadA*	VRER-nCas9 (D10A)	NGCG	–	2×	4–8	[31]
VRQR-ABEmax	TadA-TadA*	VRQR-nCas9 (D10A)	NGA	–	2×	4–8	[31]
Sa-ABEmax	TadA-TadA*	Sa-nCas9 (D10A)	NNGRRT	–	2×	4–14	[31]
SaKKH-ABEmax	TadA-TadA*	SaKKH-nCas9 (D10A)	NNNRRT	–	2×	4–14	[31]
xABEmax	TadA-TadA*	x-nCas9 (D10A)	NGN	–	2×	4–6	[31]
CP-ABEmax	TadA-TadA*	CP-nCas9 (D10A)	NGG	–	2×	4–14	[31]
ABE7.10 ^{F148A}	TadA-F148A-TadA*-F148A	nCas9 (D10A)	NGG	–	1×	5	[32]
miniABEmax	TadA*	nCas9 (D10A)	NGG	–	2×	4–7	[53]
SECURE-ABEs	TadA*-K20A/R21A or TadA*-V82G	nCas9 (D10A)	NGG	–	2×	4–7	[53]
ABE-P1S	TadA*	nCas9 (D10A)	NGG	–	1×	3–12	[54]
ABE-P2S	TadA*	Sa-nCas9 (D10A)	NNGRRT	–	1×	5–17	[54]
ABE-P5S	TadA*	SaKKH-nCas9 (D10A)	NNNRRT	–	1×	4–9	[54]

Table 2: (continued)

Name	Deaminase	Cas nuclease	PAM	UGI	NLS	Editing window	References
ABE8e	TadA*	nCas9 (D10A)	NGG	–	2×	4–7	[55]
ABE8s	TadA*	nCas9 (D10A)	NGG	–	1×	3–10	[56]
ABE9	TadA*	nCas9 (D10A)	NGG	–	2×	5–6	[57]

TadA*: mutant TadA.

Harvard University developed the first CBE base editor (BE1) [7], which can directly transform C-to-T without requiring DSBs, HDR or donor DNA rAPOBEC1 and dCas9 which is completely lost its cutting activity. Under the guidance of gRNA and dCas9, BE1 achieved effective C-to-T base transition *in vitro* (25%–40%) with the active window range from 4th to 8th of sgRNA (PAM is calculated as 21–23). However, the uracil DNA glycosylase (UDG) restricts the editing efficiency of BE1 in mammalian cells as its reverse U-G mismatches to C-G base pair through the base-excision repair (BER) in the cells. To overcome the role of UDG, the second-generation base editor BE2 (rAPOBEC1-XTEN-dCas9-UGI) was developed by fusing uracil DNA glycosylase inhibitor (UGI) to BE1. UGI inhibits the function of UDG both in mammalian cells and bacteria, resulting in efficient C-to-T conversion. The Liu laboratory developed the third-generation base editor BE3 (rAPOBEC1-XTEN-nCas9-UGI) immediate after BE2. BE3 replaces the dCas9 in BE2 with nCas9 (D10A) to nick the non-edited strand, which activated the intracellular mismatch repair (MMR) using the edited strand as template to increase the efficiency of base editing. The results showed that BE3 achieved an average editing efficiency around 37% at the 6 sites in human cells, which is two to six-fold more efficient than BE2.

Adopting a similar strategy, the Akihiko Kondo laboratory at Kobe University in Japan successfully developed Target-AID base editor which combined dCas9 with PmCDA1 deaminase from sea lamprey in August 2016 [8]. The Target-AID base editor achieved C-to-T fixed-point replacement both in yeast and mammalian cells with an efficiency of 80 and 10%, respectively. The editing window of Target-AID base editor is three to five bases surrounding the –18 position upstream of the PAM sequence. Consistently, the editing efficiency of Target-AID base editor was greatly improved when combined with UGI and D10A nickase. In October 2016, the Cas9-AID-P182X (AIDx) was invented by fusing human cytidine deaminase hAID to the C terminus of dCas9 in Chang laboratory at Shanghai Jiao Tong University in China. Paired with sgRNAs, dCas9-AID was recruited to targeted genomic loci to induce deamination of cytidines with an enhancement of editing efficiency by introducing uracil-DNA glycosylase inhibitor [9]. Notably, unlike the dCas9-APOBEC1 fusion, in the absence of UGI, AIDx converted C to all other three bases. AIDx expanded the editing window and created more mutations within and beyond the protospacers when combined with multiple sgRNAs. At the same time, the CRISPR-X was developed by Michael C. Bassik laboratory at Stanford University [10]. CRISPR-X employed MS2 modified sgRNAs to recruit hAID, which allowed larger range editing when combined with multiple sgRNAs.

In addition, to overcome PAM sequence dependency the George Church laboratory at Harvard University developed zinc finger (ZF) and transcription activator-like effectors (TALEs) nucleases-based editing systems, which had lower editing efficiency compared to the above-mentioned base editors [11].

Optimization of CBE

Product purity

In addition to C-to-T conversion, CBEs also have a certain chance to convert C to all other three nucleotides [58], which decreases the product purity. To overcome this, at the same month, David Liu laboratory developed a series of base editors to increase the product purity [17]. They firstly replaced the APOBEC1 deaminase with CDA1, AID or APOBEC3G deaminases to generate CDA1-BE3, AID-BE3 and APOBEC3G-BE3, which improved the editing efficiency. Then they fused 2xUGI to the C terminus of BE3 (BE3-2xUGI) which resulted in large increased in product purity and editing efficiency compared to BE3. They went on to optimize the linkers between BE3 components and generated BE3C, BE3D and BE3E, which further improved product purity and editing efficiency. They finally integrated all these improvements into a single base editor to create BE4 and derived versions (BE3-Gam, SaBE3-Gam, BE4-Gam, and SaBE4-Gam) which all substantially increased product purity and efficiency. Chen Jia laboratory at Shanghai University of Science and Technology optimized the expression of UGI and established eBE-S3 to improve the purity of the edited product in mammalian cells in August 2017 [16].

Editing efficiency

The expression level and localization of the intracellular base editor is key determinant factor for editing efficiency. Liu and coworkers generated efficient base editors BE4max and AncBE4max by enhancing the nuclear localization signals and optimizing the codon sequence [49]. Zafra et al. developed FNLS-BE3 with an average 15-fold improvement in editing efficiency compared to BE3 by increasing the number of NLS, changing the position of NLS, and optimizing the codon sequence of nCas9 to the codon sequence preferred by mammalian cells [26]. Recently, Li Dali laboratory at East China Normal University developed hyBE4max, hyA3A-BE4max and hyeA3A-BE4max with higher editing range and activity (up to 257-fold) by fusing a single-stranded DNA-binding protein domain between Cas9 nickase and deaminase [38].

PAM dependency

CBEs of SpCas9 protein fusions requires PAM sequence, which greatly limits the targeting range of CBE in the genome. To this end, Liu et al. replaced SpCas9 with other Cas variants with different PAM preference, which expanded the editing coverage of the CBE systems [12]. The current fusion of different variants have transformed CBE from PAM of NGG to the corresponding PAM forms such as NGA, NGCG, NNGRRT and NNNRRT. In addition, dCpf1-eBE was developed by fusing rAPOBEC1 to a catalytically inactive version of *Lachnospiraceae bacterium* Cpf1 to overcome base editors limited by G/C-rich PAM [19]. Kleinstiver et al. engineered an enhanced *Acidaminococcus sp.* Cas12a variant (enAsCas12a) which overcome the requirement of an extended TTTV PAM [29]. Similarly, Zhou et al. fused Sp-macCas9 with rAPOBEC1 to generate Spy-macnCas9-BE3 which recognized a T-rich PAM sequence [21]. Through phage-assisted evolving, Liu and coworkers invented xBE3 which recognized a broad range of PAM sequences including NG, GAA and GAT [18]. Nishimasu et al. created a SpCas9 variant that recognizes NG but not NGG [25]. The SpCas9-NG variant has increased targeting range, has similar specificity to the wild-type enzyme. Zong et al. fused nCas9 with human APOBEC3A to generate A3A-PBE which converted cytidine to thymidine efficiently in wheat, rice and potato with a 17 nt editing window at all examined sites, independent of sequence context [59].

Editing window

The base editing window of different types of CBE is slightly different and should be chosen accordingly. A large modification window will lead to editing of non-target base C around, which brings potential risk when CBE is used in gene therapy and other fields. Hence, Liu laboratory developed YE1-BE3, YE2-BE3, EE-BE3 and YEE-BE3 base editors through mutating key enzyme activation point of the role of the rAPOBEC1 cytosine deaminase, which narrowed the editing window from 5 nt to 1–2 nt with reduced editing efficiency [12]. A series of nCDA1-BE3 versions established by shortening the carboxyl-terminal sequence of PmCDA1 deaminase ensure the editing efficiency and reducing the active window of editing to 1–2 nt [28]. Using circularly permuted Cas9 variants, the Liu laboratory produced four cytosine base editors with an editing window expanded from 4–5 nucleotides to 8–9 nucleotides [31]. They successively developed phage-assisted continuous evolution of base editors (BE-PACE) to improve its editing efficiency and target sequence compatibility such as evoAPOBEC1-BE4max, evoFERNY-BE4max and evoCDA1-BE4max to illuminate the relationship between deaminase activity, base editing efficiency, editing window width and byproduct formation [33]. Liu et al. developed A3G-BE4max to solve the problem of bystander editing when there were multiple C in the editing window [40]. Keith Joung laboratory at Massachusetts General Hospital developed eA3A-BE3 by strengthening the feature that base editing efficiency depends on the sequence background and generated eA3A-HF1-BE3-2 × UGI and eA3A-Hypa-BE3-2 × UGI [23]. These base editors, with decreased editing activity, further reduced editing of adjacent non-target C.

Jiang et al. fused 10 copies of GCN4 peptide to nCas9 for recruiting scFv-APOBEC-UGI-GB1 to the target sites and developed the BE-PLUS system, which broadened the mutation window of BE3 from 5 nt (4–8) to 13 nt (4–16) [22]. Base editors based on fusion hA3A cytosine deaminase have windows of 14 nt (2–13) and 17 nt (1–17) in mammalian cells and plants, respectively [24, 59]. In November 2018, Liu et al. developed DBE-A3A and DBE-AIDmono which can widen the mutation window to positions 2–17, and rely on the CBE system to achieve antibody affinity maturation in cells cultured *in vitro* [27]. In addition, Cheng et al. screened 13 divergent CDAs and CDALs to generate NT-CBEs and CT-CBEs, which both had expanded and diversified editing scopes [34]. Wang et al. developed BE-PIGS by embedding the deaminase into the Cas9 PI domain, which significantly expanded the editing scope [35]. More recently, Chen et al.

reengineered TadA-8e to Td-CGBE with high efficiency and accurate editing window [42].

Adenine base editor (ABE)

Development of ABE

Although adenine deaminases exist in nature with the capability of converting adenine to inosine, such as *Escherichia coli* TadA (ecTadA), mouse ADA, human ADAR2 and ADAT2. However, none of them use DNA as substrate and none of them showed editing activity on ssDNA when combined with nCas9 [43]. Based on the success of CBE, researchers have further developed the Adenine Base Editor (ABE) with similar principle of action of CBE. ABE is fused by nCas9 and an evolved adenine deaminase that enables A·T-to-G·C conversion. With the guidance of sgRNA, ABE binds to the target sequence region and deaminates a certain range of adenine (A) into inosine (I), which will be treated as guanine (G) at the DNA level [60], eventually achieving the A-to-G conversion. ecTadA is a tRNA adenine deaminase [61] that converts adenine to inosine in the single-stranded anticodon loop of $tRNA^{Arg}$ [62, 63]. Through seven rounds of evolution, Liu et al. generated variants of ecTadA with the capability of acting on ssDNA. Fourteen mutations (A106V, D108 N, D147Y, E155V, L84F, H123Y, I156F, H36L, R51L, S146C, K157 N, P48A, and R152P) were identified, creating ABE7.10 [43]. ABE7.10 was fused by nCas9 to wild-type TadA and modified TadA* which forms heterodimers. ABE7.10 was shown to introduce A-to-G point mutation with an average editing efficiency of 58% and an editing window of position 4–9 within the protospacer in living cells [43], although some studies showed that wild-type TadA was not necessary [53].

Optimization of ABE

To improve the editing efficiency, minimize bystander editing, scientists further optimized the ABE system by directed evolution of ecTadA, changing the number and position of NLS, expanding the selection range of PAM sequences [58, 64, 65]. ABEmax had improved editing efficiency by increasing the number of NLS without expanding editing window. By changing the position of NLS, PABE-7 was designed to improve editing efficiency [48]. Adenine base editors such as xABE [18], ABE-P5 [46, 47], VQR-ABE [46, 47], ABE-P4 [47], and NG-ABE [25] were improved in editing efficiency and editing window by expanding the selection of PAM sequences. The directed evolution of ecTadA also improved the editing efficiency of ABE like ABE8e [55] and reduced bystander editing by narrowing the editing window, such as ABE9 [57].

Cytosine and adenine dual base editor

To maximize the editing capability, Liu et al. used a combination of ABE and SaBE3 to achieve both C to T and A-to-G base editing for the first time [66]. In June 2020, Sakata et al. also developed Target-ACEmax to edit C-T and A-G at the same time by combining cytosine and adenine base editors [39]. They also detected that ABE not only generated A-to-G mutant, but also produce unexpected C-to-T mutation with cytosine deamination activity [67].

By phage assisted continuous evolution for cytidine deaminization, David Liu laboratory developed TadCBE with the highly active deoxyadenosine deaminase Tada-8e. They also generated dual-base editor TadDE that performs equally efficient base editing of cytosine and adenine [41]. By repurposing adenine deaminase TadA-8e for cytosine conversion with introduction of the N46L mutation in TadA-8e, they developed highly specific and efficient C-to-G base editor Td-CGBE [42].

Application and perspectives

Since the development of base editing system, CBE and ABE show tremendous application potential both in basic and translational research, including establishment of various mammalian cell lines, animal modeling, agricultural breeding and disease treatment. Zong et al. at the Chinese Academy of Sciences applied base editing in regenerative rice, wheat and maize plants at frequencies of up to 43.48% [13]. They also fused cytidine deaminase and adenosine deaminase to generate engineered dual base editors STEMEs, including STEME-1, STEME-2, STEME-3, STEME-4 and STEME-NG which introduced *de novo* mutations and facilitate directed evolution of plant genes [36]. Liang et al. generated a modified high-fidelity version of base editor 2 (HF2-BE2) with 100% biallelic C-to-T editing efficiency in mouse embryos [14].

ABE was used to generate Fah mutation in mice to mimic human type I hereditary tyrosinemia (HTI) [46]. ABE was also used to create rat model of Pompe disease [46], rabbit models of X-linked dilated cardiomyopathy (XLCM), and amyotrophic lateral sclerosis (ALS) [68]. The successful applications indicate that ABEs are powerful tools for gene editing *in vivo*, providing new tools for the establishment of new models and disease treatment.

For human point mutation diseases, such as sickle cell disease and β-thalassemia, ABE8e successfully created two mutations in the promoter of *HBG* genes, which increased the expression of fetal hemoglobin [55]. ABE9 corrected missense mutations of *COL1A2* gene in autosomal dominant osteogenesis imperfecta, mutations of *CARD14* gene in psoriasis, BVES mutations in myotonic dystrophy and *KCNA5* mutations in common arrhythmia [57].

The ABE systems were also used for gene optimization in rice. ABE-P1 (ABE7.10), ABE-P4 (VERE-ABE), and ABE-P5 (SaKKH-ABE) successfully completed the A·T-to-G·C transformation without introducing any indels or bystander mutations [44]. Application of PAGE-7 resulted in increased herbicide resistance in wheat and rice, which reduced the time cost of weeding [48].

Although base editing techniques have transformed life sciences and exhibit huge potential for precise treatment of genetic disorders, how to further reduce the mutation window and truly modify genes at a single base resolution without affecting the side sequence will be the future focus of base editing system. The recently developed Prime Editing (PE) systems are more promising in accurate base replacement, short sequence insertion and large DNA deletion with a lower editing efficiency when compared to latest version of base editors in general. Well-tailored base editing tools, complementary to prime editing systems, along with cutting-edge delivery methods hold great potential in precision medicine.

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